Infection by the parasitic helminth *Trichinella spiralis* activates a Tas2r-mediated signaling pathway in intestinal tuft cells

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The parasitic helminth *Trichinella spiralis*, which poses a serious health risk to animals and humans, can be found worldwide. Recent findings indicate that a rare type of gut epithelial cell, tuft cells, can detect the helminth, triggering type 2 immune responses. However, the underlying molecular mechanisms remain to be fully understood. Here we show that both excretory–secretory products (E–S) and extract of *T. spiralis* can stimulate the release of the cytokine interleukin 25 (IL-25) from the mouse small intestinal villi and evoke calcium responses from tuft cells in the intestinal organoids, which can be blocked by a bitter-taste receptor inhibitor, allyl isothiocyanate. Heterologously expressed mouse Tas2r bitter-taste receptors, the expression of which is augmented during tuft-cell hyperplasia, can respond to the E–S and extract as well as to the bitter compound salicin whereas salicin in turn can induce IL-25 release from tuft cells. Furthermore, abolishment of the G-protein γ13 subunit, application of the inhibitors for G-protein αo/i, Gιγ subunits, and phospholipase Cζ2 dramatically reduces the IL-25 release. Finally, tuft cells are found to utilize the inositol triphosphate receptor type 2 (Iπp2) to regulate cytosolic calcium and thus Trpm5 activity, while potentiation of Trpm5 by a sweet-tasting compound, stevioside, enhances tuft cell IL-25 release and hyperplasia in vivo. Taken together, *T. spiralis* infection activates a signaling pathway in intestinal tuft cells similar to that of taste-bud cells, but with some key differences, to initiate type 2 immunity.

Significance

Intestinal tuft cells are sentinels monitoring the luminal contents and play a critical role in type 2 immunity. In this work, *Trichinella spiralis* excretion–secretion and extract were shown to directly induce interleukin 25 (IL-25) release from the intestinal villi, evoke calcium responses in tuft cells, and activate Tas2r bitter-taste receptors, whereas the bitter compound salicin was shown to activate and induce tuft cells to release IL-25. Gu-gustducin/Gιγ13 and/or Geo/Gιγ13, Pl(γ)2, Iπp2, and Trpm5 comprise the signal transduction pathways that tuft cells utilize to initiate type 2 immune responses. Potentiation of Trpm5 by a natural sweet compound, stevioside, can enhance the tuft cell–ILC2 circuit’s activity, indicating that modulating these signaling components can help devise new means of combating parasites.
Results

*T. spiralis* Infection Triggers Tuft- and Goblet-Cell Hyperplasia in the Mouse Duodenum, Jejunum, and Ileum. Since different parasitic helminths have their preferred habitats and thus evoke the host’s immune responses in different tissues (17), we set out to determine the extent to which each segment of the mouse small intestine remodels its epithelium following the helminth invasion. Two weeks postoral inoculation of 400 *T. spiralis* muscle larvae into each mouse, each small intestine was fixed, sectioned, and stained with an antibody against a tuft-cell marker, doublecortin-like kinase 1 (Dclk1), and with Aluin blue-nuclear fast red to visualize goblet cells, respectively. Significant increases in the numbers of tuft and goblet cells as well as the size of goblet cells were found in all proximal, middle, and distal segments of the small intestine (SI Appendix, Figs. S1 and S2).

