Mass cytometry-based identification of a unique T-cell signature predicting childhood allergic asthma

Short title: T-cell signature in paediatric AA

Key words:
allergic asthma, paediatric, clinical disease parameters, CyTOF, T-cell subpopulations

To the Editor,

Allergic asthma (AA) in childhood is characterized by a dominance of type 2 immunity driven by $CD4^+$ T helper 2 (Th2) cells expressing the transcription factor (TF) GATA3 and inefficient counter-regulation by Tregs among other mechanisms.[1] However, a detailed analysis of T-cells associated with paediatric AA is still needed.

To identify T-cell phenotypes associating with paediatric AA, we applied a 42-antibody mass cytometry panel in combination with unsupervised computational analyses in a cohort of well-characterized 14 treatment-naïve AA and 9 healthy children (HC) from the CLARA/CLAUS study (Table S1,S2 Figure S1A).[2] Integrating information from 12 lineage-markers we identified seven major T- and NK-cell populations within peripheral blood mononuclear cells (PBMC) (Figure S1B,C) of which $CD8^+$ T-cell abundance was reduced with underrepresented memory compartment in AA vs HC (Figure 1A,S1D,E).

Accordingly, the $CD4^+/CD8^+$ T-cell ratio was elevated in AA vs HC and positively correlated with blood eosinophil frequencies, a determinant of AA severity[3], (Figure 1B,C). To address potential disease-associated changes within the $CD4^+$ T-cell compartment, we selected 30 markers for subsequent clustering using FlowSOM algorithm. Two clusters, cluster_c6 and cluster_c30, were significantly expanded in AA vs HC (Figure 1D,E,S2A). Cluster_c6 represented Th2 cells (Figure 1F), since it expressed the Th2-specific TF GATA3 and

chemokine receptors CRTH2 and CCR4.[4] It uniquely co-expressed TIGIT and ICOS, which was not reported in AA so far and could be specific for childhood, since *TIGIT* hypomethylation has been described only in paediatric AA.[5] The frequency of the $TIGIT^{+}ICOS^{+}$Th2_cluster, correlated with eosinophilia in AA with additional allergic comorbidities and also with CD4/CD8 T-cell ratio in asthmatics having intermittent disease symptoms, while inversely in stable disease (Figure 1G,H), indicating its association with more symptomatic disease including allergic comorbidity linked to eosinophilia.

Cluster_c30 expressing markers characterizing naïve/resting Tregs (Figure S2B) matched the previously described $CD45RA^{+}FOXP3^{low}$ Treg fraction(Fr)-I,[6] while eTregs represented Fr-II ($CD45RA^{-}FOXP3^{high}$) and Fr-III ($CD45RA^{-}FOXP3^{low}$) (Figure 1I). Accordingly, the frequencies of Fr-I were enriched in AA vs HC, while Fr-II and Fr-III were similar (Figure S2C). Cluster_c30 abundance correlated partially inversely with lung function, and significantly with memory $CD8^{+}$ T-cell frequencies (Figure 1J,S2D), indicating its connection to lung function and $CD8^{+}$ T-cell alterations. The abundances of Fr-I vs Fr-II correlated inversely (Figure 1K), consistent with the linear developmental model[6], suggesting a differentiation-block from Fr-I towards Fr-II, and a possible altered eTreg compartment in paediatric AA.

Therefore, we next analyzed eTregs by FlowSOM clustering, which revealed underrepresented cluster_c2 and cluster_c10 in AA (Figure 2A,B,S2E). Considering a possible eTregs impairment and an overrepresentation of $TIGIT^{+}ICOS^{+}$Th2-cells, we asked if the two phenomena are connected. Indeed, the $TIGIT^{+}ICOS^{+}$ Th2/eTregs ratio was higher in children with AA versus HC, but failed to associate with eosinophilia (Figure 2C,D). In contrast, $TIGIT^{+}ICOS^{+}$ Th2-cell ratio to eTreg_c2 and eTreg_c10, correlated significantly suggesting a relation between their underrepresentation and $TIGIT^{+}ICOS^{+}$Th2-associated eosinophilia.

Thus, naïve/resting Tregs (cluster_c30) and eTregs are linked to two different pathological features of asthma, lung function and eosinophilia, respectively.

