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THE DYNAMICS OF HUMORAL COMPONENT OF IMMUNE SYSTEM` PARAMETERS AND THE AMOUNT OF CIRCULATING IMMUNE COMPLEXES UNDER CONDITIONS OF EXPERIMENTAL ACUTE LUNG INJURY

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Abstract

In the response to the simulation of acute lung injury up to 24 hours it was observed a significant increase of the amount of immunoglobulins A, M, G in blood serum. Up to 72 hours their amount continued to reduce, however, it didn't reach control level. Under these conditions, in parallel with the increasing of the concentration of immunoglobulins in blood serum, the amount of circulating immune complexes increases with the achievement of maximum level after 24 hours of experiment and with a subsequent decrease to 72 hours, which significantly exceeds control level.

Key words: **acute lung injury, humoral immunity, circulating immune complexes.**

Introduction. Acute lung injury (ALI) is one of the main causes of acute respiratory failure. ALI — is a diffuse inflammation of the lungs that occurs in patients under critical conditions and is one of the important components of multiple organ failure syndrome. Mortality level ranges from 40 to 60% [1]. ALI is a heterogeneous disease, as the causes of its occurrence can be both pulmonary and extrapulmonary. [2, 3]. Regardless of the causes of its occurrence, a non-specific inflammatory reaction, the destruction of the alveolar-capillary

membrane with increasing of its permeability and the development of non-cardiogenic pulmonary edema and hypoxia are main components of ALI pathogenesis [4].

As a result of endothelial and epithelial damage of the lungs there is an accumulation of neutrophils in the bronchoalveolar and interstitial space, as well as collection of exudate, rich in proteins, in the alveoli, resulting in activation of cytokine system [5]. This leads to the activation and strengthening of the immune system in the center of inflammation, primarily due to the formation of immunoglobulins [8, 11]. Binding of antigens with the subsequent formation of circulating immune complexes (CIC) is one of the main functions of immunoglobulins [6, 7]. This process proceeds continuously and is aimed to maintaining the homeostasis of the organism [10, 12]. Formation of the CIC in the body is one of the components of the normal immune response, which have to lead to the neutralization and elimination of the antigen.

However, under the conditions of excess amount of immune complexes, they are capable of being deposited on the walls of microvessels and lead to microcirculation disruption in organs and systems far from the site of injury [9]. Despite the fact, that the pathogenesis of ALI/ARDS (acute respiratory distress syndrome) has been studied by many authors [1, 2, 14, 17], the features of the immune response are still poorly understood.

The aim of the work was to establish the features of the dynamics of humoral component of immune system` parameters and CIC in the blood of laboratory animals in the simulation of ALI.

Material and methods. Experiments were conducted on white, sexually mature, non-linear male rats weighing 200-220 g, which were kept under standard vivarium conditions. Animals were selected based on their resistance to hypoxia. To evaluate the resistance to hypoxia, the technique of V. Ya. Berezovsky [19] was used. In the simulation of ALI, only the rats with medium resistance to hypoxia were used. The ALI simulation was carried out using the method of G. Matute-Bello [18]. Animals were anesthetized by intraperitoneal administration of thiopental sodium (40 mg•kg^{-1}). After cervicotomy the animals were positioned at an angle of 45° and a a solution of hydrochloric acid (pH = 1,2; 1 ml•kg^{-1}) was injected with an insulin syringe to trachea on the inspiration. A control group of animals under similar conditions was injected with a physiological solution at an equivalent dose.

After 12, 24, 48 and 72 hours after the simulation of ALI, an euthanasia of animals was carried out in the conditions of anesthesia using the method of total cardiotomy. The cellular link of immunity was evaluated by determining the content of immunoglobulins A, M, G (IgA, Ig M, Ig G) in the blood serum using an immunoassay assay kit with the reagent

set “eBioscience, Inc” [20]. The concentration of CIC in serum was determined by precipitation with a solution of polyethylene glycol-6000 [13].

