

AD695424

AFRRI SR69-17

October 1969

THE EFFECT OF X IRRADIATION ON DELAYED HYPERSENSITIVITY
AND CIRCULATING ANTIBODY

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FOREWORD
(Nontechnical summary)

Immune responses in animals are mediated primarily by the globulin fraction of the serum proteins. These serum proteins, termed antibodies, have been demonstrated to protect the animal from a multitude of foreign invaders. The immune responses not related to antibody induction are mediated by immune-sensitive cells. Delayed hypersensitivity or allergy is mediated by these immune-sensitive cells. Stimulation by antigen is required for antibody induction and allergic response.

Previous investigations have demonstrated that the primary antibody response is quantitatively more radiosensitive than the secondary antibody response (booster response). The allergic response has been characterized as being only slightly radiosensitive. On this basis the allergic response has been accorded some kinship to the secondary antibody response. Findings attesting to the relative radioresistance of the allergic response have been reported for the rabbit and guinea pig. The present investigation was designed to examine the effect of ionizing irradiation on the allergic response in rats.

Rats exposed to x radiation 24 hours prior to antigen administration exhibited detectable skin reactions to tuberculin 2 days later than nonirradiated sensitized controls. In terms of reaction sizes the irradiated rat exhibited a decreased sensitivity to tuberculin which persisted through day 14.

ABSTRACT

Rats exposed to x radiation 24 hours prior to antigen administration exhibited detectable skin reactions to tuberculin 2 days later than nonirradiated sensitized controls. Reaction sizes illustrate that the irradiated rat experienced a decreased sensitivity to tuberculin which persisted through day 14. The radiation dose (475 rads) responsible for the observed depression of the delayed skin reactions produced a greater depression of the circulating antibody response. The results obtained suggest that the cellular and humoral immune responses in the rat are radiosensitive.

I. INTRODUCTION

Prior to 1956, several investigators^{6-8, 11} reported on failures to induce a delayed tuberculin skin reaction in rats. In a later review, Crowle³ cited evidence based on methods other than the skin test that delayed hypersensitivity may be induced in this species. In 1959, Rowley⁹ reported the induction of delayed skin reactivity in the rat subsequent to intradermal sensitization with pertussis vaccine. Recently, Flax and Waksman⁵ presented data establishing that delayed tuberculin skin reactivity may be induced in the rat and noted that the reactions were similar to those classical delayed reactions observed in the guinea pig and rabbit in terms of time course (after skin testing), histological appearance, and the capacity to transfer sensitivity to normal animals.

The suppressive effect of x irradiation on humoral antibody production has been demonstrated.^{12, 13} Specifically, sublethal doses of x irradiation protracted or inhibited the onset of antibody formation when administered at critical times prior to immunization.^{10, 13, 14} The effect of x irradiation on delayed hypersensitivity reactions is less clear. Separate reports by Salvin¹⁰ and Uhr¹⁴ have indicated that x radiation sufficient to prolong or inhibit humoral antibody formation in guinea pigs and rabbits had little or no effect on the onset or intensity of delayed hypersensitivity. In contrast, Brooke² and Cummings et al.⁴ reported definite depressions of delayed skin reactivity in irradiated guinea pigs and rabbits. The experimental design in terms of antigens used, time of irradiation relative to antigen administration and radiation doses differs in each of the investigations.^{2, 4, 10, 14} The lack of agreement among the several studies may be caused by differences in methodology.

Among rodents, the guinea pig is less than ideal for radiation studies because of its extreme radiosensitivity. The rat, though quite efficient as an antibody producer, is not the animal of choice for studies on delayed cutaneous hypersensitivity. The induction of delayed hypersensitivity can be demonstrated in the rat and therefore imposes fewer limitations on the experimental design. The present study was designed to determine the effect of x irradiation, given 24 hours prior to antigenic stimulation, on the induction of delayed hypersensitivity and circulating antibody in the rat.

II. METHODS AND MATERIALS

In this study, 1304 male Sprague-Dawley rats weighing from 300-400 grams were used. Of this number of animals, 714 were used to test for delayed hypersensitivity, 140 for the radiation effect on circulating antibody titer, and 450 to obtain differential leucocyte and total cell counts.

In order to test for delayed hypersensitivity, the group of 714 rats was sensitized by injecting one hind footpad with 0.05 ml of 3 mg/ml heat-killed Mycobacterium butyricum (Difco, Detroit, Michigan) suspended in oil. Of this group 367 animals were exposed to 475 rads of x radiation ($LD_{10/30}$) a dose adequate for the suppression of detectable circulating antibody for 9-11 days when administered 24 hours prior to sensitization. The remaining 347 animals were utilized as nonirradiated controls.

