MUCOSAL IMMUNOLOGY

Made to order tuft cells by an OCA-T1 isoform switch

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A genetic mechanism accounts for the variation in tuft cell abundance seen among inbred mouse strains: alternative isoforms of OCA-T1 (Oct coactivator from tuft cells 1), a recently discovered transcriptional coactivator that specifies the tuft cell lineage (see related Research Article by Nadjsombati *et al.*).

Inbred mouse strains display marked differences in their immune responses, particularly when challenged with a parasite infection (*1*). However, the vast number of genetic differences between mouse strains has historically presented obstacles to mapping a single causal gene underlying this variability in immune cell function. In this issue of *Science Immunology*, Nadjsombati *et al.* identified significant differences in tuft abundance between different mouse strains and used positional cloning to implicate the *Pou2af2* locus as accounting for this phenotypic variation (*2*). *Pou2af2* encodes the protein OCA-T1 (Oct co-activator from tuft cells 1), a recently identified coactivator of the transcription factor POU2F3, which together form a transcriptional complex that functions as a master regulator of tuft cell development (*3*). Remarkably, the causal single-nucleotide polymorphisms (SNPs) associated with reduced tuft cells led to the expression of a nonfunctional isoform of OCA-T1 that cannot bind to POU2F3 (*2*). This important work highlights the powerful influence of coactivator isoform usage as a mechanism to regulate tuft cell differentiation from epithelial stem cells (Fig. 1).

Tuft cells are chemosensory epithelial cells that coordinate innate immune responses to pathogens in several mucosal barrier tissues, such as the intestine, upper airway, and stomach (*4*). Tuft cells also exist in the thymus, where they regulate T cell development. The most well-studied role of tuft cells is in the regulation of type 2 immune responses to parasites in the small intestine (SI) in a process termed the tuft–group 2 innate lymphoid cell (ILC2) circuit (*5*–*7*). In this circuit, tuft cells directly sense the presence of metabolites released by pathogens (e.g., succinate produced by protists) and release effectors such as the cytokine interleukin-25 (IL-25) (*5*). These effectors then activate ILC2 cells to release additional cytokines, such as IL-13, that trigger a variety of cellular responses that promote parasite clearance (*5*). One output of the tuft-ILC2 circuit is the proliferative expansion of mucus-producing goblet cells and tuft cells, which differentiate from $Lgr5^+$ epithelial stem cells in a manner that is enhanced by IL-13 (*5*–*7*). Of note, most of the studies on tuft cells have been performed in the C57BL/6J (B6) inbred mouse strain.

Nadjsombati *et al.* (*2*, *8*) observed that the Balb/cj (Balb) mouse strain had significantly fewer tuft cells at baseline in several tissues (e.g., distal SI, colon, and trachea) when compared with B6 mice, whereas similar tuft cell frequencies were found in the thymus. They focused their subsequent investigation on the SI, because the tuft cell deficit in this tissue was most striking in Balb mice. After ruling out the involvement of the microbiome, they compared the tuft-ILC2 circuit between these two strains (*2*). Their experiments suggested that ILC2 cells were functionally equivalent in B6 and Balb strains (e.g., by measuring IL-25–induced IL-13 release), whereas the tuft cells exhibited specific functional deficits in Balb mice (*2*). For example, SI tuft cells in Balb mice failed to proliferate in response to succinate or when challenged with a chronic *Tritrichomonas* protist infection. However, the tuft cell defect seen in Balb mice was not absolute: Succinate-induced tuft cell expansion in the SI could be largely rescued if the mice were pretreated with IL-25 or IL-4. In addition, the baseline differences in tuft cell abundance between B6 and Balb were preserved in SI-derived organoid cultures (*2*), further emphasizing the strain-specific difference lying within the epithelial compartment. Collectively, their analysis pointed to discrete molecular deficits (e.g., transcriptional and/or signaling) being present within the tuft cell lineage of Balb, which exists in a organ-specific manner.

