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Not just ”leuko“ after all: Epithelial leukotriene production in type 2 immunity

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New research shows that specialized epithelial cells (tuft cells) are major producers of lipid mediators (leukotrienes) that drive allergic inflammation and host defense against helminth parasites. (See the related research article by Ualiyeva *et al.*)

Introduction

Cysteinyl leukotrienes (cysLTs) are pro-inflammatory lipid mediators that are synthesized from the omega-6 fatty acid arachidonic acid by the action of 5-lipoxygenase (5-LOX/ Alox5) and leukotriene C_4 synthase (LTC_4S/ Ltc4s). Based on their slow but long-lasting bronchoconstrictor functions in asthmatic airways cysLTs, were originally termed “slow reacting substance of anaphylaxis”. Indeed cysLT-triggered responses are delayed, although being 1000-fold higher in potency compared to histamine. Following their biochemical and structural characterization, cysLTs were given their name based on their synthesis in “leuko”cytes, their characteristic “triene” (three conjugated double bonds) structure, and their glutathione (γ-Glu-Cys-Gly) moiety *(1).* CysLTs (LTC_4, LTD_4, LTE_4) initiate multiple responses involved in allergy and asthma, including bronchoconstriction and eosinophil recruitment as well as the activation of type 2 innate lymphoid cells (ILC2s) and Th2 cells. Traditionally, myeloid cells (mast cells, eosinophils, dendritic cells (DCs) and monocytes/ macrophages) have been regarded as the major - if not exclusive physiologically relevant - source of cysLTs. However, recent studies implicate a group of specialized epithelial cells, called tuft cells (or brush cells) in cysLT synthesis and function.

Tuft cells are chemosensory cells in the epithelia of multiple organs, including the thymus, the small intestine, and the airways. Intestinal tuft cells express the enzymes of the complete leukotriene cascade including cytosolic phospholipase A_2 (cPLA2), 5-LOX, LTC_4S and leukotriene A_4 hydrolase (LTA_4H) *(2)*. In addition, airway epithelial cells can contribute to cysLT production in inflammatory settings, including allergic asthma and nasal polyposis *(3, 4)*. However, these studies do not specify the epithelial cell type responsible for the synthesis of cysLTs. The identification of transcription factors and markers of tuft cells, including POU Class 2 Homeobox 3 (Pou2f3), Transient receptor potential cation channel subfamily M member 5 (Trpm5), Choline acetyltransferase (ChAT) and Doublecortin-like kinase 1 (Dclk1) now allows the ablation and characterization of these cells in the context of type 2 immune responses in allergy and helminth infection.

Tuft cells as a source of cysLTs in type 2 immunity

A key role for tuft cell-derived LTs in expulsion of the rodent nematode parasite *N. brasiliensis* is determined by using mice with a conditional deletion of the Alox5 gene in tuft cells *(5)*. CysLTs exert a synergistic effect with tuft cell-derived IL-25, a well characterized initiator of type 2 immunity. Mice with an inducible ablation of LT production in tuft cells ($Alox5^{fl/fl}$;$Pou2f3\text{-}cre^{Ert2}$) show a similar reduction in the early type 2 immune response to helminths as mice with a global deficiency in Alox5 or treated with the cysLT1 receptor ($CysLT_1R$) antagonist Montelukast. This study thus suggests a non-redundant role of tuft cell-produced cysLTs in anti-helminth immunity in the small intestine. While demonstrating cysLT generation by tuft cells cultured *ex vivo*, the amounts of cysLTs that are produced by tuft cells during type 2 immune responses *in vivo* remain uncertain. This is particularly relevant as myeloid cells, including eosinophils, macrophages and mast cells that are recruited and expand during type 2 immunity, are potent sources of cysLTs. Thus, the relative contribution of tuft cells to cysLT production *in vivo* remains subject of debate. In addition, the ELISA-based quantification fails to discriminate which of the three cysLTs (LTC_4, LTD_4, LTE_4) is predominantly produced by tuft cells. As LTC_4, LTD_4 and LTE_4 display different affinities to the receptors $CysLT_1R$ [$LTD_4 > LTC_4 >> LTE_4$], $CysLT_2R$ [$LTD_4 = LTC_4 >> LTE_4$] and $CysLT_3R$ [$LTE_4 >> LTC_4 > LTD_4$], their downstream effector functions may differ considerably depending on the receptor repertoire and composition of target cells in inflammatory settings.

