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Roles of Thyroid Hormones, Mast Cells, and Inflammatory Mediators in the Initiation and Progression of Autoimmune Thyroid Diseases

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Keywords
Autoimmune diseases · Thyroid hormones · Mast cells · Degranulation · Costimulatory molecules

Abstract
Background: Interrelation between thyrocytes and immunocytes has been established. However, the roles of mast cells and thyroid hormones in the triggering mechanism of thyroid autoimmune processes have been insufficiently investigated. This study evaluated the role of thyroid hormones and mast cells in the mechanisms of losing tolerance to thyroid autoantigens. ***Materials and Methods:*** Two groups of patients were studied: patients with Graves' disease and patients with nodular euthyroid goiter. Wistar rats with induced exogenous hypothyroidism, thyrotoxicosis, and thyrotoxicosis in parallel with administration of interleukin-2 were used. The levels of hormones, autoantibodies, and cytokines in the serum and thyroid tissue were analyzed through the enzyme-linked immunosorbent assay. Morphological verification was performed through the immune-histochemical method with antibodies against tryptase and CD86. Transmission electron microscopy and laser confocal microscopy were used. ***Results:*** In both Graves' disease and induced thyrotoxicosis, we detected a significant increase in the levels of interferon-γ, active interfollicular infiltration and degranulation of mast cells, and the intrathyroid overexpression of CD86. Complex analysis of the rat's thyroid morphofunctional state and systemic and local levels of cytokines in induced thyrotoxicosis and hypothyroidism demonstrated an increase of intrathyroid degranulation of mast cells and a drastic disruption of IFNγ/IL10 balance. ***Conclusions:*** When exposed to excessive amounts of thyroid hormones, an inflammatory response is triggered in the thyroid gland, and mast cells overexpress costimulating CD86 in the thyroid. This finding confirms their possible involvement in autoantigen presentation. Significant increase in the levels of interferon-γ shows a determining influence of cytokine on the progression of the pathological process.

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Introduction

In recent decades, research reported a high rate of autoimmune thyroid diseases (ATD) [1, 2]. It is well established that Graves' hyperthyroidism is caused directly by autoantibodies to the thyroid stimulating hormone (TSH) receptor (TRAbs), mimicking the stimulatory activity of TSH [2]. Previously, the activation of intrathyroid mast cells (MC) in the development of exogenous thyrotoxicosis was analyzed to confirm the close association between MC, thyroid hormones (TH), and the sympathoadrenal system [3, 4]. Moreover, the influence of TH and TSH on lymphoid immunocompetent cells through their specific membrane and nuclear receptors was demonstrated [5]. However, many aspects of the pathogenesis of ATD remain unknown, including the trigger mechanism, primary autoantigen, and all antigen-presenting cells (APC), as well as the activation mechanisms and pathogenic role of MC in these diseases [3, 6]. The role of microchimerism, molecular mimicry, and anti-idiotypic antibodies has been actively discussed [1, 6]. Nevertheless, none of these ATD immunopathogenic mechanisms can fully explain the pathways of losing tolerance to thyroid autoantigens. This investigation was based on a comprehensive analysis of the morphofunctional condition of the thyroid gland, systemic and local levels of cytokines, and intrathyroid expression of the costimulatory molecule CD86 by MC in induced thyrotoxicosis and Graves' disease (GD). The signaling system through CD28 and CD152 involving CD80 and CD86 plays a crucial role in the induction and regulation of immune responses [7, 8]. The CD86 and CD80 molecules are coregulators of T-cell activation [7]. Additional markers, CD80 and CD86, appear on activated B cells, as opposed to resting B lymphocytes. In addition to B lymphocytes, CD86 is constitutively expressed on interdigitating dendritic cells in the T zones of secondary lymphoid organs and in low quantities on Langerhans cells and peripheral blood dendritic cells [9, 10]. The overexpression of CD86 on monocytes was detected in peripheral blood of patients with ATD, and the expression of CD80, CD86, and CD40 was significantly increased on follicular B cells, but not on the marginal zone spleen B cells after the development of spontaneous autoimmune thyroiditis [7, 11]. Moreover, the association was found between polymorphisms of B7 molecules (CD86) and Graves' ophthalmopathy [12]. The aim of this study was to evaluate the possible role of TH, cytokines, and MC in the mechanisms of losing tolerance to thyroid gland components in response to induced thyrotoxicosis and hypothyroidism.

Methods

Clinical Study

This was a prospective, open, and observational study, based on parallel grouping principles. The observation period for each patient was 12 months. The inclusion criteria were as follows: patients with confirmed diagnosis of GD and nodular euthyroid goiter in compliance with international and federal guidelines of thyroid associations for the diagnosis and management of thyroid diseases. The patients with GD (52 patients) had stable thiamazol-induced euthyroidism prior to thyroidectomy, while 50 patients had nodular euthyroid goiter (control group). The mean age was 42.56 ± 1.9 years (range: 21–58 years). The study included 24 men (23.5%) and 78 women (76.5%). The exclusion criteria were as follows: patients with concurrent conditions, such as diabetes mellitus, exacerbation of chronic inflammatory diseases, oncological diseases, psychiatric disorders, alcoholism, smoking, pregnancy, or lactation. The study was performed in accordance with the requirements of the Helsinki Declaration of the World Medical Association titled "Ethical principles for medical research involving human subjects" (amended in 2000), and the Rules of Clinical Practice in the Russian Federation approved by the Resolution of the RF Ministry No. 266 (dated June 19, 2003). All patients independently and voluntarily provided written informed consent to participate in the study. GD was initially verified based on summarized data of clinical, immunological (TSH-receptor autoantibodies), hormonal (TSH, free triiodothyronine [FT3], and free thyroxine [FT4]), and ultrasound markers. The levels of TSH, FT3, FT4, TRAbs, and opposing cytokines (IL10, interferon γ [IFNγ]) in the blood serum were determined using the enzyme-linked immunosorbent assay (human diagnostic ELISA kits; R&D Systems Inc., Minneapolis, MN, USA). Patients received standard thionamide treatment (thiamazole at a dose of 15–40 mg daily). Clinically and laboratory-confirmed stable euthyroid state was a criterion for the adequacy of treatment (TSH: 0.25–3.2 mIU/mL; FT4 and FT3: within reference range). The patients with GD, including those with complicated thyrotoxicosis, received treatment with thionamides. Subsequently, thyroidectomy was performed after 6–12 months of treatment. The control group was matched in terms of age and sex with the GD group. The control group did not have clinical or laboratory symptoms of ATD. In all cases, the diagnosis of nodular euthyroid goiter was verified morphologically. Fine-needle aspiration biopsy was performed after ultrasound control. Morphological verification was performed according to the Bethesda classification. Vein punctures in a sterile environment of procedure rooms were performed to obtain blood samples (10 mL). The levels of TRAbs, opposing cytokines, TH, and TSH in the serum of patients from both groups were analyzed at the beginning of the study. Further measurements of these levels in patients with GD were performed after 3, 6, 9, and 12 months according to the standard therapy protocols. The present study was conducted between 2015 and 2018 at the facilities of Primorye Regional Clinical Hospital No. 1, Vladivostok, Russia; the Pacific State Medical University, Vladivostok, Russia; and the National Center of Marine Biology of the Russian Academy of Science, Vladivostok, Russia. Postsurgical materials, such as thyroid glands after thyroidectomy in patients with GD and perinodular thyroid tissues in cases of euthyroid goiter after subtotal thyroid resection, were investigated by performing histochemical and immunohistochemical (IHC) analyses.

