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Is the plasticity of the Th17 subset a key source of allergenic Th2 responses?

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Abbreviations:

CyTOF: cytometry by time of flight or mass cytometry

EGFP: enhanced green fluorescent protein

HDM: house dust mite

IFN- γ : Interferon gamma

IL: interleukin

LCMV-GP: lymphocytic choriomeningitis virus glycoprotein

PPAR- γ : peroxisome proliferator-activated receptor- γ

TCR: T cell receptor

tdRFP: tandem-dimer red fluorescent protein

TGF- β : Transforming growth factor beta

Th: T helper cells

UMAP: uniform manifold approximation and projection

The differentiation process of naïve CD4⁺ T cells relies on the strength of TCR engagement and signals triggered by polarizing cytokines, which determine the generation of distinct Thelper (Th) cell subsets. Once differentiated towards a specific subset, Th cells play essential effector functions in host defense and orchestrate the pathogenesis of many inflammatory diseases including autoimmunity, asthma, and allergy. Th subsets have been stratified based on key lineage-specific cytokines that identify Th1 (IFN- γ), Th2 (IL4, IL5, and IL13), Th9 (IL9), Th17 (IL17A and IL21), and Th22 (IL22) subsets. Recent reports of Th cell heterogeneity, plasticity, and promiscuity of their putative Th-specific transcription factors have called into question these classical categories of Th subsets^{1,2}. In this regard, novel technical innovations may allow deeper insights into Th cell identity through high-dimensional characterization at the single cell level including RNAseq, spectral flow cytometry, and cytometry by time of flight (CyTOF).

In their recent paper, Tortola et al.³ have investigated Th cell identity permanence, diversity, and plasticity utilizing CyTOF with uniform manifold approximation and projection (UMAP) and PhenoGraph with combinatorial Boolean gating for analysis. The authors employed unsupervised clustering to analyze *in vitro* polarized CD4⁺ T cells from SMARTA mice (a strain carrying a re-arranged TCR specific for LCMV-GP₆₁₋₈₀ epitope) under canonical Th1, Th2, and Th17 as well as neutral (Th0) conditions. Their results showed remarkable heterogeneity in the intracellular cytokine patterns within the Th subsets detected by CyTOF. This heterogeneity was prominent in Th2-polarized cells where they even found expression of IFN- γ (a canonical Th1 cytokine) in several Th2 subpopulations. In addition to cells polarized *in vitro*, they studied T cell diversity using established animal models that have a pronounced bias toward each lineage: influenza A virus infection (Th1), *Nippostrongylus brasiliensis* nematode infection (Th2), and T cell transfer colitis (Th17). They observed heterogeneous effector states with unique cytokine responses within each subset including exceptionally diverse cytokine expression patterns within Th2 cells. These results are concordant with studies that demonstrated a functional heterogeneity within pathogenic Th2 cells in asthma and other allergic disorders^{4,5,6}.

In order to evaluate lineage plasticity, the authors tested whether Th cells trained towards a specific Th identity permanently preserve this distinctiveness or can adopt a new Th profile with a second polarization³. Although some new cytokine patterns were observed after re-polarization, Th0-, Th1-, and Th2-trained cells showed stable cell identity despite exposure to cytokines that would otherwise polarize naïve CD4⁺ T cells towards a different lineage. This suggests that these cells are largely committed to their lineage and most of the effector states found are not transitional stages of differentiation, but rather stable entities, whose cytokine expression profile is inherited by their progeny. This stability included the vast cytokine diversity observed in Th2 cells after re-exposure to the antigen, although upon the second round of polarization, IL5⁺ Th2 cells were lost irrespective of challenge conditions, suggesting that IL5-expressing cells may be a transitional stage of differentiating Th2 cells. However, other authors have reported that IL5 expression increases after consecutive rounds of polarization and it defines a subpopulation of highly differentiated Th2 cells⁷.

In contrast, re-polarization of Th17-trained cells revealed a critical instability of this lineage. Unexpectedly, these cells were re-trainable towards a Th2 phenotype not only in the context of Th2 stimulation but also under neutral conditions (Th0), losing IL17A and gaining IL4 expression. Nevertheless, in the presence of TGF- β , IL17A expression was maintained and IL4 production was inhibited, explaining the potential role that TGF- β -producing cells may have in the maintenance of Th17 phenotype and the prevention of Th2-biased responses at tissue level. Because IL4 is the primary cytokine that mediates Th2 induction, the authors used different experimental approaches to investigate whether it was required for repolarization of Th17-trained cells toward a Th2 profile and they showed that autocrine production of IL4 may drive this repolarization. These results insinuate that *de novo*-generated Th17 cells are plastic and they may adjust their cytokine expression towards a Th2 pattern under certain inflammatory microenvironmental changes.

Lastly, they validated these results *in vivo* by using IL17A fate mapping mice for Th17 tracing (IL17A:Cre with Rosa:Cre^{tdRFP}) that were prompted to experimental asthma by epicutaneous immunization to house dust mite (HDM), a model known to promote mixed Th2- and Th17-trained cells³. Upon intratracheal HDM administration, Th17-trained cells (ex-Th17) expressed

high levels of IL4 in the lungs with mixed neutrophilic and eosinophilic infiltrates. Without prior Th17 training, they observed a purer Th2 response with eosinophilia. Consistent with *in vitro* results, these experiments supported a model of plastic Th17 cells that can acquire Th2-associated cytokine expression when exposed to allergenic conditions.

Taken together, findings reported by Tortola et al.³ suggest that during differentiation of Th1 and Th2 cells there is a molecular “point-of-no-return” event that prevents re-training towards a different cell identity and confers lineage stability. Questions remain on the reported Th17 plasticity however: Is Th17 plasticity due to lack of full maturity that could be achieved at a later time-point? Is the impermanence of Th17 cell-identity a general characteristic of the Th17 subset identity? Should the current categorization of Th subsets be reviewed? For example, Harbour et al. reported that Th17 cells give rise to Th1 cells that are required for the pathogenesis of certain types of inflammatory bowel diseases⁸. More recently, it has suggested that Th9 cells, currently categorized as an independent subset, should be considered as a unique subtype of the Th2 subset distinguished from “classical” Th2 cells based on their expression of IL9 and PPAR- γ ¹. Moreover, Th2-related cytokine inhibition has been attempted therapeutically and inhibition of IL13 as an asthma treatment has previously been shown to induce IL17 and neutrophilia⁹. While these findings were previously unexplained, these new results from Tortola et al.³ may suggest that ex-Th17 cells could be key contributors to this cell identity switch and resistance to anti-IL13 therapy. Ultimately, due to the unreliability previously found between mouse and human findings regarding plasticity and differentiation of Th subsets, it is highly important to identify how the results discussed above could be directly interpreted in the context of human Th17 immune responses.

In conclusion, given the importance of Th2 cells in development of allergies and the diversity of Th2 cell identity reported by this³ and other works^{4,5,6}, it is important to find the functional implications of this heterogeneity within the Th2 subset in order to recognize potential therapeutic repercussions. Finally, a better understanding of trajectories and plasticity of Th subset identity will continue to shape our understanding of T cell role in allergic diseases.

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