Animal management experiments were carried out in compliance with the norms of the European Convention for the Protection of Animals (Strasbourg, 18.03.1986) and the Resolution of the First National Congress on Bioethics (Kyiv, 2001).

The obtained digital material was processed in the department of systemic statistical research of State Higher Educational Institution “I.Ya. Horbachevsky Ternopil State Medical University MPH of Ukraine” using the non-parametric Mann-Whitney Criterion. STATISTICA software package (StatSoft, Inc., USA) was used.

Results and discussion. The results of study of humoral component of immune system and CIC in rats with simulated ALI are presented in Table 1.

It is known that the concentration of immunoglobulins in the blood serum reflects the balance between their synthesis and disintegration. Our studies have shown the presence of disimmunoglobulinemia in animals with ALI. Thus, in comparison with control, in blood serum of experimental animals, after 12 hours of simulation of ALI, the amount of IgM increased by 35,6% ($p < 0,05$), Ig G — by 55,2% ($p < 0,05$) and Ig A — by 38,6% ($p < 0,05$).

After 24 hours of experiment, the amount of Ig M, Ig G, Ig A in serum continued to grow up. Compared to the control group, the amount of Ig M was 44,8% ($p < 0,05$) higher, but did not significantly differ from the similar level after 12 hours of observation ($p_1 > 0,05$). The amount of IgG was 56,6% ($p < 0,05$) higher compared to control and 14,2% compared to 12 hours after the simulation of ALI ($p_1 < 0,05$). Similarly, the amount of IgA in serum was higher ($p < 0,05$, $p_1 < 0,05$).

After 48 hours of the experiment, the amount of Ig M in serum began to decrease and became significantly lower comparing previous observation periods ($p_{1-2} < 0,05$), however, it exceeded control level by 41,1 % ($p < 0,05$). Similarly, the level of Ig G ($p_{1-2} < 0,05$) in blood serum also decreased, but was 56,3 % higher comparing to control, which turned out to be statistically significant ($p < 0,05$). Also, the level of IgA was reduced in comparison with 12 and 24 hours of ALI ($p_{1-2} < 0,05$), but remained 39,7% higher compared with the control group ($p < 0,05$).

Table 1 — The dynamics of humoral component of immune system` parameters and the amount of CIC after 12, 24, 48 and 72 hours after simulation of acute lung injury (M±m)

Parameter	Experiment conditions				
		12 h (n=10)	24 h (n=10)	48 h (n=8)	72 h (n=8)
CIC, c.u.	Experiment	122,7±2,3*	147,2±2,2* $p_1<0,05$	142,5±1,8* $p_1<0,05$	122,5±2,4* $p_1>0,05$ $p_2<0,05$ $p_3<0,05$
	Control	71,00±0,91 (n=10)	72,34±0,88 (n=8)	70,13±0,78 (n=6)	68,05±1,66 (n=8)
Ig M, $g \cdot l^{-1}$	Experiment	1,278±0,080*	1,489±0,105* $p_1>0,05$	1,160±0,002* $p_1<0,05$ $p_2<0,05$	1,123±0,006* $p_1<0,05$ $p_2<0,05$ $p_3<0,05$
	Control	0,822±0,027 (n=10)	0,808±0,032 (n=8)	0,836±0,030 (n=6)	0,802±0,044 (n=8)
Ig G, $g \cdot l^{-1}$	Experiment	2,164±0,010*	2,252±0,002* $p_1<0,05$	2,220±0,003* $p_1<0,05$ $p_2<0,05$	2,182±0,006* $p_1>0,05$ $p_2<0,05$ $p_3<0,05$
	Control	0,977±0,005 (n=10)	0,968±0,008 (n=8)	0,954±0,025 (n=6)	0,992±0,015 (n=8)
Ig A, $g \cdot l^{-1}$	Experiment	1,086±0,005*	1,124±0,003* $p_1<0,05$	1,107±0,002* $p_1>0,05$ $p_2<0,05$	0,997±0,008* $p_1<0,05$ $p_2<0,05$ $p_3<0,05$
	Control	0,667±0,005 (n=10)	0,652±0,011 (n=8)	0,669±0,008 (n=6)	0,684±0,012 (n=8)