Experimental Study

The experiments were conducted on 6-month-old outbred healthy female Wistar albino rats weighing 245 ± 15 g in accordance with basic methodological principles (comprehensiveness, integrity, objectivity, and reliability). The rats were divided into 4 groups (10 animals per group) and observed for 30 days. Experimental thyrotoxicosis was induced in group I through the administration of levothyroxine (sodium levothyroxine at a dose of 50 μg/kg per day of rat body mass) 1 h before the morning feeding (i.e., on empty stomach). Group II received the same dose of sodium levothyroxine in a similar manner and for the same period. In addition, on day 15 of the experiments, the rats received subcutaneous injections of recombinant veterinary interleukin-2 (IL2) isolated from *Saccharomyces cerevisiae* yeast cells according to the following schedule: 0.1 mL (0.05 mg; 5,000 IU) every 72 h (total: 5 injections during the course) [13]. Experimental hypothyroidism was induced in group III through the daily administration of thiamazole dissolved in drinking water at a dose of 5 mg per day for 30 days. Group IV was a control group maintained under the same conditions as the experimental groups. All groups had ad libitum access to water and received the same food. Blood serum samples from all animal groups were obtained through tail vein puncture prior to the initiation of the experiment and on day 30 to determine TH, cytokines, and TRAbs. Observation of animals included daily examination of the general condition, behavior, mobility, thermometry, and administration of the drug in the morning. The thyroid glands, adrenal glands, and hypophyses of experimental animals were collected for histochemical and IHC analyses.

The levels of TSH, FT3, FT4, TRAbs, and opposing cytokines (IL1β, tumor necrosis factor-α [TNF-α], IL10, and IFNγ) in the blood serum and thyroid gland supernatants of experimental animals were determined using the enzyme-linked immunosorbent assay (rat diagnostic ELISA kits; R&D Systems Inc., Minneapolis, MN, USA). The supernatants of the thyroid glands of animals were obtained in accordance with a patented technique (patent April 10, 2015, No. 216.013.38 BB) [14]. After thyroidectomy, suspensions of thyroid tissue obtained from the treated rats were prepared and homogenized using a mortar with the addition of deionized water (0.5 mL). The resulting suspension was centrifuged at 25°C for 20 min at 10,000 rpm and stored at −18°C. In the cytokine studies, the values were calculated based on 1 g of protein in the supernatant. For the morphological verification of the postsurgical material, the following procedures were performed: histochemical staining of thyroid gland specimens with toluidine blue and silver nitrate, and IHC with host-specific IHC markers, in particular tryptase for MC (monoclonal mouse anti-human Mast Cell Tryptase; Dako, Glostrup, Denmark) and the costimulating molecule CD86 (1:200, monoclonal Rabbit Anti-CD86 antibody [EP1158Y]; Abcam, Cambridge, MA, USA). IHC markers were applied on dewaxed and dehydrated sections of postsurgical material (i.e., thyroid glands of patients and thyroid glands of rats). Unmasking of antigenic determinants was performed in a microwave oven in 0.01 M citrate buffer. Immune staining was conducted using the streptavidin-biotin peroxidase method with diaminobenzidine used as chromogen. The materials for IHC were fixed for 4 h with 4% paraformaldehyde prepared with pH 7.5 phosphate-buffered saline (PBS), washed in phosphate-buffered saline, placed in Neg-50 medium (Thermo Scientific, Waltham, MA, USA), and prepared into 14- to 16-μm frozen sections. The sections were incubated with primary monoclonal antibodies against tryptase diluted 1:200 on PBS supplemented with 1% bovine serum albumin for 24 h and monoclonal Rabbit Anti-CD86 antibody (Abcam, Cambridge, MA, USA) diluted 1:200 on PBS supplemented with 1% bovine serum albumin for 24 h; they were marked with different fluorochromes on one frozen section. Secondary antibodies were anti-host specific and labeled with Alexa 488 (Molecular Probes, Eugene, OR, USA) and 546 (Molecular Probes, Eugene, OR, USA) both diluted 1:1,000 on PBS supplemented with 1% bovine serum albumin for 1.5 h. Nuclear DNA of thyrocytes was stained with 4′,6-diamidino-2-phenylindole (DAPI) according to manufacturer's instructions (Molecular Probes, Eugene, OR, USA). Negative control was performed. The material was analyzed with laser confocal scanning microscopy (LSM 780, Carl Zeiss & MT, Oberkochen, Germany). The captured images were examined using computer-assisted morphometric analysis (ImageJ, National Institutes of Health, USA). The MC index of degranulation [15] was determined. The ImageJ software was used to calculate the surface area of the item; all detected MC were recorded with this program. Degranulating MC were isolated and the index of degranulation was calculated using the following formula: quantity of degranulating MC divided by the sum of items (unaltered MC plus degranulating MC). The staining intensity was evaluated on a 4-point scale: no staining = 0 points; weak staining (+) = 1 point; moderate staining (++) = 2 points; intensive staining (+++) = 3 points. For the CD86 marker, the degree of expression was evaluated per 100 counted cells, with at least 1,000 cells in each case.

Transmission electron microscopy was used to study the ultrastructural aspects of immunocytes, thyrocytes, and MC. The specimens of thyroid glands were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), postfixed with 1% osmium tetroxide in cacodylate buffer, dehydrated in an ethanol series, and embedded in Epon-Araldite (Sigma-Aldrich Corp., St. Louis, MO, USA). Ultrathin sections were produced using a Leica EM UC6 ultramicrotome (Leica Mikrosysteme Vertrieb GmbH, Vienna, Austria), stained with uranyl acetate and lead citrate and examined under a Zeiss Libra 120 (Carl Zeiss & MT, Oberkochen, Germany) transmission electron microscope (magnification range: ×4,000 to ×20,000).

The experiments were performed in compliance with the requirements of the World Society for the Protection of Animals and European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Directive 86/609 EU of November 24, 1986) and in strict accordance with the European Convention (ETS No. 123, Strasbourg, March 18, 1986) on maintaining, feeding, treating, and sacrificing experimental animals. In addition, the experimental study was approved by the ethics board of the Pacific State Medical University (Protocol No. 3/13).

