Effect of Soft x-Ray irradiation on Immunological Functions in Mice

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(Received 16 October 1991/Accepted 6 March 1992)

Abstract. Effect of soft x-ray irradiation on immunological functions in mice was investigated. Soft x-ray irradiation with 100R or more induced a significant reduction in the number of plaque-forming cells (PFC). The reduction in the number of PFC depended on the irradiation doses. Irradiation with 600R or more showed a significant reduction in the delayed reaction of footpad swelling. However, soft x-ray irradiation with doses ranging from 100R to 1000R did not exert significant influence on the K values of carbon clearance test. Irradiation with 100R or more of soft x-ray showed a remarkable reduction of response to concanavalinA (ConA) or lipopolysaccharide (LPS) in spleen cells, and the response to ConA was lower than that to LPS. These results suggest that in the soft x-ray-irradiated mice, antibody-producing ability, delayed type hypersensitivity reaction and mitogenic activity are sensitive to soft x-ray irradiation and furthermore, T cell is more sensitive than B cell, but phagocytic activity of reticulo-endothelial system (RES) is resistant to soft x-ray irradiation.—KEY WORDS: antibody production, delayed type hypersensitivity, mitogenic activity, phagocytic activity, soft x-ray irradiation.


Soft x-ray is often used in the non-destructive examination of light metals and chemical products. Moreover, soft x-ray has widely been applied to take skeletal photogram of experimental animals in the research of teratology [8]. However, the effect of soft x-ray irradiation on the physiological features of mice, especially immunological function, has not yet been clarified. In the previous experiments, we demonstrated that soft x-ray irradiation decreased lymphocyte, neutrophil and total leukocyte counts, plaque forming cell counts and inhibited the tumor growth in mice [13–15]. The present study was performed to see whether soft x-ray irradiation causes changes in the immunological functions (antibody production, delayed type hypersensitivity reaction, phagocytic activity and mitogenic activity) of mice.

Materials and Methods

Mice: Female mice of C57BL/6 strain at 8 weeks of age were used. Five mice were subjected to each expose dose in all experiments. The mice obtained from Japan SLC, Inc. (Shizuoka).

Antigen: Sheep red blood cells (SRBC) were obtained from Nakarai Chemical Co. (Kyoto). SRBC in Alsever's solution were washed 3 times with saline before use.

Immunization: Mice were injected intravenously with $1 \times 10^8$ or $1 \times 10^9$ SRBC in saline. In primary immunization in irradiated mice, SRBC were injected 1 day after soft x-ray irradiation.

Irradiation: Irradiation with doses of 100, 300, 600 and 1000R of soft x-ray were done on the back of animals 1 day before intravenous administration of antigen or carbon by using an apparatus generating soft x-rays, a SOFRON NST-100SC (Souken Co., Tokyo). Its conditions were as follows: distance (focus-skin distance) 50 cm, voltage 70KVP, current 5 mA, both with and without a 100% acryl filter (10 mm in thickness). Exposure doses were determined on the skin surface of the mouse with Victoreen 666. From the results in Table 1, the dose rate used was 66.7R/min for irradiation without filter and 10.8R/min for irradiation with filter. Half-value layer was 0.15 mmAl.

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<th>Distance (cm)</th>
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Table 1. Irradiation dose rate of soft x-ray at 70 KVP (R/min.)
Assay of hemolytic plaque-forming cells: Hemolytic antibody-forming cells (PFC) as an indicator of antibody production. Spleens were obtained 4 days after primary immunization and squeezed between two glass slides in medium Eagle MEM supplemented. The cell suspensions were passed through a layer of gauze to remove residuallarge fragments. The PFC against SRBC antigen were determined, for individual mice, by the method of Cunningham and Szenberg [6] and were reported as PFC per spleen.

Assay of delayed type hypersensitivity reaction: Delayed footpad reaction (DFR) on the SRBC antigen is generally accepted as indicative of delayed type hypersensitivity [19]. For elicitation of DFR, $1 \times 10^6$ SRBC in 50 $\mu$l saline were injected into the left hind footpad 4 days after primary immunization. As a control, 50 $\mu$l saline was injected into the right hind footpad. Swelling of these footpads were measured 24 hours later with a dial thickness gauge. The degree of reaction was expressed as the difference in thickness between the left and right hind footpad.

