Morphofunctional Analysis of Development of Nonthyroid Disease Syndrome in Experimental Endotoxicosis

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The development of the nonthyroid disease syndrome in acute endotoxicosis involves mechanisms related to polarization of the immune response by the Th1 type. Thyroid status of rats with pronounced and weak polarization of the immune response was evaluated. Morphofunctional changes in the thyroid gland in animals with the central and peripheral mechanisms of the development and regression of the nonthyroid disease syndrome were detected.

Key Words: *nonthyroid disease syndrome; thyroid; morphology*

The nonthyroid disease syndrome (NTDS) is an acutely developing dysfunction of the thyroid gland (TG) observed in many somatic diseases of different severity, which is characterized by reversible reduction of thyroid hormone production [1]. The specific features and regularities of morphofunctional changes in TG during its transitory dysfunction are little studied. The morphological and functional changes in TG during activation of the immune system and different types of immune response to pathogens are particularly interesting.

We studied the morphofunctional parameters of NTDS development and regression in experimental endotoxicosis with consideration for individual reactions of the immune system.

MATERIALS AND METHODS

Experiment was carried out on 45 male Wistar rats (200-220 g). Experimental rats (n=30) received a single intraperitoneal injection of *E. coli* strain 026:B6 LPS (Sigma) in a dose of 20 mg/kg. Controls (n=15) were injected with the same volume of saline. The animals were sacrificed by zoletil overdosage 1 and 7 days after LPS injection. Serum levels of thyrotropic hormone (TTH), total thyroxin (T4) and triiodothyronine (T3), free fractions of these hormones (fT4 and fT3), and the concentrations of IL-2, -10, -12, IFN-γ, TNF-α in splenocyte culture fluid were measured by solid phase EIA with commercial kits (Monobind Inc., Invitrogen).

After fixation in Bouin fluid and standard processing, the preparations of TG were embedded in paraffin. The sections were stained with hematoxylin and eosin and with toluidine blue for selective detection of mast cells. Morphometric analysis was carried out using Image ProPlus software. The areas of follicles, percentage of epithelial area in a follicle, height of follicular thyrocytes, and areas of their nuclei were evaluated. The content of colloid in follicles was evaluated by the proportion of the colloid area to the follicle lumen area and expressed in percent. Mast cell function was evaluated separately in the peripheral and central zones of TG by the following parameters: cell count per mm^2 of section area, degranulation index, and the mean histochemical coefficient, reflecting cell saturation with secretory material.

The data were statistically processed using cluster analysis and Mann–Whitney test. The differences were considered significant at p<0.05.

RESULTS

One day after LPS injection, all rats developed NTDS characterized by reduced serum levels of thyroid

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hormones. Cluster analysis revealed two variants of NTDS development differing by biochemical and morphological parameters (Table 1).

One day after LPS injection, the production of TNF-α, IL-2, and IFN-γ by splenic cells was low in cluster 1 rats, not differing from that in the controls. Serum concentrations of T4 and fT4 reduced significantly in comparison with the control (p=0.00016). The level of T3 dropped to the lowest threshold level in the control, while fT3 was the same as in the control. Serum TTH level decreased significantly (p=0.00085). Hence, the central type of NTDS development was observed in this cluster of rats due to reduction of the pituitary functional activity.

Study of the histological preparations of TG from cluster 1 animals showed stromal edema and plethoric vessels with stasis and sludge primarily in the peripheral zones of the gland. The height of the follicular thyrocytes and the percentage of the epithelium in the follicle decreased (p=0.002) in the central zones; the content of colloid in follicles also decreased (p=0.0043; Fig. 1, *a*, *b*). These data reflect activation of resorption processes and their predominance over thyroglobulin synthesis. Evaluation of the status of mast cells in TG showed that after LPS injection the molecular mode of their secretion predominated over the microapocrine mode, this being more pronounced in the central zone. The decrease in the mean histochemical coefficient of mast cells in comparison with the control (p=0.0004), reflecting this secretion mode, inversely correlated with thyrocyte height (R=-0.5). The count of mast cells did not change in comparison with the control.