Statistical Analysis

Statistical analysis was conducted with parametric and nonparametric methods using the SPSS version 17.0.1 (SPSS Inc., Chicago, IL, USA) software (ROC analysis). A p value of <0.05 denoted statistical significance for all hypotheses. Descriptive statistics analysis of indicators was represented in absolute values and standard errors. Preliminary testing for the normality of value distribution for all indicators in all groups was performed with the Shapiro-Wilk test. Analysis of differences between 3 groups in the case of abnormal data distribution was based on the Mann-Whitney U test with Bonferroni correction for small groups. Average values

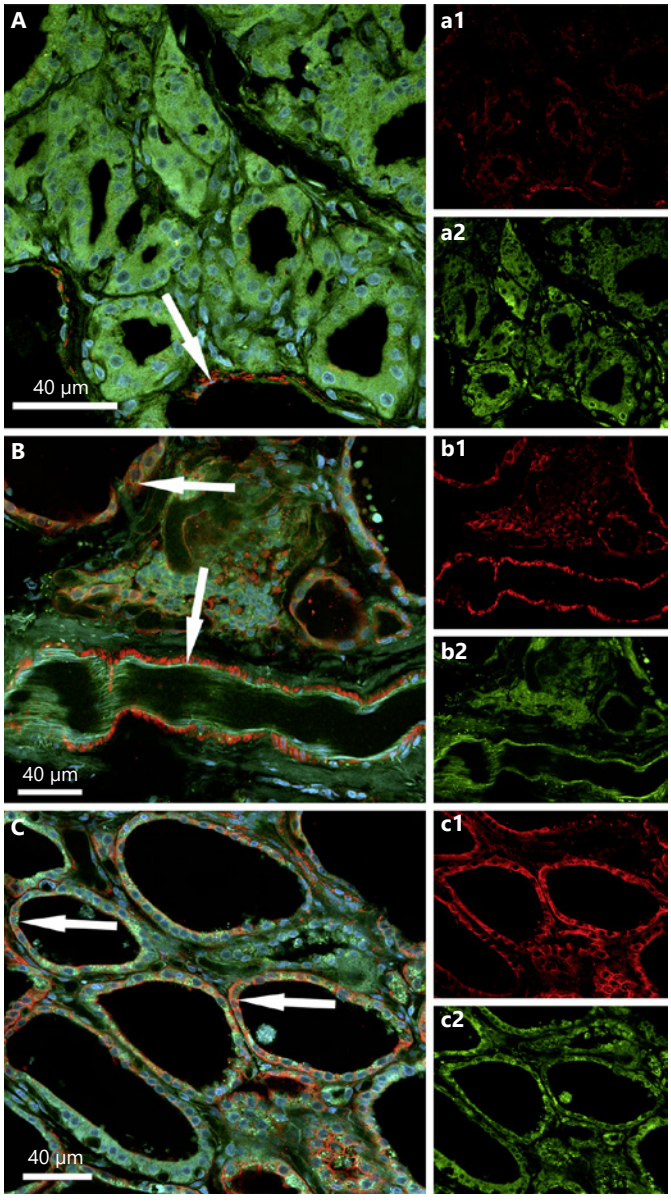

Fig. 1. Immunohistochemical analysis of thyroid in the case of Graves' disease ($n = 52$). **A** Normal thyroid tissue (postsurgical material, unchanged perinodular thyroid tissue; $n = 50$) – expression of CD86 is weak and paravasal predominantly. **B**, **C** Postsurgical material of the thyroid gland in Graves' disease; arrows point to evident overexpression of CD86 paravasally and intrafollicularly. Antibodies against CD86 – red immunofluorescence; antibodies to tryptase – green immunofluorescence; nuclear DNA is stained with 4′,6-diamidino-2-phenylindole – blue immunofluorescence.

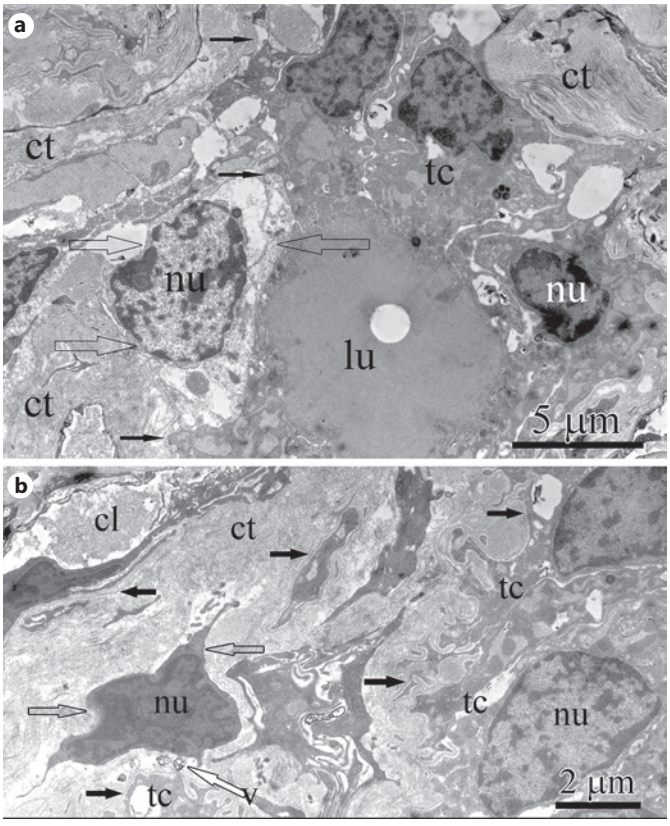

Fig. 2. Electron microscopy of the thyroid in Graves' disease, postsurgical material of the thyroid gland ($n = 10$). **a** Mast cells after degranulation (transparent arrows) are near the basal membrane (black arrows) surrounding a follicle. **b** Dendritic cells (transparent arrows) in connecting tissue of the thyroid gland. Basal membrane surrounds follicles and capillaries (black arrows) and separates them from connective tissue stroma unlike the dendritic cells located directly in the main substance of connecting tissue. The cytoplasm of dendritic cell processes contacting with thyroid cells contains peculiar microvesicles (a white arrow). cl, a capillary; ct, connecting tissue; lu, cavity of a follicle; nu, nucleus; tc, thyrocyte; v, microvesicles. 119 × 139 mm (300 × 300 DPI).

in small samples were compared using the nonparametric criterion of the Wilcoxon-Mann-Whitney test (W-criterion). Correlation analysis and the Pearson coefficient (r) were applied for the description of significant interrelations between values. Receiver operating characteristic analysis was employed to evaluate the quality of binary classification and area under the receiver operating characteristic curve, showing a qualitative interpretation of indicators in the groups.

Results

In the group of patients with autoimmune thyrotoxicosis (GD), significantly higher level of serum IFNγ was recorded compared to the group with nodal euthyroid goiter (72.98 ± 6.21 pg/mL vs. 12.10 ± 0.87 pg/mL, $p < 0.01$). In autoimmune thyrotoxicosis, a significant increase in serum IL10 level was detected compared with

Table 1. Values of thyroid hormones and TSH-receptor antibodies in blood serum of experimental animals and in the control group

Value	Values of thyroid hormones and antibodies in groups (Me; Q_{25}–Q_{75})			
	group I (n = 10)	group II (n = 10)	group III (n = 10)	control (n = 10)
Free T3, pmol/L	8.27[a] (7.05–9.34)	10.59[b] (8.24–11.30)	1.98[a] (1.24–2.09)	5.25 (3.60–6.90)
Free T4, pmol/L	52.43[a] (39.73–64.60)	62.70[a] (42.70–68.24)	8.04[a] (2.21–9.16)	15.73 (13.44–18.02)
TSH-receptor antibodies, mIU/mL	4.27[a] (3.33–7.12)	3.52 (2.80–3.71)	4.31 (3.21–6.10)	2.11 (2.26–3.31)

[a] Statistical significance of differences between values in experimental animals and in the control group ($p < 0.05$ by U-criterion of Mann-Whitney). [b] $p < 0.01$ by U-criterion of Mann-Whitney.

