Immune system response after stress in the background of ionizing radiation

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Abstract

We studied the combined effect of gamma radiation (6 Gy, remote period) and emotional stress on immunological reactivity. At the early period of emotional stress, all indicators of the immune system, such as cellular, humoral, and nonspecific phagocytic links of immunity were activated, indicating the activation of the general adaptive syndrome of the organism. The suppressive action of gamma radiation on the immune system was preserved in a remote period, with this suppression being revealed not only on the T-cellular link, but also on nonspecific phagocytic links, and the functional-metabolic activity of neutrophils. In the remote period after the combined effect of sublethal gamma radiation and emotional stress in the early stage of the adaptation syndrome, there was a decrease in all cells of the T-system of immunity, in the functional ability of leukocytes, and in the mononuclear phagocytic system of the body. The experimental emotional-radiation pathological process was accompanied by disorders of the functional activity of the essential adaptive systems of the body. Based on our results, we could conclude about the dominant role of ionizing radiation in immunological reactivity.

Introduction

The occurrence of leukemia and malignant tumors are the most often remote effects of radiation. It has also been shown that the health effects of radiation might depend on the duration of the exposure [1, 2]. Damage by ionizing radiation is known to be mainly due to lipid peroxidation caused by active oxygen forms. The higher the level of the active oxygen form, the greater the oxidative damage [3, 4]. In the long term, even a small dose of radiation could cause genetic changes, oncogenesis, and physiological changes, associated with a compromised immune system and increased cellular stress [5, 6]. In contrast, it is also believed that a low dose of radiation could induce both positive and negative bioeffects, thereby reducing carcinogenesis, increasing longevity, and increasing fertility [7, 8, 9].

Absorption of energy from ionizing radiation by genetic material in the cell is known to lead to DNA damage, which in turn leads to chromosome aberrations and gene mutations. Both early and late effects of radiation has been reported to lead to organ and tissue damage caused by cell death, which in turn leads to the development of cancer. Epidemiological studies have demonstrated a dose-response relationship for the induction of cancer [10]. For instance, aberrations of the chromosomal complex of peripheral blood lymphocytes were revealed in the long term following the incidence of the leaked ionizing radiation at the Chernobyl nuclear power plant (ChNPP) [11].

The results of this study revealed a high prevalence of chronic diseases among the population living in the vicinity of radioactive waste storage facilities. The population was reported to also receive chronic irradiation as a consequence of the long term exposure to radiation, with the results of the study pointing to

an almost complete absence of healthy people living in the area. Diseases of the cardiovascular, hematopoietic, respiratory, and musculoskeletal systems occupied the top places in the prevalence of diseases among the population exposed to radiation [12, 13, 14]. According to the results obtained from field and laboratory analytical studies, the authors identified the negative impact of the tailings dump (radioactive waste storage) of the Stepnogorsk mining and chemical plant (uranium mining enterprise, Republic of Kazakhstan) on the environment of adjacent areas, expressed as radionuclides contaminating the soil, water and vegetation. The population living in local radioactive areas was estimated to receive an annual effective dose of about 6.5 mSv/y, whereas the normal level from natural sources should be 1 mSv/y [15].

Recently, much has been said about the "untargeted effects" of ionizing radiation in a remote period. Briefly, "targetless effects" is considered the ability to transfer changes from irradiated cells (target cells for radiation exposure) to unirradiated cells. This could be considered as one of the directions of the distant effects of ionizing radiation. The authors also received new data related to the radiation "witness effect" and adaptive response. It was found that there are 2 mechanisms - intercellular interactions and secret factors that could be transmitted at a distance. The importance of studying "non-targeted effects" is related to the use of research results in theoretical radiobiology, as well as in radiation ecology, when studying the long-term consequences of human-made disasters [16, 17]. It has been shown that "in radiation disasters, mankind for the first time has encountered the complex, multifactorial stress," which includes not only the biological effects of radiation but also the psychological stress of a complex structure. Psychological manifestations and consequences of this stress have been associated with the expectation of a health catastrophe [18].

