Immunophenotyping of regulatory T-cells in adult systemic autoimmune diseases, pediatric asthma and immunodeficiency

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Introduction

Regulatory T cells (Tregs) are a specialized subset of lymphocytes essential for preserving immune homeostasis by suppressing excessive immune responses and promoting self-tolerance. These cells have emerged as critical regulators of inflammation and are key to preventing autoimmunity.
The aim of this study was to characterize the complex and heterogeneous Treg subpopulations using single-cell mass cytometry (CyTOF) in therapy-naive autoimmune and immune-mediated diseases: adult rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) (n=16), childhood asthma (n=33) and immunodeficiency (n=18). Age- and sex-matched healthy controls were also recruited (n=9 children, n=12 adults).

Methods

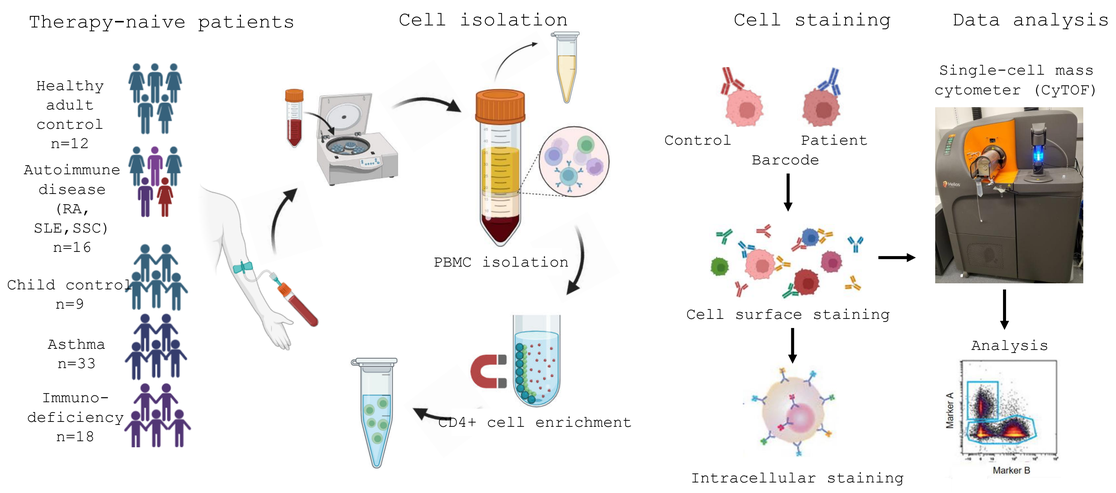

Figure 1. After blood collection, PBMCs were isolated and plasma was stored for later experiments, CD4+ cells were enriched, then barcoded and stained with lanthanide metal isotope-labeled antibodies. Our Treg panel contains 13 marker: CD49D, CD4, CCR4,CD45RA, CD45RO,CD3, CD39, Foxp3, CD95, CD25, HLA-DR, CD127, IKZF1, CD69, PD-1 for population identification and immunophenotyping. The samples were aquired by CyTOF and analyzed using Cytobank.

Subpopulations of regulatory T-cell

In our study, to investigate the dysfunction of Tregs in the periphery, we divided them into subgroups. Naive Tregs remain in an undifferentiated state until they encounter an antigen, at which point they can differentiate into effector Tregs. Effector Tregs have a powerful suppressive function and are able to migrate to inflammatory environments, where they exert their high cytokine-producing activity. Terminal effectors are similar to effectors, but exhaustion can also be obs

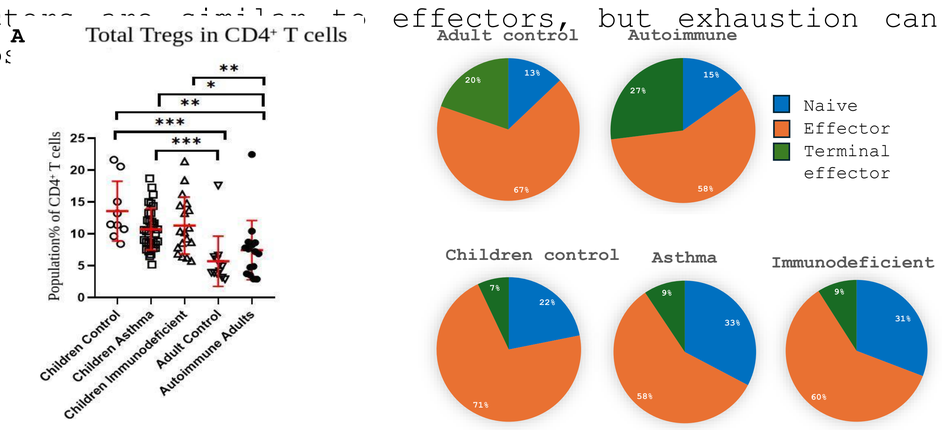

Figure 2. A) The percentage of total Tregs within CD4+ cells. B) The pie charts show the percentage of different subpopulations in groups.

Our experiments showed that the total Treg population in children was 14% in the control group and around 11% in the patient groups, indicating a reduced regulatory capacity. These ratios decrease significantly further in adulthood. We observed differences in the proportion of Treg subpopulations and marker expression in autoimmune patients compared to healthy controls. In both age groups, the proportion of active, suppressive effector Tregs is highest in the control group, while in patients more cells are still undifferentiated or already exhausted.