Table 2. Comparative analysis of cytokine levels in blood serum and supernatants of rat thyroid glands on the 30th day of experiment

Values	Values of cytokines in groups (Me; Q_{25}–Q_{75})			
	control group (n = 10)	group I with thyrotoxicosis (n = 10)	group II with thyrotoxicosis associated with IL-2 (n = 10)	group III with hypothyroidism (n = 10)
Serum, pg/mL				
IL-10	5.62 (2.68–9.24)	16.59 (11.80–28.08)[a]	34.23 (28.41–45.19)[b]	8.40 (2.70–27.20)
IL-1β	1.90 (0.02–1.98)	15.42 (5.80–38.20)[a]	3.90 (2.35–42.24)[a]	4.76 (4.10–4.09)[a]
TNF-α	0.28 (0.26–1.52)	0.32 (0.28–16.6)	0.29 (0.28–0.85)	13.72 (11.1–36.93)[b, c]
IFN-γ	5.96 (4.41–10.06)	19.58 (10.26–68.32)[b]	26.41 (10.14–47.15)[a]	16.30 (13.91–23.88)[a]
Supernatant, pg/100 μg of protein				
IL-10	25.07 (10.94–40.52)	8.19 (5.39–10.48)[a]	20.48 (2.74–35.61)	110.18 (82.39–165.23)[b, c]
IL-1β	17.31 (16.69–64.24)	19.26 (12.83–42.82)	21.36 (6.21–41.45)	516.08 (450.37–748.69)[b, c]
TNF-α	44.91 (6.10–58.09)	42.58 (11.98–126.45)	50.17 (22.56–121.72)	478.68 (430.63–591.48)[b, c]
IFN-γ	16.40 (14.87–29.21)	92.91 (29.84–133.13)[a]	112.89 (32.51–151.29)[a]	154.92 (106.44–591.48)[b]

[a] Statistical significance of differences between values in experimental animals and in the control group ($p < 0.05$ by U-criterion of Mann-Whitney). [b] $p < 0.01$ by U-criterion of Mann-Whitney. [c] Statistical significance of differences between groups with hypothyroidism and thyrotoxicosis ($p < 0.05$ by U-criterion of Mann-Whitney).

the group with nodular euthyroid goiter (131.94 ± 5.84 pg/mL vs. 11.06 ± 0.39 pg/mL, $p < 0.01$), and there was an imbalance of IFNγ/IL10 compared with nodular goiter. Balance IFN/IL10 was decreased ~2-fold in the group of patients with Graves' disease (0.55 vs. 1.09), compared with nodular euthyroid goiter. The average fluorescence intensity of expression of the CD86 marker in thyroid cells in patients with GD was increased 3 times (+++ vs. ±) than in control subjects with nodular euthyroid goiter ($p < 0.01$). Thyroid expression levels of CD86 were increased in patients with GD (78.05 ± 2.41% vs. 25.43 ± 0.85%, $p < 0.01$) compared with perinodular thyroid tissue. The strong overexpression of CD86 observed around thyroid epithelial cells is associated with the presence of intra- and perifollicular CD86-positive MC. MC were identified using their specific marker, tryptase (Fig. 1). IHC test and electron microscopy of thyroid tissues in patients with GD showed a similar perifollicular and interfollicular infiltration with MC and their active degranulation (Fig. 1, 2). In contrast, few inactive MC were found in the perinodular thyroid tissues of patients with nodular euthyroid goiter (Fig. 1).

Analysis of thyroid hormones in experimental animals showed significant difference versus control (Table 1). These groups demonstrated a tendency toward increase in TSH-receptor antibodies. However, a significant difference compared with the control group was found only in group I ($p < 0.05$; Mann-Whitney criterion: 21). Intake of TH per os significantly increased the levels of opposing cytokines (IL1β, IFNγ, and IL10) in the serum of experimental animals (Table 2). Blockade of TH synthesis with thiamazole in induced hypothyroidism also resulted in significantly increased serum levels of IL1β, IFNγ, and TNF-α (Table 2). Reliable deviations in the concentration

Table 3. Morphofunctional characteristics of MC in thyroid glands in control and experimental groups

Animal groups	Morphofunctional indicators	
	amount of MC in 1 mm^2	MC degranulation index
Control (n = 10)	2.75±0.31	3.14±0.02
Group I (n = 10)	12.56±1.20[a]	5.56±0.03[b]
Group II (n = 10)	5.75±0.21[b, c]	3.90±0.11[b, c]
Group III (n = 10)	7.32±0.27[b]	5.97±0.15[b]

[a] $p < 0.01$ by U-criterion of Mann-Whitney. [b] Statistical significance of differences between values in experimental animals and in the control group ($p < 0.05$ by U-criterion of Mann-Whitney). [c] Statistical significance of differences between groups with hypothyroidism and thyrotoxicosis ($p < 0.05$ by U-criterion of Mann-Whitney).

of IL1β ($p < 0.05$) were recorded between groups with hypothyroidism and thyrotoxicosis (Table 2). Median values of cytokines in group I was maximal and exceeded those of the control group by 8-fold. In hypothyroidism, this difference was 3-fold. In the groups of animals with thyrotoxicosis combined with injections of recombinant IL2, this difference was 2-fold. Strong inverse correlations were found between the values of IL1β and IL10 in the group with thyrotoxicosis ($r_I = -0.75$; $p < 0.001$); these correlations became direct after stimulation with IL2 ($r_{II} = 0.69$; $p < 0.001$).

In all experimental groups, significantly higher levels of serum and local IFNγ were recorded in comparison with the control group (Table 2). In thyrotoxicosis, both groups showed a significant increase in the levels of IL10 in the serum compared with the control group (increased by 3-fold and more after stimulation with IL2) and the group with hypothyroidism (Me I and II increased by 2-fold and 3-fold, respectively; $p < 0.01$). The MC degranulation index was significantly higher in groups I and III (Table 3). Direct moderate correlation was revealed between the levels of FT3 and IL10 ($r_s = 0.52$; $p < 0.05$). Strong and moderate direct correlations were found between IL10 and TRAbs in both groups with thyrotoxicosis ($r_I = 0.78$; $p < 0.001$; $r_{II} = 0.63$; $p < 0.05$).

Serum and local levels of TNF-α significantly exceeded those of the control group only in the group with hypothyroidism (Table 2). An inverse moderate correlation ($r_{III} = -0.57$; $p < 0.001$) was found between the concentration of TNF-α and the levels of TH (FT4), which may correspond to the local levels of this cytokine and hypothyroidism (Table 2). The group with recombinant IL2 showed direct moderate correlations between the levels of TNF-α and IL10 ($r_{II} = 0.56$; $p < 0.001$). Correlations between the levels of TRAbs and IFNγ ($r_{II} = -0.50$; $p < 0.01$) in group II and in the group with hypothyroidism ($r_{III} = -0.67$; $p < 0.01$) demonstrated a specific interaction between thyrocytes and immunocytes through TSH receptors and via cytokines (i.e., TNF-α and IL2). The levels of IL1β in group II were significantly decreased (Me 3.90 pg/mL; range: 2.35–42.24 pg/mL) in comparison with those observed in group I (Me 15.42 pg/mL; range: 5.80–38.20 pg/mL; $p < 0.01$). This finding is consistent with recent research data on the immune regulatory properties of IL2 aimed at the differentiation of regulatory T cells [16, 17]. The balance of IFNγ/IL10 in animal blood serum increased to 1.9 and 1.2 in the groups with hypothyroidism and thyrotoxicosis, respectively. In contrast, it decreased to 0.77 in the group with thyrotoxicosis associated with IL2, versus 1.06 in the control group (Fig. 3). Cytokine regulation was investigated at the local level in the thyroid supernatants of the animals (Table 2). There was a decrease in the local levels of IL10 in the thyroid supernatants of group I compared with the control group. Group I showed an increase in the levels of IFNγ and a significant imbalance of opposing cytokines IFNγ/IL10 versus the control group (11.35 vs. 0.66, respectively; $p < 0.01$). Administration of IL2 in rats with thyrotoxicosis resulted in a decrease in the imbalance of IFNγ/IL10, owing to the increased levels of IL10 to almost normal values (Table 2; Fig. 3). However, the levels of IFNγ were also higher in comparison with those observed in the control group. The most significant differences in cytokine concentrations at the local level were recorded in induced hypothyroidism: the levels of IL1β, IFNγ, and TNF-α were significantly different from those of the control group ($p < 0.01$). Notably, the levels of IL10 exceeded those of the control group by 4-fold. In hypothyroidism, these levels exceeded those of the corresponding markers in thyrotoxicosis: IL1β (by 27-fold), TNF-α (by 11-fold), and IL10 (by 5- to 13-fold). The local levels of IFNγ in induced hypothyroidism were maximal; the median value was 1.7-fold higher than that obtained in induced thyrotoxicosis (Table 2).