*T. spiralis* Activates Bitter-Taste Receptors (Tas2rs) on Tuft Cells. Tuft cells are found to express many taste signal transduction components and have been postulated to act as sentinels to monitor and respond to infectious pathogens (18). We hypothesized that the Tas2r bitter-taste receptors may be able to sense the parasitic helminths. To test this hypothesis, we prepared mouse small intestinal villi, stimulated them with the excretion-secretion (E–S) and extracts of *T. spiralis* muscle larvae and adult worms, and then measured the IL-25 released from the villi. The results showed that both the extracts and E–S elicited significantly more IL-25 than the vehicle-treated control (Fig. 1A and SI Appendix, Fig. S3). However, when the villi were pretreated with a bitter-taste receptor inhibitor, allyl isothiocyanate (AITC), a component of mustard oil (19, 20), *Ts* extract-induced release of IL-25 was significantly reduced (Fig. 1A). To further confirm the activation of tuft cells by *Ts* products, we prepared intestinal organoids from a gene knock-in mouse line, Trpm5-lacZ, in which the lacZ-encoded β-galactosidase is faithfully expressed in the cells normally expressing Trpm5. Tuft cells in the organoids from the Trpm5-lacZ/+ heterozygous mice containing one copy of an intact Trpm5 gene and one copy of the lacZ gene were then identified by their red fluorescence from the compound 2-dodecylresorufin in the cells after incubation with the ImaGene Red β-galactosidase substrate dodecylresorufin β-d-galactopyranoside (Fig. 1 B and C, Insets). Cells were then loaded with the Ca^2+/-sensitive dye Fluo-4 AM and stimulated with *Ts* extract or E–S products. Transient increases in intracellular Ca^2+ concentrations were observed in the red cells, indicating that Trpm5-expressing tuft cells responded to both *Ts* extract and E–S products (Fig. 1 B and C and SI Appendix, Fig. S4).

To assess Tas2r expression in the small intestine and any changes in its expression during type 2 immune response to *Ts* infection, we carried out reverse transcription–qPCR (RT-qPCR) assays with RNAs prepared from the intestines with or without *Ts* infection. The results showed that, among all 35 mouse Tas2r genes, the expression of 8 Tas2r108—Tas2r114, Tas2r117, Tas2r122, Tas2r130, Tas2r136, and Tas2r143—was significantly up-regulated, 8 others significantly down-regulated, and the remaining 19 unchanged (Fig. 1D and SI Appendix, Figs. S5 and S6).

Small mouse small intestinal villi contain not only epithelial cells but also immune cells and others (21), small intestinal organoids possess much enriched epithelial cells originating from the Lgr5-expressing stem cells in the gut (22). To obtain more accurate expression data from tuft cells, we again performed qPCR assays with the cultured small intestinal organoids following the IL-13 treatment that remarkably increases tuft-cell abundance (SI Appendix, Fig. S4). The results showed that IL-13 treatment significantly up-regulated the expression of 3 Tas2r108—Tas2r117, Tas2r136, and Tas2r143—11 others significantly down-regulated, and the remaining 21 *Ts*2rs unchanged (Fig. 1E and SI Appendix, Fig. S5). Meanwhile, expression of the tuft-cell marker genes Par2, Dclk1, and Sucnr1 was also significantly up-regulated (SI Appendix, Fig. S5), consistent with the tuft-cell hyperplasia (SI Appendix, Fig. S1). To confirm the expression these Tas2rs in tuft cells, in situ hybridization was performed on small intestinal sections followed by immunostaining with the Dclk1 antibody. The results indicated that individual *Tas2r* gene transcripts were localized to subsets of tuft cells (SI Appendix, Fig. S7).

![Fig. 1.](image)

