

## Dynamics of indicators of cellular immunity in conditions of acute generalized peritonitis in rats

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### ABSTRACT

The course of peritonitis depends on the state of the immune system and the adequacy of the immune response. The aim of this investigation was to explore the features of the cellular immune response cell in rats with simulated acute generalized peritonitis (AGP). The study was conducted on 32 rats, divided into two groups: the main group – 24 animals with simulated peritonitis; control– 8 intact animals. In the animals of the main group AGP were modeled by injecting of 10 % filtered stool suspension into the abdominal cavity of the study rats at a dose of 0.5 ml per 100 g of body weight. Removal of material for histological examination was carried out on days 1, 3 and 7. Indicators of cellular immunity were determined by a method that was based on the interaction of fluorescently labeled monoclonal antibodies with lymphocyte surface antigens. In the main group of animals, all indicators of the cellular immunity gradually decreased from 1 to 7 days of the experiment. The concentration of CD3+ - cells decreased by 1.90 times per day, by 1.97 times by 3 days, and by 2.10 times by 7 days, compared with intact animals. Suppression of the cellular immunity was observed after modeling of AGP, which is combined with a decrease in the number of both CD3+ lymphocytes and the main subpopulations of CD4+, CD8+ and CD16+ cells. The greatest decrease in the cellular level of immunity was observed on day 7 of the experiment.

**Abbreviation:** AGP – acute generalized peritonitis.

**Keywords:** *cellular immunity; acute generalized peritonitis; simulated peritonitis.*

### 1. INTRODUCTION

AGP remains a medical and social problem since the high risk of mortality depends on timely diagnosis and severity [1]. The course of peritonitis depends on the state of the immune system, the adequacy of the immune response and is an important criterion in the progression of the inflammatory process in the abdominal cavity [2]. The correct immune response and sufficient compensation reserves contribute to the localization of inflammation [3].

A considerable number of complications and fatal cases in peritonitis are caused by various pathogenetic mechanisms involved in this pathological process, many of which have not been sufficiently studied to date. The idea of the leading peritonitis mechanisms over time has undergone a transformation [4-5]. The severity of peritonitis depends on the adequacy of the immune response. Correct immune response and sufficient reserves of compensation of the body contribute to the localization of the inflammation [6]. Immune deficiency causes an adverse course of

peritonitis. It is characterized as a secondary acquired immunodeficiency state, which causes the development of complications, sepsis, and death of patients [7-8].

The state of the immune system is main determinant of the development of the inflammatory process in peritonitis. During cell destruction, the cellular immune response is created, mediated by macrophages, T-lymphocytes, natural killer cells, which exhibit a specific (by direct cytolysis) and nonspecific (through the production of inflammatory mediators) cytotoxic effect [9-10]. The potentiation or summation of pathogenetic effects, one of which is the immune response, is decisive in the course of the development of AGP and this remains an unresolved question [11]. Although some progress has been made in the study of the pathogenesis of AGP in recent years, the role of the cellular immune system in the development of complications of the investigated pathology has not yet been fully elucidated.

### 2. MATERIALS AND METHODS

#### 2.1. Animals and Experimental Protocol.

In the experiment, 32 white rats were used, which were divided into 2 groups: the main group – 24 animals with simulated peritonitis; the control group consisted of 8 intact animals, which were kept under standard vivarium conditions. All groups of compared animals were representative by weight, sex, and age.

The AGP was studied on the model proposed by V.A. Lazarenko et al [12]. This model is close to a similar process in humans in

terms of etiological factors, clinical manifestations, and phase of the course. On the 14th day after administration of streptozotocin, animals of the main group were injected with 10 % filtered stool suspension into the abdominal cavity of the study rats at a dose of 0.5 ml per 100 g of body weight. Rats of the comparative group received only subcutaneous injection of fecal suspension. Rats of the comparison group received only subcutaneous injection of fecal suspension. The stool suspension was obtained by mixing

isotonic saline and fecal matter the caecum of 2-3 intact animals, then filtered twice through a double layer of gauze. The resulting suspension was injected into the intact rats in a puncture manner no later than 20 min after preparation. In order to avoid damage to the internal organs when the fecal suspension was introduced into the abdominal cavity, the animals were kept upright, with a caudal end up. Using the method of puncture of the ventral wall in the center of the midline of the abdomen, directing the end of the needle alternately into the right and left iliac areas, introduced the same amount of stool suspension. The observation dates were 1, 3, and 7 days from the beginning of peritonitis modeling.

