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Biochemical and Immunological Markers of Autoimmune Thyroiditis

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Abstract—Correlations between biochemical and immunological markers of programmed cell death (apoptosis), and the functional state of the thyroid gland (hyperthyroidism, euthyroidism, hypothyroidism) have been investigated in autoimmune thyroiditis (AT) (also known as chronic autoimmune thyroiditis). Annexin V, TRAIL and TNFα, as well as DNA-hydrolyzing antibodies were used as the main markers. Increased levels of TRAIL were found in the serum of AT patients (hyperthyroidism > hypothyroidism > euthyroidism) compared with healthy individuals. The highest frequency of antibodies to denatured DNA (Abs-dDNA) had the highest frequency in AT patients (97%) compared with healthy controls. Among these patients, 75% had hyperthyroidism, 85% had hypothyroidism, and 84.7% had euthyroidism. Abs hydrolyzing activity demonstrated correlation dependence with symptoms of the thyroid dysfunction.

Keywords: apoptosis, autoantibodies, antibodies to DNA, abzymes, antibodies to thyroid tissue components, autoimmune thyroiditis

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INTRODUCTION

This study has been focused on the investigation of some biochemical and immunological markers associated with the problem of impaired process of programmed cell death, particularly apoptosis, as one of the main factors in the development of autoimmune diseases of the thyroid gland known as autoimmune thyroiditis (AT). This is one of the most common diseases of the thyroid gland, which has been used as a model to study the features of programmed cell death (apoptosis). This pathology is characterized by the formation of several variants that differ in the functional state of the thyroid gland (hypothyroidism, euthyroidism, hyperthyroidism) and its structure (atrophy, hypertrophy, nodulation). Studies performed in recent decades indicate the multifactorial nature of this disease [1], which is caused by complex interactions of genetic predisposition, trigger factors and endogenous mechanisms.

There is also evidence that in AT apoptosis of thyrocytes is induced by antibodies (Abs) [2] to the components of thyroid tissue, and also to autoantigens of apoptotic bodies [3]. T and B lymphocytes as well as signals stimulating thyroid-stimulating hormone receptors play a leading role in apoptosis of thyrocytes [2].

Some authors demonstrated that autoantibodies to DNA (Abs-DNA) are the markers of impaired immune function; they induce the development and progression of autoimmune diseases, which probably result in destruction of tissues and organs, by changing the physiological level of apoptosis [4, 5]. Differences in clinical and immunological picture of AT seem to be related to the phenomenon of Abs heterogeneity [4], demonstrating different levels of Abs specificity. However, to date, no a common viewpoint on the causes and nature of the immune changes that lead to loss of control over the formation of antibodies and the appearance of either abzymes with catalytic and cytotoxic effects or Abs-DNA devoid of these properties has been elaborated yet.

Interest in apoptosis is determined by the fact that the mechanism of its dysregulation under conditions of autoimmunity still need better understanding [6, 7].

The need of better understanding of the clinical importance of the autoimmune process in AT in integrated assessment of biochemical factors underlying impairments of autoimmunity, especially in regions with iodine deficiency, which include the Republic of Tatarstan, determine the relevance of our study.

In this context, it was important to characterize levels of specific (annexin V, TRAIL and TNFα) and non-specific (antibodies to native and denatured DNA, determination of their DNA-hydrolyzing activity) markers of apoptosis in AT patients with dif-

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ferent clinical and immunological picture (euthyroidism, hypothyroidism, and hyperthyroidism) and clinically healthy individuals.

MATERIALS AND METHODS

Serum samples of venous blood of 298 women aged 20 to 60 years were investigated in this study. A group of patients with diagnosed AT included 161 women, and a control group included 137 clinically healthy women without autoimmune pathology.

AT was diagnosed on the basis of results of ultrasound examination and evaluation of the functional state of the thyroid gland, the content of thyroid-stimulating hormone, free thyroxine and free triiodothyronine and titration of antibodies to thyroid peroxidase (Abs-TPO) and thyroglobulin (Abs-TG) in the blood serum. The degree of activity of the autoimmune process was assessed by the concentration of antibodies to TPO in serum.

An additional criterion for diagnosis included results of lymphocyte immunophenotyping (determined by expression of differentiation markers ($CD4^+$ $CD8^+$) on the surface of venous lymphocytes) flow cytometry on a flow FACSCalibur cytometer (BectonDickinson, USA) using the MultiSetTM software and a commercial kit MultitestIMKKit (BectonDickinson).

