CHAPTER 9

POST-RADIATED AND POST-STRESSED VOLATILE SECRETIONS: SECONDARY IMMUNE AND BEHAVIORAL REACTIONS IN GROUPS OF ANIMALS

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Abstract: It was shown that the irradiated mice gave off with urine immunosuppressive components which possessed of high volatility. With their help even the one irradiated individual can induce in intact mice the disturbance of immunity and alteration of number of blood cells. Moreover, the predominant mouse from exposed group of mice also was capable to elicit disturbance of immunity in next group of intact animals. The same effects could be received by transfer of the urine samples from irradiated mice or from intact mice exposed with urine of irradiated animals to the box with intact individuals. It was established that ionizing radiation (4–6 Gy) of male mice increase their scent attractiveness to intact singenic male conspecifics. The carried out experiments have shown existence of the mediated by volatile chemosignals mechanism of multiplication second post-radiated disturbances of immunity in the groups of animals. The immunosuppressive volatile components were induced also by stress and some immunodepressants (dexametasone and cyclophosphamide).

Keywords: immunosuppressive volatile components; irradiation; stress; immunodepressants; olfactory behavior of mice

Introduction

It is known that in physiological conditions animals give off secretion including urine volatiles (pheromones). These substances cause specific physiological behavioral reactions in the recipients and provide them with information about the social, sexual and reproductive status of other individuals within the species (Halpin, 1986; Penn and Potts, 1998; Yamazaki et al., 1999; Moshkin

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et al., 2002). There are practically no findings suggesting that animals possess any chemical signals of pathologic conditions. The exception is provided by some reports on alterations in the scent attractiveness of female mice to chemosignals of infected male mice (Penn and Potts, 1998; Moshkin et al., 2002), "the smell of fear" produced by stressed animals (Zalcman et al., 1991).

Previous experiments have shown that mice and rats exposed to radiation or stress can induce some disorders of the immune reactivity and the content of formed elements of blood in intact individuals (Surinov et al., 1998). Such effects have been designated as communicative. Subsequently, they were found to be conditioned by volatile components (VC) produced with urine (Surinov and Dukhova, 2004). These VC appear to function to attract intact individuals.

These VC can cause not only mediated secondary post-radiation reactions not associated with direct exposure of animals to radiation but also contribute, as proved, to their multiplication and diffusion (Surinov et al., 2005). This effect is similar to the bystander effect, which is well known in radiobiology and manifests itself as attenuation of the genome of intact cells adjoining the irradiated cell (Mothersill and Seymour, 1998–2001).

Materials and Methods

IMMUNOLOGICAL INVESTIGATIONS

CBA and C57Bl/6 (B6) and F1 (CBAxC57Bl6) strains of male mice (weighted 22–24 g) were used in this study. They were maintained under vivarium conditions, provided standard food, water, and kept under a usual light/dark cycle. Before the experiment, mice were housed in tens in standard plastic boxes for 2 weeks. They were given whole-body irradiation of 4, 6 Gy using a 60Co source with a dose-rate of 3.2 mGy/sec on "Gamma-Cell-220" equipment (Atomic Energy Canada Limited, Canada).

Mice were exposed to stress by one-time swim within 1 h at 30°C.

Immunodepressants (dexametasone or cyclophosphamide) were injected introperitoneal.

In 1–2 days after irradiation, stress or injection of immunodepressants to mice, on bottom of box put a sheet of filter paper (bedding). Access to this bedding was limited by the second perforated bottom, lifted above basic bottom on 0.5 cm. Such paper bedding, containing of absorbed 24 hrs urine was transferred in box with individuals (recipients) also under perforated bottom. In 24 hs after exposition with bedding animals immunized by sheep red blood cells (SRBC) in dose 1×10^8 cells/mouse. In 4 days, mice were decapitated of under ester narcosis. The number of nuclear cells and antibody-forming cells (AFCs) was determined by Cunningham's method. Statistic analysis of the results of the study was performed with the use of Student's *t*-test.

