Streszczenie w języku angielskim

Introduction: In recent years, there has been a growing trend in the incidence of autoimmune diseases. Autoimmune thyroid diseases (ATD) with chronic Hashimoto's thyroiditis (HT) and Graves' disease (GD) are a significant therapeutic problem in the pediatric group of patients. The view that the pathogenesis of ATD is based on the presence of a triad of triggering factors, which are: genetic predispositions, environmental factors and immune dysfunction, is still valid. Despite this knowledge, researchers are constantly exploring this topic in order to find new methods of prevention and therapy of ATD.

In the field of immunology, regulatory B cells are currently of great interest, which, according to previous reports, allow the maintenance of immune homeostasis by neutralizing the negative effects of effector cells.

On the other hand, in terms of new environmental factors that may play a role in the pathogenesis of autoimmune diseases, attention has recently been paid to the problem of zinc deficiency, which is known to have properties that support the maturation and functionality of T and B lymphocytes. Zinc transporter 8 (ZnT8) is already known as one of autoantigens in type 1 diabetes, hence the suspicion that it may also be involved in the pathogenesis of other autoimmunopathies, including ATD.

The objectives of the conducted research included:

1. Assessment of the percentage of regulatory B cells with the phenotype CD19+CD24hCD27+IL-10+ and CD19+IL-10+ (B10) in children with ATD compared to the control group.

2. Evaluation of the effect of thyrostatic treatment (in the case of hyperthyroidism) and levothyroxine (in the case of hypothyroidism) on the percentage of Breg cells.

3. Evaluation of the correlation of the above-mentioned subpopulations of Breg cells with the concentration of hormones and antithyroid antibodies.

4. Assessment of ZnT8 expression in thyroid tissue in patients after thyroidectomy due to GD.

Material and methods: Due to the relatively low incidence of autoimmune diseases in children, this study referred to 3 different research groups consisting of patients suffering from ATD. In each of the mentioned study groups, there was an almost identical scheme of clinical qualification for the study, based on the history, physical examination, biochemical tests of thyroid hormones and the presence or absence of specific autoantibodies, and ultrasound assessment of the thyroid gland.

The first study group consisted of 53 pediatric patients diagnosed with autoimmune thyroid disease (12 patients with Graves' disease and 10 with Hashimoto's thyroiditis), aged 5-19 years. The control consisted of 15 patients without any autoimmune diseases. All patients underwent standard laboratory tests evaluating thyroid function and started typical pharmacotherapy - methimazole for GD and levothyroxine for HT. Breg assessment by flow cytometry was performed on admission, after 3 and after 12 months of therapy.

In the case of the assessment of ZnT8 expression, the study group consisted of patients aged 8-18: 17 with non-toxic nodular goitre (NTNG) and 20 patients with Graves' disease. Patients underwent total or subtotal thyroidectomy.

The third and last study group consisted of patients with Graves' disease (n=22) treated with methimazole at an initial dose of 0.5-1.0 mg/kg/body weight/day. These patients were followed before and during treatment: 3 months and 1 year. In addition, a control group (n=31) consisting of pediatric patients without autoimmune and inflammatory diseases and with a negative family history in this direction was also collected.

To assess the frequency of individual populations of regulatory B lymphocytes in pediatric patients with ATD, whole blood in a volume of 2.7 ml was collected with the use of an anticoagulant (dipotassium edetate, EDTA-K2) by puncture of the vein of the forearm.

Peripheral blood mononuclear cells (PBMC) after incubation with appropriate stimulants were subjected to the procedure of staining of extra- and intracellular receptors using monoclonal antibodies combined with appropriate fluorochromes. Cells were analyzed using a BD FACS Calibur cytometer (BD Bioscience) and FlowJo software (Tree Star Inc.).

The obtained data were processed using the statistical software GraphPad Prism 5.0 (GraphPad Prism Inc., San Diego, CA, USA) and Statistica 12.0 (Stat Soft, Poland). The normality of the distribution of numbers was confirmed or excluded using the Shapiro-Wilk test. For groups of data with a normal distribution, parametric tests such as the t-test or ANOVA were used. However, in the absence of parametric distribution, the Mann-Whitney and Wilcoxon tests were used, respectively. In addition, the levels of correlation between individual cell populations were calculated using the Spearmann test. The obtained data were presented using mean values and standard deviation.

ZnT8 expression in human thyroid tissues from patients with immune-mediated and non-immune thyroid diseases was investigated by simultaneous immunohistochemical, Western Blot and immunofluorescence analyses.

Results: There was a reduced number of IL-10-producing (B10) B cells relative to both B cells and total lymphocytes in each treatment group compared to healthy controls. There was a decrease in IL-10 production by Breg cells expressing CD19+CD24+CD27+IL-10 and CD1d+CD5+CD19+IL-10+ in both untreated and treated ATD patients.

In the study group of patients, higher levels of Foxp3+ and IL-10+ Breg with the CD38(-) phenotype and a reduced number of CD38+Foxp3+IL-10+ were observed in children with GD. In addition, selected Breg subgroups were found to significantly correlate with TSH and TRAb levels.

Significantly higher levels of Th1, Th17 and Th22 effector cells were found in patients with Graves' disease. Thiamazole did not significantly affect the changes within the examined cells, with a tendency only to reduce Th22 lymphocytes after 1 year of therapy. In contrast to healthy controls, patients with GD showed significant correlations between the studied Th and Breg cells only in the context of Th1 and Th17 cells. Initiation of treatment with thiamazole resulted in small but statistically insignificant changes in these interactions.

Expression of the ZnT8 transporter was identified by immunohistochemistry in thyroid tissues of pediatric patients with GD (per +++) and NTNG (per ++). This expression was found in both thyroid follicular cells (cytoplasm and cytoplasmic membrane in follicular cells) and C cells (membrane-cytoplasmic reaction) by fluorescence method.

Conclusions: The conducted research revealed further aspects of the complex pathomechanisms leading to the development of ATD. The reduction in the level of Breg lymphocytes with the CD19+CD24+CD27+IL-10+ and CD19+IL-10 phenotype may be responsible for the violation of immune tolerance in the course of GD. Analysis of the involvement of Breg in the course of ATD should focus on the effects caused by these cells, and not on the presence of specific phenotypes. The presence of ZnT8 expression in the thyroid tissue of patients with GD and NTNG may suggest the potential role of this transporter as another autoantigen in the pathogenesis of ATD. The obtained results may be the foundation for further research on immunocompetent cells and autoantigens and the use of their potential in new methods of therapy of autoimmune diseases.