AT patients as well as healthy individuals included in the control group represented a mixed population of the Republic of Tatarstan were not connected in kinship. They all gave their informed consent to participate in the study.

Serum levels of annexin V, TNFα, and TRAIL were determined by the enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to manufacturer's instructions (Bender-MedSystem, Austria). Product registration was performed using an Awareness technology INC stat fax-2100 spectrophotometer (USA) at 450 nm and expressed as optical density units (OD_{450}).

Antibodies to native DNA (Abs-nDNA) and denatured DNA (Abs-dDNA) were determined by ELISA using genomic DNA (Human Genomic DNA, Promega, USA) [8]. Product registration was performed using the same spectrophotometer Awareness technology INC stat fax-2100 at 450 nm and expressed as optical density units (OD_{450}).

DNase activity of Abs-DNA was determined in vitro, using a commercial DNA preparation of pBR322 plasmid DNA of the *E. coli* XL-1Blue strain (SibEnzyme, Russia) as a substrate. The hydrolysis reaction was carried out at 37°C and samples were taken after certain time intervals. DNA-hydrolyzing activity of antibody preparations was assayed by monitoring the conversion of supercoiled plasmid DNA (scDNA) in the relaxed circular or linear form; the results were visualized using agarose gel electrophoresis followed by visualization with ethidium bromide using the Gel ChemiDoctmXRS documenting system (Bio-Rad Laboratories, USA).

The statistical analysis included data analysis of variance, using the Mann-Whitney and Kruskal-Wallis test with Bonferroni correction. The relationship between the parameters was assessed by the Spearman correlation coefficient [9]. The statistical analysis was performed using the Excel Office 2003 software package.

RESULTS

One of the integrative criteria of thyroid gland pathology, in particular, AT, is the detection of specific antithyroid Abs such as antithyroglobulin Abs (Abs-TG) and Abs-TPO [10]. In our study, high titers of Abs-TG and Abs-TPO (462.2 Me (2.5P 1.45; 97.5P 3150) and 382.6 Me (2.5P 1; 97.5P 1050) IU/mL, respectively) were found in all AT patients. An additional criterion in characterizing AT data included immunophenotyping of lymphocytes (definition on the surface of T-lymphocytes differentiation markers ($CD4^+/CD8^+$)). Results of lymphocyte immunophenotyping (determination of the differentiation markers ($CD4^+/CD8^+$) on the surface of T-lymphocytes) were also used as an addition characteristics of AT.

The analysis showed a significant increase in the proportion of subpopulations of CD4 + T-lymphpocytes in AT patients and a significant decrease in the proportion of subpopulation of T-suppressors compared with healthy individuals ($p < 0.05$). The average ratio of T-lymphpocytes $CD4^+$ $CD8^+$ in patients was twofold higher (Me 2.05; CI 1.22–0.89) than in healthy subjects (Me 0.99; CI 0.009–0.01) (Fig. 1).

Some authors [4, 5] demonstrated the presence of DNA-binding antibodies exhibiting DNAse activity in serum of patients with autoimmune thyroid diseases. In this context, we analyzed the levels of anti-DNA antibodies (nDNA and dDNA) in AT patients and in healthy subjects of the the control group (Table 1); we also investigated Abs associated DNA-hydrolyzing activity in dependence of the functional state of the thyroid gland.

It was found, that in 97% of AT patients Abs-dDNA was higher than in control ($p = 0.001$; 95% CI 0.34–0.41). These included hyperthyroidism (75%), euthyroidism (84.7%), and hypothyroidism (85%). Analysis of the whole set of AT patients revealed a trend to the increase in Abs-nDNA, which, however, did not reach the level of statistical significance.

The study of DNA-hydrolyzing activity of antibodies usually raises a reasonable question, whether the catalytic properties may be actually attributed to the antibodies [4]. It is known that the activity of serum DNase is thermolabile and is inactivated by heating to 56°C [11]. Thus, to exclude the possible influence of serum DNases on the hydrolysis of plasmid DNA,

