AJPMHS GLOBAL RESEARCH NETWORK

AMERICAN Journal of Pediatric Medicine and Health Sciences
Volume 3, Issue 3, 2025 ISSN (E): 2993-2149

Experimental Assessment of the Effect of Acute Radiation on Immune System Indicators and the Efficiency of Biocorrection

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Abstract: The aim of the study was to determine the effect of acute radiation on the immune status of experimental animals and to demonstrate the degree of influence of biocorrection on this process. It was found that the relative and absolute indicators of immune system cells in male white rats that received and did not receive acute radiation were different. This was observed in the total number of lymphocytes, the number of CD3+-, CD4+- and CD8+-cells. A decrease in IRI in the main group by 2.01 times was recognized as the main sign of the development of secondary immunodeficiency. It was found that there were no obvious changes in CD20+-cells, CD16+-cells significantly increased by 1.35 times in the main group of laboratory animals, and CD95+-cells significantly decreased by 1.51 times. In the biocorrected group, the immune system failure was not relatively deep, it was proved that the used biopreparation has an immunostimulating effect, its use reduces the negative effect of acute radiation on the indicators of immune system cells.

Keywords: acute radiation, immunocompetent cells, laboratory animals, secondary immunodeficiency, biocorrection.

Introduction.

It is known that radiation, when it negatively affects all systems and organs of the body, leads to irreversible consequences [3, 10]. It has been proven that radiation selectively affects organs and tissues of the body, including the hematopoietic system and the immune system, the negative, and in many cases fatal, effects of radiation on these organs have been proven by data presented in numerous sources [5, 9, 12].

Acute radiation - depends on the frequency and duration of ionizing radiation and develops to varying degrees depending on the sensitivity of organs to radiation. The most sensitive organs to acute radiation are the organs of the immune system, the mucous membranes of the gastrointestinal tract, exocrine and endocrine glands, and gonads. Organs with low sensitivity to radiation include the heart, kidneys, liver, brain and spinal cord, bone tissue, and joints [4, 11].

The purpose of the research work was to determine the effect of acute radiation on the immune status of experimental animals and to show the level of influence of biocorrection on this process.

Material and methods. To achieve this goal, 60 adult females , weighing 160-180 g , were selected . Male white outbred rats were recruited for the study. Laboratory animals were housed in plastic cages under standard vivarium conditions with relative humidity (50-60%), temperature (19-22 ^{0} C) , and 12-hour dark and 12-hour light regime. The care of laboratory animals was carried out according to the recommendations of Nuraliev N.A. et al . [6] . When

working with laboratory animals, the rules of biological safety [2, 6] and the ethical principles of working with laboratory animals were strictly observed .

All laboratory animals were divided into the following groups:

The main group was white outbred rats (n=30) that received a single acute radiation dose of 5 Gy, fed a standard vivarium diet;

Control group - intact white outbred rats (n=30) fed a standard vivarium diet and not exposed to acute radiation.

The main group was divided into 2 subgroups: group 1a - white crossbred rats (n=15) that received a single acute radiation dose of 5 Gray, with the addition of the biologically active supplement "Lactopropolis-AWL" as a biocorrection to the standard vivarium diet; group 1b - white crossbred rats (n=15) that received a single acute radiation dose of 5 Gray, without biocorrection.

In the experiment, irradiation of laboratory animals was carried out using the AGAT-R1 (Estonia) gamma-therapeutic apparatus, in which the radiation source was So-60. Studies related to irradiation of animals were conducted at the Bukhara branch of the Republican Specialized Scientific and Practical Center of Oncology and Radiology of the State Medical University of the Republic of Uzbekistan.

The drug "Lactopropolis-AWL" was administered to all laboratory animals every morning, based on their weight. Those who received acute radiation were given the drug for 20 days, irradiated on the last day, and then sacrificed on the 5th day, and immunological studies were conducted. The composition of the biologically active supplement "Lactopropolis-AWL" consists of an extract of probiotic bacteria *Lactobacillus rhamnosus 925, Enterococcus durans* , and biologically active compounds of propolis, which has antimicrobial, immunostimulating, and anti-inflammatory properties (product of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan and "AllWellLab" LLC).