By using LC-MS/MS-based eicosanoid profiling, type 2 immune responses associated with allergic asthma or helminth infection are shown to result in broadly altered eicosanoid profiles, including distinct patterns of cysLT synthesis *(6, 7)*. Bankova and colleagues now provide detailed insights into eicosanoid- and cysLT production by tuft cells by means of targeted lipidomic (LC-MS/MS) analyses. They show that when stimulated with Ca^{2+} ionophore to trigger release of the substrate arachidonic acid, tuft cells generate similar amounts of cysLTs as airway macrophages. Their study further identifies LTC_4 as the major tuft cell-derived eicosanoid, while other arachidonic or linoleic metabolites, including PGD_2 or 9- and 13-HODE are synthesized in low amounts by tuft cells isolated from the airways of naïve or *Alternaria*-challenged mice. Given that prostanoid production can be induced by allergenic stimuli *(6)* or helminth products *(7)* future studies should further investigate the plasticity of eicosanoid pathways in tuft cells, e.g. during acute and chronic exposure to allergens or during infection with helminth parasites.

Surprisingly the authors find no cysLT generation by lung eosinophils, a cell type generally known to synthesize large amounts of cysLTs. This may suggest that eosinophils, which are prone to degranulate and undergo apoptosis, are harmed during isolation from the airways. Such possible *ex vivo* artifacts could be circumvented by future *in vivo* studies providing a side-by-side comparison of cysLT generation in the airways (or intestine) in $ChAT^{Cre}Ltc4s^{fl/fl}$ mice versus mice with a selective deletion of cysLT production in eosinophils or other myeloid cell types.

Roles of tuft cell-derived cysLTs in type 2 airway inflammation

In addition to demonstrating an important contribution of tuft cells in the early cysLT response following allergen challenge, Ualiyeva *et al.* dissect the functional synergy between LTC_4 and IL-25 in the initiation of type 2 airway inflammation. In line with previous findings in helminth infection *(5)* the authors demonstrate that the efficient induction of a type 2 immune response requires the presence of both mediators (cysLTs and IL-25). Thus, mice that are co-administered LTC_4 and IL-25 by inhalation develop airway eosinophilia and exhibit ILC2 accumulation and

increased type 2 (IL-4, IL-5, IL-13) cytokine production, while the same amounts of IL-25 or LTC_4 alone fail to induce these responses. The amplifying effect of LTC_4 on IL-25-triggered type 2 inflammation is largely mediated by the $CysLT_1R$ and to a smaller extend by $CysLT_2R$. The involvement of $CysLT_1R$ and $CysLT_2R$ in tuft cell-driven type 2 airway inflammation is in line with the essential role of these receptors in the tuft cell-triggered type 2 immune response against helminth parasites *(5)*. Thus, while autocrine effects of tuft cell-derived cysLTs may largely depend on $CysLT_3R$ *(8)*, paracrine effects on leukocytes, including eosinophils, dendritic cells and ILC2s, require signaling via $CysLT_1R$ and $CysLT_2R$, the high-affinity receptors for LTD_4, and LTC_4. Despite these different receptor requirements, cysLT signaling via $CysLT_3R$ also acts in synergy with IL-25 to drive tuft cell expansion. Albeit showing abrogated airway tuft cell expansion in $LTC_4S^{-/-}$ mice *(8)*, the authors' previous work does not provide direct proof for airway tuft cells as the source of cysLTs during *Alternaria*-induced type 2 airway inflammation. By studying the initiation of type 2 inflammation in mice with a conditional deficiency in *Ltc4s* in airway tuft cells ($ChAT^{Cre}Ltc4s^{fl/fl}$), the current study now identifies tuft cell-derived cysLTs as non-redundant mediators of allergic airway inflammation. A lack of cysLT synthesis in tuft cells abrogates allergen-induced tuft cell expansion and attenuates ILC2 and eosinophil responses, demonstrating both autocrine and paracrine functions of tuft cell-derived cysLTs. In addition, *Alternaria*-challenged $ChAT^{Cre}Ltc4s^{fl/fl}$ mice show reduced DC recruitment and impaired T- and B-cell responses in local lymph nodes. Thus, in addition to triggering early innate type 2 inflammation, tuft cell-derived cysLTs likely govern adaptive (Th2) immune responses that determine the development of chronic airway inflammation in allergy or protective immune memory following helminth infection.

