

# Postirradiation Volatile Secretions of Mice: Syngeneic and Allogeneic Immune and Behavioral Effects

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The immune response in immunologically and olfactorily high- and low-reactive CBA and C57Bl/6 mice is almost similarly decreased after exposure to volatile secretions of syngeneic animals exposed to ionizing radiation in a dose of 4 Gy. In the preference/avoidance test intact animals prefer secretions of irradiated syngeneic and allogeneic animals to those of intact animals, while without irradiation animals of both strains prefer syngeneic secretions. C57Bl/6 mice differ from CBA animals by lower sensitivity.

**Key Words:** *volatile postirradiation secretions; behavior; immunity; mouse genotype*

Mice and rats exposed to ionizing radiation in sublethal doses induce disorders in immunological reactivity in intact animals housed together with them over 10-15 days [5]. This effect is similar to the bystander effect demonstrated on cultured cells and manifesting in genome instability in intact cells surrounding the irradiated cell [9]. In our experiments this effect was observed in animals and was determined by urinary excretion of volatile components reducing humoral immune reactivity in intact animals even after a short-term exposure (1 h or 1 day) [6-8].

Many vital functions are regulated by chemocommunication in groups of animals [4,10,11]. There are no publications about chemical signals of pathological states and their effects on intact animals. The only exception is reports about "fear smell" release by stressed animals; this smell modified the immunity of intact animals [6,13].

Immunosuppressive effects of postirradiation volatile secretion were observed in CBA mice with high immune and olfactory reactivity.

## MATERIALS AND METHODS

The study was carried out on male CBA and C57Bl/6 mice (23-25 g). The animals were exposed to whole-

body  $\gamma$ -irradiation ( $^{60}\text{Co}$ ) in a dose of 4 Gy on a Gamma-cell-220 device (Atomic Energy Canada Ltd.) at 0.7 cGy/sec power. Two days after the exposure a sheet of filter paper was put on the bottom of the box; this sheet was protected by a second grid-bottom elevated by 0.5 cm above the base. On the next day this filter paper with absorbed urine of exposed or intact (control) animals was put into boxes with intact recipient animals (similarly under a grid bottom). After 24-h exposure with this sheet the mice were immunized with sheep erythrocytes ( $1 \times 10^8$  cells) and after 4 days were decapitated under ether narcosis. The count of antibody-producing cells (APC) in the spleen was evaluated by the method of Cunningham. The significance of differences was evaluated using Student's *t* test.

The preference/avoidance test with intact mice-testers of volatile compounds was carried out in a modified T-maze (a plastic 50×50 cm box with 35-cm walls and a 100 W electric lamp 1.5 m above the box). Tester mice entered through openings in opposite walls into "shelters" (dark plastic 15×10×5 cm boxes). Filter paper from boxes with compared mouse groups was layered on the bottom of the "shelters". Every tester mouse ( $n=10$ ) was 6 times placed into the center of the maze and preferred "shelter" was determined. Delayed exit of the tester animal from the "shelter" was recorded. The results were expressed in percent ratio of preference of compared "shelters".

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**TABLE 1.** APC Counts in Spleens of Recipient CBA and C57Bl/6 Mice after Exposure to Volatile Compounds Released by Intact and Irradiated (4 Gy) Donor Mice ( $\times 10^3$ ;  $M \pm m$ )

Donors of volatile compounds	CBA recipients	C57Bl/6 recipients
Intact CBA	428 $\pm$ 40 (100.0 $\pm$ 9.4)	78 $\pm$ 2 (51.0 $\pm$ 1.3) <sup>+</sup>
Irradiated CBA	266.0 $\pm$ 23.8 (62.0 $\pm$ 5.6) <sup>*</sup>	66 $\pm$ 12 (43.1 $\pm$ 7.8) <sup>+</sup>
Intact C57Bl/6	377 $\pm$ 24 (88.0 $\pm$ 5.6)	153 $\pm$ 4 (100.0 $\pm$ 2.7)
Irradiated C57Bl/6	296.0 $\pm$ 6.8 (69.2 $\pm$ 1.6) <sup>**</sup>	92 $\pm$ 12 (73.0 $\pm$ 9.5) <sup>+</sup>

**Note.** <sup>\*</sup> $p < 0.05$  compared to intact CBA, <sup>+</sup>intact C57Bl/6. Percentage of syngeneic control is shown in parentheses.

The significance of differences was estimated using Wilcoxon and Student's tests.

## RESULTS

Volatile compounds released on day 3 after irradiation possess a high immunosuppressive activity [6]. Immune reactivity of highly reactive recipient CBA mice decreased equally after exposure to postirradiation secretion of syngeneic CBA mice (62.0 $\pm$ 5.6%) and allogeneic C57Bl/6 mice (69.2 $\pm$ 1.6%) (Table 1). Immunologically low-reactive C57Bl/6 mice differed from CBA animals by immunosuppressive reaction to syngeneic and allogeneic postirradiation secretion (73.0 $\pm$ 9.5 and 43.1 $\pm$ 7.8%, respectively).

Effects of the postirradiation volatile signals were also observed in behavioral experiments. The tester mice preferred boxes with postirradiation secretion in the preference/avoidance test during 4-7 days after irradiation (Table 2); later this regularity was less expressed. This preference was genetically not strictly specific, because the testers virtually similarly reacted to syngeneic and allogeneic postirradiation secretion. The difference between the testers of different strains consisted in lower sensitivity of C57Bl/6 mice ( $p < 0.05$ ) to postirradiation allogeneic secretions of CBA mice.