In cluster 2 rats, the systemic inflammatory reaction one day after LPS injection manifested in a significant increase in the levels of TNF-α (p=0.000052), IL-12 (modulating Th0 lymphocyte differentiation into Th1 lymphocytes; p=0.00011), IL-2 (p=0.00011), and IFN-γ (p=0.0032), the main Th1 lymphocyte cytokines, in comparison with the control. Serum levels of T4 and fT4 were reduced (p=0.00016), the concentration of fT3 was extremely low (p=0.000089), while the levels of T3 and TTH were normal. Hence, cluster 2 rats developed the peripheral type of NTDS, manifesting in isolated involvement of TG.

Morphological analysis showed that stromal edema and microcirculatory disorders were more pronounced in rats with manifest inflammatory reaction. The size of follicles increased, especially in the peripheral zone. The percentage of the epithelium in the follicles and thyrocyte height in the central and peripheral zones were significantly higher than in rats with the central type of NTDS development. The content of colloid in the follicles was also higher, surpassing the control values in the peripheral zones (p=0.0010). These data indicate intensification of synthetic processes and reduction of colloid resorption processes, which was responsible for reduced serum levels of thyroid hormones. The counts of mast cells in TG and the mode of secretory material release by them did not differ from those in cluster 1 rats. The intensity of secretion of bioactive substances by mast cells in rats with the peripheral NTDS was lower than in rats with the central type of NTDS development (Fig. 1, *a*, *b*).

Seven days after injection of LPS, the cytokine profile of rats was characterized by increased content of all studied cytokines. Serum hormone levels increased. Cluster analysis revealed differences in the cytokine profile and in the thyroid status of rats (Table 1). High level of cytokine production was detected in cluster 1 rats. The concentrations of T4, fT4, and fT3 increased in comparison with day 1, but did not reach the control levels. Serum content of T3 corresponded to the values in the control. The production of thyroid hormones increased in the presence of low serum TTH concentration. Examination of TG preparations showed stromal edema, microfollicular restructuring of the central parenchyma, reduction of follicular thyrocyte height and size of their nuclei in comparison with the control group. In the peripheral zones, the content of colloid in the follicular cavity decreased compared to that in the central zones. Mast cell count did not change. Their mean histochemical coefficient increased significantly, this indicating more intense deposition of secretory material (Fig. 1, *c*, *d*). In cluster 2 rats, the production of TNF-α, IL-12, IL-2, and IFN-γ by splenocytes was significantly lower than in cluster 1 rats. Serum TTH level was extremely low. Serum T4 and fT4 levels were similar to those in cluster 1 rats, while the levels of T3 and fT3 were significantly lower than in the control (Table 1). No stromal edema was detected in TG. Microfollicular rearrangement of the parenchyma was observed in the central zones. The follicles in the peripheral zones were much smaller than in cluster 1. The percentage of the epithelium in the follicles and thyrocyte height in the central zones decreased compared to the control. Thyrocyte nuclei shrank in both zones. A characteristic feature of cluster 2 was a significant increase in colloid content in the follicular lumen in the central and peripheral zones, indicating reduced resorption of the colloid (Fig. 1, *c*, *d*).

High LPS doses polarize the immune response by the Th1 type [5]. One day after injection of the sublethal dose of LPS, the immune response was polarized (with different intensity) by the Th1 type. Rats with high production of Th1 cytokines developed the "classical" variant of NTDS characterized by reduced serum levels of T4 and T3 and of their free fractions against the background of normal TTH concentration. These changes in the thyroid status indicate predominant isolated involvement of TG in the development of

NTDS in this group of rats and functional dissociation of the central and peripheral components of the hypothalamic—pituitary—thyroid axis. The TTH intensified all stages of the secretory cycle of thyrocytes, including colloid resorption. More intense synthesis of thyroglobulin and its reduced resorption support the fact that thyrocyte sensitivity to TTH decreased significantly, while the synthetic stage of hormonogenesis was stimulated by another factor, most likely LPS.

