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STUDIES ON WHOLE-BODY X-IRRADIATION EFFECTS ON ANAPHYLACTIC HYPERSENSITIVITY IN GUINEA PIGS

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One of the early proofs for the inhibitory effects of X-irradiation on antibody synthesis was the demonstration that guinea pigs sensitized with a single dose of horse serum and irradiated afterwards did not manifest an anaphylactic reaction on subsequent intravenous challenging injection of horse serum. The depressive effect of X-irradiation on the anaphylactic response was shown to be the same when sensitization took place one or eight days before exposure (1). However, in mice receiving X-irradiation two or more days after sensitization and challenged 14 days after sensitization (2) this suppression was not observed, which is in agreement with the more or less generally observed radioresistance of immunological response if antigenic stimulation was applied before irradiation (3, 4).

Since we intended to use the anaphylactic response in guinea pigs as a model for studying different immunologic effects of whole-body X-irradiation, it was first necessary to establish the temporal relation between the sensitizing injection and the depressive effect of irradiation. Guinea pigs were sensitized in different time intervals before or after X-ray exposure challenged 14 days after sensitization.

It was later of interest to determine whether X-irradiation influences the anaphylactic reactivity in guinea pigs. This report gives a comparison of the reactions between passively sensitized normal and irradiated animals.

MATERIALS AND METHODS

Guinea pigs of both sexes from a heterozygous random bred colony were used. Body weights ranged between 300 and 400 g.

The antigen used in sensitizing and challenging procedures was horse serum. For sensitization each guinea pig received 0.1 ml of undiluted horse serum subcutaneously. The challenging dose (0.15 ml of horse serum) was injected rapidly into the vena jugularis under light local anesthesia (2 % Novocain).

Criteria for the anaphylactic response were clinical symptoms shown in the first ten minutes after the challenging injection. The degree of reaction was expressed by means of a scoring system in which animals showing no symptoms were marked with 0, very weak or uncertain symptoms with 1, mild symptoms with 2, moderate symptoms with 3, severe symptoms with 4, and fatal symptoms with 5 (or Ex).

Irradiated animals were exposed to whole-body X-irradiation (350, 400 or 450 r) under the following conditions: 220 kV, 15 mA, at 40 cm distance, with 0.5 mm Cu and 1.0 mm AL added filtration, the dose rate was about 83 r per minute). Unirradiated animals were included as controls in all the experiments.

RESULTS

Seventeen guinea pigs were sensitized and challenged with horse serum 14 days later. The anaphylactic reactions of this group were taken as control and compared with the anaphylactic reactions observed in 22 animals sensitized from 30 minutes to 14 days before irradiation (350 or 400 r), and with the reactions observed in 44 animals sensitized from 30 minutes to 14 days after the exposure to the same doses of X-rays. Cumulative data of the observed anaphylactic response are shown in Fig. 1.

The reactions observed in animals sensitized after irradiation were much less than those observed in the controls. No lethal response was observed. However, the anaphylactic res-

