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Oral immunotherapy directs systemic transcriptomic changes in children with hen's egg allergy

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Abstract

Background: Hen's egg (HE) is a major food allergen in children. Oral immunotherapy (OIT) has emerged as a promising therapeutic option for hen's egg allergy (HEA), but the precise immunological mechanisms underlying HE-OIT are not fully understood.

Objective: We aimed to investigate the systemic immune phenotype in children with HEA and to examine transcriptomic changes during HE-OIT.

Methods: We enrolled 16 children, aged between 3 and 12 years, diagnosed with HEA (median age, 4.5 years). Peripheral blood mononuclear cells were collected before the initiation of HE-OIT and after the completion of the build-up phase. The transcriptomics of the samples were analyzed using single-cell RNA sequencing.

Results: All eight patients (8/8) whose blood samples were collected after the build-up phase achieved desensitization to 60 g of boiled HE white (6.0 g of HE proteins). Following the OIT build-up phase, significant reductions in total $CD4^+$ T cells and early activated $CD4^+$ T cell were observed (P = 0.001 and 0.045, respectively), while the frequencies of late activated $CD4^+$ T cells and fully activated $CD8^+$ T cells were increased (P = 0.019 and 0.038, respectively). Clonal analysis revealed proliferation within the late activated $CD8^+$ T cell subset following OIT, indicative of the exhausted state of $CD8^+$ T cells. Additionally, the population of regulatory T cells with abundant *IKZF2* expression was significantly increased after the OIT build-up phase.

Conclusion: HE-OIT was associated with systemic immune cell transcriptomic changes, suggesting that its efficacy derives from these immune alterations.

Key words: Anaphylaxis, Food Allergy, Egg allergy, Immunotherapy, Transcriptomics

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Abbreviations:

CCR4	CC chemokine receptor 4
EW	egg white
HE	hen's egg
HEA	hen's egg allergy
FA	food allergy
GATA3	GATA-binding protein 3
OFC	oral food challenge
OIT	oral immunotherapy
PA	peanut allergy
PBMC	peripheral blood mononuclear cell
PD-1	programmed cell death-1
scRNA-seq	single-cell RNA sequencing
Th2	T helper 2
Treg	regulatory T
UMAP	uniform manifold approximation and projection

Introduction

Hen's egg (HE) is a major food allergen globally and the leading cause of IgE-mediated food allergy (FA) in 6–7-year-olds in Korea, with an estimated prevalence of 0.25%.[1] Hen's egg allergy (HEA) negatively affects nutritional status, economic resources, and psychological well-being in children and strict avoidance of HEs in the diet is often challenging and burdensome.[2,3] Recently, oral immunotherapy (OIT) has emerged as a promising approach for managing IgE-mediated FA by inducing desensitization and sustained unresponsiveness to allergens.[4] HE-OIT could increase thresholds for allergic reactions in 40%–96% of patients with HEA after the build-up or maintenance phase.[5] Despite its clinical efficacy, the precise immunological mechanisms underlying HE-OIT and its associated immune cell phenotypes are not fully understood.[6]

Previous studies have highlighted OIT-induced immunomodulatory effects in peanut allergy (PA) patients, showing compromised regulatory T (Treg) cell development and increased T helper 2 (Th2) cells, which promote IgE class switching and expansion of allergic effector cells.[7] During OIT, allergen-specific Th2 anergy, along with increases in Tregs and IL-10-producing $CD4^+$ T cells, is observed, resulting in reduced specific IgE (sIgE) levels, elevated allergen-specific IgA and IgG4 levels, and subsequent desensitization.[8] Circulating allergen-specific IgG plays a crucial role in neutralizing allergens and inhibiting mast cell and basophil degranulation, supporting the effectiveness of desensitization in OIT-treated patients.[6] Recent advancements in single-cell RNA sequencing (scRNA-seq) have revealed the immunological complexities of PA, revealing the existence of highly differentiated Th2 cells that produce IL-4, IL-5, IL-9, and IL-13.[9] Another study identified a diverse range of peanut-reactive Th cell phenotypes beyond the traditional effector Th2 profile and that are associated with outcomes in OIT-treated PA patients.[10]

Despite the prevalence of HEA in Asian countries, scRNA-seq studies have predominantly focused on PA.[9-11] To address this discrepancy, we conducted a comprehensive single-cell analysis using flow cytometry and RNA-seq to investigate the phenotype of T-cell responses underlying HEA and the immune alterations during HE-OIT.

Methods

Study population

We enrolled 16 patients aged 3–12 years with IgE-mediated HEA. The diagnosis of HEA was based on a positive oral food challenge (OFC) test and elevated serum-specific IgE (sIgE) level ≥ 0.35 kU/L. The exclusion criteria were 1) history of autoimmune or systemic diseasis; 2) a history of eosinophilic gastrointestinal disorders; 3) uncontrolled atopic dermatitis (AD) or asthma; and 4) previous use of systemic corticosteroids, immunosuppressants, or biologics within 12 weeks prior to blood sample collection. Clinical and demographic information for each patient was collected, including past medical history, clinical symptoms after HE exposure, age at OIT initiation, and symptoms during OIT. The severity of symptoms was assessed using the Consortium of Food Allergy Research (CoFAR) grading system for allergic reactions.[12] Peripheral blood samples were obtained from all 16 subjects before initiating OIT (T1). After the build-up phase of OIT (T2), peripheral blood was collected from 8 subjects. Eosinophil counts were measured in peripheral blood, and the levels of total IgE, HE-sIgE, ovomucoid (OM)-sIgE, HE-specific IgG4, and OM-specific IgG4 were determined using ImmunoCAP (ThermoFisher Scientific Inc., Waltham, MA, USA). This study was approved by the Institutional Review Board of Samsung Medical Center, Seoul, Korea (SMC 2020-02-164). Written informed consent was obtained from all parents and/or patients before participation in this study.

Oral immunotherapy protocol

The OIT protocol consisted of a build-up phase and a maintenance phase.[13,14] Before starting OIT, detailed instructions were given to guardians regarding symptom management and the proper use of epinephrine auto-injectors in case of anaphylaxis. An OFC was conducted at the hospital to diagnose HEA and assess the threshold dose for egg white (EW) intake before initiating OIT. Daily intake of boiled EW at home began with half the maximum tolerated dose determined by the OFC with an increment of 0.1 g per week for the first four weeks and then 0.1 g every three days until reaching 1.0 g of boiled EW. Subsequently, the dose was further increased by 5% daily or 25% weekly, ultimately reaching a maximum of 40 g (4.0 g of HE proteins) of boiled EW.[13,14]

Dose adjustments were made based on symptom severity. For mild symptoms (CoFAR grade 1), the same dose was maintained the next day. For moderate to severe symptoms (CoFAR grade 2 or higher), the dose was reduced to the previous level and only increased when symptoms resolved. After consuming 40 g of boiled EW daily, an OFC was performed and patients proceeded to the maintenance phase. Desensitization was defined as no reactions with a cumulative dose of 60 g (6.0 g of HE proteins) during OFC after the build-up phase. The maintenance phase required patients to consume at least 40 g of EW at least 4 times a week for a minimum of 12 months.

Patient sample preparation and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from blood collected before the initiation of OIT (T1) and after the completion of the build-up phase (T2) by density centrifugation (Ficoll-paque Plus; GE Healthcare, Chicago, IL, USA) and were cryopreserved until use. These cells were thawed and labeled with TotalSeq C antibodies (BioLegend, San Diego, CA, USA) for multiplexing according to the manufacturer's instructions and the demonstrated protocol (CG000149) of 10x Genomics (Pleasonton, CA, USA). After washing, pooled cells were loaded onto the Chromium X system (10x Genomics). cDNA generation followed by gene-expression library and T cell receptor (TCR) library construction were conducted according to the manufacturer's instructions (user guide CG000424; 10x Genomics). The resulting cDNA and libraries were checked for quality using TapeStation 4150 (Agilent Technologies, Santa Clara, CA, USA). Libraries were sequenced using the NovaSeq 6000 system (Illumina, San Diego, CA, USA).

Analysis of single-cell RNA-seq data

scRNA-seq reads were mapped to the human reference genome (GRCh38) and processed using the 10x Genomics Cell Ranger. We used the Seurat package in R software for the downstream analyses. The integrated gene expression data were normalized and scaled and variable genes were identified for the unsupervised clustering. Principal component analysis (PCA) was carried out on the variable genes, and we embedded the data into a two-dimensional space using uniform manifold approximation and projection (UMAP) with the first principal components (PCs). The clusters were identified with a resolution of 0.5 and marker genes were calculated using the FindAllMarkers function with a minimum percentage (min.pct) of 0.25 and a log threshold (logfc.threshold) of 0.25 to determine the cellular identity of each cluster. The clusters were manually annotated using the known marker genes.

Statistical analysis

Data are presented as numbers with percentages or medians with ranges. Fisher's exact and Kruskal–Wallis tests were used to identify differences in baseline characteristics and laboratory data between the two groups. The Wilcoxon rank-sum test was used to compare laboratory data between time points T1 and T2 within the same group. For analyses, concentrations of specific IgE > 100 kU/L and specific IgG4 > 30 mg/L and < 0.07 mg/L were assigned values of 101 kU/L, 30 mg/L, and 0.07 mg/L, respectively. Data were analyzed using SPSS for Windows (version 27.0; IBM Corporation, Armonk, NY, USA) and GraphPad Prism (version 10.2.3; GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics

The median age of the patients was 4.5 years (range, 3–12 years). Among the 16 study subjects, 56.3% (9/16) were diagnosed with AD and 50.0% (8/16) had a history of HE-induced anaphylaxis (**Table 1**). Eight patients whose blood samples were collected at T2 completed the build-up phase at a median duration of 7 months (range, 5–9 months), and all achieved desensitization. To date, these 8 patients have been regularly ingesting at least 10 g of boiled EW (1.0 g of HE proteins) without adverse events for a median duration of 27.5 months (range, 25–33) following the maintenance period. HE- and OM-sIgE levels were reduced after the build-up phase (P = 0.043 and 0.017), and significant increases in specific IgG4 levels to HE and OM were observed (both P = 0.018) (**Table 2**). Among the remaining 8 patients who did not provide T2 blood samples, 7 successfully achieved desensitization, while 1 patient failed to reach desensitization due to anaphylaxis after consuming 13 g of EW during the build-up phase.

