ANALYSIS OF T REGULATORY CELLS IN THE PERIPHERAL BLOOD OF CHILDREN WITH NEWLY RECOGNIZED TYPE 1 DIABETES

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Background: Type 1 diabetes (T1D) is one of the most common endocrine and metabolic conditions in childhood, with overall annual increase of 3% in pediatric population. T1D is characterized by autoimmune destruction of insulin producing pancreatic islet beta cells. A growing interest has recently been directed toward a population of regulatory T-cells (Tregs), which play a crucial role in maintaining homeostasis and self-tolerance. Abnormalities of Tregs, either in cell number or in function, are associated with initiation and progression of T1D.

Objective: The aim of the study was to investigate the percentage of Treg cells, Tregs apoptosis, interleukin IL-10 and TGF-beta production and CD137, CD134 expression on Tregs in the peripheral blood of children with newly diagnosed T1D in comparison with healthy controls.

Methods: 34 children (15 girls and 19 boys) with new onset of T1D, mean age 6.9 +/-5.2 yr (range 0,9-17,5 yr) and 18 healthy controls (8 girls, 10 boys), with mean age 7.3 +/-4.6 (1,9-17,5 yr) were included into the study. Flow cytometric analysis of T regulatory cells was performed using the following markers: anti-CD4, anti-CD25, anti-CD127 (IL-7R) and transcription factor FoxP3. CD4+CD25high cells were analyzed for interleukin IL-10 and TGF-beta production. Apoptosis was measured using anti-active caspase 3 monoclonal antibody. Percentage of apoptotic cells was measured within CD4+CD25highFoxP3+ cells. CD4+CD25high cells were analyzed for CD137 and CD134 surface markers. Samples were evaluated within 24 hours on Cytomics FC500 flow cytometer (Beckmann Coulter).

Results: Flow cytometry data showed significantly lower percentage of CD4+CD25highCD127lowfoxp3+ cells in children with T1D compared to control (median values 0.96 [range 0.32-7.79] vs. 3.08 [range 0.81-9.29] respectively, $p<0.0001$). There were no differences in the percentages of CD4+CD25high cells with IL-10 expression between diabetic children and control (median values 1.07 [range 0-8.4] vs. 0.87 [range 0-4.8] respectively, $p=0.986$). No differences were noted in the percentages of CD4+CD25high cells with TGF-beta expression between diabetic and healthy children (median values 1.08 [range 0-9.76] vs. 1.29 [range 0-7.6] respectively, $p=0.477$). There was significantly higher

percentage of CD4+CD25highCD137 cells in diabetic children compared to control (median value 5,76 [0.6-26.11] range vs. 3.40 [range 0.37-9.48] respectively, p=0.025). There was no difference in the percentage of CD4+CD25highCD134 cells between diabetic and healthy children (median value 3.58 [range 0-21.16] vs. 2.03 [range 0.45-11.73], respectively, p=0.278), and no statistical difference in the percentage of apoptotic CD4+CD25highFoxP3+ cells between children with diabetes and healthy subjects (median value 0 [range 0-26.83] vs. 0 [range 0-2.56] respectively, p=0.302.

Conclusions: The results of our study showed lower percentages of T regulatory cells defined as CD4+CD25highCD127lowfoxp3+ and higher percentage of CD4+CD25highCD137 cells in children with newly recognized T1D compared to healthy controls. The difference in the percentage of Treg cells and increased percentage of 4-1BB cells which down-regulate suppression mediated by Tregs may influence on initiation and progression of T1D. Future clinical studies in the large cohort of children with T1D are needed to determine the role of T regulatory cells in type 1 diabetes.