Triggers and regulators of tuft cell activation and cysLT synthesis

The essential functions of tuft cell-derived cysLTs in type 2 immune responses necessitate a detailed understanding of the pathways that trigger and regulate tuft cell activation and expansion. Ualiyeva *et al.* identify ATP as a trigger of cysLT production in tuft cells *(9)*. House dust mite or *Alternaria* allergens, which trigger ATP release, induce cysLT production by airway tuft cells *(9)*. The study identifies the nucleotide sensor P2Y2 as the major receptor mediating ATP-driven cysLT generation by tuft cells. Thus, tuft cell-derived cysLTs may represent an early damage signal that leads to the induction of a tissue reparative type 2 immune response.

In line with this function, products of the helminth *N. brasiliensis*, a parasite causing extensive lung damage, trigger cysLT generation by *ex vivo* cultured intestinal tuft cells *(5)*. Thus, the early induction of cysLT synthesis by tuft cells is a common feature of allergens and helminth parasites, which may aid to rapidly repair the epithelial barrier albeit at the risk of causing pathological type 2 inflammation. In contrast to *N. brasiliensis*, which causes a rapid type 2 immune response and tuft cell hyperplasia, products of the helminth *H. polygyrus* inhibit tuft cell activation and gene expression under type 2 inflammatory conditions *(10)*. Of note, *H. polygyrus* excretory/secretory products (HpES) also reverse tuft cell expansion in response to the TCA metabolite succinate, which induces type 2 immunity and helminth expulsion. Future studies should thus investigate whether helminth products can regulate cysLT synthesis in tuft cells similar to their LT-suppressive effects on myeloid cells *(7)*. The induction of Alox12 and cPLA2 gene expression by HpES in tuft cells *(10)* may suggest that *H. polygyrus* products induce anti-inflammatory and reparative eicosanoids in line with the immune regulatory potential of this

parasite. Indeed, helminth products and allergens modulate the expression of eicosanoid pathway enzymes in macrophages, thus inducing changes in eicosanoid synthesis *(6, 7)*. In contrast, the current study shows that the tuft cell transcriptome, including the expression of LT synthesis enzymes is not altered following *in vivo Alternaria* exposure. Thus, increased cysLT production in *Alternaria*-challenged mice is likely driven by alternative pathways, including tuft cell expansion, ATP signaling or other yet to be identified signaling mechanisms.

Conclusions and future directions

Tuft cells are important producers of leukotrienes. While tuft cell-derived cysLTs are implicated in early innate type 2 immune responses it is unclear what the relative contribution of tuft cell cysLTs in chronic type 2 inflammation is, e.g. in allergic asthma or nasal polyposis. Additionally, a potential plasticity of tuft cell eicosanoid profiles in different organs or immunological settings should be resolved. This would shed light on potential additional functions of tuft cells in inflammation, repair and host defense.

Most studies on tuft cells are conducted in mice despite the presence of these cells in human epithelia. Expression of eicosanoid biosynthesis enzymes including cyclooxygenase 1 (COX1) and 5-lipoxygenase activating protein (FLAP) suggests that tuft cell-derived eicosanoids are important mediators in human immune responses. Future studies should thus investigate how the synthesis of cysLTs or other eicosanoids by airway or intestinal tuft cells contributes to human disease.

