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RESEARCH NOTE

Allergen exposure boosts peripheral Th9 responses in patients with local allergic rhinitis

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Key points:

- Intranasal allergen exposure increases peripheral total Th2 and Th9 cells in patients with local allergic rhinitis (LAR).
- Peripheral T-cell response seems dominated by Th9 cells in patients with LAR, whereas Th2 responses prevail in patients with allergic rhinitis.
- Our results identify Th9 cells as potential therapeutic targets for patients with LAR.

KEYWORDS
allergens, allergic rhinitis, biomarker, inhalation allergen challenge, rhinitis, therapeutics

Francisca Palomares and Almudena Testera-Montes share first authorship.

Carmen Rondon and Ibon Eguiluz-Gracia share senior authorship.

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1 | INTRODUCTION

Local allergic rhinitis (LAR) is defined by a clinical history suggestive of allergy, a positive response to the nasal allergen challenge (NAC), and the absence of atopy,[1] whereas the allergic rhinitis (AR) phenotype shares the first two features but is characterized by the positivity of atopy tests. A recent study showed that a 3-day NAC protocol with *Dermatophagoides pteronyssinus* (NAC-DP) increases the proportion of total peripheral plasmablasts and plasma cells in patients with AR and those with LAR.[1] Similarly, a 3-day NAC induces an accumulation of T-helper (Th) and Th2 lymphocytes in the nasal mucosa of patients with AR,[2] together with an increase of circulating eosinophils. Nevertheless, to date, no study has investigated the involvement of T cells in LAR pathophysiology. Here, we analyze the effect of a 3-day NAC-DP on peripheral T-cell subsects from patients with LAR, those with AR, and healthy controls (HCs).

2 | METHODS

We implemented a 3-day NAC-DP using a standardized DP extract (100 HEP/mL, Leti Pharma) in nine patients with LAR, five patients with AR, and five HCs (Table S1 and Appendix S1). All patients had a previous positive NAC-DP performed according to diagnostic protocols[3] (Appendix S1). Blood samples were taken before (day 1) and 24 h after the last allergen dose (day 4) to isolate peripheral blood mononuclear cells (PBMCs), immunophenotype T-cell subpopulations,[4] and analyze DP-specific proliferation by 5,6-carboxyfluorescein diacetate N-succinimidyl ester (CFSE) dilution assay. The results of CFSE assay were expressed as proliferation index (PI). The PI was calculated for each cell subset as (% CFSElow stimulated PBMC)/(% CFSElow unstimulated PBMC), and the cutoff for proliferation was set at 2.[1] The immunological response was assessed by determining intracellular cytokines by flow cytometry and cytokine secretion using a multiplex immunoassay kit (Appendix S1). Further methodological details can be found in the supplementary material.

3 | RESULTS

At baseline, the proportion of T-cell subpopulations was comparable among the three study groups. The NAC-DP (in vivo) increased significantly the total peripheral T-cell proportion in both rhinitis phenotypes but not in HCs (Figure 1A). The provocation increased the percentage of T cells expressing C-C chemokine receptor 4 (CCR4; chemokine receptor related to type 2 responses[5]) in patients with LAR but not in patients with AR or HCs (Figure 1B). NAC-DP induced a significant increase in total peripheral IL-9$^+$Th9 cells in patients with LAR but not in patients with AR or HCs, whereas IL-4$^+$Th2 cells increased in a significant and comparable manner in both rhinitis phenotypes but not in HCs (Figure 1C). No changes were observed for total peripheral IFNγ^+Th1 and IL-10$^+$Treg cells in any study group (Figure 1C), and the provocation did not change C-X-C motif chemokine receptor 3 (CXCR3) expression (chemokine receptor associated with type 1 responses[5]) in peripheral T cells from any study group.

In a second step, we examined the effect of DP stimulation on the proliferation and cytokine production of peripheral T cells. To this end, PBMCs (including T-cell subpopulations) were isolated from post–NAC-DP samples and stimulated in vitro with DP. The CFSE assay

