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IMMUNOLOGICAL FEATURES OF THE MODEL OF RADIATION-INDUCED ANTI-INFECTIOUS RESISTANCE DEFECT

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Introduction

The problems of influence of ionizing irradiation on the living organism preserve their relevance. In the last decades attention of radiobiologists has radically shifted towards the study of low doses of irradiation [1-2].

The literature analysis shows that the changes in the immune system resulting from the influence of the low-dose irradiation may lead to a decrease in the anti-infectious resistance in the experimental animals [3-6].

It is proven that in mice exposed to the low-intensity irradiation in the postnatal period the concentration of anti-inflammatory cytokines was increased, except interleukin - 1β - (IL-1 β) and interferon -γ - (INF-γ) and macrophages activation is observed [7], as well as an enhanced sensitivity to bacterial infection [8].

The reaction to the pathogen is complex. T- and B cells, macrophages and the range of such cytokines as interferon-γ (IFN-γ), interleukin -12 (IL-12), interleukin-18 (IL-18), tumor necrosis factor-α (TNF-α) are taking part in its development. The activation of macrophages plays an important role in in the course of reaction development as this cell type provides the killing of the intracellular agent. Therefore, macrophages play the key role in the immune protection from Salmonella typhimurium on the salmonellosis model [9-10] and are affected by low-dose irradiation in the first place.

In summary, it should be noted that because of the complex nature of the influence of low-intensity ionizing irradiation and great quantity of the aspects, connected with the process a progressive, systematical study of influence of the ionizing irradiation in certain doses on the immune system state and the long-term consequences of the former is needed and the study of the increased sensitivity to the bacterial infection is one of the most important issues of the problem [11].

According to the mentioned above data, the aim of our study was to develop a model of radiation-induced defect of anti-infectious resistance in Wistar rats. The developed model was intended to have characteristic features of functional decompensation of the anti-infectious resistance system through the selection of the optimal conditions of irradiation and additional functional load in order to be used for the immunomodulator evaluation.

Materials and methods

Animal models. The model objects were female Wistar rats aged two months, weight 250-270 g. The animals in course of the experiment were raised in the vivarium of SE "Mechnikov Institute for Microbiology and immunology". The animals were fed on the usual diet with free access to water. The experiment was carried out according to the principles of Helsinki declaration, ratified by the General Assembly of the World medical associate and to the national "General ethical principles of experiments on animals" (Ukraine, 2001), that were in accordance with the principles of "European convention for protection of the vertebrate animals, that are used for experimental and other scientific purposes" (Strassbourg, 18.03.1986). All painful and stressful operations were carried out inder light ether anesthesia; the killing was performed by decapitation under ether anesthesia.

Irradiation. Antenatal irradiation was performed on the primipara pregnant female Wistar rats, aged 2 months, weight 250 - 270 g. The irradiation was carried out on the 3rd day of pregnancy in the dose of 0,5 Gy for the 40 seconds, the skin-focus distance 40 cm, current strength 20 mA, pipe voltage 160 kV. The second group of pregnant female rats was irradiated on RUM-17 in the dose of 1 Gy for 2 minutes, skin-focus distance 40 cm, current strength 10 mA, voltage 180 kV, filters 0,5 mm copper and 1,0 mm aluminum. The rats aged 30 days, obtained from non-irradiated females formed the experimental group for study of the postnatal irradiation. The rats were irradiated on the RIM– 17 (radiation - inducing machine) in the dose of 1 Gy for 2 minutes. Skin-focus consisted of 40 cm, current strength 10 mA, voltage 180 kV, filters 0,5 mm copper and 1,0 mm aluminum.

Inoculation. For infection model a group B strain № 36 of Salmonella typhimurium was used. The culture was obtained from an acute gastroenterocolitis patient before therapeutical management, and was kept in the museum of live microorganisms of the laboratory for specific prophylaxis of respiratory infection of SE "Mechnikov Institute of Microbiology and Immunology of AMS of Ukraine".

The infection of the rats was performed through intraperitoneal inoculation of 0, 5 ml of daily Salmonella culture with the determined concentration of microbial cells. The animals of the control groups were inoculated with 0, 5 ml of sterile saline solution.

Bacteriological analysis. In order to evaluate the bacterial population in the organs, the brain, heart, liver and intestine were studied. The organs were obtained in the aseptic conditions, weighted (the weight of every probe was 1 g.), then homogenate in the sterile conditions. The obtained homogenates were cultured in the accumulative medium and incubated for 18 hours at the temperature of 37 ^{0}C.