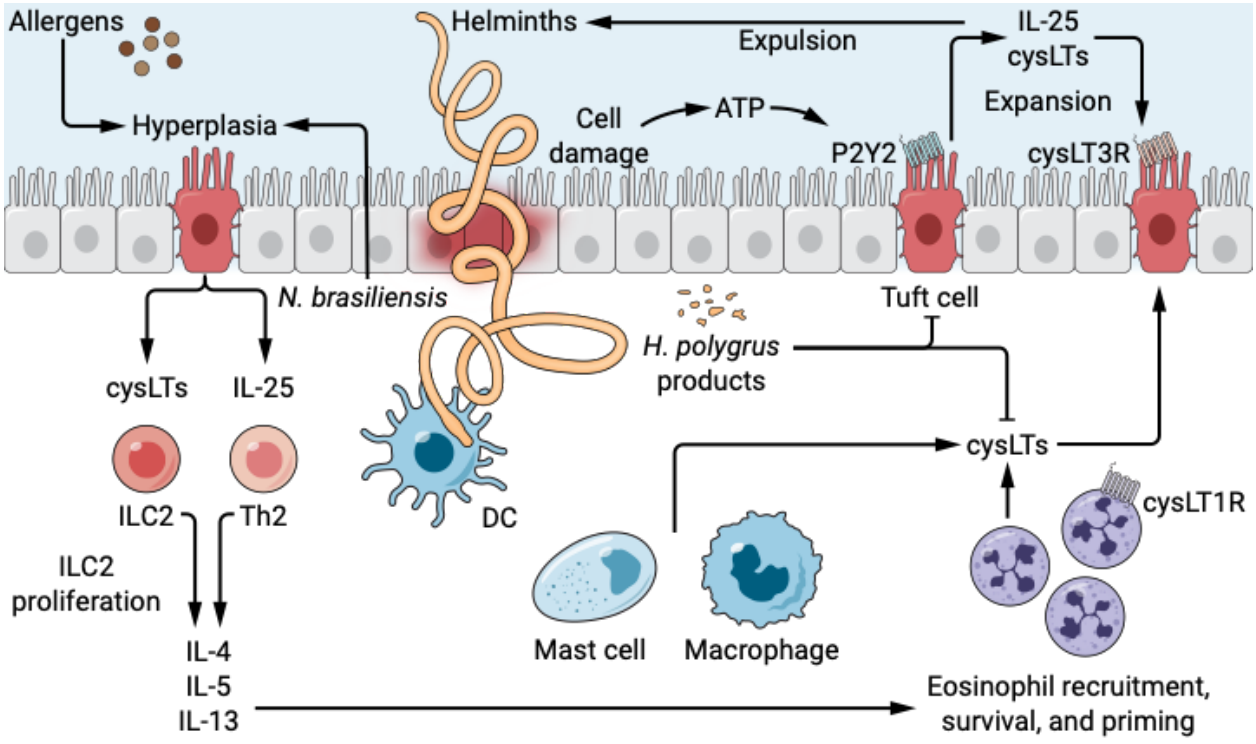

Fig. 1. Tuft cell–derived CysLTs drive the initiation of type 2 immune responses. Allergens or helminth parasites trigger the release of the alarmin IL-25 and ATP and the synthesis of CysLTs by tuft cells in the epithelium of the air-ways or the small intestine. Tuft cell–derived CysLTs synergize with IL-25 to drive tuft cell expansion (via CysLT3R) or ILC2 activation, proliferation, and type 2 cytokine production largely via CysLT1R. N. brasiliensis infection causes tuft cell hyperplasia, whereas H. polygyrus products reverse tuft cell expansion. Tuft cell–derived CysLTs promote eosino-phil recruitment and survival directly via CysLT1R or indirectly by triggering IL-5 production by ILC2s. By activating migratory (CD301b+) DCs, tuft cell–derived CysLTs may also promote the activation of T_H2 cells. CysLTs produced by recruited and expanded myeloid cells, including macrophages, mast cells, and eosinophils, further amplify and per-petuate the type 2 immune response initiated by tuft cell–derived mediators. CREDIT: A. MASTIN/SCIENCE IMMUNOLOGY

References and notes

1. B. Samuelsson, Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation, *Science* **220**, 568–575 (1983).

2. C. Bezençon, A. Fürholz, F. Raymond, R. Mansourian, S. Métairon, J. Le Coutre, S. Damak, Murine intestinal cells expressing Trpm5 are mostly brush cells and express markers of neuronal and inflammatory cells, *J. Comp. Neurol.* **509**, 514–525 (2008).

3. A. J. Jame, P. M. Lackie, A. M. Cazaly, I. Sayers, J. F. Penrose, S. T. Holgate, A. P. Sampson, Human bronchial epithelial cells express an active and inducible biosynthetic pathway for leukotrienes B4 and C4, *Clin. Exp. Allergy* **37**, 880–892 (2007).