The authors next sought to understand whether these strain-specific tuft cell differences could be attributed to a specific genetic locus. By interbreeding B6 and Balb mice and quantifying succinate-induced tuft cell frequencies in the SI of F1 and F2 progeny, it became evident that a single recessive genomic locus accounted almost entirely for this phenotype (*2*). To map the relevant locus, they profiled SNPs in 84 F2 mice using low-coverage whole-genome sequencing. By correlating SNPs with tuft cell frequencies in these hybrid animals, they identified a region on chromosome 9 that accounted for the majority of tuft cell differences. Through a series of backcrosses, they were able to generate congenic mouse strains and further reduce this genomic interval to 17 megabases (*2*). Last, they used an RNA sequencing analysis to nominate *Pou2af2* as the most down-regulated gene in Balb versus B6 tuft cells (*2*).

SNPs that render a *Pou2af2* deficiency provided a compelling explanation for the tuft cell phenotypes observed in Balb mice, because recent studies had shown that the OCA-T1 protein (encoded by *Pou2af2*) is a critical master regulator of tuft cell development by forming a heterodimeric complex with POU2F3 (*3*). Moreover, the recent characterization of OCA-T1–deficient mice (on a B6 background) revealed striking tuft cell deficiencies in the SI, colon, and trachea but normal tuft cell abundance in the thymus, a tissue-specific pattern that closely resembled observations in wild-type Balb mice (*3*). To definitively show that OCA-T1 deficiency is the cause of the Balb tuft cell defect, the authors showed that expressing an OCA-T1 cDNA in Balb-derived organoid cultures was sufficient to restore B6 levels of tuft cell abundance (*2*).

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However, the OCA-T1 mRNA deficiency in the SI of Balb mice was modest (only ~3-fold) relative to B6, which prompted the authors to deepen their genomic analysis to account for the severity of the tuft cell phenotypes. Using 5′ rapid amplification of cDNA ends, they found that the residual OCA-T1 mRNA of Balb SI tuft cells was initiated from a downstream transcriptional start site, resulting in a shortened isoform of the encoded OCA-T1 protein (*2*). Importantly, this shorter isoform lacks the critical binding site needed to complex with POU2F3 and hence would be incapable of supporting tuft cell differentiation. Although the exact SNPs that account for this preferential isoform usage were not defined, it is likely that transcription factor binding sites are being altered at the *Pou2af2* locus by these SNPs in a strain-specific manner to influence the promoter activity. Quite remarkably, the treatment of Balb organoids with IL-13 was sufficient to restore expression of the full-length OCA-T1 mRNA (Fig. 1) (*2*). This provocative finding suggests that expression of the long isoform can be acutely regulated by signaling responses downstream of immune cell activation within the tuft-ILC2 circuit.

This work provides compelling evidence that genetic variation of the *Pou2af2* locus is a naturally occurring mechanism that sets thresholds for the tuft-ILC2 circuit in mice. What might be the evolutionary pressures that drive strain-specific differences in tuft cell function? Although tuft cells are of clear benefit in response to parasite infections, it is clear that tuft cells are also a reservoir for noroviruses. Thus, trade-offs may exist that determine tuft cell abundance/activity and that balance having an optimal level of mucosal immunity with viral susceptibility. Remarkably, a recent genome-wide association study independently pointed to the *POU2AF2* locus as a source of variation of tuft cell frequencies in human populations (*9*). In this study, it was also noted that colon cancer risk was also linked to this locus, suggesting that tuft cells might have functional links to this malignancy. A clear area of future investigation will be to determine whether other disease associations exist in humans with inherited deficiencies in *POU2AF2* or of other tuft cell master regulators (e.g., *POU2F3* and *POU2AF3*).