BEHAVIORAL INVESTIGATIONS

We used also a choice assay to test the VC preferences of irradiated mice. For this purpose, we used a modified T-maze (Bures et al., 1983) with a "field of choice" represented as an open plastic 30 × 35 cm cage with a wall height of 35cm. On its external opposite sides, there were two "hiding boxes" (lightproof plastic 10 × 10 × 5 cm boxes), where mice (testers) could freely go out through the holes in the "field" walls. At a distance of 0.5 m above the "field", there was an electrical halogen 50 wt lamp. Male mice used as testers (10 individuals) were individually placed six times each in the middle of the "field" before irradiation (several series of observations at 1–2 days intervals) and at different intervals after irradiation to record which of "hiding boxes" will be chosen by a given individual. Within first 1–3 min, the testers went round the "field" and alternately entered both "hiding boxes". Finally, the most testers spent more than 0.5 min inside one of the boxes. The latter was considered as preference for VC cues outgoing from bedding containing a certain urine sample. Each series of observations was conducted within the first half of the light day and contained 60 preference assessments.

The results were expressed in the percentage of the preferences for compared samples (number of the preferences for one sample in relation to the total number of assessments in a series). We used Wilcoxon Signed-Ranks test to assess the significance of the VC preferences by one of the groups in comparison with another group in each period of observations. All data were subjected to Student's *t*-test to assess the significance of the differences between preference rates.

Results and Discussion

IMMUNOLOGICAL INVESTIGATIONS

As a result of investigation of influence 24 h bedding from the irradiated in doses 4Gy mice on immunological parameters of intact singenic mice were obtained the following data (Table 1).

The bedding from a cage with irradiated animals transferred to a box with intact ones and then these mice immunized by the SRBC. As control used mice which transferred bedding from intact singenic conspecifics. In 5 days at experimental animals defined a content of AFC in a spleen. Immunosuppressive effect was observed in that case when the bedding was from mice in 3–7 days after an irradiation. In other periods of research of essential suppression, antibody genesis was not defined. According to the numerous data just for 3–7 days the greatest postradiation disturbance of immunity takes place.

TABLE 1. Immunosuppressive activity ($M \pm m$) of volatile components of urine of mice CBA in different periods after irradiation (4 Gy)

Animals–donors of VC	Time after irradiation, days	Spleen		Thymus
		Number of cells, 1×10^6	AFC, 1×10^3	Number of cells, 1×10^6
Control	1	123 ± 12.6 (100 ± 10.3)	178 ± 12.9 (100 ± 7.2)	53.0 ± 3.9 (100 ± 7.3)
Irradiated		100 ± 9.5 (81.0 ± 7.7)	151 ± 10.5 (84.8 ± 5.7)	39.3 ± 1.8* (74.2 ± 3.4)
Control	2	125 ± 14.8 (100 ± 11.9)	173 ± 21.0 (100 ± 12.0)	39.0 ± 1.3 (100 ± 3.3)
Irradiated		122 ± 17.5 (97.6 ± 14.0)	122 ± 8.5* (70.6 ± 4.9)	32.5 ± 7.1 (83.0 ± 18.0)
Control	3	103 ± 6.7 (100 ± 6.5)	187 ± 11.2 (100 ± 6.0)	35.5 ± 1.5 (100 ± 4.2)
Irradiated		100 ± 12.2 (97.0 ± 11.8)	126 ± 13.7* (67.7 ± 7.3)	36.1 ± 7.3 (101 ± 20.6)
Control	7	162 ± 9.2 (100 ± 5.7)	148 ± 13.6 (100 ± 9.0)	45.2 ± 3.8 (100 ± 8.4)
Irradiated		133 ± 5.4* (82.0 ± 3.3)	111 ± 6.0* (75.0 ± 4.0)	38 ± 2.2 (84 ± 4.9)
Control	14	130 ± 9.0 (100 ± 7.0)	143 ± 22.0 (100 ± 15.0)	53.2 ± 4.3 (100 ± 8.1)
Irradiated		170 ± 19.3 (131 ± 14.8)	135 ± 20.0 (94.4 ± 14.0)	31.6 ± 4.5* (59.4 ± 8.5)
Control	21	130 ± 7.1 (100 ± 5.5)	260 ± 34.0 (100 ± 13.0)	44.0 ± 6.3 (100 ± 14.3)
Irradiated		120 ± 10.5 (92.0 ± 8.0)	191 ± 41.2 (73.5 ± 15.8)	38.4 ± 4.2 (87.3 ± 9.5)

Note: In brackets – % to control

*Significant different ($p < 0.05$) from control.

Thus mice subjected to single action of ionizing radiation in sublethal doses secreted with urine of volatile components possessing by immunosuppressive activity reference to intact animals. The time of appearance of such components coincided with period the greatest postradiation disturbance of immunity.