The further study and identification of the obtained microorganism's cultures was carried out according to the morphological, cultural, biochemical and serological features with the help of generally accepted methods. [12-14].

Immunological methods. For determination of cytokines level in blood serum diagnostical ELISA assays were used ("Vector-Best", Russia). The determination of circulating immune complexes was performed with the standard precipitation method in

3,5% polyethyleneglycol solution (M.m/ 6000) (AppliChem Gmb) followed by spectrometry in optical density values [15]. The serum complement activity was determined according to the 50 % hemolysis with optical density CH50. The hemolysis level was measured on the photoelectrocolorimeter at 541 nm. The determination of the peripheral blood neutrophil phagocytic activity was performed according to the evaluation of Right and Hamburger indexes [16].

Results and discussion

Female Wistar rats aged 30 days were γ-irradiated in the dose range 0,75 - 1,5 Gy and infected with S.typhimurium in the dose of 5 x 10^8 CFU intraperitoneally. The inoculation period was chosen according to the aim of the study: as in the present work the early effects of irradiation were studied, the inoculation was performed immediately after the irradiation.

The intraperitoneal inoculation of Salmonella typhimurium culture in the intact Wistar rats has caused salmonellosis peritonitis and septic condition that was accompanied with infection generalization, hematogenic dissemination and pathogen persistence in the blood and internal organs (liver, spleen, large intestine). The clinical symptoms (fever, the decrease in motor activity and) were observed for 3 -5 days.

The inoculation dose was chosen experimentally in order to achieve zero lethality level in the groups on the background of the minimal percentage of the animals that developed no septic condition due to the localization of infection. The experimental studies have shown that the dose of 5 x 10^8 CFU provides 90 – 100 % level of infection process generalization on the 7 day of the infection with the positive pathogen dynamics during the second week after inoculation (table 1.). The decrease in the dose led to the increase in the percentage of animals that didn't have infection generalization which makes it impossible to observe the process dynamics. The increase in the dose led to the increase in the mortality level.

The salmonellosis peritonitis was created in 5 groups of rats aged 1 month. There were 10 animals in each group. I group – non – irradiated animals; II group – antenatally irradiated animals in the dose of 0,5 Gy; III group – postnatally irradiated animals in the dose of 0,75 Gy; V group – postnatally irradiated animals in the dose of 1 Gy; VI group – irradiated postnatally in the dose of 1,5 Gy.

The bacterial load of internal organs was investigated on the 7-th and 14-th days after inoculation. The results are shown in the table 1.

Table 1. Bacterial load levels in the salmonellosis sepsis in different groups of Wistar rats

Animal Groups	The study period	Animals with the generalized form of the disease, %	Bacterial load of the internal organs < CFU/g			
			1 - 20	21 - 50	51 – 100	>100
I	7 days	90	0	0	20	80
(n = 10)	14 days	60	0	40	60	0
II	7 days	90	0	0	20	80
(n = 10)	14 days	70	0	40	60	0
III	7 days	100	0	0	0	100
(n = 10)	14 days	70	0	40	60	0
IV	7 days	100	0	0	0	100
(n = 10)	14 days	80	0	0	30	70
V	7 days	100*	0	0	0	100
(n = 10)	14 days	100	0	0	0	100

Note: * - two animals died during the first 5 days after inoculation.

According to the previously described data, the animals, irradiated in the dose of 0, 5 Gy (group II), as well as the animals, irradiated in the dose of 0,75 Gy (III group), have not shown statistically valid differences in the dynamics of bacterial load decrease with the non-irradiated animals (I group).

Postnatal irradiation in the dose of 1 Gy (IV group) influences the susceptibility of the rats to the chosen infection but does not increase the mortality level and allows the study of process dynamics during 14 days.

The increase of the irradiation dose up to 1,5 Gy leads to an increase in mortality level, which decreases the possibility to study the infectious process dynamics: for two animals of the V group the consequences of infection were lethal; the animals, that survived have shown physical symptoms of more severe disease course with longer period of disease manifestation. There was no positive dynamics in bacterial load on the 7-th and 14-th days in this group.

The irradiation repetition was minimal, as it allowed obtaining the desirable technical effect according to the obtained data.

The comparative analysis of anti-infectious resistance condition is shown in the table 2.

