ALLERGY

Zooming in on T cell clones: Are we heading to personalized treatment of allergy?

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Single-cell RNA and TCR sequencing of peripheral blood and esophageal cells of human eosinophilic esophagitis uncovers antigen-restricted T_H2 cells.

The term "food allergy" typically refers to immunoglobulin E (IgE)–mediated immediate immune hypersensitivity to food antigens, but there is now increased attention on a variety of other diseases associated with food-induced allergic inflammation involving mechanisms that are not simply IgE-dependent and may have relatively chronic features (*1*). Accordingly, it remains difficult to predict whether a response to food allergens will manifest as IgE-mediated anaphylaxis and/or an allergic inflammatory tissue-specific response, such as eosinophilic esophagitis (EoE). EoE is a food antigen–driven chronic type 2 immune response associated with esophageal eosinophilic inflammation and dysfunction. Although multiple common foods (e.g., milk, eggs, peanut, and wheat) can trigger the disease, why specific individuals develop EoE in response to one or multiple food allergens remains largely unknown (*2*). The field now understands that the tissue-specific manifestation of EoE is mediated, at least in part, by variations in the genes that encode thymic stromal lymphopoietin (TSLP) and calpain 14 (CAPN14) (*3*). These are gene products of esophageal epithelial cells that provide upstream innate immune cues for initiating a type 2 immune response, because CAPN14 regulates epithelial cell homeostasis and barrier function, dysregulation of which elicits the release of pro-atopy innate alarmins that include TSLP (*3*).

These findings set the stage for understanding the subsequent mechanisms of adaptive immunity, in which preclinical studies have provided evidence for a primary role for adaptive T cell–mediated rather than B cell–mediated immunity (*3*). Clinical studies have shown the lack of benefit of anti-IgE therapy (*4*) yet have supported the role of pathogenic effector T helper 2 (T_H2) cells secreting large amounts of interleukin-5 (IL-5) and IL-13 (*5*). IL-5 is a key eosinophil growth and activating factor, whereas IL-13 potently induces the production of disease-relevant mediators including the eosinophil chemoattractant chemokine (C-C motif) ligand 26 (CCL26) (for which the gene is highly overexpressed in the esophagus of patients with EoE compared with control individuals) and CAPN14 itself (*3*). In addition, antibody blockade of type 2 responses [e.g., anti–IL-4 receptor (dupilumab) and anti–IL-13 (cendakimab)] has shown early efficacy in clinical trials (*4*). Together, there is a timely need to better understand the adaptive T_H2 response associated with EoE.

Recently developed single-cell sequencing technologies have enabled the elucidation of the inflammatory environment of a variety of diseases based on the molecular markers of various cell types (*6*). In a recent study, we focused on esophageal T cell populations using the C1 Fluidigm platform, a droplet-based single-cell technology that uses a unique, barcoded bead capture technology (*5*). Of eight tissue-resident T cell populations identified, one subpopulation was present exclusively in the esophagus of patients with active EoE, exhibited an overactivated pathogenic effector memory T_H2 cell transcriptional profile, and was identified as the predominant source of the key disease-associated cytokine IL-13. In this issue of *Science Immunology*, Morgan *et al.* (*7*) profiled the cellular composition of the esophageal tissue in active and inactive EoE by using the Seq-Well platform for single-cell RNA and T cell receptor (TCR) sequencing. This gravity-based technology is considered less harmful to cells compared with other technologies (including the C1 Fluidigm platform), which may explain why the authors were able to detect tissue-resident eosinophils that have been challenging to identify by single-cell RNA sequencing in the past (*8*). The gene signature of the esophageal eosinophils was defined by expression of *SIGLEC8*, *CCR3*, *IL5RA*, *CLC*, and *RNASE2*, and evidence for a nuclear factor κB–mediated activation pathway was demonstrated.

Consistent with our prior single-cell RNA sequencing study, tissue-resident esophageal T cells included *GATA3*-expressing pathogenic effector T_H2 cells that also expressed the surface markers *IL17RB* and *IL1RL1*, T_H2 cytokines, and lipid metabolism–related genes (*5*). Morgan *et al.* (*7*) also report the expression of an unappreciated homing receptor GPR15, which was enriched in the tissue-resident and circulating $CRTH2^+CD161^+$ memory $CD4^+$ T cells that have previously been associated with EoE. Specific expression of the chemoattractant receptor GPR15 by T_H2 cells led the authors to propose a mechanism of T cell homing to the inflamed esophagus driven by the interaction of GPR15 with its ligand, GPR15L, that was expressed by the basal esophageal epithelium.