OIT modifies the frequencies of major circulating immune cell frequencies in HEA patients

Integration of blood sample data obtained from both time points T1 and T2, followed by quality-control procedures, yielded a total of 106,955 cells suitable for subsequent scRNA-seq analysis. These cells were subsequently subjected to computational clustering using UMAP, which identified 8 distinct clusters corresponding to major immune cell populations of $CD4^+$ T cells, $CD8^+$ T cells, B cells, monocytes, NK cells, NK T cells, platelets, and plasmablasts (**Figure 1B**).

We compared immune cell proportions according to patient age, history of anaphylaxis, and threshold dose before initiation of OIT. Patients with a history of anaphylaxis showed significant elevations in the B cell population compared to those without a history of anaphylaxis (P = 0.001). No significant differences were found in the proportions of $CD4^+$ T cells, $CD8^+$ T cells, NKT cells, NK cells, platelets, and plasmablasts according to age, anaphylaxis history, or between patients with a threshold dose ≤ 15 mg and those with a threshold dose > 15 mg as determined by OFC.

Table 1. Baseline characteristics of subjects.

Characteristics	Patients (n = 16)	Patients (n = 8)*
Male sex	14 (87.5)	8 (100.0)
Age (years)	4.5 (3-12)	6.5 (3-12)
Comorbid conditions		
Atopic dermatitis	9 (56.3)	4 (50.0)
Asthma	3 (18.8)	2 (25.0)
Allergic rhinitis	3 (18.8)	2 (25.0)
Allergic conjunctivitis	1 (6.3)	1 (12.5)
Family history of allergic diseases	12 (75.0)	7 (87.5)
Anaphylaxis history before OIT	8 (50.0)	4 (50.0)
Absolute eosinophil count (/μL)	379.1 (179.5–981.8)	343.9 (196.8–552.9)
Eosinophil cationic protein (μg/L)	42.8 (5.9–120.0)	30.9 (5.9–79.6)
Total IgE (kU/L)	1280.0 (229.0–3348.0)	795.0 (247.0–3348.0)
Specific IgE to egg white (kU/L)	78.2 (4.3–101.0)	33.4 (4.3–101.0)
Specific IgE to ovomucoid (kU/L)	27.5 (4.4–101.0)	18.8 (4.4–101.0)
Specific IgG4 to egg white (mg/L)	1.1 (0.1–6.8)	0.4 (0.1–3.6)
Specific IgG4 to ovomucoid (mg/L)	0.8 (0.0–7.2)	0.1 (0.1–2.8)

Data are presented as numbers (%) or median (range) values.
*Patients whose blood samples were collected at both T1 and T2.

Table 2. Immunologic changes between t1 and t2 in 8 subjects who achieved desensitization.

Variables	T1	T2	*P* value
Absolute eosinophil count (/μL)	344.0 (196.9–553.0)	335.4 (77.8–637.2)	0.575
Eosinophil cationic protein (μg/L)	12.7 (5.9–79.6)	17.5 (5.3–90.4)	0.735
Total IgE (kU/L)	1034 (247.0–3348.0)	1148 (218.0–4674.0)	0.093
Specific IgE to egg white (kU/L)	29.9 (4.3–101.0)	25.8 (1.8–101.0)	0.043
Specific IgE to ovomucoid (kU/L)	14.9 (4.4–87.9)	8.3 (1.4–6.7)	0.017
Specific IgG4 to egg white (mg/L)	0.6 (0.1–4.1)	5.2 (0.5–30.0)	0.018
Specific IgG4 to ovomucoid (mg/L)	0.09 (0.07–2.87)	6.11 (0.31–30.00)	0.018

Data are presented as median (range) values.

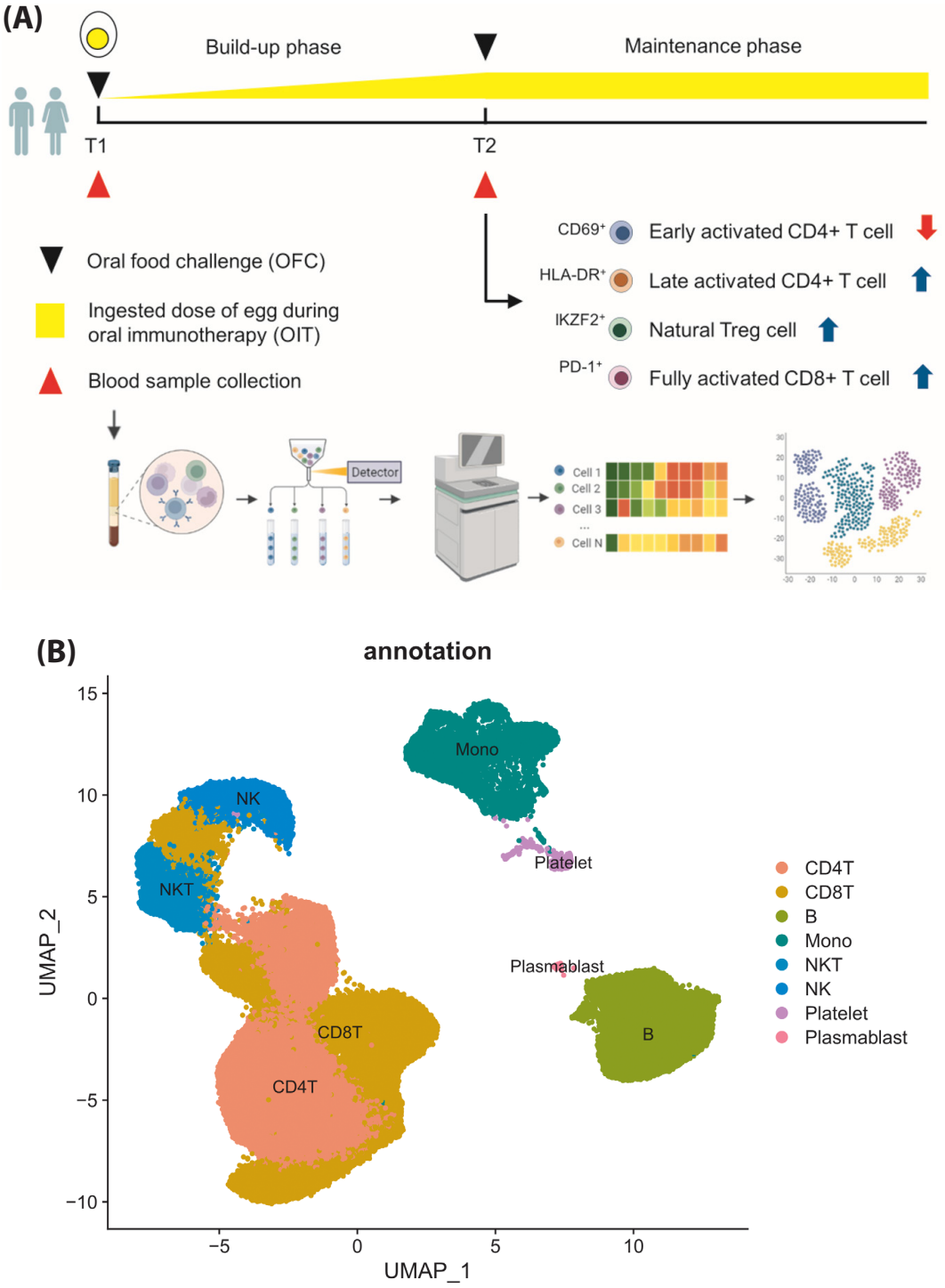


Figure 1. Schematic representation of immunological changes after HE-OIT and global scRNA-seq data analysis in patients with HEA. (A) Experimental design, analysis workflow and changes in key immune cells after HE-OIT. (B) UMAP plot of HEA patient blood samples labeled by cell type. (C) Boxplot showing differences in cell type proportions at T1 and T2. *P* values were calculated by Wilcoxon rank-sum test.

Abbreviation: HE, hen's egg; OIT, oral immunotherapy; HEA, hen's egg allergy.

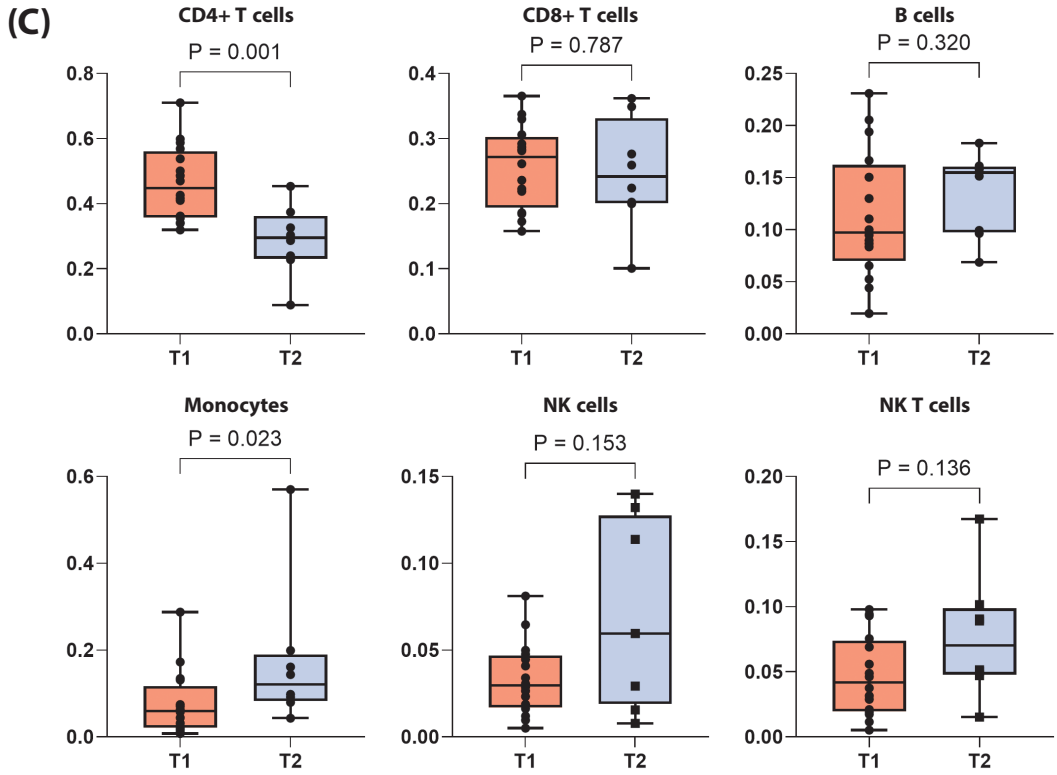


Figure 1. (Continued)

A comparative analysis of the proportions of each immune cell type between the two time points revealed significant reductions in the CD4⁺ T cell subset and elevations in the circulating monocyte population at T2 (P = 0.001 and = 0.023) (**Figure 1C**). In contrast, there were no significant differences in the proportions of CD8⁺ T cells, B cells, NK cells, and NK T cells between the two time points (P = 0.787, 0.320, 0.153, and 0.136, respectively).