Experiments on rat thyrocyte cultures showed that they express functionally active toll-like receptors 4 and a complex of adaptor proteins MD2/CD14, providing the signal transmission during the receptor interaction with LPS [6]. *In vitro* experiments detected an increase of thyroglobulin gene transcription by thyrocytes under the effect of LPS against the background of TTH and the absence of this reaction without TTH [8]. The increase in synthetic activity of follicular thyrocytes 1 day after LPS injection in the presence of normal serum TTH level, observed in our study, confirms the *in vitro* data indicating the possibility of stimulation of thyroglobulin synthesis by LPS in the presence of TTH. Rats with low level of cytokine production also developed NTDS, but it differed by a number of parameters. Morphological changes in TG indicate attenuation of the synthetic processes in follicular thyrocytes, which also confirms the data on the absence of the LPS stimulatory effect on thyrocytes without TTH. More intense resorption of the colloid does not mean increased production of

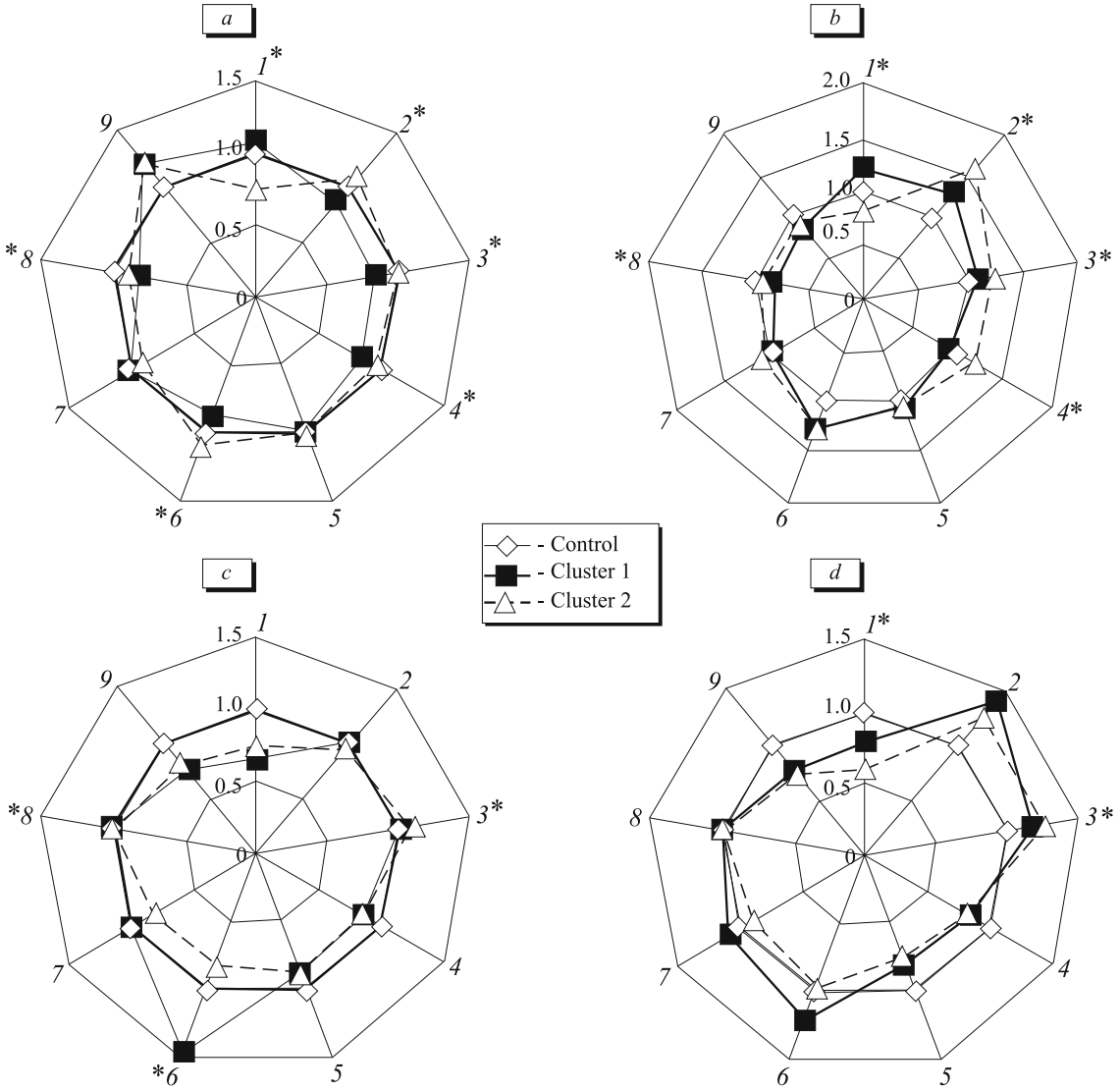

Fig. 1. Results of cluster analysis of morphometric parameters of TG 1 and 7 days after LPS injection. *a*) central and *b*) peripheral zones of the gland 1 day after LPS injection; *c*) central and *d*) peripheral zones of the gland 7 days after