Tropical urban environments reveal a strong association of CD45RB lo CD161+ Th2 subset to allergic rhinitis

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To the Editor:

Allergic airway diseases such as allergic rhinitis (AR) affects more than 400 million individuals worldwide and afflicts substantial health and economic morbidity. [1] AR is strongly associated with a type 2 response, characterized by the cytokines IL-5, IL-4 and IL-13. However, the key drivers behind AR immunopathogenesis remained to be elucidated. This study aims to identify critical pathogenic cell populations associated with AR using the Singapore System Immunology Cohort (SSIC) [2] and a clinician-diagnosed paediatric cohort with active AR manifestation (Supplementary Table 1). In both cohorts, the eosinophilic nature of AR was confirmed by higher blood eosinophil numbers (Supplementary Figure 1).

Whole blood gene expression analysis revealed a total of 23 probes representing 20 unique genes were associated with AR in the SSIC (**Table 1A**). To account for ethnicity and environmental influences we validated our findings in BAMSE population-based cohort comprising of Swedish adolescents. **Table 1B** shows 11 DEGs which was also associated with AR, confirming the transferability of our findings to other populations. For the top DEGs that reached nominal significance in the SSIC we performed an Ingenuity Pathway Analysis (IPA). **Supplementary Table 2** revealed important pathways related to hypersensitivity and inflammation and also functional enrichment for eosinophils, basophils and mast cells. In particular, functional activation of Th2 was highlighted as a key pathway for AR pathogenesis. As CRTH2 was reported to be expressed by cell types involved in the eosinophilic response, [3] an unsupervised cluster analysis was performed on the CRTH2+ subset in PBMC of individuals from SSIC (**Figure 1 A and B**) to determine CRTH2+ subsets associated with AR. We found that CD161+Th2 subsets in particularly to be strongly associated with AR (**Figure 1C and D**) (Supplementary Figure 2). Further characterization found that the marker CD45RB to be significantly downregulated on CD161+Th2 cells of AR individuals (**Figure 1E**). Low CD45RB expression on T cells is indicative of a mature phenotype. Interestingly, significantly higher circulatory plasma IL-5 levels (**Figure 1F)** . Furthermore we could also demonstrate AR individuals produced significantly higher IL-5 in an *in vitro* PMA-stimulation assay (**Figure 1G**).

While we noted a small population of IL-5 secreting conventional CD161-Th2 (cTh2), IL-5 secretion was significantly elevated in CD161+Th2 cells (**Figure 1H**). Strikingly, IL-5 was found to be predominantly secreted by CD45RB^{lo} subset in both cTh2 and CD161+Th2 (**Figure 1H and I**). There was also a significant increase in the IL-5 producing CD45RB^{lo}CD161+Th2 population from the AR individuals (**Figure 1I**). These findings confirm CD45RB^{lo}CD161+Th2 as the main producers of IL-5.

We further validated our findings in a second paediatric cohort with clinically diagnosed active AR manifestations. To further refine CD161+Th2 subset that is associated with AR, we performed unsupervised PhenoGraph and UMAP clustering on CD161+Th2 (Supplementary Figure 3A and B). Amongst the UMAP clusters, "cluster 3" was found to be significantly associated with active AR (Supplementary Figure 3C and D). Deep characterization reveals "cluster 3" to be an IL-5 secreting CD45RB^{lo}population, confirming our earlier observation (Supplementary Figure 3E). Furthermore, this cluster appeared to be a highly differentiated population of mature CD161+Th2 cells with an activated phenotype secreting IL-2, IL-3, IL-4, IL-9 and IL-13 concomitantly (Supplementary Figure 3E and F). Thus, the severity of eosinophilic airway allergies such as AR seems to be driven by an activated terminally differentiated CD161+Th2 subset that is able to secrete a complex set of inflammatory cytokines.

The presence of CD45RB^{lo}CD161+Th2 population in both cohorts shows the persistence and pertinence of this population in the pathogenesis of AR. Both cohorts described in this study were collected in Singapore, whereby majority of the individuals are sensitized against HDM. HDM is a perennial allergen in tropical nations such as Singapore, thus T cells in atopic individuals undergo constant stimulation. This could explain the strong association observed between CD45RB expression on CD161+Th2 cells and atopy markers despite the fact that not all subjects demonstrated active AR symptoms during the collection of SSIC cohort. Taken together, our current study unifies the markers previously reported for allergic-specific Th2 subsets and provides clarity for the pathogenic Th2 subset previously reported in different allergic diseases.[4-6] Neutralizing the CD45RB^{lo}CD161+Th2 subset should disrupt the allergic response pathway, thus providing a target for lasting therapeutic interventions. Moreover, these cells may also be leveraged as a biomarker for the effectiveness of immunotherapy as well as a potential biomarker of public health surveillance of allergic individuals.