Skin tests using at least 15 irradiated and 15 nonirradiated animals were conducted on successive days from day 2 through day 14 postsensitization. Five irradiated and 5 nonirradiated animals were tested on days 15 and 16 while 15 of each were tested on day 21. All rats were skin tested by intradermal injections of 2500 μ g

Old Tuberculin (O.T.). Comparative skin tests using O.T. and purified protein derivative of tuberculin (P.P.D.) were conducted on one group of animals. Each animal of this group received 2 to 3 dilutions of each preparation as intradermal injections.

Observations were made at time of maximum skin reaction, 24 hours after intradermal injections. At this time, two diameters, one bisecting the other at the midpoint at right angles, were measured through the area of erythema and induration. Only reactions in which the product of the two diameters exceeded 36 mm^2 were considered as positive since the reactions of 85 percent of the nonsensitized controls never exceeded these diameters. No animals were skin tested more than once. No skin reactivity was observable at 6 hours.

In those experiments in which the radiosensitivity of the circulating antibody titer was evaluated, 140 rats were injected intraperitoneally or subcutaneously in the footpads with 0.5 to 1.0 ml of an emulsion as complete or incomplete Freund type adjuvant. The incomplete adjuvant (FA) was composed of equal volumes of an aqueous phase containing 1 mg of ovalbumin per ml of saline and an oil phase containing 8.5 parts Drakeol and 1.5 parts Arlacel per ml. Complete adjuvant (CFA) required the addition of 4 mg Mycobacterium tuberculosis (Difco, Detroit, Michigan) to each ml of the oil phase.

Qualitative antibody determinations were carried out using the technique of agar-gel diffusion, while quantitative determinations were performed by the method of passive hemagglutination as modified from Boyden.¹

Differential leucocyte and total cell counts were done on blood samples obtained from 450 rats which were bled only once. The blood was obtained via jugular vein puncture on ether anesthetized animals. Clotting was prevented by the thorough mixing of 1 ml of blood with 1 mg dry EDTA. Bleeding for purposes of antibody determination was always performed prior to skin testing. Animals were exposed to x rays from a General Electric Maxitron x-ray machine at a distance of 110 cm. The machine was operated at 250 kVp and 30 mA and the beam was filtered through 1.2 mm beryllium and 0.95 mm copper. Half value layer was 1.9 mm copper and the midline exposure rate was calculated to be 20 R per minute in air.

The "t" test was utilized to evaluate the difference among the group means.

III. RESULTS

Following intradermal challenge with 2500 μg tuberculin, maximally intense skin reactions indicating delayed hypersensitivity were seen at 24 hours. No detectable skin reactions were observable at 6 and 12 hours. Comparisons between various preparations of O.T. and P.P.D. are shown in Table I. As depicted, the test doses of Veterinary O.T. (25 to 2500 μg) used in this study are approximately equivalent to P.P.D. in the range of 5 to 50 μg .

At day 4 after sensitization, 13 of 26 non irradiated rats demonstrated positive delayed tuberculin reactions in excess of 36 mm^2 . By day 5, the mean reaction size to tuberculin was in excess of 100 mm^2 in the nonirradiated animals (Figure 1). Exposure to 475 rads of x ray 24 hours prior to sensitization results in a significant depression of the immune response from day 3 to day 14 ($p < .05$). At day 15, 16, and 21, the response of the irradiated animals is not significantly different from that

of the nonirradiated. Adhering strictly to the criteria of reaction size larger than 36 mm² for positive reactions, the nonirradiated group demonstrates positive reactions by day 4, while the irradiated group does not give a positive response until day 6 (Figure 1).