The state of the immune system of laboratory animals was assessed by the expression of CD-differentiating and activating antigens. The following markers of immunocompetent cells were determined: CD3+-, CD4+-, CD8+-, CD16+-, CD20+-, CD95+-lymphocytes. The expression of CD receptors was determined by the method of Garib F.Yu. et al. (1995) in the rosette reaction using monoclonal antibodies of the LT series manufactured by Sorbent LLC (RF). The immunoregulatory index (IRI , CD4+/CD8+) was calculated.

The materials were statistically processed using traditional variational statistics methods. For this purpose, a software package for medical and biological research was used on a personal computer based on the Pentium IV processor. The principles of evidence-based medicine were used in the organization and conduct of the research.

Results and discussion. To study the effects of acute radiation, the main parameters of the immune system of intact white male rats that were not exposed to this effect were first studied and the results were analyzed. A total of 9 parameters were interpreted and analyzed (Table 1).

Table 1. Basic parameters of the immune system of intact white outbred rats involved in the study, n=30

Indicators	Relative (%)	Absolutely
Leukocytes, x10 ^{9} /l	-	4680±36
The total number of lymphocytes	49.8±1.1	2331±51
CD3+ cells	50.3±1.2	1172±28
CD4+ cells	32.7±0.9	762±21
CD8+ cells	12.9±0.8	301±19
IRI, unity	2.53±0.01	2.53±0.01

CD16+ cells	18.1±1.3	422±30
CD20+ cells	19.6±1.4	457±33
CD95+ cells	17.8±1.2	415±28

Quantitative and relative (%) parameters of these indicators are presented in Table 1. These results were similar to the data reported by previous researchers [1].

Table 2 shows the results obtained on the fifth day post-irradiation in the basic indicators of the immune system of white non-bred rats that received a single acute radiation dose of a total of 5 Gray .

Table 2. Quantitative parameters of the main immunocompetent cells of white outbred rats that received acute radiation, n=30

Indicators	Relative (%)	Absolutely
Leukocytes, x10 ^{9} /l	-	4600±49
The total number of lymphocytes	35.3±1.4	1624±64
CD3+ cells	31.8±1.5	516±24
CD4+ cells	22.8±1.1	370±18
CD8+ cells	18.1±1.2	294±19
IRI, unity	1.26±0.02	1.26±0.02
CD16+ cells	24.2±1.6	396±26
CD20+ cells	21.9±1.7	356±28
CD95+ cells	11.8±1.5	192±24

The results showed that the quantitative indicators of leukocytes in the main and control groups of laboratory animals did not differ significantly from each other (P>0.05). In our opinion, this is explained by the short period after irradiation (5 days).

When the quantitative and relative amounts of lymphocytes were compared, we witnessed a completely different picture. It was recognized that their relative index reliably decreased to 1.41 times in experimental animals that received acute radiation compared to the control group (intact) (R<0.05).

When comparing the absolute parameters of these cells, a practically similar trend was observed, the decrease was 1.44 times (P<0.05). The decrease in the relative and absolute numbers of lymphocytes is explained by the effect of acute radiation on the proliferation and differentiation of these cells, as well as a decrease in their activity.

Regarding the analyzed immunocompetent cells of the body's immune system, changes in T-lymphocytes (SD3+-cells) and their main subpopulations (SD4+ and SD8+-cells) were different. Relative and quantitative parameters of SD3+-cells showed a convincing decrease compared to the parameters of the control group (Figure 1).