For comparison post-radiated and post-stressed dynamics of a secretion of volatile components with their immunosuppressing properties the following research was carry out. A 24 h bedding from a box with stressed mice transferred to a box with the intact singenic recipients just as was made in experiences with an irradiation.

As has appeared within the first 2 days the stressed animals produced volatile components, which twice reduced the immune response to SRBS at intact individuals (Table 2).

TABLE 2. Immunological parameters ($M \pm m$) at intact male mice F1(CBA × C57Bl6) after transfer to them of bedding received in different time from stressed mice

Animals–donors of VC	Time after stress, days	Number of cells in spleen, 1×10^6	AFC in spleen, 1×10^3
Control	2	146 × 10 (100 ± 6.8)	86.6 ± 7.8 (100 ± 9)
Stress		109 ± 3.1 (74.7 ± 2.1)*	44 ± 5.6 (50.8 ± 6.5)*
Control	7	111 ± 15.6 (100 ± 14)	63.6 ± 4.7 (100 ± 7.4)
Stress		113 ± 11.7 (102 ± 10.5)	54.4 ± 7.7 (85.5 ± 12.1)
Control	14	112 ± 7.5 (100 ± 6.7)	78.4 ± 5.1 (100 ± 6.5)
Stress		125 ± 5.7 (112 ± 5.1)	51 ± 4 (65.1 ± 5.1)*
Control	21	125 ± 12.6 (100 ± 11)	85.7 ± 4.6 (100 ± 5.4)
Stress		128 ± 15.2 (102±12.2)	55 ± 4 (64.2 ± 4.7)*
Control	30	110 ± 12.3 (100 ± 12)	132 ± 14 (100 ± 11)
Stress		106 ± 11.6 (96.4 ± 10.5)	95.6 ± 14.1 (72.4 ± 10.7)

Note: In brackets – % to control
*Significant different ($p < 0.05$) from control.

By 7 days after a stressing, the suppressing activity of bedding was minor. By 14 days the suppressing activity of components to some extent was restored and by 30 days it was again reduced.

Described earlier dynamics of immunosuppressing activity of a bedding from stressed mice practically completely coincident with dynamics of alteration of the antibodygenesis at mice subjected single stress, which was investigated and reported by us earlier. The greatest suppression of the immune response took place just for 1–2 days after a stress.

Thus a post-stressed dynamics of secretion with urine of mice volatile components coincide with dynamics of post-stressed suppression of immunity.

Ability to induce secretion of immunosuppressive volatile components possessed also the synthetic glucocorticoid dexametasone. Under its direct influence since a dose 4 mg/kg in mice-donors there was a dose-dependent reduction of the number of AFC in spleen and nuclear cells.

At the recipients the reduction of antibody formation was observed only in that case when the donors of volatile components received high doses of the drug (16 and 32 mg/kg). The immunosuppressive state elicited by direct action of dexametasone at the donors was replicated somewhat with the help volatile secrets at the mouse-recipient. However, at the recipients of VC the inhibition of the immune response was less expressed and was not accompanied by decrease of number of cells in lymphoid organs.

For research of dynamics of secretion of volatile immunosuppressive components the samples of urine of donors obtained through 1–28 days after injection of dexametasone in dose 16 mg/kg exposed to the recipients. It was showed that the suppressive activity of volatile components is observed at least within 3 days after injection of the drug (Table 3).

TABLE 3. Secretion of immunosuppressive components by mice-donors after injection of dexametasone (16 mg/kg)

