Pathogenetic interdependence of thyroid endocrine dysfunction and disturbancies of thymic-dependent immunoregulation

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Abstract

Close functional connections between neuroendocrine homeostasis and thymic dependent regulation of immunity constitute the grounds for supposition that thymic-dependent immunoregulatory dysfunction may participate in pathogenesis of thyroid endocrine hyper- or hypofunction. The suggestion has been confirmed by the results of hormonal and immunological examinations performed in the groups of hyper- and hypothyroid patients. The serum level of TSH, fT3 and fT4, the ATPO and ATG antibodies and cytokine (TNF-α, IL-1β, IL-1ra, IL-6, IL-8, IL-10) concentrations were determined. In population of mononuclear cells isolated from the blood (PBMC) the quantities of CD3, CD4, and CD8 phenotypes were measured and in the PBMC microcultures the parameters of T lymphocyte immunocompetence (response to mitogens, T cell suppressive activity) and monokine production (LM index, IL-1β/IL-1ra ratio) were estimated. The tests were performed in all the patients three times, once before and twice after the one and two months of the course of routine substitutive or modulatory hormonal treatment. The results indicate that the kind and range of immune dysfunctions observed in hyper- and hypothyroid patients were different and dependent on the kind of thyroid malfunction. As a result of routine hormonal treatment the partial improvements of the values of tested immune parameters were achieved, more distinct in the group of hyperthyroid patients. Conclusions: 1) distorted function in pituitary-thyroid axis and thymic-dependent immunoregulatory abnormalities are pathogeneticaly interdependent. 2) immunocorrective treatment with thymic hormones can be expected to further improve the therapeutic results of the routine hormonal treatment of hyper- and hypothyroid patients.

Key words: *thyroid endocrine malfunction, thymus-dependent immunoregulatory disorders.*

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Introduction

Cooperation of the neural, endocrine and immune systems integrated in the neuro-endocrine-immune functional network [1-3] are decisive for maintenance of homeostatic balance and healthy status of the organism. In this network the endocrine thyroid influencing the tissue metabolism, may affect also the T lymphocyte maturation and function, otherwise dependent on the endocrine activity of thymic epithelial cells. These two endocrine activities remaining under control of hypothalamo-pituitary axis appear to be mutually interdependent [4-8]. The endocrine and lymphopoietic function of the thymus is supported by growth hormone, prolactine and enkefalins and is suppressed by corticosteroids and sex hormones [5-11]. Moreover, the positive correlation has been observed between the serum concentration of trijodothyronine (T3) and the endocrine activity of thymic epithelial cells [9, 12-16]. On the other hand, the thymic hormonal products appear to activate the hormonal cascade of hypothalamo-pituitary-peripheral endocrine organs [5, 17, 18].

The quantitative and qualitative features of multipotent T lymphocyte population (TCD4, TCD8, TCD4CD25 Foxp3reg, Th17) and their ability to cooperate with other lymphoid cells, including immunogenic antigen presenters (dendritic cells, monocytes, macrophages) are commonly

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accepted manifestations of immunoregulatory efficiency of immune system subdued to the superior control of neuroendocrine regulation.

In the light of possible potential interdependence between the thymic dependent functions of immune system and the endocrine influences of the thyroid, the supposition arose that distorted thymic-dependent immunoregulatory functions may represent an important pathogenetic elements in development of thyroid hypo- or hyperactivity. Reciprocally, the thyroid malfunctioning my lead to the distortion of thymic lymphopoiesis.

The aim of undertaken investigations was to check if the thymic dependent immunoregulatory dysfunctions are appearing in parallel with manifestations of thyroid hormonal abnormalities in hypo- and hyperthyroid patients and how they behave under the influence of routine hormonal substitutive or modulatory treatment.

Materials and methods

Patients

The investigations were performed in two groups of patients in age from 28 to 40 years, with equal participation of both sexes, in whom hypofunction (n = 15) or hyperfunction (n = 15) of the thyroid was recognized on the grounds of anamnestic, physical and endocrinological examination All the patients signed document of conscious agreement for participation in the investigations which were approved by the Ethical Committee of Medical University in Łódź.