One of the most radiosensitive functions of human and animal organisms is immunological reactivity. A characteristic feature of exposure to radiation is known to be the long preservation of damages in separate parts of the immune system and its associated remote consequences and complications manifested by the development of malignant tumors [19]. Ionizing irradiation in different doses in remote periods might lead to the suppression of the innate and acquired humoral and cellular immunity. Whole-body radiation exposure at doses > 2 Gy (Gray) could cause different clinical symptoms; higher doses could be so acute that they would become life-threatening [20].

Studies on the health effects of remote exposure to ionizing radiation are relevant worldwide, including Kazakhstan; some areas have been exposed to local fallout from nuclear tests at the Semipalatinsk nuclear test site; the populations living in these areas have been exposed to internal and external exposure. People who received a specific dose of radiation while living in these areas, might also face many other daily difficulties, causing further emotional distress. Given the importance of the immune system in the formation of pathological processes, its high sensitivity, as well as the significant consequences following its compromise, we were interested in its role in the formation of pathological processes in combination with the action of emotional stress and remote period exposure to ionizing radiation.

Objective

To study the immunological reactivity under the combined effect of γ-radiation (6 Gy, remote period) and emotional stress.

Materials and methods

Subjects

To address this, we carried out experiments on 40 white laboratory male Wistar rats weighing 220 ± 20 g (Kennel laboratory animals "Pushchino", Moscow region, Russia), which were divided into 4 groups: group I - intact animals, group II - animals exposed to gamma radiation (dose of 6 Gy), group III - animals exposed to emotional stress, and group IV - animals exposed to combined exposure to gamma radiation (dose of 6 Gy) and emotional stress.

Irradiation

Animals in groups II and IV animals were irradiated 90 d before the study on the TERAGAM radiothera-

peutic cobalt unit ("ISOTREND spol. S.r.o.", Czech Republic); the radionuclide source used was cobalt-60 (Co60). Experimental animals received a single sublethal dose of 6 Gy. All experiments on animals were conducted according to the Geneva Convention (1990), the Helsinki Declaration on Humane Treatment of Animals, and the ethical standards of the local ethics committee of the JSC "Astana Medical University," Kazakhstan under the protocol No. 4 of 07.09.2017.

Before irradiation, we carried out a topometric and dosimetric preparation of all experimental animals for radiation. Briefly, the object was placed on the isocentric therapeutic table of the "Terasix" (X-ray simulator Czech Republic), which based on its design and parameters corresponded to the therapeutic table of a gamma-irradiator. After being displayed on screens, the pattern of irradiated animals was directly entered into the planning system via the network connection of the computer using the digitizer. We calculated the isodose with the help of the "PlanW-2000" (UJP Praha) planning system with the topometric-dosimetric map containing technical parameters and planned doses of irradiation. Animals were exposed once to a total gamma radiation dosage of 6 Gy: SSD - 97.2 cm, SAD - 100.0 cm, field 40 × 40 cm, t = 356 s (SSD - distance from the source of ionizing radiation in the device to the conventional center of the irradiated pathological focus; SAD - distance from the source of ionizing radiation in the device to the nearest surface of the irradiated object) [21]. During irradiation, animals were in a specially designed organic glass cell, with multiple isolated cells being employed for each animal [22].

In groups III and IV, animals were modeled for acute emotional stress by immobilization for 6 h in bright light. For this, experimental animals were placed in individual plastic compartments adapted for immobilization (length - 20 cm, width - 6 cm). Animals were euthanized 1 h after the induction of acute emotional stress, by incomplete decapitation under mild ether anesthesia.

