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LETTER

Early expansion of allergen-responsive LAP$^+$ B regulatory cells in allergic rhinitis but not in allergic asthma subjects during allergen immunotherapy

To the Editor,

Allergen immunotherapy (AIT) is used successfully for treatment of allergic rhinitis (AR). There is also strong evidence of AIT being effective in allergic asthma (AA), when used as an add-on treatment to pharmacotherapy such as oral corticosteroids.[1] There is limited data available on immunological effects of AIT in AA compared to AR and the impact of pharmacotherapy on mechanisms of AIT. Induction of B-regulatory (Bregs) and T-regulatory (Tregs) cells are key components in tolerance induction to allergens in AIT. Bregs produce immunosuppressive cytokines IL-10 and TGF-β, which not only suppress Th2 cell responses, but also mediate induction of Tregs.[2] In this study, allergen-responsive B-cells were monitored in 7 AA and 8 AR subjects prior to and after 4, 9, and 24 months of subcutaneous allergen immunotherapy (Figure 1A). Allergen-responsiveness was determined through in vitro proliferative cell responses, indicated by loss of CFSE, to house dust mite (HDM) allergen at day 5 (Table S1). In total, 10 clusters of allergen-responsive B-cells were identified (Figure 1B). Four clusters expressed the memory marker CD27 and six did not, mostly representing naïve/transitional B-cell populations. Within those B-cell populations, a CD71$^+$CD73$^-$CD25$^+$LAP$^+$ cluster was identified, consistent with a Breg phenotype. In AR subjects, the frequency of LAP$^+$ Bregs within allergen-responsive B-cells increased at 24 months of AIT with nominal significance and was already significantly increased at 4 months (with fdr-correction, Figure 1C). This increased LAP-expression was however not observed in AA subjects. LAP is 'latency associated peptide' and serves as a marker for TGF-β expression. TGF-β is vital for induction of Tregs[2] and has important roles in Breg-induced control of autoimmunity and allergy.[3,4] Intracellular IL-10 showed a minimal overlap with LAP-expression (Figure S1), suggesting that LAP$^+$ Breg cells produce only minimal amounts of IL-10. The increased percentage of LAP$^+$ Bregs in AR subjects at 4 months was accompanied with a (nominal) significant decrease in CD25$^+$CD71$^-$CD27$^+$ memory B-cell clusters (Cluster 1 and 5; Figure 1D). Again, this change was not observed in AA subjects. The frequency of LAP$^+$ B-cells was markedly lower in non-HDM responsive B-cells (CFSE^{hi} cells) compared to HDM-responsive B-cells (CFSE^{low} cells) and was not increased in AR or AA at 24 months of AIT (Figure S1). Enhanced HDM- or Der p 1-responsive LAP$^+$ B-cells were also observed in two independent cohorts of 14 untreated AR patients[5] or 7 AA patients,[6] respectively, and 23 age/sex-matched healthy controls. No differences were observed between patients and their matched controls. This suggests that AIT forms a dominant trigger for the development of LAP$^+$ B-cells, and that this may be transient.

Changes also occurred within the transcriptome of allergen-specific memory B-cells (Figure 2A). In AR subjects, there was a change in gene expression at 4 months of AIT, which did not occur in AA patients (Figure 2B). The gene ontology pathways included small GTPases-mediated signal transduction, important in cellular processes (signal transduction, cell adhesion, chemotaxis and motility, cell growth, and division), along with plasma cell, immunoglobulin (Ig) and MHC-TLR7-TLR8 pathways, necessary for germinal center forming and Ig production (Figure 2C). This was further reflected in reduced Ig heavy chain transcripts in AR subjects at 4 and 24 months of AIT, but not in AA patients (Figure 2D). Of interest, TLR7/8 pathways have been implicated in Breg function.[7]

In summary, AR subjects displayed early changes in B-cell populations during the first 4 months of AIT consisting of increased HDM-responsive LAP$^+$ B-cells and reduced Ig-related transcripts of allergen-responsive memory B-cells, which do not occur in allergic asthma subjects. Asthma and quality of life scores showed improvement at 24 months of AIT (Figure S2); while allergen challenges post-immunotherapy confirming clinical efficacy were lacking. However as other studies showed variations in clinical efficacy between AR and AA,[8] the differences observed in B-cells at 4 months of AIT between these groups may shed light on immunological mechanisms involved in achieving clinical efficacy and how it may differ between diseases. Additionally, use of steroid-based medication may prevent changes that would otherwise occur during AIT. Indeed, steroids were shown to suppress Breg cell(s) (development) in patients with myasthenia gravis.[9] Overall, the observed differences in LAP$^+$ B-cells between AR and AA patients may provide a first hint as why AIT in AA patients shows more variations in clinical efficacy. Further investigation is warranted to determine the consequences of these discrepancies between AR and AA on clinical outcome of AIT.

