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LETTER

Deciphering Dysfunctional Regulatory T Cells in Atopic Dermatitis

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To the Editor,

Atopic dermatitis (AD) is a chronic inflammatory skin disease whose immune pathophysiology is primarily explained by Th2 cell biology. However, considering the immune dysregulation polyendocrinopathy enteropathy X-linked syndrome and the scurfy mice phenotype, we reasoned that regulatory T cells (Tregs) play central roles in AD pathology. Herein, we present compelling evidence of the dysfunction in cutaneous lymphocyte-associated antigen-positive (CLA^+) Tregs in the peripheral blood of AD patients. We isolated $CLA^+CD45RO^+TCR\alpha\beta^+CD4^+CD25hiCD127lo$ cells from peripheral blood mononuclear cells (PBMCs) of 11 AD patients and 11 healthy controls (Figure 1A). FOXP3 expression analysis using real-time PCR confirmed the identity of isolated $TCR\alpha\beta^+CD4^+$+CD25hiCD127lo cells as Tregs (Figure S1A). We found that the proportion of total Tregs among $CD4^+$ T cells was comparable between AD patients and healthy controls (Figures S2A,B). When we analyzed Treg subpopulations, the distribution analysis revealed differences in $CLA^+CD45RO^+$, CLA-CD45RO+, and CLA-CD45RO− Treg subsets between groups (Figure S2C,D), with a significant increase specifically in the $CLA^+CD45RO^+$ Treg population in AD patients (Figure 1B). However, in vitro Treg suppression assays using samples from our cohort (total $n=8$ for HC, $n=9$ for AD) demonstrated that CLA^++$CD45RO^+$ Tregs from AD patients exhibited significantly reduced suppressive capacity compared to those from healthy controls (Figure 1C). The impaired suppressive function of AD patient Tregs showed a dose-dependent pattern across multiple Treg:Teff ratios (1:1, 1:4, 1:16, and 0:1) (Figure S3A–D). To elucidate the molecular mechanisms underlying this dysfunction, single-cell RNA sequencing (scRNA-seq) was performed on isolated CLA^+ Tregs from five subjects per group. Unsupervised clustering revealed three distinct subgroups: Tregs, Tconvs, and proliferating T cells (Figure 2A,B). The Treg population was defined by using a public Cellular Indexing of Transcriptomes and