4. K. Dietz, M. de Los Reyes Jiménez, E. S. Gollwitzer, A. M. Chaker, U. M. Zissler, O. P. Rådmark, H. A. Baarsma, M. Königshoff, C. B. Schmidt-Weber, B. J. Marsland, J. Esser-von Bieren, Age dictates a steroid-resistant cascade of Wnt5a, transglutaminase 2, and leukotrienes in inflamed airways, *J. Allergy Clin. Immunol.* **139**, 1343-1354.e6 (2017).

5. J. W. McGinty, H.-A. Ting, T. E. Billipp, M. S. Nadjsombati, D. M. Khan, N. A. Barrett, H.-E. Liang, I. Matsumoto, J. von Moltke, Tuft-Cell-Derived Leukotrienes Drive Rapid Anti-helminth Immunity in the Small Intestine but Are Dispensable for Anti-protist Immunity, *Immunity* **52**, 528-541.e7 (2020).

6. F. D. R. Henkel, A. Friedl, M. Haid, D. Thomas, T. Bouchery, P. Haimerl, M. de Los Reyes Jiménez, F. Alessandrini, C. B. Schmidt-Weber, N. L. Harris, J. Adamski, J. Esser-von Bieren, House dust mite drives proinflammatory eicosanoid reprogramming and macrophage effector functions, *Allergy* **74**, 1090–1101 (2019).

7. M. de Los Reyes Jiménez, A. Lechner, F. Alessandrini, S. Bohnacker, S. Schindela, A. Trompette, P. Haimerl, D. Thomas, F. Henkel, A. Mourão, A. Geerlof, C. P. da Costa, A. M. Chaker, B. Brüne, R. Nüsing, P.-J. Jakobsson, W. A. Nockher, M. J. Feige, M. Haslbeck, C. Ohnmacht, B. J. Marsland, D. Voehringer, N. L. Harris, C. B. Schmidt-Weber, J. Esser-von Bieren, An anti-inflammatory eicosanoid switch mediates the suppression of type-2 inflammation by helminth larval products, *Sci Transl Med* **12** (2020), doi:10.1126/scitranslmed.aay0605.

8. L. G. Bankova, D. F. Dwyer, E. Yoshimoto, S. Ualiyeva, J. W. McGinty, H. Raff, J. von Moltke, Y. Kanaoka, K. Frank Austen, N. A. Barrett, The cysteinyl leukotriene 3 receptor regulates expansion of IL-25-producing airway brush cells leading to type 2 inflammation, *Sci Immunol* **3** (2018), doi:10.1126/sciimmunol.aat9453.

9. S. Ualiyeva, N. Hallen, Y. Kanaoka, C. Ledderose, I. Matsumoto, W. G. Junger, N. A. Barrett, L. G. Bankova, Airway brush cells generate cysteinyl leukotrienes through the ATP sensor P2Y2, *Sci Immunol* **5** (2020), doi:10.1126/sciimmunol.aax7224.

10. C. Drurey, H. T. Lindholm, G. Coakley, M. C. Poveda, S. Löser, R. Doolan, F. Gerbe, P. Jay, N. Harris, M. J. Oudhoff, R. M. Maizels, Intestinal epithelial tuft cell induction is negated by a murine helminth and its secreted products, *J Exp Med* **219**, e20211140 (2022).

Figure Legend:

Tuft cell-derived cysteinyl leukotrienes drive the initiation of type 2 immune responses
Allergens or helminth parasites trigger the release of the alarmin IL-25, ATP and the synthesis of cysteinyl leukotrienes (cysLTs) by tuft cells in the epithelium of the airways or the small intestine. Tuft cell-derived cysLTs synergize with IL-25 to drive tuft cell expansion (via CysLT3R) or ILC2 activation, proliferation and type 2 cytokine production largely via CysLT1R. *N. brasiliensis* infection causes tuft cell hyperplasia, while *H. polygyrus* products reverse tuft cell expansion. Tuft-cell derived cysLTs promote eosinophil recruitment and survival directly via CysLT1R or indirectly by triggering IL-5 production by ILC2s. By activating migratory ($CD301b^+$) DCs, tuft cell-derived cysLTs may also promote the activation of Th2 cells. CysLTs produced by recruited and expanded myeloid cells, including macrophages, mast cells and eosinophils further amplify and perpetuate the type 2 immune response initiated by tuft cell-derived mediators.

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