Immunofluorescence, phytohemagglutinin, and immunodiffusion

Blood was taken in heparin tubes (25 unit/mL) in order to assess their immunological status. Accordingly, we determined the total number of leukocytes and lymphocytes in the peripheral blood of all experimental animals. The number of CD19+ and CD3+ lymphocytes and their subpopulation were determined by immunofluorescent cell staining using antibodies conjugated with FITC, explicitly adapted for experiments with rats. CD3+, CD4+, CD8+, and CD19+ FITC monoclonal antibodies were acquired from GALTAG Laboratories (5791 Van Allen Way Carlsbad, CA 92008 USA). Stained cells were examined with a fluorescent microscope (EVOS^{TM} FL, Thermo Fisher Scientific). Surveillance was conducted in a darkened room. We determined both the inhibition reaction of leukocyte migration (LMIR) with phytohemagglutinin (PHA-M, Gibco^{TM}) [23], as well as the concentration of circulating immune complexes (CIC) [24]. We determined the content of immunoglobulins A, M, G, using radial immunodiffusion on Mancini agar gel [25]. We also carried out the determination of the phagocytic activity (PA) of neutrophils [26] and performed nitro blue tetrazolium tests (NTT) [27]. Obtained results were statistically processed; the differences were evaluated using the t criterion of the Student's t -test.

Results & Discussion

The immune system in irradiated animals at a dose of 6 Gy

Our study showed that exposure of experimental animals to a high dose of radiation in a remote period acted as an immunosuppressive agent, revealing a significant sensitivity of leukocytes and T-lymphocytes and their subpopulations. Hence, we observed the occurrence of leukopenia in exposed animals; the number of leukocytes was reduced by 18.72 % ($p < 0.05$) (**Table 1**).

At the same time, both the percentage and the absolute number of lymphocytes were shown to be increased by 8.58 % ($p < 0.05$) and 5.74 % ($p < 0.05$), respectively. Regarding T-lymphocytes, we identified the following changes: the total number of lymphocytes was reduced by almost 45,03 % ($p < 0,001$), and the relative number by 35,12 % ($p < 0,05$), leading to a significant reduction of cells with CD4+ helper activity: the total number was reduced by 26,32 % ($p < 0,05$), and the relative number by 30,27 % ($p < 0,05$). Thus, the immunoregulatory index (IRI = CD4+/CD8+) in animals exposed to radiation was reduced in

comparison with the intact group. In the case of T-suppressors, an inhibition of their activity was observed, also exhibiting a decrease in the total and relative number by 22.22 % ($p < 0.05$)and 36.86 % ($p < 0.01$), respectively. It is known that the activity of the CD3+ lymphocyte lymphokine-producing cells reflects the functional activity of the T-system of immunity. Our study revealed a decrease in the production capacity of CD3+ lymphocytes in irradiated animals, as well as a 32.56 % increase in the LMIR migration index in the PHA-treated compared with the control group ($p < 0.05$). We found that the ability of cells to produce cytokines suppressing the migration of leukocytes was expressed in the control group. Whereas, in animals under the influence of gamma radiation, the production of cytokines was reduced, thus indicating the immunosuppressive action of radiation, which led to the suppression of the synthesis of cytokines, as the migration index was higher than that in intact animals. According to several researchers [28,29], ionizing radiation could cause a decrease in DNA synthesis, and the number of cells transformed into blasts under the influence of PHA, reducing the production of the factor inhibiting the migration of macrophages (MIF) and the associated cytotoxic effect.

Against the background of the explicit suppression of cell immunity, we obtained the result of the immunoregulatory index (CD4+/CD8+), exhibiting a shift to the left towards a decrease. The decrease in IRI is considered to be typical in immunodeficiency states, and cancers. Thus, the pathognomonic laboratory symptom of immune deficiency was shown to gradually include incomplete numbers of CD4+ and a decrease of IRI. The decrease observed in IRI in our example was assumed to be due to a sharp decrease in the number of T-cells with helper activity compared with those of T-cells with suppressor activity. As is known, CD4+ cells stimulate the CD19+ lymphocyte population for production of antibodies. Insufficient helper function of T-lymphocytes might lead to a decrease in the sensitivity of the body to antigenic stimulation, contributing to the development of severe infectious complications, and pathological conditions of radiation genesis.