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**A.** Tas2r receptors sense and initiate the response to the helminth *T. spiralis*. (A) *Ts* extract of muscle larvae (*Ts* ext.) stimulated the small intestinal villi to release significantly more IL-25 than the vehicle treatment; preincubation with AITC (*Ts* ext.+AITC) significantly reduced IL-25 release (*n* = 4). Representative traces of Ca^2+ responses to *Ts* ext. (B) and to *Ts* E–S (C) are shown from tuft cells of small intestinal organoids derived from a Trpm5-lacZ heterozygous mouse. Red fluorescence was used to identify tuft cells (Insets). (D) qPCR showed that *Ts* infection increased expression of eight Tas2r108—Tas2r114, Tas2r117, Tas2r122, Tas2r130, Tas2r136, and Tas2r143—and down-regulated eight others in the small intestinal villi. (E) qPCR showed that IL-13 treatment increased expression of 3 Tas2r108—Tas2r117, Tas2r136, and Tas2r143—and down-regulated 11 others in the intestinal organoids. (F) Fluorescent images of the Ca^2+/-sensitive dye Fluo-4 AM-loaded HEK293 cells cotransfected with *Tas2r143* and G-actin tagged with a cotransfection efficiency of 80%, before (HBSS) and after *Ts* ext. stimulation (Ts ext.). About 28% of the Fluo-4 AM-loaded cells responded to *Ts* ext. Three representative responding cells are marked by arrowheads. (G) Traces of Ca^2+ responses from the three marked cells in F. (H) Salcin stimulation released significantly more IL-25 than the vehicle treatment from the small intestinal villi; preincubation with AITC (Salcin+AITC) significantly reduced IL-25 release. *P* < 0.05; **P < 0.01; ***P < 0.001. (Scale bars, 50 μm.)
In an attempt to predict the up-regulated ’Tas2rs’ ligands, we performed phylogenetic analysis against human TAS2Rs. The results revealed that the eight mouse Tas2rs up-regulated by T. muris infection show high diversity in amino acid sequences from one another but display certain similarity with some human bitter receptors; in particular, mouse Tas2r143 has a high-level identity to human TAS2R16 (SI Appendix, Fig. S8). Further, we carried out receptor protein structure modeling and found that all these 8 Tas2rs have very different predicted receptor structures (SI Appendix, Fig. S8). However, the predicted mouse Tas2r143 has a nearly identical receptor structure to that of human TAS2R16. Since Tas2r143 is expressed in tuft cells (23) and human TAS2R16 can be activated by a bitter compound, salicin (24), we reasoned that salicin may be able to activate Tas2r143 on mouse intestinal tuft cells. Indeed, both Ts extract and salicin activated heterolo-
gously expressed mouse Tas2r143 receptors, which were inhibited by AITC (Fig. 1 F and G and SI Appendix, Fig. S9). Furthermore, salicin evoked calcium responses from tuft cells of small intestinal organoids and induced IL-25 release from the intestinal villi, which were Trpm5-dependent and inhibited by AITC (Fig. 1H and SI Appendix, Fig. S10).

Tuft Cells Use the Trimeric G Proteins Go,-Gustducin/Gja113 and Go/-
Gja13 to Mediate the Responses to the Parasitic Helminths. Previous studies have shown that the Gja3-encoded G-protein α-subunit α-gustducin is involved in the response to the protist T. muris and to the bacteria-produced succinate (8, 12). To determine whether other G-protein subunits also contribute to tuft-
cell responses, we performed qPCR to analyze the expression of the genes for all known α, β, and γ subunits as well as their variants Gnao1-A and Gnao1-B in the villi isolated from the mouse duodenums with or without Ts infection. Comparative analyses revealed that Ts infection resulted in significant in
creases in expression of three Ga genes—Gna1-B, Gna3, and Gja13—but in decreased expression for nine other Ga genes with the exception of four Ga genes unchanged (Fig. 2A and SI Appendix, Fig. S11). As to G-protein β γ subunits, Ts infection significantly increased the expression of Gnb5, Gng7, and Gng13, but reduced 4 others with the remaining 9 unaffected (Fig. 2B and SI Appendix, Fig. S11).

The qPCR analysis with the intestinal organoids showed that IL-13 treatment dramatically increased the expression of Gna13, Gna3, and Gnao1-B and Gnb5, and Gng13 in the duodenal organoids (Fig. 2B). In addition, IL-13 treatment also augmented the expression of Gnb1, which was unchanged in the Ts-infected duodenums. Further comparison of the data revealed that Gnaz and Gna13 were down-regulated in both the Ts-
infected villi and the IL-13–treated organoids, but an additional four genes were down-regulated only by IL-13 treatment; and conversely, 11 other genes were down-regulated only in the Ts-infected villi. Gna1 and Gng1 expression was undetectable in the villi but detectable in the organoids; nevertheless, their expression was unaffected by the IL-13 treatment (Fig. 2 B and SI Appendix, Fig. S11).