## 2.2. Determination of indicators of cellular immunity.

Indicators of cellular immunity were determined by a method that was based on the interaction of fluorescently labeled monoclonal antibodies with lymphocyte surface antigens. T- and B-lymphocyte subpopulations were determined using Rat ELISA Kits («NeoScientific» та «MyBioSource», USA). 20  $\mu$ l of antibodies (CD3+/CD4+/CD8+, CD16+) and ethylenediamine tetraacetic acid were introduced into each tube

## 3. RESULTS

As shown by our studies, the concentration of total T-lymphocytes (CD3+ cells) decreased by 1.90 times compared to intact animals as early as 1 day in animals with simulated AGP (Table 1). On the 3rd day after peritonitis modeling, this index also decreased (by 1.97 times). A more significant decrease in the level of CD3+ cells was obtained on day 7 of the experiment. During this period, CD3+ was statistically significantly lower than the intact animal level by 2.10 times.

Subpopulations of regulatory T-lymphocytes, namely: T-helper cells (CD4+ cells) and T-suppressors (CD8+ cells) are constituents of the total fraction of the T-cell system. Therefore, it is quite natural that the same dynamics of the level of regulatory T-lymphocytes as the dynamics of the level of CD8+ cells as the AGP develops (Figure 1). When determining CD4+ cell fraction at days 1, 3, and 7, we recorded a decrease of 1.5, 1.6, and 1.7 times, respectively. At the same time, the content of T-suppressors / cytotoxic lymphocytes (CD8+ cells) was already decreased by 1.4 times for 1 day, and for 3 and 7 it had a similar tendency. The presented data showed that, against the background of significant intoxication in the simulated AGP, the content of natural killer cells (CD16 + cells) decreased by 1, 3 and 7 days by 1.4, 1.5 and 1.6 times, respectively.

### 3.1. Discussion.

It is known that the pathogenesis of peritonitis, including AGP, plays an important role in bacterial intoxication. This condition, which is essentially a significant stress factor, causes structural and functional restructuring of the immune system. The accumulation of pathogenic microflora in these conditions is more characterized as an immunosuppressive syndrome. The results obtained show that in this dysfunctional state are all the levels of cellular immunity evaluated by us. Inflammatory processes of any level are realized with the coordinated interaction of all components of the immune system.

It is well known that the functional potential of cells of the monocyte-phagocytic system significantly influences the state of the cellular level of immunity. When assessing the phenotypic characteristics of the main populations and subpopulations of the

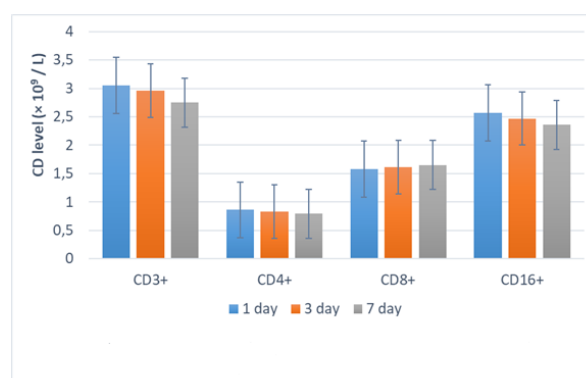
without touching the tip of the tube walls. The samples were stirred in a vortex and incubated in the darkness for 15-30 min at room temperature. The recommended number of leukocytes is 3.5-9.4 g / L. For erythrocyte lysis, 500  $\mu$ l of lysis solution was added to each tube, shaken on a vortex, and incubated for 10-15 min at room temperature. 500  $\mu$ l of buffer solution was added to the tubes.

The samples were analyzed on an Epics-XL flow cytometer manufactured by Beckman Coulter (USA).

### 2.3. Statistical analysis.

Statistical processing of the received data was performed on a personal computer using standard software packages of Microsoft Excel and with the help of the computer program Statistica for Windows version 6.0 (Stat Soft inc., USA). The results were presented as mean values (M)  $\pm$  the error of the mean (m) and were tested by one-way ANOVA, followed by Fisher's least significant difference procedure as a post-hoc test. A level of  $P < 0.05$  was considered significant.

cellular level of immunity, their significant differences between groups of intact animals and animals that were modeled by acute peritonitis were established. An important point in our case is a regular change in the indicators of T-cell immunity as the terms of the study prolong. The concentration of total T-lymphocytes (CD3+ cells) a day after the AGP was 1.9 times lower than the control.



