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, Gβγ subunits, and phospholipase Cβ2 dramatically reduces the IL-25 release. Finally, tuft cells are found to utilize the inositol triphosphate receptor type 2 (Ip_3r2) 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.

α-gustducin | Gαo | Gβ1γ13 | Ip_3r2 | type 2 immunity

The mammalian gut epithelium is a single layer of cells that covers the luminal surface of the intestine. The function of the epithelial cells includes not only absorbing nutrients and forming a barrier to protect the rest of the body but also communicating with the gut microbiota that comprises an enormous number of commensal, symbiotic, and pathogenic microorganisms such as viruses, archaea, bacteria, fungi, and parasitic helminths (1, 2). A growing body of evidence has shown that the crosstalk between the gut epithelial cells and microbiome has profound impact on the host's physiology and health (3–6).

Recent studies indicate that a rare type of intestinal epithelial cells, tuft cells, provides a critical link to the infection of viruses, protozoa, and helminths (7–11) as well as to the alterations in the gut microflora (12). Upon activation by some unknown signals from parasitic nematodes such as *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus* or the protozoan *Trichuris muris*, sparse tuft cells initiate an intracellular signaling cascade, leading to the release of interleukin 25 (IL-25), which directly acts on intestinal type 2 innate lymphoid cells (ILC2s) in the lamina propria to secrete interleukin 13 (IL-13). In turn, IL-13 directs stem and progenitor cells in the crypt to proliferate and preferentially differentiate into tuft and goblet cells, leading to the "weep and sweep" response including mucus secretion and parasite expulsion from the gut.

Thus, this feed-forward circuit of the immune response consists of tuft cells, IL-25 release, ILC2s, IL-13 production, tuft- and goblet-cell hyperplasia, and final elimination of the parasites (8–13). How the initial few sentinel tuft cells detect and respond to the parasite invasion appears to be the key to the steps to switch on the circuit. Gene expression analyses found that tuft cells express some taste-signaling proteins, suggesting that tuft cells are chemosensory cells that may employ taste-signaling pathways to sense and transduce parasitic signals (14–16). Indeed, the heterotrimeric G-protein α-subunit α-gustducin is critical to mounting a type 2 immune response to *T. muris* (8) and to the succinic acid-producing bacteria (12) whereas a transient receptor potential ion channel, Trpm5, is required for tuft cells to turn on the circuit in response to *T. muris* and to the altered microflora (8, 12). It is, however, still unknown how the low number of tuft cells are maintained during the rapid intestinal epithelial cell turnover in the absence of any parasites or their metabolites.

In this study, we identified and functionally characterized Tas2r receptors and other key signaling components utilized by tuft cells in response to one of the parasitic helminths, *Trichinella spiralis* (*Ts*), and provided a more comprehensive understanding of the molecular mechanisms that may underlie the initiation of type 2 immune responses upon infection.

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. Gα-gustducin/Gβ1γ13 and/or Gαo/Gβ1γ13, Plcβ2, Ip_3r2, 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.

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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 *Ts* 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 Alnin 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 *Ts* 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. 1*A* 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. 1*A*). 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-PCR) 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 *Tas2r*s—*Tas2r108, Tas2r110, Tas2r114, Tas2r117*, *Tas2r122, Tas2r130, Tas2r136*, and *Tas2r143*—was significantly up-regulated, 8 others significantly down-regulated, and the remaining 19 unchanged (Fig. 1*D* and *SI Appendix*, Figs. S5 and S6).

Since 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 *Tas2r*s—*Tas2r117*, *Tas2r136*, and *Tas2r143*—11 others significantly down-regulated, and the remaining 21 *Tas2r*s unchanged (Fig. 1*E* 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).

