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SCIENTIFIC PROOF OF IMMUNOLOGICAL SIGNS OF THE EFFECTS OF ACUTE RADIATION IN EXPERIMENTAL ANIMALS

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Abstract: The aim of the study was to determine the effect of acute irradiation on the immune status of experimental animals, taking into account the degree of influence of biocorrection on this process. It was found that in white outbred male rats that received and did not receive acute irradiation, distinctive results were obtained in the relative values of immune system cells. This was observed in the total number of lymphocytes, cells - CD3+-, CD4+- and CD8+. A key indicator of the development of secondary immunodeficiency is a decrease in IRI by 2.01 times in the main group. No changes were found in CD20+ cells, in the main group of laboratory animals the number of CD16+ cells increased by 1.35 times, and in CD95+ cells it decreased by 1.51 times. The deficiency of the immune system in the biocorrection group was relatively shallow, the biological product used had an immunostimulating effect. It has been proven that its use reduced the negative impact of acute irradiation on the quantitative indicators of immune system cells.

Key words: Acute irradiation, immunocompetent cells, laboratory animals, secondary immunodeficiency, biocorrection.

It is known that the negative impact of irradiation on all organs and systems of the body leads to irreversible consequences [3,10]. often detrimental effect on these organs. [5,9].

Acute exposure depends on the frequency and duration of exposure to ionizing radiation and develops at different levels depending on the radiation sensitivity of the organs. The organs of the immune system, mucous membranes of the gastrointestinal tract, exo- and endocrine glands, and sex glands are the most sensitive organs to acute irradiation. 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 is to determine the effect of acute irradiation on the immune status of experimental animals and to show the degree of influence of biocorrection on this process.

Materials and methods. To achieve the goal, 60 adult white male rats weighing 160-180 grams took part in the study. Laboratory animals were kept in plastic cages under standard vivarium conditions at relative humidity (50-60%), temperature (19-220C), with a light regime of 12 hours of darkness and light. The care of laboratory animals was carried out according to 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 consisted of white rats (n=30) on a standard vivarium diet, which received a single acute irradiation at a dose of 5 Gy;

the control group consisted of intact white outbred rats kept under standard vivarium conditions that did not receive acute irradiation (n=30).

The main group, in turn, was divided into two small groups: 1a subgroup - white outbred rats that received a single acute irradiation at a dose of 5 Gray with the addition of a biologically active additive "Lactopropolis-AWL" as a biocorrection (n=15); 1b subgroup - white outbred rats without biocorrection, who received a single acute irradiation at a dose of 5 Gray (n=15).

In the course of the experiment, laboratory animals were irradiated with the AGAT-R1 gamma-therapeutic apparatus (made in Estonia), while the source of irradiation was Co-60. Studies on animal irradiation were carried out in the Bukhara regional branch of the Republican Specialized Scientific and Practical Center of Oncology and Radiology, Ministry of Health of the Republic of Uzbekistan.

The drug "Lactopropolis-AWL" was administered once, every morning, based on the body weight of all laboratory animals. Those who received acute irradiation were given the drug for 20 days, on the last day they were irradiated, and then on the 5th day they were mortified and immunological studies were carried out. The biologically active additive “Lactopropolis-AWL” contains probiotic bacteria Lactobacillus rhamnosus 925, Enterococcus durans and an extract of biologically active compounds of propolis with antimicrobial, immunostimulating, anti-inflammatory properties (product of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan and LLC “AllWellLab”).

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 identified: CD3+-, CD4+-, CD8+-, CD16+-, CD20+-, CD95+-lymphocytes. The expression of CD receptors was carried out according to the rosette formation reaction with monoclonal antibodies of the LT series according to the method of Garib F.Yu. et al. (1995) developed by Sorbent LLC (RF). The immunoregulatory index (IRI, CD4+/CD8+) was calculated.

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

Obtained results and discussion. In order to study the effect of acute irradiation, first of all, the main parameters of the immune system of intact white outbred male rats that were not exposed to this effect were studied, the results were analyzed and interpreted, a total of 9 indicators (table-1).

