Peanut oral immunotherapy (OIT) was recently approved by the US FDA. However, not all patients respond to OIT, and there is a high likelihood of regaining sensitization to peanuts after cessation of treatment. It is important, therefore, to identify biomarkers that impact and predict OIT outcomes. In this issue of the *JCI*, Monian, Tu, and colleagues describe distinct subsets of peanut-reactive CD4+ Th cell phenotypes and gene signatures with relevance to OIT outcomes using single-cell RNA-Seq and paired T cell receptor (TCR) α/β sequencing. The insights obtained will inform the development of therapeutics that target these Th cell phenotypes or deplete peanut-specific Th2 cells to achieve sustained nonresponsiveness in food allergy.

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Reducing the severity of anaphylactic reactions
Palforzia was approved by the US FDA in January 2020 as the first treatment for peanut allergy. The treatment includes daily oral administration of a controlled amount of peanut protein with escalating doses over a prolonged period to desensitize patients and reduce the severity of anaphylactic reactions (1). The treatment goal is to render patients bite-proof to accidental exposure, such that the primary efficacy endpoint is the ability to consume a challenge dose of 600 mg peanut protein without an adverse reaction (1). The treatment has a high efficacy of 67%; specifically, participants who received active treatment passed the exit food challenge (1). In addition, continued maintenance can improve the rate of efficacy (2). However, some patients do not respond to oral immunotherapy (OIT), and discontinuation can increase the likelihood of re sensitization to peanuts (3). For these reasons, it is important to understand the mechanisms underlying the failure of some patients to respond to OIT.

Food allergies are mediated by a Th2 immune response (4, 5). The Th2 cytokines IL-4 and IL-13 promote B cell class switching to IgE; binding of allergen-specific IgE to FcεRI receptors on mast cells or basophils initiates and propagates a hypersensitivity reaction (6). OIT has been shown to suppress the circulation of Th2 effector cells (6–8). For example, Blumchen et al. reported that peanut OIT resulted in a reduction in the amount of IL-4 and IL-5 produced by PBMCs in response to stimulation with peanut extract in vitro (7). Ryan et al. demonstrated that successful OIT caused allergen-specific Th2 cells to expand and shift toward an anergic and more tolerogenic status, with increased expression of genes such as TGF-β1 (8). However, most of these findings were not correlated with the clinical outcomes of OIT treatment. Recent studies have evaluated the role of T follicular helper (Tfh) cells as an alternate source of IL-4 and IL-13 in food allergy pathogenesis (9, 10); whether OIT affects Tfh cells remains unclear. In this issue of the JCI, Monian, Tu, and colleagues used single-cell RNA-Seq and paired T cell receptor (TCR) α/β sequencing to analyze allergen-specific T cell populations collected from the peripheral blood of 12 patients with peanut allergy longitudinally during the course of OIT (11) (Figure 1). The authors identified distinct Th cell phenotypes and gene signatures that were relevant to OIT efficacy.

Peanut OIT and Th cells
Monian, Tu, and co-authors first briefly activated antigen-specific T cells from the peripheral blood by in vitro stimulation with peanut extract. They then sorted peanut-reactive CD4+ memory T cells on the basis of CD154 and CD137 expression (11). This method enriched for antigen-specific T cells that were activated during the peanut stimulation, although the authors could not exclude the possibility that the selected cell populations still contained nonspecific, activated T cells. The T cell transcriptomes from these subsets formed distinct clusters, separated by differentially expressed genes, including CD40LG and TNFRSF9. Using sparse principal component analysis (PCA), the investigators identified gene modules that were consistent with the phenotypes of Th1, Th2, Th17, and Treg cell subsets. TCRβ was used for the subsequent clonotype analysis, since the TCRβ sequencing data covered most cells, were uniform, and paired well with a single TCRα. The TCRβ repertoire diversity of CD154+ and CD137+ cells was lower than that of the CD154–CD137– cells. This result suggests that the CD154+CD137+ subset was associated with clonally expanded T cells activated by in vitro stimulation, effectively lessening the influence that may have derived from nonspecifically activated T cells on this analysis.

Six phenotypically distinct cell populations were further identified within Th1, Th2, and Th17 cells: Thh2-like, Th2 regul-
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COMMENTARY

Figure 1. Association between clinical responses of OIT and peanut-reactive CD4+ T cells. Monian, Tu, and co-authors (11) assessed the transcriptomes of CD154+ and CD137+ peanut-reactive CD4+ Th cells from peripheral blood of patients with peanut allergy undergoing OIT. Suppression of Th1-conv and Th2A-like cell populations was associated with positive outcomes of OIT. Gene expression by Thf2-like cells correlated with peanut-specific IgE levels, supporting the role of Thf2 cells in class switching to IgE. Finally, the authors identified baseline inflammatory gene signatures, mostly present in Th1 and Th17 cell populations, that associated with treatment failure. These signatures suggest a potential role for these genes and Th1 and Th17 cells as predictors or influencers of OIT outcomes (dashed arrows).

Conclusions and clinical implications

The study by Monian, Tu, and colleagues suggests that OIT acts predominantly via Th2A-like cell suppression rather than through clonal deletion, providing additional insight into why some patients revert to an allergic phenotype after treatment (11). Thf2-like cells described in the study by Monian, Tu, and co-authors (11) were identified in peripheral blood. Future studies should examine possible direct or indirect links between these Thf2-like cells and B cell class switching.

Interestingly, the investigators assessed the impact of OIT on Th cell subtypes at different time points (i.e., at baseline and during buildup, maintenance, and avoidance; ref. 11). First, the authors showed that, while OIT decreased the abundance of peanut-reactive CD154+ and CD137+ cells in the blood overall, there was no evidence for deletion of specific TCR clones over time. However, individuals in the treatment group showed significant suppression of the Th2 module over time (P = 0.036). Suppression in the Th1 modules between the baseline and maintenance time points was nearly significant (P = 0.117), and no difference was observed for deletion of specific TCR clones over time. The study by Monian, Tu, and colleagues established that, although OIT changes the T cell repertoire, it has a limited impact on Th fates.

The study by Monian, Tu, and colleagues (11) points to a potential role for inflammatory gene signatures and Th1 and Th17 cells as predictors or influencers of OIT outcomes. Further studies with optimized methods for analyzing peanut-specific Tregs are needed to explore the predictive role of these cells in OIT.
like cell gene expression correlated with peanut-specific IgE, but OIT did not suppress Th2-like cells, which may provide another explanation for why it is difficult to achieve sustained nonresponsiveness through OIT. This study also established that certain gene modules broadly related to inflammation pathways at baseline were associated with the failure to respond to OIT, revealing the potential to predict success or failure of OIT before treatment begins. Future studies with larger samples and deeper sequencing approaches may reveal additional details about predictive gene signatures. Additionally, characterizing the transcriptomes of tissue-resident cell populations, particularly in the gut, will be critical to understanding how OIT influences Th2 cells and the resultant B cell responses. In summary, Monian, Tu, and co-authors (11) demonstrated that OIT modulated distinct Th2A-like and Th1-conv cell phenotypes and identified gene signatures that could potentially predict OIT efficacy. These clues to cellular mechanisms of OIT may provide insight into targets for the treatment of food allergy.

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