Taking into account the complexity of the character of changes in the of humoral immunity with the radiation lesion of the organism, we aimed to study the long term reaction of humoral immunity in interrelation with the nonspecific phagocytic link of immunity at the same dose of 6 Gy gamma-irradiation. To determine the state of humoral and nonspecific phagocytic links of immunity in the case of gamma-irradiation, we studied the number of CD19+ lymphocytes, the concentration of CIC, serum immunoglobulins, phagocytic activity, phagocytic number, and also performed a NTT-test.

The suppressed levels of CD4+ cells, as well as the persistent level of CD19+ cells indicated the implementation of the immune response, which, thus, activated the B-cells; proof of this was a reliable increase in the absolute and relative number of CD19+ lymphocytes in the blood by 37.5 % and 41.99 %, respectively compared with control ($p < 0.05$). B-lymphocytes, in turn, are known to switch from IgM synthesis to IgG and IgA synthesis as they mature, but in this case we observed a suppression in the levels of IgG and IgA , whereas the concentration of IgM was shown to be increased by 43,11 % ($p < 0{,}05$) in irradiated animals. The decrease of IgA (by 16,29 %, $p < 0{,}05$) and IgG (by 32,66 %, $p < 0{,}01$) was an important criterion of the immunosuppressive action of radiation in humoral immunity. Irradiation had also a significant impact on the concentrations of CIC. As such, we identified a reliable decrease of 21.64 % ($p < 0.05$) in the blood serum of experimental rats.

Serious changes were observed in the nonspecific phagocytic immunity link in irradiated organisms. We observed a reduction of the phagocytic activity of blood cells by 17,31 % ($p < 0{,}05$) in irradiated experimental animals. The results of the calculation of the average number of latex absorbed by one phagocyte (phagocytic number) in the group of irradiated animals revealed a decrease by 9.35 % ($p < 0.05$).

Evaluating the oxygen-dependent phagocytic killing in the recovery test of NTT is known to serve as an indicator of the phagocytic and metabolic activity of neutrophilic granulocytes. A NTT-test might reflect the final reaction of one of the critical enzymatic systems responsible for the effector potential of phagocytes. Our study showed that the index of the NTT-test in experimentally irradiated animals was decreased by 13.12 % ($p > 0.05$), indicating a suppression of the functional activity of neutrophils. Collectively, the revealed tension of the phagocytic link in the form of the suppression of the phagocytic activity, reduction of the phagocytic

number, and obtained NTT-test results in irradiated animals further testified for the immunodepressant effect of radiation.

Thus, we revealed a number of changes in experimental rats irradiated with gamma radiation, which were characterized, first of all, by a decrease of the absolute and percentile number of T-lymphocytes and their subpopulation (CD4+, CD8+), decrease in the functional activity of T-lymphocytes, and a decrease in the protective and adaptive mechanisms of the organism in response to the action of long term exposure to a high dose of radiation. The decrease in the quantity of CD3+ cells and their subpopulation, which are known to serve as the response of the immune system to developing pathological processes could be regarded as a general physiological response of an organism in response to a stress irritant [30]. However, there were also changes in qualitative and quantitative indicators of humoral and nonspecific phagocytic links of immunity, which constitute a fact of developed immunodeficiency of radiation genesis. Accordingly, we found that the proliferative activity of T-cells, and quantitative and functional states of the T-system of immunity were also decreased.

The analysis of the factual material showed that the suppressive action of gamma radiation on the immune system was preserved in the long term; suppression was detected not only on the T-cell link, but also on nonspecific phagocytic links, and on the functional-metabolic activity of neutrophils.