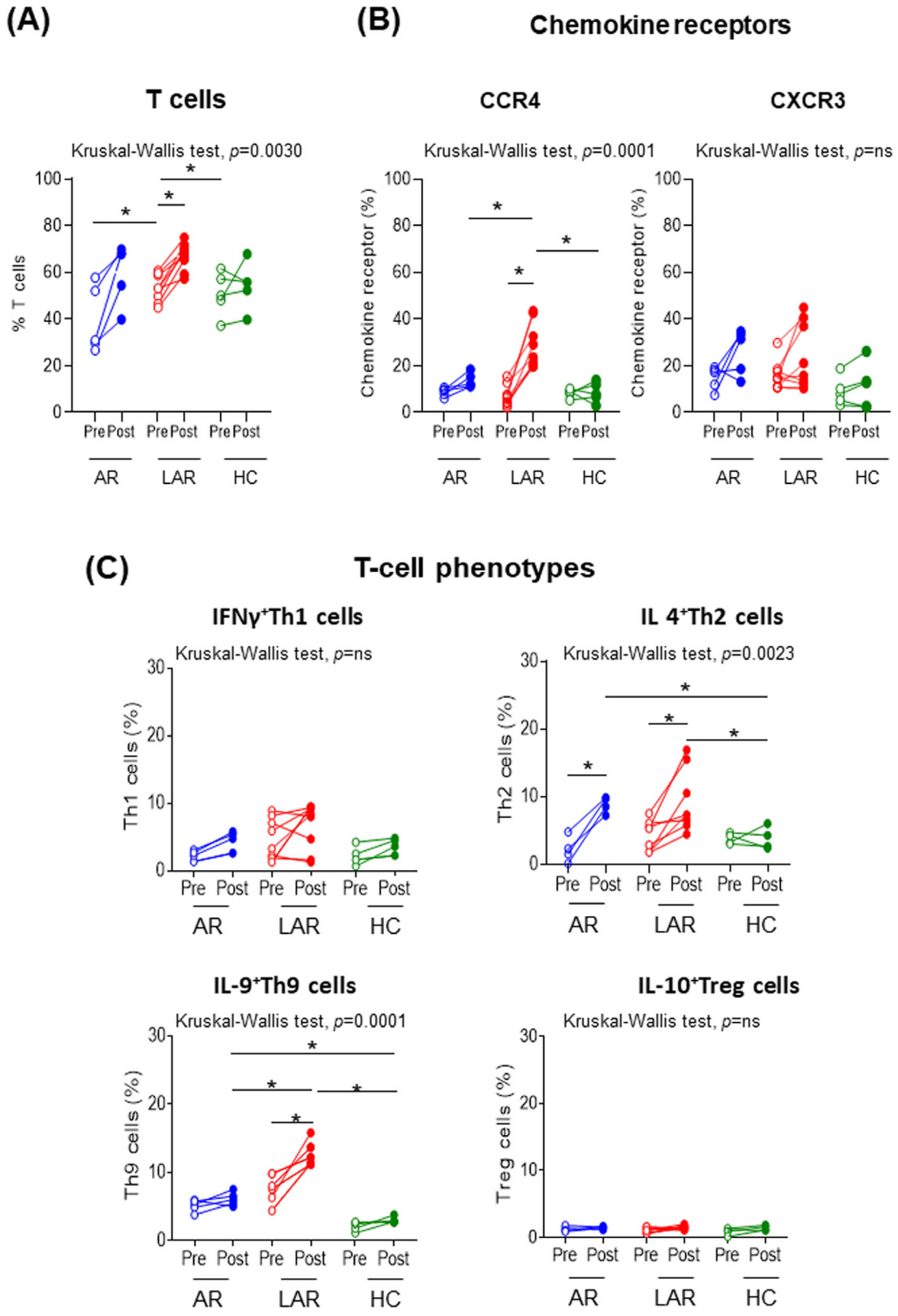

FIGURE 1 (A) Proportion (%) of T cells in peripheral blood mononuclear cells (PBMCs), (B) percentage of chemokine receptors in T cells before and after the provocation, and (C) different T-cell subsets percentage before and after the provocation. Patients with allergic rhinitis (AR): $N = 5$. Patients with local AR (LAR): $N = 9$. Healthy controls (HCs): $N = 5$. The unfilled symbols represent the percentage before the provocation and filled symbols after the provocation. Kruskal-Wallis test was used to detect differences in unrelated samples across multiple comparisons in representing significant *p*-values. Mann–Whitney test for pairwise comparisons in unrelated samples and Wilcoxon test was used for pairwise comparisons in related samples, representing significant *p*-values as *($p < 0.05$), respectively. CCR4, C-C chemokine receptor 4; CXCR3, C-X-C motif chemokine receptor 3; IFNγ, interferon γ; IL, interleukin.