Table-1 The main parameters of the immune system of intact white rats involved in the study, n=30

Indicators	Relative (%)	Absolute
Leukocytes, x10^9/л	-	4680±36
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
Measure, IRI	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

Table-1 shows the quantitative and relative (%) parameters. These results were identical to those previously reported by the investigators. [one].

The results obtained on the fifth day after irradiation on the main indicators of the immune system of white outbred rats that received a single acute irradiation in the amount of 5 Gray are shown in table -2.

Table-2 Quantitative indicators of the main immunocompetent white outbred rats treated with acute irradiation, n=30

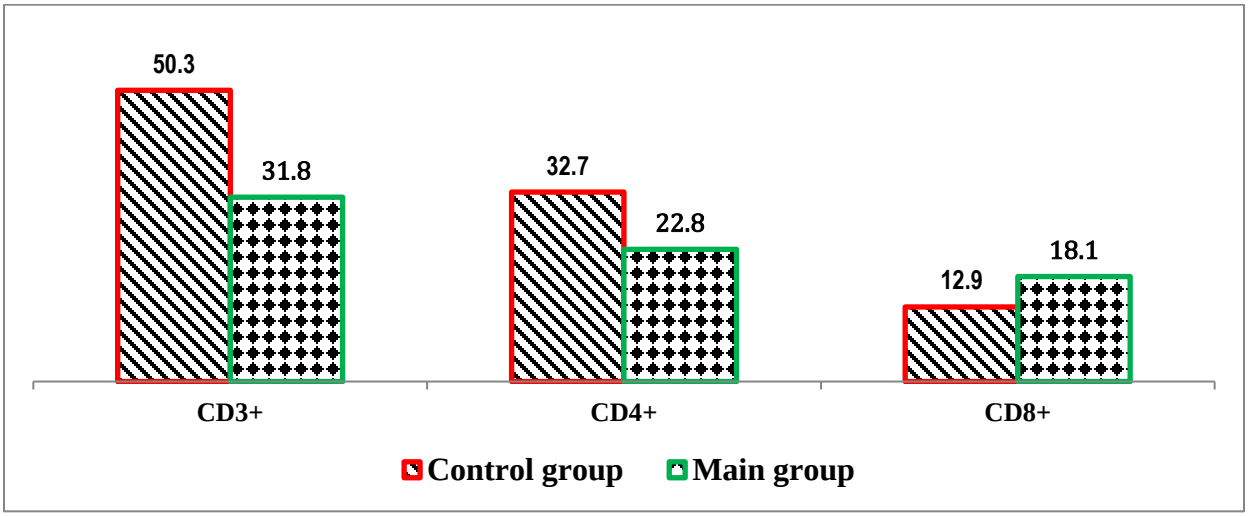
Indicators	Relative (%)	Absolute
Leukocytes, x10^9/л	-	4600±49
total number of lymphocytes	35,3±1,4	1624±64
CD3+ cells	31,8±1,5	516±24
CD4+ Cells	22,8±1,1	370±18
CD8+-Cells	18,1±1,2	294±19
Measure, IRI	1,26±0,02	1,26±0,02
CD16+ Cells	24,2±1,6	396±26
CD20+ Cells	21,9±1,7	356±28
CD95+ Cells	11,8±1,5	192±24

The results obtained showed that the quantitative index of leukocytes in the main and control groups of laboratory animals did not have a significant difference ($P>0.05$). In our opinion, this condition is explained by a short period after irradiation (5 days).

When comparing the quantitative and relative amounts of lymphocytes, we observed a completely different picture. A decrease in relative indicators by 1.41 times ($P<0.05$) was established in experimental animals that received acute irradiation compared with the relative indicators of the control group (intact) experimental animals.

When comparing the absolute indicators of this cell, an almost similar trend was observed, the decrease was 1.44 times ($P<0.05$). The decrease in the relative and absolute number of lymphocytes is explained by the effect of acute irradiation on the proliferation, differentiation of these cells, and a decrease in their activity.