Table I. Comparison of Various Tuberculin Preparations*

Rat #	Lilly O.T. [*] 10,000 µg mm x mm	Lilly O.T. 100 µg mm x mm	Vet. O.T. [†] 2500 µg mm x mm	Vet. O.T. 250 µg mm x mm	Vet. O.T. 25 µg mm x mm	P.P.D. [‡] 50 µg mm x mm	P.P.D. 5 µg mm x mm
1	12 x 10	3 x 3	9 x 10	0	0	8 x 8	6 x 6
2	11 x 13	3 x 3	10 x 10	4 x 4	3 x 3	15 x 15	8 x 8
3	11 x 11	0	9 x 11	0	0	10 x 12	8 x 9
4	11 x 14	3 x 3	11 x 13	7 x 7	2 x 3	12 x 13	9 x 10
5	10 x 12	0	9 x 10	4 x 5	0	12 x 13	9 x 10
6	11 x 14	0	10 x 10	0	0	12 x 14	9 x 9
7	10 x 12	0	10 x 10	4 x 4	3 x 3	11 x 13	7 x 7
8	12 x 14	0	12 x 12	3 x 3	0	13 x 16	9 x 10
9	10 x 10	0	9 x 10	0	0	10 x 11	7 x 8
10	11 x 11	0	10 x 12	4 x 4	0	11 x 11	8 x 9
11	10 x 11	0	10 x 14	5 x 5	0	13 x 14	9 x 10
12	9 x 11	0	10 x 11	0	4 x 4	11 x 14	10 x 10
13	13 x 14	4 x 4	11 x 11	5 x 5	0	12 x 13	9 x 9
14	11 x 11	0	9 x 12	0	0	12 x 13	8 x 10
15	10 x 12	0	11 x 11	5 x 6	0	7 x 10	13 x 14
16	13 x 13	3 x 3	12 x 14	6 x 6	0	11 x 12	9 x 10

* Skin tested at day 14 postsensitization

† O.T. Lot 2092 - 794599PH, Eli Lilly and Company

‡ O.T. Lot 98C (each ml contains 250 mg Koch's O.T.), Jensen-Salsbery Laboratories

§ P.P.D. Lot 35564, Merck, Sharp and Dohme

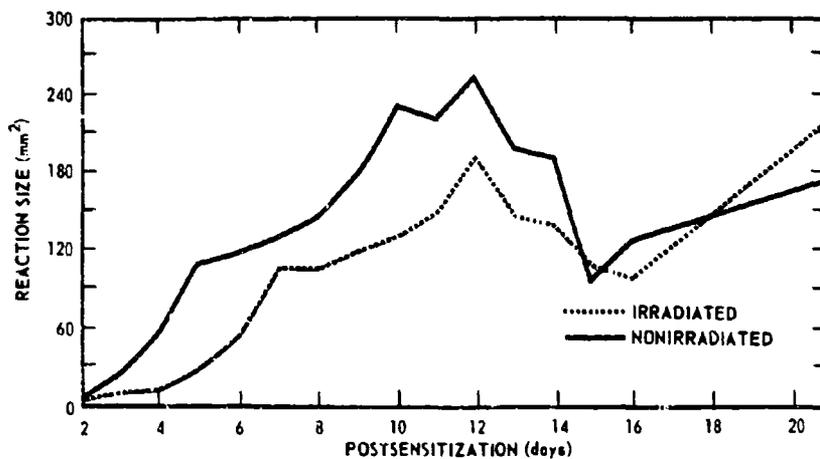


Figure 1. The depressive effect of x irradiation (475 rads) given 24 hours before intradermal sensitization with mycobacteria in oil on delayed skin reactions to tuberculin

The presence of circulating antibody as determined by agar diffusion tests was seen on day 12 in the 4 of 10 nonirradiated rats sensitized to ovalbumin. By day 19 and 20 respectively, 5 of 5 nonirradiated animals showed precipitation bands while 10 of 10 of the 475-rad group failed to show these bands. In the irradiated groups, precipitin reactions were not seen until day 21.

Circulating antibody as measured by passive hemagglutination is compared in the nonirradiated and irradiated rat (Figure 2). The titer to ovalbumin incorporated in incomplete or complete adjuvant is respectively 3.5 and 5.6 log (log₂) more in the nonirradiated animal. Precise comparisons between the groups receiving ovalbumin as complete adjuvant were not possible since only 3 of 20 irradiated animals survived to day 37. The survival in the nonirradiated group receiving ovalbumin as complete adjuvant was 16 of 20 on day 37. The overall survival rate was higher in both groups receiving ovalbumin as incomplete adjuvant. In the irradiated group 43 of 50 survived

through day 37 compared to a survival rate of 19 of 20 in the nonirradiated group over the same time period. The data (Figure 2) show clearly an incapacity of the immunized irradiated rat to produce a titer equal to that of the immunized nonirradiated animal even at 37 days postirradiation.