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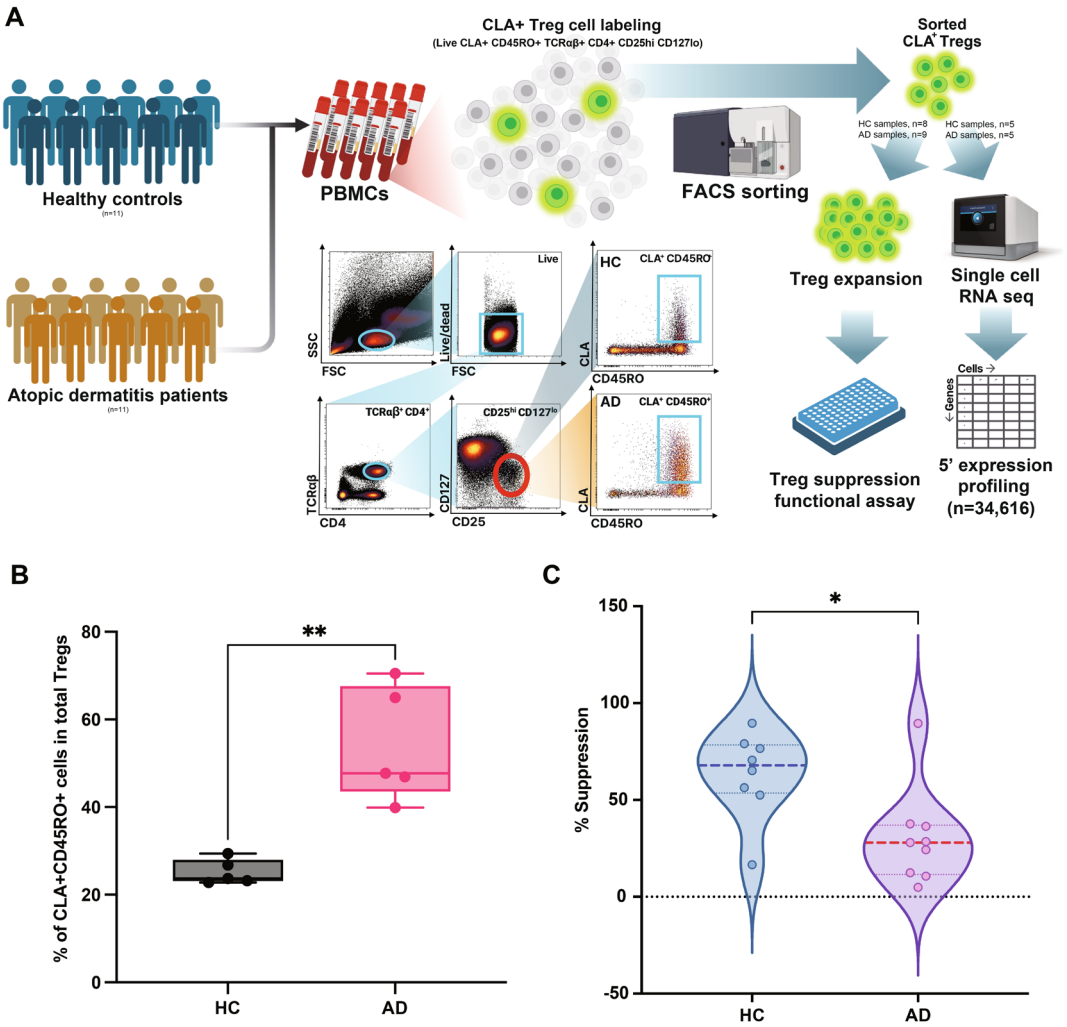

FIGURE 1 | Increased frequency but impaired function of CLA$^+$ Tregs in AD patients (A) Experimental workflow for isolation and analysis of Live CLA$^+$ Treg cells from healthy controls ($n = 11$) and atopic dermatitis patients ($n = 11$). PBMCs were isolated and CLA$^+$ Tregs (Live CLA$^+$ TCRαβ$^+$ CD4$^+$ CD25hi CD127lo) were labeled and sorted by FACS. Representative dot plots show gating strategy. Sorted CLA$^+$ Tregs underwent Treg suppression functional assay (HC sample, $n = 8$; AD sample, $n = 9$), and single cell RNA sequencing (HC sample, $n = 5$; AD sample, $n = 5$). (B) Box plots comparing the frequency of CLA$^+$ CD45RO$^+$ Tregs in PBMCs of HC versus AD patients ($n = 5$ per group, $p = 0.0079$). (C) Violin plots showing results of in vitro Treg suppression assays, demonstrating reduced ability of CLA$^+$ Tregs from AD patients to inhibit T responder cell (Tresp) proliferation (Treg: Tresp = 1: 1) compared to HC. *Y*-axis represents % suppression of Tresp proliferation ($n = 8$ for HC, $n = 9$ for AD, $p = 0.012$). Statistical analysis was performed using the Mann–Whitney *U* tests or unpaired *t*-tests based on data distribution. $p < 0.05$ (*) and < 0.01 (**) were considered statistically significant. Treg, regulatory T cells; HC, healthy control; AD, atopic dermatitis.

Epitopes by sequencing (CITE-seq) dataset and Treg-associated gene scores [1] (Figure 2C, Figure S4A–D).

