Background: This study was undertaken to analyze subsets of lymphocytes in peripheral blood in the early phase and in the thyroid tissue in the late phase of Graves' disease (GD) in children.

Methods: The study included 30 children with GD and 30 healthy children. Monoclonal antibodies were used to define peripheral blood lymphocyte subsets and they were analyzed using the flow cytometer Ortho Diagnostic System. After thyroidectomy, T cells were detected by CD3, CD4, CD8 antibodies, B cells by CD79a antibodies, and the antigen presenting dendritic cells (APCs) by CD1α antibodies (DakoCytomation) in the thyroid tissue.

Results: Before the treatment, an increased percentage of CD4+ T helper cells and B cells and decreased CD8+ T suppressor/cytotoxic cells were observed in peripheral blood in all the GD children. The number of lymphocytes and dendritic cells in the thyroid tissue increased in the children with GD in comparison to the control group, especially T cells subsets CD4+ and CD8+ and CD79a+ B cells. The percentage of T cells in the thyroid tissue was lower and that of B cells was higher than in peripheral blood. In their structure, thyrocytes can have components similar to α-chains connected with β-microglobulins, which were characteristic for APCs.

Conclusions: The primary defect of immunoregulation in children with GD is probably dependent on a large number and the activity of T helper cells and on a small number and hypofunction of T suppressor cells. The amount of lymphocytes decreased proportionally to the duration of methimazole treatment. The thyrocytes probably can present antigens.
Lymphocytes in children with Graves’ disease

Autoimmunological reactions increase with age. In the pediatric population, thyroid diseases occur less frequently than in adults and they are usually not distorted by other illnesses. The knowledge on the mechanisms of autoimmunological reactions in the thyroid tissue facilitates the use of drugs that selectively block lymphocyte subpopulations responsible for GD development.[10-12]

The aim of this study was to analyze subsets of lymphocytes in peripheral blood in the early phase and in the thyroid tissue in the late phase of GD in children.

Methods

Patients

The prospective study included 30 children with GD (6 boys, 24 girls), aged 16.1±2.2 years. All the children were treated at the Department of Pediatric Endocrinology and Neurology in Lublin (n=24) and Pediatric Department in Rzeszow (n=6) in the years from 1994 to 2007. The symptoms and laboratory examinations of thyrotoxicosis in these patients were noticed: a large goiter, tachycardia, sleeplessness, irritability, exophthalmos, an increase in systolic blood pressure, non-homogenous thyroid picture on ultrasonography, an increase in free T4 (FT4) (mean: 3.8 ±0.7 ng/dl) and total T3 (TT3) (mean: 363±175.3 ng/dl), and also a decrease in thyroid stimulating hormone (TSH) (mean: 0.004±0.003 mU/L).

The levels of TRAb (mean 71 U/ml) and usually the levels of TPO Ab (mean 1098 U/ml) and TG Ab (mean 1226 U/ml) were increased. TSH, FT4 and TT3 hormones were assayed by microparticle enzyme immunoassay (MEIA, Abbott Ireland Diagnostic Division Lisnamuck Longford). The levels of TRAb were measured by radioimmunoassay and luminescence immunoassay (TRAK assay, LumiTest BRAHMS Diagnostica GmbH, Berlin, Germany). TPO Ab and TG Ab were assayed by LIA (LumiTest BRAHMS Diagnostica GmbH, Berlin, Germany).

The patients were treated with methimazole at an initial dose of 0.5-0.9 mg/kg body weight per day during 4-6 weeks and after that time, when in euthyroidism, they got a maintenance dose of c.a. 0.1 mg/kg body weight per day (mainly 5 mg/day) in combination with a low dose of L-thyroxin (25 μg/day) during 18-24 months.

Only those children with GD in whom early relapses of hyperthyroidism were observed and thyroidectomy after 18-36 months was performed were qualified for investigations. The investigation was approved by the local Ethical Committee in Medical University in Lublin.

Lymphocyte subsets in peripheral blood

Lymphocyte subpopulation investigations were conducted in 15 children during the first visit at our department before thyrostatic treatment. Venous blood samples were collected and lymphocyte subsets were assayed not later than 5 hours after sampling. Next, the subsets of lymphocytes in peripheral blood were investigated within 6 months of therapy with methimazole when the patients were in euthyroidism. Monoclonal antibodies were used to define peripheral blood lymphocyte subsets, using the flow cytometer Cytoron Absolute Ortho Diagnostic System before starting and after 6 months of the treatment.

As the control group, 30 healthy children without autoimmune disease or goiter were assayed; they were the children who had behavioral therapy of obesity with FT4, TSH, TRAb, TPO Ab and TG Ab in normal ranges.

Lymphocyte subsets in thyroid tissue

Paraffin thyroid specimens obtained from the 15 children with GD after methimazole treatment and 15 children who were observed from the onset of GD, but without the initial lymphocyte investigations.

As the control group, thyroid specimens of 30 children who died in accidents or had been operated on due to thyroglossal cysts, neck injuries and surgery for parathyroid glands were investigated. The consents were obtained from the parents of the patients before blood samples and specimens were collected.

After thyroidectomy, histological examinations of 4 μm thyroid slices were performed using hematoxyline-eosin (HE) staining. Immunohistochemical reactions in paraffin specimens with monoclonal antibodies against T cell markers CD3+, CD4+, CD8+ as well as against CD79α+ B cells and the antigen presenting dendritic cells-CD1α+ antibodies (DakoCytomation Denmark) were performed.

The specimens were observed with a microscope Axiosstar plus. The lymphocytes were counted in Sony Colour Camera ExwaveHAD, and lymphocyte subsets were analyzed by MultiScan 5 software and hardware with Show Time Plus, S-VHS frame grabber using the IBM Pentium computer. We determined the number of lymphocyte subpopulations in the thyroid tissue by counting lymphocytes, marked with CD3+, CD4+, CD8+, CD79α+ and CD1α+ monoclonal antibodies, in every 1000 cells present in 10 vision fields of the microscope and by estimating their content percentage.

Statistical analysis

The results were expressed as means ± SD. The percentage of lymphocytes expressing receptors to