PRIMATOLOGY

Effects of Low-Dose γ-Irradiation on the Counts of Immunocompetent Cells in a Model Experiment on Primates

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Cellular immunity was studied by flow cytometry with Becton Dickinson monoclonal antibodies in clinically healthy *Macaca mulatta* males before and after low-dose exposure to ionizing radiation. It was shown that T and B cells are radiosensitive, B cells being more sensitive, which is seen from a significant drop of their count. Natural killers are radioresistant. The count of immunocompetent cells recovers sooner after single compared to fractionated irradiation in the same summary dose.

Key Words: *monoclonal antibodies; low dose; γ-radiation; primates*

In the days of technological revolution a human being is sometimes exposed to extreme environmental conditions, such as elevated basal radiation, ecological hazards, changes in barometric pressure or temperature, *etc.* Problems in provision of the optimal vital activity under these conditions and maintenance of high working capacity stimulate comprehensive studies of human functional status.

One of the important problems we face now is the effect of low-dose radiation. This problem received little attention. Opinions on the direction and severity of biomedical aftereffects of this exposure vary [7,8,10,14]. Some authors regard all effects of low doses as stochastic, realized in the form of cancer, genetic, immune, and other disorders of different severity [2,11,13,16]. Of all physiological systems of human organism, the immune system is one of the most sensitive to radiation exposure. Studies aimed at detection of immune disorders early after radiation exposure, determining the formation of immunopathological processes and diseases in subjects exposed to low-dose ionizing radiation during a long period, are therefore very important.

The problem of radiation and its consequences are studied in experiments on animals. Monkeys as "laboratory twins" of human beings are the most adequate model for solving these problems [4,6].

We studied cellular immunity in *Macaca mulatta* 24-85 days after exposure to low-dose ionizing radiation.

MATERIALS AND METHODS

Experiment was carried out in 12 clinically healthy *Macaca mulatta* males aged 3-5 years. The animals were kept in common cages without limitation of their motor activity on rations consisting of natural foodstuffs. The psychoemotional sphere, somatic status, and physiological secretions of monkeys were normal. Quarantine and adaptation to experiment conditions were carried out during 3 months before experiment.

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The model of exposure has been developed by physicists from Biomedical Institute for Mars 500 experiment [5].

The animals were distributed into 3 groups. Group 1 (n=4) animals were exposed according to the following protocol: 5 sessions 25 cGy each, 2-day interval, and 5 more sessions of 25 cGy. The effective residual dose by the end of exposure was 100 cGy.

Group 2 (n=4) animals were exposed according to the following protocol: 1 session in a dose of 66 cGy, 6-day interval, and one more session of 66 cGy. The effective residual dose by the end of exposure was 100 cGy.

Group 3 animals (controls, n=4) were not exposed to radiation.

Immunophenotypical analysis of cells was carried out by flow cytofluorometry based on reaction between monoclonal antibodies with fluorescent label and lymphocyte surface antigens and subsequent analysis of the samples on a FAGCalibur flow cytometer (Becton Dickinson). Double labeling of cells was used [3].

Blood for immunophenotyping was collected after overnight fasting. Monoclonal antibodies (20 μl) were added to 100 μl blood treated by heparin (20 U/ml, Microgen), mixed, and the sample was incubated during 20 min in the darkness at ambient temperature. Lysis and fixation of the samples were carried out manually. Lysing solution (2 ml) was added into the sample which was then incubated (10-15 min) in the darkness at ambient temperature till the sample clarification. Two washing procedures were then carried out by the standard method [3]. The samples were analyzed on the same day. Levels of expression of CD3, CD4, CD8, CD16, CD20, CD25, CD45 RA, and HLA-DR molecules on cell surface were studied. The cell population gate was set up from a combination of direct and lateral light diffusion and cell size. The results were recorded using CellQuestPro software in the presence of 5000 cells in the gate. The data were statistically processed using Microsoft Excel software. In order to evaluate the effect of exposure on immunocompetent cells (ICC), absolute counts of lymphocytes were estimated by a two-platform method, including evaluation of the percentage of lymphocyte subpopulations on a flow cytometer using monoclonal antibodies. Cell counts were evaluated by common blood analysis. Leukocyte and lymphocyte counts in the peripheral blood were determined, after which the result was obtained by mathematical calculations [15]: $X=(WBC\times A)/100$, where X is absolute count of lymphocytes, WBC is leukocyte count, and A is lymphocyte percentage; $Y=(X\times B)/100$, where Y is absolute count of cells carrying this or that CD and B is the percentage of cells carrying this or that CD.

RESULTS

The mean absolute counts of lymphocytes before and at different periods after radiation exposure are presented in Table 1.

