Relationship between Functional Activity of the Thyroid Gland and Levels of Proinflammatory and Immunoregulatory Cytokines in Acute Experimental Endotoxicosis

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Functional activity of the thyroid gland is reduced in Wistar rats with acute experimental endotoxicosis developing in response to sublethal dose of LPS and paralleled by polarization of the immune response by the Th-1 type. This decrease does not depend on the level of thyrotropic hormone and correlates with the production of IL-2 and IFN-γ.

Key Words: *lipopolysaccharide; endotoxicosis; thyroid hormones; cytokines*

Interactions of the neuroendocrine and immune systems in health and disease remain a pressing problem of modern biology and medicine, despite many-year history of their research. The reaction of the hypothalamus—pituitary—thyroid axis associated with the immune response to pathogenic microorganisms attracts special scientific and practical interest. The production of thyroid hormones is reduced in many acute diseases of different origin. This status is called the nonthyroid disease syndrome or euthyroid weakness syndrome, thus emphasizing the absence of a history of thyroid gland (TG) disease in the patients. Evaluation of this status was the object of numerous discussions for almost 40 years. Transitory hypothyroidism was described in patients with various infectious diseases, sepsis, myocardial infarction, burn disease, and injuries [2]. Stereotypical reaction of TG in acute diseases was hypothesized in this connection. However, new data on the duration and severity of the developing thyroid status disorders show that the etiological factor somehow affects the pathogenetic mechanisms of TG dysfunctions.

We studied thyroid status of rats with acute inflammatory process developing in response to injection of a sublethal dose of LPS and associated with polarization of the immune response by the Th1 type.

MATERIALS AND METHODS

The study was carried out on 45 male Wistar rats (200-220 g) in winter. The rats were randomly divided into control (n=15) and experimental (n=30) groups. Experimental rats were intraperitoneally injected with a single sublethal (20 mg/kg) dose of strain 026:B6 *E. coli* LPS (Sigma). Controls were injected with 20 mg/kg saline. The animals were sacrificed by zoletil overdosage 1 and 7 days after LPS injection. All manipulations with rats were carried out in accordance with the regulations on studies on experimental animals approved by the Order No. 577 of the Ministry of Health (August 12, 1977). The thyroid status was evaluated by serum levels of thyrotropic hormone (TTH), total thyroxin (T4), total triiodothyronin (T3), free fractions of these hormones (fT4 and fT3), and corticosterone measured by ELISA using commercial kits (DSL, Monobind). Splenocytes were isolated from rat spleen for evaluation of the cytokine profiles. The production and release of cytokines was induced by culturing cell suspension (10^6/ml) for 24 h in 1 ml complete growth medium with concanavalin A (in a final concentration of 5 μg/ml) in 24-well culture panels at 67°C and 5% CO_2. The concentrations of IL-2,

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-4, -10, -12p40, IFN-γ, and TNF-α in culture medium were measured by ELISA using commercial kits (Bio Source International).

The data were statistically processed using parametric and nonparametric tests. The groups were compared using ANOVA, Kruskal—Wallis and Mann—Whitney tests. Analysis of correlations was carried out using Spearman test. The differences were considered significant at $p<0.01$.

RESULTS

Serum corticosterone level increased 24 h after LPS injection from 216.5±5.9 to 301.1±5.6 ng/ml ($p=0.00005$). Significant shifts in the thyroid status of experimental rats were detected (Table 1). Minor reduction of TTH and statistically significant (virtually 4-fold) drop of T4 and 2-fold drop of fT4 in comparison with the control were observed. The percent content of fT4 increased 2-fold in comparison with the control. The concentrations of T3 and fT3 and hence, the percent of fT3/T3 concentrations did not change 24 h after LPS injection. On day 7, the concentration of corticosterone decreased significantly, reaching 234.9±9.0 ng/ml ($p=0.0001$). Serum concentration of T4 increased 2-fold, but was significantly lower than in the control. Serum fT4 concentration virtually did not change, while the percent of fT4 decreased 2-fold, reaching the basal level. The levels of T3 and fT3 did not change, while the percent of fT3 decreased in comparison with the control. Serum concentration of TTH continued to decrease and on day 7 was 2-fold lower in comparison with the control level. A significant direct correlation between serum T4 and fT4 concentrations was detected, more pronounced on day 1 after LPS injection ($R=0.81$; $p<0.01$) than on day 7 ($R=0.65$; $p<0.01$). Analysis of correlations revealed no relationship between TTH concentration and levels of T4, fT4, T3, and fT3.

The cytokine profile 24 h after LPS injection was characterized by a significant increase in TNF-α, IFN-γ, IL-2, and IL-12p40 levels (Table 2), which was in line with previously noted polarization of the immune response by the Th1 type in response to high doses of LPS [5]. The production of proinflammatory cytokines in these animals exhibited a pronounced correlation ($R=0.68$-0.96). The highest relationship was detected for IL-2 and IFN-γ production ($R=0.96$; $p<0.00001$). The production of antiinflammatory cytokine IL-10 did not increase, and the level of IL-4 in culture medium was extremely low in the control and experimental groups. The levels of proinflammatory cytokines (TNF-α, IFN-γ, IL-2, IL-12p40) continued to increase on day 7. The relationship between the concentrations of IL-2 and IFN-γ did not change ($R=0.9$; $p<0.00001$), similarly as the direct relationships between the levels of other proinflammatory cytokines. The production of antiinflammatory cytokines IL-4 and IL-10 did not change either, but a direct relationship between the production of IL-10 and proinflammatory cytokines developed on day 7: moderate for IL-12 ($R=0.76$; $p<0.01$) and TNF-α ($R=0.75$; $p<0.01$) and strong for IL-10 and IFN-γ ($R=0.83$; $p<0.001$) and IL-2 ($R=0.81$; $p<0.001$).