CD4⁺ T cell transcriptomics suggest an exhausted phenotype and distinctive differentially expressed gene signatures after OIT.

Our focus was directed towards the characterization of CD4⁺ T cell populations. CD4⁺ T cells were classified into 6 major clusters, including central memory CD4⁺ T cells, effector CD4⁺ T cells, naive CD4⁺ T cells, early activated CD4⁺ T cells, Tregs, and late activated CD4⁺ T cells (**Figure 2A**). Significant changes in the composition of CD4⁺ T cell subpopulations were observed at T2, with a reduction in early activated CD4⁺ T cells (P = 0.019) characterized by CD69 expression and an increase in HLA-DR expressing late activated CD4⁺ T cells (P = 0.005) (**Figure 2B**). Additionally, the frequency of Tregs at T2 did not show a statistically significant increase (P = 0.264). The gene expression levels of the late activated CD4⁺ T subset were analyzed and selectively represented on the unsupervised heatmap in **Figure 2C**. Several Th2-associated genes were listed and compared according to their expression levels in CD4⁺ T cells before and after OIT, as illustrated in the UMAP plots (**Figure 2D**). The expression of both GATA-binding protein 3 (GATA3) and CC chemokine receptor 4 (CCR4) were significantly increased after the build-up phase of OIT.

Exhausted CD8⁺ T cell expression is upregulated after OIT

To isolate CD8⁺ T cells for additional examination, we clustered them into 4 groups according to functional annotation, identifying naive, early activated, intermediate, and fully activated CD8⁺ T cells (**Figure 3A**). Proportion analysis revealed no difference in the populations of early activated, naive, and intermediate CD8⁺ subgroups between T1 and T2 (**Figure 3B**). However, a significant increase was observed in the fully activated CD8⁺ T cell subgroup with enhanced expression of programmed cell death-1 (PD-1) after OIT (P = 0.038). Pseudotime analysis and clonal analysis of CD8⁺ T cells suggested clonal expansion of CD8⁺ T cells potentially specific to HE (**Figures 3C and 3D**). The relative expression levels of the most differentially expressed genes in fully activated CD8⁺ T cells are illustrated in the unsupervised heatmap (**Figure 3E**).

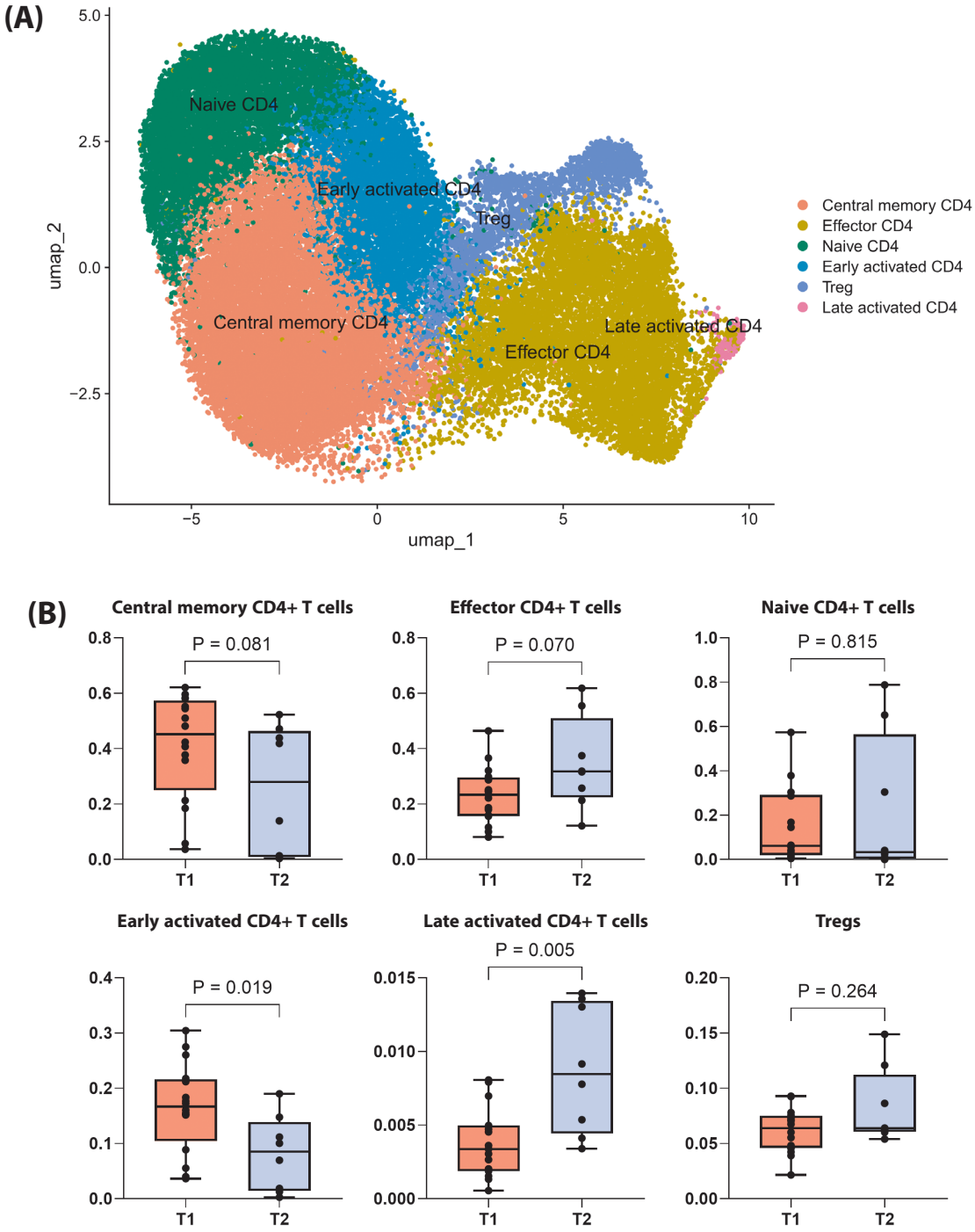
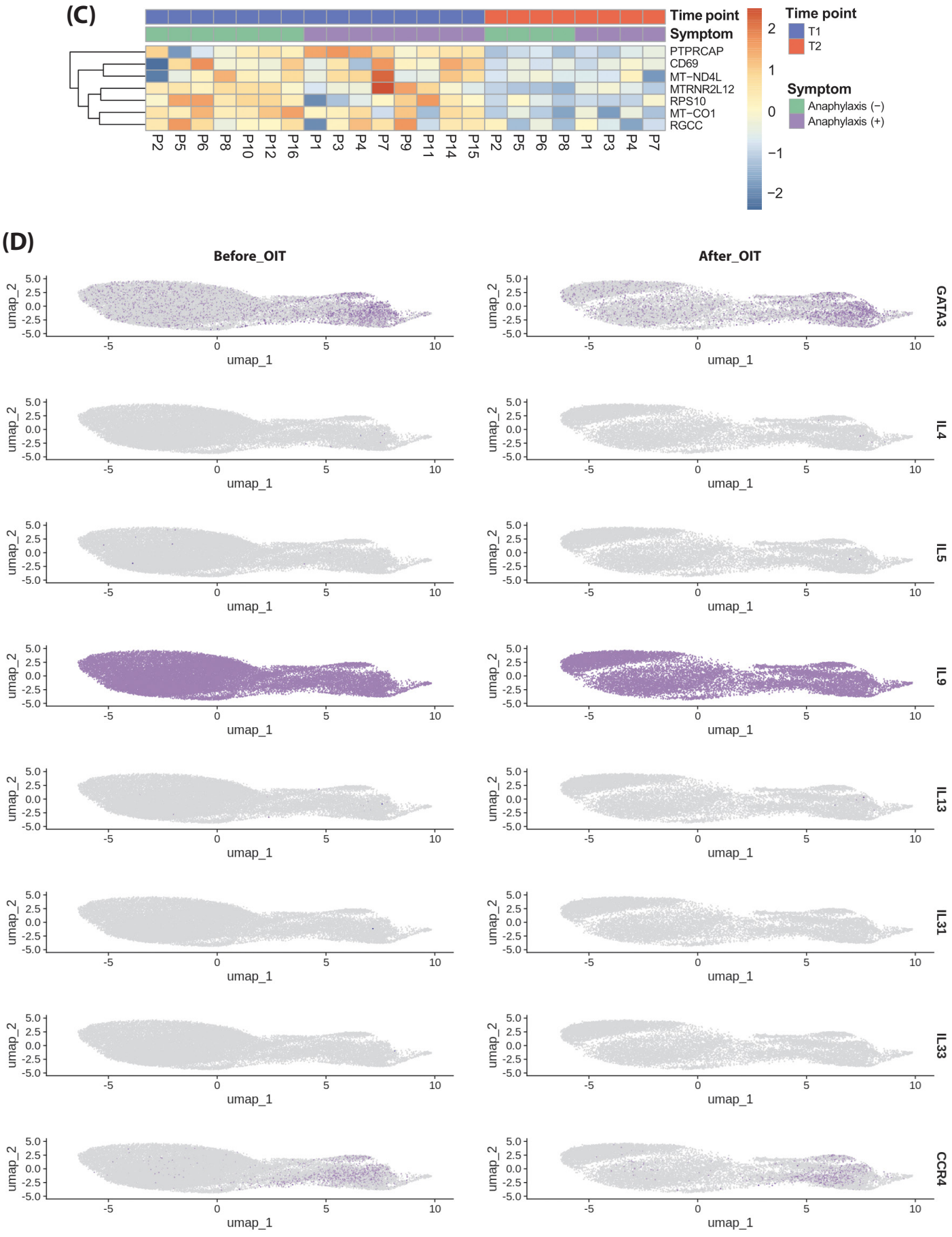