As for the analyzed immunocompetent cells of the body's immune system, changes in T-lymphocytes (CD3+ cells) and their main subpopulations (CD4+ and CD8+ cells) had different forms. There was a significant decrease in the relative and quantitative indicators of CD3+ cells compared with the control group. (picture 1)



Picture-1. Comparative characteristics of relative indicators in the system of T-lymphocytes in white outbred rats that received (basic) and did not receive (control) acute irradiation in %

In quantitative terms, the decrease in CD3+ cells was 1.58 times (P<0.001), and the relative number of CD4+ cells decreased 1.43 times (P<0.05). We observed the opposite picture in terms of the relative number of CD8+ cells; it was found that these cells significantly increased relative to the control in the main group - 1.40 times (P<0.05). Both lymphocytes responded to the same exposure with different changes.

The decrease in the relative number of CD3+ and CD4+ cells in the group of white outbred rats that received acute irradiation is explained by a decrease in the total number of lymphocytes, immunodeficiency in the T-lymphocyte system, and this condition was recognized as an exposure to acute irradiation, because other factors affecting laboratory animals were eliminated. Given that one of the main functions of CD8+ cells is to reduce the immune response, an increase in the number of these cells relative to other cells is one of the reasons for the development of secondary immunodeficiency.

Similar results were obtained for the quantitative indicators of the above-mentioned immunocompetent cells (except for CD8+ cells). If a significant difference of 2.27 times in favor of the control group (P<0.001) was found between the absolute values of CD3+ cells between the main and control groups, then the same trend remained for CD4+ cells (difference by 2.06 times, P< 0.001). However, it was noted that no such trend was found in -CD8+ cells. No results were obtained in one or the other group of these data (P>0.05). This distinction between relative and quantitative measures raises the question of what indicators should be relied upon for inference based on interpretation and analysis. If we take into account that the quantitative indicator is more dependent on the quantitative indicators of leukocytes and lymphocytes, it becomes clear that the trend of change in relative indicators allows us to obtain reliable results and draw reasonable conclusions. Therefore, in experimental studies it is recommended to use relative indicators in assessing the activity of the immune system, the state of immunocompetent cells.

Another scoring parameter used to evaluate the T-system of the immune system is IRI. This indicator indicates the ratio of T-lymphocytes to the main immunoregulatory cells in the same case, the higher the IRI, the less pronounced immunodeficiency in the body, the lower the level of secondary immunodeficiency [7]. Therefore, it is recommended to constantly use IRI when assessing the immune status. Studies have established that IRI in the control group is significantly higher than in the main group by 2.01 times (P<0.001). The fact that this unit shows the same result both in relative and absolute parameters indicates that it can be used to assess the degree of depth of immunodeficiency. To assess the

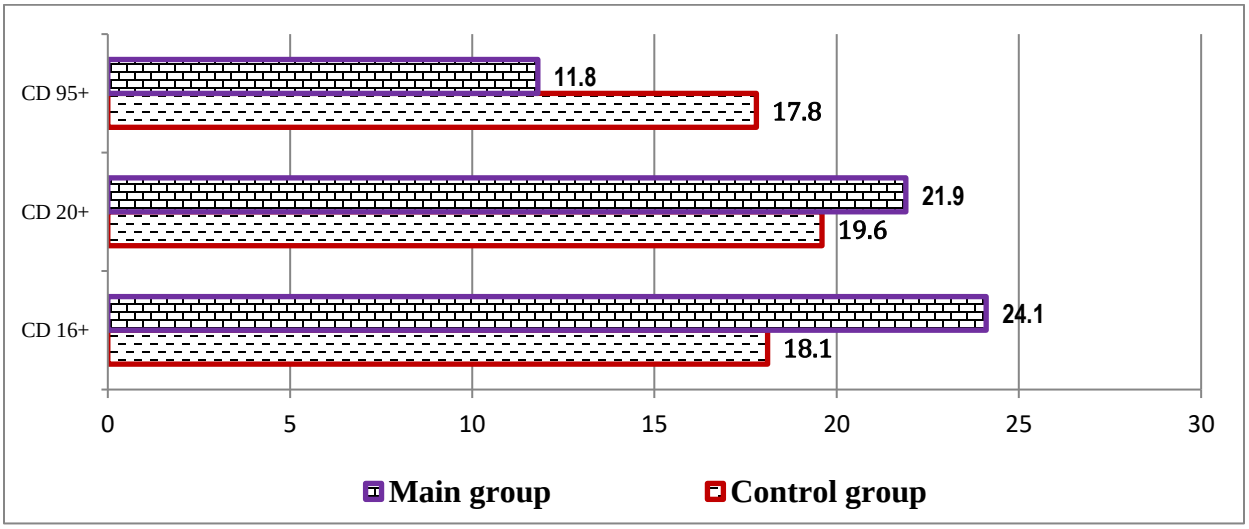
activity of the immune system, we believe that a comparative assessment of the relative parameters of immunocompetent cells and IRI is sufficient.