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FIGURE 1 Allergen-responsive B-cell populations in allergic rhinitis (AR) and allergic asthma (AA) subjects during allergen immunotherapy (AIT). (A) Allergen immunotherapy study set-up and dosing schedule. (B) Allergen-responsive B-cell identification (CFSE^{low} cells; example is an AR patient at pre-dose) and expression clustering-based phenotyping represented by heatmap. (C) Cluster density plots and cluster 4 (LAP^{+} B cells) frequency in AR and AA groups during AIT. (D) Cluster density plots and cluster 1&5 (CD27^{+} memory B cells) frequency in AR and AA groups during AIT. For AR ($n=6$): at pre-dose 6 donors, at 4 months 5 donors, at 9 months 3 donors and at 24 months 6 donors are included. For AA ($n=6$): at pre-dose 6 donors, at 4 months 4 donors, and at 9 and 24 months both 6 donors were included. *fdr < 0.05.

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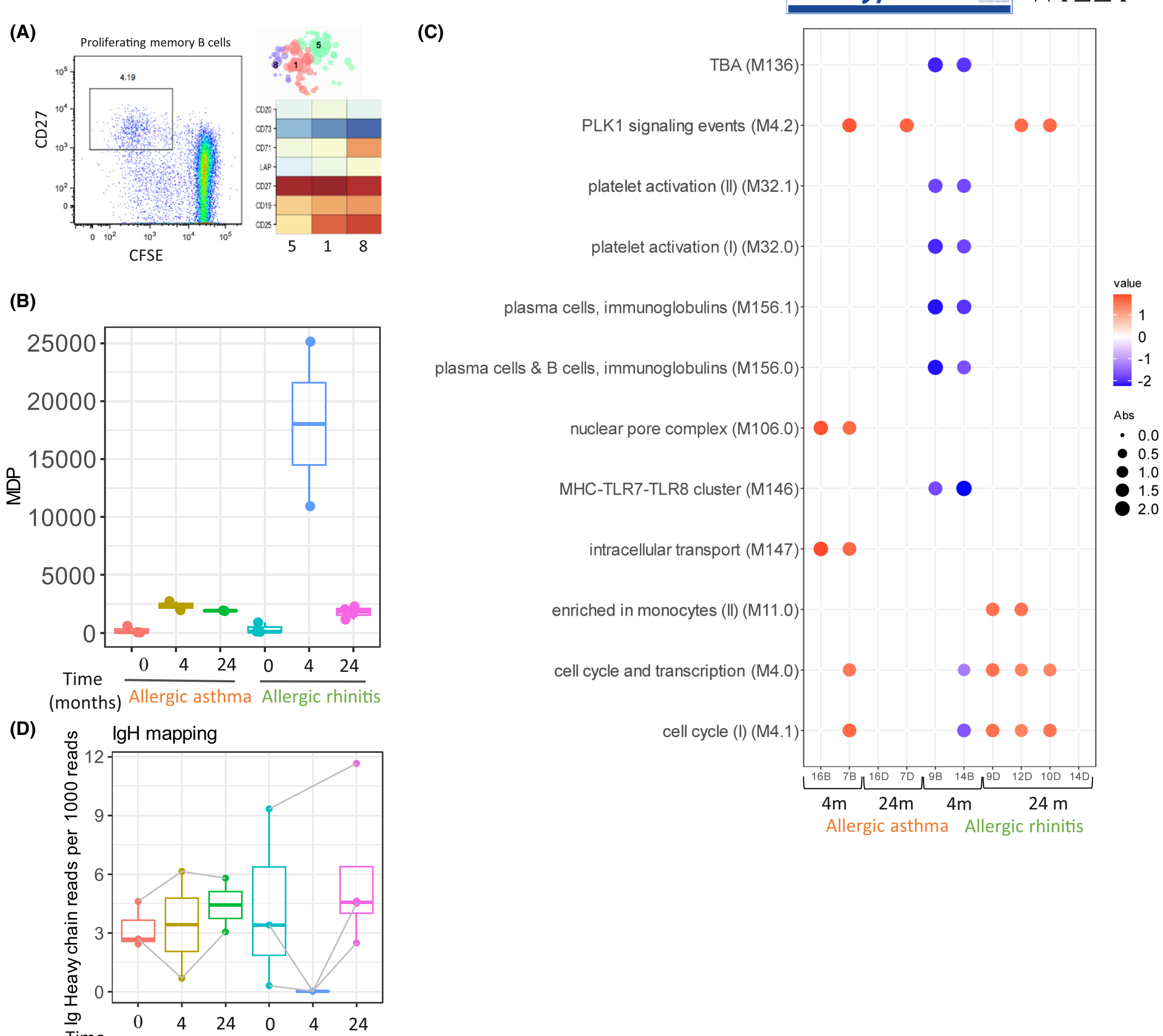