Changes in CD95 marker

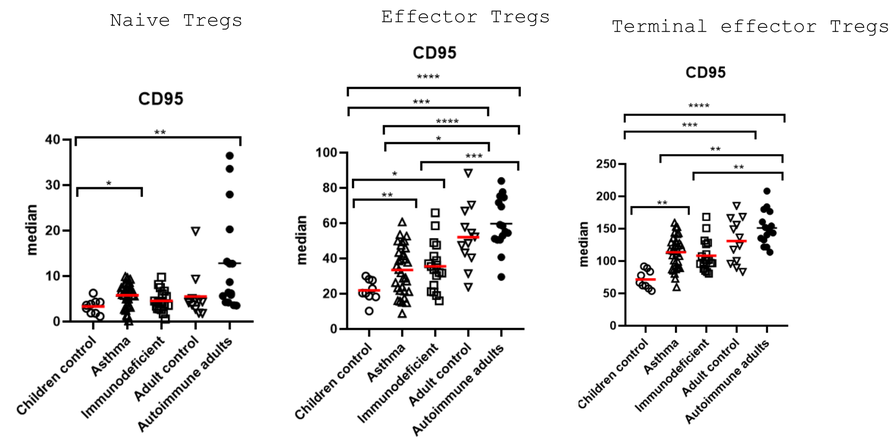

Figure 3. CD95 expression in different Treg population

CD95 (Fas) is a death receptor involved in apoptosis and immune regulation. Naive Tregs exhibit very low CD95, reflecting a resting, unactivated phenotype. In asthma and autoimmunity, CD95 increases, indicating early activation. Effector Tregs in all groups show higher CD95 expression than naive Tregs, which is further enhanced in terminal effector Tregs. Particularly high in autoimmune adults, indicating chronic activation, and potential functional exhaustion or susceptibility to apoptosis.

Marker expression patterns

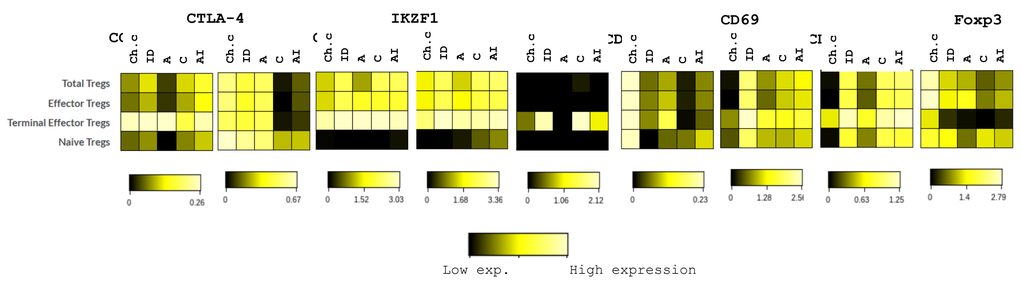

Figure 4. The heatmaps show how the marker expression patterns of different Treg subpopulations change in the 5 groups. Black indicates low expression, light yellow indicates high expression. Ch.c.: Children control, ID: Immundeficient, A: Asthma, C: Adult control, AI: Autoimmune

Terminal effector Tregs simultaneously show signs of exhaustion with high expression of CTLA-4 and a strong suppressive effect with high Foxp3, CD25 and low CD127 markers. In immunodeficient children cells often express high levels of functional markers such as the anti-inflammatory enzyme CD39, suggesting active regulatory function, despite a reduced overall frequency of total Tregs, as shown in figure 2. In adults, autoimmune disease is associated with increased expression of activation markers, such as CD69, compared to healthy controls, reflecting elevated immune activation.

T-SNE analyis of total Tregs in children and adults

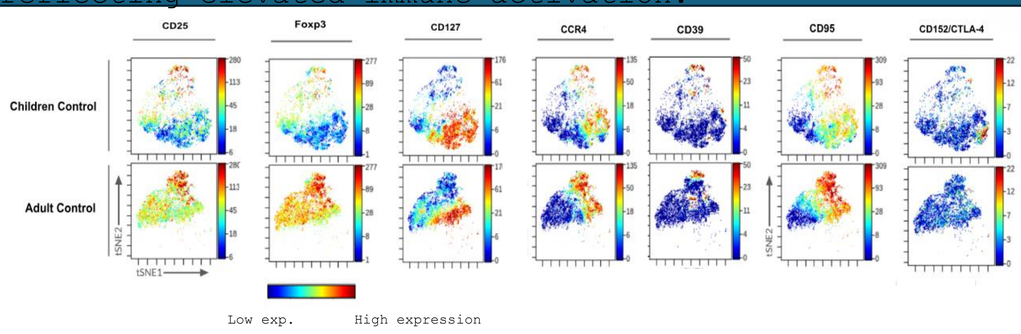

Figure 5. To visualize Treg heterogeneity, we used t-SNE analysis to compare healthy children and adults. A color scale indicates the intensity, blue indicates low, red indicates high expression.

The figures clearly demonstrate the heterogeneity within Tregs, with certain markers highly expressed in one cell population but absent in another. Additionally, the algorithm distinctly separates children and adults into two clusters, highlighting the markedly different Treg phenotypes between healthy children and healthy adults.

Conclusion

Immunophenotyping of Tregs reflects their activation state, suppressive capacity and exhaustion, dynamically adapting to immune status, age and disease. Our results revealed disease-specific alterations in Treg subset distribution and marker expression. The $CD25^{+}Foxp3^{+}$ Treg proportion was reduced in children with asthma and immunodeficiency compared to controls. Effector Tregs were more frequent in healthy controls, while patient groups exhibited shifts toward naive or terminally differentiated states. Elevated CD95 in disease states and with age likely reflects increased Treg activation and susceptibility to apoptosis, which may compromise their regulatory capacity.
This CyTOF-based profiling highlights Treg dysfunction as a key phenomenon in immune dysregulation and may support the development of novel diagnostic and therapeutic strategies.