Using gene set enrichment analysis (GSEA), we identified a reduced expression of genes associated with Treg function in patients with AD (Supporting Information). In addition, the AD patients exhibited lower expression levels of FOXP3-regulated genes than healthy individuals (Figure 2D). These findings suggest two potential mechanisms: either a reduction in FOXP3 expression or an impairment in the transcriptional activity of FOXP3 [2]. Given that FOXP3 expression was not diminished in peripheral blood (data not shown), we revealed that Treg dysfunction in AD is likely due to impaired epigenetic regulation of FOXP3 [3]. In line with the publication by Zhang et al., which demonstrated that OX40 (TNFRSF4) regulates FOXO1 to epigenetically control Treg function via FOXP3 [4], we examined OX40 expression. Indeed, we found a significant increase in OX40 levels in Tregs from the AD patients compared to healthy controls (Figure 2E). Further analysis showed that the dysfunctional markers OX40 (TNFRSF4) and GITR (TNFRSF18) were upregulated [5]. Their expressions in AD patients were around twice higher than HC (TNFRSF4: 1.6-fold difference, $p = 3.11 \times 10^{-55}$, TNFRSF18: 1.9-fold difference, $p = 4.88 \times 10^{-82}$, Wilcoxon rank-sum test). In contrast, the functional markers ETS1, IKZF2, and STAT5A were downregulated in AD patients (Figure 2F). Flow cytometry analysis further confirmed the increased protein level expression of OX40 in AD patient CLA$^+$ Tregs (Figures S5A,B). These findings support the dysregulated state of CLA$^+$ Tregs in AD.

Next, to investigate if this systemic observation is related with the target tissue event, we analyzed public AD skin scRNA-seq

data. Reannotation of Tregs (Data not shown) reveals comparable expression patterns (Figure 2G) with blood. Furthermore, spatial transcriptomics data showed increased OX40/OX40L expression in AD skin (Figure 2H). Deconvolution analysis indicated that this increased expression was primarily enriched in immune cells (Figure 2I).

Our findings help resolve the complex relationship between Treg frequencies and their function in AD. Previous studies have reported increased frequencies of circulating Tregs in AD patients [6], while skin lesion analysis revealed impaired $CD4^+CD25^+FOXP3^+$ T cell infiltration [7]. Our study specifically addresses this complexity by demonstrating that CLA^+