The animals were divided into 5 groups in the course of the study: Wistar rats, aged 1 months irradiated postnatally in the dose of 1 Gy (Group A), Wistar rats, infected intraperitoneally S.typhimurium culture (Group B), irradiated with the dose of 1 Gy and infected with S. typhimurium (group C), irradiated antenatally in the dose of 0,5 Gy and infected with S. typhimurium (Group D) and intact animals (control group).

Table 2. The parameters of non-specific resistance in the Wistar rats aged 1 month

Parameters	M/ m	Group A (n = 10)	Group B (n = 10)	Group (n = 10)	Group D (n = 10)	Control (n = 10)
General complement, h.u.	M	92,8	62,88**	36,75**	60,01**	96,61
	m	±3,41	±2,78	±1,75	±2,2	±2,2
Circulating immune complexes,optical density	M	0,005	0,007**	0,009**	0,007**	0,005
	m	±0,0004	±0,0005	±0,0007	±0,0006	±0,0006
TNF-α, pg/ml	M	42,28	111,8**	94,07	100,3**	39,18
	m	±0,52	±3,64	±2,67	±3,33	±0,33
IL-4, pg/ml	M	21,11*	100,1**	17,9**	91,89**	35,89
	m	±0,29	±1,53	±0,54	±0,47	±0,3
TNF-α/IL-4	M	2,00	1,11	5,2	1,08	1,09
	m	±0,1	±0,2	±0,1	±0,03	±0,05
Neutrophil phagocytosis index, %	M	46,2*	71,1	62,3	69,8	67,1
	m	±1,8	±3,5	±1,8	±3,9	±3,2
Neutrophil phagocytic count, %	M	5,1*	9,8	6,8	7,1	8,7
	m	±0,1	±1,3	±0,1	±0,3	±0,3
Phagocytosis index of peritoneal macrophages, %.	M	34,2**	69,4**	39,9*	61,3	52,8
	m	±2,3	±2,8	±1,4	±2,1	±2,5
Phagocytic count of peritoneal macrophages, %.	M	4,3**	12,7**	5,2**	7,3	9,5
	m	±0,2	±1,3	±0,3	±0,5	±0,2

Notes:
*** - significant difference from the control group p<0,1;**
**** -significant difference from the control group p<0,05.**

In the group A the condition of non-specific immunity in the animals could be evaluated as fully compensated.Yet it was shown that the TNF-α/IL-4 ratio is two time higher compared to the control (2,00±0,1) against(1,09±0,05) and the parameteres of phagocytic activity of circulating blood neutrophils (neutrophil phagocytosis index: (46,2±1,8) against (67,1±3,2); neutrophil phagocyic count: (5,1±0,1) against (8,7±0,3)) and peritoneal macrophages (phagocytosis index (34,2±2,3) against (52,8±2,5)); phagocytic count (4,3±0,2) against(9,5±0,2)) were decreased. The decrease in phagocytosis parameters and cytokine profile disturbance points to the possibility of system decompensation on the background of functional load .

The studies, that were carried out on the 5 day after S.typhimurium infection in the non-irradiated animals (group B) have shown features of immune system activation, characteristic for infectious process, such as an increase in phagocytic activity of peripheral blood neutrophils (phagocytosis index (71,1±3,5) against (67,1±3,2)and peritoneal macrophages (phagocytosis index (69,4±2,8) against (52,8±2,5)). Also characteristic changes in cytokine profile were observed (TNF-α level was (111,8±3,64) against (39,18±0,33); IL-4 level was (100,1±1,53) against (35,89±0,3)), as well as a decrease in general complement level (62,88±2,78) against (96,61±2,2) and an increase in circulating immune complexes (0,007±0,0005) against (0,005±0,0006).

Postnatal irradiation in the dose of 1 Gy (group C) leads to characteristic changes in the non-specific resistance, while no substantial changes were observed in the animals irradiated antenatally at 0,5 Gy (group D) compared with the non- irradiated animals.

In all infected animals an excessive activation of circulating immune complexes was observed. In group C the activation level was the highest. In addition, the decrease in the complement level in this group was twice as high compared to the infected non-irradiated animals (26,75±1,75) against (96,61±2,2). Taken together with relatively low neutrophil activity such condition leads to the increase in circulating immune complexes concentration (0,009±0,0007) against (0,005±0,0006).