Since we considered that together with the T-system of immunity it is important to define the B-system of immunity, the relative and absolute values of cells - CD20+ were studied and analyzed. The results obtained showed no significant differences between groups compared to this immunocompetent cell ($P>0.05$). Apparently, the difference of 1.12 times was in favor of the main group.

Although the results did not differ significantly from each other, white outbred rats exposed to acute irradiation showed a tendency to multiply in -CD20+ cells compared with intact laboratory animals. This situation showed that changes in the T-system of the immune system develop faster than the B-system seeks to compensate for the deficiency that occurs in the B-system of immunity. It is noteworthy that the results obtained in absolute terms differ from the relative parameters, the data in the control group were higher than in the main group ($P<0.001$).

CD16+ cells, which are part of the nonspecific defense of the immune system, are allogeneic and xenogeneic cells that multiply in the body regardless of the antigen and perform the function of detecting and destroying tumor cells. Taking into account the need to eliminate tumor cells formed as a result of external influences (irradiation), taking into account the increase in their number and increase in their activity, the real reasons for the quantitative and relative changes in CD16+ cells become clear. The observations showed that in laboratory animals that received acute irradiation, the relative index of these cells increased significantly compared to the control group - 1.35 times ($P<0.05$). No significant difference was found between the absolute values ($P>0.05$).

The CD95+ receptor is one of the apoptosis receptors, which is expressed on the surface of all cells of the immune system and is involved in the control (regulation) of the immune system. In our study, the relative number of -CD95+ cells was significantly reduced in white outbred rats of the control group - 1.5 times, $P<0.05$ (Figure-2).



Picture-2. Comparative indicators of the relative number of immunocompetent cells of laboratory animals that received (basic) and did not receive (control) acute irradiation, %

Considering that a decrease in the number of lymphocytes with the expression of cells-CD95+ marker was observed in autoimmune and oncological pathologies [8], and this indicates a decrease in the level of readiness of lymphocytes for apoptosis, and a gradual decrease in immunity.

The next stage of the study was the assessment of the degree of influence of biocorrection on the cells of the immune system of experimental animals that received acute irradiation.

Biocorrection was carried out with the preparation "Lactopropolis-AWL", taking into account the serious condition of laboratory animals, it was prescribed once every morning, depending on the weight of the animals. The drug was administered for 20 days, on the last day, acute irradiation was performed at a dose of 5 Gray, on the fifth day after irradiation, laboratory animals were mortified, blood was taken, and immunological studies were performed.

The results obtained are shown in table-3.

Table -3 Comparative indicators of the main indicators of the immune system of laboratory animals that received acute irradiation in a biocorrected and uncorrected state

Indicators	Those who received biocorrection, n=30		Those who did not receive biocorrection, n=30	
	%	Absolute	%	Absolute
Leukocytes, x10^9/l	-	4600±49	-	5650±6,1*↑
Total number of lymphocytes	35,3±1,4	1624±64	44,5±1,6*↑	2514±90*↑
CD3+ cells	31,8±1,5	516±24	39,9±1,7*↑	1003±43*↑
CD4+ cells	22,8±1,1	370±18	23,9±1,2↔	601±30*↑
CD8+ cells	18,1±1,2	294±19	16,3±1,1↔	410±28*↑
IRI	1,26±0,2	1,26±0,2	1,47±0,1*↑	1,47±0,1*↑
Cells-CD16+	24,4±1,6	396±26	22,3±1,5↔	561±38*↑
Cells-CD20+	21,9±1,7	356±28	23,6±1,8↔	593±45*↑
Cells-SD95+	11,8±1,5	192±24	15,5±1,0*↑	390±30*↑

Note: * - signs of confidence between biocorrected and non-biocorrected groups: ↑ - direction of changes; ↔ - no significant difference.