FIGURE 2 Allergen-responsive memory B-cell populations in allergic rhinitis (AR) and allergic asthma (AA) subjects during allergen immunotherapy (AIT). (A) Allergen-responsive memory B-cells (CFSE^{low} cells) selected for FACS isolation for RNA-sequencing. (B) Molecular degree of perturbation quantifying the heterogeneity between samples to indicate overall changes in gene expression. For AR ($n=4$): at pre-dose 3 donors, at 4 months 2 donors, and at 24 months 4 donors are included. For AA ($n=3$): at pre-dose 3 donors, at 4 months 2 donors, and at 24 months 2 donors were included. (C) Gene set enrichment analysis indicating increased (red dots) or decreased (blue dots) expression of gene pathways compared to time point 0. Dot size indicated fold change. (D) IgH mapping of B cell receptors.

AUTHOR CONTRIBUTIONS

ALV, NWdJ, GMM, RGvW, and HHS conceived and designed the analysis; LEPMvdV, MvdB, MCN, and GJB contributed to data or analysis tools; ALV, SPJ, AOF, ORJvH, and KAS performed the analysis; ALV and HHS wrote the paper; all authors reviewed the paper.

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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. Zhang W, Lin C, Sampath V, Nadeau K. Impact of allergen immunotherapy in allergic asthma. *Immunotherapy*. 2018;10:579-593.
2. Jansen K, Cevhertas L, Ma S, Satitsuksanoa P, Akdis M, van de Veen W. Regulatory B cells, A to Z. *Allergy*. 2021;76(9):2699-2715. doi:10.1111/all.14763
3. Huai G, Markmann JF, Deng S, Rickert CG. TGF-β-secreting regulatory B cells: unsung players in immune regulation. *Clin Transl Immunol*. 2021;10(4):e1270. doi:10.1002/cti2.1270
4. Lee JH, Noh J, Noh G, Choi WS, Cho S, Lee SS. Allergen-specific transforming growth factor-β-producing CD19+CD5+ regulatory B-cell (Br3) responses in human late eczematous allergic reactions to cow's milk. *J Interferon Cytokine Res*. 2011;31(5):441-449. doi:10.1089/jir.2010.0020
5. Voskamp AL, Tak T, Gerdes ML, et al. Inflammatory and tolerogenic myeloid cells determine outcome following human allergen challenge. *J Exp Med*. 2023;220(9):e20221111.
6. Vieira Braga FA, Kar G, Berg M, et al. A cellular census of human lungs identifies novel cell states in health and in asthma. *Nat Med*. 2019;25(7):1153-1163. doi:10.1038/s41591-019-0468-5
7. Liu BS, Cao Y, Huizinga TW, Hafler DA, Toes REM. TLR-mediated STAT3 and ERK activation controls IL-10 secretion by human B cells. *Eur J Immunol*. 2014;44(7):2121-2129. doi:10.1002/eji.201344341
8. Gerth van Wijk R, Dahl R. Pros and cons: should allergen immunotherapy be considered in all patients with allergic asthma? *Allergy*. 2022;77(3):1070-1072. doi:10.1111/all.15123
9. Yilmaz V, Maillard S, Truffault F, et al. Regulatory B cells in myasthenia gravis are differentially affected by therapies. *Ann Clin Transl Neurol*. 2018;5(11):1408-1414.

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