The count of T cells on day 24 after exposure decreased by 1.7 times in group 1, by 1.4 times in group 2, and by 1.1 times in group 3. It seems that the slight decrease in T cell count in group 3 (control) can be

TABLE 1. Absolute Count of ICC in the Peripheral Blood of Monkeys before and after Irradiation

Group	Period of study	Differentiation clusters		
		CD3	CD16	CD20
1	Before exposure	1387.3	262.7	757.3
	After 24 days	797.8	264	159.2
	After 50 days	849.2	324.8	290.6
	After 85 days	1044.9	241.2	322.7
2	Before exposure	1589.6	207.9	950.7
	After 24 days	1171.4	286.1	395.9
	After 50 days	1532	217.9	723.5
	After 85 days	1696	175.8	940.8
3 (control)	Initial	1508.8	200.8	448.6
	After 24 days	1345.3	220	352.4
	After 50 days	1488.1	201.9	418.6
	After 85 days	1455.1	176.8	336.2

neglected. The "radiation immunodeficiency" after the exposure was the most pronounced in group 1 animals. Reduced counts of T cells persisted in them on days 50 and 85 after the exposure. In group 2 receiving single sessions the counts of T cells were also decreased on day 24; on day 50 cell count normalized, and on day 85 even somewhat increased in comparison with the initial level (Fig. 1).

The peripheral blood counts of NK cells on day 24 after irradiation did not differ from the initial levels in groups 1 and 3, while in group 2 the count increased 1.4 times. By day 50 after the exposure, the NK cell count increased in group 1 and decreased to the initial level in group 2. After 85 days, the parameters virtually normalized in all experimental groups (Fig. 2).

On day 24 after irradiation, the count of B cells in groups 1 and 2 decreased by 4.8 and 2.4 times, respectively. The changes were more pronounced in group 1 after fractionated irradiation. Significant decrease in B cell count 24 days after the exposure was followed by a slight increase in this parameter after 50 and 85 days. By day 85, the count of B cells was still 2.3 times lower than before the exposure. In group 2, a drop of cell count on day 24 was followed by its increase by day 50 and complete recovery by day 85 (Fig. 3).

Hence, our results are in line with the findings of other authors indicating radiosensitivity of T- and B cells [1,12]. The B cells are characterized by higher radiosensitivity, which is shown by a significant decrease in their count, while NK cells are radioresistant. In addition, it was previously shown that ICC counts ìstore sooner after single exposure compared to fractionated irradiation [8]. Our results have confirmed this fact in a long-term experiment at different irradiation modes.

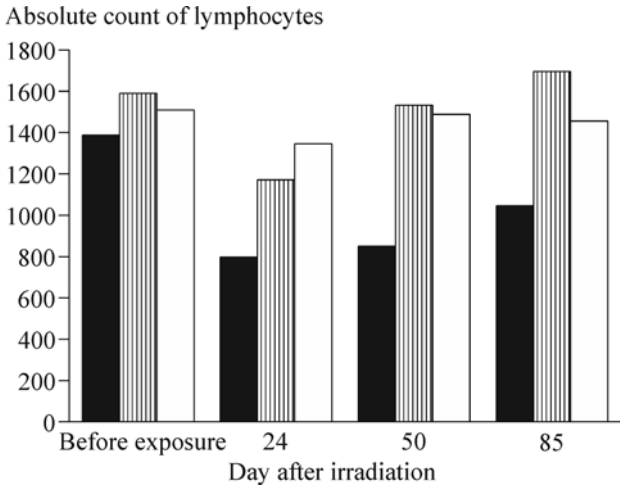

Fig. 1. Effect of ionizing radiation on T cell (CD3) count. Here and in Figs 2, 3: light bars: control (group 3); dark bars: group 1; vertically hatched bars: group 2.

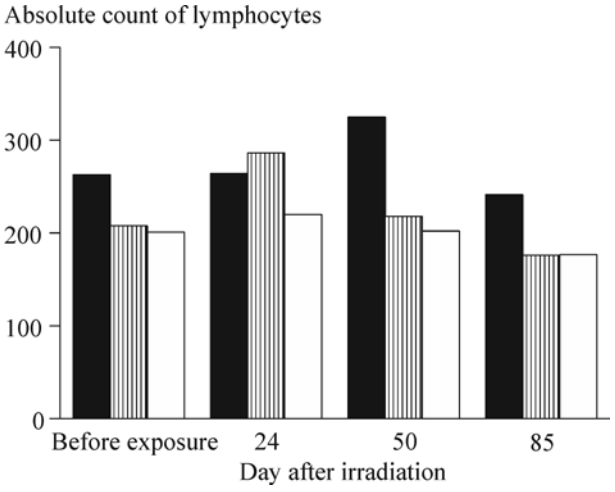

Fig. 2. Effect of ionizing radiation on NK cell (CD16) count.

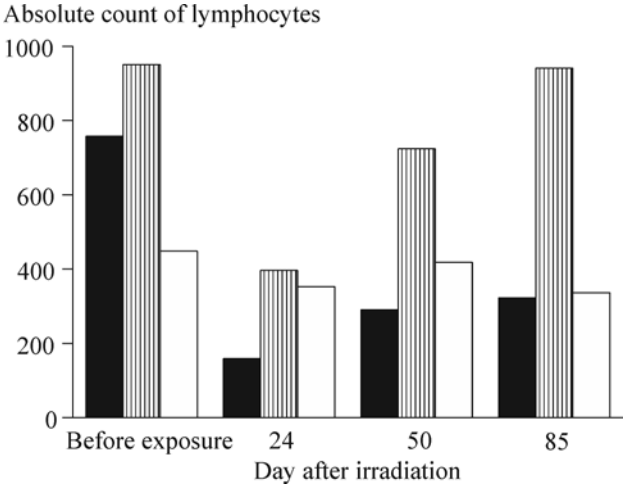

Fig. 3. Effect of ionizing radiation on B cell (CD20) count.

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