Groups of mice-recipients	Time after stress, days	Number of cells in spleen, 1×10^6	AFC in spleen, 1×10^3	Number of cells in Thymus, 1×10^6
Control	1	151.9 ± 10.8 (100 ± 7.1)	76.3 ± 4.8 (100±6.3)	66.2 ± 2.6 (100±3.9)
Dexametasone		138.6 ± 12.4 (91.2 ± 8.2)	51.0 ± 4.7 (66.8 ± 3.2)*	62.8 ± 5.1 (94.9 ± 7.7)
Control	3	152.3 ± 9.5 (100 ± 6.2)	129.2 ± 11.7 (100 ± 9.1)	82 ± 9.5 (100 ± 11.6)
Dexametasone		150.0 ± 14.5 (98.5 ± 9.5)	89.1 ± 3.8 (69.7 ± 2.9)*	62.2 ± 7.5 (75.9 ± 9.1)
Control	7	137.2 ± 9.0 (100 ± 6.6)	107.2 ± 10.0 (100 ± 9.3)	92.2 ± 6.1 (100 ± 6.6)
Dexametasone		154.4 ± 10.5 (112.5 ± 7.7)	134.9 ± 15.6 (125.8 ± 14.6)	95.8 ± 8.3 (103.9 ± 9.0)
Control	15	173.4 ± 13.8 (100 ± 7.9)	44.0 ± 6.3 (100 ± 14.3)	43.0 ± 3.0 (100 ± 7.1)
Dexametasone		143.3 ± 10.4 (82.6 ± 6.0)	41.1 ± 9.9 (93.4 ± 22.5)	40.5 ± 3.3 (94.2 ± 7.7)
Control	21	227.8 ± 24.5 (100 ± 10.8)	39.7 ± 3.7 (100 ± 9.3)	63 ± 1.0 (100 ± 1.6)
Dexametasone		170 ± 14.2 (74.6 ± 6.2)	36.4 ± 1.4 (88.3 ± 3.5)	63.5 ± 3.4 (100.8 ± 5.4)
Control	28	172.2 ± 12.6 (100 ± 7.3)	53.1 ± 8.5 (100 ± 16.0)	49.8 ± 2.11 (100 ± 4.2)
Dexametasone		165.6 ± 9.6 (96.2 ± 5.6)	52.2 ± 3.0 (98.3 ± 5.7)	48.7 ± 2.1 (97.8 ± 4.2)

Note: In brackets – % to control
*Significant different ($p < 0.05$) from control.

By ability to induce secretion of the immunosuppressive volatile components except for a synthetic glucocorticoid dexametasone had also the cytostatic cyclophosphamide. However, the effect of this drug took place only at the large doses.

According to results of present research at mice-donors under influence of cyclophosphamide in dose 300 mg/kg took place deep suppression of the immune response up to 24.0 ± 2.1% which accompanied by a reduction of a number of cells in a spleen and thymus up to 35.7 ± 3.2% and 73.6 ± 9.3% respectively. The volatile secretions of these animals elicited in the recipients a decrease in number of AFC in a spleen up to 63.4 ± 6.3% and a number of cells in a thymus up to 69.0 ± 8.6%. A number of cells in spleen remained without an alteration.

Earlier we formulated the supposition about a possibility of multiplication and spreading of mediated secondary post-radiated disturbances in groups of

animals with the help of volatile components. As the basic model the co-content in the same cage of irradiated and intact mice or rats in the ratio 5:5 was used. The presence in this cage among intact animals only of one irradiated individual did not ensure steady effect. We have assumed that the effect of one individual can depend upon its hierarchical position in group. The experiments with an irradiation of the dominant mouse were carried out. For this purpose from group of outbred mice have chosen individuals with dominant behavior according to described in the literature procedure (Bures et al., 1983). The research have shown that the dominant mouse returned in the group after an irradiation induced at intact mice augmentation of number of neutrophilic leucocytes in peripheral blood up to 181 ± 28% as compared with control (in control group the dominant individual did not subject an irradiation). Then from groups of animals 1 and 2 took out the next dominant individual (having contact with irradiated mouse) and put it for 24 h in group of other intact mice (Table 4). After taking out of this dominant individual at the stayed 4 mice the decrease in number of blood cells in particular of erythrocytes (44.0 ± 8.7%) and leucocytes (77.0 ± 5.0%) was observed.

TABLE 4. Effect of dominant individual-inductor irradiated in dose 4Gy or dominant individual-inductor exposed by irradiated one on parameters of peripheral blood of intact outbred mice