LPS injection. The values in the control group are taken for one unit. *1*) follicular area; *2*) percentage of epithelium; *3*) colloid content in a follicle; *4*) height of follicular thyrocytes; *5*) area of follicular thyrocyte nuclei; *6*) percentage of stroma; *7*) count of mast cells per section area unit; *8*) mean histochemical coefficient of mast cells; *9*) mast cell degranulation index. *p<0.05 compared to cluster 1.

TABLE 1. Concentrations of Serum Hormones and Cytokines in Rat Splenocyte Culture Medium 1 and 7 Days after LPS Injection (*M*±*SD*)

Parameter	Control	One day after LPS injection		Seven days after LPS injection	
		cluster 1	cluster 2	cluster 1	cluster 2
TTH, μU/ml	0.14±0.05	0.07±0.02	0.20±0.14**	0.07±0.02	0.04±0.02*
T4, μg/dl	4.05±0.85	1.10±0.80	0.89±0.72	2.72±1.15	2.50±0.89
fT4, pg/ml	2.05±0.64	0.91±0.12	1.15±0.66	1.10±0.69	1.15±0.37
T3, μg/dl	0.88±0.19	0.72±0.09	0.89±0.51**	0.89±0.13	0.71±0.07**
fT3, pg/ml	1.75±0.47	2.25±1.58	0.39±0.35**	1.27±0.18	0.76±0.11**
IL-2, pg/ml	152.0±42.7	674.3±21.3	1512.5±525.5	2214.67±564.20	1589.4±76.8
IL-10, pg/ml	61.8±29.9	49.7±4.0	45.7±13.8	63.6±29.9	39.3±3.9
IL-12, pg/ml	14.2±5.0	40.7±37.2	37.7±6.9	312.8±192.2	63.3±10.8**
TNF-α, pg/ml	37.8±8.9	32.0±12.1	113.8±42.6*	277.7±201.5	111.6±61.7**
IFN-γ, pg/ml	<12.0	<12.0	50.6±33.9**	225.4±143.7	69.4±26.8**

Note. *p<0.05, **p<0.01 compared to cluster 1.

