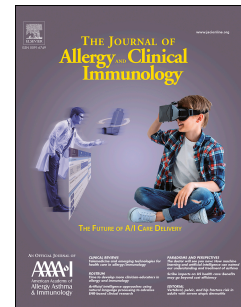


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T helper cell diversity and response to Dupilumab in atopic dermatitis patients

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Capsule Summary

Our results show the dynamic variations of eight T helper subsets in atopic dermatitis patients during treatment with Dupilumab and suggest the implication of other populations than just Th2 cells.

Key words: Atopic Dermatitis, Dupilumab, T helper cells, T follicular helper cells, biomarker

Abbreviations: AD: Atopic Dermatitis, Th: T helper, Tfh: T follicular helper

To the Editor:

We report here on a systematic assessment of T helper (Th) subsets and their dynamic changes, in the context of Dupilumab treatment, a recently approved biotherapy in atopic dermatitis (AD).

Dupilumab targets the interleukin (IL)- 4 receptor alpha chain, common to the IL-4 and IL-13 receptors. Although the IL-4 pathway is critical for Th2 differentiation, it may also affect other Th pathways directly or through cross-regulation.

In this study, we used multicolor flow cytometry to measure the evolution of eight Th and T follicular helper (Tfh) cell populations in peripheral blood of chronic AD patients before and after Dupilumab, with or without concomitant treatment (as detailed in the Online Repository Table E2). Samples were obtained from 29 patients with moderate-to-severe AD at different time-points from therapy (Month (M) 0 (baseline), M1, M3, M6, and $\geq$ M12), and 25 age- and gender-matched healthy subjects (Online Repository Table E1). Using a validated five-parameter surface staining (1) (Online Repository Figure E1), we measured the proportions of all eight major memory Th and Tfh cell populations: Th1, Th2, Th17, Th1/17, Tfh1, Tfh2, Tfh17, and Tfh1/17. AD patients at baseline (M0) had similar percentages of CXCR5⁻ (total Th) and CXCR5⁺ (total Tfh) CD4 T cells as compared to healthy donors (HD), with no significant variations along Dupilumab treatment (Online Repository Figure E2A), supporting the importance of a detailed analysis.

Addressing the Tfh cell populations, Tfh1, Tfh2 and Tfh1/17 percentages were not significantly different between AD at M0 and HD, but Tfh17 cells were lower in AD as compared to HD. Additionally, we did not detect any significant variation of the Tfh2, Tfh17 and Tfh1/17 cell percentages during Dupilumab treatment. Only Tfh1 cell percentages decreased between M0 and M3, but returned to baseline levels at M12 (Online Repository Figure E2B).

The biggest baseline differences and dynamic variations were measured on the Th cell populations. When comparing AD at M0 and HD, there was no significant difference in Th1 cells, but higher percentage of Th2 and Th17 cells, and lower percentages of Th1/17 (Figure 1A). Dynamically, we detected a significant increase in Th1 and Th1/17 cell percentages between M0 and M12, paralleling a gradual decrease in Th2 cells (Figure 1A). Changes in Th17 cell percentage were more subtle, with an initial increase (M0 to M1 and M3), and

return to baseline at M12. Evolution of Th2 and Th17 cell percentages for individual patients are also provided (Online Repository Figure E3).

Among routine blood cell count variables, total lymphocytes slightly increased over Dupilumab treatment, while eosinophils increased from M0 to M6 and returned to baseline (Figure 1B). These were minor changes as compared to modifications in Th subpopulations. Other studies have measured Th cell populations in peripheral blood of AD patients (2-5), bringing new insights on the proportions of Th subsets between skin-homing CLA⁺ and CLA⁻ CD4⁺ T cells. Complementarily, our study focuses on the total Th and Tfh subsets in circulating blood of AD patients.

Two interesting studies used transcriptomic analysis in whole lesional skin biopsies comparing baseline to four weeks Dupilumab treatment, and found that Th2-associated chemokines were downregulated in response to Dupilumab (6, 7). Our study significantly extends those findings by providing a detailed and dynamic analysis of Th cell diversity in AD patients along 12 months Dupilumab treatment.

In parallel to the immunological variables, we monitored the baseline value and dynamics of standard clinical scores: EASI and SCORAD at each timepoint. As previously reported (8), both scores significantly decreased from M0 to M12 during patient treatment with Dupilumab (Figure 1C). Administration of concomitant therapies did not influence EASI score (data not shown).