	Number of cells in 1 mL of peripheral blood			
Experiments	Erythrocytes, 1×10^6	Leucocytes, 1×10^3	Lymphocytes, 1×10^3	Neutrophils, 1×10^3
Control 1 – contained with intact individual	8.72 ± 0.8 (100 ± 9.2)	6.2 ± 0.8 (100 ± 13.0)	4.3 ± 0.7 (100 ± 15.9)	0.9 ± 0.1 (100 ± 14.6)
Experiment 1 – contained with irradiated individual	8.3 ± 0.4 (95.1 ± 4.9)	6.8 ± 0.6 (110.0 ± 9.2)	3.9 ± 0.6 (92.5 ± 12.8)	1.7 ± 0.3 (181 ± 26.8)*
Control 2 – contained with individual from group of control 1	8.6 ± 0.7 (100 ± 8.1)	7.9 ± 0.5 (100 ± 6.8)	5.7 ± 0.7 (100 ± 11.9)	1.33 ± 0.1 (100 ± 9.4)
Experiment 2 – contained with exposed individual from group of experiment 1	3.8 ± 0.7 (44.0 ± 8.7)*	6.1 ± 0.4 (76.9 ± 5.0)*	4.1 ± 0.4 (71.6 ± 7.7)	1.0 ± 0.1 (77.0 ± 8.9)
Control 3 – contained with individual from group of control 2	8.1 ± 0.2 (100 ± 2.9)	9.8 ± 0.6 (100 ± 6.1)	7.1 ± 0.5 (100 ± 7.1)	1.4 ± 0.2 (100 ± 11.2)
Experiment 2 – contained with exposed individual from group of experiment 1	8.0 ± 0.2 (99.6 ± 2.7)	7.9 ± 0.9 (81.0 ± 9.4)	5.2 ± 0.5 (72.8 ± 6.8)*	1.3 ± 0.1 (93.0 ± 7.0)

Note: In brackets – % to control
*Significant different ($p < 0.05$) from control.

A repetition of the earlier described procedure – translocation of one by one dominant individual from exposed groups 3 and 4 in next groups of intact mice – group 5 and 6 – also induced a decrease in number of blood cells mainly of lymphocytes (72.8 ± 7.0%).

The sequential "transmission" of disturbances from one group of animals in other one can be realized not only with the help of separate individuals but also with the help of samples of urine absorbed in a paper bedding within 24 h.

So transfer of bedding from a box with the irradiated singenic mice into box with group of intact mice (male F1(CBAxC57Bl6) resulted in reduction of their ability to immune response up to 74.8 ± 4.0% versus control (bedding from a box with intact animals). When from these exposed by urine of mice received a bedding saturated with their own urine then it also caused an immunosuppression in other intact animals. The ability to the immune response was reduced practically up to the same level (73.0 ± 7.9%) as well as in the previous group. Thus even one irradiated mouse can cause of spreading of secondary indirect disturbance of an immune reactivity and decrease a number of blood cells in group contacting with its individuals.

The absence at individual, group (it is showed in the present work), specific restrictions for reactions on post-radiated secretions obviously can have ecological consequences.

The phenomena found by us remind the bystander effect which is widely considered in the literature with only one difference that in the later case there is a disturbance of a genome stability in intact cells under influence of the factors outgoing from irradiated cells (Mothersill and Seymour, 1998; 2000; 2001). Whereas, we study a distant action of one organism to others in groups of animals. Namely influence of pheromone-like volatile components induced by damaging actions on immunity of intact animals.

BEHAVIORAL INVESTIGATIONS

One of the important problems at study of properties of post-radiated volatile secretion is their similarity or difference from post-stressed volatile secretion which also have immunosuppressive activity (Surinov and Dukhova, 2004)

The comparative research of dynamics of the reaction of intact testers on post-radiated and post-stressed volatile secretions in test on olfactory preference-avoidance has shown the following. At comparison secretion of the intact and irradiated mice the intact mice-testers preferred post-radiated secretions. At comparison secretion of the intact and stressed mice the same mice-testers preferred volatile secretions of stressed individuals. Hence, as post-radiation secretions and post-stressed these of mice CBA have the high scent attractiveness for intact mice. These attractive properties of the volatile

components were found only during first week after an irradiation or stressing action. Later such attractiveness was not found.

In the same experiments the comparative research of reaction of preference-avoidance by intact testers of the post-radiated and post-stressed volatile secretions was carried out (Table 5).

It was supposed that in the case of identity of compared secretions the mice-testers cannot distinguish. However testers with the greater frequency chose cover with bedding from stressed individuals than from irradiated ones.

Thus obtained data do not exclude that post-irradiated and post-stressed secretions are not identical.

In our other experiment, intact male mice significantly preferred VC produced by individuals of the congenial genotype while choosing between hiding boxes containing VC of CBA and B6 males. Before irradiation, intact CBA males preferred VC of syngeneic males with an average daily rate of 59.67 ± 0.48%. Males of the B6 strain preferred VC of B6 males with an average daily rate of 59.50 ± 0.50%. This olfactory behavior of male mice has been previously designated as homing, i.e. yearning for their males (Surinov and Dukhova, 2004; Surinov et al., 2005) unlike the reproductively conditioned attractiveness to females with a strange genotype (Yamazaki et al., 1999; Moshkin et al., 2002).