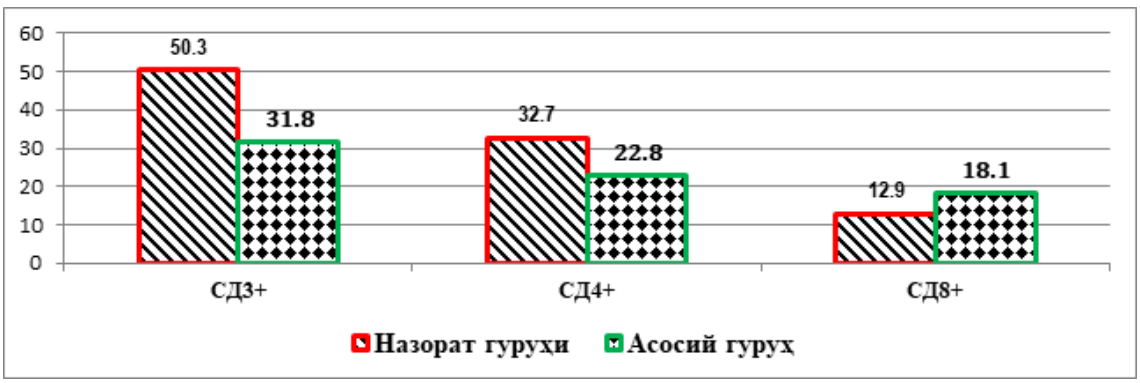

Figure 1. Comparative description of relative parameters in the T-lymphocyte system of white rats that received acute radiation (primary) and did not receive (control), %

If we summarize the figures, the decrease in CD3+ cells was 1.58 times (P<0.001), while the relative amount of CD4+ cells was 1.43 times (P<0.05). We witnessed the opposite picture in the relative amount of CD8+ cells - these cells were significantly increased in the main group compared to the control - by 1.40 times (P<0.05). Both lymphocytes responded to the same effect with different changes.

The decrease in the relative number of CD3+ and CD4+ cells in the group of acutely irradiated white outbred rats compared to intact laboratory animals was explained by a decrease in the total number of lymphocytes, immunodeficiency in the T-lymphocyte system, and this phenomenon was also recognized as an acute radiation effect, since other factors affecting laboratory animals were eliminated. If we take into account that one of the main functions of CD8+ cells is to reduce the strength of the immune response, the increase in these cells relative to other cells is one of the reasons for the development of secondary immunodeficiency.

Similar results were obtained for the above-mentioned quantitative indicators of immunocompetent cells (except for CD8+ cells). If a significant difference of 2.27 times was found in the absolute number of CD3+ cells between the main and control groups in favor of the control group (P<0.001), then the same trend was observed for CD4+ cells (difference of 2.06 times, P<0.001). However, it was noted that no such trend was found for CD8+ cells. No significant differences were found in the two compared groups (P<0.05). This difference between relative and quantitative indicators raises the question of which indicators should be relied on when drawing informed conclusions based on interpretation and analysis. If we take into account that the quantitative indicator depends more on the quantitative parameters of leukocytes and lymphocytes, it becomes clear that the trend of changes in relative indicators allows us to obtain accurate results and draw reasonable conclusions. Therefore, it is recommended to use relative indicators in experimental studies to assess the functioning of the immune system and the state of immunocompetent cells.

Another evaluation parameter used to assess the T-system of the immune system is the IRI. This index shows the ratio of the main immunoregulatory cells, T-lymphocytes, to each other at the same time, the higher the IRI, the less pronounced the immune deficiency in the body, the lower it is, the greater the depth of secondary immunodeficiency [7]. Therefore, it is recommended to always use the IRI when assessing the immune status. In studies, the IRI in the control group differed significantly from the main group - by 2.01 times (P<0.001). The fact that this unit shows the same results in both relative and absolute parameters indicates that it can be used to assess the depth of immunodeficiency. We believe that to assess the functioning of the immune system, a comparative assessment of the relative indicators of immunocompetent cells and the IRI is sufficient.

Since we found it appropriate to describe the T-cell of the immune system along with the B-cell, the relative and absolute numbers of CD20+ cells were also studied and analyzed. The results showed that there were no significant differences between the groups compared in terms of this immunocompetent cell (P > 0.05). It can be seen that the 1.12-fold difference was in favor of the main group.

Although the results were not significantly different, SD20+ cells from acutely irradiated albino rats showed a proliferative tendency compared to intact laboratory animals. This situation showed that changes in the immune system's T-joint develop faster than in the V-system, and the V-joint tends to fill the deficit that occurs in the immune system. It is noteworthy that results different from relative parameters in terms of absolute indicators were obtained, the data in the control group was higher than the main group (R<0.001).

SD16+ cells, which are included in the non-specific defenses of the immune system, perform the task of identifying and destroying allogeneic and xenogeneic cells, tumor cells, which multiply in the body independently of antigens. If we take into account that when there is a need to destroy tumor cells formed as a result of external influences (irradiation), their number increases

and their activity increases, the true reasons for the quantitative and relative changes of SD16+-cells become known. In the observations, it was observed that the relative parameter of these cells in the laboratory animals that received acute radiation increased convincingly compared to the control group - by 1.35 times, (R<0.05). No significant difference was found between the absolute values (R>0.05).