Figure 2. Transcriptomic difference in CD4⁺ T cell induced by OIT. (A) UMAP plot of isolated CD4⁺ T cells labeled by subpopulation. (B) Boxplot showing difference in each CD4⁺ T cell subtype proportions at T1 and T2. *P* values were calculated by Wilcoxon rank-sum test. (C) Unsupervised clustering heatmap showing relative expression levels of the most differentially expressed genes in late activated CD4⁺ T cells (stratified by diagnosis of anaphylaxis vs. none) with each row representing a gene and each column representing a patient sample at T1 and T2. (D) Expression of Th2-associated genes illustrated in CD4⁺ T cell UMAP at T1 and T2.

Abbreviation: OIT, oral immunotherapy.



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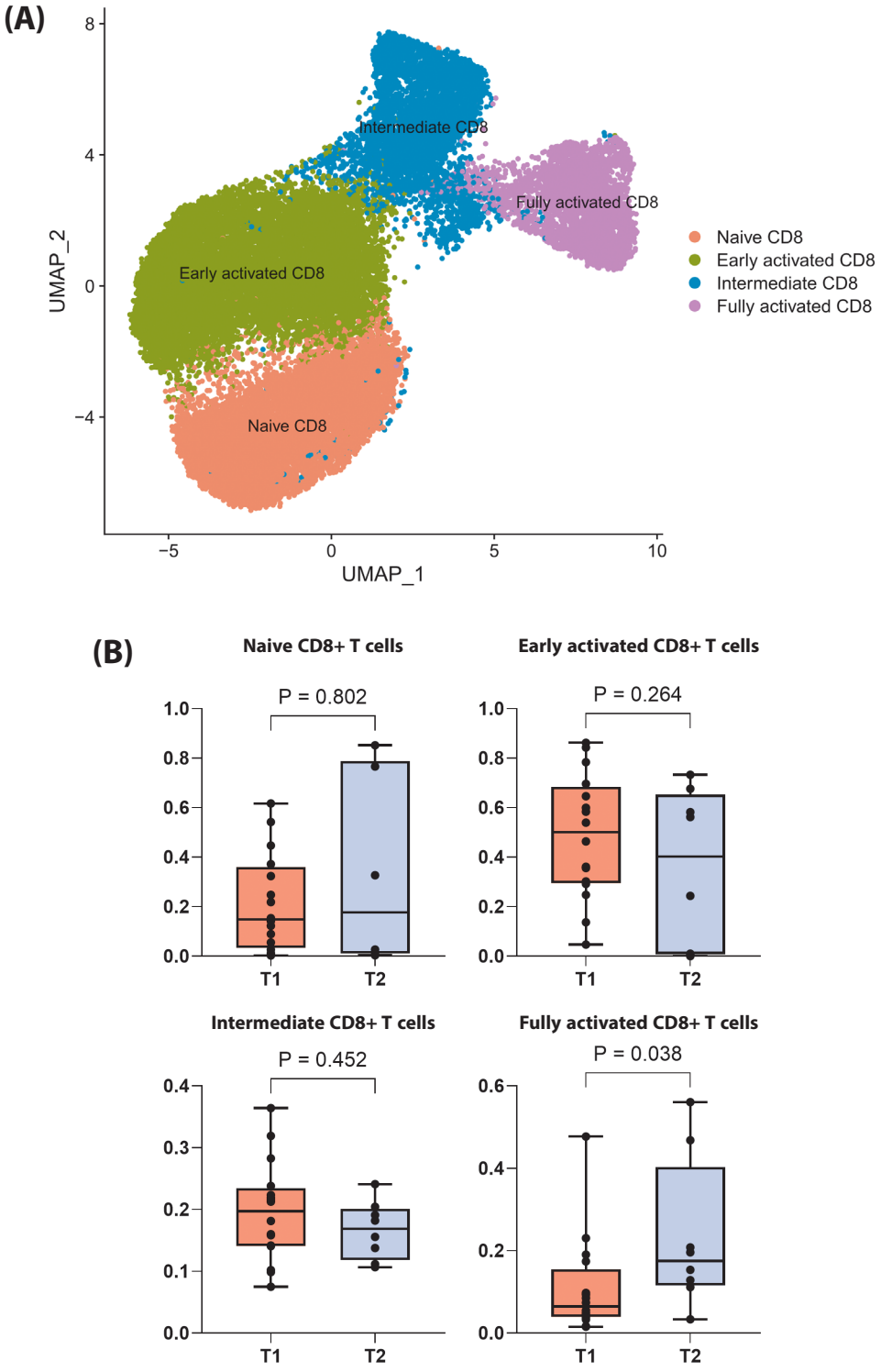
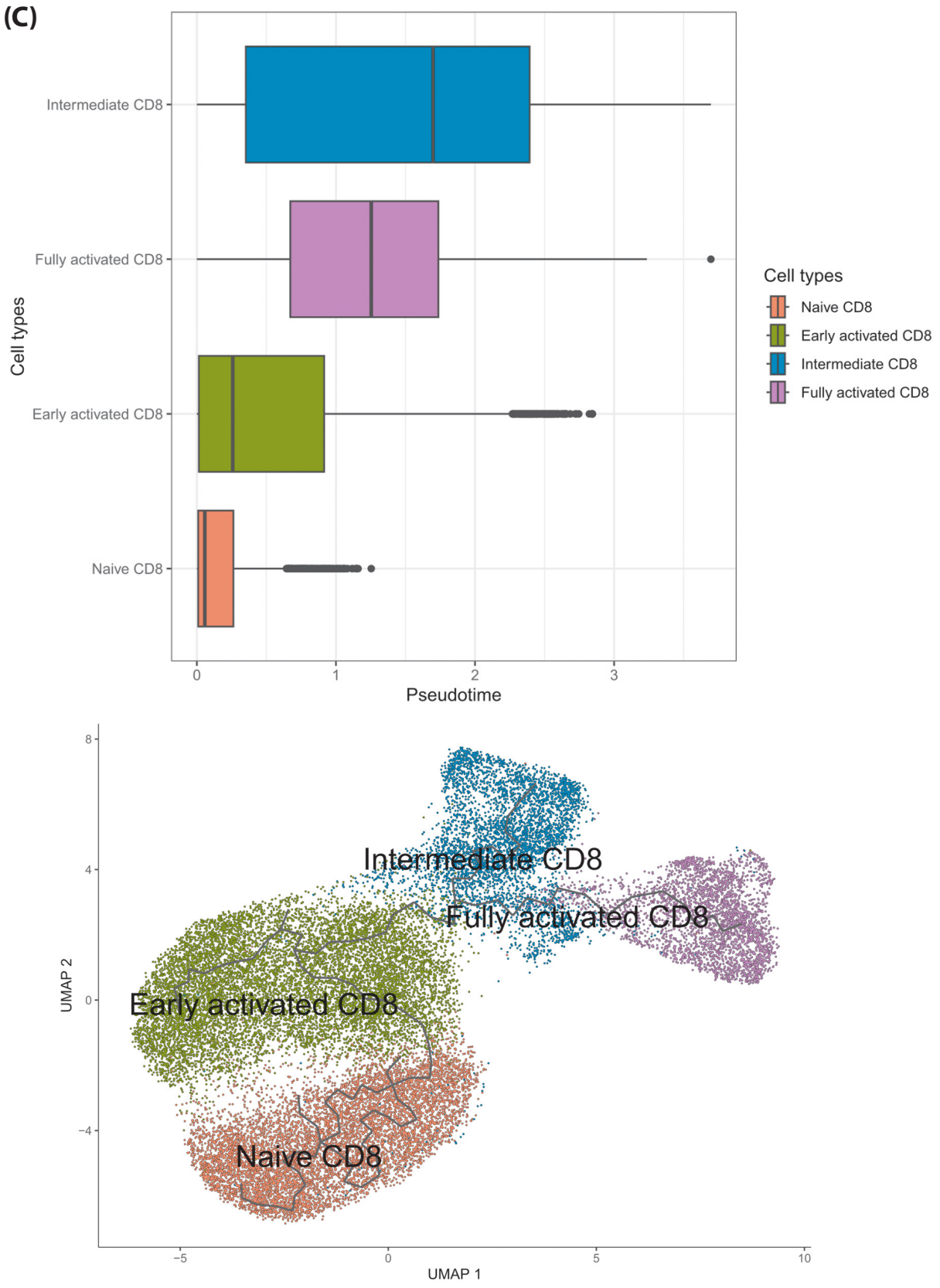


Figure 3. Transcriptomic difference in CD8⁺ T cell induced by OIT. (A) UMAP of isolated CD8⁺ T cells labeled by subpopulation. (B) Boxplot showing differences in each CD8⁺ T cell subtype proportions at T1 and T2. *P* values were calculated by Wilcoxon rank-sum test. (C) Pseudotime analysis of CD8⁺ T cell subpopulations. (D) Analysis of CD8⁺ T cell clonal expansion at T1 and T2. (E) Unsupervised clustering heatmap showing relative expression levels of the most differentially expressed genes in fully activated CD8⁺ T cells.