The immune system under emotional stress

We found that the number of leukocytes in animals under emotional stress (withdrawn from the experiment after 1 h of emotional stress) was increased by 41.49 % ($p < 0.05$) compared with the intact group (**Table 1**). Furthermore, the total number of lymphocytes in the animals of group III was increased by 60.07 % ($p < 0.01$), as did the relative number of lymphocytes (p ¿ 0.05). The absolute number of CD3+ lymphocytes in experimental animals was increased by 1,18 times ($p < 0.05$), whereas the percentage of lymphocytes remained within the levels of those in the intact group (p ¿ 0.05). Regarding the CD4+ cells, their total number was shown to be increased by 31,57 % ($p < 0.05$) in group III after 1 h of stress, with their relative number also exhibiting an increasing tendency (p ¿ 0.05). In the case of CD8+ lymphocytes, a similar picture was revealed: increase of their absolute number by 24,07 % ($p < 0.05$), and relative number by 26,90 % ($p < 0.05$). We also noted an increas of IRI in group III by 6,42 % (p ¿ 0.05). We also identified an increase of the production capacity of CD3+ lymphocytes, and a 24,42 % decrease of the LMIR migration index in PHA in experimental animals in comparison with the intact group ($p < 0.05$). Thus, it was suggested that production of cytokines was stimulated on the background of stress.

To determine the state of humoral and non-specific phagocytic links of immunity under stress we evaluated the number of CD19+ lymphocytes in peripheral blood, the concentration of CIC, immunoglobulins in serum, and determined phagocytosis, phagocytic number, as well as performed a NTT-test. Our results showed that 1 h after stress, the absolute and relative number of CD19+ lymphocytes in the blood of animals increased by 1.65 and 1.91 times, respectively, compared with those in the control ($p < 0.001$). The serum concentration of CIC was observed to be decreased from 1.34 ± 0.04 to 0.71 ± 0.07 g/L ($p < 0.001$). Against the background of the activation of cellular immunity in the investigated animals, we observed the increase in the levels of IgA and IgG: the concentration of IgA increased by 60 % ($p < 0.01$), while that of IgG by 88,52 % ($p < 0.001$), demonstrating the importance of these indicators for assessing the level of humoral immunity under stress.

In animals exposed to emotional stress, the phagocytic activity of blood cells was shown to be increased by 64,32 % ($p < 0.01$), with the phagocytic number being increased by 82,01 % ($p < 0.01$). We also found that the NTT-test index was increased by 76.67 % ($p < 0.01$), indicating the activation of the functional activity of neutrophils.

Thus, we demonstrated here the changes that occurred in humoral immunity of experimental rats undergoing emotional stress, which were primarily characterized by an increase in the number of white blood cells, the absolute and percentile of CD3+ lymphocytes and their subpopulation (CD4+, CD8+), as well as an increase in the functional activity of CD3+ lymphocytes and the protective mechanisms of the body in response to

stress. The currently conducted study exhibited the occurrence of activation in all indexes, both in cellular, humoral, and non-specific phagocytic links of immunity, and thus activation of the general adaptive syndrome of an organism at the early period of emotional stress.

The immune system under combined exposure to gamma radiation (dose of 6 Gy, remote period) and emotional stress

Our study showed that combined exposure of experimental animals to a high dose of radiation in the remote period and emotional stress acted as an immunosuppressive agent, revealing the dominant role of ionizing radiation. We noted the occurrence of leukopenia in experimental animals, with their number of leukocytes being reduced by 19.65 % ($p < 0.05$) (**Table 1**). A significant reduction was shown in the absolute number of lymphocytes in blood from 2,68 ± 0,11 × 10^9/L to 2,24 ± 017 × 10^9/L ($p < 0.05$), whereas their relative number was not markedly changed (p ¿ 0.05).