T3 and T4, because resorbed thyroglobulin can be released into circulating blood or back into the colloid cavity without removal of iodinated thyrosyls from it [4]. These changes are characteristic of hypothyrosis developing predominantly by the central mechanisms and indicate an adequate reaction of TG to significant reduction of TTH production. Comparison of the cytokine production and serum TTH levels in rats of both groups indicate that more pronounced Th1 and proinflammatory response do not modify the production of TTH. However, normal serum TTH level can be maintained by not only pituitary, but also extrapituitary TTH produced by the immune system cells [3]. Injection of LPS causes TTH release into the blood by the immune system cells (TTH is a potent modulator of phagocytic activity and together with IL-2 a costimulator of natural killer activity [7]). The reaction of mast cells (also involved into natural immunity reactions) was less pronounced in rats with high level of cytokines and normal serum content of TTH in comparison with rats with low cytokine and TTH production. Hence, the intensity of the mediator release by mast cells stimulated by LPS was reciprocally related to the level of cytokine production by splenic cells. The inverse relationship between activity of mast cells and height of follicular thyrocytes detected in our experiment indicates the involvement of mast cells into suppression of functional activity of thyrocytes via paracrine modulation of the specific spectrum of biological mediators.

On day 7, the concentration of TTH decreased, while the levels of T4 and fT3 increased in rats with higher levels of proinflammatory and Th1 cytokines, while in rats with low levels of cytokine production, the level of TTH decreased in parallel with the increase in the levels of T4 and fT4 and reduction of fT3. Hence, with the development of the underlying disease the peripheral mechanism of NTDS development is paralleled by the central mechanism in rats with high level of Th1 cytokines. Starting recovery of TG function on day 7 is explained by discontinuation of the peripheral mechanism functioning. In rats with low levels of proinflammatory and Th1 cytokines, the central mechanism is supplemented by the peripheral one with disease development, because hormone content increases against the background of very low TTH levels; however, the central mechanism remains the leading one.

Morphological changes in rat TG were also peculiar. In rats with high levels of cytokines, the microfollicular restructuring involved mainly the central zone of TG and functional activity increased at the expense of an increase in the number of hormone-producing cells and recovery of colloid resorption processes in the central zone. In rats with low cytokine levels, the follicles shrank also in the peripheral zones, but the intensity of resorption processes was lower, and hence, thyroid hormone production was also lower. Morphological studies of TG from patients dead from acute and somatic diseases revealed microfollicles in the gland; their appearance is considered as a sign of NTDS [2]. Our findings disagree with this conclusion, because the microfollicular rearrangement of the parenchyma is a universal reaction observed during NTDS regression.

Hence, NTDS in acute endotoxemia develops as a result of reduced functional activity of the hypothalamic—pituitary complex (central mechanism) and selective involvement of TG (peripheral mechanism). The peripheral mechanism predominates if the immune response is markedly polarized by the Th1 type, while the central mechanism is predominating in less pronounced reaction of the immune system. The main morphological parameters of NTDS development are dissociation of thyroglobulin synthesis and resorption by follicular thyrocytes with predominance of synthetic processes over resorption in cases with the peripheral mechanism and of resorption processes over synthetic ones in central mechanism and the transition of mast cells to mainly molecular mode of the mediator release. The main morphological indicators of NTDS regression are more intense resorption of the colloid by follicular thyrocytes and reduced release of mediators by mast cells of TG.

REFERENCES

1. L. J. De Grorot, *Crit. Care Clin.*, **22**, No. 1, 57-86 (2006).
2. F. E. De Jongh, A. C. Jobsis, and J. W. Elte, *Eur. J. Endocrinol.*, **144**, No. 3, 221-226 (2001).
3. J. R. Klein, *Exp. Biol. Med.* (Maywood), **231**, No. 3, 229-236 (2006).
4. Z. Kostrouch, F. Bernier-Valentin, and Y. Munari-Silem, *Endocrinology*, **132**, No. 6, 2645-2653 (1993).
5. L. Li, *Curr. Drug Targets Inflam. Allergy*, **3**, No. 1, 81-86 (2004).
6. J. P. Nicola, M. L. Velez, A. M. Lucero, *et al.*, *Endocrinology*, **150**, No. 1, 500-508 (2009).
7. M. Provinciali, G. Di Stefano, and N. Fabris, *Int. J. Immunopharmacol.*, **14**, No. 5, 865-870 (1992).
8. M. L. Vélez, E. Costamagna, E. T. Kimura, *et al.*, *Endocrinology*, **147**, No. 7, 3260-3275 (2006).