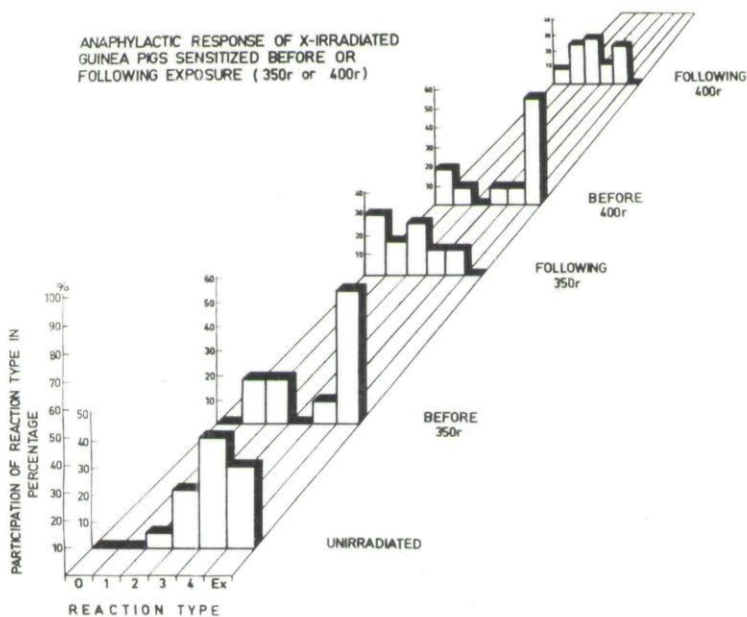


Fig. 1.

ponse in animals sensitized before irradiation showed a higher percentage of fatal reaction than in the unirradiated group.

Data on the lethal anaphylactic response depended on how long before irradiation, the sensitization was performed as shown in Fig. 2. Eighty percent of guinea pigs sensitized from 1 to 14 days before irradiation gave a fatal reaction on subsequent challenge. Animals sensitized 30 minutes, 6 hours or 12 hours before irradiation showed about the same number of fatal anaphylactic reactions as the control unirradiated group.

In experiments on the passive transfer of anaphylactic hypersensitivity, pooled blood serum obtained from normal or irradiated guinea pigs 14 days after sensitization, viz. 15 days after irradiation, was injected intraperitoneally into normal or irradiated animals. Table 1 shows the results of two experiments. In the first experiment, blood serum obtained from normal guinea pigs 14 days after sensitization was injected intraperitoneally into normal or irradiated animals (5 days after exposure to 400 or 450 r). As seen from the table, all

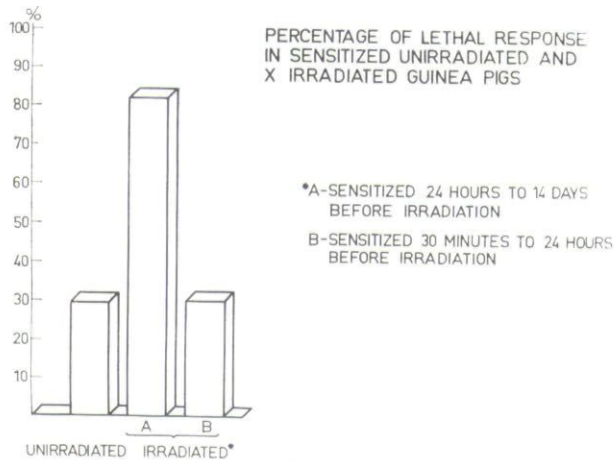


Fig. 2.

passively sensitized animals showed on anaphylactic reaction after challenge (24 hours after receiving the sensitizing serum). Although the anaphylactic indices used to evaluate differences between the groups are somewhat greater in the irradiated than in the control group, the difference is too small to suggest that the anaphylactic reaction in irradiated animal groups were more pronounced.

TABLE 1

Sensitized donor animals	Injected amount of serum (ml)	Recipient animals	Number of react/tested	Anaphylactic index*
Normal	2	Normal	17/17	2.6
Normal	1	Normal	8/8	1.5
Normal	2	Irradiated 400r	12/12	3.2
Normal	2	Irradiated 450r	17/17	2.9
Irradiated	2	Normal	4/6	1.1
Irradiated	1	Normal	3/8	0.5

In the second experiment, blood serum was obtained from guinea pigs sensitized 24 hours after irradiation with 300 r. Normal recipient animals manifested an appreciably weaker

anaphylactic response to challenge than after the acceptance of the same amount of serum from normal sensitized animals. Obviously, the blood of irradiated sensitized animals contained fewer circulating antibodies.