Abbreviation: OIT, oral immunotherapy.



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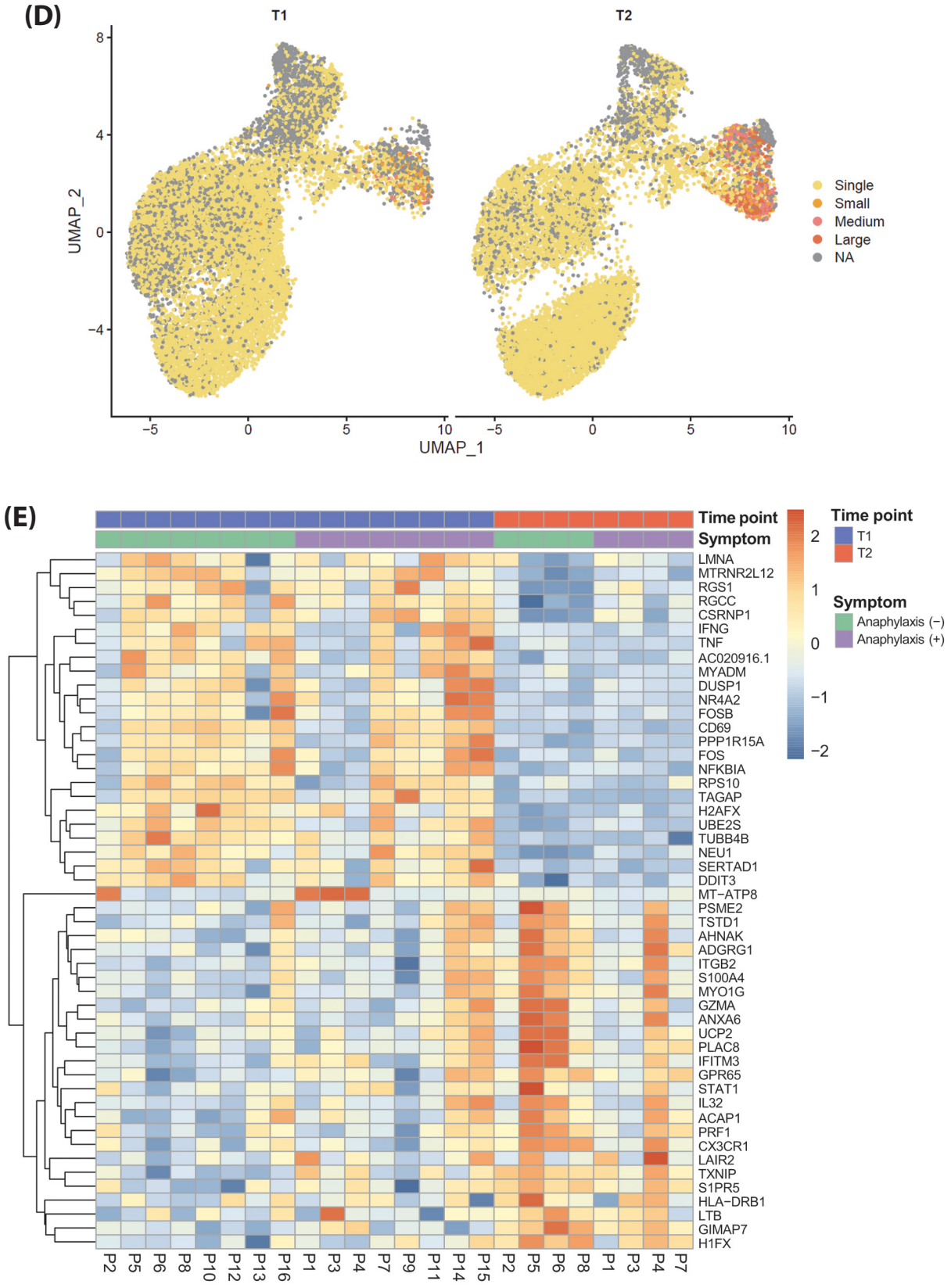


Figure 3. (Continued)

Treg subpopulation analysis shows increased natural Treg (nTreg) proportion after OIT

Using the Treg population defined in **Figure 2A**, we conducted differential gene-expression (DEG) analysis to identify genes with heightened expression within this subset. Based on this gene set, a Treg scoring system was developed and visually represented in the global annotation (**Figure 4A**), with gene expressions used in the Treg scoring displayed in **Figure 4B**. Within the Treg populations, certain subsets exhibited higher Treg scores, highlighted in red. We isolated these Tregs and classified them into four subgroups, including one nTreg group and three peripheral-induced Treg (pTreg) groups. Upon reapplying the Treg scoring system, the nTreg subpopulation recorded the highest Treg score, indicating that it best displays Treg properties (**Figure 4C**). Proportion analysis at T1 and T2 revealed a significant increase in the nTreg subpopulation, consistent with the Treg scoring system findings (P = 0.045) (**Figure 4D**). One subcluster within the potential Treg groups showed significant enrichment of Helios (IKZF2) (P < 0.001) (**Figure 4E**). The relative expression levels of the most differentially expressed genes among nTreg cells are displayed in an unsupervised heatmap (**Figure 4F**).

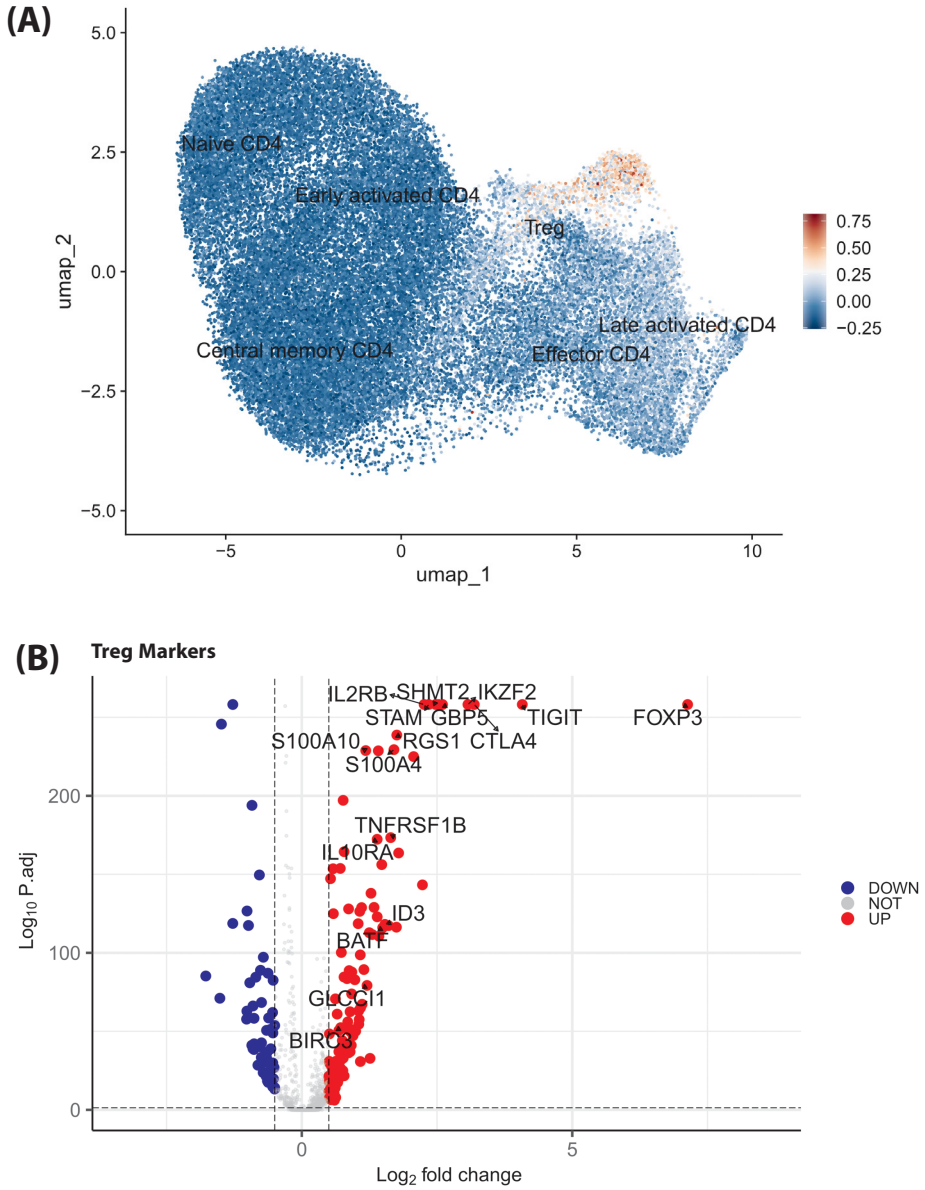
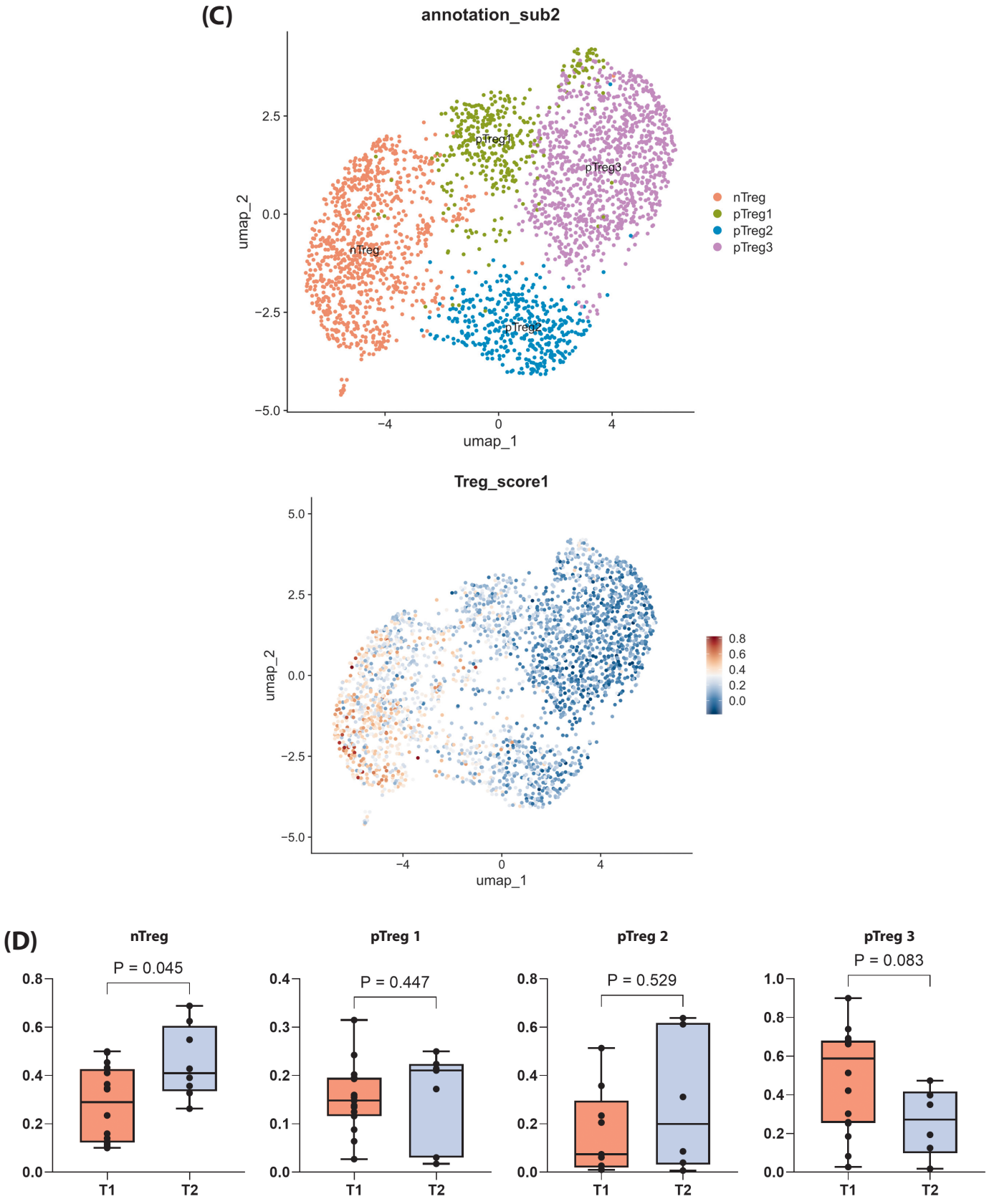


