Lymphocytes in peripheral blood and thyroid tissue in children with Graves' disease

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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 CD79α 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 CD79α+ 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.


Key words: Graves' disease; lymphocyte subsets; thyroid

Introduction

In autoimmune thyroid disorders (AITD), Graves' disease (GD), Hashimoto thyroiditis and others are characterized by reactivity to self-thyroid antigens. It has been known for years that hyperthyroidism in GD is induced by immunological reaction, in which TSH receptor antibodies bind to the receptors on the surface of thyrocytes, activate them and initiate thyroid hormone production independent of hypothalamic-hypophyseal control. Numerous examinations were performed to analyze autoimmunological reactions in GD and to estimate the levels of TSH receptor antibodies (TRAb), sodium-iodide symporter antibodies, thyroid peroxidase antibodies (TPO Ab) and thyroglobulin antibodies (TG Ab). The latest research has been focused on examining the processes of regulation of autoimmunological reactions in this disease by estimating lymphocytes, adhesion protein and cytokine subpopulation in peripheral blood before and during hyperthyroidism treatment. It is known that probably for environmental or endogenous reasons, GD may develop in genetically predisposed individuals. When immune tolerance to thyroid antigens is broken, endothelial cells of regional postcapillary venules are activated, allowing extravasation of blood leukocytes.

Antigens of self-thyrocytes are presented in such a way that they are recognized by self-T-helper lymphocytes. An increase in T helper lymphocytes in peripheral blood, especially in Th1 lymphocytes, leads to activation of B lymphocytes and their transformation into plasmatic cells which produce thyroid antibodies: TRAb, TPO Ab and TG Ab. These infiltrating cells attack thyrocytes with different proinflammatory
cytokines, mainly chemokines. 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) \((\text{mean: 3.8±0.7 ng/dl)}\) and total T3 (TT3) \((\text{mean: 363±175.3 ng/dl)}\), and also a decrease in thyroid stimulating hormone (TSH) \((\text{mean: 0.004±0.003 mU/L)}\).

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

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 euthyrosis, 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 \((25 \mu \text{g/day)}\) during 18-24 months.

Only those children with GD in whom early relapses of hyperthyroidosis 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 euthyrosis. 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 \(\mu\)m 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 CD79a+ B cells and the antigen presenting dendritic cells-CD1α+ antibodies \((\text{DakoCytomation Denmark)}\) 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+, CD79a+ 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
monoclonal antibodies was compared using Students' \( t \) test or confirmed by the Mann-Whitney test. \( P<0.05 \) was considered statistically significant. For correlation analysis Spearman's test was used. Mathematical and statistical calculations were performed with Statistica 5.0.

Results

Lymphocyte subsets in peripheral blood

The total amount of T-cells was equal in the control and GD groups in an early phase before and after treatment with methimazole.

The percentage of total T helper subsets (CD4+) was increased in GD after a 6-month therapy compared to the control group (\( P=0.098 \)) and GD before treatment (\( P=0.004 \)). After a 6-month therapy, the increase was statistically significant (\( P<0.05 \)). A statistically significant increase was observed in the percentage of active T-helper cells (CD4 HLA DR+) in GD patients before treatment with methimazole and a decrease after the therapy (Table 1).

CD8+ T suppressor/cytotoxic cells remained decreased before and after methimazole therapy. The active subset of CD8 HLA DR+ suppressor/cytotoxic cells was increased significantly at the onset of the disease. Methimazole therapy led to normalization of the percentage of this subset (Table 1). In the subset of T suppressor/cytotoxic cells, the percentage of T suppressor cells (CD8+11b+) was decreased in the children with GD compared with the control group. The subset of T cytotoxic cells (CD8+11b-) was decreased at the onset of GD and increased during the treatment (Table 1).

The subpopulation of CD19+ B cells (which produced TRAb, TPO Ab and TG Ab) increased in GD especially at its beginning and was normalized after methimazole therapy. The subset of B-native plasma cells was increased significantly in GD and decreased during the medication (Table 1). A correlation was observed between the levels of thyroid TPO Ab and TG Ab levels and percentage of lymphocyte subsets. Correlation between the levels of TRAb and CD19+ B lymphocytes was observed (\( r=0.62, P=0.002 \)).