The work by Nadjsombati *et al.* also highlights how little we currently understand about the transcriptional and signaling mechanisms that govern tuft cell development and function in different tissues. It is clear that tuft cells are highly responsive to their microenvironmental signals, which include specialized functions in distinct organs. The mechanistic link between IL-13 signaling and OCA-T1 isoform usage identified here is likely only scratching the surface of how various inflammatory signals can adjust the transcriptome of tuft cells to achieve their context-specific functions. For example, how are tuft cell master regulator transcription factors regulated in the thymus, a site in which tuft cells do not depend on OCA-T1? Another question raised by this study is regarding the functionality of the newly identified OCA-T1 short isoform. Although this isoform is incapable of binding to POU2F3, it is possible that it still carries out a nontranscriptional function. The paralog protein OCA-B, a coactivator of POU2F1/POU2F2, is known to perform a cytoplasmic signaling function downstream of the B cell receptor by binding to SYK kinase (*10*). Future efforts should be aimed at clarifying whether OCA-T1 also performs a tuft cell signaling function via its short isoform.

In summary, this study by Nadjsombati *et al.* makes a significant advance in our molecular understanding of tuft cell biology by leveraging the power of forward mouse genetics. Their work underscores the centrality of POU2F3 and OCA-T1 as master regulators that drive tuft development and the evolution of effective mucosal immunity.

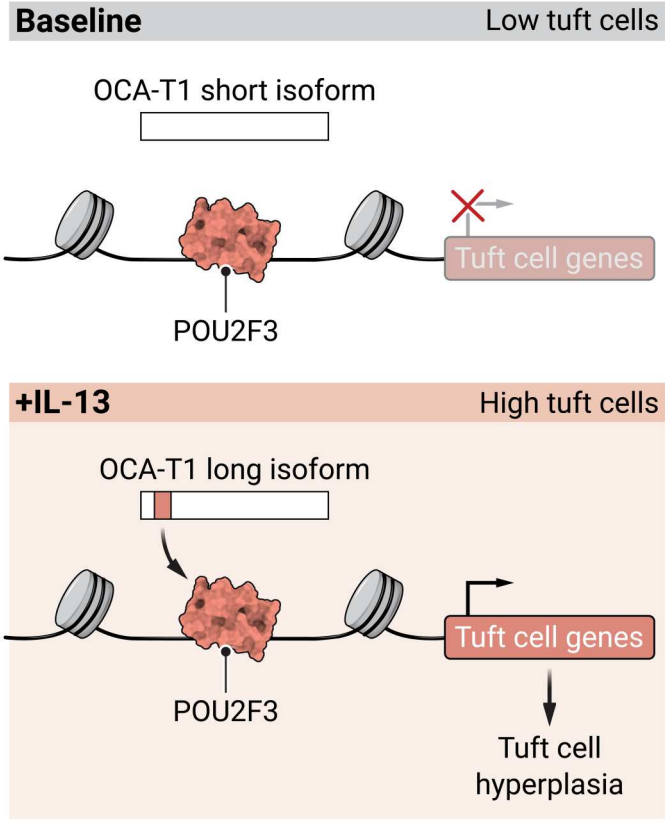

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Fig. 1. *Pou2af2*/OCA-T1 isoform usage is dynamically regulated to tune tuft cell differentiation and type 2 immunity. Balb mice have a low baseline number of tuft cell numbers in the distal SI because of the expression of a nonfunctional short isoform of OCA-T1. Upon IL-13 treatment, epithelial stem cells activate the expression of a functional OCA-T1 isoform that is capable of binding to POU2F3 to promote transcription of genes that stimulate tuft cell expansion.

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Acknowledgments
Funding: This work was supported by NIH grant CA242919.
Author contributions: X.S.W. and C.R.V. wrote the manuscript. **Competing interests:** C.R.V. has received consulting fees from Flare Therapeutics, Roivant Sciences, and C4 Therapeutics; has served on the advisory boards of KSQ Therapeutics, Syros Pharmaceuticals, and Treeline Biosciences; has received research funding from Boehringer-Ingelheim and Treeline Biosciences; and owns equity in Treeline Biosciences. 10.1126/sciimmunol.adh3123

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Sci. Immunol., **8** (83), eadh3123.
DOI: 10.1126/sciimmunol.adh3123

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