The related content of CD3+ lymphocytes in experimental rats with an absolute reduction in their number by 48,34 %, $p < 0.001$), was demonstrated to correspond to that of the intact value. The subpopulation of T-lymphocytes under these conditions exhibited the following changes: the absolute number of CD4+ was decreased by 38,16 % (p<0,01), the elative by 28,9 % ($p < 0.05$), the absolute number of CD8+ by 33,33 % ($p < 0.05$), and the relative by 33,24 % ($p < 0.05$). At the same time, the immunoregulatory index was shown to be accordingly decreased due to a decrease in the number of lymphocytes with helper activity. During this period, we noted a reliable increase in the index of leukocyte migration in the LMIR reaction to PHA from 0.86 ± 0.05 to 1.11 ± 0.09, indicating a decrease in the functional activity of the T-system of immunity ($p < 0.05$).

Collectively, our results indicated that following the combined effect of remote period sublethal gamma radiation and emotional stress there was a decrease in the total number of lymphocytes; this decrease in some CD3+ and several CD4+ lymphocytes, which appeared to play a considerable role in immunological reactivity, was also reflected in the low indicators of IRI. In addition, we found that in parallel with a decrease in the number of CD8 + lymphocytes, the functional capacity of T-lymphocytes was also decreased.

Our study results showed that under the combined effect, the absolute and relative number of CD19+ lymphocytes in the blood of animals was increased by 20 % and 48.46 %, respectively, compared with the control ($p < 0.05$). This combination had also a significant impact on the concentrations of CIC. Thus, we observed a 26.12 % decrease ($p < 0.05$) in the concentration of CIC in the serum of experimental rats. At this background of the increase in the concentration of CD19+ in investigated animals, we identified a suppression in the levels of IgA (14,89 %, p ¿ 0.05) and IgG (on 23,37 %, $p < 0.05$), whereas the concentration of IgM was shown to be increased to 40,17 % ($p < 0.05$). Regarding the phagocytic activity of blood cells, we noted a suppression by 23,77 % ($p < 0.05$) and decrease in the phagocytic number by 12,95 % (p ¿ 0.01).

Moreover, the NTT-test indicator exhibited a downward trend. It should be noted that the implementation of recovery processes in the body is known to be facilitated in the remote period after exposure with a decrease in the dose rate. Under irradiation of the organism with different power, the degree of the response of its reserve adaptive capacity would be different. The revealed changes in the remote period characterizing the immunodepression of the irradiated organism could be considered as an adaptive-compensatory reaction of the organism in response to radiation alone and in combination with nonradiative factors [31].

Thus, in the remote period after the combined exposure to sublethal gamma-irradiation and emotional stress in the early stage of the adaptation syndrome, all cells of the T-system of immunity, such as CD4+/CD8+, as well as the functional ability of leukocytes and mononuclear phagocytic system of the organism decrease. Moreover, we noted a significant depression of immunoglobulins A and G, whereas IgM was shown to be activated. The decrease in the content of IgA and G testified to the occurrence of immunodeficiency, which is known to be accompanied by chronic inflammation and the presence of high tendencies for the development of neoplasm at high doses of ionizing radiation. Based on the results of this study, we could conclude about the dominant role of ionizing radiation in the immunological reactivity of organisms.

Conclusion

The effect of remote period gamma radiation remained suppressive on the immune system, including non-specific phagocytic links, and the functional-metabolic activity of neutrophils. Hence, the mechanisms of development of stress response to radiation genesis might depend on the adaptation and insufficiency of physiological measures for the protection of the irradiated organism. At the early period of emotional stress, all indexes of the immune system, such as cellular, humoral, and non-specific phagocytic links of immunity were activated, indicating the activation of the general adaptive syndrome of the organism. In the remote period after the combined action in the early stage of the adaptation syndrome, there was a decrease in all cells of the T-system of immunity, in the functional ability of leukocytes, and in the mononuclear phagocytic system of the organism. Ionizing radiation, combined with emotional stress had a more pronounced effect on the formation of immunodeficiency syndrome in a remote period. Moreover, the dominant agent affecting the developing pathology under the combined effects of the above mentioned 2 factors was shown to be ionizing radiation.