Lymphocyte subsets in thyroid tissue

Lymphocytes were present in the interstitium of thyroids from the control group. The thyroids of children with GD were available for microscopical studies in the late phase of GD after long thyrostatic treatment in euthyrostatus status. In the thyroid glands from patients with GD, lymphocytes were observed in the interstitium and formed infiltrations, frequently accompanied by lymphatic follicles.

Dendritic cells marked with CD1α antibodies were present in a low percentage (0.16±0.01%) in normal thyroids of the children from the control group in the perifollicular or connective tissues. In the children with GD, APCs were observed in the interstitium of the thyroid tissue (0.36±0.33%) and in thyroidal lymphatic follicles (0.5±0.81%). Dendritic cells, as CD1α+ APCs, were observed in the lymphatic follicles in the thyroid in the germinal center or at the edge. It was interesting that some thyrocytes revealed positive reaction with CD1α monoclonal antibody, a characteristic for dendritic cells, which detected transmembrane α-chain connected with β-l microglobulin (Table 2, Figs. 1, 2).

CD3+ T cells were noticed in the thyroid interstitium between thyroid follicles in the control group (1.02±0.28%) and as infiltrates in the interstitium (5.07±6.90%) and in the lymphatic follicles (30.39±19.60%) in the patients with GD. The differences in the amount of these lymphocyte subsets in the patients with GD compared to the control group were statistically significant (Table 2, Fig. 3).

| Table 1. The subsets of lymphocytes of peripheral blood in children with Graves' disease during methimazole treatment (as the percentage of all lymphocytes) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Subsets of lymphocytes | Control group (%) | Control group | Patients with Graves’ disease before treatment (%) | Patients with Graves’ disease after 6 mon treatment in euthyrosis (%) |
| | Means ± SD | | Means ± SD | | Means ± SD | | Means ± SD | |
| CD3+ | T | 72.20±5.83 | 70.70±7.11 | 0.734 | 72.35±4.23 | 0.067 |
| CD4+ | T helper | 39.44±5.83 | 40.60±8.76 | 0.098 | 43.07±5.19 | 0.004 |
| CD4+HLA-DR+ | T helper active | 2.72±0.77 | 4.45±1.76 | 0.003 | 2.96±0.88 | 0.096 |
| CD8+ | T suppressor/cytotoxic | 33.48±3.16 | 28.84±6.21 | 0.064 | 29.01±2.83 | 0.032 |
| CD8+HLA-DR+ | T suppressor/cytotoxic active | 2.17±0.71 | 3.44±1.84 | 0.001 | 1.94±0.80 | 0.036 |
| CD8+11b+ | T suppressor | 15.55±4.03 | 12.41±5.66 | 0.043 | 12.50±2.72 | 0.043 |
| CD8+11b- | T cytotoxic | 17.38±3.64 | 15.12±3.67 | 0.086 | 16.00±1.91 | 0.089 |
| CD19+ | B | 11.98±2.48 | 13.80±5.10 | 0.056 | 12.37±3.58 | 0.065 |
| CD19+CD5+ | B native plasma cells | 4.74±1.67 | 7.75±3.32 | 0.004 | 4.20±1.86 | 0.780 |

*: Comparison of lymphocyte subsets percentage of control group and GD patients before treatment showed significant changes (\( P<0.05 \)); †: Comparison of lymphocyte subsets percentage of control group and GD patients after 6 months treatment showed significant changes (\( P<0.05 \)).
CD4+ T helper lymphocytes were infiltrated in the lymphatic follicle and between thyroid follicles in the thyroid. The percentage of this lymphocyte subset was lower in the control group than in the patients with GD, but the difference was not statistically significant (Table 2, Fig. 4). The percentage of this subset was lower in the thyroid tissue compared to that of blood.

CD8+ T suppressor/cytotoxic cells infiltrated into the thyroid follicles and were found in the lymphatic follicles. They were in close contact with thyrocytes and other lymphocytes. CD8+ T cells were observed with a high percentage (11.39±6.39%), especially in infiltrations in the thyroid tissue in GD patients (Table 1, Fig. 5). During methimazole treatment the percentage of CD8+ T cells was decreased, and a remarkable correlation coefficient between time of treatment and the percentage of CD8+ T cells was noticed (Table 3). The subset of T cells in the thyroid was more numerous, but the percentage of this subset was lower than in the blood (Tables 1, 2).