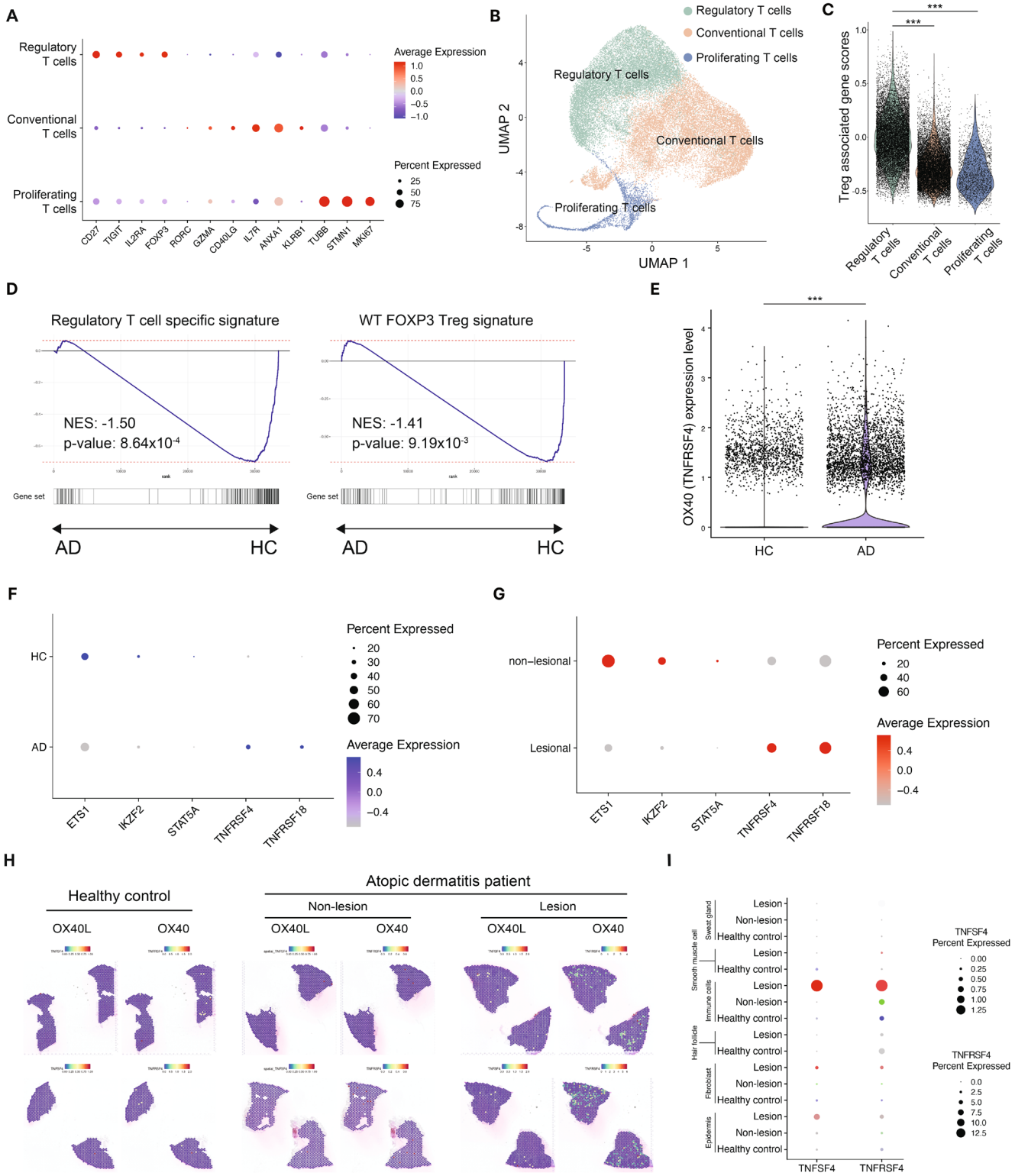

FIGURE 2 | Legend on next page.

FIGURE 2 | Molecular characterization of CLA$^+$ Tregs reveals dysregulation in AD (A) Dot plot showing differentially expressed genes (DEGs) across three subgroups identified by unsupervised clustering: Tregs, conventional T cells (Tconv), and proliferating T cells. (B) UMAP plot illustrating clustering of the three subgroups. (C) Violin plots comparing Treg-associated gene scores across subgroups, validated using a public CITE-seq dataset. (D) GSEA plots for Treg-specific genes, FOXP3-regulated genes. (E) Violin plot demonstrating increased OX40 expression in CLA$^+$ Tregs from AD patients. (F) Dot plot showing differential expression of functional (ETS1, IKZF2, and STAT5A) and dysfunctional (TNFRSF4, TNFRSF18) Treg markers between HC and AD Tregs. (G) Dot plot comparing expression of the same marker genes between non-lesional and lesional skin-resident Tregs from AD patients using publicly available single-cell transcriptomics data. For (F and G) dot size and color intensity indicate percent of expressing cells and average expression level, respectively. (H) Spatial transcriptomics analysis showing increased OX40 and OX40L expression in AD lesional skin compared to non-lesional skin and HC. (I) Deconvolution analysis of OX40 and OX40L expression across different cell types in spatial transcriptomics data, highlighting elevated expression in immune cells. Statistical analysis was performed by log-rank test with $p < 0.001$ (***) considered statistically significant. UMAP, uniform manifold approximation and projection; CITE-seq, Cellular Indexing of Transcriptomes and Epitopes by Sequencing.

Tregs, despite their increased frequency, show compromised suppressive function in vitro. The impaired suppressive function we observed can be explained by previous findings that IL-4 can modulate both Treg numbers and their suppressive function through CTLA-4 expression [8]. Our single cell transcriptomic analysis reveals the central role of the OX40/OX40L signaling axis in this dysfunction, with reanalysis of publicly available spatial transcriptomic data [9] further supporting the tissue-level relevance of this pathway in AD skin.

In conclusion, this comprehensive analysis of CLA$^+$ Tregs in AD reveals a paradoxical increase in their frequency coupled with functional impairment, driven by dysregulation of the OX40/OX40L axis. In line of this notion, the promising clinical outcomes of OX40-targeted therapeutics [10] validate the central role of OX40/OX40L signaling in AD pathogenesis, particularly through the modulation of Treg function.