To confirm the qPCR data and to localize the proteins of the genes up-regulated by the Ts infection and IL-13 treatment, we carried out immunostaining on mouse small intestinal sections with antibodies against α-gustducin, Dclk1, and other proteins encoded by the up-regulated genes (Fig. 2 and SI Appendix, Fig. S12). As reported previously, α-gustducin and Dclk1 are colo-
calized to a subset of small intestinal epithelial cells (Fig. 2C) (8). Interestingly, an antibody that can bind to both A and B isoforms of Goo, encoded by Gnaol-A and -B, respectively, stained some tuft cells with the Dclk1 antibody while some epithelial cells were stained by the Goo antibody alone, indicating that Goo is expressed not only in tuft cells but also in some other Dclk1-negative epithelial cells (Fig. 2D). In contrast, the antibody against Gna13-encoded Gx15 stained cells in the lamina propria without overlapping with Dclk1 staining at all, indicating that Gx15-expressing cells were not tuft cells or even epithelial cells (Fig. 2E). Double immunostaining also showed that both Gj11 and Gx13 but not Gj5 are colocalized with Dclk1, indicating that Gj113 subunits are expressed in tuft cells (Fig. 2 F–H). Notably, Gj11 proteins seemed to be concentrated at the tip of tuft cells (Fig. 2F).

To validate the role of these up-regulated G-protein subunits in tuft-cell physiology, we performed IL-25 ELISAs on the small intestinal villi with the Ts extract in combination with G-protein–specific pharmacological agents. Similar to the results shown in Fig. 1A, Ts extract evoked the small intestinal villi to release IL-
25. However, preincubation with pertussis toxin, an inhibitor specific for the G-protein αi/subfamily, or with a myr-
istyaled peptide inhibitor specifically for Gαo (25), blocked Ts extract-induced release of IL-25 (Fig. 3 A and B). Similarly, the Gj5-subunit inhibitor gallex4 also inhibited the IL-25 release from Ts extract-evoked villi (Fig. 3C).

To genetically verify Gja13’s role in the regulation of IL-25 release from tuft cells, we took advantage of a conditional gene knockout mouse line, Lgr5-EGFP-IRES-CreERT2/Gng13flox/flox which was generated by crossing the Lgr5-EGFP-IRES-CreERT2 (26) mouse with the Gng13flox/flox mouse (27). Tamoxifen ad-
mistration rendered CreERT2 recombinase activity in the Lgr5-
expressing stem cells in the gut, abolishing Gj3 expression in tuft cells (SI Appendix, Fig. S13). ELISAs showed that Ts extract-evoked release of IL-25 from the Gng13−/− villi was significantly

![Image](https://example.com/image.png)
red tested compared with WT control (Fig. 3D), indicating that Gβ13 is critical to the IL-25 release from tuft cells. To further confirm Gβ13’s contribution to type 2 immune response in vivo, another Gng13−/− mouse, Vil1-Cre;Gng13floxfloxflox mouse, was generated by deleting the Vil1-Cre mouse (28) with the Gng13floxfloxflox mouse, nullifying the Gng13 expression in the small intestine constitutively. Ts infection resulted in much reduced tuft-cell hyperplasia in the knockout small intestines compared with WT control (SI Appendix, Figs. S13 and S14).