Next, we performed principal component analysis (PCA) based on significantly changed ratio and subset-frequencies, which separated AA from HC children at the first PC, indicating that the detected dysbalanced T-cell composition allows a discrimination between AA and HC (Figure 2E). Additionally, ROC analyses revealed a relation of resting/naïve Tregs (CD4_c30), eTreg_c2 and eTreg_c10 to the paediatric AA phenotype (sensitivity, true positive rate) (Figure 2F), further supporting the relevant involvement of the Treg dysbalance in childhood AA.

Summarizing, our approach identifies a unique T-cell signature of childhood AA and provides insights for pathophysiological involvement of dysbalanced Tregs, $TIGIT^{+}ICOS^{+}$ Th2 and memory $CD8^{+}$ T-cells. This can be useful for immunomonitoring, immunomodulation and for further studies in childhood AA.

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Figure Legends

Figure 1. Dysbalanced CD4$^+$ and CD8$^+$ T-cell compartment in paediatric AA. A. T- and NK-cell frequency. **B.** CD4/CD8 T-cell ratio. **C,G,H,J,K** Linear regression analysis in AA children of: (C,G) blood eosinophil frequency versus (C) CD4/CD8 T-cell ratio, (G) cluster_c6 frequency with/without comorbidity, (H) CD4/CD8 T-cell ratio versus c6(TIGT$^+$ICOS$^+$) with intermittent symptoms or stable disease, (J) cluster_c30 frequency versus percent-predicted FEV1/FVC, (K) Fr-I versus Fr-II frequency. **D** tSNE-visualisation of 30 CD4$^+$ T-cell cluster. **E.** Cluster_c6 and cluster_c30 frequency. **F**. Cluster_c6 mean marker expression. **I.** Dot-plot with cluster_c30 and eTregs overlay**.** A,B,E. mean ± SD (A), *P* value by Mann-Whitney test.

Figure 2. Integrated T-cell signature distinguishes children with AA from HC. A. eTregs tSNE-visualization with cluster_c2 and cluster_c10. **B.** eTregs cluster_c2 and cluster_c10 frequency **C.** Cluster_c6TIGIT$^+$ICOS$^+$/eTreg ratio. **D.** Linear regression analysis of the respective ratios versus blood eosinophil frequency in AA children. **E.** Principal component analysis of the AA and HC samples based on the significantly regulated features (CD8$^+$ T-cells, CM CD8$^+$ T-cells, CD4$^+$ T-cell_c6, CD4$^+$ T-cell_c30, eTreg_c2, eTreg_c10 and CD4/CD8 T-cell ratio). **F.** Receiver operating characteristic (ROC) curves were calculated for CD4$^+$ T-cell cluster_c30, eTreg cluster_c2 and eTreg_c10. B,C (*P* value by Mann-Whitney test).