We evaluated the association between Th and Tfh cell populations and EASI score.

Th2 cell percentage significantly correlated to EASI score at all timepoints except M6 (Figure 2A). This was expected, since the decrease in Th2 cell percentage from M0 to M12 (Figure 1A), paralleled the decrease of EASI score (Figure 1C). It also validated blood Th2 cells as a robust pharmacodynamic biomarker of Dupilumab treatment.

Our working hypothesis was that Th cell cross-regulation may induce more complex dynamic changes in Th subsets than just Th2. Indeed, Th17 cell percentage significantly correlated with EASI score at M0 and very strongly at M12 (pearson correlation = 0.7867) (Figure 2B). Also, correlation between relative difference of EASI score between M12 and M0 and relative difference of Th17 cell percentage between M12 and M0 showed that the largest decreases in Th17 cell percentages were found in patients with EASI score largest decreases (Figure 2C). This is in line with a possible role for Th17 cells in the physiopathology of AD (9).

Finally, we grouped the patients according to their response to treatment: high responders displaying an improvement of the EASI score at $\geq$ M12 of at least 85%, low responders showing a decrease of the EASI score of less than 60%, and the remaining intermediate responders. Low responders displayed significantly lower levels of CXCR5⁺ total Tfh cells at M12, as compared to strong and intermediate responders. This difference was also identified for Tfh1 and Tfh2 cells. Conversely, low responders displayed higher levels of CXCR5⁻ cells

at M12, as compared to high/intermediate responders. Tfh17 and Tfh1/17 cell percentages were not significantly different between the two groups (Figure 2D).

Reductions in CCL26 and CCL13 (Th2-associated chemokines) expression were previously correlated with improvement in the EASI score (7). Here, we were able to show that EASI score correlated not only with Th2 cell percentage but also with Th17 cell percentage, and that total Tfh, Tfh1 and Tfh2 cell percentages could allow separation of low versus high responders.

Our detailed and systematic study further established a strong link between Th2 cells and response to Dupilumab. However, it also suggests an important role of other Th and Tfh cell populations in AD physiopathology and treatment response. Although established on a limited number of patients, our results warrant validation in larger independent cohorts. This should set the ground for the exploitation of fine-grained Th cell subsets as biomarkers in future clinical observation studies, or in the context of controlled therapeutic trials.

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Figure legends

Figure 1: Systematic analysis of clinical scores and Th cell populations in AD patients during Dupilumab treatment. A) Percentages of Th1, Th2, Th17 and Th1/17 cells among CD4⁺CD45RO⁺ cells in HD and AD patients at each timepoint during Dupilumab treatment. B) Values of lymphocytes and eosinophils numeration in blood at each timepoint. C) Values of EASI and SCORAD measured by clinicians at each timepoint. Median $\pm$ interquartile range are plotted for each graph.

Figure 2: EASI score association with Th2, Th17, and Tfh cell percentages in AD patients treated with Dupilumab. A) Correlation of Th2 cell percentage with EASI score at M0, M1, M3, M6 and $\geq$ M12 of Dupilumab treatment. B) Correlation of Th17 cell percentage with EASI score at M0 and $\geq$ M12 of Dupilumab treatment. C) Correlation of EASI score relative difference between $\geq$ M12 and M0 and Th17 cell percentage relative difference between $\geq$ M12 and M0. D) Comparison of CXCR5⁺, CXCR5⁻, Tfh1, Tfh2, Tfh17 and Tfh1/17 cell percentage at $\geq$ M12 between low responders and high/intermediate responders.