The Plcβ2-Ip3p2 Axis Is Part of the Tuft-Cell Intracellular Signaling Cascade. The G-protein Gβ13 dimer is known to activate Plcβ2 to generate the second messengers inositol 1,4,5-triphosphate (IP3) and diacylglycerol whereas IP3 binds to its receptor on the endoplasmic reticulum (ER), releasing Ca2+ ions from the ER into the cytosol and elevating the cytoplasmic free Ca2+ concentration (29). Plcβ2 is found to be expressed in tuft cells (Fig. S4). To test Plcβ2’s role in tuft cells, we evaluated the effect of the Plcβ2 inhibitor U73122 on the Ts extract-induced IL-25 release. Preincubation with U73122 significantly reduced the amount of IL-25 released from the villi in response to Ts extract compared with that without U73122 (Fig. 4B).

To determine which IP3 receptor subtype is activated by IP3, we performed immunostaining with the antibodies against the receptor subtypes Ip3r2 and Ip3r3. Double immunostainings showed that Ip3r2 was nearly completely overlapped with Dclk1 in tuft cells (Fig. 4C). In contrast, no overlapping was observed between Ip3r3 and Dclk1 on the intestinal epithelial cells, and Ip3r3 immunostaining was restricted mostly to the lamina propria (Fig. 4D and SI Appendix, Fig. S15). Thus, unlike taste-bud cells that employ Ip3r3 to transduce taste signals, tuft cells use Ip3r2 to convey the parasitic signals forward.

Potentiation of Trpm5 Facilitates the Activation of the Tuft Cell–ILC2 Circuit. Trpm5 is coexpressed with Dclk1 in tuft cells (8); thus, the LacZ gene in the Trpm5-lacZ knockin mice is colocalized with Dclk1 in tuft cells (SI Appendix, Fig. S16). To determine whether Ts-induced IL-25 release from tuft cells requires Trpm5, we quantified the released IL-25 from the small intestinal villi of WT vs. Trpm5−/− knockout/lacZ knockin mice (Trpm5−/−lacZ). Results showed that Trpm5−/− significantly reduced Ts extract-induced IL-25 release compared with WT control (Fig. 4E). Furthermore, Ts infection significantly increased tuft-cell abundance in WT but not in Trpm5−/− mice (SI Appendix, Fig. S17), indicating that Trpm5 is required for Ts-induced IL-25 release and tuft-cell hyperplasia.

To further elucidate Trpm5’s role in type 2 immune response, we incubated the small intestinal villi with a Trpm5 potentiator, stevioloside, a noncaloric sweet-tasting compound present in the plant Stevia rebaudiana (30). The results showed that stevioloside elicited significantly more IL-25 from WT than from Trpm5−/− villi, which is similar to the effect of Ts extract on these two types of villi (Fig. 4 E and F). Furthermore, oral administration of stevioloside engendered tuft- and goblet-cell hyperplasia in the small intestine, similar to the effect of 5 mM succinate (12) (Fig. 4G and SI Appendix, Figs. S18–S22).

Recent studies have revealed that many cytokines are released from the intracellular stores via vesicular secretion (31). To determine whether IL-25 is also secreted from tuft cells, a vesicular transport inhibitor, brefeldin A (BFA) (32), was used to preincubate the small intestinal villi. The results showed that BFA significantly reduced the IL-25 release from tuft cells in response to Ts extract (SI Appendix, Fig. S23).

Discussion

Pathogens and Tuft Cells. Recent studies have shown that tuft cells can detect and respond to multiple types of pathogens, including bacteria, protists, and nematodes. Parasitic helminths may have different preferred habitats inside their hosts, thus stimulating tuft cells residing in particular locations (33). Our data show that Ts infection incurred tuft- and goblet-cell expansion throughout the proximal, middle, and distal segments of the small intestine (SI Appendix, Figs. S1 and S2), which is consistent with the expansion caused by the other two helminths studied so far, N. brasiliensis and H. polygyrus. However, both Ts and N. brasiliensis can provoke a 10-fold increase in tuft-cell abundance whereas H. polygyrus is able to provoke a 5-fold increase (9). The difference in effectiveness can be a result from tripartite interactions among parasitic worms, enteric microflora, and gut cells (12, 33–36). It is also possible that tuft cells express multiple sets of receptors to sense different infectious agents, prompting host cell responses at different intensities. Further studies are needed to understand the underlying molecular mechanisms.

