

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

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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 corticosteroids¹. 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 Tregs². 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 HDM allergen at day 5. 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 increase 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 Tregs² and has important roles in Breg-induced control of autoimmunity and allergy^{3,4}. 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 compared to HDM-responsive B-cells and was not increased in AR or AA at 24 months of AIT (**Supplementary figure 1**).

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 9 months of AIT, but not in AA patients (**figure 2D**). Of interest, TLR7/8 pathways have been implicated in Breg function⁵.

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 (**Supplementary figure 2**); while allergen challenges post-immunotherapy

confirming clinical efficacy were lacking. However as other studies showed variations in clinical efficacy between AR and AA⁶, 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 gravis⁷. Further investigation should determine consequences of immunological discrepancies between AR and AA on clinical outcome of AIT.

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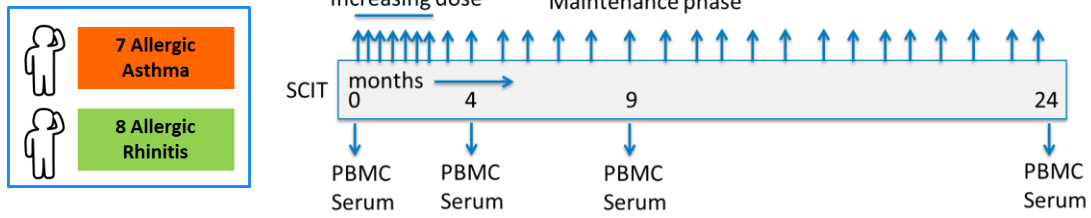
Figure legends

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 and clustering-based phenotyping. (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. * fdr < 0.05

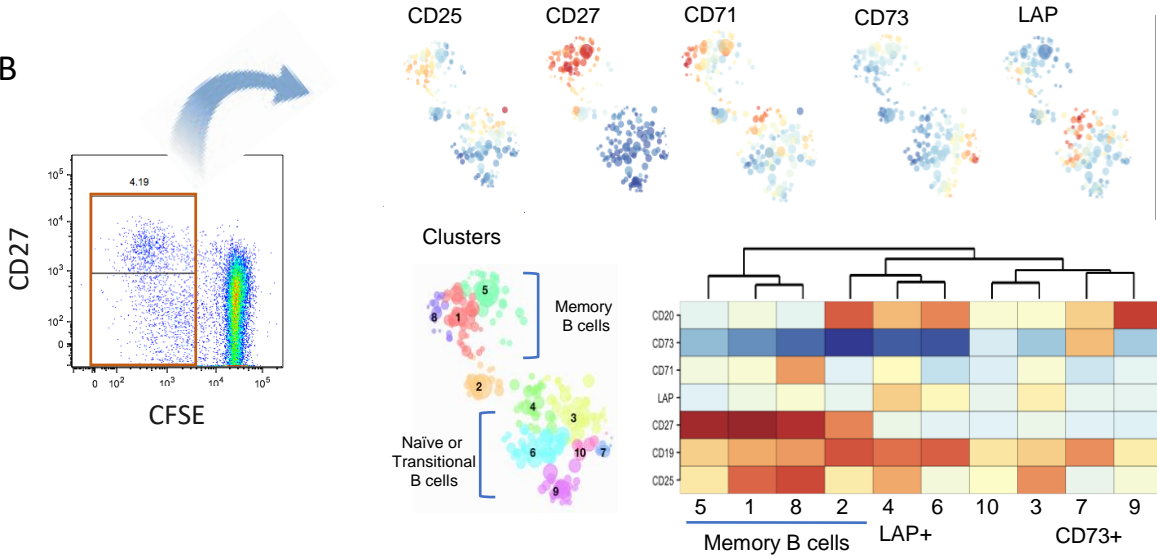
Figure 2. Allergen-responsive memory B-cell populations in allergic rhinitis (AR) and allergic asthma (AA) subjects during allergen immunotherapy (AIT). (A) Allergen-responsive memory B-cells selected for FACS isolation for RNA-sequencing. (B) Molecular degree of perturbation quantifying the heterogeneity between samples to indicate overall changes in gene expression. (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.

Figure 1

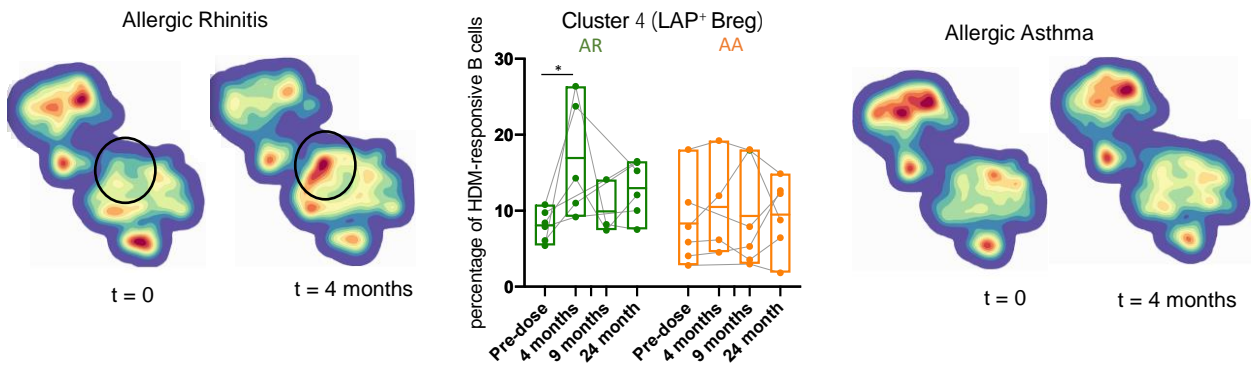
A



B



C



D

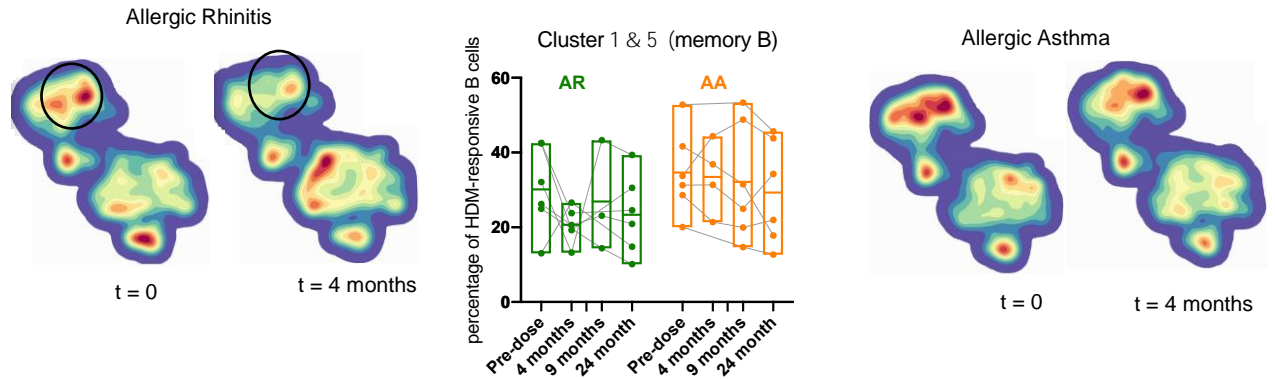


Figure 2.