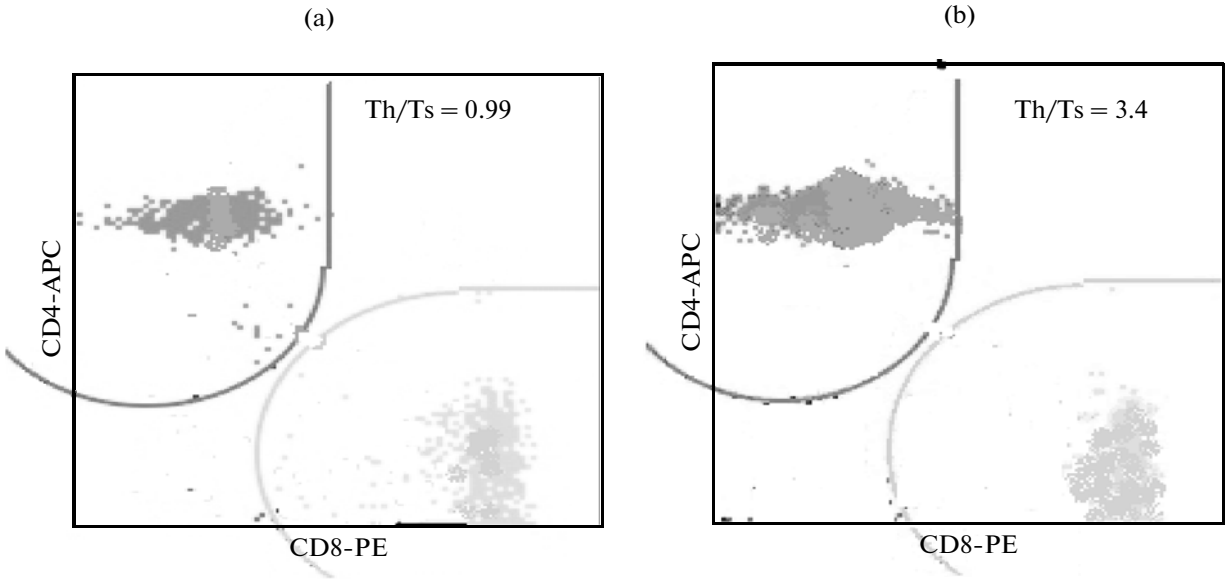

Fig. 1. Characteristics of circulating T-lymphocytes in blood of healthy individuals and patients with autoimmune thyroiditis. Immunophenotyping of T-lymphocytes was performed by the content of T-helpers (Th) ($CD4^+$ –APC) and T-suppressors (Ts) ($CD8^+$ – PE) in the blood in healthy persons (A), and AT patients (B). APC and PE are types of monoclonal antibody dyes conjugated with a fluorescent label to identify and membrane and cytoplasmic antigens of the cell.

serum samples from healthy individuals and AT patients were pre-heated at + 56°C for 40 min. After heating the serum samples of healthy subjects lost their ability to convert the substrate – scDNA pBR322 (form I) in a circular (form II) and linear (form III) forms.

Figure 2 shows existence of Abs characterized by different specificity to the DNA substrate (pBR322) and DNA-hydrolyzing activity in dependence of the functional state of the thyroid gland.

The main pool of serum Abs of AT patients with hyperthyroidism includes Abs-DNA with DNA-hydrolyzing activity specific to a single DNA break in the supercoiled pBR322 molecule. Accumulation of the relaxed circular form of pBR322 DNA occurred during 24 h of the substrate hydrolysis. Resultant relaxed circular DNA molecules (form II) were more resistant to Abs DNA hydrolyzing activity, because adding of excess of serum Abs did not result in complete transition of the relaxed circular form in the linear form (form III).

In AT patients with hypothyroidism the pool of circulating serum Abs includes Abs that possess higher hydrolytic activity, because after 2 h 50% of scDNA was hydrolyzed to the relaxed circular form, whereas in AT patients with hyperthyroidism hydrolysis of 50% scDNA occurred only after 7 h of incubation.

After 12 h incubation we observed appearance of hydrolysis products in the form of linear DNA (form III) accompanied by reduction in the amount of relaxed circular DNA.

The pool of Abs circulating in blood of euthyroid AT patients is the most interesting one. The densitogram of Fig. 2b demonstrates formation of three groups of products of pBR322 hydrolysis: a relaxed circular DNA (form II), a linear DNA (form III), and discrete DNA fragments, exhibiting time-dependent decrease during incubation (Fig. 2b, see 12 h and 24 h). This suggests that various Abs characterized by

Table 1. Levels of circulating antibodies to nDNA (Abs-nDNA) and dDNA (Abs-dDNA) in patients with autoimmune thyroiditis and in the control group

@Выборка (*n*)	Abs-nDNA, OD units		Abs-dDNA, OD units	
	M ± SD	95% CI	M ± SD	95% CI
Patients (161)	0.89 ± 0.25	0.2–0.5	**0.94 ± 0.33[1]**	0.34–0.41
Healthy controls (137)	0.84 ± 0.18	0.16–0.48	0.79 ± 0.19	0.23–0.34

Here and in Table 2 data represent mean (M) ± standard deviation (SD). 1 – Statistical significance of differences ($p < 0.05$) in comparison with the control group.