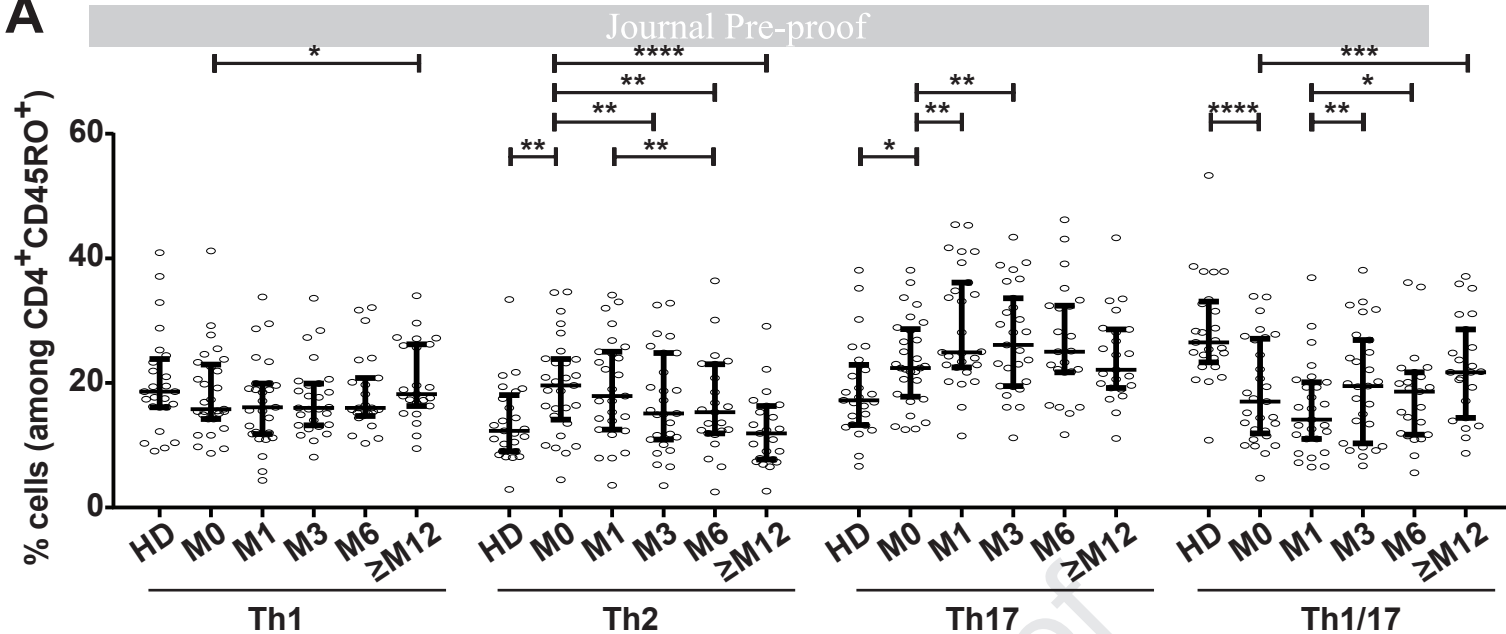
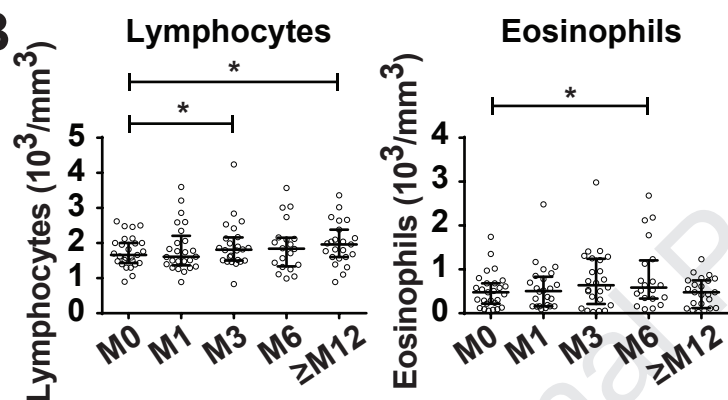
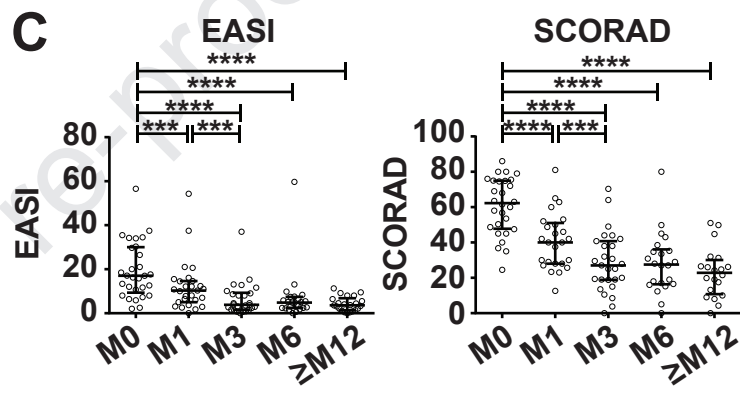
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FIGURE 1: Systematic analysis of clinical scores and Th cell populations in AD patients during Dupilumab treatment