The absence of genotype specificity in the postirradiation secretion was confirmed by the absence of

stable preferences in CBA and C57Bl/6 testers comparing syngeneic and allogeneic secretions (sometimes allogeneic secretion was preferred; Table 3). Due to this, the mean daily number of preferences of allogeneic (C57Bl/6) postirradiation secretions to syngeneic one was significantly higher for CBA testers. Under physiological conditions (without irradiation) the mice release chemical signals attracting syngeneic animals. This effect can be denoted as homing (striving to one's own group, to one's relatives). It was demonstrated in comparison of preferences of volatile secretions from syngeneic and allogeneic intact mice: CBA mice preferred (with high incidence: statistical mean for 5 series of experiments was 62.5 $\pm$ 0.5%;  $p < 0.05$ ) secretions of another group of CBA mice to those of C57Bl/6 mice, while C57Bl/6 mice more often (54.5 $\pm$ 2.5%;  $p < 0.05$ ) preferred syngeneic, but not allogeneic secretions (this value was significantly lower than for CBA mice). Hence, homing is impaired after exposure to ionizing radiation, presumably because of postirradiation volatile secretions.

The capacity of mice with different genotypes to react to secretions of each other under physiological conditions manifests not only by behavioral reactions, but by changed immune reactivity as well. In CBA mice exposed to secretions of intact CBA animals the number of APC in the spleen only slightly decreased (Table 1), while in C57Bl/6 mice the immune response

**TABLE 2.** Incidence of Preferences (%) of Secretions of Intact and Irradiated Mice by Tester Mice

Day of observation	CBA testers		C57Bl/6 testers	
	CBA/CBA, 4 Gy	C57Bl/6/C57Bl/6, 4 Gy	C57Bl/6/C57Bl/6, 4 Gy	CBA/CBA, 4 Gy
1	43/67 <sup>*</sup>	22/78 <sup>*</sup>	32/68 <sup>*</sup>	47/53
2	47/63 <sup>*</sup>	45/55 <sup>*</sup>	52/48	45/55 <sup>*</sup>
3	42/68 <sup>*</sup>	45/55 <sup>*</sup>	45/55 <sup>*</sup>	38/62 <sup>*</sup>
4	35/65 <sup>*</sup>	43/67 <sup>*</sup>	40/60 <sup>*</sup>	42/58 <sup>*</sup>
Mean daily percentage of preferences ( $M \pm m$ )	44.2 $\pm$ 1.1/65.8 $\pm$ 1.1 <sup>+</sup>	46.2 $\pm$ 5.5/63.8 $\pm$ 5.5 <sup>+</sup>	42.2 $\pm$ 4.2/57.8 $\pm$ 4.2 <sup>+</sup>	43.0 $\pm$ 1.9/57.0 $\pm$ 1.8 <sup>+</sup>

**Note.** Here and in Table 3:  $p < 0.05$  <sup>\*</sup>compared to the ratio according to Wilcoxon's test, <sup>+</sup>compared to the ratio according to Student's test.

**TABLE 3.** Incidence of Preferences (%) of Volatile Secretions of Irradiated Mice (4 Gy) by CBA and C57Bl/6 Tester Mice

Day of observation	CBA testers CBA/C57Bl/6	C57Bl/6 testers CBA/C57Bl/6
1	42/58*	53/47
2	47/53	55*/45
3	43/57*	57*/43
4	50/50	43/57*
7	43/57*	58*/42
Mean daily preference ( $M \pm m$ )	45.0 $\pm$ 1.7/55.0 $\pm$ 1.7 <sup>+</sup>	53 $\pm$ 3/47 $\pm$ 3

considerably decreased after exposure to volatile compounds released by intact allogeneic CBA mice (51.0 $\pm$ 1.3%). Irradiation of CBA mice did not increase immunosuppressive activity of their volatile secretion towards C57Bl/6 mice.

Differences in the reactions of two mouse strains are explained by a lower immunoreactivity of C57Bl/6 vs. CBA animals [2] and by lesser olfactory sensitivity and productivity of the former strain, at least by the reproduction-regulating feromones [1]. Their radiosensitivity is also different: the bone marrow form of acute radiation disease is characteristic of CBA mice and the enteric form is typical of C57Bl/6 animals [3]. The latter fact seems to be negligible for our study, because despite some differences in their sensitivity, the mice similarly reacted to allogeneic postirradiation secretions during the same periods after exposure.

Volatile compounds released by infected animals can reduce the contacts between animals in the same group and thus prevent dissemination of the infection [4,12]. Our findings indicate that contrary to expectations, postirradiation secretions were attractive for intact animals irrespective of the genotype of mice pro-

ducing these secretions. On the other hand, postirradiation secretion is characterized by immunosuppressive effects on intact animals. Under physiological conditions syngeneic secretions are attractive, while allogeneic secretions are characterized by immunosuppressive activity. Presumably, this is a manifestation of emission of different chemical signals: in one case they denote individuality, relation or difference, while in the other they inform about aftereffects of radiation injury. Biological purpose of these chemical signals can be associated with the natural selection [4].

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