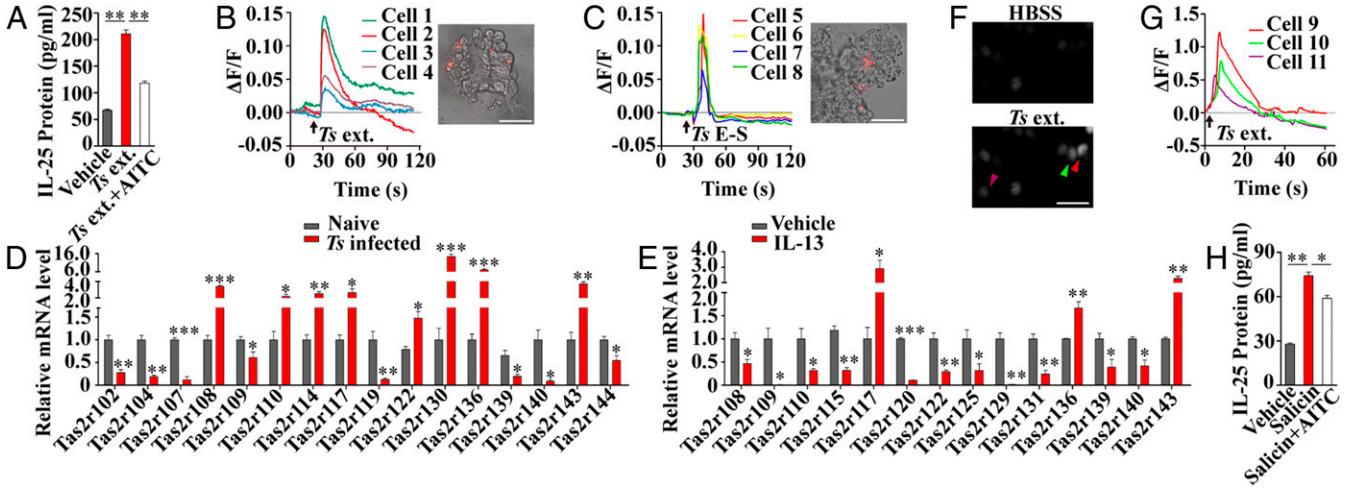

Fig. 1. Tas2r receptors sense and initiate the response to the helminth *T. spiralis*. (*A*) *Ts* extract of muscle larvae (*Ts* ext.) stimulated the small intestinal villi to release significantly more IL-25 than the vehicle treatment; preincubation with AITC (*Ts* ext.+AITC) significantly reduced IL-25 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 *Tas2rs*—*Tas2r108*, *Tas2r110*, *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 *Tas2rs*—*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*/Gα16-gust44 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*) Salicin stimulation released significantly more IL-25 than the vehicle treatment from the small intestinal villi; preincubation with AITC (Salicin+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 *Ts* 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 heterologously 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. 1*H* and *SI Appendix*, Fig. S10).

Tuft Cells Use the Trimeric G Proteins Gα-Gustducin/Gβ1γ13 and Gαo/Gβ1γ13 to Mediate the Responses to the Parasitic Helminths. Previous studies have shown that the *Gnat3*-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 increases in expression of three Gα genes—*Gnao1-B*, *Gnat3*, and *Gna15*—but in decreased expression for nine other Gα genes with the remaining four Gα genes unchanged (Fig. 2*A* and *SI Appendix*, Fig. S11). As to G-protein βγ subunits, *Ts* infection significantly increased the expression of *Gnb5*, *Gng*7, and *Gng13*, but reduced 4 others with the remaining 9 unaffected (Fig. 2*A* and *SI Appendix*, Fig. S11).

The qPCR analysis with the intestinal organoids showed that IL-13 treatment dramatically increased the expression of *Gnao1-B*, *Gnat3*, *Gna15*, *Gnb5*, and *Gng13* in the duodenal organoids (Fig. 2*B*). In addition, IL-13 treatment also augmented the expression of *Gnb1* by 20.9-fold, which was unchanged in the *Ts*-infected duodenums. Further comparison of the data revealed that *Gnaz* and *Gnat2* 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. *Gnat1* 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 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 colocalized to a subset of small intestinal epithelial cells (Fig. 2*C*) (8). Interestingly, an antibody that can bind to both A and B isoforms of Gαo, encoded by *Gnao1-A* and *-B*, respectively, costained some tuft cells with the Dclk1 antibody while some epithelial cells were stained by the Gαo antibody alone, indicating that Gαo is expressed not only in tuft cells but also in some other Dclk1-negative epithelial cells (Fig. 2*D*). In contrast, the antibody against *Gna15*-encoded Gα15 stained cells in the lamina propria without overlapping with Dclk1 staining at all, indicating that Gα15-expressing cells were not tuft cells or even epithelial cells (Fig. 2*E*). Double immunostaining also showed that both