The hypothyroid patients received substitutive treatment with Euthyrox in a dose of 0.025 mg which was increased gradually until the deficiency of thyroid hormones was compensated. The patients suffering from hyperthyroidism were treated with Metizol-Polfa in devided doses of 60 mg daily for the first month, 20 mg for the second and 10 mg for the third month. They also received Propranolol (3 × 20 mg daily).

The immunological and endocrinological examinations were performed in all the patients before commencing the treatment and again after one and two months.

Immunological tests

Quantities of CD3+ CD4+ and CD8+ lymphocytes in mononuclear cell population isolated from the blood (PBMC) were determined by flow cytometry with the use of Simultest™ IMK Plus kit (Becton Dickinson).

Microcultures of PBMC. The microcultures were set up like described earlier [19-21] with appropriate rearrangements of microculture contents at 24 h in aim to estimate indexes of T-cell suppressive activity (SAT index) and monocyte immunogenic activity (LM index) [20, 21]. The Nuncoln plates containing several triplicates of microcultures (10^5 PBMC/0.2 ml RPMI 1640 + 15% autologous inactivated serum) were incubated at 37°C in ASSAB 5% CO_2 incubator for 72 h. Particulate microculture triplicates were left without stimulation or stimulated with phytohaemagglutinin (PHA Buroughs-Wellcome, 0.4 μg/cult.) or concanavalin A (Con A, Sigma, 8 μg/cult.). For the last 18 h of culturing 3H-thymidine (3HTdR, Ammersham, spec. act. 5 C/mM) was added into respective cultures in a dose of 0.4 μC/cult. After harvesting the incorporation of 3HTdR was measured in Packard Tri-carb 2100 TR scintillation counter. The results were presented as a mean values of desintegration per minute (dpm) for every labelled triplicate.

Determination of cytokine contents. The serum samples or not stimulated microculture supernatants harvested at 24 h of incubation were frozen (–70°C) and the concentration (pg/ml) of cytokines (TNF-α, IL-1β, IL-1ra, IL-4, IL-6, IL-8, IL-10) were determined by ELISA method with the use of respective R&D (Mineapolis, USA) kits.

The serum levels of anti-thyreoglobulin antibodies (ATG) and anti-thyroid peroxidase antibodies (ATPO) were determined using the Autostat II kits (Cogent Diagnostics Ltd.).

Endocrine examinations

The serum levels of TSH, fT3 and fT4 were measured in ELECSYS 2010 (Roche) apparatus using electro-chemiluminescence method (ECLIA) with commercial kits: Elecsys module for TSH estimation, Cobas for fT3 estimation and Elecsys module for determination of fT4.

The results of conducted immunological and endocrinological determinations are presented as a mean values ± SD for particulate groups of patients. For statistical analysis of the results the t-Student test and computer Statistica program were used.

Results

Immunological tests

Quantitative determination of T lymphocyte phenotypes

Table 1 presents percentage values of CD3, CD4 and CD8 T cell phenotypes in PBMC populations of hypothyroid and hyperthyroid patients. In the first group of patients values tested before and after one month of treatment remained at the normal level. Significant increase of percentages of CD3 and CD4 cells was observed in his group at the end of second month of treatment. In the group of hyperthyroid patients the first test showed higher than normal value of CD8 cells (28.0 ±3.5), the percentage values of CD3 and CD4 cells in the first and the second test were normal. At the end of second month of treatment (third test) percentages of CD3 and CD4 cells increased and percentage value of CD8 cells decreased to the normal value.

Table 1. Percentage values of CD3, CD4 and CD8 T lymphocytes in PBMC population of hypo- and hyperthyroid patients

T lympho-cytes	Group of patients					
	hypothyroid			hyperthyroid		
	test 1	test 2	test 3	test 1	test 2	test 3
CD3	65.0 ±7.8	57.2 ±5.1	69.3 ±6.4 *	64.1 ±9.5	57.8 ±9.6	73.0 ±7.1*
CD4	37.0 ±5.8	32.1 ±4.1	40.7 ±2.9 *	32.0 ±4.7	35.0 ±7.1	42.0 ±3.1*
CD8	21.0 ±7.2	19.5 ±6.1	23.5 ±3.4	28.0 ±3.5	22.0 ±4.1	21.0 ±3.2*