Comparison of the proportions of opposing cytokines (IFNγ/IL10) in blood serum samples and thyroid gland supernatants (Table 2; Fig. 3) suggests that higher concentrations of IFNγ were mostly associated with its excessive synthesis in the thyroid glands of the animals. In addition, IFNγ was significantly elevated in the blood serum of experimental animals (groups I, II, and III). However, it was markedly higher in the thyroid supernatants ob-

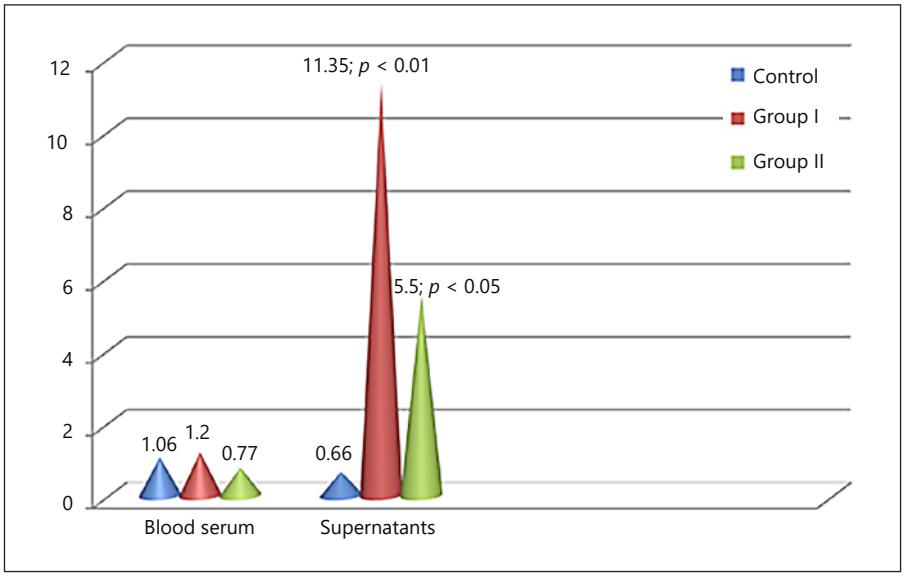

Fig. 3. Balance of opposing cytokines (IFNγ/IL10) in blood serum and in thyroid supernatants of rats with experimental thyrotoxicosis (group I) and thyrotoxicosis associated with interleukine-2 (group II) and in the control group.

tained from these groups. High levels of IL10 were mostly induced by hyperproduction of cytokines in immunocytes of peripheral blood. Of note, there was an increase in the concentration of IL10 primarily in blood serum samples of groups I and II. However, in hypothyroidism, the levels of IL10 in thyroid supernatants were significantly higher, and the marker was inversely correlated with the levels of TRAbs ($r_{III} = -0.55$; $p < 0.001$; Table 2). Groups I, II, and III showed significantly high levels of IL1β (Table 2) in the blood serum of animals. This finding may indicate a considerable activation of immunocytes in both cases of induced dysfunction of the thyroid. However, similar to the control group, groups I and II showed significantly lower levels of IL1β in the thyroid supernatants compared with the levels of cytokine in the thyroid supernatants of Group III (Table 2).

The ratio of opposing cytokines (IFNγ/IL10) was close to 1.0 at the systemic level in the control group and in thyrotoxicosis (group I). In thyrotoxicosis (groups I and II), this ratio (IFNγ/IL10) was drastically increased (9.5- to 11.0-fold) at the local level – it changed toward IFNγ in the thyroid supernatants. However, IL10 was slightly predominant at the thyroid level in healthy animals (Table 2). Administration of IL2 in rats with induced thyrotoxicosis lowered this balance (IFNγ/IL10) in blood serum to 0.77. In hypothyroidism (group III) at the local level, similar to blood serum, the proportion of IFNγ/IL10 showed a statistically significant increase; it changed toward T helper type 1 (Th1)-marker cytokines (Table 2). The content of TNF-α in the thyroid gland in experimental hypothyroidism demonstrated the most significant change (10-fold). This observation is important because TNF-α plays a leading role in the activation and proliferation of lymphocytes, increases the expression of adhesion molecules, and induces the activity of nitric oxide synthase phagocytes and proliferation of fibroblasts in combination with IFNγ [18].

Histochemical analysis revealed that proliferation of thyroid epithelia of animals in group I mostly occurred with active formation of microfollicules; MC were present in the stroma and perifollicular space (Fig. 4). Microfocal lymphoid infiltration of thyroid parenchyma was recorded in 2 cases of induced thyrotoxicosis. The thyroid glands of animals in group II exhibited medium and large follicular structures (Fig. 4, 5). The majority of samples contained differentiated MC grouped interfollicularly and perifollicularly in the state of active cytoplasm degranulation (partial and standard) and with formation of desmosomal bridges between MC and thyrocytes. Furthermore, 3 samples showed local lymphoid infiltration of the thyroid gland without formation of lymphoid follicles and light centers. Thyroid glands of rats with hypothyroidism (group III) contained multiple dissociated MC located interfollicularly and intrastromally. Follicle sizes were mostly increased due to the increasing volume and thickness of their colloid. The group with hypothyroidism showed prevalence of MC thyroid infiltration in the capsular zone, perivascularly and interfollicularly. In the capsular and perivascular zones, the MC exhibited polygonal and oval shapes, while the interfollicular MC