The CD4/CD8 ratio was higher in infiltrations of the thyroid (9.77±1.97) and in the interstitium of the thyroid (1.85±1.12) in the GD patients than that in the control group (0.34±0.01) (P=0.0007).

CD79α+ B lymphocytes infiltrated into the thyroid among the thyroid follicles and lumen. B lymphocytes were predominant in the infiltrates of the thyroid with a higher percentage (39.86±28.02%) compared to the control group (P=0.00009) (Table 2, Fig. 6). B lymphocytes were observed in the thyroid in GD patients with a percentage higher than in the blood (Tables 1, 2).

A correlation was observed between the number of lymphocytes and the time of methimazole administration. In the patients with a long treatment with methimazole, the number of lymphocytes in all the subsets was lower and infiltrations into the lymphatic follicles rarely occurred (Table 3). No correlation was observed between the lymphocyte count in peripheral blood in the early phase of the disease and the amount of lymphocytes in the thyroid tissue after surgery.

A negative correlation was observed between the duration of therapy with methimazole and the amount of CD3+ T cells in the thyroid interstitium (r=-0.59) and in infiltrations (r=-0.38). The negative correlation was observed between the length of methimazole treatment, the amount of CD8+ T suppressor/cytotoxic cells in infiltration (r=-0.69), and the amount of CD79α+ B cells (r=-0.58). CD4+ T helper cells and CD1α+ dendritic cells were not correlated with the duration of this therapy (Table 3). No correlation

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**Table 2.** Subsets of lymphocytes as the percentage of cells in the vision fields in the thyroid tissue

<table>
<thead>
<tr>
<th>Lymphocyte subsets (%)</th>
<th>Control group</th>
<th>Graves’ disease interstitium of the thyroid</th>
<th>Graves’ disease infiltrations in the interstitium of the thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means ± SD</td>
<td>Means ± SD</td>
<td>P</td>
</tr>
<tr>
<td>CD1α       Dendritic cells</td>
<td>0.16±0.01</td>
<td>0.36±0.33</td>
<td>0.052</td>
</tr>
<tr>
<td>CD3        T</td>
<td>1.02±0.28</td>
<td>5.07±6.90*</td>
<td>0.002</td>
</tr>
<tr>
<td>CD4        T helper</td>
<td>0.19±0.001</td>
<td>1.17±1.94</td>
<td>0.080</td>
</tr>
<tr>
<td>CD8        T suppressor-cytotoxic</td>
<td>0.65±0.67</td>
<td>1.59±1.63</td>
<td>0.234</td>
</tr>
<tr>
<td>CD79α      B</td>
<td>4.07±3.62</td>
<td>6.54±2.12*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*: Comparison of lymphocyte subsets percentage of GD patients after 18-24 and 25-36 months of methimazole therapy showed significant changes (P<0.05); †: Comparison of lymphocyte subsets in thyroid interstitium of the control group and infiltrations of interstitium of GD patients showed significantly different changes (P<0.05)

**Table 3.** The correlation coefficients between the duration of methimazole therapy and the lymphocyte subsets in the thyroid tissue

<table>
<thead>
<tr>
<th>Lymphocyte subsets</th>
<th>The percentage of lymphocyte subsets in infiltrations of lymphocytes and lymphatic follicles in different duration of methimazole treatment (min-max)</th>
<th>The percentage of lymphocyte subsets in interstitium of the thyroid in different duration of methimazole treatment (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18-24 mon</td>
<td>25-36 mon</td>
</tr>
<tr>
<td>CD1α    Dendritic cells</td>
<td>0.85-1.61</td>
<td>0.18-0.69</td>
</tr>
<tr>
<td>CD3    T</td>
<td>11.15-52.22</td>
<td>21.27-35.24</td>
</tr>
<tr>
<td>CD4    T helper</td>
<td>5.05-7.01</td>
<td>0.21-5.99</td>
</tr>
<tr>
<td>CD8    T suppressor-cytotoxic</td>
<td>8.22-24.13</td>
<td>5.10-12.31</td>
</tr>
<tr>
<td>CD79α    B</td>
<td>52.66-69.12</td>
<td>15.03-45.21</td>
</tr>
</tbody>
</table>

*: Comparison of lymphocyte subsets percentage of GD patients after 18-24 and 25-36 months of methimazole therapy showed significant changes (P<0.05).
was detected between lymphocyte subpopulations and thyroid hormones before treatment and between lymphocyte subpopulations in blood in the early stage of GD. Nor correlation was noticed between levels of TRAb, TPO Ab and TG Ab and the percentage of lymphocyte subsets in the thyroid tissue.

