

Th17 cells in children with Graves' disease during methimazole treatment



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INTRODUCTION

Graves disease (GD) is the most common cause of hyperthyroidism in the pediatric population. T helper 17 (Th17) IL-17A+CD3+CD4+ cells represent a novel subset of T helper cells that play an active role in inflammatory and autoimmune diseases. Although methimazole (MMI) lowers the levels of thyroid autoantibodies, little is still known about its influence on cell-mediated immune response. The role of Th17 cells in GD pathogenesis remains uncertain and the impact of MMI treatment on these cell subset has not been investigated.

The aim of this research was to describe the percentages and absolute counts of Th17 lymphocytes in children with GD and to assess changes in the amount of these cell subset during MMI treatment. The relationships between Th17 and selected clinical parameters were also assessed.

MATERIAL AND METHODS

Patients and controls

Ethics statement

The research protocol was approved by the Ethics Committee at the Medical University of Lublin. All caregivers or guardians gave their written informed consent on the behalf of the minors/children participating in our study.

Peripheral blood (PB) was obtained from 60 previously untreated adolescent patients with hyperthyroidism due to GD (mean age 14.07±1.94, median 14, range 11–17.4 years; 48 females, 12 males), hospitalized at the Department of Paediatrics, Division of Paediatric Endocrinology and Diabetology, Medical University of Lublin, Poland, and from 20 healthy donors (mean age 14.47±2.21, median 14.65, range 11–17.5 years; 15 females, 5 males). The patients were diagnosed on the basis of clinical examination as well as morphological and immunological criteria. The patients were treated with MMI at 0.51±0.05 mg/kg body weight/day for approximately 4–6 weeks, and after that time, after euthyroidism was confirmed, blood samples were collected again to assess lymphocyte subsets.

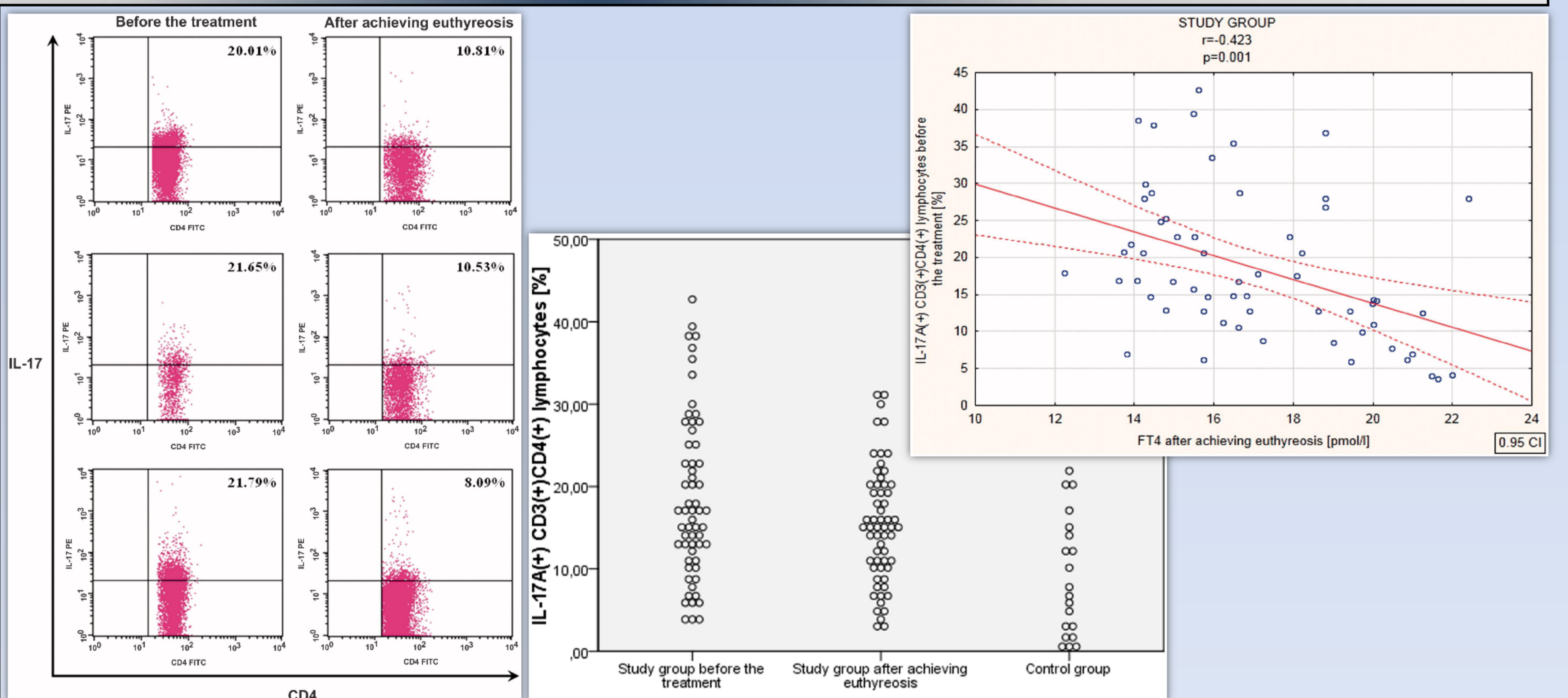
None of the patients and controls had signs of infection at the time of investigation and for a month before sampling and none had been taking drugs with known influence on the immune system, including oral contraceptives. None of the patients or healthy participants had undergone a blood transfusion. Persons with a history of allergic diseases were excluded from the study.