After irradiation (in dose of 4 Gy), testers of both strains showed changes in the short-term attractiveness to syngeneic chemosignals when choosing hiding boxes with VC of syngeneic or allogeneic male mice as compared with data before irradiation. The attractiveness of CBA testers was significantly

TABLE 5. Comparative evaluation of preference rate (%) by intact testers (CBA-mice) of volatile secretions of intact irradiated (4Gy) or stressed mice CBA.

Time after irradiation, days	CBA male testers		CBA male testers		CBA male testers	
	VC control	VC irradiated	VC control	VC stressed	VC irradiated	VC stressed
1	40 ± 4.62	60 ± 4.62*	44 ± 2.78	56 ± 2.78	46 ± 4.34	54 ± 4.34*
2	43 ± 4.34	57 ± 4.34*	39 ± 4.45	61 ± 4.45*	44 ± 2.78	56 ± 2.78*
3	38 ± 3.56	62 ± 3.56*	42 ± 4.34	58 ± 4.34*	42 ± 3.56	58 ± 3.56*
4	40 ± 4.45	60 ± 4.45*	42 ± 2.78	58 ± 2.78*	46 ± 3.69	54 ± 3.69
7	43 ± 4.62	57 ± 4.62*	42 ± 4.62	58 ± 4.62*	44 ± 3.56	56 ± 3.56
9	48 ± 4.34	52 ± 4.34	46 ± 3.69	54 ± 3.69	46 ± 4.62	54 ± 4.62
11	48 ± 3.56	52 ± 3.56	44 ± 4.62	56 ± 4.62*	48 ± 3.56	52 ± 3.56
14	48 ± 4.62	52 ± 4.62	50 ± 4.34	50 ± 4.34	46 ± 4.34	54 ± 4.34
16	45 ± 4.45	55 ± 4.45	48 ± 3.56	52 ± 3.56	44 ± 4.45	56 ± 4.45
18	45 ± 4.34	55 ± 4.34*	46 ± 4.62	54 ± 4.62	54 ± 3.69	46 ± 3.69
21	48 ± 2.78	52 ± 2.78	48 ± 3.69	52 ± 3.69	46 ± 4.34	54 ± 4.34

*Significantly (according to Wilcoxon) preferred odortype.

TABLE 6. Preference rate ($M \pm m$, %) in CBA and B6 male testers exposed to 4 Gy irradiation for volatile secretions produced by intact syngeneic and allogeneic males

Period after irradiation, days	CBA male testers		B6 male testers	
	VC of CBA	VC of B6	VC of B6	VC of CBA
Before irradiation	65.00 ± 9.61*	34.00 ± 9.61	62.38 ± 2.14*	37.62 ± 2.14
1	68.33 ± 4.66*	31.67 ± 4.66	71.67 ± 3.56**	28.33 ± 3.56
2	73.33 ± 3.69***	26.67 ± 3.69	71.67 ± 4.34*	28.33 ± 4.34
3	70.00 ± 4.16*	30.00 ± 4.16	80.00 ± 5.44***	20.00 ± 5.44
4	65.00 ± 4.62*	35.00 ± 4.62	76.67 ± 4.45***	23.33 ± 4.45
7	73.33 ± 5.67*	26.67 ± 5.67	68.33 ± 4.62*	31.67 ± 4.62
8	73.33 ± 4.45*	26.67 ± 4.45	76.67 ± 4.45***	23.33 ± 4.45
10	78.33 ± 5.00*	21.67 ± 5.00	68.33 ± 5.24*	31.67 ± 5.24
11	73.33 ± 4.45*	26.67 ± 4.45	73.33 ± 3.69***	26.67 ± 3.69
14	68.33 ± 5.24*	31.67 ± 5.24	70.00 ± 4.84*	30.00 ± 4.84
17	68.33 ± 3.89*	31.67 ± 3.89	71.67 ± 4.34*	28.33 ± 4.34
19	66.67 ± 4.30*	33.33 ± 4.30	75.00 ± 3.73***	25.00 ± 3.73
23	60.00 ± 2.72*	40.00 ± 2.72	75.00 ± 2.78***	25.00 ± 2.78
28	61.67 ± 4.34*	38.33 ± 4.34	68.33 ± 2.99*	31.67 ± 2.99
32	61.67 ± 3.56*	38.33 ± 3.56	68.33 ± 3.89*	31.67 ± 3.89

*Significantly (according to Wilcoxon) preferred odortype;

**Significant differences (according to Student, $p < 0.05$) as compared with the attractiveness of testers before the exposure to radiation.

enhanced on the second day (Table 6). Interestingly, this effect lasted considerably longer (up to 23 days after irradiation) in irradiated B6 males.