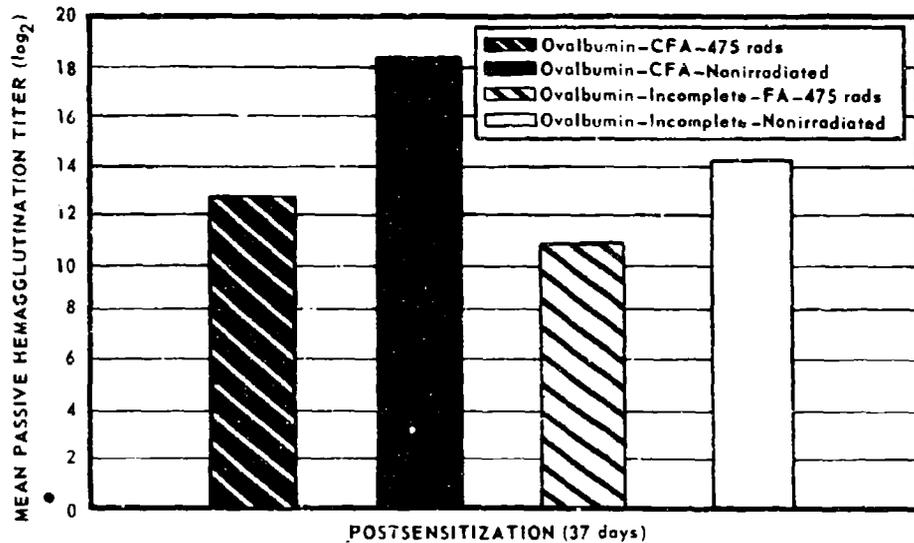


Figure 2. The depressive effect of x irradiation (475 rads) given 24 hours before immunization with ovalbumin on circulating antibody responses

The graphs (Figures 3 and 4) giving the absolute leucocyte and lymphocyte count are typical of rats receiving 475 rads of x irradiation. Note that irradiated animals, whether immunized and irradiated or irradiated alone, present a similar recovery curve. Full recovery to control values requires about 30 days. Reversals of leucocyte-lymphocyte ratios were not observed. Irradiation seemingly depresses both counts to the same degree, and the recovery to control values reflects nearly identical curves for both cell types.

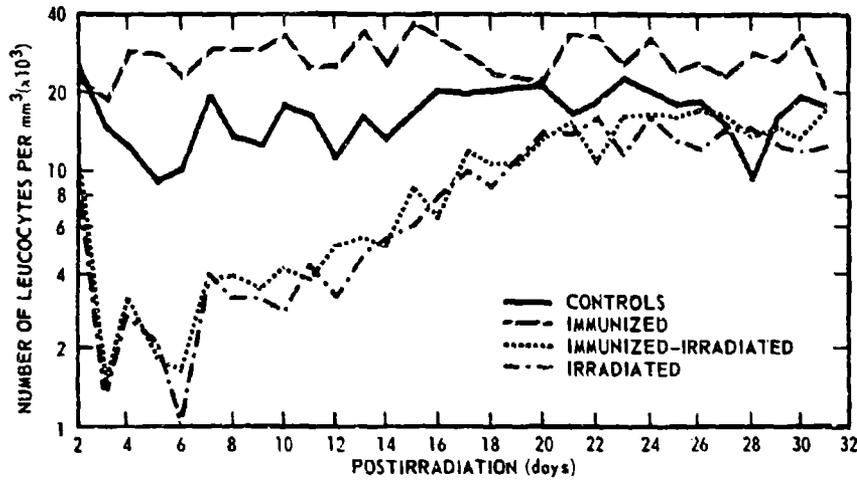


Figure 3. Effect of x irradiation (475 rads) on peripheral blood leucocytes

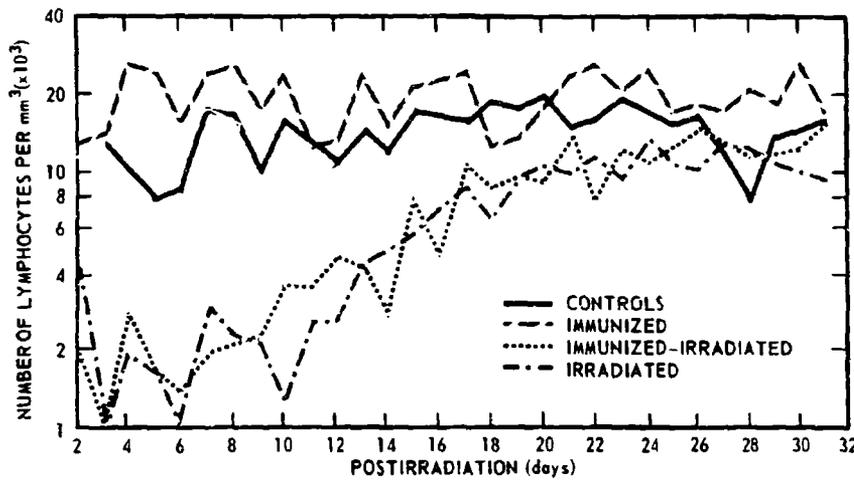


Figure 4. Effect of x irradiation (475 rads) on peripheral blood lymphocytes

IV. DISCUSSION

In rats exposed to antigen 24 hours postirradiation, circulating antibody response was observed to be more radiosensitive than delayed tuberculin skin reactivity. Irradiated immunized animals which required an additional 9 to 10 days to regain the