Assay of phagocytic activity: The phagocytic activity was measured by carbon clearance test according to Biozzi et al. [4]. Pelican carbon particles (C11/1431a, Gunter Wagner, Hanover, Germany) were suspended at 25 mg/ml in saline. Mice were injected intravenously with a gelatin-stabilized suspension of carbon in a dose of 0.1 mg/10 g on the 1 day before irradiation. The kinetics of disappearance of the carbon from the blood were determined by a series of bleedings from the retro-orbital venous plexus. Each sample of 25 $\mu$l obtained by pipette was diluted and hemolysed in 2 ml of 0.1% Na$_2$CO$_3$ and concentration of carbon measured electrophotometrically at 675 nm. K (phagocytic index) was determined as the slope of the straight line derived by plotting the optical density values expressed as logarithms against time in minutes, according to the equation $K = \log C_2 - \log C_1$ $\overline{T_2 - T_1}$ in which $C_1$ and $C_2$ were the carbon concentrations at time $T_1$ and $T_2$. The average value for a group of five mice was determined.

Assay of mitogenic activity: Spleens in irradiated mice were obtained at 1 day after irradiation. Spleen cell suspensions were prepared by pressing through a screen. Spleen cell suspensions were washed and resuspended in RPMI 1640 culture medium containing 5% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 $\mu$g/ml streptomycin. Aliquots of 100 $\mu$l containing $5 \times 10^5$ spleen cells were dispensed into individual flat bottom wells of microculture plates. Then, 100 $\mu$l of media containing the mitogens was added to obtain a final concentration of 4 $\mu$g/ml concanavalin A (ConA: Difco Labs, Detroit) as T cell mitogen and 10 $\mu$g/ml lipopolysaccharide (LPS: E. coli 055, Difco) as B cell mitogen were used [12]. The cell cultures were incubated for 72 hours in a humidified incubator at 37°C in the presence of 5% CO$_2$ in air. Twenty-four hours before harvest, the cultures were pulsed with 0.5 $\mu$Ci $^3$H-thymidine (New England Nuclear, Boston). The cultures were harvested with a multiple sample harvester and counted in liquid scintillation counter. All experiments were carried out in triplicate cultures.

Statistical analysis: All data were expressed as mean ± S.E.. Comparison between soft x-ray irradiated group and non-irradiated group (control) was made with a two-tailed Student t-test. Any p-value less than 0.05 was considered significant.
RESULTS

Effect of soft x-ray irradiation on antibody production: Mice were given whole body irradiation of doses from 100 to 1000R of soft x-ray, administered intravenously SRBC on the 1 day after irradiation. Antibody production was assessed on days 4.

Soft x-ray irradiation with filter reduced dose-dependently the number of PFC at the doses from 100 to 1000R. In the experiments without filter, a dose-dependent reduction in the number of PFC was observed with 300R or more (Fig. 1). The irradiation with filter produced a much stronger drop in this number of PFC than that without filter, as shown in Fig. 1.

Effect of soft x-ray irradiation on the delayed type hypersensitivity reaction: Soft x-ray irradiated mice and non-irradiated mice were immunized intravenously with $10^6$ SRBC on 1 day after irradiation and delayed footpad reaction was elicited on day 4.

In the experiments with filter or without filter, the soft x-ray irradiation reduced dose-dependently the swelling of footpad at the doses from 600 to 1000R (Fig. 2). The irradiation with filter produced a much stronger drop in this swelling than that without filter.

Effect of soft x-ray irradiation on the phagocytic activity: The phagocytic activity of the reticuloendothelial system (RES) was investigated by measuring the rate of clearance of carbon particles on 1 day after soft x-ray irradiation.

The soft x-ray irradiation did not affect on the K values of carbon clearance test at the doses from 100 to 1000R. The K values of soft x-ray irradiated mice were almost to the level of the non-irradiated mice (Fig. 3). There was no difference between irradiations with filter and without filter.

Effect of soft x-ray irradiation on mitogenic activity: Mitogenic activities to ConA or LPS of spleen cells were measured at 1 day after irradiation.

Stimulation Index (S.I.) of ConA or LPS in

![Graph](image1)

Fig. 2. Effect of soft x-ray irradiation on the swelling of footpad reaction (mean ± S.E.). Significant difference from control: *p<0.05, **p<0.01.