FIGURE 1

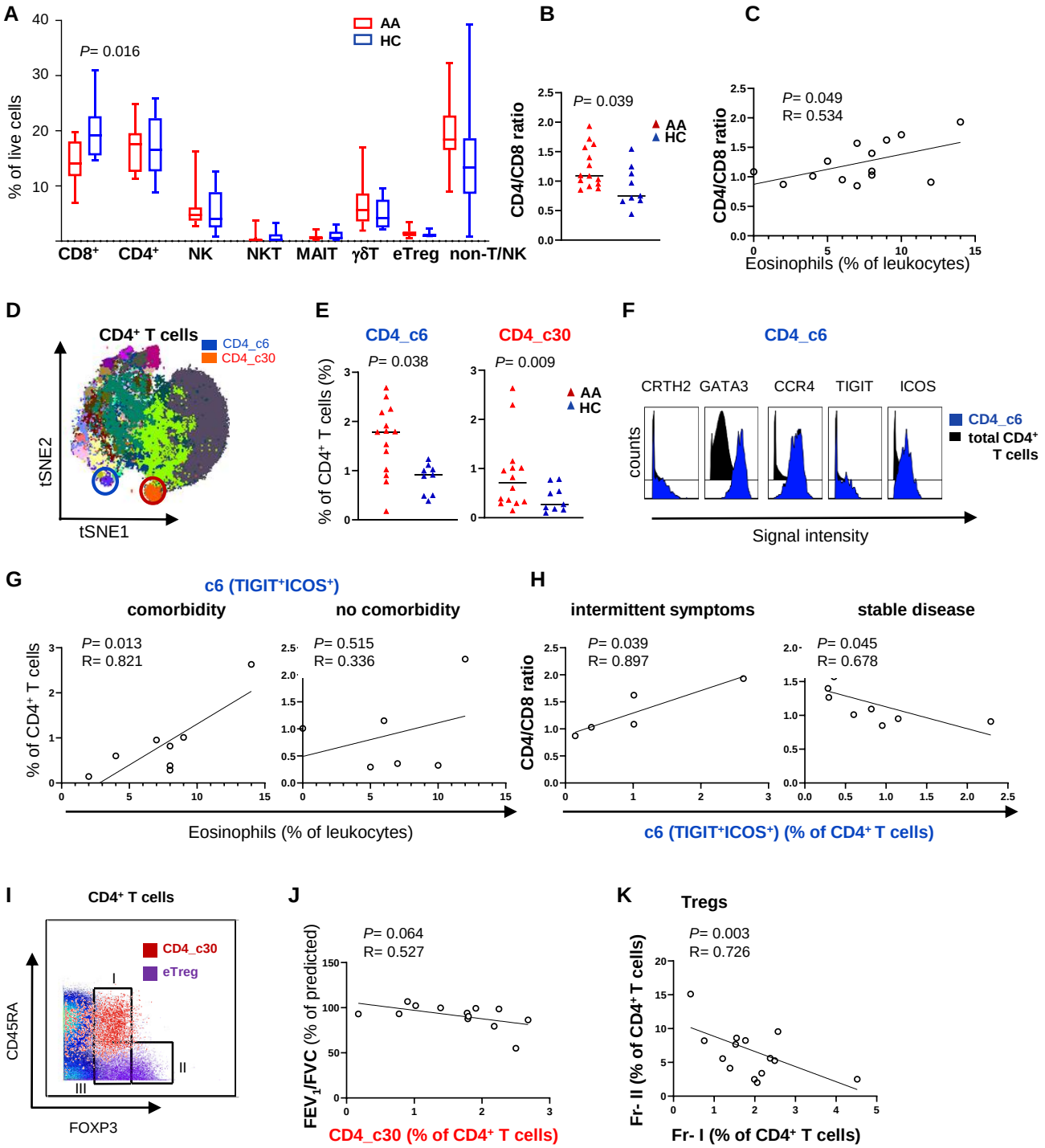
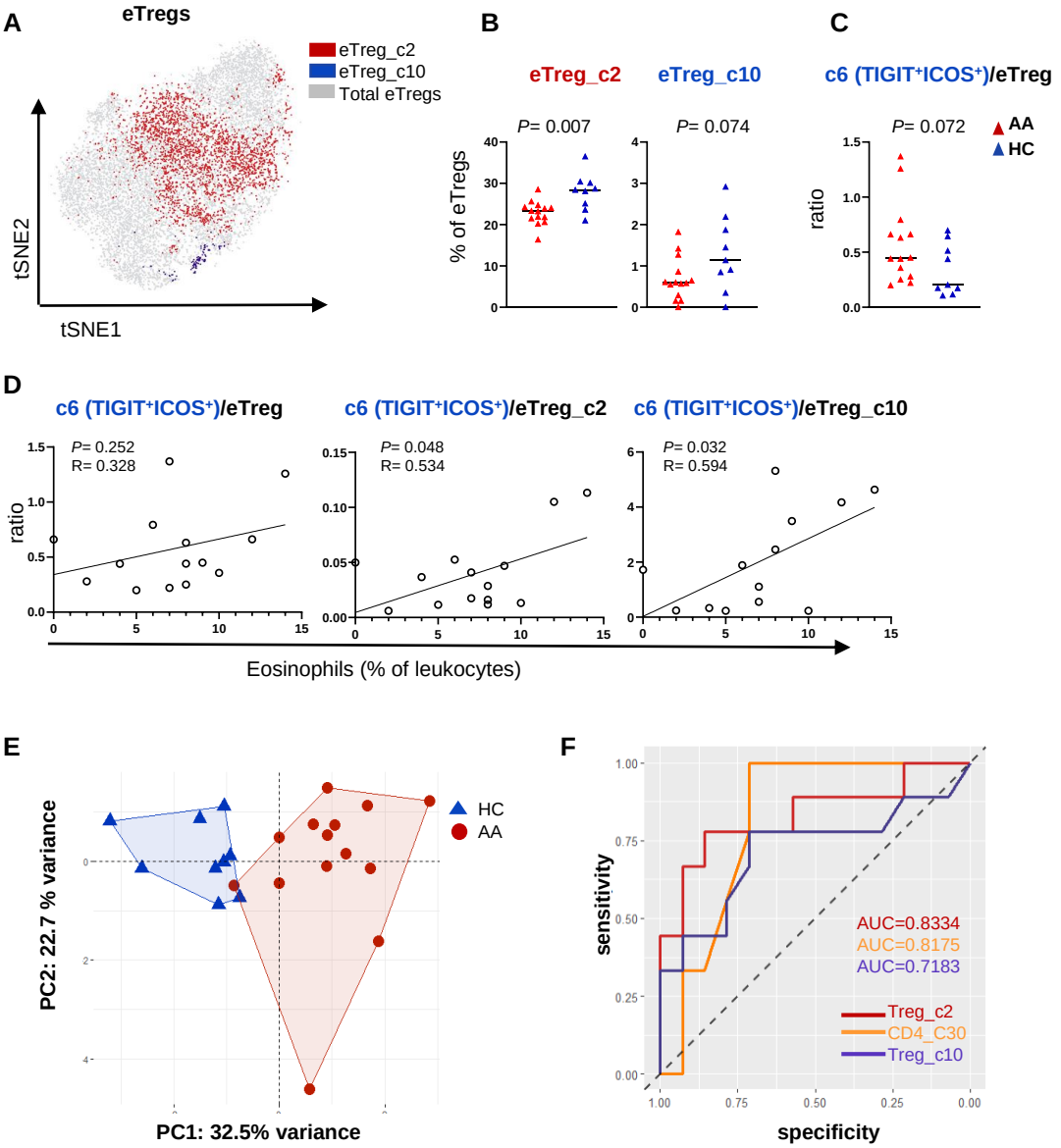