Notes:

1. * - differences are statistically significant in comparison with the control group ($p < 0,05$);
2. p_1 - the significance of differences compared to 12 hours after the simulation of ALI;
3. p_2 - the significance of differences compared to 24 hours after the simulation of ALI;
4. p_3 - the significance of differences compared to 72 hours after the simulation of ALI.

After 72 hours of ALI, the amount of Ig M in blood serum continued to decrease and was statistically significantly lower than in 12-48 hours ($p_{1-3} < 0,05$). Compared to the control group, the amount of Ig M remained significantly higher by 26,7% ($p < 0,05$). The level of serum Ig G, which reached its values after 12 hours of observation ($p_1 > 0,05$) and was statistically significantly lower than in 24 and 48 hours ($p_{2-3} < 0,05$) was decreased but, compared with the control group, its level remained significantly higher — by 55,5% ($p < 0,05$). The level of serum IgA in 72 hours of observation significantly decreased, which turned out to be statistically significant in comparison with other terms of observation

($p_{1-3} < 0,05$), but the parameter did not reach the level of control and remained statistically significantly higher by 33, 1% ($p < 0,05$).

The violation of immunoglobulins` amount in the blood serum of experimental animals reflects the reaction of primary and secondary lymphoid organs. It is well-known that an adequate immune response is based on the balance of such reactions as humoral-immune and cell-mediated. One of the main functions of immunoglobulins is both antigens and autoantigens neutralization with the subsequent formation of CIC and their excretion from the organism. At certain pathological conditions, the CIC are fixed under the basal membranes and in the wall of the internal organs, which lead to an inflammatory reaction [14]. The investigations have shown that after 12 hours after the ALI simulation, a significant increase in serum CIC level was detected — by 1,7 times compared with the control group ($p < 0,05$).

After 24 years of experiment, it was observed an increase of serum CEC level by 2,1 times ($p < 0,05$) compared to control and by 1,2 times compared with 12 hours of follow-up ($p_1 < 0,05$). The level of serum CIC 48 hours after ALI simulation decreased by 1,2 times compared to 12 hours ($p_2 < 0,05$), but remained significantly higher than in the control group (2,0 times, $p <0,05$). After 72 hours after the ALI simulation, the amount of CIC decreased by 1,2 times compared to 24 hours ($p_2 < 0,05$) and by 1,1 times compared to 48 hours ($p_3 < 0,05$), however, it was 1,7 times greater, than in control ($p < 0,05$).

The detected changes indicate on the increased reactivity of B-cells and the violation of mechanisms of CIC neutralization. However, we can assume that the increase of serum CIC may be an indirect sign of complement activation, resulting in tissue damage. CIC interact with almost all cells in the bloodstream, complement system and the receptors of many tissues and organs [15]. Interacting with immunocompetent cells they stimulate an immune response, which results in the withdrawal of proteolytic enzymes, increasing production of kinins, and anafilotoxins and opsotoxins [16], which is accompanied by harmful effect on the tissue, creating a syndrome of multiple organ dysfunction and failure.

Conclusions 1. In response to the simulation of ALI up to 24 hours it was observed a significant increase in the amount of immunoglobulins A, M, G. Up to 72 hours their levels continued to reduced, however, they didn’t reach the level of control.

2. Under the conditions of experimental ALI, proportional to the level of immunoglobulins, the amount of CIC increases with the maximum after 24 hours and the subsequent decreasing to 72 hours, which significantly exceeds the control level.

Prospects for further researches. It is suggested to conduct an in-depth study of systemic manifestations of accumulation of CEC in serum under conditions of experimental ALI and the search for methods of its correction.

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