It can be seen that the results obtained by relative and absolute indicators have differences, 4 of the relative indicators (out of 8 parameters) 50.0% of the indicator changed confidently in a positive direction, and no significant changes were detected for the remaining 4 indicators (50.0%), however, they tend to shift in the positive direction.

Reliable relative values are the total number of lymphocytes (increase by 1.26 times P<0.05), the number of CD3+ cells (increase up to 1.25 P<0.05), IRI (increase by 1.17 times P<0.05) and the number of CD95+ cells (increase up to 1.31 times, P<0.05) was not observed.

Significantly relative values were the total number of lymphocytes (R<0.05, an increase of 1.26 times), the number of SD3+ cells (R<0.05, an increase of up to 1.25 times), IRI (R<0.05 , increase by 1.17) and SD95+ cell count (R<0.05, increase to 1.31).

In cells subjected to acute irradiation for the first time, sensitivity partially decreased after biocorrection.

Significant changes were observed in absolute amounts (100%) of 9 studied immunocompetent cells (P<0.05-P<0.001). The indicators of the group of laboratory animals that underwent biocorrection prior to acute irradiation showed a positive shift in immune system cells from 1.17 to 2.03 times compared to non-biocorrected white outbred rats, we want to emphasize once again that all changes were significant.

If we compare the indicators of the biocorrected subgroup 1b with those of intact laboratory animals (control group), we observe that there are the following differences from this subgroup:

firstly, the absolute values of 9 studied immunocompetent cellular parameters of the immune system were significantly higher than in laboratory animals of the control group;

the second aspect was that the relative number of 9 immunocompetent cells studied by the immune system was close to that in intact laboratory animals, it was especially clearly manifested in the total number of lymphocytes and CD25+ cells;

the third aspect is that -CD4+ cells before and after biocorrection with the biologically active additive "Lactopropolis-AWL" are almost identical, far from normal;

the fourth aspect is that -CD20+ cells differ more than the normative values given. In general, the quantitative deficiency of the immune system in the group of outbred rats (subgroup 1b) that underwent biocorrection was observed to a lesser extent than in the comparison group (subgroup 1a).

Findings

1. Distinctive results were obtained on the relative and absolute values of the cells of the immune system of white outbred rats that received and did not undergo acute irradiation. These results were mainly observed in the ratio of the total number of lymphocytes, cells - CD3+-, CD4+- and CD8+, which were significantly reduced by 1.41, 1.58 and 1.43 times, respectively, in laboratory animals exposed to acute radiation (P<0 .05), the number of cells - CD8+ increased statistically significantly only 1.40 times (P<0.05).
2. The main indicator of the development of secondary immunodeficiency is recognized as a decrease in IRI by 2.01 times in the main group. To assess the activity of the immune system, it was considered sufficient to compare the relative indices of immunocompetent cells and IRI.
3. No changes were observed in the B-link of the immune system, there was no significant difference, but in the main group there was a tendency for their growth. Cells - CD16+ significantly increase by 1.35 times in the main group of laboratory animals, which is a sign of increased activity of the immune system in relation to allogeneic and xenogenic cells. A significant decrease in the number of -CD95+ cells by 1.51 times in the main group was explained by a decrease in the readiness of lymphocytes for apoptosis, an increase in the likelihood of an increase in the number of tumor cells in the body.
4. When comparing the parameters of the biocorrected subgroup 1b with those of intact laboratory animals, we observe the following differences: absolute values of the absolute values of 9 studied parameters of the immune system relative to the control group; approximation of the relative number of immunocompetent cells to that of intact laboratory animals, the relative number of cells - CD4+ cells is almost the same before and after biocorrection with the biologically active additive "Lactopropolis-AWL" (P> 0.05) are far from normal. A large deviation of cells - CD20+ from the specified normative values. In the group of white outbred rats, the quantitative deficiency of the immune system was less pronounced than in the comparable group.
5. The immune system deficiency in the biocorrected group was relatively shallow, which indicated the immunostimulating effect of the biological product used, it was proved that its intake reduced the negative impact of acute exposure on the quantitative indicators of immune system cells.

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