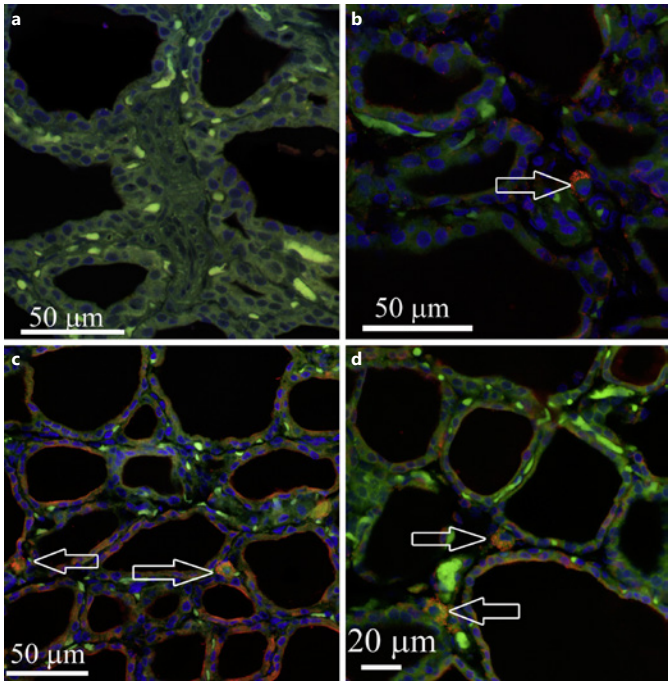

Fig. 4. Immunohistochemical analysis of rat thyroid tissue with monoclonal antibodies against CD86 (red immunofluorescence) and tryptase (green immunofluorescence). Absence of immune-positive cells in control (**a**; $n = 10$); immune-positive cells – overexpression of CD86 on mast cells in rat thyroid tissue with exogenous thyrotoxicosis (**b**; $n = 10$) and with thyrotoxicosis associated with interleukine-2 stimulation (**c**, **d**; $n = 10$). Nuclear DNA is stained with 4′,6-diamidino-2-phenylindole (blue immunofluorescence). Immune-positive cells are marked with arrows. Note: there is a green autofluorescence of erythrocytes in addition to specific immunofluorescence on sections of thyroid tissue. 129 × 129 mm (300 × 300 DPI).

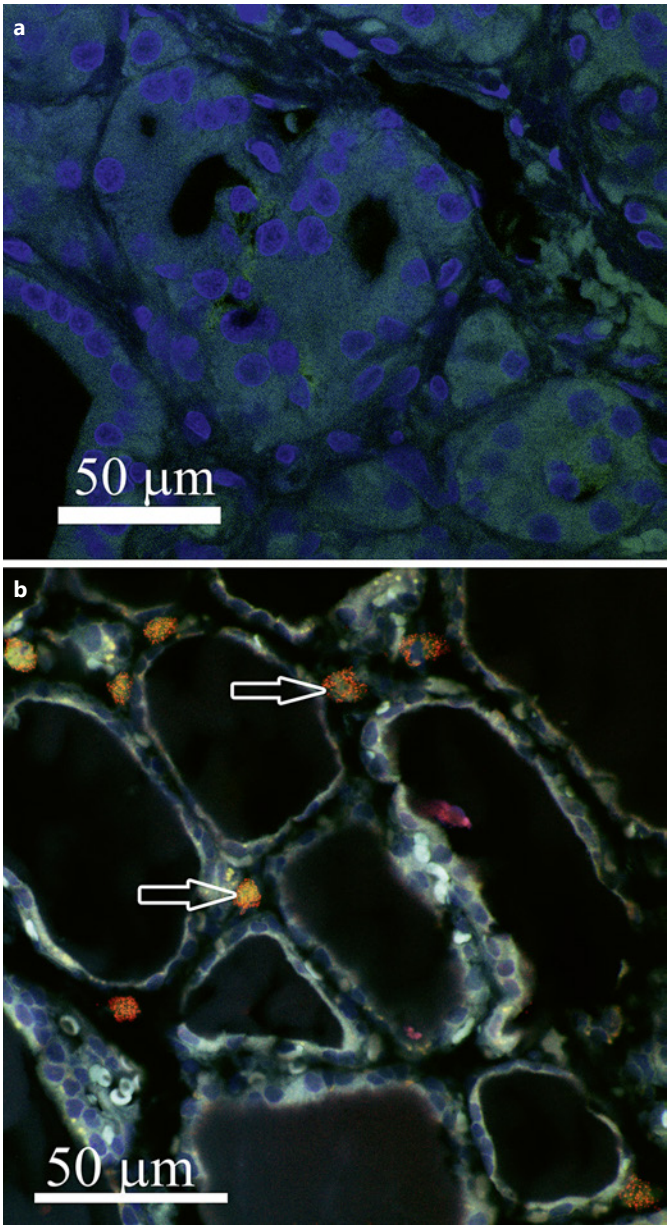

Fig. 5. Immunohistochemical staining of rat thyroid tissue in the case of thyrotoxicosis associated with interleukine-2 stimulation (**b**) with monoclonal antibodies against CD86 (red immunofluorescence) and tryptase (green fluorescence) reveals immune-positive cells – overexpression of CD86 on mast cells in the connective tissue of the thyroid (arrows). Nuclear DNA is stained with 4′,6-diamidino-2-phenylindole (blue immunofluorescence). Absence of immune-positive cells in control (**a**); the number of animals in each group was 10.

were mostly linear and fusiform. Experimental groups I and II showed interfollicular MC closely adjacent and degranulating among thyrocytes (Fig. 4, 5). The vast majority of thyrocyte nuclei did not show increased nucleolus organizer regions to indicate the lack of increased proliferative properties of A-cells. Local infiltration of follicle stroma MC was detected both with GD and with induced thyrotoxicosis (Fig. 1, 2, 4, 5). The highest degranulation index was observed in animals of groups I and III (Table 3). Group I showed local MC infiltration of the thyroid stroma and peripheral adipose tissue with partial MC degranulation, which was significantly changed after stimulation with IL2 in group II (Fig. 5). Animals in groups I and II showed local MC infiltration of the thyroid stroma and peripheral adipose tissue with partial MC degranulation that was also recorded in group III. Electron microscopy and confocal laser microscopy with antibodies against tryptase confirmed the presence of activated MC in thyroid tissues in GD and induced thyrotoxicosis. The activity of MC manifested itself in intercellular contacting with follicular epithelium and

cells of the stroma, especially in the focuses of the microfollicular proliferation of thyrocytes (Fig. 1, 2, 4, 5). In comparison with the control group (29.86 ± 1.25% vs. 74.92 ± 3.53%, $p < 0.01$) immunopositive MC with increased overexpression of CD86 were identified in the thyroid gland in exogenous thyrotoxicosis (group I) associated with the simultaneous use of monoclonal antibodies against CD86 and tryptase (Fig. 2–4). Overexpression of CD86 in group II after administration of IL2 compared with the control group was also recorded by us (82.72 ± 3.76% vs. 29.86 ± 1.25%, $p < 0.01$). Compared with the control (0 points), the average fluorescence intensity of CD86 expression in thyroid cells with induced thyrotoxicosis in group I was increased ~2.5-fold (++/+++; $p < 0.01$) and in group II with induced thyrotoxicosis and IL2 by 3 times (+++; $p < 0.01$).