One of the most interesting aspects of the study by Morgan *et al.* (*7*) was the analysis of T cell clonality. Clonally expanded pathogenic effector T_H2 cells, defined by sharing common alpha or beta chain CDR3 sequences, were detected in the esophagus, consistent with an antigen-specific response. Circulating conventional T_H2 cells also demonstrated clonal expansion, albeit not as substantially as seen in the esophagus. Morgan *et al.* (*7*) demonstrated a specific reactivity of $CRTH2^+CD4^+$ pathogenic effector T_H2 cells from the peripheral blood after ex vivo exposure to milk protein. Milk-responsive pathogenic effector T_H2 cells expressed GPR15, suggesting that these cells acquire enhanced homing potential to the esophagus. Collectively, the authors propose that peripheral blood pathogenic effector T_H2 cells undergo allergen-specific clonal expansion and

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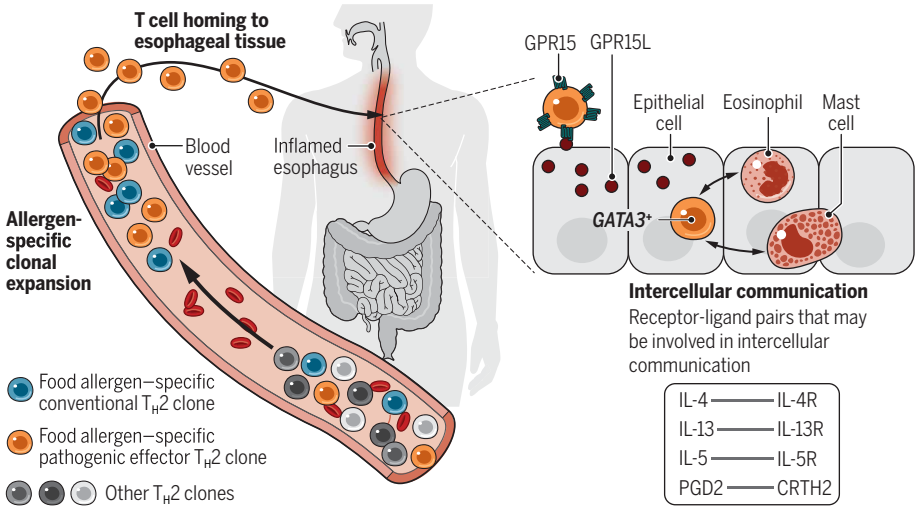

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Fig. 1. Role of pathogenic effector T_H2 cells in the tissue-specific manifestation of EoE. After exposure to food allergens, some individuals in the population develop EoE. Mechanistically, food allergen–responsive conventional and pathogenic effector T cells undergo clonal expansion and are detected in the bloodstream of patients with EoE. Subsequently, pathogenic effector T_H2 cells home into the esophagus potentially via the interaction between chemokine receptor GPR15 and its ligand GPR15L expressed by the basal epithelial cells. In the tissue, $GATA3^+$ pathogenic effector T_H2 cells secrete proallergic T_H2 cytokines and prostaglandin, thus recruiting and activating other cell types involved in local inflammatory response such as eosinophils and mast cells.

subsequently migrate to the esophagus using a GPR15-mediated homing mechanism.

Should the study by Morgan *et al.* (*7*) shift our view of the adaptive immune system in EoE and other related diseases? Possibly, but there is still more to learn, in part because the investigators focused their analysis on a small number of patients and mainly concentrated on milk-driven EoE. In addition, the authors did not include analysis of a food allergic, non-EoE group to determine whether the clonally expanded circulating T cells are specific to EoE or more broadly associated with food allergy. Future work should also more directly assess the functional role of GPR15. GPR15 is an interesting molecule, being first found as an HIV receptor and more recently shown to be involved in T cell homing to the intestine. Genetic ablation of GPR15 in mice leads to colonic inflammation that is rescued by GPR15-sufficient regulatory T cells (*9*). GRP15L, and likely GPR15 itself, is expressed by several different cell types, including colonic epithelial cells, but the reason for the apparently specific role of this homing pathway in the inflamed esophagus (e.g., lack of intestinal involvement in EoE) remains unclear.

These findings, together with other emerging data, expand our understanding of the tissue-specific manifestations of EoE. In particular, circulating, food allergen–driven T_H2 clones home to the esophagus, perhaps via the receptor-ligand interaction between $GPR15^+$ T cells and GPR15L-expressing basal epithelial cells. Local concentration of these pathogenic effector T_H2 cells leads to elevated levels of IL-5, IL-13, and eicosanoids (specifically PGD2), thereby attracting and activating allergic inflammatory cells, such as eosinophils and mast cells (Fig. 1). The finding that disease-causing pathogenic effector T_H2 cells have a limited TCR repertoire, including limited enrichment of antigen-specific clones, opens strategies for diagnosing and treating EoE and possibly other similar food-allergic diseases. The findings by Morgan *et al.* (*7*) imply that future therapies may focus on the specific elimination of allergen-specific T cell clones, perhaps by using recently emerging approaches, such as cytotoxic tetramers, which have been used to ablate $CD8^+$ T cell clones (*10*). However, several questions remain unanswered. First and foremost is why specific food antigens are recognized by the pathogenic effector T_H2 cells. Another highly relevant question is whether the findings also apply to non–milk-driven responses in EoE, which is important because a substantial proportion of patients with EoE are not sensitive to milk (*2*). Understanding the mechanism of T cell clonal expansion in response to different food allergens offers a potential opportunity to develop personalized medicine based on clonal T cell responses and should be a direction for future research.

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