Evaluation of the relationship between the production of TTH, thyroid hormones, and cytokines showed that the decrease in TTH synthesis did not correlate with the release of proinflammatory cytokines, while reduced production of T4 correlated with the increase of IL-2 production ($R=-0.70$; $p<0.01$) and IFN-γ ($R=-0.86$; $p<0.01$).

According to published data, production of T3 is the first to reduce in acute disease, while combined reduction of T3 and T4 is observed in severe pathologies. Serum TTH level can increase or decrease. Clinical and experimental studies revealed a reduction of TTH, T4, fT4, T3, and fT3 in sepsis [1,3]. It is assumed that

TABLE 1. Changes in the Thyroid Hormone Status of Rats Injected with a Sublethal Dose of LPS (*M*±*m*)

Hormones	Serum hormone concentrations		
	control	experiment	
		day 1	day 7
TTH, μU/ml	0.144±0.012	0.109±0.032	0.067±0.025^{xx}
T4, ng/ml	40.5±2.6	11.4±2.1*	25.8±2.9^{+xx}
fT4, pg/ml	20.5±1.9	11.3±1.8**	11.7±1.5^{x}
fT4 percentage	0.050±0.004	0.11±0.01**	0.05±0.01^{xxx}
T3, ng/ml	0.89±0.06	0.90±0.16	0.86±0.05
fT3, pg/ml	1.75±0.14	1.89±0.6	1.28±0.16^{x}
fT3 percentage	0.20±0.01	0.18±0.05	0.14±0.01^{x}

Note. *$p<0.001$, **$p<0.01$, $^{x}p<0.01$, $^{xx}p<0.001$, $^{xxx}p<0.0001$ compared to the control; $^{+}p<0.01$ compared to day 1.

TABLE 2. Changes in Cytokine Profile of Rat Splenocytes after Injection of a Sublethal Dose of LPS (*M*±*m*)

Parameter	Cytokine concentration in culture fluid, pg/ml		
	control	experiment	
		day 1	day 7
TNF-α	37.8±2.4	80.6±17.0*	178.5±32.0^{+}**
IFN-γ	0	47.9±19.5*	219.1±95.2^{+}**
IL-2	152.0±24.7	1066.9±272.5*	1838.0±245.2*
IL-10	61.8±8.3	60.1±11.2	59.8±11.2
IL-12p40	14.2±1.4	28.8±7.1	183.0±40.2^{++}**

Note. *p<0.01, **p<0.00001 compared to the control; ^{+}p<0.01, ^{++}p<0.0001 compared to day 1.

the main cause of TG dysfunction is reduced synthesis of thyrotropin releasing hormone by the hypothalamic paraventricular nuclei and hence, reduced synthesis of TTH by the pituitary [4]. Our data indicate that the reduction of serum T4 and fT4 concentrations are not proportional to reduction of TTH concentration on day 1. On day 7, the concentrations of T4 and fT4 increase in the presence of continuing reduction of TTH level, which indicates a direct effect of the inflammatory process on TG. Study of the thyroid status of rats 24 h after LPS injection showed that the systemic inflammatory response was not associated with T3 and fT3 reduction. The maintenance of normal T3 level in the serum in the presence of the 4-fold reduction of T4 concentration indicates preserved high activity of deiodinases and reduced penetration of T3 into the target cells. Functional activity of TG partially recovered on day 7, which together with reduced T3 level in the serum indicated increased need of target cells in thyroid hormones.

Study of the relationship between the cytokine profile and the production of thyroid hormones in infections as one of the main elements in the pathogenesis of the nonthyroid disease syndrome was based on experimental data indicating an inhibitory effect of some cytokines on the thyrocyte secretory activity *in vitro* [6]. These data could not be reproduced *in vivo* in the majority of cases [7,8]. The cytokines are an intricate highly integrated system, and evaluation of their effects on functional activity of TG is a difficult problem. Study of cytokine production by splenocytes showed a significant increase in the production of proinflammatory TNF-α and IL-12 (initiating differentiation of zero lymphocytes towards the Th1 direction) and of the central regulatory cytokines IL-2 and IFN-γ. No production of IL-4 (the main factor of zero lymphocyte differentiation towards Th2 cells) was detected on day 1 or on day 7. The concentration of antiinflammatory cytokine IL-10 virtually did not change in comparison with the control, but on day 7 its production was in strong correlation with the production of proinflammatory cytokines, particularly IL-2 and IFN-γ. The detection of the involvement of IL-2 and IFN-γ in the development and changes in T-cell response prompted study of their effects on the production of thyroid hormones by TG. Evaluation of the mutual dependence of IL-2 and IFN-γ and of T4 secretion showed a strict relationship between the production of these cytokines and reduced functional activity of TG.

Hence, the thyroid status is changed in severe acute LPS-induced endotoxicosis associated with polarization of the immune response by the Th1 type. These changes consist mainly in reduction of functional activity of TG, not depending on the content of TTH and correlating with the production of IL-2 and IFN-γ in the presence of normal T3 levels of mainly extrathyroid origin.

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