Ungar Benjamin (Orcid ID: 0000-0003-0882-8163)
Lozano-Ojalvo Daniel (Orcid ID: 0000-0002-3524-2929)
Guttman-Yassky Emma (Orcid ID: 0000-0002-9363-324X)

Revised clean version

Title: The impact of dupilumab treatment on SARS-CoV-2 T cell responses in atopic dermatitis patients

Benjamin Ungar, MD*[1]; Susan Hartzell, MS*[2]; Daniel Lozano-Ojalvo, PhD*[2]; Sabrina Ghalili, BS[1]; Swaroop Bose, MS[1]; Alexandra K. Golant, MD[1]; Kathryn Tan, MD[1]; Yeriel D. Estrada, BS[1]; Giselle K. Singer, BS[1]; Ana B. Pavel, PhD[1,4]; Paolo Cravedi, MD, PhD**[2], Emma Guttman-Yassky, MD, PhD**[1]

* Contributed equally to this work

** Contributed equally to this work

[1] Department of Dermatology and Laboratory of Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, New York, NY

[2] Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States

[3] Translational Transplant Research Center, Department of Medicine, Icahn school of Medicine at Mount Sinai, New York, NY

[4] Department of Biomedical Engineering, the University of Mississippi, University, MS

Corresponding Authors:

Emma Guttman-Yassky, emma.guttman@mssm.edu

Department of Dermatology and Laboratory of Inflammatory Skin Diseases

Icahn School of Medicine at Mount Sinai

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5 E. 98th Street, New York, New York 10029

Paolo Cravedi, MD, PhD, paolo.cravedi@mssm.edu

Department of Medicine and Translational Transplant Research Center

Icahn School of Medicine at Mount Sinai

1 Gustave Levy Place, New York, New York 10029

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Galderma, Ichnos Sciences, Incyte, Janssen Biotech, Kyowa Kirin, Leo Pharma, Pandion Therapeutics, Pfizer, Ribon, RAPT Therapeutics, Regeneron Pharmaceuticals, Inc., Sanofi, SATO Pharmaceutical, Siolta Therapeutics, Target PharmaSolutions, UCB, and Ventyx Biosciences. B. Ungar is an employee of Mount Sinai and has received research funds (grants paid to the institution) from: Incyte, Rapt Therapeutics, and Pfizer. He is also a consultant for Arcutis Biotherapeutics and Castle Biosciences. ABP is an employee of the University of Mississippi and has a research contract with Mount Sinai. The other authors have no conflicts of interest to disclose.

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Abbreviations:

COVID-19: coronavirus disease 2019

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

AD: atopic dermatitis

IgG: Immunoglobulin G

mRNA: messenger RNA

PBMC: Peripheral blood mononuclear cell

To the Editor,

Studies on the pathophysiology of immune responses in COVID-19 point at a critical role for Th1 cells in viral clearance. Therefore, it has been postulated that abnormally elevated Th2 cytokines in individuals with atopic dermatitis (AD), a dermatological condition characterized by Th2-driven skin inflammation, impairs appropriate Th1 responses to viral infection and that specific Th2-targeting therapies are corrective.[1] In line with this hypothesis, we previously showed that dupilumab, a monoclonal antibody that blocks the IL-4Rα subunit, thereby inhibiting Th2-associated IL-4 and IL-13 signaling, is associated with milder COVID-19 severity in AD patients.[1] Importantly, dupilumab does not affect SARS-CoV-2 IgG antibody levels after vaccination.[2] However, the effect of dupilumab on T cell responses after infection or vaccination is not known.

We prospectively collected PMBC samples from ≥ 12 year-old moderate-to-severe AD patients either after COVID-19 infection or after SARS-CoV-2 mRNA vaccination in the Department of Dermatology at the Icahn School of Medicine, New York, between June 2020 and October 2021. Fifty-five samples from patients with prior SARS-CoV-2 infection confirmed by positive anti-SARS-CoV-2 Spike IgG (unvaccinated at the time of sample collection), and 125 post-vaccination samples from different subjects were analyzed. PBMCs were incubated for 24 hours with peptides covering the immune-dominant regions of the S glycoprotein of SARS-CoV-2 and IFNγ and IL-2 antigen-specific responses were quantified using IFNγ/IL-2 Double-Color FluoroSpot (see Supplement and Supplementary Figure 1 for further details). Spike antigens were used to provide comparisons between treatment groups

as a measure of T cell responses for both post-infection and post-vaccination samples (vaccine contains only spike antigen of SARS-CoV-2). Comparisons on log_{10}-transformed spot counts to minimize the impact of outliers were made with unpaired Student's t-tests and correlations were calculated with Spearman correlation coefficient. Patients were stratified into three cohorts based on their treatment strategy: 1) Dupilumab alone (Dupilumab); 2) other systemics immunomodulators (JAK-inhibitors, prednisone, phototherapy; Systemic), and 3) untreated or topical medications only (Limited).