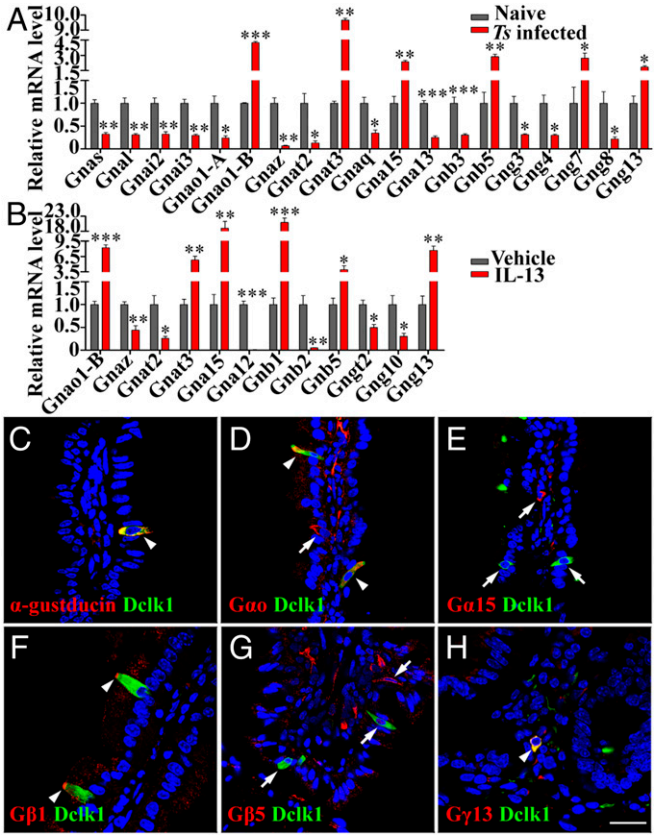

Fig. 2. Intestinal tuft cells highly express Gαo, α-gustducin, Gβ1, and Gγ13. (*A*) *Ts* infection altered G-protein subunit expression in the small intestinal villi. qPCR analyses show that *Ts* infection for 2 wk significantly increased the expression of *Gnao1-B*, *Gnat3*, *Gna15*, *Gnb5*, *Gng7*, and *Gng13*, but decreased 13 others. (*B*) IL-13 treatment of the intestinal organoids up-regulated the expression of *Gnao1-B*, *Gnat3*, *Gna15*, *Gnb1*, *Gnb5*, and *Gng13* but decreased 6 others. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. (*C–H*) Images of double immunostaining show that Dclk1 (green) is overlapped with α-gustducin, Gαo, Gβ1, and Gγ13 (arrowheads in *C*, *D*, *F*, and *H*) but not with Gα15 or Gβ5 (arrows in *E* and *G*). Note: one epithelial cell expressed Gαo but not Dclk1 (*D*, arrow) while Gβ1 was enriched at the tips of tuft cells (*F*, arrowhead). (Scale bar, 20 μm.)

Gβ1 and Gγ13 but not Gβ5 are colocalized with Dclk1, indicating that Gβ1γ13 subunits are expressed in tuft cells (Fig. 2 *F–H*). Notably, Gβ1 proteins seemed to be concentrated at the tip of tuft cells (Fig. 2*F*).

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. 1*A*, *Ts* extract evoked the small intestinal villi to release IL-25. However, preincubation with pertussis toxin, an inhibitor specifically for the G-protein αo/i subfamily, or with a myristoylated peptide inhibitor specifically for Gαo1 (25), blocked *Ts* extract-induced release of IL-25 (Fig. 3 *A* and *B*). Similarly, the Gβγ-subunit inhibitor gallein also inhibited the IL-25 release from *Ts* extract-evoked villi (Fig. 3*C*).

To genetically verify Gβ1γ13'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:Gng13$^{flox/flox}$, which was generated by crossing the Lgr5-EGFP-IRES-CreERT2 (26) mouse with the Gng13$^{flox/flox}$ mouse (27). Tamoxifen administration rendered CreERT2 recombinase activity in the Lgr5-expressing stem cells in the gut, abolishing Gγ13 expression in tuft cells (*SI Appendix*, Fig. S13). ELISAs showed that *Ts* extract-evoked release of IL-25 from the Gng13$^{-/-}$ villi was significantly

