PReS-FINAL-1002: Dissecting the dissociation of foxp3 and cd25 expression on cd4+ t cells in synovial fluid identifies three distinct subpopulations of human t regulatory cells present at the chronically inflamed site

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Introduction

T regulatory cells (Treg), vital to prevent autoimmunity, are defined by expression of FoxP3 in combination with high CD25 and low CD127 expression. It has been reported, however, that upon in vitro activation, conventional T cells (Tconv) can manifest phenotypic marks associated with Treg and, as such, expression of these markers alone is insufficient to determine Treg commitment. A unique feature of Treg is the presence of a Treg specific demethylated region (TSDR) in intron 1 of the FOXP3 gene. This distinguishes activated Tconv from bona fide Treg. In CD4+ T cells from the joints of children with JIA we have observed a clear dissociation of CD25 and Foxp3 expression. The relationship between CD25, CD127 and Foxp3 expression and commitment to Treg lineage at the inflamed site is unclear; meaningful investigation is hampered by the technical difficulties in isolating cells based on FoxP3 status.

Objectives

To analyze phenotype and frequency of CD4+ T cells isolated from synovial fluid (SF) from JIA patients based on expression patterns of Foxp3, CD25 and CD127, their in vivo turnover, and degree of commitment to the Treg lineage.

Methods

Peripheral blood mononuclear cells (PBMC) from JIA patients and controls and SF mononuclear cells (SFMC) were analyzed ex vivo for the expression of FoxP3, CD25, CD127, Ki67 and PD-1. In addition, SF CD4+ T cells, which were fixed and stained for Foxp3, were sorted into 4 distinct populations based on CD25, Foxp3 and CD127 expression. DNA was extracted using a modified phenol-based protocol, bisulfite-treated, the TSDR amplified, cloned and sequenced to quantify methylation levels.

Results

3 populations of CD4+ T cells displaying Treg lineage commitment were identified: Population I (PI), CD4+CD127loCD25loFoxp3hi (median of 5.03% of CD4+ T cells in SFMC vs. 0.94% in control, and 1.22% in JIA, PBMC); population II (PII), CD4+CD127loCD25hiFoxp3hi, (median 11.69% CD4+ T cells in SFMC vs. 5.74% in control, and 4.93% in JIA, PBMC); and population III (PIII), CD4+CD127loCD25hiFoxp3bi (median 4.13% of CD4+ T cells in SFMC vs. 0.82% in control, and 0.63% in JIA, PBMC); all 3 were significantly enriched compared to controls and displayed low levels of TSDR methylation (median methylation rates: PI, 19.73%; PII, 3.8%; PIII, 15.65%; Tconv, 95.55%). PD-1 expression was higher on all 3 populations when compared to controls, with highest expression PIII, which also had a lower frequency of Ki67+ cells compared to PI and PII (% ki67+ median: PI, 18.4%; PII, 20.2%; PIII, 8.96%).

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Conclusion
The presence of CD25 and/or FoxP3 alone is insufficient to define Treg populations in the inflamed joint. We propose 3 populations: PI & PII represent Treg expressing high levels of FoxP3 differing in their levels of CD25 but have robust turnover in vivo. PIII, with high levels of CD25 but low FoxP3 may represent an exhausted population of Treg (indicated by downregulated FoxP3, high PD-1 and low turnover in vivo). Understanding turnover and fate of regulatory cells in JIA will aid definition of how tolerance is lost in this autoimmune disease.

Disclosure of interest
None declared.

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