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Table 1. Immune system indices in the blood of experimental animals under the influence of stressogenic factors, M ± m

Exponents	**Exponents**	**Group I Intact animals**	**Group II - animals exposed to gamma radiation (dose of 6 Gy)**	**Group III - animals exposed to emotional stress**	**Group IV - animals exposed to combined exposure to gamma radiation and emotional stress**
Leucocytes, $\times 10^9$/L	Leucocytes, $\times 10^9$/L	6.41 ± 0.17	5.21 ± 0.34 *	9.07 ± 0.75 *	5.15 ± 0.33 *
Lymphocyte, $\times 10^9$/L	Lymphocyte, $\times 10^9$/L	2.68 ± 0.11	2.91 ± 0.21	4.29 ± 0.32 **	2.24 ± 0.17 *
Lymphocyte, %	Lymphocyte, %	37.81 ± 3.31	35.64 ± 2.45	44.37 ± 3.24	40.22 ± 3.75
CD3+	Abs.n.°	1.51 ± 0.08	0.83 ± 0.05 ***	1.79 ± 0.11 *	0.78 ± 0.06 ***
	%	30.61 ± 2.53	19.86 ± 1.24 *	33.15 ± 2.37	26.63 ± 2.42
CD4+	Abs.n.°	0.76 ± 0.06	0.56 ± 0.04 *	1.00 ± 0.08 *	0.47 ± 0.03 **
	%	19.72 ± 1.57	13.75 ± 0.71 *	21.45 ± 1.65	14.01 ± 1.24 *
CD8+	Abs.n.°	0.54 ± 0.04	0.42 ± 0.03 *	0.67 ± 0.05 *	0.36 ± 0.03 *
	%	11.04 ± 0.76	6.97 ± 0.53 **	14.01 ± 1.25 *	7.37 ± 0.71 *
CD19+	Abs.n.°	0.40 ± 0.03	0.55 ± 0.03 *	0.66 ± 0.04 ***	0.48 ± 0.04
	%	6.81 ± 0.54	9.67 ± 0.73 *	13.07 ± 1.07 ***	10.11 ± 0.73 *
CD4+/CD8+	CD4+/CD8+	1.40 ± 0.10	1.33 ± 0.08	1.49 ± 0.12	1.30 ± 0.11
CIC, y.e.	CIC, y.e.	1.34 ± 0.04	1.05 ± 0.08 *	0.71 ± 0.07 ***	0.99 ± 0.08 *
ITML	ITML	0.86 ± 0.05	1.14 ± 0.08 *	0.65 ± 0.05 *	1.11 ± 0.09 *
Phagocytes, %	Phagocytes, %	36.10 ± 2.46	29.85 ± 1.87 *	59.32 ± 4.36 **	27.52 ± 2.51 *
Cytophagous number	Cytophagous number	1.39 ± 0.11	1.26 ± 0.08	2.53 ± 0.20 **	1.21 ± 0.13
NTT-test, %	NTT-test, %	4.80 ± 0.37	4.17 ± 0.22	8.48 ± 0.64 **	4.08 ± 0.31
Ig A g/L	Ig A g/L	5.71 ± 0.40	4.78 ± 0.21 *	9.14 ± 0.72 **	4.86 ± 0.32
Ig M g/L	Ig M g/L	6.82 ± 0.43	9.76 ± 0.73 *	6.26 ± 0.47	9.56 ± 0.67 *
Ig G g/L	Ig G g/L	7.93±0.53	5.34±0.37 **	14.95 ± 1.02 ***	5.68 ± 0.42 *

Note: changes statistically significant in comparison with intact control * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ° absolute number of cells $\times 10^9$/L.