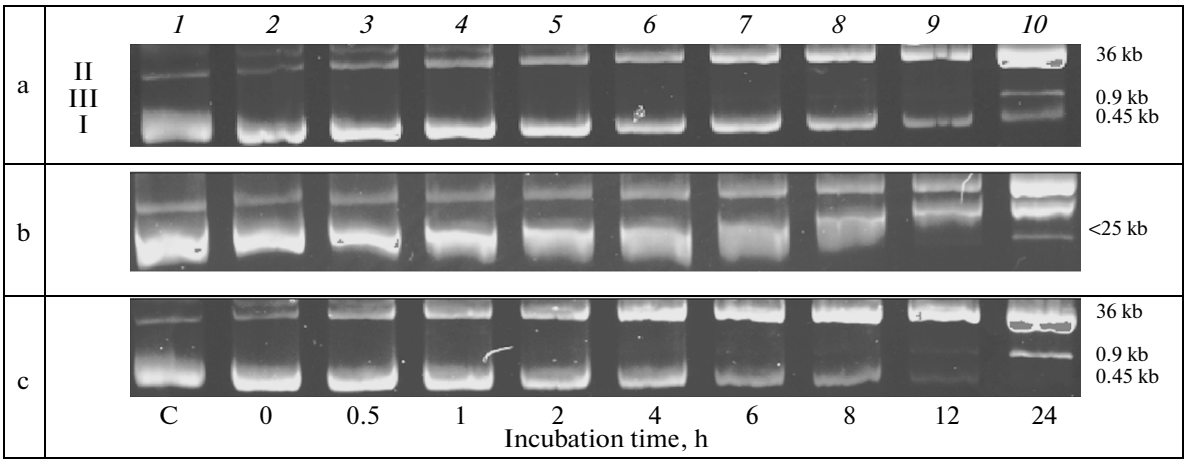

Fig. 2. Characteristics of DNA-hydrolyzing ability of circulating antibodies in serum of patients with autoimmune thyroiditis in dependence of the physiological state of the thyroid gland (A – electrophoretogram, B – densitogram).

A – : 1 – control (substrate pBR322 + hydrolyzing TE buffer (0.025 M Tris-HCl buffer, pH = 7.5)), 2–10 – substrate pBR322 + serum of AT patients (a – with hyperthyroidism, b – with euthyroidism, c – with hypothyroidism) (I-, II-, III- forms of pBR322 DNA, respectively), the parameters of electrophoresis – 0.8% agarose gel, working voltage of 80V; B –Densitogram demonstrating degree of hydrolysis of pBR322 DNA by circulating antibodies in serum of a AT patient (▲ – supercoiled, ● – circular, ■ – linear, ◆ – short oligonucleotides). The results of a typical experiment are shown.

different ability to hydrolyze various plasmid DNA in certain sites do exist in the serum. It is reasonable to suggest existence of two pools of Abs: one pool is specific to single-strand breaks at the site DNA superhelical tension and the other one includes Abs with DNA-hydrolyzing activity specifically cleaving double-stranded scDNA.

It should be noted that DNA hydrolyzing activity of Abs specific to single strand DNA was lower in serum of AT patients with euthyroidism than that in serum of AT patients with hyperthyroidism or hypothyroidism, because the rate of accumulation of hydrolysis products of relaxed circular DNA was lower. It is possible that Abs hydrolyzing DNA in AT patients with euthyroidism do not act due to their low activity compared with Abs in AT patients with hypothyroidism [12, 13].

On the other hand, electrophoretically scDNA was completely hydrolyzed after 8 h of the DNA hydrolyzing reaction and this may account for appearance of discrete liner DNA fragments.

Thus, changes in the degree of the hydrolytic activity of Abs-DNA and their substrate specificity in AT patients may reflect the dynamics and the severity of the autoimmune process.

Correlation analysis in the total group of AT patients revealed a direct correlation between the level of Abs-TG and Abs-nDNA ($Rs = 0.38$, $p < 0.05$), and an inverse correlation between the level of Abs-TPO and Aba-nDNA ($Rs = 0.2$, $p < 0.05$), whereas no correlation was found between the levels of Abs-dDNA and Aba-TG, as well as between Abs-nDNA and Abs-TPO antibodies.