After COVID-19 infection, IFNγ$^+$ T cell-reactive counts were non-significantly higher in the Dupilumab (n=24) vs. Limited (n=23) groups (p=0.072 [FDR=0.144]; Figure 1A). IL-2$^+$ and dual IFNγ$^+$IL-2$^+$ T cell counts did not differ between these two cohorts (not shown), but the IFNγ$^+$/IL-2$^+$ T cell count ratio trended toward an increase in the Dupilumab vs. Limited cohorts (p=0.091 [FDR=0.182]; Figure 1B). No differences were identified between Systemics (n=8) and the other groups in terms of IFNγ$^+$ or IL-2$^+$ T cell counts or the IFNγ$^+$/IL-2$^+$ ratio.

In post-vaccination samples, there were significantly greater IFNγ$^+$ T cell counts in Dupilumab vs. Systemics patients (p=0.048 [FDR=0.071]; Figure 2A) and a trend toward greater IFNγ$^+$ T cell counts vs. Limited patients (p=0.068 [FDR=0.068]; Figure 2A). The IFNγ$^+$/IL-2$^+$ ratio was not significantly between Dupilumab vs. Limited (p=0.181 [FDR=.362]; Figure 2B) or vs. Systemics. We observed no correlation between IFNγ$^+$ or IL-2$^+$ T cell counts or IFNγ$^+$/IL-2$^+$ ratio and time after vaccination, consistent with previous reports.[3]

To validate these results, in a subset of patients we determined the specificity of the memory ($CD45RA^-$) IL-2- and IFNγ-producing $CD4^+$ T cells by flow cytometry using CD154 (CD40L) as an early activation marker upregulated upon Spike antigen recognition. While no significant differences were observed in $CD154^+IL\text{-}2^+$ T cells between groups, there was a higher percentage of IFNγ-producing Spike-specific ($CD154^+$ $IFN\gamma^+$) $CD4^+$ T cells in dupilumab-treated patients vs. the Limited group after vaccination (Supplementary Figure 2A-B). Consistent with previous studies,[4] we observed an augmentation of IFNγ-producing Th1 cells after SARS-CoV-2 mRNA vaccination that was significantly higher in subjects treated with Dupilumab.

This study supports the hypothesis that specific Th2-targeting by dupilumab promotes a more balanced Th1/Th2 response to COVID-19 infection in AD individuals (as shown in comparison to the Limited group, consisting of patients not receiving systemic treatments), with trends toward increased SARS-CoV-2-specific $IFN\gamma^+$ T cell counts and a greater IFNγ/IL-2 ratio, potentially an indicator of a more specific Th1 response given that IL-2's involvement in multiple Th pathways.[5] Furthermore, by blunting the abnormal activity of Th2 cells, dupilumab appears to promote Th1-prone T cell responses to mRNA vaccination. Of note, IFNγ counts were greater in the post-infection group than the post-vaccination group across all treatments. Further work is necessary to evaluate this, raising the question of whether Spike protein in the presence of other viral antigens may elicit a more robust response than Spike mRNA vaccination. Limitations of this study include small sample size, unknown COVID-19 infection dates (prior infection was identified by IgG serologies), and

lack of serial samples. Future studies in larger populations are needed to characterize T cell phenotypes more thoroughly.

Overall, this study suggests that Th2-inhibition with dupilumab does not hinder, and may possibly even improve, Th1-specific T cell responses to COVID-19 infection and mRNA vaccination.