Discussion

Multidirectional induced changes in the thyroid status resulted in a significant increase in the serum levels of IFNγ. This effect implies the presence of mediator immunocytes, such as innate immune cells with nonspecific responses (MC) and target cells (thyrocytes) with subsequent overproduction of IFNγ. This was confirmed by the significantly high levels of this cytokine in blood and at the local level of animals with experimental thyrotoxicosis and hypothyroidism. These findings indicated a leading role of this cytokine in the pathogenesis of ATD and conclusively shows a general vector of immune disorders, that is, the cellular Th1-type response of the immune system following multidirectional changes in the thyroid status [12]. The significant increase in the levels of IFNγ at the local level and increased proportion of IFNγ/IL10 in induced thyrotoxicosis and hypothyroidism (Table 2) confirms the important role of intrathyroid synthesis of IFNγ in the mechanisms of ATD development. Moreover, it shows the prevalence of a proinflammatory cellular type of immunological response in immunopathogenesis [18]. The influence of the increased levels of IL1 is potentially directed at thyroid epithelial cells. This change may cause chronic inflammation of the thyroid gland owing to specific stimulation of thyrocytes through Th1-cytokine-induced effects [18–20]. High levels of TNF-α can suppress the activity of regulatory T cells and activate apoptosis of thyrocytes by strengthening the aberrant expression of adhesion molecules and class II human leukocyte antigen on thyrocytes. By stimulating the synthesis of CXCL10 in thyrocytes, TNF-α can amplify the immune response and initiate autoimmune thyroiditis [19, 21]. Change in the balance of IL10/IL1β in the rat's blood serum and thyroid gland supernatants, as well as a proportion of cytokines after administration of IL2 in rats with thyrotoxicosis, confirms the hypothesis regarding the immune regulatory role of IL2 and its possible involvement in the pathogenesis of ATD. Comparative data concerning the levels and correlation of opposing cytokines in blood and the thyroid gland confirm the secondary changes in immunocyte activity following alterations in the content of TH in blood. These data are consistent with the recorded MC local intrafollicular infiltration, active degranulation of MC in the thyroid, and increased expression of the costimulatory molecule CD86 in GD (Fig. 4, 5).

The absence of follicular hyperplasia with reactive foci in lymphatic node tissues of rats with thyrotoxicosis shows the lack of stimulation of the B-cell population of lymphocytes. However, the local infiltration by MC of follicle stromata found in thyroid gland samples of induced thyrotoxicosis and GD (autoimmune thyrotoxicosis) can lead to the activation of T-cell lymphocytes. Our hypothesis regarding the regulatory role of MC in induced thyrotoxicosis and hypothyroidism is based on the fact that their degranulation was directly related to changes in the concentration of TH in animals with a subsequent increase in the synthesis of opposing cytokines. It is well established that, following integration into tissues, MC progenitor cells are differentiated in a microenvironment that defines their final phenotype [19]. In recent years, a molecular mechanism of MC secretion has been investigated. This mechanism does not involve damage to the integrity of the plasmalemma and emission of secretory granules. The mechanism of this "gradual degranulation" can also be triggered by bacterial factors that activate MC via the Toll-like receptor [22]. By means of degranulation or molecular interaction, MC generate poly-functional cytokines, histamine, and other mediators, thus affecting the immunocytes, thyrocytes, and APC [23, 24]. Therefore, activated MC can participate in a violation of immunological tolerance. One mechanism that can assist MC in activating the function of T cells is mediated by the secretion of opposing cytokines. This may explain the more than 5-fold increase in the levels of IFNγ in supernatants of animals versus the background of infiltration and active degranulation of MC in the thyroid gland of animals (Table 3; Fig. 4, 5). This fact was confirmed in our research: healthy animals with short-term exogenous thyrotoxicosis and hypothyroidism showed a significant increase both in the serum and/or local levels of opposing cytokines and in the ratio of Th1/Th 2 – marker cytokines in blood serum and in thyroid supernatants (Ta-

ble 2; Fig. 3). In addition to the identified costimulatory molecule CD86, the surface of MC contains major histocompatibility complex class II molecules [19, 24]. This offers an opportunity for MC to perform the functions of APC in the thyroid gland in the case of change in the levels of TH. Moreover, MC can be viewed as a future target for the pathogenetic therapy of ATD.

In thyrotoxicosis, the significant disproportion of the IFNγ/IL10 content in the thyroid gland was eliminated by administering IL2. However, the character of MC degranulation changed, indicating the involvement of MC in the inflammatory response in the thyroid glands. It is possible that MC also perform protective functions, since they are known to counteract the differentiation of regulatory T cells into the Th17 type, thus contributing to the suppression of autoimmune inflammation. In addition, there is evidence of MC involvement in the adaptive immune response by recruiting lymphocytes from regional lymph nodes [25]. By studying the role of MC in the pathogenesis of ATD, the dynamics of their functional activity during different thyroid statuses and conditions of follicular thyroid epithelium will shed new light on the pathogenesis and therapy of ATD.

Jung and co-authors [26] demonstrated that IL10 gene polymorphisms are associated with susceptibility to ATD. Data of Rodriguez-Munoz and co-authors [27] indicate that although CD69 NKG2D cells are significantly increased in patients with ATD, the poor suppressive function of these cells is not able to modify the clinical course and the inflammatory in this pathology. Moreover, these lymphocytes are able to downregulate the immune response, mainly through mediation of IL-10 and TGF-β [27]. It is highly likely that this increase in IL-10 production acts as a compensatory mechanism of the immune system, in an attempt to control the autoimmune process, as these lymphocytes exhibit weak immunosuppressive function. This may explain the imbalance of cytokines that we found in patients with GD.

We established that MC, as part of the regulatory system of physiological functions [24], are directly involved in the adaptation to changes of the thyroid status through active degranulation in the thyroid interfollicular space, synthesis of opposing cytokines, and expression of CD86 in the thyroid. The morphological data of the thyroid gland in induced thyrotoxicosis and GD, in terms of increased intrathyroid expression of CD86 by MC, prove the involvement of MC in antigen-presenting function in ATD. This may occur via the influence of MC on the process of dendritic cell maturation and functioning. Based on these findings and previous evidence, MC promote further proliferation of CD4+ cells, release of cytokines by T cells, and intensification of Th1- and Th17-responses [19, 25]. The hypothesis regarding the reception of TH by MC is additionally confirmed by the active degranulation and expression of CD86 in MC in induced thyrotoxicosis and hypothyroidism, as well as the recorded correlation between the levels of some cytokines and TH. A drastic increase in the ratio of opposing cytokines at the local level, aside from Th1-marker cytokines, in experimental thyrotoxicosis proves the direct involvement of TH in the mechanisms of system cellular response by influencing the balance of Th1-/Th2-marker cytokines. The administration of TH caused local infiltration of the thyroid gland by MC and active degranulation in MC was associated with a significant increase in proinflammatory cytokines at the local level. Therefore, TH may participate in the initiation of the systemic inflammatory response. In thyroid dysfunction, IL2 may play a regulatory role in maintaining the ratio of opposing cytokines and directing the vector of the immune response. This is attributed to significant differences in the balance of Th1-/Th2-marker cytokines in the blood serum of experimental animals with induced thyrotoxicosis associated with the administration of IL2 versus that observed in those with induced thyrotoxicosis and hypothyroidism.

Conclusions

The results of this experimental research demonstrated that TH are involved in the initiation of inflammatory responses in the thyroid gland. Specifically, the administration of TH or blockade of their synthesis in healthy animals resulted in infiltration of the thyroid gland MC, active interfollicular and intrafollicular MC degranulation, and significant changes in the synthesis of opposing IFNγ/IL10 cytokines.

Significant change in the balance of opposing cytokines with an increase of Th1-marker cytokines in the thyroid of rats with experimental thyrotoxicosis and hypothyroidism revealed the direct involvement of TH in the initiation of proinflammatory mechanisms of the systemic cellular response and contributes to the theory of hormone reception by immune system cells. Therefore, alterations in the levels of TH can trigger an inflammatory response in the thyroid gland due to disruption of the opposing cytokine balance under certain genetic or epigenetic conditions. This effect is followed by processing and presentation of APC autoantigens.