**Figure 1.** Indicators of cellular immunity in rats on 1, 3, 7 days after modeling of AGP. AGP – acute generalized peritonitis.

The development of any systemic pathology, including AGP, is a powerful stress-inducing factor for the body. T-lymphocytes, both the general population and its subpopulations, express on the membrane a significant number of receptors for glucocorticoids, which causes cell death in apoptosis at increased concentrations of these hormones in the body. The mechanism of such death of T-lymphocytes is realized at least 40-48 hours after the development of stress. Therefore, we obtained a significant decrease in the content of T-lymphocytes at this time programmed phenomenon of cell death in apoptosis.

Subpopulations of regulatory T-lymphocytes, namely: T-helper cells (CD4+ cells) and T-suppressors (CD8+ cells) are constituents of the total fraction of the T-cell system. Therefore, it is quite natural that the same dynamics of common T lymphocytes as the AGP develops. However, there are clearly different degrees of change in each subpopulation. The concentration of T-helper

cells (CD4+ cells) decreased from 1 to 7 days of experiment 1.5 and 1.7 times, respectively. From the 1st to the 3rd day, the rate of decline of T-helpers was significantly lower. At the same time, the content of T-suppressors / cytotoxic lymphocytes decreased by 1.4 times by 1 day, and by 3 and 7 had a similar tendency. Such redistribution of subpopulations with a predominant content of T-suppressors/cytotoxic lymphocytes indicates a pronounced dysregulatory state in the AGP of those cells that largely determine the level of implementation of the immunoinflammatory response. It is the cells with the suppressor response that ensure its realization within the "physiological corridor". Against the background of insufficient number (and function), this process becomes "unmanageable" with those features that we have identified and presented in Figure 1.

In recent years, attention has been focused on cells that exert effector protective function of the body on natural killer cells (CD16+ cells). The presented data showed that, against the background of significant intoxication with the simulated AGP, the content of these cells decreased from 1.4 to 1.6 times from 1 to 7 days, respectively, which is explained by the violation of water, electrolyte, carbohydrate and vitamin metabolism. However, expressed intoxication leads to impaired protein metabolism and liver function – accumulate intermediate metabolites of metabolism. In such conditions, the immune system shows its failure, which can be a consequence of the imbalance of the neuroimmune vector.

**Table 1.** Indicators of cellular immunity in rats with simulated acute generalized peritonitis (m±M).

Research term	CD3+, ×10 <sup>9</sup> / L	CD4+, ×10 <sup>9</sup> / L	CD8+, ×10 <sup>9</sup> / L	CD16+, ×10 <sup>9</sup> / L
control group	5,82±0,22	1,35±0,14	2,23±0,19	3,81±0,20
1 day (n=8)	3,05±0,23	0,86±0,12	1,58±0,24	2,57±0,13
3 day (n=8)	2,96±0,31	0,83±0,11	1,61±0,28	2,47±0,17
7 day (n=8)	2,75±0,13* <sup>#</sup>	0,79±0,13	1,65±0,23*	2,36±0,24* <sup>#</sup>

Notes: \* – significance of the difference of indicators in comparison with 1 day;

# – significance of the difference of indicators in comparison with 3 day.

#### 4. CONCLUSIONS

Suppression of the cellular immunity was observed after modeling of AGP, which is combined with a decrease in the number of both CD3+ lymphocytes and the main subpopulations of CD4+, CD8+ and CD16+ cells. A more significant decrease in CD3+ lymphocytes was obtained on day 7 of the experiment. During this time, the level of CD3+ lymphocytes was statistically significantly lower than the level of intact animals 2.1 times. The

concentration of T-helper cells (CD4+ - cells) gradually decreased from 1 to 7 days of experiment 1.5 and 1.7 times, respectively. The presented data showed that, against the background of significant intoxication with the simulated AGP, the content of natural killer cells (CD16+ cells) decreased from 1.4 to 1.6 times from 1 to 7 days, respectively.

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