Fig. 1. The CD1α+ dendritic cells in the thyroid tissue from GD patient after the treatment (original magnification × 200).

Fig. 2. The immunochemical reaction with CD1α in some thyrocytes in the thyroid tissue from GD patient after the treatment (original magnification × 200).

Fig. 3. The CD3+ T cells in the thyroid tissue from GD patient after the treatment (original magnification × 200).

Fig. 4. The CD4+ T helper cells in the thyroid tissue from GD patient after the treatment (original magnification × 200).

Fig. 5. The CD8+ T suppressor/cytotoxic cells in the thyroid tissue from GD patient after the treatment (original magnification × 200).

Fig. 6. The CD79α+ B cells in the thyroid tissue from GD patient after the treatment (original magnification × 200).
Lymphocytes in children with Graves' disease

Discussion

Numerous investigations including ours suggested that autoimmunological reaction in GD is dependent on antigen presentation by dendritic cells and probably on some thyrocytes, which have traits of APC.\(^\text{[13,14]}\)

It is known that the circulating lymphocytes migrate from blood vessels to the thyroid in GD. This process proceeds as follows: first, the lymphocyte comes into contact with the thyroid vascular endothelium, in which selectins participate (L-on the lymphocyte surface and E-on endothelial cells); second, the activated lymphocyte adheres tightly to the vascular endothelium attended by adhesion proteins and alpha and beta integrins, and finally, as a result of diapedesis, it infiltrates into the thyroid endothelium towards the thyroid interstitium.\(^\text{[7]}\)

Two molecular complexes VLA-4/VCAM-1 and LFA-1 ICAM -1,-2,-3 regulate adhesion and migration of lymphocytes and monocytes through the vascular wall, similarly to L-selectin (CD62L), which is expressed mainly on lymphocytes and neutrophils and plays the main role in homing T-cells into lymphoid organs and to the area of the local inflammation.\(^\text{[15-18]}\)

Bossowski et al\(^\text{[5]}\) described the decrease in beta 1 and beta 2 integrins in peripheral blood of patients with GD, probably associated with the increased migration of lymphocytes with these surface antigens into the thyroid gland. After the appearance of antithyroid autoantibodies in the circulation, T and B cells start to infiltrate into the thyroid and at the same time there is a remarkably sharp increase in the number of thyroid dendritic cells.\(^\text{[19,20]}\)

In our investigation, likewise in other studies,\(^\text{[20,21]}\) T cells, B cells and dendritic cells accumulating in the thyroid do not form destructive infiltrates, but they are organized as a peripheral lymphatic tissue. Thyrocytes adjacent to areas of the intra-thyroid lymphatic tissue start to express MHC class II molecules,\(^\text{[19-21]}\) probably as a consequence of the cytokines produced in the intra-thyroid lymphatic tissue. Auto-antigen specific B-cells play a role as APC in the late phase of autoimmune reactivity, too.\(^\text{[22]}\)

In the microscopical picture of the thyroid in GD patients, there are areas of intra-thyroid lymphatic tissue and areas in which the thyroid follicles are intact with significantly elevated numbers of perifollicularly located dendritic cells.\(^\text{[19,15,23]}\)

Most perifollicular dendritic cells have an immature phenotype CD10.\(^\text{[13]}\)

The presentation of autoantigens with MCH II complex leads to activation of T-helper cells (CD4+): Th1 subsets and Th2 subsets. GD patients seem to have mixed Th1/Th2 profiles.\(^\text{[6]}\)

Th2-cells dependent B cells activation leads to over-production of autoantibodies and cytokines.\(^\text{[24,25]}\)

It is known, and we observed it too, that patients with untreated GD have an increased number of circulating B cells, which are involved in the production of autoantibodies against thyroid antigens, including CD5+ B cells producing natural autoantibodies.\(^\text{[24,26,27]}\)

The B cells observed in the thyroid were mainly scattered as single cells or appeared in small groups in the T-lymphocyte infiltrates characterized by CD20+\(^\text{[2,28]}\) Similarly to us, Armengol et al\(^\text{[29]}\) found lymphocytic infiltrates containing germinal centers in 54% patients with GD. B cells were abundant in the germinal centers and they are believed to be of oligoclonal origin. In the germinal centers memory B-cells and plasma cells are formed.\(^\text{[30]}\)