The sufficient misbalance of cytokine profile on the infectious background should be mentioned as the most characteristic feature and possibly a key parameter of the induced destabilization of the immunological resistance: if in intact animals on the 5-th day after infection TNF-α/IL-4 ratio was about 1 (1,09±0,05), then in group C in the same period the level of TNF-α was 5 times higher than IL-4 level (5,2±0,1) . This effect was not observed in the antenatally irradiated animals.

Summary

In summary, the analysis of non-specific resistance has shown the characteristic features of immune system activation: phagocytosis stimulation, the increase in proinflammatory cytokines concentrations, the decrease in the general complement level and increase in circulating immune complexes. The severity of disease course and its consequences (the duration of persistence of S typhimurium in the organism, the level of internal organs lesions) was determined by the state of anti-infectious resistance of the animals and also depended on the bacterial load, received during the inoculation.

Therefore, the application of the selected biological object (Wistar rats aged 1 month), irradiation scheme (1 time, dose 1 Gy), infection model (peritoneal

sepsis, caused by inoculation with S.typhimurium in the dose of 5 x 10^8 CFU) are necessary and sufficient conditions for creating a model of radiation induced anti-infectious resistance defect. This model allows evaluating the consequences of irradiation at any time after exposition.

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In course of the present work a model of radiation-induced anti-infectious resistance defect was developed. The research has shown that infection with Salmonella typhimurium of the postnatally irradiated Wistar rats in the dose range of 0,75 - 1,5 Gy leads to the complex defect of anti-infectious resistance, characterized with the following features of immune system activation: phagocytosis depression, the increase in proinflammatory cytokines levels -TNF-α level was (94,07±2,67) pg/ml, the decrease in the general complement level (36,75±1,75) h.u. and an increase in circulating immune complexes (0,009±0,0007) o.d.u. The developed model can be used in order to evaluate the consequences of irradiation at any time after exposition and methods of their correction.

Key words: low-dose irradiation, anti-infectious resistance, Salmonella typhimurium, immunological parameters.

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ІМУНОЛОГІЧНІ ПАРАМЕТРИ МОДЕЛІ РАДІАЦІЙНО-ІНДУКОВАНОГО ДЕФЕКТУ ПРОТИІНФЕКЦІЙНОЇ РЕЗИСТЕНТНОСТІ

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В даній роботі була розроблена модель радіаційно-індукованого дефекту протиінфекційної резистентності. В ході дослідження було показано, що інфікування Salmonella typhimurium щурів лінії Wistar, опромінених у постнатальному періоді розвитку в дозовому діапазоні 0,75 - 1,5 Гр призводить до комплексному дефекту протиінфекційної резистентності, який характеризувався наступними ознаками порушення діяльності імунної системи: депресією фагоцитарної активності, підвищення рівню прозапальних цитокінів - рівень ФНП-α становив (94,07±2,67) пг/мл, зниженням рівню загального комплементу (36,75±1,75) г.о. та підвищенням циркулюючих імунних комплексів (0,009±0,0007)о.о.щ. Розроблена модель може бути застосована для оцінки наслідків опромінення та їх корекції в будь-який період після впливу.

Ключові слова: низькодозове опромінення, протиінфекційна резистентність, Salmonella typhimurium, імунологічні параметри.

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ИММУНОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ РАДИАЦИОННО-ИНДУЦИРОВАННОГО ДЕФЕКТА ПРОТИВОИНФЕКЦИОННОЙ РЕЗИСТЕНТНОСТИ

Коляда Т.И., Брусник С.В., Скляр Н.И., Михайличенко М.С., Нестеренко А.М., Коляда О.Н.

В данной работе была разработана модель радиационно-индуцированного дефекта противоинфекционной резистентности. В ходе исследований было показано, что инфицирование Salmonella typhimurium крыс линии Wistar, облученных в постнатальном периоде развития в дозовом диапазоне 0,75 - 1,5 Гр приводит к формированию комплексного дефекта системы противоинфекционной резистентности, который характеризуется следующими признаками нарушения деятельности иммунной системы: стимуляцией фагоцитарной активности, повышением уровня провоспалительных цитокинов - уровень ФНО-α составлял (94,07±2,67) пг/мл, снижением уровня общего комплемента (36,75±1,75) г. е. и повышением уровня циркулирующих иммунных комплексов (0,009±0,0007) е.о.п. Разработанная модель может быть использована для оценки последствий облучения в любой момент после воздействия, а также методов их коррекции.

Ключевые слова: низкодозовое облучение, противоинфекционная резистентность, Salmonella typhimurium, иммунологические параметры.