Figure 4. OIT induces enrichment of specific Treg subclusters. (A) Treg scoring based on DEG illustrated in global UMAP. (B) Volcano plot of Treg markers used for scoring. (C) UMAP of isolated Treg cells labeled by subpopulation (upper) and Treg score (lower). (D) Boxplot showing differences in Treg cell subtype proportions at T1 and T2. *P* values were calculated by Wilcoxon rank-sum test. (E) $CD4^+$ T cell UMAP illustrating *IKZF2* expression level (upper) and a violin plot representing *IKZF2* expression level in Tregs and non-Tregs (lower). (F) Unsupervised clustering heatmap showing relative expression levels of the most differentially expressed genes in nTreg cells.

Abbreviations: OIT, oral immunotherapy; Treg, regulatory T.



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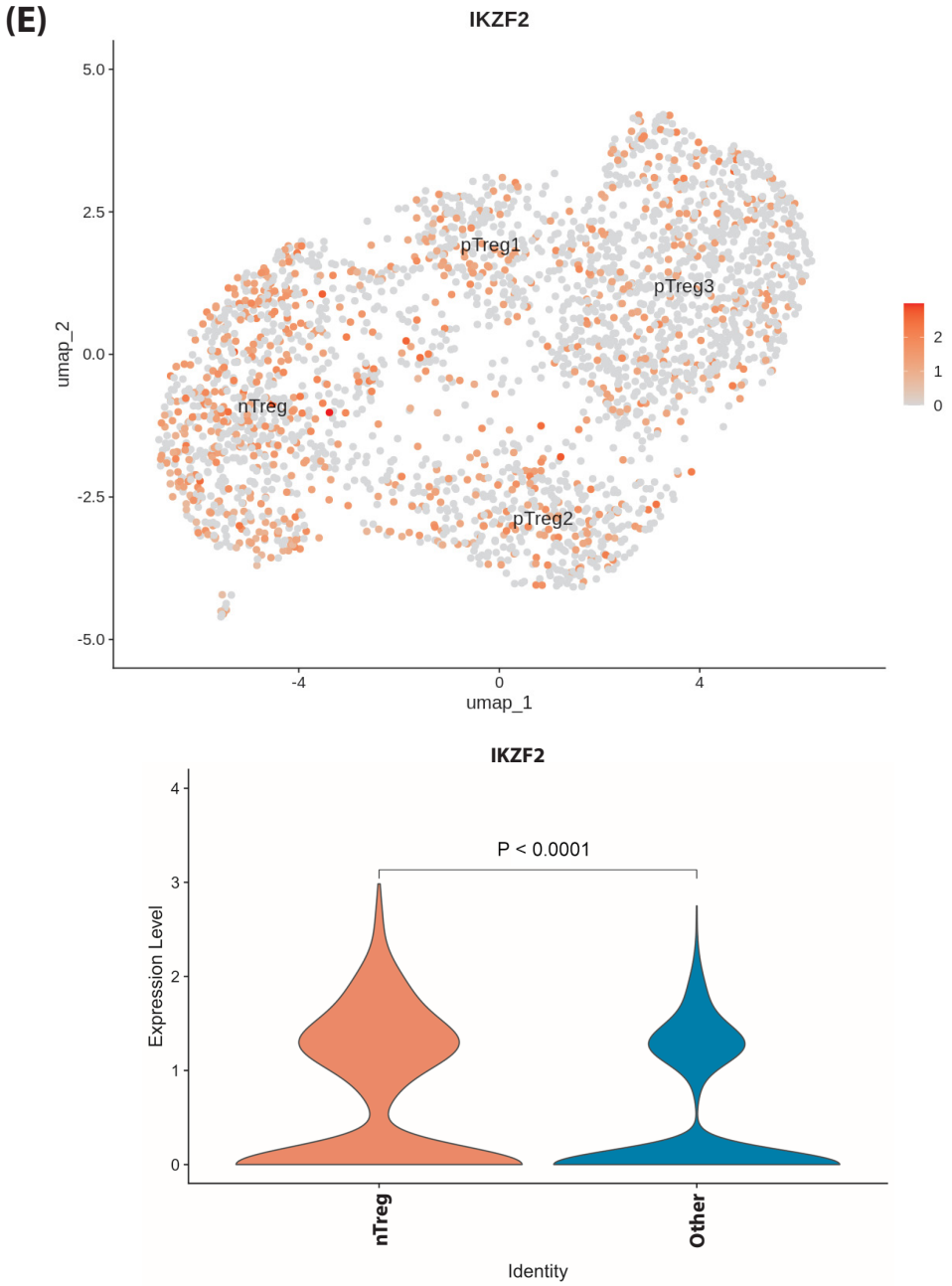

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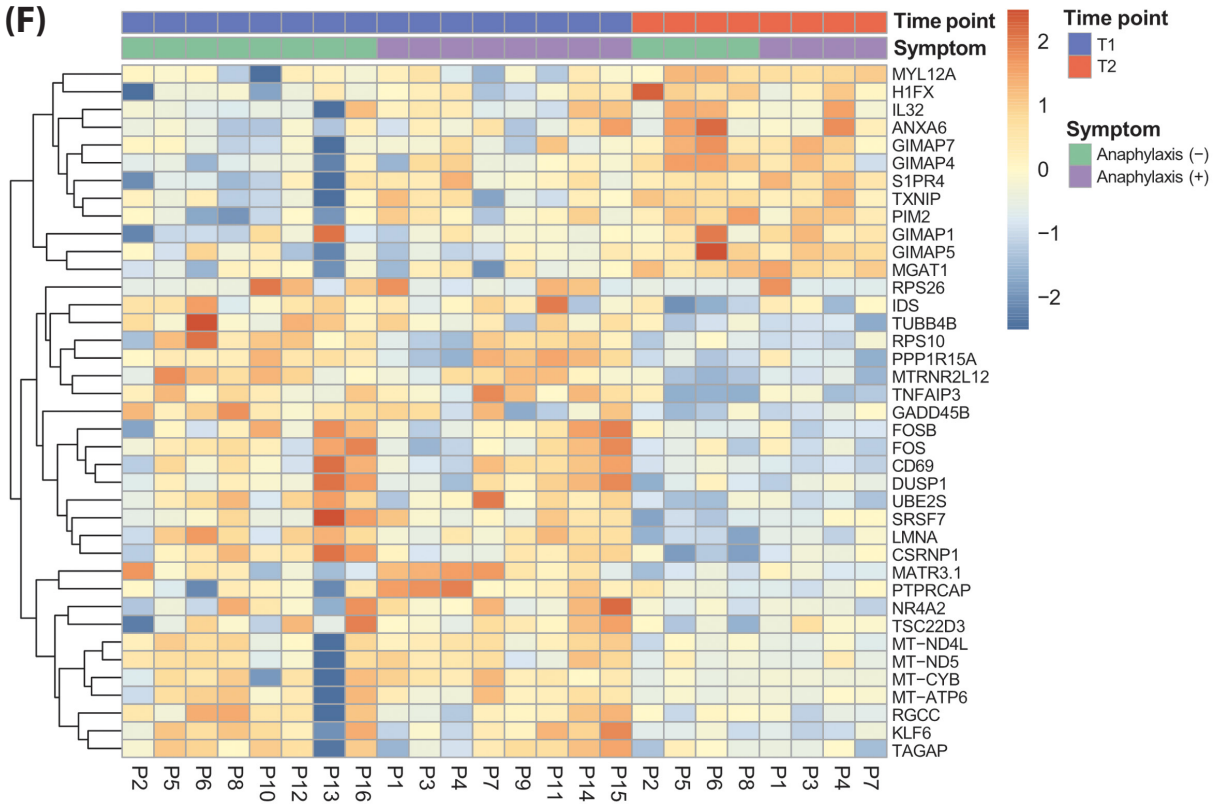

Figure 4. (Continued)

Discussion

To our knowledge, this is the first comprehensive study using sc-RNAseq to investigate specific immune cell modifications associated with HE-OIT. Our analysis revealed significant reductions in $CD4^+$ T cells, including early activated subsets, and an increase in late activated $CD4^+$ T cells. Additionally, we observed an increase in fully activated $CD8^+$ T cells and elevated expression of Th2-associated genes, GATA3 and CCR4 in $CD4^+$ T cells, reflecting substantial immunological reprogramming. Furthermore, an increase in the nTreg population, marked by enhanced expression of Helios (*IKZF2*), suggests important changes in Treg profiles.