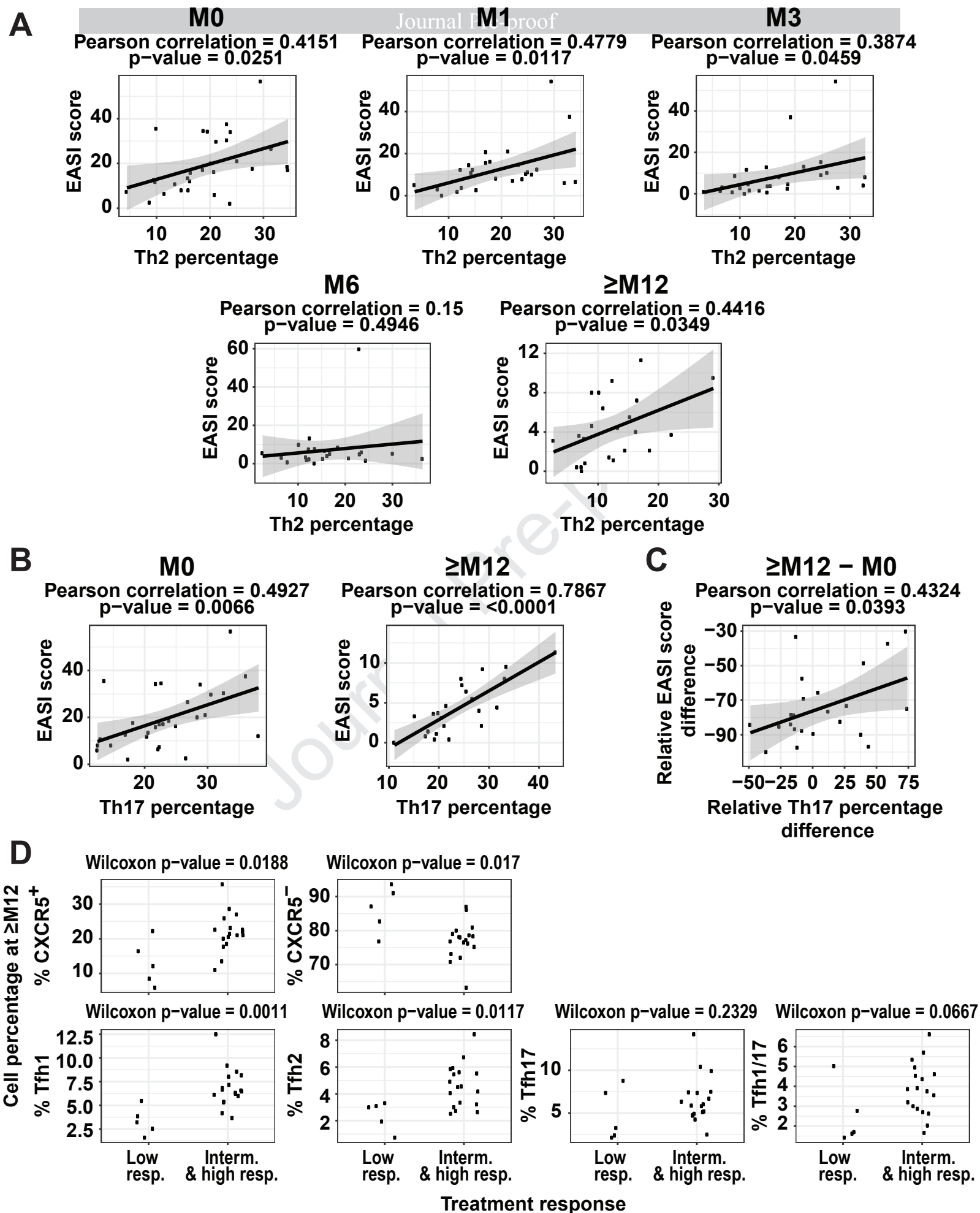


FIGURE 2: EASI score association with Th2, Th17, and Tfh cell percentages in AD patients treated with Dupilumab