It is well known that whole-body X-irradiation leads to the dilatation and engorgement of capillaries in the lung (6) and increases the capillary permeability (9). If those factors which theoretically make it possible that antibodies come into contact with shock organs quicker or in greater amount, really play a role then irradiated animals should manifest more severe reactions upon challenge performed in the same time intervals after passive sensitization. Irradiated (5 days after 400 r) or normal animals were injected intraperitoneally with 3.0 ml of pooled serum obtained from a group of sensitized normal guinea pigs. In different time intervals after the serum transfer (8, 24 or 48 hours) animals were challenged. In each time interval four animals of each group were used. Results shown in Table 2 indicate that only a small difference exists between the control and the irradiated group.

TABLE 2

Recipient group		Time of challenge (in hours) following serum transfer		
		8	24	48
Normal	Anaph. index*	0.5	2.8	2.8
Irradiated	Anaph. index*	0.7	3.5	3.0

* Anaphylactic index is expressing the relative degree of anaphylactic state. It is calculated by multiplying the number of animals showing fatal symptoms by 5, severe symptoms by 4, moderate symptoms by 3, mild symptoms by 2, very weak or uncertain symptoms by 1, without symptoms by 0, and dividing the sum of this product by the total number of animals challenged. That is a slightly modified system described by Weigle et al. (5).

DISCUSSION

The results with animals sensitized before X-ray exposure indicate clearly that with the dosages employed the anaphy-

lactic response was not only diminished, but increased. These data could indicate that sensitized and afterwards irradiated animals generally respond with a stronger anaphylactic reaction. As a possible reason for this stronger reaction after the challenge one could think of the dilatation and engorgement of capillaries in the lung (6). This has been described as occurring in a matter of hours after exposure to radiation of laboratory animals. But if this were the reason, passively sensitized animals should manifest more intense symptoms upon challenge, too. Since the difference in response between passively sensitized normal and irradiated animals was much less pronounced than between actively sensitized animals, it seems that irradiation did not significantly increase the animal's susceptibility to the consequence of the antigen-antibody reaction. In a few preliminary trials irradiated guinea pigs did not manifest greater susceptibility to histamine intoxication than normal animals. Accordingly, some additional factors would be responsible for the stronger reaction in actively sensitized irradiated animals. Between them it could be mentioned: anamnestic increase of anaphylactic antibodies by the direct immunocytolytic effect of X-rays (7), and/or by the indirect effect due to the increase in cortical hormonal secretion after irradiation (7, 8). It is further not impossible that in irradiated animals, because of the increased capillary permeability in almost all organs of the body (9), more antibodies can in a shorter time be connected with shock organs so as to elicit a stronger reaction upon challenge. Results on the passive transfer, however, do not indicate that this latter mechanism plays a major role.

Taking each factor independently, it is not obvious which is the more important in strengthening the anaphylactic response in sensitized irradiated animals. Nevertheless, all factors together as well as many others not mentioned here, but which could simultaneously occur in irradiated animals, may probably cause irradiated animals previously sensitized to be more susceptible to the challenging injection of antigen.

Our results corroborate the observation reported in an old

report (1) as to the inhibitory effect of X-ray exposure immediately after sensitization on the anaphylactic reaction and antibody synthesis, but differ in respect of the anaphylactic depression in animals sensitized one or more days before irradiation. These results however, in respect of the time relation between the antigenic stimulation and the influence of X-ray exposure, agree well with the irradiation effects on immunologic response in other sorts of animals, as seen in antibody synthesis (4, 5), anaphylactic response in mice (2), and antibody response in guinea pigs immunostimulated with another antigen (10).

SUMMARY

Guinea pigs sensitized with horse serum before whole-body X-ray exposure (300 or 400 r) manifested stronger anaphylactic response than unirradiated animals, except when sensitized a few hours before irradiation. Animals sensitized after irradiation (up to 21 days) showed weaker response than unirradiated guinea pigs. Passively sensitized irradiated animals showed that irradiation did not impair the ability of guinea pigs to react anaphylactically. Moreover, they always manifested a somewhat stronger reaction than control unirradiated animals.

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