Correlation analysis in groups of AT patients subdivided by their functional state of the thyroid gland (hypothyroidism, euthyroidism, and hyperthyroidism) revealed different correlations between specific and non-specific Abs. In hyperthyroidism a direct high correlation was observed between the levels Abs-DNA (both Abs-nDNA and Abs-dDNA), and the level of Abs-TG and Abs-TPO. In hypothyroidism a moderate direct correlation was observed between the Abs-nDNA and Abs-TG, and the inverse correlation between antibodies to Abs-dDNA and Abs-TG, while in euthyroidism inverse correlations between Abs-nDNA and Abs-dDNA as well as between Abs-nDNA and Abs-TPO increased.

The physiological state of the thyroid gland is associated with the process of apoptosis. Biochemical changes during apoptosis include phosphatidylserine (PS) translocation from the inner side to the outer side of the plasma membrane. Binding to PS on the cell surface, annexin V, conjugated with a fluorochrome also serves as a marker of apoptosis [14]. We did not find significant differences in the content of annexin V in serum of AT patients with different functional state of the thyroid gland and the control group (Table 2).

We also found a significant increase in the serum level of TRAIL in AT patients with hyperthyroidism as compared to healthy people. There was a trend to the decrease of the serum TRAIL in AT, depending on the functional state of the thyroid gland: hyperthyroidism > hypothyroidism > euthyroidism, and individual variations of the TRAIL level in AT patients with hypothyroidism and euthyroidism.

Correlation analysis between the levels of TRAIL and annexin V in the total set of AT patients revealed a

Table 2. Levels of apoptosis markers in the serum of patients with autoimmune thyroiditis, depending on the functional status of the thyroid gland

AT	M ± SD		
	Annexin V, pg/mL	TNFα, pg/mL	TRAIL, ng/mL
Hyperthyroidism	0.258 ± 0.0012	6.8 ± 1.02	**60.2 ± 20.07**[1]
hypothyroidism	0.257 ± 0.0014	2 ± 13.18	45.5 ± 19.3
Euthyroidism	0.257 ± 0.0014	3.05± 18.44	41.6 ± 14.7
Control	0.258 ± 0.001	1.89 ± 16.48	39.38 ± 11.6

direct weak correlation (Rs = 0.07; ($p < 0.05$) between these parameters. We found a tendency to increase of the serum TNFα content in AT patients (which, however, did not reach the level of statistical significance, $p > 0.05$). The maximum increase in the serum TNFα level was observed in AT patients with hyperthyroidism. In addition, high individual variations of the TNFα levels were observed in patients with hypothyroidism (95% CI 0.99–5.61) and euthyroidism (95% CI: 2.59–5.9).

DISCUSSION

According to modern concepts, AT is considered as a disease determined by a genetic defect in T-suppressors that results in the T-suppressor interaction with B-lymphocytes, which induce the synthesis of autoantibodies (Abs-TG and Abs-TPO) [15]. According Weetman et al., a deficit of suppressor T-lymphpocytes ($CD8^+$) and activation of T-helpers ($CD4^+$) [16] are the the central pathogenic link in the development of autoimmune endocrine diseases.

According to our data, AT patients have an imbalance in $CD4^+/CD8^+$ lymphocytes; this suggests that impairments in T-cell immunity play an important role in the pathogenesis of this disease. This can be expressed as hyperactivation of the immune system, in particular through changes in the level of specific and non-specific Abs (found in serum of AT patients), this is supported by the evidence that apoptosis of thyrocytes may be induced by Abs to thyroid tissue components, and also by Abs to apoptotic cell components. Thus, Abs-DNA are used as a sensitive but nonspecific test for AT screening [17, 18].

Functional impairments of the thyroid gland in AT may include hyperthyroidism, euthyroidism, and hypothyroidism. Hyperthyroidism is determined by thyrocyte destruction followed by release of excessive amounts of peripheral thyroid hormones. Subsequently, hyperthyroidism is replaced by a period of euthyroidism, and then hypothyroidism gradually develops [19].

Analyzing levels of nonspecific Abs in dependence of the functional state of the thyroid gland in AT patients, we found a tendency to the increase of the DNA-binding antibodies specific for nDNA ($p > 0.05$) and a significant increase in Abs-dDNA compared with the control group ($p < 0.05$). The prevalence of Abs-dDNA was found in groups of patients with euthyroidism and hypothyroidism (84.7% and 85%, respectively) and in 75% of patients with hyperthyroidism.

