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Gender Differences in Pulmonary and Immune Response in Acute Experimental Endotoxicosis

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Differences in the immune and inflammatory response were revealed in the lungs of male and female Wistar rats on day 1 after administration of LPS in a dose inducing the development of acute bacterial endotoxemia. Females showed more pronounced morphofunctional signs of immune system activation than males: this was characterized by more severe accidental involution of the thymus, devastation of the splenic white pulp, and enhanced production of IL-4, IL-12, and TNF-α by splenocytes. In males, production of the above cytokines decreased and inflammation in the lungs was more pronounced at these terms.

Key Words: *gender differences; lipopolysaccharide; immune system; cytokines*

Published data on the effect of sex steroids on the immune system are contradictory [8]. According to experimental and clinical studies, estrogens can either inhibit or activate the immune response. Women are more resistant to viral, bacterial, and parasitic infections than men, but they are more predisposed to severe autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, *etc*. [3,9,11]. Female susceptibility or resistance to infectious-inflammatory and autoimmune diseases is largely determined by sex steroids; plasma levels of sex steroids vary depending on the postnatal period on the menstrual phase [3,15]. Gender differences are usually not taken into account when treating several infectious, inflammatory, and autoimmune diseases, which may reduce the effectiveness of therapy.

Here we studied gender differences in pulmonary and immune response in Wistar rats with acute endotoxicosis induced by administration of high dose of LPS.

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MATERIALS AND METHODS

The studies were carried out on adult Wistar rats of both sexes weighing 220-270 g obtained from Stolbovaya nursery. All animal experiments were performed in accordance with the Order of the Russian Ministry of Health No. 755 of August 12, 1977. The animals were kept in cages (7 rats per cage) at 20-22°C under natural day-night cycles with free access to water and food.

For evaluation of pulmonary and immune response of male (n=10) and female (n=10) Wistar rats to endotoxin, they were intraperitoneally injected with LPS from *E. coli* (strain O26:B6) in a dose of 1.5 mg/kg, causing the inflammatory process and degenerative changes in the target organs (liver and lung) [2]. The animals were sacrificed by overdose of general anesthetic zoletil (10 mg/kg intraperitoneally) on day 1 after LPS administration, because this term corresponds to more pronounced pathological changes in the target organs [2]. Controls (10 males, 11 females) were intraperitoneally injected with saline.

The blood was taken from the cervical veins and centrifuged at 200g for 20 min. The serum was

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separated, frozen at -70°C, and stored for 2 months. Serum corticosterone concentration was assessed by ELISA (Assay designs); endotoxin levels were determined using chromogenic LAL endotoxin assay kit (HBT).

The thymus, spleen and lungs were isolated. Immune organs were fixed in Bouin's fixative for 24 h and the lungs in Carnoy's fluid for 2 h, then embedded in paraffin, sliced, and stained with hematoxylin and eosin. On histological sections, volume density of functional areas of the spleen and thymus was assessed by light microscopy using an Avtandilov grid [1]. The width of the subcapsular layer of the thymus was measured ×640. On histological sections of the lungs, the number of neutrophils in the interalveolar septae was counted (field area of 25,000 μ^2).

To induce cytokine synthesis and secretion, a suspension of splenic cells (10^6/ml) was cultured for 20 h in 1 ml of complete growth medium with concanavalin A (5 μg/ml) in 24-well culture plates at 37°C and 5% CO_2. The culture medium consisted of RPMI-1640 (PanEko) with 5% inactivated FBS, 2 mM glutamine, and 50 μg/ml gentamicin. After incubation, the supernatant was stored for 2 months at -70°C. The concentrations of IL-2, IL-4, IL-12, TNF-α, IFN-γ in the supernatant were measured by ELISA (Bender MedSystems test-systems).

The data were processed statistically. Significance of differences was evaluated using parametric and nonparametric tests (Statistica 7.0). Differences were considered significant at $p<0.05$.

RESULTS

In acute endotoxicosis, male and female Wistar rats exhibited an inflammatory response in the lungs characterized by increased number of neutrophils in the interalveolar septae in comparison with controls (Table 1). At these terms, endotoxin level in the serum significantly increased to 5.2±2.1 U/ml *vs*. 0.7±0.2 U/ml in the control in males and to 11.5±4.5 U/ml *vs*. 3.0±0.4 ME/ml in the control in females. On day 1 after LPS administration, only males showed significantly increased corticosterone level (340.1±22.2 ng/ml *vs*. 200.5±32.3 ng/ml in the control), while in females this parameter did not differ significantly (171.2±40.3 and 158.8±45.8 ng/ml in the experimental and control groups, respectively).

Morphological and morphometric study of the immune organs on day 1 after LPS administration revealed signs of accidental thymus involution, stages I-III, and changed width of the subcapsular zone. In the spleen of males and females, devastation of the white pulp was observed characterized by decreased volume fraction of lymphoid nodules and PALS zones (periarteriolar lymph sheath; Table 1).