Further studies should be conducted with larger cohorts and focus on exploring the long-term impact of CLA$^+$ Treg dysfunction on AD progression.

Author Contributions

Seong-Jun Kang planned and performed the experiments, analyzed the in vitro data, interpreted the findings, prepared in vitro figure sets, and wrote the manuscript. Jeong-Ryeol Gong analyzed the single cell RNA-seq data, interpreted the findings, prepared single cell RNA-seq figure sets, and wrote the manuscript. Seon-Pil Jin analyzed the data, wrote the manuscript. Jin-Mi Oh and Kyung Yeon Han did single cell RNA-seq experiments. Hyunjin Jin conducted flow cytometry. Yuji Lee, Yewon Moon, Dongjun Kim, Hyo Jeong Nam, and Sanha Hwang performed in vitro experiments. Hyun Seung Choi and Jihwan Moon analyzed the single cell RNA-seq data. Yun Jung Huh collected clinical samples. Jongsuk Chung, Woong-Yang Park, and Chung-Gyu Park support the project, reviewed manuscript. Hyun Je Kim designed the study, interpreted the findings, wrote and reviewed the manuscript, and give substantial comments for scientific evidence. Jeong Eun Kim provided clinical samples, interpreted the findings, wrote and reviewed the manuscript, and give substantial comments for clinical evidence.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

1. S. Sakaguchi, M. Miyara, C. M. Costantino, and D. A. Hafler, "FOXP3$^+$ Regulatory T Cells in the Human Immune System," *Nature Reviews. Immunology* 10, no. 7 (2010): 490–500.

2. G. Lal, N. Zhang, W. van der Touw, et al., "Epigenetic Regulation of Foxp3 Expression in Regulatory T Cells by DNA Methylation," *Journal of Immunology* 182, no. 1 (2009): 259–273.

3. M. He, X. Zong, B. Xu, et al., "Dynamic Foxp3-Chromatin Interaction Controls Tunable Treg Cell Function," *Journal of Experimental Medicine* 221, no. 9 (2024): e20232068.

4. X. Zhang, X. Xiao, P. Lan, et al., "OX40 Costimulation Inhibits Foxp3 Expression and Treg Induction via BATF3-Dependent and Independent Mechanisms," *Cell Reports* 24, no. 3 (2018): 607–618.

5. J. Shimizu, S. Yamazaki, T. Takahashi, Y. Ishida, and S. Sakaguchi, "Stimulation of CD25$^+$CD4$^+$ Regulatory T Cells Through GITR Breaks

Immunological Self-Tolerance," *Nature Immunology* 3, no. 2 (2002): 135–142.

6. L. M. Roesner, S. Floess, T. Witte, S. Olek, J. Huehn, and T. Werfel, "Foxp3^{+} Regulatory T Cells Are Expanded in Severe Atopic Dermatitis Patients," *Allergy* 70, no. 12 (2015): 1656–1660.

7. J. Verhagen, M. Akdis, C. Traidl-Hoffmann, et al., "Absence of T-Regulatory Cell Expression and Function in Atopic Dermatitis Skin," *Journal of Allergy and Clinical Immunology* 117, no. 1 (2006): 176–183.

8. B. Wang, Z. Yu, J. Liu, et al., "IL-4-Induced Decrease in Both the Number and CTLA-4 Expression of T(Reg) Impairs Suppression of Th2 Type Inflammation in Severe Atopic Dermatitis," *Journal of Dermatological Science* 114, no. 2 (2024): 54–63.

9. Y. Mitamura, M. Reiger, J. Kim, et al., "Spatial Transcriptomics Combined With Single-Cell RNA-Sequencing Unravels the Complex Inflammatory Cell Network in Atopic Dermatitis," *Allergy* 78, no. 8 (2023): 2215–2231.

10. E. Guttman-Yassky, E. L. Simpson, K. Reich, et al., "An Anti-OX40 Antibody to Treat Moderate-to-Severe Atopic Dermatitis: A Multicentre, Double-Blind, Placebo-Controlled Phase 2b Study," *Lancet* 401, no. 10372 (2023): 204–214.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.