There are limited data on the mutual influence of organ specific (Abs-TG, Abs-TPO) and non-specific (Abs-DNA) antibodies and their complex diagnostic significance, especially at different clinical variants of AT. Analysis in the total group of patients with AT revealed a direct correlation between the level of Abs-TG and Abs-nDNA, and the inverse correlation between the level of Abs-TPO and Abs-nDNA; no correlation dependence was found between the levels of Abs-dDNA and specific antithyroid antibodies.

Thus, the data on the correlation between the levels of Abs specific to thyroid tissue and non-specific Abs-DNA, are very interesting, as they both are not only parameters of activity, but also the generalization of autoimmune diseases.

Previously, Pedro et al., studied the Abs-DNA in Graves' disease (GD) and AT in the stage of euthyroidism; they found Abs-DNA in 50% of euthyroid AT patients with and in 82% of GD patients. The frequency of Abs-dDNA was 90% of the total set (AT and GD), in which AT patients represented 75% [20]. The results of our studies of serum of AT patients also demonstrated the prevalence of Abs-dDNA.

It is suggested that during autoimmunization to the genomic DNA, Abs-dDNA appear first in the serum pool of Abs; later accumulation of somatic mutations in Ab-producing B-lymphocyte results in maturation of Ab specificity from anti-dDNA to anti-nDNA [21, 22]. Consequently, using determination of Abs-dDNA it is possible to reveal appearance of the disease at earlier stages [22]. It is believed that Abs-DNA, exhibiting catalytic hydrolyzing properties can act as a powerful regulator of apoptosis, which rate is multiplied in AT [4, 5].

Screening of serum samples for the presence of Abs with DNA hydrolyzing activity in AT patients (Fig. 2) revealed heterogeneity of substrate specificity of circulating Abs-DNA, in dependence of the physiological condition of the thyroid activity and the level circulat-

ing Abs. In other words, the hydrolytic activity can affect the functional state of the thyroid gland in the course of the disease and also apoptosis of thyrocytes.

Biologically active substances, primarily cytokines (Fas, TRAIL, TNFα) attract much interest in the context of regulation of apoptosis [6]. TRAIL mediates apoptosis of both thyrocytes and autoreactive cytotoxic lymphocytes [23]. TNFα is a homologue of FasL, and this suggests its high apoptotic activity [24]. In this regard, we have evaluated the process of apoptosis on the basis of the quantitative analysis of three specific markers: annexin V, TNFα, and TRAIL. Annexin V as well as other annexins is not released from normal cells; destructed cells are the source of extracellular annexin V. One of the early signs of apoptosis is the exposure of PS on the cell membrane. This process is an integral part of apoptosis regardless of the type of cells and the trigger of the activation of the cell death program. The literature data on the induction of apoptosis are controversial. Previously no differences were found in the induction of apoptosis in lymphocytes of euthyroid AT patients and healthy individuals, whereas a significant decrease in the induction of apoptosis was found in hypothyroid and hyperthyroid AT patients (4 times and 2 times, respectively) [25].

Some authors found increased number of thyrocytes undergoing apoptosis in the thyroid gland of AT patients [26]. We did not find any significant differences in the content of annexin V in the serum of AT patients with different functional state of the thyroid gland and the control group of healthy individuals. This suggests normal (from a physiological viewpoint) duration of apoptosis in AT.

Death receptors (DR) are the key molecules involved in signal transduction of death ligands inside cells. According to the model of receptor-mediated apoptotic thyroid gland cell death in AT, TRAIL (TNF-related apoptosis-inducing ligand) induces apoptosis through DR-4, DR-5. Mature T cells acquire sensitivity to TRAIL-dependent apoptosis after exposure to IL-2; this indicates that TRAIL plays a certain role in the control of immune responses. Furthermore, it was shown that in patients with some autoimmune pathologies cells demonstrate increased sensitivity to this type of apoptosis [27].

Results of our study demonstrate a significant increase in the level of TRAIL in the serum of AT patients with hyperthyroidism compared with healthy individuals. We found a tendency of reduction of the serum TRAIL in AT patients in dependence of the functional state of the thyroid gland: hyperthyroidism > hypothyroidism > euthyroidism.

Several authors reported about increased concentrations of serum TRAIL and TNFα as a marker necrobiotic states [28, 29]. Necrosis is always caused by rough pathology. Cell death during necrosis is associated with impairments in the cell membrane or cytoplasm and it does not affect significantly the cell nucleus. The reason is the attack of the cell by Abs and complement; death of target cells induced by the mature $CD8^+$ T-cells.