Hence, LPS administration to both females and males after 1 day resulted in a severe inflammatory reaction in the lungs, increased levels of endotoxin and corticosterone in males, and reactive changes in the central and peripheral organs of the immune system. The above changes were accompanied by increased production of proinflammatory cytokine IL-2 by splenocytes (Table 2) indicating predominant Th_1 polarization of the immune response.

The analysis of morphology and functional state of immune organs and lungs under conditions of experimental acute endotoxicosis revealed a number of gender differences.

In males, the number of neutrophils in the interalveolar septae after LPS administration was significantly higher than in females (Table 1). According to published data, sex hormones modulate migration and functioning of neutrophils in the respiratory department of the lungs [4,10]. Estrogens are known to attenuate maturation and migration of neutrophils from

TABLE 1. Morphometric Characteristics of Immune Organs and Lungs in Male and Female Wistar Rats under Normal Conditions and on Day 1 after LPS Injection (*M*±*m*)

Gender, group		Lungs	Thymus		Spleen		
		number of neutrophils in the interalveolar septa	ratio of cortex to medulla	width of the subcapsular zone, μ	volume fraction of PALS, %	volume fraction of the lymphoid nodules, %	ratio of white pulp to red pulp
Females	control	1.33±0.13	1.47±0.05	36.56±1.37	39.20±1.37	28.09±1.31	2.71±0.34
	LPS	5.71±0.64*	1.03±0.03*	26.80±1.03*	23.17±0.39*$^{+}$	14.89±0.42*	0.63±0.02*
Males	control	1.6±0.17	1.67±0.06^{++}	30.25±1.61^{+++}	33.88±1.72^{++}	24.76±2.00	1.51±0.10^{+++}
	LPS	10.44±1.07*$^{+}$	1.29±0.03*$^{+}$	39.74±0.72**$^{+}$	24.08±0.35*	16.99±0.33*$^{+}$	0.71±0.02*$^{+++}$

Note: *$p<0.001$, **$p<0.01$ in comparison with controls; $^{+}p<0.001$, $^{++}p<0.05$, $^{+++}p<0.01$ in comparison with females.

TABLE 2. Cytokine Production by Splenocytes in Male and Female Wistar Rats under Normal Conditions and on Day 1 after LPS Administration (pg/ml; *M*±*m*)

Gender, group		IL-2	IL-4	IL-12	IFN-γ	TNF-α
Females	control	69.70±16.89	15.45±4.50	35.11±8.45	11.33±4.86	30.73±6.21
	LPS	371.73±134.87*	29.70±7.45*	41.09±27.00	22.91±9.21	65.23±11.75*
Males	control	91.51±24.19	30.73±7.16^{+}	39.14±9.86	48.29±5.18^{+}	39.75±2.23^{+}
	LPS	380.90±126.82*	5.38±1.01**$^{+}$	3.00±0.03*	33.09±5.47	29.38±8.59^{+}

Note. *p<0.01, **p<0.05 in comparison with controls; ^{+}p<0.05 in comparison with females.

the red bone marrow [14] and decrease their adhesion to the vascular endothelium [6].

On day 1 after LPS administration, the females demonstrated more pronounced activation of the immune system characterized by accidental thymus involution (stages II-III) with devastation of the cortical substance and "starry sky" appearance of the thymus, significantly decreased volume fraction of lymphoid nodules and PALS in the spleen (Table 1) apparently due to migration of lymphocytes into barrier organs. More severe functional activation of the immune system in females was apparently caused by direct effects of endotoxin, because its blood level in females increased by several times in comparison with males. According to the literature, endotoxin binds to specific receptors, expressed by cells of the immune system including thymocytes and splenocytes, which may promote apoptosis and changes in cytokine production [7].

The study of cytokine profile in male and female Wistar rats under conditions of acute endotoxicosis revealed gender differences in the levels of cytokine production by splenocytes. It was found that LPS administration reduced the level of IL-4, IL-12, and TNF-α production in males and increased the level of above cytokines in females (Table 2). The more pronounced activation of the immune system in females is due to the fact that estrogens, in contrast to androgens, activate the expression of TLR4 on the surface of immunocompetent cells [12,13]. This triggers MyD88-dependent and MyD88-independent signal pathways with subsequent production of Th_1-cytokines (IL-2, IFN-γ), Th_2-cytokines (IL-4), and proinflammatory cytokine TNF-α [5].

Thus, differences in the immune response and severity of inflammatory process in the lungs were reported in male and female Wistar rats on day 1 after LPS administration. Females showed more pronounced activation of the immune system and less severe inflammatory changes in the lungs than males. Functional activation of the immune system in females is characterized by increased cytokine level in response to administration of high doses of LPS. At these terms, males exhibited immunosuppression, which manifested in lower IL-4, IL-12, and TNF-α levels. Gender differences in morphofunctional changes depend on the pre-existing immune status determined by the level of sex hormones and expression pattern of estrogen and androgen receptors in immunocompetent cells.

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