A
P= 0.016
AA
HC
% of live cells
CD8+
CD4+
NK
NKT
MAIT
γδT
eTreg
non-T/NK
B
P= 0.039
AA
HC
CD4/CD8 ratio
C
P= 0.049
R= 0.534
CD4/CD8 ratio
Eosinophils (% of leukocytes)
D
CD4+ T cells
CD4_c6
CD4_c30
tSNE2
tSNE1
E
CD4_c6
CD4_c30
P= 0.038
P= 0.009
AA
HC
% of CD4+ T cells (%)
F
CD4_c6
CRTH2
GATA3
CCR4
TIGIT
ICOS
counts
CD4_c6
total CD4+ T cells
Signal intensity
G
c6 (TIGIT+ICOS+)
comorbidity
no comorbidity
P= 0.013
R= 0.821
P= 0.515
R= 0.336
% of CD4+ T cells
Eosinophils (% of leukocytes)
H
intermittent symptoms
stable disease
P= 0.039
R= 0.897
P= 0.045
R= 0.678
CD4/CD8 ratio
c6 (TIGIT+ICOS+) (% of CD4+ T cells)
I
CD4+ T cells
CD4_c30
eTreg
I
II
III
CD45RA
FOXP3
J
P= 0.064
R= 0.527
FEV1/FVC (% of predicted)
CD4_c30 (% of CD4+ T cells)
K
Tregs
P= 0.003
R= 0.726
Fr- II (% of CD4+ T cells)
Fr- I (% of CD4+ T cells)

FIGURE 2


A
eTregs
eTreg_c2
eTreg_c10
Total eTregs
tSNE2
tSNE1
B
eTreg_c2
eTreg_c10
P= 0.007
P= 0.074
% of eTregs
C
c6 (TIGIT+ICOS+)/eTreg
P= 0.072
AA
HC
ratio
D
c6 (TIGIT+ICOS+)/eTreg
c6 (TIGIT+ICOS+)/eTreg_c2
c6 (TIGIT+ICOS+)/eTreg_c10
P= 0.252
R= 0.328
P= 0.048
R= 0.534
P= 0.032
R= 0.594
ratio
Eosinophils (% of leukocytes)
E
HC
AA
PC2: 22.7 % variance
PC1: 32.5% variance
F
sensitivity
specificity
AUC=0.8334
AUC=0.8175
AUC=0.7183
Treg_c2
CD4_C30
Treg_c10