The above shown data on the deficit of suppressor T-lymphocytes ($CD8^+$) are consistent with indications by Weetman [30] that this deficit is the central pathogenetic link in the development of autoimmune endocrine diseases during activation of T-helper cells ($CD4^+$) observed in the total set of AT patients. This suggests that the ratio of apoptosis/necrosis determines the functional state of the thyroid gland in AT (hyperthyroidism > hypothyroidism > euthyroidism). The highest levels of TRAIL, found in AT patients with hyperthyroidism may reflect predominance of necrotic processes over apoptotic mechanisms in this group of patients. On the other hand, a lower level TRAIL in serum in patients with hypothyroidism and euthyroidism may reflect intensity of DR-5 formation, which actively binds TRAIL and therefore reduces its serum concentrations. This results in induction of TRAIL-mediated apoptosis and death of thyrocytes.

TNFα may regulate both apoptosis [6] and necrosis, by triggering specific intracellular signaling pathways [31].

We found a tendency to the increase of the serum TNFα content in AT patients with hyperthyroidism (Table 1). Increased concentrations of TNFα, along with increased serum TRAIL content are considered by many investigators as a marker necrobiotic states. Our data are consistent with the work by Kotovich [28]. Thus, the level of this cytokine reflects the dependence of the functional state of the thyroid gland on the nature of the pathological process (apoptosis or necrosis) in the thyroid tissue.

CONCLUSIONS

Our study has shown that AT is accompanied by TRAIL induced apoptosis. Detection of the maximal increase of TRAIL in AT patients with hyperthyroidism may reflect the predominance of necrotic processes over apoptotic mechanisms. These results are consistent with the data obtained by many researchers that indicate the destructive nature of hyperthyroidism during AT [15, 32].

It is possible that the simultaneous increase of the specific and nonspecific Abs seen in hyperthyroidism demonstrates high activity of the autoimmune process with predominance of necrotic processes over apoptotic, which in turn may cause damage to thyroid cells and amplify the autoimmune responses. The lack of correlation between all Ab parameters observed in two other clinical manifestations of AT suggests transition of remission and normalization of programmed cell death processes towards apoptosis of various intensities (e.g., lack of correlation between Abs-TG and

Abs-DNA in euthyroidism). Pedro et al. [20] also demonstrated the lack of correlation between Abs-nDNA and Abs-TG in euthyroid patients. The hydrolyzing activity of Abs demonstrated correlation with the severity of the clinical manifestation of AT, as well as with symptoms of dysfunction and destruction of the thyroid gland [33], which was also detected in our study.

However, this issue requires further detailed study.