capacity for the production of detectable circulating antibody also failed to match the antibody production of nonirradiated immunized rats 37 days later. In contrast, irradiated sensitized rats required only 2 more days for recovery of a detectable delayed skin reaction, and by day 14, skin reactivity matched that of the nonirradiated rats. Unlike the previous studies by Salvin¹⁰ and Uhr¹⁴ using the guinea pig and rabbit, the present study used the rat. For all species there is general agreement that the induction of delayed hypersensitivity is depressed by x irradiation to a lesser extent than that of circulating antibody. However, the present study emphasizes that delayed skin reactivity is more than marginally sensitive to x irradiation. The significance of this radiosensitivity becomes more evident when it is considered that the radiation dose causing the inhibition in this study is approximately an LD_{10/30}, a noteworthy contrast to the LD₅₀ doses used in the Salvin¹⁰ and Uhr¹⁴ studies. As a first approximation, the increased radiosensitivity of the delayed response seen in rats might be attributed to species difference. Additionally the observed radiosensitivity of delayed hypersensitivity could be referable to the difficulty of establishing this response in the rat.

Depression of the circulating antibody responses noted in this study seems comparable to those reported in the previous studies by Salvin¹⁰ and Uhr.¹⁴ In this investigation, the 475 rads of x irradiation, adequate for the inhibition of the circulating antibody response, was equally inhibitory and depressive for the delayed tuberculin response. That species difference may account for the more complete depression of antibody and cellular immune responses in the rat as contrasted to only the antibody response in the guinea pigs and rabbits would seem unique.

That there is a difference in radiosensitivity between delayed hypersensitivity and circulating antibody is undisputed. This study underscores the relativity of this difference.

Uhr¹⁴ interpreted the difference in radiosensitivity of delayed hypersensitivity compared to antibody formation as being due to the dependence on a different mechanism for the two phenomena, or to the fact that the serological detectability is less sensitive than in the case of delayed hypersensitivity.

While the present investigation fails to rule out either interpretation, it does note that the delayed response, like antibody formation can be inhibited by exposure to radiation. This finding makes one question whether the two phenomena, hypersensitivity and humoral antibody response, are controlled by different mechanisms.

V. SUMMARY

Rats exposed to x radiation 24 hours prior to antigen administration exhibited detectable skin reactions to tuberculin 2 days later than nonirradiated sensitized controls. Reaction sizes illustrate that the irradiated rat experienced a decreased sensitivity to tuberculin which persisted through day 14.

The radiation dose (475 rads) responsible for the observed depression of the delayed skin reactions produced a greater depression of the circulating antibody response.

The results obtained suggest that there is no apparent difference in radiosensitivities of the cellular and humoral immune responses in the rat.

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DOCUMENT CONTROL DATA - R&D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author) Armed Forces Radiobiology Research Institute Defense Atomic Support Agency Bethesda, Maryland 20014	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	
	2b. GROUP N/A	
3. REPORT TITLE THE EFFECT OF X IRRADIATION ON DELAYED HYPERSENSITIVITY AND CIRCULATING ANTIBODY		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (Last name, first name, initial) Bowser, R. T., Spence, D. B. and Exum, E. D.		
6. REPORT DATE October 1969	7a. TOTAL NO OF PAGES 18	7b. NO OF REFS 14
8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S) AFRI SR69-17	
b. PROJECT NO.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
c. MC 3 90301		
d.		
10. AVAILABILITY/LIMITATION NOTICES Distribution of this document is unlimited		
11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Defense Atomic Support Agency Washington, D. C. 20305	
13. ABSTRACT Rats exposed to x radiation 24 hours prior to antigen administration exhibited detectable skin reactions to tuberculin 2 days later than nonirradiated sensitized controls. Reaction sizes illustrate that the irradiated rat experienced a decreased sensitivity to tuberculin which persisted through day 14. The radiation dose (475 rads) responsible for the observed depression of the delayed skin reactions produced a greater depression of the circulating antibody response. The results obtained suggest that the cellular and humoral immune responses in the rat are radiosensitive.		

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