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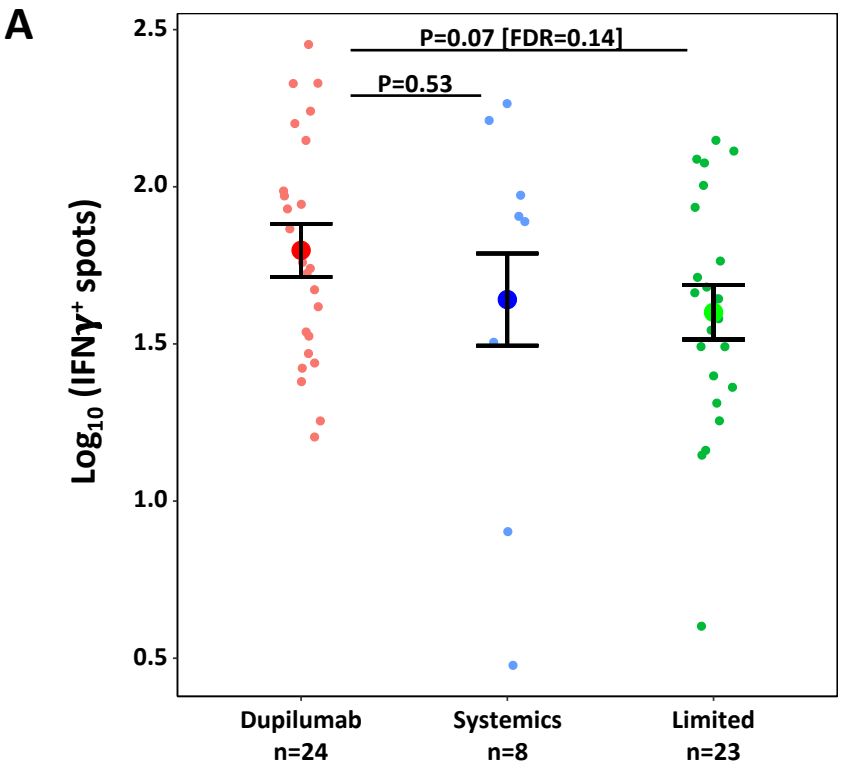
Figure 1. A. Log_{10} (IFNγ^+ T cell spots) and **B.** Log_{10} (IFNγ^+ spots/IL-2^+ spots) for Dupilumab (n=24), Systemics (n=8), and Limited (n=23) samples after COVID-19 infection. FDR, False Discovery Rate

Figure 2. A. Log_{10} (IFNγ^+ T cell spots) and **B.** Log_{10} (IFNγ^+ spots/IL-2^+ spots) for Dupilumab (n=64), Systemics (n=9), and Limited (n=52) samples after SARS-CoV-2 mRNA vaccination. FDR, False Discovery Rate

Figure 1



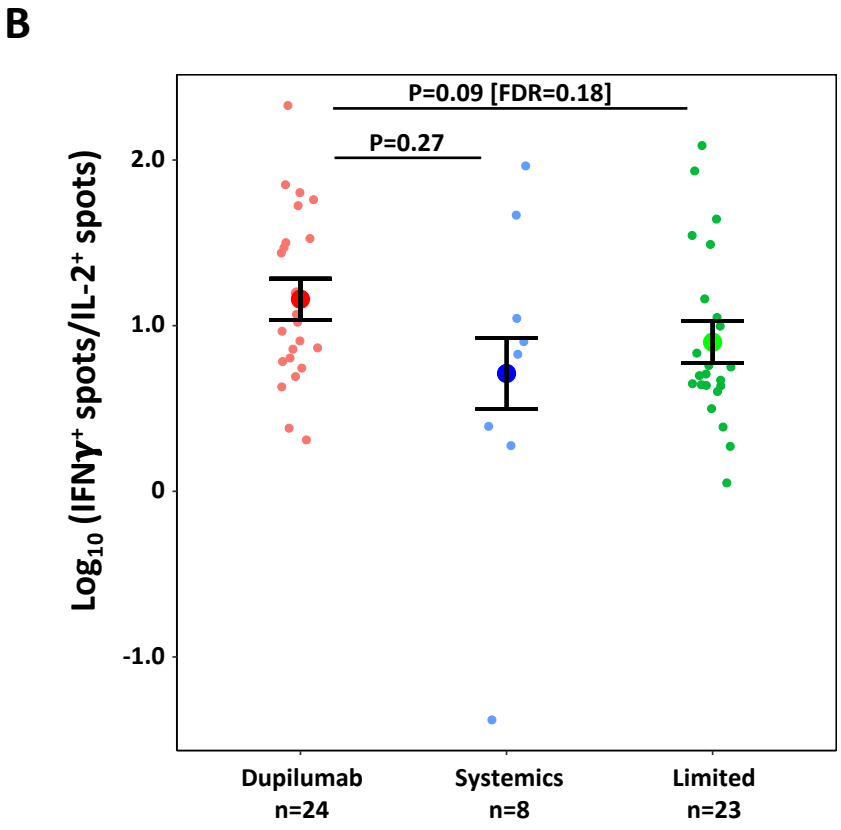


Figure 2