The SD95+-receptor is one of the apoptosis receptors, which is expressed on the surface of all cells of the immune system and participates in the control (regulation) of the activity of the immune system. In our studies, the relative amount of SD95+-cells was convincingly reduced in non-white rats belonging to the control group - by 1.51 times, R<0.05 (Fig. 2).

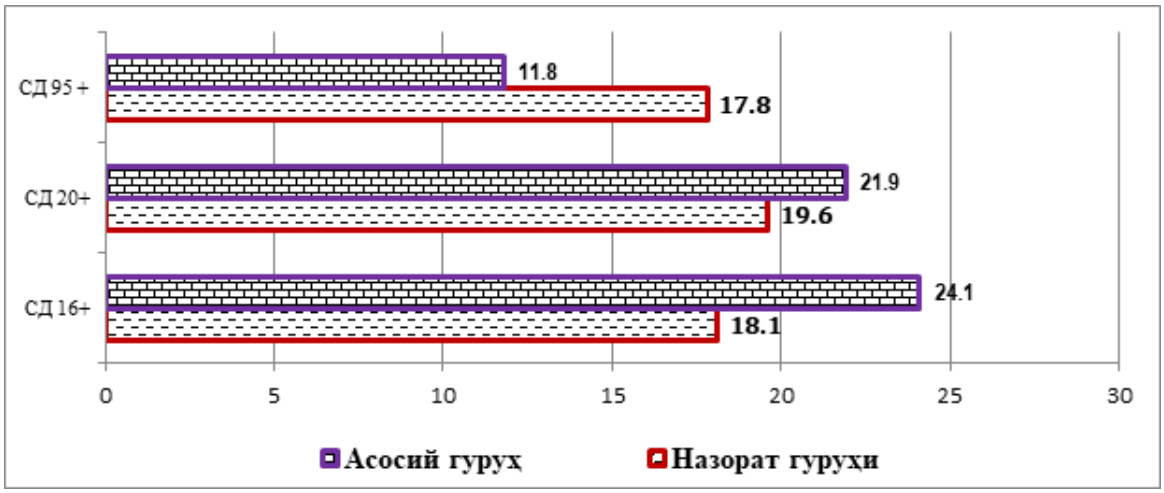

Figure 2. Comparative indicators of the relative amounts of immunocompetent cells of laboratory animals that received (primary) and did not receive (control) acute radiation, %

If we take into account that the decrease in the number of lymphocytes expressing the SD95+ marker is observed in autoimmune and oncological pathologies [8], it indicates that the level of preparation of lymphocytes for apoptosis has decreased, and the activity of the immune system is gradually decreasing.

The next stage of the work was to evaluate the effect of biocorrection on cells of the immune system in experimental animals that received acute radiation.

Biocorrection was carried out with the drug "Lactopropolis-AWL", which was administered daily in the morning, depending on the weight of the laboratory animals. This drug was administered for 20 days, and on the last day, acute total radiation was performed once at a dose of 5 Gray. On the 5th day after irradiation , the laboratory animals were euthanized, their blood was taken, and immunological studies were conducted.

The obtained results are presented in Table 3.

Table 3. Comparative indicators of the main parameters of the immune system of laboratory animals that received acute radiation in the state with and without biocorrection

Indicators	No biocorrection, n=30		Biocorrected, n=30	
	%	Absolutely	%	Absolutely
Leukocytes, x10 ^{9} /l	-	4600±49	-	5650±6.1* ↑
The total number of lymphocytes	35.3±1 , 4	1624±64	44.5±1.6* ↑	2514±90* ↑
CD3+ cells	31.8±1.5	5 16±24	39.9±1.7* ↑	1003±43* ↑
CD4+ cells	22.8±1.1	370±18	23.9±1.2 ↔	601±30* ↑
CD8+ cells	1 8.1±1.2	294±19	16.3±1.1 ↔	410±28* ↑

BIG	1.26±0.2	1.26±0.2	1.47±0.1* ↑	1.47±0.1* ↑
CD16+ cells	24.4±1.6	396±26	22.3±1.5 ↔	561±38* ↑
CD20+ cells	21.9 ± 1.7	356±28	23.6±1.8 ↔	593±45* ↑
CD95+ cells	11.8±1.5	192±24	15.5±1.0* ↑	390±30* ↑

Note: * - reliability sign between the groups with and without biocorrection: ↑ - direction of change; ↔ - no reliable difference.