![Graph](image2)

Fig. 3. Effect of soft x-ray irradiation on the K-value of carbon clearance test (mean ± S.E.).
control were 10.1 or 9.8, respectively. ConA or LPS showed remarkable mitogenic activity on spleen cells of non-irradiated mice. However, soft x-ray irradiation reduced dose-dependently the response to ConA or LPS in the incorporation of $^3$H-thymidine in spleen cells at the doses from 100 to 1000R. The response to ConA of spleen cells in soft x-ray irradiated mice at the all irradiation dose was considerably lower in contrast to the response to LPS (Figs. 4, 5). The irradiation with filter produced a much stronger drop in this incorporation of $^3$H-thymidine in spleen cells of the response to ConA or LPS than that without filter.

**DISCUSSION**

X-rays of short wavelength are usually referred to hard x-rays, and those of long wavelength to soft x-rays. Hard x-rays are often used in immunological studies. They are known to inhibit the antibody production [10], to suppress the delayed-type hypersensitivity [17] and to inhibit the proliferation of T cell in irradiated animals [1]. Whereas, several reports show that the phagocytic activity of the RES is not affected in hard x-ray irradiated rats [3, 16]. Recently, soft x-rays of long wavelength are often used non-destructive radiological examination [11]. However, the effect of soft x-ray irradiation on immunological functions has been less well elucidated. Therefore, we examined the effect of soft x-ray irradiation on immunological functions in mice. PFC assay is measured as an indicator of antibody-producing ability [6]. Makidono et al. [9] reported that the number of PFC in spleen against SRBC antigen decreased by about 60% after irradiation with 100R of hard x-ray in mice. In the present study, the number of PFC decreased by 70% or more by irradiation with 300R of soft x-ray with or without filter. This result suggested that soft x-ray irradiation either with or without filter might have suppressed the antibody producing ability of mice to almost the same extent as irradiation with a similar dose of hard x-ray.

The validity of the footpad test in mice as a sensitive measure of delayed type hypersensitivity to a variety of other antigens is also well established.
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[19]. Volkman and Collins [18] reported that the swelling of footpad of delayed-type reactivity to tuberculin in irradiated mice with 400 rads of hard x-ray was noticed progressive suppression on the 2 days after irradiation. In our present experiment, the swelling of DFR to SRBC antigen was decreased by 30% or more by irradiation of 600R of soft x-ray with or without filter.

Carbon clearance test is generally accepted as an indicator of the phagocytic activity of RES. The phagocytic index K, which is thought to reflect the phagocytic capacity of RES, was determined by carbon clearance test [4]. Benacerraf et al. [3] reported that a dose of 600R to mice and 850R to rats of hard x-ray did not interfere with the phagocytic activity of the RES. In the present study of soft x-ray, 1000R of soft x-ray irradiation did not affect the phagocytic activity of the RES as hard x-ray irradiation did not.

Several reports described that the functions of T cell and B cell in mitogenic activity were suppressed by hard x-ray irradiation [2, 7]. In these experimental studies of hard x-ray irradiation, B cells were more sensitive than T cells to hard x-ray. However, sensitivities of T cells and B cells to hard x-ray have led to conflicting conclusions [5]. It has been reported that ConA acts as mitogens on T cells, LPS acts as mitogen on B cells [12]. We examined the effect of soft x-ray irradiation on mitogenic activity to ConA or LPS of spleen cells in irradiated mice. In our results, the mitogenic activity to ConA of spleen cells in soft x-ray irradiated mice were lower than the mitogenic activity to LPS at the same soft x-ray irradiation dose. This result suggests that T cell function is more sensitive than B cell function to soft x-ray irradiation.

These results show that in the soft x-ray irradiated mice, antibody-producing ability, DFR and mitogenic activity are sensitive to soft x-ray irradiation, but phagocytic activity of RES is resistant to soft x-ray irradiation as well as hard x-ray. Furthermore, antibody-producing ability was more sensitive than DFR to soft x-ray irradiation.

The wavelength of soft x-ray generated directly from the SOFRON is much longer than that of hard x-ray. The mean wavelength of soft x-ray selected through filter is shorter than that of soft x-ray generated directly from the apparatus. In the present study, the soft x-ray irradiation with filter produced a much stronger decrease in the number of PFC, the swelling of footpad reaction and the mitogenic activity to ConA or LPS than that without filter. These results indicate that x-ray of short wavelength can cause a more marked inhibition in antibody-producing ability, delayed type hypersensitivity and mitogenic activity than that of long wavelength.

ACKNOWLEDGEMENTS. The authors wish to thank Dr. O. Matsuoka for his valuable suggestions and Souten Co., Ltd., Tokyo for the determination of irradiation dose of soft x-ray.

REFERENCES