The observed reduction in early activated $CD4^+$ T cells and increase in late activated $CD4^+$ T cells indicate a shift towards a more regulated immune environment, potentially reducing the availability of effector cells that could mediate allergic reactions.[15] CD69 plays a crucial role in promoting tissue residency, particularly in allergen-specific $CD4^+$ tissue-resident memory T cells.[16] Repeated exposure to inhalant allergens, such as house dust mites or *Aspergillus*, has been shown to induce Th2 cells and IL-9-producing T cells that express high levels of CD69 and persist in the lungs.[16] Recent studies have found that $CD4^+ST2^+$ T cells expressing CD69 during the early stages of tissue-resident memory T cell development in mice exposed to *Alternaria* remained in the lung tissue longer compared to their CD69− counterparts.[17] Furthermore, elevated HLA-DR expression is critical for antigen presentation, subsequent T cell activation, and allergen-induced late phase reactions.[18,19]

The increase in the proportion of fully activated $CD8^+$ T cells suggests a potential link to the observed $CD8^+$ T cell exhaustion and clinical improvements in our patients. The increased frequencies of fully activated $CD8^+$ T cells following HE-OIT suggest a potential enhancement in cytotoxic responses, contributing to desensitization. The shift from early activated to fully activated $CD8^+$ T cells implies that egg antigen-specific $CD8^+$ T cells may have clonally expanded due to repeated stimulation of the gastrointestinal tract by the HE antigen, although the possibility of clonal expansion from other factors such as vaccinations or recent infections should also be considered. In alignment with our findings, a peanut OIT trial also demonstrated an increase in the proportion of terminally differentiated $CD8^+$ T cells among patients who achieved desensitization.[20] Interestingly, however, patients who achieved sustained unresponsiveness showed a decrease in terminally differentiated $CD8^+$ T cells, suggesting that the immunological mechanisms underlying temporary desensitization may differ from those involved in long-term sustained unresponsiveness. These observations emphasize the need for long-term studies to further investigate the mechanisms distinguishing transient from permanent immune tolerance.

Given that repeated antigenic stimuli without inflammation can lead to T cell anergy or dysfunction,[21] it is plausible HE-specific CD4$^+$ T cells in peripheral circulation may have become anergic or dysfunctional, supporting the efficacy of HE-OIT as a therapeutic strategy. The exhausted T cell phenotype is often observed in chronic infections and cancer, where T cells show impaired function with upregulation of inhibitory receptors like PD-1 and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4).[22,23] Although blocking these pathways has become a key therapeutic strategy in cancer treatment, the role of T cell exhaustion in the context of OIT requires further investigation. A recent study on peanut OIT patients demonstrated a decrease in certain T cell populations with high PD-1 expression following OIT.[24] This finding differs from our results, suggesting that PD-1-mediated T-cell exhaustion may not uniformly contribute to all processes of immune tolerance. In that study, these T cells produced significant amounts of Th2 cytokines upon peanut antigen stimulation, indicating that distinct functional subsets exist within PD-1-expressing T cells. The differential responses observed across studies highlight the need for further studies employing antigen stimulation to better understand the functional dynamics of antigen-specific T cells throughout the course of OIT.

The differentiation of naive CD4$^+$ T cells toward Th2 cells, rather than Th17 or Treg cells, is driven by key transcription factors such as GATA3, which are crucial for Th2 cell differentiation.[25] Naive T cells express low levels of GATA3, which are upregulated by TCR and the IL-4 receptor, with GATA3 expression increasing during Th2 development and decreasing during Th1 development.[26,27] In accordance with the Th2-predominant expression of CCR4, the significance of CCR4 and its ligands in allergic diseases has been documented in patients with asthma or PA.[9,28,29] However, in the current study, the upregulation of Th2-associated genes, GATA3 and CCR4 suggests a possible role of Th2 cells in the desensitization process. While our findings align with recent research indicating that peanut-OIT upregulates CCR4 in both CD4$^+$ and CD8$^+$ T effector memory cells, a notable distinction is that the previous study involved multifood OIT combined with omalizumab, which may have influenced the immunological changes before and after treatment.[30] Therefore, it remains unclear whether the upregulation of CCR4 represents a generalizable effect of OIT or is influenced by the specific OIT protocol used. Supporting this uncertainty, a study on venom immunotherapy reported no significant changes in CCR4 T cell marker expression, suggesting that the immunological effects of immunotherapy may vary depending on the allergen and treatment approach.[31] Additionally, soluble CD83 can restrict the development of Th2 cells and inhibit antigen-specific Th2 cell activation by suppressing GATA3 activities, suggesting a method for regulating skewed Th2 responses.[32] This may indicate a temporary shift toward Th2 immunity during the early stages of OIT, and further long-term studies are needed to observe changes over time.[33,34]

In our study, patients with a history of anaphylaxis showed significant elevation in their B cell population compared to those without a history of anaphylaxis. This suggests that a history of anaphylaxis may influence immune responses to OIT, and increased B cell frequency in patients who have experienced anaphylaxis seems to be partially related to an increase in B cells producing IgE.[35] However, it is known that the relative abundance of IgE-producing B cells among the total B cell population is low, reported to be 104-fold less than that of IgG-producing B cells.[36] Further comprehensive research to compare the frequency of each subpopulation of B cells according to clinical phenotype is warranted.

Treg cells constitute a T helper cell lineage that originates from the thymus as nTreg cells or is induced in the periphery as pTreg cells.[37] In our study, we observed a significant increase in the nTreg subpopulation, which corresponds to the findings indicated by the scoring system. Notably, a subcluster within these Treg groups demonstrated significant enrichment of Helios (*IKZF2*), a member of the Ikaros transcription factor family commonly associated with nTregs, suggesting these cells are thymically derived.[38] These findings are consistent with the known role of Foxp3$^+$Helios$^-$ T cells in the periphery, which are generally induced, contrasting with that of thymically derived Foxp3$^+$Helios$^+$ T cells.[38] Helios^{high} Tregs have been reported to possess a greater suppressive capacity and better lineage stability, possibly due to Helios's role in epigenetically silencing the IL-2 gene, thereby regulating IL-2 production in Tregs.[39,40] The increase in nTregs, particularly those with high Helios expression, seen in our present study, may contribute to enhanced immune regulation and stability, which is relevant in the setting of OIT. However, since much of the existing research on Helios has been conducted in murine models,[38,40] further studies are needed to explore these mechanisms in humans, particularly in pediatric populations, to fully understand their implications for treatment strategies in allergic diseases.

In the case of the patient who failed to achieve desensitization due to anaphylaxis during OIT, we identified differences in gene expression patterns in fully activated CD8$^+$ T cells at T1. This patient's blood sample was not available at T2, but as shown in the heatmap (**Figure 3E, P14**), they exhibited a markedly higher expression of *NR4A2* (nuclear receptor subfamily 4 group A2) at T1 compared to OIT responders. Although the role of *NR4A2* in allergic reactions is not fully understood, an animal study suggests its involvement in mast cell activation and enhancement of *TNF-α* gene expression in mucosal mast cells, which may contribute to anaphylactic reactions.[41] While the absence of longitudinal data at T2 limits direct interpretation, our findings suggest that pre-existing immune signatures, such as heightened *NR4A2* expression in fully activated CD8$^+$ T cells, may be associated with an increased risk of severe allergic reactions during OIT. Future studies incorporating a larger cohort and a broader spectrum of OIT responses will be essential for validating these findings and identifying potential biomarkers for predicting OIT outcomes.

Our study has limitations related to its small sample size and the absence of a control group, which may affect the generalizability of its findings. To identify potential mechanistic insights, we initially focused on a smaller cohort of patients with favorable responses to OIT. The findings from this study provide a basis for future research with larger cohorts, including well-matched control groups and patients with poor responses to OIT to further refine our understanding of the immunological mechanisms underlying desensitization. Considering the natural history of HEA, some patients may experience spontaneous resolution, highlighting the need for further research on the immunological differences between desensitization resulting from natural outgrow and that induced by OIT. Additionally, the lack of allergen specificity in our findings stems from the absence of allergen stimulation in this study. While we provide insights into the baseline distribution and status of immune cells, we cannot assess how these cells respond to allergens or how OIT influences immune responses following exposure. Moreover, we did not examine immune profiles across different stages of OIT or in relation to adverse reactions during the therapy, which limits our understanding of temporal changes and specific immune mechanisms. Nevertheless, the immune cell changes observed in our patients with a good response to OIT may serve as potential biomarkers for monitoring treatment response. A reduction in early activated $CD4^+$ T cells and an increase in late activated $CD4^+$ T cells may indicate a positive treatment response and could provide valuable insights when determining whether to escalate the dose during OIT. As more data become available on the role of terminally differentiated $CD8^+$ T cells in desensitization and sustained unresponsiveness, these findings may help determine the optimal timing for discontinuing regular allergen consumption while maintaining desensitization.

In conclusion, our study demonstrated significant changes in immune cell populations following HE-OIT, including reduced early activated $CD4^+$ T cells, increased late activated $CD4^+$ T cells, fully activated $CD8^+$ T cells, and nTreg populations with enhanced Helios expression. These findings suggest substantial immunological reprogramming, offering valuable insights to improve therapeutic strategies for HEA and potentially other FAs.