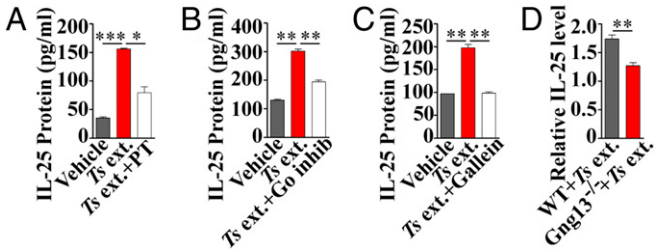

Fig. 3. G-protein inhibitors and Gγ13 knockout abolish tuft-cell response to *Ts* extract. *Ts* ext. evoked the release of significantly more IL-25 from the intestinal villi compared with vehicle control. Preincubation with the Gαi/o inhibitor pertussis toxin (PT) (*A*), Gαo peptide inhibitor (Go inhib) (*B*), or Gβγ inhibitor gallein (*C*) significantly reduced *Ts* ext.-induced IL-25 release. (*D*) Tamoxifen-induced *Gng13* knockout in the Lgr5-EGFP-IRES-CreERT2: $Gng13^{flox/flox}$ mice significantly reduced *Ts* ext.-induced IL-25 release compared with WT control. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

reduced compared with WT control (Fig. 3*D*), indicating that Gγ13 is critical to the IL-25 release from tuft cells. To further confirm Gβ1γ13's contribution to type 2 immune response in vivo, another $Gng13^{-/-}$ mouse line, Vil1-Cre:$Gng13^{flox/flox}$, was generated by crossing the Vil1-Cre mouse (28) with the $Gng13^{flox/flox}$ 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-Ip_3r2 Axis Is Part of the Tuft-Cell Intracellular Signaling Cascade. The G-protein β1γ13 dimer is known to activate Plcβ2 to generate the second messengers inositol 1,4,5-triphosphate (IP_3) and diacylglycerol whereas IP_3 binds to its receptor on the endoplasmic reticulum (ER), releasing Ca^{2+} ions from the ER into the cytoplasm and elevating the cytoplasmic free Ca^{2+} concentration (29). Plcβ2 is found to be expressed in tuft cells (Fig. 4*A*). 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. 4*B*).

To determine which IP_3 receptor subtype is activated by IP_3, we performed immunostaining with the antibodies against the receptor subtypes Ip_3r2 and Ip_3r3. Double immunostainings showed that Ip_3r2 was nearly completely overlapped with Dclk1 in tuft cells (Fig. 4*C*). In contrast, no overlapping was observed between Ip_3r3 and Dclk1 on the intestinal epithelial cells, and Ip_3r3 immunostaining was restricted mostly to the lamina propria (Fig. 4*D* and *SI Appendix*, Fig. S15). Thus, unlike taste-bud cells that employ Ip_3r3 to transduce taste signals, tuft cells use Ip_3r2 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 ($Trpm5^{-/-}$). Results showed that $Trpm5^{-/-}$ significantly reduced *Ts* extract-induced IL-25 release compared with WT control (Fig. 4*E*). 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, stevioside, a noncaloric sweet-tasting compound present in the plant *Stevia rebaudiana* (30). The results showed that stevioside 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 stevioside engendered tuft- and goblet-cell hyperplasia in the small intestine, similar to the effect of 5 mM succinate (12) (Fig. 4*G* and *SI Appendix*, Figs. S18–S22).

Previous studies have shown 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