Isolation of peripheral blood cells and the detection of Th17 cells

Venous blood samples were collected from the study patients and controls by venipuncture using sterile, lithium heparin-treated tubes (S-Monovette, SARSTEDT). PB mononuclear cells (PBMCs) were aseptically separated by a standard density gradient centrifugation (Gradisol L, Aqua Medica). For the detection of Th17 cells, PBMCs were resuspended in RPMI-1640 culture medium (Sigma Aldrich) containing 10% heat-inactivated fetal calf serum (FCS, Sigma Aldrich), 2 mM L-glutamine, 100 U/ml penicillin (Sigma Aldrich), and 100 µg/ml streptomycin (Sigma Aldrich). We stimulated mononuclear cells for 5 h at 37 °C in 5% CO₂ with 25 ng/ml of phorbol 12-myristate 13-acetate (PMA, Sigma Chemical C.) and 1 µg/ml of ionomycin (Sigma Aldrich) in the presence of 10 µg/ml of Brefeldin A (Sigma Aldrich), which blocks the intracellular transport processes resulting in the accumulation of cytokine proteins on the Golgi complex. Next, PBMCs were collected, washed with PBS solution and prepared at a final concentration of 10⁶ cells/ml. The number of viable leukocytes was determined using 1% trypan blue exclusion. Subsequently, the mononuclear cells were stained with anti-CD3 CyChrome and anti-CD4 fluorescein-isothiocyanate (FITC) conjugated monoclonal antibodies (Becton Dickinson). The permeabilization of cell membranes was obtained by Cytofix/Cytoperm Kit (BD Pharmingen), which was added for 15 min at a temperature of 4 °C. After this procedure, cells were washed twice with PBS. The permeabilized cells were stained with PE-conjugated anti-human IL-17A monoclonal antibody (eBioscience). Cells were then washed twice with PBS. Cells were immediately analyzed with the use of Becton Dickinson Canto II flow cytometer (Becton Dickinson) and analysed with FACSDiva™ Software (Becton Dickinson). The results were presented as percentage of CD45+ cells stained with antibody. The percentage of positive cells was calculated via comparison with the control. Background fluorescence was determined using isotype-matched directly conjugated mouse anti IgG1/IgG2a monoclonal antibodies. The samples were gated on forward scatter versus side scatter to exclude debris and cell aggregates.

RESULTS

Higher absolute counts of Th17 lymphocytes were found in hyperthyroid adolescents before the treatment initiation and after achieving euthyroidism than in healthy individuals ($p=0.0001$ and $p=0.047$). Treatment with MMI caused a significant decrease in the percentages and absolute counts of Th17 lymphocytes ($p=0.047$ and $p=0.043$). After achieving euthyroidism, the serum concentration of FT4 correlated inversely with the percentages of Th17 cells ($r=-0.423$, $p=0.001$).



CONCLUSIONS

Despite the observed high frequencies of Th17 cells in GD adolescents in comparison with controls, and its tendency to decrease after MMI administration, treatment with MMI did not lead to the normalization of Th17 levels. Obtained results suggest also that severe immune system deregulation in the form of extremely high numbers of Th17 cells may lead to rapid GD relapse after the treatment.