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Conflict of Interest

The authors have no conflicts of interest to declare for this article.

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References

1. Kim M, Lee JY, Jeon H-y, Yang H-k, Lee K-J, Han Y, et al. Prevalence of Immediate-Type Food Allergy in Korean Schoolchildren in 2015: A Nationwide, Population-based Study. Allergy Asthma Immunol Res. 2017;9:410-6.
2. Kim S, Kim M, Kim J, Park B, Min N, Jung M, et al. Quality of Life in Food Allergy: Validation of the Korean Version of the Food Allergy Quality of Life Questionnaire Parent Form (K-FAQLQ-PF) and Risk Factor Analysis. Allergy Asthma Immunol Res. 2023;15:43-54.
3. Jung M, Kang U, Kim S, Yoo HW, Kim HY, Kim M, et al. Psychological Distress and Perceived Burden in Parents of Korean Children With IgE-Mediated Food Allergy. J Korean Med Sci. 2023;38:e208.
4. Patrawala S, Ramsey A, Capucilli P, Tuong LA, Vadamalai K, Mustafa SS. Real-world adoption of FDA-approved peanut oral immunotherapy with palforzia. J Allergy Clin Immunol Pract. 2022;10:1120-2.e1.
5. Özdemir PG, Sato S, Yanagida N, Ebisawa M. Oral Immunotherapy in Food Allergy: Where Are We Now? Allergy Asthma Immunol Res. 2023;15:125-44.
6. Kulis MD, Patil SU, Wambre E, Vickery BP. Immune mechanisms of oral immunotherapy. J Allergy Clin Immunol. 2018;141:491-8.
7. Tordesillas L, Berin MC, Sampson HA. Immunology of Food Allergy. Immunity. 2017;47:32-50.
8. Barshow SM, Kulis MD, Burks AW, Kim EH. Mechanisms of oral immunotherapy. Clin Exp Allergy. 2021;51:527-35.
9. Chiang D, Chen X, Jones SM, Wood RA, Sicherer SH, Burks AW, et al. Single-cell profiling of peanut-responsive T cells in patients with peanut allergy reveals heterogeneous effector T(H)2 subsets. J Allergy Clin Immunol. 2018;141:2107-20.
10. Monian B, Tu AA, Ruiter B, Morgan DM, Petrossian PM, Smith NP, et al. Peanut oral immunotherapy differentially suppresses clonally distinct subsets of T helper cells. J Clin Invest. 2022;132:e150634.
11. Jeong K, Kim J, Ahn K, Lee SY, Min TK, Pyun BY, et al. Age-Based Causes and Clinical Characteristics of Immediate-Type Food Allergy in Korean Children. Allergy Asthma Immunol Res. 2017;9:423-30.
12. Chinthrajah RS, Jones SM, Kim EH, Sicherer SH, Shreffler W, Lanser BJ, et al. Updating the CoFAR Grading Scale for Systemic Allergic Reactions in Food Allergy. J Allergy Clin Immunol. 2022;149:2166-70 e1.
13. Jeong HI, Lee B, Kim S, Kyung Y, Jung M, Kim M, et al. Home-Based Up-Dosing in Build-Up Phase of Oral Immunotherapy of Egg Allergy Is Safe and Feasible in Real-World Practice. Allergy Asthma Immunol Res. 2021;13:791-8.
14. Shin S, Jang S, Kim J, Song J, Park S, Kim Y, et al. Initial updosing phase of oral immunotherapy improves quality of life and psychological burden in parents of children with food allergy. Allergy Asthma Proc. 2024;45:128-36.

15. Tsuda M, Arakawa H, Ishii N, Ubukata C, Michimori M, Noda M, et al. Dietary Fructo-Oligosaccharides Attenuate Early Activation of CD4+ T Cells Which Produce both Th1 and Th2 Cytokines in the Intestinal Lymphoid Tissues of a Murine Food Allergy Model. Int Arch Allergy Immunol. 2017;174:121-32.
16. Ulrich BJ, Kharwadkar R, Chu M, Pajulas A, Muralidharan C, Koh B, et al. Allergic airway recall responses require IL-9 from resident memory CD4(+) T cells. Sci Immunol. 2022;7:eabg9296.
17. Kobayashi T, Iijima K, Matsumoto K, Lama JK, Kita H. Lung-resident CD69(+)ST2(+) T(H)2 cells mediate long-term type 2 memory to inhaled antigen in mice. J Allergy Clin Immunol. 2023;152:167-81.e6.
18. Eberlein-König B, Jung C, Rakoski J, Ring J. Immunohistochemical investigation of the cellular infiltrates at the sites of allergoid-induced late-phase cutaneous reactions associated with pollen allergen-specific immunotherapy. Clin Exp Allergy. 1999;29:1641-7.
19. Polak D, Hafner C, Briza P, Kitzmüller C, Elbe-Bürger A, Samadi N, et al. A novel role for neutrophils in IgE-mediated allergy: Evidence for antigen presentation in late-phase reactions. J Allergy Clin Immunol. 2019;143:1143-52.e4.
20. Kaushik A, Dunham D, Han X, Do E, Andorf S, Gupta S, et al. CD8(+) T cell differentiation status correlates with the feasibility of sustained unresponsiveness following oral immunotherapy. Nat Commun. 2022;13:6646.
21. Kalekar LA, Schmiel SE, Nandiwada SL, Lam WY, Barsness LO, Zhang N, et al. CD4(+) T cell anergy prevents autoimmunity and generates regulatory T cell precursors. Nat Immunol. 2016;17:304-14.
22. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. 2006;443:350-4.
23. Ye B, Liu X, Li X, Kong H, Tian L, Chen Y. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. Cell Death Dis. 2015;6:e1694.
24. Calise J, DeBerg H, Garabatos N, Khosa S, Bajzik V, Calderon LB, et al. Distinct trajectories distinguish antigen-specific T cells in peanut-allergic individuals undergoing oral immunotherapy. J Allergy Clin Immunol. 2023;152:155-66 e9.
25. Peng H, Ning H, Wang Q, Lu W, Chang Y, Wang TT, et al. Monocyte chemotactic protein-induced protein 1 controls allergic airway inflammation by suppressing IL-5-producing T(H)2 cells through the Notch/Gata3 pathway. J Allergy Clin Immunol. 2018;142:582-94.e10.
26. Pot C, Jin H, Awasthi A, Liu SM, Lai CY, Madan R, et al. Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. J Immunol. 2009;183:797-801.
27. Zheng WP, Flavell RA. Pillars Article: The Transcription Factor GATA-3 Is Necessary and Sufficient for Th2 Cytokine Gene Expression in CD4 T Cells. Cell. 1997. 89: 587-596. J Immunol. 2016;196:4426-35.
28. Yoshie O, Matsushima K. CCR4 and its ligands: from bench to bedside. Int Immunol. 2015;27:11-20.
29. Yu W, Zhou X, Dunham D, Lyu SC, Manohar M, Zhang W, et al. Allergen-specific CD8(+) T cells in peanut-allergic individuals. J Allergy Clin Immunol. 2019;143:1948-52.
30. Manohar M, Dunham D, Gupta S, Yan Z, Zhang W, Minnicozzi S, et al. Immune changes beyond Th2 pathways during rapid multifood immunotherapy enabled with omalizumab. Allergy. 2021;76:2809-26.
31. Cabrera CM, Urra JM, Alfaya T, Roca Fde L, Feo-Brito F. Expression of Th1, Th2, lymphocyte trafficking and activation markers on CD4+ T-cells of Hymenoptera allergic subjects and after venom immunotherapy. Mol Immunol. 2014;62:178-85.
32. Song W, Li H, Jia B, Wang Z, Liu Q, Yang G, et al. Soluble CD83 suppresses experimental food allergy via regulating aberrant T helper 2 responses. Immunol Res. 2020;68:141-51.
33. Ryan JF, Hovde R, Glanville J, Lyu SC, Ji X, Gupta S, et al. Successful immunotherapy induces previously unidentified allergen-specific CD4+ T-cell subsets. Proc Natl Acad Sci U S A. 2016;113:E1286-95.
34. Karisola P, Palosuo K, Hinkkanen V, Wisgrill L, Savinko T, Fyhrquist N, et al. Integrative Transcriptomics Reveals Activation of Innate Immune Responses and Inhibition of Inflammation During Oral Immunotherapy for Egg Allergy in Children. Front Immunol. 2021;12:704633.
35. Michelet M, Balbino B, Guilleminault L, Reber LL. IgE in the pathophysiology and therapy of food allergy. Eur J Immunol. 2021;51:531-43.
36. Dullaers M, De Bruyne R, Ramadani F, Gould HJ, Gevaert P, Lambrecht BN. The who, where, and when of IgE in allergic airway disease. J Allergy Clin Immunol. 2012;129:635-45.
37. Ernerudh J, Berg G, Mjösberg J. Regulatory T helper cells in pregnancy and their roles in systemic versus local immune tolerance. Am J Reprod Immunol. 2011;66 Suppl 1:31-43.
38. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. J Immunol. 2010;184:3433-41.
39. Baine I, Basu S, Ames R, Sellers RS, Macian F. Helios induces epigenetic silencing of IL2 gene expression in regulatory T cells. J Immunol. 2013;190:1008-16.
40. Szurek E, Cebula A, Wojciech L, Pietrzak M, Rempala G, Kisielow P, et al. Differences in Expression Level of Helios and Neuropilin-1 Do Not Distinguish Thymus-Derived from Extrathymically-Induced CD4+Foxp3+ Regulatory T Cells. PLoS one. 2015;10:e0141161.
41. Wang X, Hayashi S, Umezaki M, Yamamoto T, Kageyama-Yahara N, Kondo T, et al. Shikonin, a constituent of Lithospermum erythrorhizon exhibits anti-allergic effects by suppressing orphan nuclear receptor Nr4a family gene expression as a new prototype of calcineurin inhibitors in mast cells. Chem Biol Interact. 2014;224:117-27.