When affected by excessive levels of TH and IL2, MC can express the costimulating molecule CD86 in the thy-

roid. This observation confirms the involvement of MC in the loss of tolerance and autoantigen presentation. Administration of recombinant IL2 in cases of exogenous thyrotoxicosis enhances the synthesis of IL10 and IFNγ; however, it reduces the balance of IFNγ/IL10 at the systemic and organ levels, thus intensifying the Th2-response.

Significant systemic and intrathyroid hyperproduction of IFNγ observed in induced thyrotoxicosis and hypothyroidism indicates a possible determining influence of this cytokine on the intensification of antigen presentation in the thyroid by APC and, consequently, on the progression of the pathological process of ATD.

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Statement of Ethics

The experiments were performed in compliance with the requirements of the World Society for the Protection of Animals and European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Directive 86/609 EU of November 24, 1986) and in strict accordance with the European Convention (ETS No. 123, Strasbourg, March 18, 1986) on maintaining, feeding, treating, and sacrificing experimental animals. The clinical and experimental study was approved by the ethics board of the Pacific State Medical University (Protocol No. 3/13). The study was performed in accordance with the requirements of the Helsinki Declaration of the World Medical Association titled "Ethical principles for medical research involving human subjects" (amended in 2000), and the Rules of Clinical Practice in the Russian Federation approved by the Resolution of the RF Ministry No. 266 (dated June 19, 2003). All patients independently and voluntarily provided written informed consent to participate in the study.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

Zdor V.: designed and conducted the experiments, collected the data, analyzed and interpreted the data, and wrote the manuscript. Geltser B., Markelova E., and Karaulov A.: supervised and coordinated the study and reviewed the manuscript. Plekhova N., Eliseykina M., and Tikhonov Y.: conducted experiments, analyzed the data, and prepared the figures. All authors reviewed and approved the manuscript.

References

1 Weetman AP. The immunopathogenesis of chronic autoimmune thyroiditis one century after Hashimoto. Eur Thyroid J. 2013;1(4):243–50.
2 De Leo S, Lee SY, Braverman LE. Hyperthyroidism. Lancet. 2016;388(10047):906–18.
3 Brown MA, Hatfield JK. Mast cells are important modifiers of autoimmune disease: with so much evidence, why is there still controversy? Front Immunol. 2012;3:147.
4 Walker ME, Hatfield JK, Brown MA. New insights into the role of mast cells in autoimmunity: evidence for a common mechanism of action? Biochim Biophysica Acta-Molecular Basis Dis. 2012;1822(1):57–65.
5 Csaba G. Hormones in the immune system and their possible role. A critical review. Acta Microbiol Immunol Hung. 2014;61(3):241–60.
6 Wiersinga WM. Thyroid autoimmunity. Endocr Dev. 2014;26:139–57.
7 Watanabe A, Inoue N, Watanabe M, Yamamoto M, Ozaki H, Hidaka Y, et al. Increases of CD80 and CD86 expression on peripheral blood cells and their gene polymorphisms in autoimmune thyroid disease. Immunol Invest. 2020;49(1–2):191–203.
8 Salmaso C, Olive D, Pesce G, Bagnasco M. Costimulatory molecules and autoimmune thyroid diseases. Autoimmunity. 2002;35(3):159–67.
9 Dedecjus M, Stasiolek M, Brzezinski J, Selmaj K, Lewinski A. Thyroid hormones influence human dendritic cells' phenotype, function, and subsets distribution. Thyroid. 2011;21(5):533–40.
10 Simons PJ, Delemarre FG, Drexhage HA. A functional and phenotypic study on immune accessory cells isolated from the thyroids of Wistar and autoimmune-prone BB-DP rats. J Autoimmun. 2000;15(4):417–24.
11 Hong SH, Braley-Mullen H. Follicular B cells in thyroids of mice with spontaneous autoimmune thyroiditis contributes to disease pathogenesis and are targets of anti-CD20 antibody therapy. J Immunol. 2014;192(3):897–905.

12 Liao WL, Chen RH, Lin HJ, Liu YH, Chen WC, Tsai Y, et al. The association between polymorphisms of B7 molecules (CD80 and CD86) and Graves' ophthalmopathy in a Taiwanese population. Ophthalmology. 2011; 118(3):553–7.
13 Moiseyev A, Sakharova E, Egorova V, Romanova O. Ronkoleykin: experience of clinical application for small pets. SPb.: Alter ego. 2012;1:11–8.
14 Olifirova OS, Proklov NI, Talchenkova TE, Ilyushenok AS, Knalyan SV, Trynov NN. Method for the diagnosis of highly differentiated thyroid cancer. Patent No. 216.013.38 BB. 2015 Oct 4.
15 Yaglova NV, Yaglov VV. Ultrastructural characteristics of molecular release of secretory products from thyroid mast cells induced by lipopolysaccharide. Bull Exp Biol Med. 2013;155(2):260–3.
16 Eshaghkhani Y, Sanati MH, Nakhjavani M, Safari R, Khajavi A, Ataei M, et al. Disturbed Th1 and Th2 balance in patients with Graves' disease. Minerva Endocrinol. 2016;41(1):28–36.
17 Colin IM, Poncin S, Levêque P, Gallez B, Gérard AC. Differential regulation of the production of reactive oxygen species in Th1 cytokine-treated thyroid cells. Thyroid. 2014; 24(3):441–52.
18 Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. Autoimmun Rev. 2015;14(2): 174–80.
19 Xu Y, Chen G. Mast cell and autoimmune diseases. Mediators Inflamm. 2015;2015:246126.
20 Sun L, Zhang X, Dai F, Shen J, Ren C, Zuo C, et al. Elevated interleukin-1β in peripheral blood mononuclear cells contributes to the pathogenesis of autoimmune thyroid diseases, especially of Hashimoto thyroiditis. Endocr Res. 2016;41(3):185–92.
21 Sayed BA, Christy A, Quirion MR, Brown MA. The master switch: the role of mast cells in autoimmunity and tolerance. Ann Rev Immunology. 2008;26:705–39.
22 Ma YY, Yang MQ, Wang CF, Ding J, Li JY. Inhibiting mast cell degranulation by HO-1 affects dendritic cell maturation in vitro. Inflamm Res. 2014;63(7):527–37.
23 Piconese S, Gri G, Tripodo C, Musio S, Gorzanelli A, Frossi B, et al. Mast cells counteract regulatory T-cell suppression through interleukin-6 and OX40/OX40L axis toward Th17-cell differentiation. Blood. 2009;114(13): 2639–48.
24 Rao KN, Brown MA. Mast cells: multifaceted immune cells with diverse roles in health and disease. Ann N Y Acad Sci. 2008;1143:83–104.
25 Dudeck A, Suender CA, Kostka SL, von Stebut E, Maurer M. Mast cells promote Th1 and Th17 responses by modulating dendritic cell maturation and function. Eur J Immunol. 2011;41(7):1883–93.
26 Jung JH, Song GG, Kim J-H, Choi SJ. Association of interleukin 10 gene polymorphisms with autoimmune thyroid disease: meta-analysis. Scand J Immunol. 2016;84(5):272–7.
27 Rodriguez-Munoz A, Vitales-Noyola M, Ramos-Levi A, Serrano-Somavilla A, González-Amaro R, Marazuela M. Levels of regulatory T cells CD69(+)NKG2D(+)IL-10(+) are increased in patients with autoimmune thyroid disorders. J Endocrine. 2016;51(3):478–89.