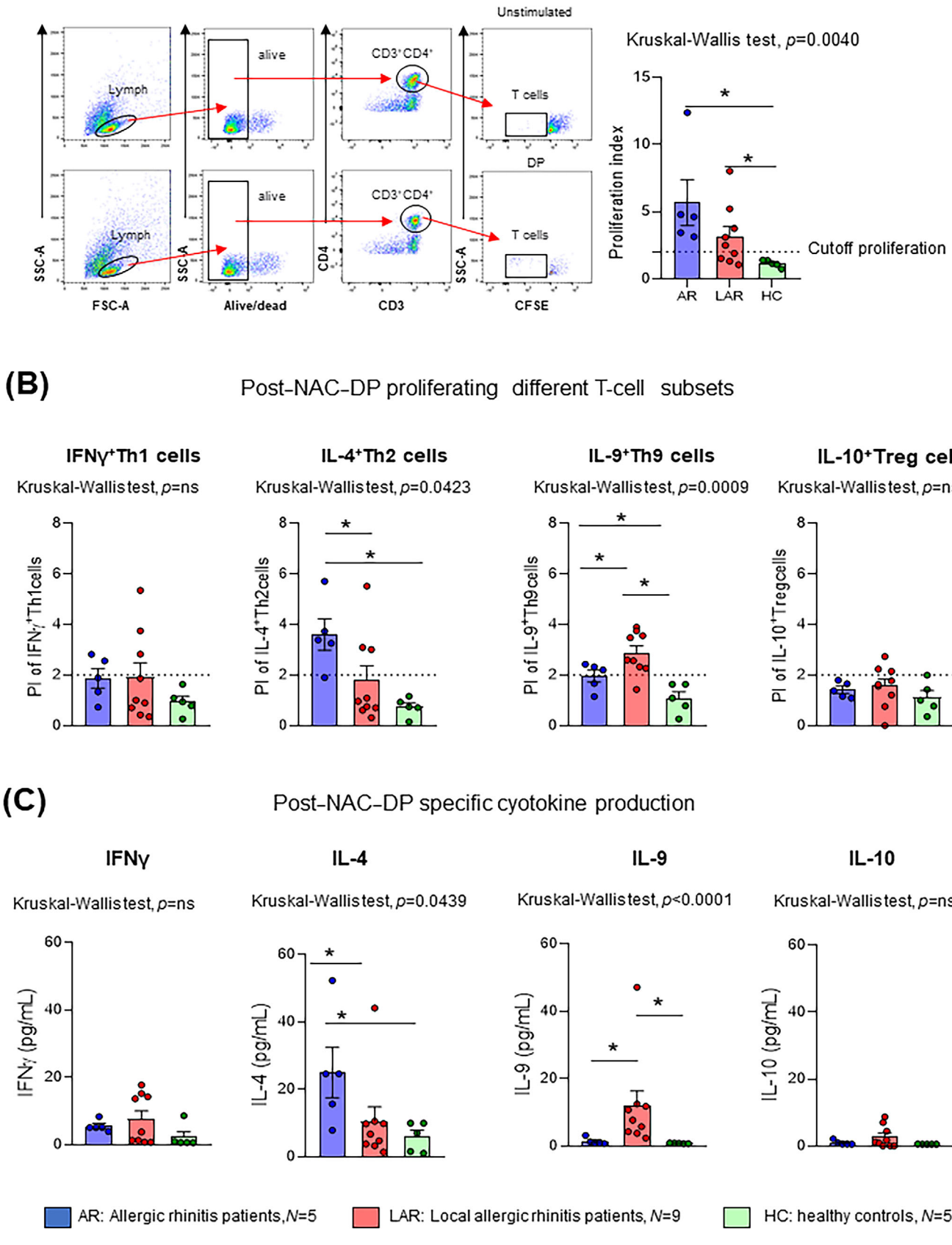

FIGURE 2 (A) Gating strategy for flow cytometry and proliferation index (PI) of T cells from peripheral blood mononuclear cells (PBMCs) after in vitro allergen (*Dermatophagoides pteronyssinus* [DP], at 10 μg/mL) stimulation of PBMC, and (B) PI of different T-cell subsets after in vitro allergen stimulation among PBMCs isolated from the blood sample taken after the provocation. The dotted line represents the cutoff indicative of proliferation at 2. Gate strategy is depicted using post–nasal allergen challenge protocol with DP (NAC-DP) samples obtained from patients with local allergic rhinitis (LAR), in whom the proliferative response after DP stimulation was observed. (C) Cytokine levels produced by PBMCs after stimulation with DP in samples post–NAC-DP. The bars with symbols represent mean and standard error of the mean of PI and cytokines levels. Kruskal-Wallis test was used to detect differences in unrelated samples across multiple comparisons, representing significant *p*-values. Mann–Whitney test for pairwise comparisons in unrelated samples, representing significant *p*-values as *($p < 0.05$). CFSE, 5,6-carboxyfluorescein diacetate N-succinimidyl ester; FSC-A, forward scatter area; IFNγ, interferon γ; IL, interleukin; ns, not significant; SSC-A, side scatter area.