It can be seen that the results obtained for relative and absolute indicators are different, in 4 of the relative indicators (8 parameters) (50%) the indicators have changed to a positive level, and in the remaining 4 indicators (50.0%), no reliable changes have been detected, however, a tendency to shift in the positive direction has been observed.

Reliable relative indicators were not observed for the total number of lymphocytes ($R<0.05$, up to 1.26-fold increase), the number of SD3+-cells ($R<0.05$, up to 1.25-fold increase), IRI ($R<0.05$, up to 1.17-fold increase) and the amount of SD95+-cells ($R<0.05$, up to 1.31-fold increase). It was observed that cells that were first exposed to acute radiation were partially less sensitive after biocorrection.

There were significant changes ($R<0.05$ - $R<0.001$) in the absolute amounts (100%) of the 9 studied immunocompetent cells. The results of the group of laboratory animals that underwent biocorrection before acute irradiation showed a positive shift of immune system cells from 1.17 to 2.03 times compared to white rats without biocorrection, we want to emphasize again that all the changes were convincing.

If we compare the parameters of the 1b subgroup that underwent biocorrection with those of intact laboratory animals (control group), we observe that there are some differences from this subgroup:

the first aspect is the convincingly higher absolute values of the studied 9 immunocompetent cells of the immune system compared to the control group of laboratory animals;

the second aspect is that the relative amounts of the studied 9 immunocompetent cells of the immune system approached the parameters of intact laboratory animals, especially the total number of lymphocytes, SD25+ cells;

The third aspect is that CD4+ cells are practically the same before and after biocorrection with the biologically active supplement “Lactopropolis-AWL” and are far from the norm;

the fourth aspect is that the SD20+-cells deviate even more from the given norm values. In general, it was observed that the quantitative deficiency of the immune system was less pronounced in the group of white rats that underwent biocorrection (group 1b) compared to the control group (group 1a).

Conclusion.

1. The results of the study showed that the relative and absolute values of immune system cells in male white rats that received and did not receive acute radiation differed from each other. These results were mainly observed in the total number of lymphocytes, CD3+-, CD4+- and CD8+-cells, which in laboratory animals that received acute radiation significantly decreased by 1.41, 1.58 and 1.43 times, respectively ($P<0.05$), while the number of CD8+-cells increased by a total of 1.40 times ($P<0.05$).

2. A decrease in IRI to 2.01 times in the main group was recognized as the main sign of the development of secondary immunodeficiency. To assess the functioning of the immune system, it was considered sufficient to compare the relative indicators of immunocompetent cells and IRI.

3. It was found that the changes in the V-joint of the immune system (SD20+ cells) were not evident, there was no significant difference, but there was a trend of their increase in the main

group. SD16+-cells were significantly increased by 1.35 times in the main group of laboratory animals, indicating increased activity of the immune system against cells of allogeneic and xenogeneic nature. The amount of SD95+-cells in the main group was convincingly reduced by 1.51 times, which explained the decrease in readiness of lymphocytes for apoptosis, and the increase in the probability of tumor cells in the body.

4. Comparing the parameters of the 1b subgroup that underwent biocorrection with the parameters of intact laboratory animals, we observe the following differences: the absolute values of the 9 studied indicators of the immune system are significantly higher than in the control group; the relative amounts of immunocompetent cells are close to the values of intact laboratory animals; the relative amount of CD4+ cells before and after biocorrection with the biologically active additive “Lactopropolis-AWL” is practically the same (P>0.05), far from the norm; the CD20+ cells differ even more from the given norm indicators. It was observed that the quantitative deficiency of the immune system in the group of white outbred rats that underwent biocorrection was less pronounced than in the comparison group.

5. In the biocorrected group, the immune system deficiency was not relatively deep, which proved that the used biopreparation has an immunostimulating effect, its consumption reduces the negative effect of acute radiation on the quantitative parameters of the immune system cells.

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