** statistically significant difference ($p < 0.01$) between the results of final and earlier tests*

Table 2. Response to mitogens and immunoregulatory properties in microcultores of PBMC from hypo- and hyperthyroid patients

Tested parametr	Group of patients					
	hypothyroid			hyperthyroid		
	test 1	test 2	test 3	test 1	test 2	test 3
Spont. incorp. of 3HTdR dpm × 10^3	1.4 ±0.3	1.5 ±0.2	1.3 ±0.2	1.2 ±0.3	1.6 ±0.4	1.4 ±0.3
PHA dpm × 10^3	37.6 ±19.9	29.8 ±17.0	51.2 ±19.8*	57.9 ±30.1	35.2 ±18.2	69.7 ±21.5*
Con A dpm × 10^3	24.9 ±11.2	27.8 ±10.8	21.1 ±9.6	32.0 ±19.6	31.6 ±14.1	27.6 ±9.3
Ratio of PHA/ConA responses	1.1 ±0.4	1.0 ±0.5	2.5 ±0.7*	2.0 ±0.7	2.1 ±1.2	2.3 ±1.1
SAT index	6.9 ±2.9	12.8 ±2.1	23.0 ±10.1*	19.5 ±12.5	20.4 ±14.9	17.0 ±6.7
LM index	16.7 ±4.3	14.0 ±8.1	7.0 ±2.1*	14.4 ±3.2	9.4 ±6.2	4.0 ±3.1*
IL-1β [pg/ml]	872 ±213	378 ±219	310 ±187*	631 ±148	438 ±116	422 ±32
IL-1ra [pg/ml]	1870 ±945	3198 ±998	3414 ±710*	2252 ±598	2929 ±346	3252 ±598*
IL-1ra/IL-1β ratio	2.5 ±0.9	15.4 ±6.6	16.9 ±7.5*	4.0 ±1.0	7.4 ±1.5	7.8 ±1.1*

** statistically significant difference ($p < 0.01$) between the final and one or two earlier tests.*

Functional assessment of T lymphocytes and monocytes in microculture system

The functional properties of T cells and monocytes, observed in microcultures, are presented in Table 2. In the group of hypothyroid patients significant increase of lymphocyte response to PHA, value of suppressive activity (SAT index) and normalization of ratio of PHA/ConA response were observed in the tests performed at the end of second month of treatment (third test). The second and third tests revealed decrease of excessive value of LM index (immunogenic activity of monocytes), decrease of excessive concentration of IL-1β, increase of concentration of IL-1ra and normalization of IL-1ra/IL-1β ratio.

In the group of hyperthyroid patients the third test showed increase of lymphocyte response to PHA, normalization of the value of LM index which in the first and second tests showed excessive values, decrease of concentration of IL-1β, increase of contents of IL-1ra and improved value of the ratio IL-1ra/IL-1β concentrations.

Cytokines in the serum

The results are presented in Table 3. Many times lower values of the concentration of IL-1β and IL-1ra were observed in the serum than those observed in the microculture supernatants (Table 2). The contents of remaining tested cytokines in the serum of patients of the both groups remained also at the low level in the range of values between 0 and about 100 pg/ml. The only exception was the contents of IL-1ra demonstrating wider range of values. In the group of hypothyroid patients the

Table 3. Cytokine concentrations (pg/ml) in the serum of hypo- and hyperthyroid patients

Cytokine	Group of patients					
	hypothyroid			hyperthyroid		
	test 1	test 2	test 3	test 1	test 2	test 3
Spont. incorp.	1.4 ±0.3	1.5 ±0.2	1.3 ±0.2	1.2 ±0.3	1.6 ±0.4	1.4 ±0.3
IL-1β	21.9 ±20.1	9.2 ±4.1	18.1 ±9.2	25.4 ±31.1	20.1 ±10.9	10.7 ±5.2*
IL-1ra	158.0 ±97.9	110.1 ±90.2	198.2 ±28.0	421.5 ±247.0	92.3 ±21.2	219.0 ±143.2
TNF-α	19.2 ±10.6	22.6 ±11.8	4.8 ±2.9*	8.9 ±16.8	21.1 ±9.8	5.2 ±6.9*
IL-4	76.2 ±45.1	51.3 ±28.0	52.1 ±45.7	25.6 ±16.6	82.0 ±37.5	15.3 ±2.6*
IL-6	67.0 ±21.2	49.2 ±29.2	18.5 ±9.4*	50.9 ±19.9	41.8 ±20.1	45.2 ±21.9
IL-8	24.2 ±19.8	16.1 ±9.2	15.2 ±4.1	18.3 ±10.4	20.2 ±16.7	18.1 ±4.8
IL-10	67.9 ±54.0	61.2 ±40.8	49.5 ±38.8	56.1 ±18.9	42.2 ±26.0	32.8 ±21.5