Our studies indicate that within short times after irradiation in sublethal dose the attractiveness of CBA and B6 male mice to volatile secretions of syngeneic males changes and exceeds the physiological level, showing no disorder of the olfactory sensitivity. Together, these findings support the idea that male mice experience selective changes in chemosignals-induced behavioral reactions. It might be caused by disturbances of metabolic and regulatory processes suggestive of a "syndrome of disease" (Barnett, 1975).

The group of animals with the absorbed dose of 6 Gy showed some different patterns (Table 7).

Within a short period after exposure to radiation, the animals lost their olfactory selectivity in respect to volatile cues from syngeneic and allogeneic individuals. For the CBA strain, that time period was 2 days and for the B6 strain – 4 days. The average of the preferences of CBA testers for volatile cues from syngeneic individuals achieved the physiological level on the third day, and the attractiveness in B6 testers regenerated up to the background level on the seventh day. Subsequently, testers of both strains showed a temporary enhancement of the attractiveness versus intact mice. In CBA testers, the attractiveness enhanced significantly over the period of 11 days, and in B6 testers – up to 14 days.

TABLE 7. Relative preference rate ($M \pm m$, %) in CBA and B6 male testers exposed to 6 Gy irradiation for volatile secretions produced by intact syngeneic and allogeneic males

Period after irradiation, days	CBA male testers		B6 male testers	
	VC of CBA	VC of B6	VC of CBA	VC of B6
Before irradiation	59.67 ± 0.48*	40.33 ± 0.48	40.5 ± 0.50	59.5 ± 0.50*
1	48.33 ± 2.99	51.67 ± 2.99	48.33 ± 2.99	51.67 ± 2.99
2	51.67 ± 2.99	48.33 ± 2.99	48.33 ± 5.24	51.67 ± 5.24
3	55.00 ± 2.55*	45.00 ± 2.55	46.67 ± 5.44	53.33 ± 5.44
4	73.33 ± 2.72***	26.67 ± 2.72	50.00 ± 3.51	50.00 ± 3.51
7	76.67 ± 2.72***	23.33 ± 2.72	46.67 ± 2.22	53.33 ± 2.22*
8	75.00 ± 2.78***	25.00 ± 2.78	28.33 ± 2.55	71.67 ± 2.55***
9	78.33 ± 3.56***	21.67 ± 3.56	26.67 ± 2.72	73.33 ± 2.72***
10	71.67 ± 4.34***	28.33 ± 4.34	31.67 ± 3.89	68.33 ± 3.89***
11	65.00 ± 1.67***	35.00 ± 1.67	31.67 ± 5.24	68.33 ± 5.24*
14	65.00 ± 2.99*	35.00 ± 2.99	28.33 ± 2.55	71.67 ± 2.55***
16	56.67 ± 2.72*	43.33 ± 2.72	38.33 ± 3.56	61.67 ± 3.56*
18	58.33 ± 2.78*	41.67 ± 2.78	36.67 ± 3.33	63.33 ± 3.33*
21	60.00 ± 2.72*	40.00 ± 2.72	36.67 ± 4.16	63.33 ± 4.16*

*Significantly (according to Wilcoxon) preferred odortype;
**Significant differences (according to Student, $p<0.05$) as compared with the attractiveness of testers before the exposure to radiation.

These studies have provided experimental evidence illustrating a clear relationship between olfactory conditioned behavioral reactions of male mice of both strains and the absorbed dose of ionizing radiation.

Conclusions

Thus, the data obtained in this study provide evidence that, at early stages after exposure to sublethal doses of ionizing radiation, stress and administration of immunodepressants the animals secrete volatile substances with urine, which attract intact animals, inducing immune disturbances in them. Apparently, they may cause the development of secondary, reactive disturbances in groups of animals that were but contacted with damaging animals. The ability of volatile substances to induce the distribution of them in groups of animals may probably have ecological consequences.

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