revealed that T cells from patients with AR and those with LAR but not from HCs proliferated after DP stimulation (Figure 2A). The analysis of DP-triggered proliferation showed that the proportion of IL-4$^+$Th2 cells increased significantly in patients with AR compared with patients with LAR and HCs. Interestingly, IL-9$^+$Th9-cell proliferation was higher in patients with LAR as compared with patients with AR and HCs, whereas individuals with AR showed significant proliferation as compared with HCs only (Figure 2B). No proliferative response was observed for IFN-γ^+Th1 or IL-10$^+$Treg cells after in vitro stimulation for any group. Thereafter, we analyzed cytokine production in culture supernatants from DP-stimulated PBMC in post–NAC-DP samples. The results paralleled those observed in the proliferative response analysis. Interestingly, IL-4 levels were higher in patients with AR than in patients with LAR and HCs, whereas patients with LAR displayed higher IL-9 levels than patients with AR and HCs (Figure 2C). No significant changes in IFNγ or IL-10 levels were observed for any group.

4 | DISCUSSION

Our results indicate that, in both patients with LAR and those with AR, repetitive intranasal allergen exposure induces the accumulation of Th9 and Th2 cells in peripheral blood. Of note, a recent work from our group showed that a 3-day NAC-DP protocol triggered a similar increase of peripheral plasmablasts in patients with LAR and those with AR.[1] The shorter provocation period might account for the lack of effect observed in other studies investigating peripheral lymphocyte responses in AR.[6] In any case, our findings are consistent with a previous work showing an increase of circulating eosinophils in individuals with AR subjected to a 3-day NAC protocol.[2] Peripheral T cells from patients with LAR expressed significantly more CCR4 after the provocation. This finding suggests that allergen exposure licenses peripheral T cells to migrate to the nasal mucosa of patients with LAR. Interestingly, a previous work identified an accumulation of recently recruited Th2 cells in the nasal mucosa of individuals with AR subjected to a 3-day NAC protocol.[2,7] Moreover, CCR4 upregulation in T cells has been associated with their migration to the airway mucosa,[8] and CCL18 (CCR4 ligand) expression increases in mucosal monocytes from patients with AR subjected to NAC.[2] Our study also suggests that T-cell response in patients with LAR are dominated by Th9 cells, whereas Th2 cells prevail in patients with AR, as previously described.[9] Nevertheless, we cannot exclude that the discrepancy between the effect of in vivo and in vitro allergen exposure on Th2 cells from patients with LAR is related to the divergent stimulation period (3 vs 7 days, respectively), among other method-specific factors. In any case, the NAC induces a more homogeneous increase of peripheral Th9 cells among individuals with LAR, as compared with Th2 cells. Therefore, it is tempting to speculate that allergen exposure induces the recruitment of allergen-specific Th2 and Th9 cells to the nasal mucosa of individuals with AR and LAR, where they contribute to the sequential class switch recombination to IgE of allergen-specific IgG1$^+$B cells, possibly through IL-4 and IL-9 release. This hypothesis is consistent with the allergen-triggered accumulation of IgE$^+$ plasmablasts in the nasal mucosa of patients with LAR that was previously reported.[1] The limited sample size, the exclusive inclusion of women in the AR group, and the lack of evaluation of lymphocyte specificity and mucosal T cells represent limitations of our study. Moreover, further works are required in order to extrapolate our results to LAR due to allergens other than house dust mites. In any case, the analysis of cell proliferation and cytokine release following in vitro allergen stimulation suggests that T-cell responses were at least partially allergen-specific. Further studies are warranted to elucidate whether allergen-specific Th9 cells are relevant therapeutic targets for patients with LAR.

AUTHOR CONTRIBUTIONS

Cristobalina Mayorga, Carmen Rondon, and Ibon Eguiluz-Gracia designed the study. Almudena Testera-Montes, Carmen Alba-Linero, Guillermo Bentabol-Ramos, Rocío Sáenz de Santa María-García, and Maria Jose Torres

evaluated and recruited the study individuals. Francisca Palomares and Carmen Alba-Linero performed the analysis of peripheral cell subpopulations and antibodies. Cristobalina Mayorga, Carmen Rondon, and Ibon Eguiluz-Gracia supervised the work of all of the authors. Francisca Palomares and Almudena Testera-Montes prepared the manuscript, which was reviewed by the rest of the authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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