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REFERENCES

1. Davies, T.F., *J. Clin. Endocrinol. Metab.*, 1998, vol. 83, pp. 3391–3393.
2. Morshed, S.A., Latif, R., and Davies, T.F., *Immunol. Res.*, 2012, vol. 54, nos. 1–3, pp. 191–203.
3. Lorenz, H.M., Herrmann, M., Winkler, T., Gaipl, U., and Kalden, J.R., *Apoptosis*, 2000, vol. 5, no. 5, pp. 443–449.
4. Nevinsky, G.A., Kanyshkova, T.G., and Buneva, V.N., *Biochemistry* (Moscow), vol. 65, pp. 1473-1478
5. Suchkov, S.V., Gabibov, A.G., Alekberova, Z.S., and Gnuchev, N.V., *Ter. Arkhiv*, 2001, no. 10, pp. 58–65.
6. Popov, L.S. and Korochkin, L.I., *Genetika*, 2004, no. 2, pp. 149–166.
7. Nedosekova, Yu.V., Urazova, O.I., Kravetz, E.B., and Tchaikovskii, A.V., *Byul. Sibirskoi Meditsiny*, vol. 1, pp. 64–71.
8. Sattarova, L.I., Gafiatullina, D.G., Agiullina, L.A., and Winter, V.G., *Biotekhnologiya*, nos. 11–12, pp. 38–41.
9. Lakin, G.F., *Biometriya* (Biometry), Moscow: Vysshaya Shkola, 1990.
10. Dedov, I.I., *Klin. Tireoidol.*, 2003, vol. 1, no. 1, p. 24.
11. Baranovskii, A.G., Matyushin, V.G., Vlassov, A.V., Zabara,V.G., Naumov ,V.A.,Giege R., Buneva, V.N., and Nevinsky, G.A., *Biochemistry* (Moscow) 1997, vol. 62, no. 12, pp. 1590–1599.
12. Bubnov, N.V., Basnak'yan, A.G., and Votrin, I.I., *Biochemistry* (Moscow) 1986, vol. 51, no. 7, pp. 1194–1202.
13. Khodarev, N.N., Morozov, A.Yu., and Sokolova, I.A., *Mol. Genet. Mikrobiol. Virusol.,* 1988, vol. 10, no. 9, pp. 26–32.
14. Gerke, V. and Moss, S.E., *Physiol. Rev.*, 2002, vol. 82, no. 2, pp. 331–371.
15. Volpe, R., in *Autoimmune thyroiditis*, Burrow, G.N. and Oppenheimer, J.H., Eds., Philadelphia: W.B. Sounders Company,1989, pp. 191–207.
16. Weetman, A.P., *Clin. Endocrinol. (Oxf.)*, 1992, vol. 36, no. 4, pp. 307–323.
17. Abramova, N.A., Fadeev, V.V., Gerasimov, G.A., and Mel'nichenko, G.A., *Klin. Eksper. Tireoidol.*, 2006, vol. 2, no. 1, pp. 21–32.
18. Naumova, T.E., Ogneva, E.A., Ageev, V.A., Alekberova, Z.S., Khitrov, A.N., Gabibov, A.G., Ponomarenko, N.A., and Suchkov, S.V., *Klin. Med.*, 2005, no. 6, pp. 128–133.
19. Abaturov, A.E., Petrenko, L.L., Gerasimenko, O.N., Agafonova, E.A., Vysochina, I.L., Krivusha, E.L., and Ermolaeva, O.A., *Zhurn. Zdorov'e rebenka* 2009, vol. 1, no. 16, pp. 25–28.
20. Pedro, A.B., Romaldini, J.H., Americo, C., and Takei, K., *Exp. Clin. Endocrinol. Diabet.*, 2006, vol. 114, no. 1, pp. 35–38.
21. Suchkov, S.V., Naumova, T.E., and Tret'yak, E.B., *Immunologiya*, 2004, vol. 2, pp. 115–119.
22. Kubota, T., *Mod. Rheumatol.*, 2002, vol. 12, pp. 300–304.
23. Nakahara, M., Nagayama, Y., Saitoh, O., Sogawa, R., Tone, S., and Abiru, N., *Endocrinology*, 2009, vol. 150, no. 3, pp. 1545–1551.
24. Nakkuntod, J., Wongsurawat, T., Charoenwong-se, P., Snabboon, T., Sridama, V., and Hirankarn, N., *Asian Pac. J. Allergy Immunol.,* 2006, vol. 24, pp. 207–211
25. Kosmacheva, S.M., Goncharova, N.V., and Shpak, I.R., *Med. Immunol.*, 2005, vol. 7, nos. 2–3, pp. 143–144.
26. Bretz, J.D., Rymaszewski, M., Arscott, P.L., Myc, A., Ain, K.B., Thompson, N.W., and Baker, J.R. Jr. *J. Biol. Chem.*, 1999, vol. 274, no. 33, pp. 23627–23632.
27. Jeremias, I. and Debatin, K.M., *Eur. Cytokine Netw.*, 1998, vol. 9, no. 4, pp. 687–688.
28. Kotovich, M.M. *Rol' etiotropnoi i patogeneticheskoi terapii v klinicheskoi i morfologicheskoi evolutsii khronicheskikh gepatitov u detei* (The role of etiothropic and pathogenetic therapy in clinical and morphological evolution of chronic hepatitis in children). Doctoral dissertation, Moscow: Russian State Medical University, 2003.
29. Kharaeva, Z.F., Ivanova, M.R., and Shevchenko, A.A., *Fundamental'nye Issledovaniya*, 2011, vol. 7, pp. 152–154.
30. Weetman, A.P., *Clin. Immunol. Immunopathol.*, 1989, vol. 51, no. 2, pp. 303–310.
31. Uzdenskii, A.B., *Biol. Membrany*, 2010, vol. 27, no. 1, pp. 7–17.
32. Balabolkin, M.I., Klebanova, E.M., and Kreminskaya, V.M., *Fundamental'naya i klinicheskaya tireoidologiya. Uchebnoe posobie*. (Basic and Clinical Thyroidology. A textbook), Moscow: Meditsina, 2007.
33. Andreeva, A.V., Suchkova, E.N., Gadzhieva, S.I., Mkrtumyan, A.M., Grishina, T.I., Suchkov, S.V., and Notkins, A.L., *Klin. Eksper. Tireoidol.*, 2011, vol. 7, no. 2, pp. 19–27

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