Tas2r Receptors Are Tuft-Cell Sensors for T. spiralis. Many taste-signaling proteins have been found in tuft cells. However, there was no strong evidence supporting the role of Tas2rs in the tuft

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cell–ILC2 circuit. In this study, we have shown that both Ts muscle larva and adult worm extracts as well as E–S products can elicit not only Ca2+ responses from tuft cells but also stimulate small intestinal villi to release IL-25, which was blocked by AITC (Fig. 1 A–C and SI Appendix, Figs. S3 and S4), strongly suggesting that the Tas2rs are critical to sensing and initiating type 2 immunity. qPCR assays indicated that Ts infection and IL-13 treatment up-regulated the expression of 8 and 3 Tas2rs, down-regulated 8 and 11 others, and left 19 and 21 unaltered in the small intestinal villi and organoids, respectively, whereas expression of tuft-cell marker genes was significantly increased, indicating robust tuft-cell hyperplasia in both Ts-infected intestinal villi and IL-13-treated organoids (Fig. 1 D and E and SI Appendix, Figs. S4 and S5). The up-regulated Tas2rs were likely expressed in the expanded tuft cells, while the down-regulated ones were expressed in the lesser represented cells with the unchanged ones possibly expressed in the cells of unchanged representation. However, Ts infection augmented the expression of eight Tas2rs in the villus cells while IL-13 treatment increased only three of these eight Tas2rs in the organoids. This discrepancy may be attributable to the differences in tuft-cell types contained in the two samples: the intestinal villus sample may contain more types of tuft cells while the organoids had fewer types due to their limited number of organoid cells. Our in situ hybridization data in the present study showed that individual Tas2r genes are expressed in subsets of tuft cells that can be divided into additional types (SI Appendix, Fig. S7) (16, 23, 37). In addition, the villus sample also contained other cells, such as immune cells, whereas the organoids consisted of mostly epithelial cells. The same reasons can also explain the discrepancies in the down-regulated Tas2rs between the Ts-infected intestinal villi and the IL-13–treated organoids.

Homolog analysis found that the eight up-regulated Tas2rs possess very diverse sequences (SI Appendix, Fig. S8), but some of them share high similarities with some human TAS2Rs. Computational modeling showed that mouse Tas2r143 is structurally similar to human TAS2R16 (SI Appendix, Fig. S8). Interestingly, Ts extract can indeed stimulate the heterologously expressed Tas2r143, which can also be activated by the human TAS2R16’s ligand salicin and inhibited by AITC (Fig. 1 F and G and SI Appendix, Fig. S9). Furthermore, salicin can activate tuft cells in the organoids and stimulate IL-25 release from the intestinal villi in an AITC-inhibitable, Trpm5-dependent manner (Fig. 1 H and SI Appendix, Fig. S10). Together, these data indicate that Tas2r143 plays an important role in Ts-evoked tuft-cell release of IL-25. It is possible that the other seven up-regulated Tas2rs also respond to Ts. However, it remains to be determined whether these receptors also contribute to the initial sensing of Ts infection.

Tas2rs have been found in many extracellular tissues (38–40), where they detect irritants and bacterial metabolites and regulate immune responses (41, 42). In the gut, these receptors play key roles in the tuft–ILC2 circuit. In addition to Tas2rs, however, other receptors, such as those for short-chain fatty acids or succinate, are also essential for intestinal type 2 immunity (12, 43). How these different signaling components orchestrate in tuft cells can be important to the effectiveness of type 2 immunity against pathogens.