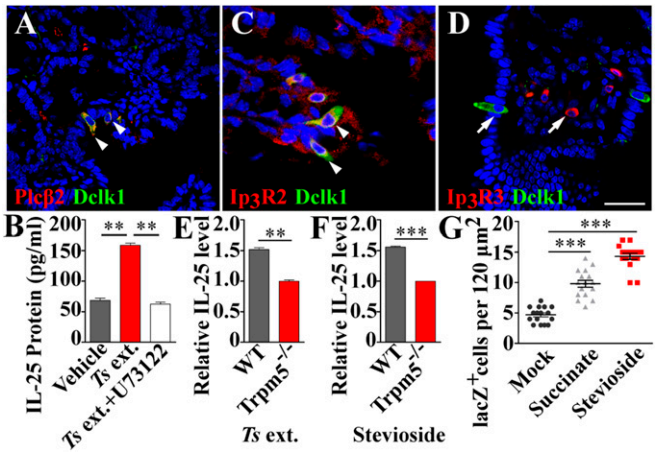

Fig. 4. Tuft cells utilize the Plcβ2-Ip_3r2-Trpm5–signaling pathway to transduce parasitic signals. (*A*) Double immunostaining on intestinal sections shows the colocalization of Plcβ2 (red) with Dclk1 (green) to tuft cells. (*B*) *Ts* ext. significantly increased the release of IL-25 from the intestinal villi compared with the vehicle control whereas preincubation with the phospholipase C inhibitor U73122 significantly reduced the IL-25 release. (*C* and *D*) Double immunostaining shows that Ip_3r2 (*C*, red), but not Ip_3r3 (*D*, red), is coexpressed with Dclk1 (green) in intestinal tuft cells. Instead, Ip_3r3 is expressed in the lamina propria. (Scale bar, 20 μm.) (*E* and *F*) ELISAs show that both *Ts* ext. and 124 μM stevioside stimulated WT but not $Trpm5^{-/-}$ duodenal villi to release IL-25. (*G*) Both 5 mM succinate and 0.5 mM stevioside, but not water in the mock treatment, significantly increased tuft-cell abundance in the duodenums of Trpm5-$lacZ^{+/-}$ mice. $**P < 0.01$; $***P < 0.001$.

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 Ca^{2+} 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 intestine 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 *Tas2r*s 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 and previous reports have shown 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 extraoral 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 Gαo/Gβ1γ13 and Gα-Gustducin/Gβ1γ13 Mediate Signal Transduction. Using the RT-qPCR approach, we discovered that *Ts* infection increased expression of three Gα genes (*Gnat3*, *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 *Gnat3*, *Gnao1-B*, *Gna15*, *Gnb5*, and *Gng13*. 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, Gαo, Gβ1, and Gγ13 are colocalized with Dclk1 and expressed in tuft cells (Fig. 2). However, Gα15 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 Gαo-expressing intestinal epithelial cells do not express Dclk1, suggesting the existence of Dclk1-negative tuft cells (16).

The Gαo/i-, Gαo-, and Gβγ-specific inhibitors, including pertussis toxin, Gαo 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 Gαo, as well as Gβ1γ13 subunits, transduce the parasitic signals and stimulate tuft cells to produce IL-25. Finally, knockout of *Gng13* significantly reduced both *Ts* extract-evoked IL-25 release from the intestinal villi and *Ts* infection-induced tuft-cell hyperplasia, indicating that Gγ13 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 Gαo/Gβ1γ13 and Gα-gustducin/Gβ1γ13 are the heterotrimeric G proteins transducing parasitic signals. Gγ13 has been known to form functional trimeric G proteins with both Gαo and Gα-gustducin, respectively (44–46). And Gα-gustducin has been known to be involved in the succinate- and *T. muris*-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-Ip$_3$r2 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 Gβ1γ13, as in taste-bud cells (45). Activated Plcβ2 generates IP_3, which in turn binds to and opens the channel receptor Ip$_3$r2, instead of Ip$_3$r3 found in taste-bud cells (Fig. 4 *C* and *D*) (29). One difference between Ip$_3$r2 and Ip$_3$r3 is that Ip$_3$r2 has a higher affinity for IP_3 but a lower affinity for Ca^{2+} (47, 48), suggesting that it can be more readily activated by a smaller amount of IP_3 produced by Plcβ2. From these data, we are inclined to conclude that signaling pathways in tuft cells are more sensitive to sense and to transduce parasitic stimuli than to taste-bud cells to detect sweet, bitter, and umami tastants and that Ip$_3$r2 makes tuft cells more readily or spontaneously active to maintain a basal level of IL-25 and a minimal number of tuft cells in naive mouse intestine and to more rapidly launch a type 2 immune response upon parasite invasion.

Trpm5 Is a Key Component in the Tuft Cell–ILC2 Circuit. Trpm5 is critical to *T. muris* 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, stevioside, 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 Ip$_3$r2's intrinsic activity and constant exposure to the plethora of gut microbes, which can be significantly amplified by stevioside. Indeed, 0.5 mM stevioside

seemed to be more potent than 5 mM succinate in launching tuft- and goblet-cell hyperplasia (Fig. 4*G* and *SI Appendix*, Figs. S20 and S21). Interestingly, stevioside 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 Trpm5 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): *Ts* molecules activate Tas2rs, which in turn stimulate Gα-gustducin/Gβ1γ13 or Gαo/Gβ1γ13; the G proteins dissociate into Gα and Gβ1γ13 moieties, and the latter acts on Plcβ2, generating IP_3; IP_3 binds to Ip_3r2, releasing Ca^{2+} from the endoplasmic reticulum into cytosol; the Ca^{2+} ions open Trpm5, 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 weep 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

C57/BL6 mice and Sprague-Dawley rats were purchased from the Shanghai SLAC Laboratory Animal Co. Trpm5-lacZ, Vil1-Cre, 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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