** statistically significant difference ($p < 0.01$) between the results of final and one or two earlier tests*

Table 4. Anti-thyreoglobulin (ATG) and anti-thyroid peroxidase (ATPO) antibidies in the serum of hypo- and hyperthyroid patients

Antibodies	Group of patients					
	hypothyroid			hyperthyroid		
	test 1	test 2	test 3	test 1	test 2	test 3
ATG [U/ml]	629	967	615	57	53	50
ATPO [U/ml]	413	410	458	75	48	48

** positive values: ATG > 325 U/ml, ATPO > 50 U/ml*

Table 5. TSH, fT3 and fT4 in the serum of hypo- and hyperthyroid patients

Hormone	Group of patients					
	hypothyroid			hyperthyroid		
	test 1	test 2	test 3	test 1	test 2	test 3
TSH [μU/ml]	54.3 ±39.5	24.1 ±19.9	8.3 ±9.3*	0.02 ±0.03	0.14 ±0.30	2.87 ±6.88*
fT3 [pg/ml]	2.3 ±1.2	2.4 ±1.1	3.4 ±0.5	30.1 ±66.5	5.1 ±2.7	4.2 ±1.9*
fT4 [pg/ml]	1.2 ±1.2	0.7 ±0.4	1.2 ±0.3	3.4 ±1.7	1.5 ±0.7	1.9 ±2.4

** statistically significant difference ($p < 0.01$) between the results of final and one or two earlier tests*

concentrations of proinflammatory cytokines TNF-α and IL-6 decreased significantly at the end of second month of treatment. In the group of hyperthyroid patients the concentrations of IL-1β, TNF-α and IL-4 were significantly lower at the end of treatment than earlier, but concentration of IL-6 remained at the stable, relatively high level.

Anti-thyreoglobulin antibodies (ATG) and anti-thyroid peroxidase antibodies (ATPO)

The results are presented in Table 4. In the group of hypothyroid patients median values of ATG and ATPO considerably exceeded the threshold values (for ATG > 325

U/ml, and for ATPO > 50 U/ml). These values remained unchanged in the consecutive tests.

In the group of hyperthyroid patients the values of ATG were lower than threshold values and did not change in consecutive tests. The ATPO values were at the threshold level and also did not change during the treatment.

Endocrinological tests

Table 5 presents results of estimations of concentration of TSH, fT3 and fT4 in the serum of both groups of patients. In the hypothyroid group the level of TSH was considerably elevated in the first and the second test and decreased significantly in the third test. The concentrations of fT3 and fT4 remained below the normal values and did not change in the consecutive tests. In the hyperthyroid group TSH represented low value at the beginning of observation which has improved a bit during the treatment. The very high value of fT3 observed before the treatment decreased considerably and normalized in consecutive tests. The concentrations of fT4 remained at the normal level during the whole period of observation.

Discussion

Immunological disorders observed in hypothyroid and in hyperthyroid patients were different in terms of particulate parameters. Hypothyroid patients demonstrated decreased immunoregulatory functions of T cells (response to PHA, SAT value), excessive production of proinflammatory IL-1β, high immunogenic activity of monocytes (LM value) and high level of ATG and ATPO autoantibodies in the serum. The concentration of anti-inflammatory IL-1ra was low and the value of IL-4, which activate humoral activity of B lymohocytes and IgM/IgG conversion [22], was elevated. In the context of superior immunoregulatory function of T lympohocytes (including Treg cells and their suppressive activitry) in immune system [4, 5, 23-26] the observed picture suggests the existence of autoaggressive mechanisms in which deficient immunoregulatory activity of T cells was not able to prevent the development of excessive immunogenic function of monokines and final production of autoantibodies.