**Both Gao/Gpα13 and Gα-Gustducin/Gpα13 Mediate Signal Transduction.** Using the RT-qPCR approach, we discovered that Ts infection increased expression of three Gz genes (Gna13, Gnao1-B, and Gna15), one Gβ gene (Gnb5), and two Gγ genes (Gng7 and Gng13) in the small intestine whereas IL-13 treatment also up-regulated gene expression of Gna13, Gnao1-B, Gβ, Gγ, and Gγ3. But Gnb1, instead of Gng7, was up-regulated in the IL-13–treated organoids. The reason that the significant increase of Gng7 expression in the infected intestine was not observed in the IL-13–treated organoids could be that Gng7 is expressed in the cells unaffected by IL-13 in the organoids. On the other hand, Gnb1 is probably expressed in tuft cells as well as in many other intestinal cells; thus, the increase in its expression contributed by the Ts-induced tuft-cell hyperplasia was diluted by its constitutive expression in many other cells and did not reach a statistical significance. In contrast, the intestinal organoids are enriched with epithelial cells including tuft cells, and the increases in the expression of the genes expressed in tuft cells are more readily detected during tuft-cell hyperplasia (SI Appendix, Fig. S5).

Immunohistochemical studies showed that Gα-gustducin, Gao, Gβ1, and Gγ13 are colocalized with Dclk1 and expressed in tuft cells (Fig. 2). However, Gdi15 and Gβ5 are not expressed in tuft cells and thus probably not involved in tuft-cell signal transduction. Notably, similar to sialic-acid–binding Ig-type lectin F (9), Gβ1 proteins are enriched at the tip of tuft cells, facilitating coupling of G-protein–coupled receptors (GPCRs) to G proteins. In addition, some Gao-expressing intestinal epithelial cells do not express Dclk1, suggesting the existence of Dclk1–negative tuft cells (16).

The Gao-β-, Gao-γ-, and Gβγ-specific inhibitors, including per-tussis toxin, Gao peptide inhibitor and gallein, respectively, significantly blocked Ts extract-induced IL-25 release from the intestinal villi (Fig. 3 A–C), indicating that Gα-gustducin and Gao, as well as Gβγ13 subunits, transduce the parasitic signals and stimulate the paracrine IL-25 release from the intestinal villi. Gγ3 significantly reduced both Ts extract-evoked IL-25 release from the intestinal villi and Ts infection-induced tuft-cell hyperplasia, indicating that Gγ3 is a key component of the tuft-cell signaling pathway (Fig. 3 D and SI Appendix, Fig. S14).

Taken together, our data indicate that both Gao/Gpα13 and Gα-gustducin/Gpα13 are the heterotrimeric G proteins transducing parasitic signals. Gγ3 has been known to form functional trimeric G proteins with both Gao and Gα-gustducin, respectively (44–46). And Gα-gustducin has been known to be involved in the sialic-acid- and T. murre-evoked type 2 immunity. Given the diversity of pathogens, tuft cells may utilize multiple GPCRs and G proteins to detect and transduce pathogenic signals.

**The Plcβ2-Ip2r2 Axis of the Intracellular Signaling Pathway.** Plcβ2 is expressed in tuft cells (8) (Fig. 4 A). Pharmacological inhibition of Plcβ2 activity with U73122 significantly reduced Ts extract-induced IL-25 release from the intestinal villi (Fig. 4 B), indicating that Plcβ2 is an intermediary signaling protein. Plcβ2 in tuft cells is likely to be activated by Gβγ13, as in taste-bud cells (45). Activated Plcβ2 generates the second messenger IP3, which in turn knock out of Gng13 significantly reduced both Ts extract-evoked IL-25 release from the intestinal villi and Ts infection-induced tuft-cell hyperplasia, indicating that Gγ3 is a key component of the tuft-cell signaling pathway (Fig. 3 D and SI Appendix, Fig. S14).