Depletion of B-lymphocyte leads to improvement of the thyroid function in GD.\(^\text{[30]}\)

In GD patients, a decrease in suppressor cells defined as CD8+ T cells was described.\(^\text{[12,53]}\)

It is widely agreed that in thyrotoxic patients with GD a decrease in CD8+ T cell number is characteristically present, and that a similar abnormality exists in the thyroid. CD8+ cells return gradually toward normal during therapy and are usually, but not always, normal at the end of therapy.\(^\text{[14,35]}\)

Our observations of the decrease of CD8+ T subsets (especially CD8 HLA DR+ cells) in peripheral blood confirmed this process. The decreased number of CD8+ T cells in the thyroid tissue in comparison to this subset in blood arose from therapy with methimazole. Patients had to be in euthyreosis before thyroidectomy.

The latest investigations\(^\text{[36,37,38]}\) suggested that a crucial role in peripheral tolerance of autoreactive T cells is played by T regulatory cell subsets (Tregs). Tregs can be largely divided into two subpopulations: naturally occurring versus inducible.\(^\text{[30]}\)

Tregs so far identified as participating in the pathogenesis of GD include naturally occurring CD4+CD25+ T cells,\(^\text{[37]}\) CD8+CD122+ T cells,\(^\text{[40,41]}\) and natural killer T (NKT) cells.\(^\text{[42]}\)

CD4+CD25+ Tregs are derived from the thymus and also generated in the periphery, constitute 5%-10% of peripheral CD4+ T cells, and have been demonstrated to play a preventive role from autoimmune diseases in humans and mice.\(^\text{[38]}\)

Depletion of CD4+CD25+ T cells and CD8+CD122+ T cells enhances the development of Graves' hyperthyroidism in the mouse model.\(^\text{[42]}\)

NKT cells polarize anti thyroid stimulating hormone receptor (TSHR) immune response to T helper type 2 (Th2) phenotype and suppress induction of GD in an animal model.\(^\text{[40]}\)

Hyperthyroidism connected with depletion of CD4+CD25+ Tregs and CD8+CD122+ Tregs is accompanied by intrathyroidal lymphocyte infiltration.\(^\text{[37]}\)

The data on CD4+CD25+ Tregs in autoimmune thyroid diseases in humans are controversial. Marazuela et al\(^\text{[43]}\) described an increased number of CD4+CD25+ T cells in Graves' thyroid glands, but these cells exhibited an impaired...
suppressor function. Nakano et al. reported decreased proportions of intrathyroidal CD4+CD25+ Tregs in patients' thyroiditis. It is possible that GD may involve a different type of immune response rather than the classical Th1/Th2 immune responses. One candidate is Th17 cells-T cells producing IL-17; they can be derived from native CD4+ T cells by IL-6 generated by dendritic cells.

Th1-cell activates the antibody dependent cellular cytotoxicity (ADCC). Numerous reports have shown that T cell immunity can be detected in GD, although the response is usually weak and not present in many patients. However, CD8+ do display a degree of restriction, although their autoreactive potential is not known at present. While T cell immunization is conventionally recognized by a stimulatory effect of antigen, direct T cytotoxicity of thyroid cells has been recognized in a few studies. An interesting potential consequence of T lymphocytic adherence to thyroid cells (observed in our microscopical investigations), via ICAM 1/LFA-3 interaction, is the stimulation of thyroid cell proliferation, which could lead to goiter formation.

Many studies have reported on natural killer cell activity and ADCC. ADCC of thyroid cells was induced by anti-TPO antibody positive sera, but other unknown antibody-antigen systems also contributed.

The analysis of many investigations and our observations suggested that GD methimazole treatment leads to reduction in the activation of T helper cells due to direct immunosuppressive effect and amelioration of hyperthyroidism, but another mechanism of thyroid damage ADCC can lead to antigen presentation and is connected with relapses of GD.

In conclusion, primary defect of immunoregulation in children with GD is probably dependent on a large number and activity of T-helper cells and on a small number and hypofunction of T suppressor cells. The amount of lymphocytes is decreased proportionally to the duration of methimazole treatment. Thyrocytes can have components similar to α-chains connected with β-microglobulins in their structure, which are characteristic for APCs.

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Received January 15, 2008
Accepted after revision May 14, 2008