In the group of hyperthyroid patients no symptoms of autoimmunity were observed. Immunoregulatory properties of T cells remained at relatively normal level but immunogenic activity of monocytes and production of proinflammatory cytokines (IL-1β, TNF-α, IL-4, IL-6) was excessive. The present symptoms of immunostimulation could be due to the increased thyroid endocrine activity. Triiodothyronine (T3) is known to activate the expression of recepotors and ligands (fibronectin, laminie, VLA-5, VLA-6) in extracellular matrix increasing cell adherence, interaction and activation [14]. Moreover, thyroid hormones activate cellular cooperation of thymic epithelial cells (TEC) with maturing thymocytes and increase production of thymic hormone (thymulin) prompting the process of thymic lymphopoiesis and replenishment of peripheral immune system with the new cohorts of multipotent T lymphocyte population [8, 9, 12-17].

The endocrine status of two groups of our patients was quite different. Excessive activity of pituitary TSH and extremely low levels of thyroid hormones were observed in the serum of hypothyroid patients. The opposite situation, low TSH and high concentrations of T3 and T4, characterized the group of hyperthyroid patients. The administered hormonal therapy, substitutive (Euthyrox) in hypothyroid and modulatory (Metizol, Propranolol) in hyperthyroid patients resulted in almost full compensation of their hormonal pituitary-thyroid balance (Table 5). It is difficult to decide if the hormonal dysfunction of pituary-thyroid axis observed in our patients was a primary cause of their immune disorders, or if the thymic-dependent deficit of immunoregulation was primarily responsible for the secondary endocrine imbalance. The positive results of hormonal treatment which improved also the immunoregulatory efficiency of immune systems in the both groups of our patients seem to indicate that thymic-dependent immune functions and hypothalamo-pituitary hormonal regulation are functionally interconnected in the neuro-endocrine-immune network.

Different kind of hormonal disorders and less severe immune deficit in hyperthyroid patients, when compared to the hypothyroid group, is predictive for expectations that compensative hormonal therapy (modulatory or substitutive, respectively) would bring better results in hyperthyroid patients in respect to their immune efficiency. The results of immune tests confirmed the suggestion. Hyperthyroid patients after treatment demonstrated quantitative normalization of T lymphocyte population (increase of CD4, decrease of CD8 and improvement of CD4/CD8 ratio), increase of T cell response to PHA, good level of T cell suppressive activity (SAT index) and decrease of excessive immunogenic activity of monocytes exemplified by lowered values of LM index, better proportion of IL-1ra/IL-1β and decreased concentration of IL-1β, TNF-α and IL-4.

The hormonal treatment administered in the both groups of patients partially improved, albeit to different extent, an immunoregulatory capacity of immune systems. The treatment did not result in a complete restoration of all immune parameters which were tested. In the hypothyroid patients anti-thyroid antibodies remained at the same level as in the initial tests. The T cell suppressive activity (SAT index) increased but did not normalize, and concentrations of some pro-inflammatory cytokines (IL-1β, IL-4) remained at high level in the serum. In the group of hyperthyroid patients T cell suppressive activity remained below the normal values and no decrease of elevated serum level of IL-6 has been observed. Interleukin 6 is known to play an important role in differentiation of naive T lymphocytes.

These cells exposed to transforming growth factor β alone (TGF-β) express forkhead box P3 (Foxp3) – the transcription factor that induces development of T cells regulatory ability to suppress inflammation and prevent autoimmunity [27]. In contrast to that, naive T cells if exposed to TGF-β together with IL-6 become Th17 cells producing IL-17, a cytokine with strong proinflammatory properties [28-30].

The results of our investigations confirm the conception that disorders in pituitary-thyroid endocrine axis are bidirectionally connected with the insufficient function of thymic dependent immunocompetent T cell population. This may suggest that complex therapy including not only hormonal compensation but also immunocorrective treatment with thymic hormones could bring the better therapeutic results in patients suffering from thyroid endocrine disorders.

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