The Plcβ2-Ip2r2 axis of the intracellular signaling pathway. Plcβ2 is expressed in tuft cells (8) (Fig. 4 A). Pharmacological inhibition of Plcβ2 activity with U73122 significantly reduced Ts extract-induced IL-25 release from the intestinal villi (Fig. 4 B), indicating that Plcβ2 is an intermediary signaling protein. Plcβ2 in tuft cells is likely to be activated by Gβγ13, as in taste-bud cells (45). Activated Plcβ2 generates the second messenger IP3, which in turn knock out of Gng13 significantly reduced both Ts extract-evoked IL-25 release from the intestinal villi and Ts infection-induced tuft-cell hyperplasia, indicating that Gγ3 is a key component of the tuft-cell signaling pathway (Fig. 3 D and SI Appendix, Fig. S14).

**Trpm5 Is a Key Component in the Tuft Cell–ILC2 Circuit.** Trpm5 is critical to T. murre or succinate-elicited type 2 immunity (8, 12). Here we have shown that Trpm5 is also essential for Ts-induced IL-25 release from the intestinal villi as well as for Ts infection-evoked tuft-cell hyperplasia (Fig. 4 E–G and SI Appendix, Fig. S17). Interestingly, stevioloside, a sweet-tasting Trpm5’s potentiator, could also elicit IL-25 release from WT but not Trpm5–/– intestinal cells and trigger tuft-cell hyperplasia in WT but not in Trpm5–/– KO mice (Fig. 4 and SI Appendix, Figs. S18 and S19). This effect can be explained by the fact that tuft cells maintain certain levels of basal activity due to Ip2r2’s intrinsic activity and constant exposure to the plethora of gut microbes, which can be significantly amplified by stevioloside. Indeed, 0.5 mM stevioloside
seemed to be more potent than 5 mM succinate in launching tuft- and goblet-cell hyperplasia (Fig. 4G and SI Appendix, Figs. S20 and S21). Interestingly, stevioloside has been used to treat obesity and stomach burn and to increase immune activity (49). But the underlying molecular mechanisms are yet to be elucidated. Our data indicate that TrpML5 is likely the stevioside’s target.

Cytokines are known to be released from the cells via several ways, but many of them are through vesicular secretion (31, 32). Our data show that preincubation of the intestinal villi with the vesicular transport inhibitor BFA significantly blocked IL-25 release (SI Appendix, Fig. S23), indicating that IL-25 is also secreted via vesicles.

In summary, our results have shown that tuft cells utilize a similar but different signaling pathway from that used by taste-bud cells (SI Appendix, Fig. S24): Ts5 molecules activate Tas2rs, which in turn stimulate Gqα-gustducin/Goβγ13 or Gαo/Gβγ13; the G proteins dissociate into Gq and Goβγ13 moieties, and the latter acts on Pkcβ2, generating IP3; IP3 binds to IP3R2, releasing Ca2+ from the endoplasmic reticulum into cytosol; the Ca2+ ions open TrpML5, leading to the influx of positively charged Na+ ions, depolarizing the membrane potential, eventually triggering the vesicular release of IL-25 from tuft cells; IL-25 activates ILC2s, which produce IL-4 and IL-13; and the cytokines promote the proliferation and differentiation of stem/progenitor cells preferentially into tuft and goblet cells, resulting in tuft- and goblet-cell hyperplasia and consequently wheeze and sweep responses. Fuller understanding of the molecular mechanisms underlying the tuft cell–ILC2 circuit can provide novel insights into type 2 immunity and help devise new ways to combat widespread parasites.

Materials and Methods

CS7BL/6 mice and Sprague-Dawley rats were purchased from the Shanghai SLAC Laboratory Animal Co. TrpML5−/−, C57BL/6, and Lgr5−/−EGFP-IRES-CreERT2 mice (Jax stock numbers 005848, 021504, and 008875, respectively) were obtained from the Jackson Laboratory. Studies involving animals were approved by the Zhejiang University Institutional Animal Care and Use Committee. More details about experimental materials and methods are described in SI Appendix. The primers used for qPCR are listed in SI Appendix, Table S1.

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