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Conflict of interest.

All authors have no conflict of interest.

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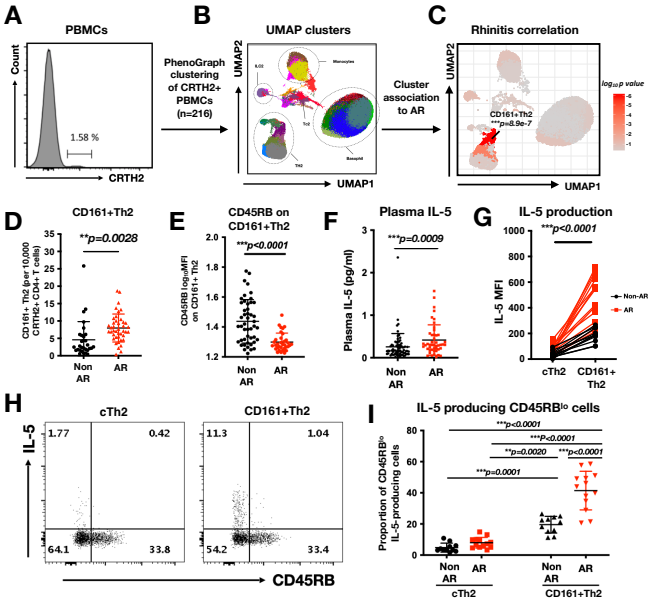

Figure 1

Table 1. Differential gene expression (DEGs) comparison between non-AR and AR from whole blood samples in (A) SSIC and (B) BAMSE cohorts

A) Differentially Expressed Genes (DEGs) for AR from SSIC				
Gene	**logFC**	**Avg_Expr**	**P-value**	**P.eosadj**
IL5RA	0.475	13.46	4.01E-07	4.04E-05
PYROXD2	0.202	12.48	2.72E-06	7.11E-05
SIGLEC8	0.575	11.92	2.22E-07	2.25E-04
THBS4	0.390	8.93	2.43E-05	2.64E-04
ALOX15	0.526	8.95	4.59E-07	3.49E-04
	0.411	12.53	9.78E-06	8.25E-04
OLIG2	0.614	9.61	1.16E-06	5.45E-04
ADGRE4P	0.411	11.97	1.16E-05	5.75E-04
CLC	0.189	14.8	1.29E-05	1.06E-03
LINC00323	0.422	7.68	1.98E-06	1.23E-03
CCL23	0.678	9.95	1.30E-05	2.24E-03
	0.604	11.95	7.57E-06	1.33E-02
HES1	0.356	10.84	2.95E-06	2.42E-03
SMPD3	0.356	9.89	1.74E-06	4.53E-03
HRASLS5	0.367	8.07	9.05E-06	5.51E-03
PRSS33	0.595	10.22	2.59E-05	1.05E-02
SLC29A1	0.411	12.47	3.63E-05	1.19E-02
	0.275	13.29	3.30E-05	3.32E-02
TFF3	0.614	10.03	8.55E-06	1.68E-02
CRTH2	0.401	11.96	3.57E-05	1.78E-02
IL17RB	0.345	8.92	3.49E-05	1.90E-02
P2RY2	0.310	9.24	1.47E-05	2.58E-02
RASL11B	-0.286	8.39	8.70E-06	2.88E-02
B) DEGs for AR replicated in BAMSE				
Gene	**logFC**	**AveExpr**	**P-value**	**P.eosadj**
SLC29A1	0.125	5.496	6.23E-06	9.97E-05
IL5RA	0.366	5.142	6.26E-05	4.27E-04
ALOX15	0.313	5.401	8.64E-05	4.27E-04
SMPD3	0.137	5.424	1.07E-04	4.27E-04
CLC	0.415	5.688	2.13E-04	6.82E-04
HRASLS5	0.109	4.045	1.16E-03	2.70E-03
SIGLEC8	0.148	4.851	1.18E-03	2.70E-03
OLIG2	0.085	4.792	1.87E-03	3.74E-03
P2RY2	0.09	5.772	4.72E-03	8.39E-03
PRSS33	0.069	5.589	8.99E-03	1.44E-02
HES1	0.058	3.107	1.46E-02	2.12E-02

logFC - logarithm base 2 of fold change effect size for AR phenotype; **Avg_Expr** - mean expression across all samples; **P-value** – False discovery rate (FDR) corrected p-value of gene expression to AR; **P.eosadj -** P-value for AR after correcting for eosinophil percentages. In the case of *ALOX15*, *CCL23* and *SLC29A1*, there were dual probes present that tagged to the same gene where expression values of both probes achieved FDR-significance.