Prolonged Suppression by X-Ray of Adaptation for the Secondary Antibody Response.* (27861)

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We have shown previously(1) that sublethal X-radiation destroyed or markedly depressed the adaptation of rabbits for a secondary antibody response to bovine gamma globulin (BGG). In those studies rabbits were exposed to 550 r X-rays 58 days after initial injection of BGG. Reinjected with BGG 49 days after irradiation, they exhibited antibody responses of primary type or, at best, much less vigorous than the typical secondary response. Unirradiated controls or animals injected with BGG 24 hours after irradiation, on the other hand, gave intense secondary responses to the test reinjection of BGG.

Our previous experiments, directed at the mechanism of adaptation for successive secondary responses, did not determine whether or not the observed effect of X-radiation was temporary. The present studies were designed to test whether or not recovery of secondary responsiveness could occur during a prolonged interval after irradiation. No sign of such recovery was seen.

Materials and methods. New Zealand white rabbits were used. Each had previously exhibited a primary and a secondary response following 10-mg doses of BGG i.v. For all injections the antigen was Armour fraction II, lot BP-201-204. Table I shows the schedule of injections, irradiation and tests for antibody. Intervals between previous exposure to BGG and X-radiation ranged from 137 to 305 days.

The animals of Groups A and C were irradiated on the same day with a GE Maxitron 300 Kv X-ray machine delivering 300 Kv at 20 ma through 1 mm Al and .025 mm Cu. Animals were exposed at 150 cm, half the time from each side, at a dose rate of 19.4 r per minute. The total dose was 550 r.

Rabbits of X-rayed Group A and its control Group B were reinjected with 10 mg BGG 73 days after irradiation. X-rayed Group C and their controls D were reinjected 171 days after irradiation, and at the same time the rabbits of Group A were given a second test injection.

As in our previous work, tests for antibody utilized a modification of the Boyden-Stavitsky hemagglutination reaction performed in 6 × 50 mm tubes with 0.1 ml serum dilution and 0.01 ml coated tanned cell suspension (1). Dilutions were made in a separate series of tubes, transferred to the small tubes already containing the cell suspension and thoroughly mixed by shaking. With this method, as we have reported previously(1), primary and secondary antibody responses can usually be differentiated on the basis of diffeernces in the patterns of hemagglutination. These differences have been confirmed by Benedict *et al.*(2), and what appears to be a similar effect was recently mentioned by Bauer and Stavitsky(3). They presumably reflect differences in avidity and physical properties of early and late antibody reported by various workers(4,5,3).

Experimental results. Table I shows the course of hemagglutinin titers in X-irradiated and control rabbits after reinjection with BGG. The responses in rabbits of Groups A and C to their first reinjection of BGG 10 and 24 weeks, respectively, after irradiation were depressed to the level of normal primary responses. Primary-type hemagglutination patterns were common in sera from the rabbits in Groups A and C. All control rabbits showed exclusively secondary-type patterns except 2 in Group D which exhibited primary-type patterns on one day each. Rabbits of Group A showed typical secondary responses when reinjected a second time along with Groups C and D.

Discussion. The data show that 550 r whole body X-radiation destroyed the adaptation for a secondary antibody response to BGG in rabbits. That there was no significant recovery from this effect is indicated by the

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TABLE I. Hemagglutinin Titers of Rabbits Reinjected with BGG 73 or 171 Days after 550 r X-radiation.

Group	No.	Interval in days between previous treatments and test BGG injection: BGG (1st)	BGG (2nd)	X-ray	BGG (3rd)	Reciprocal hemagglutinin titer: Days after test BGG injection: 0	3	5	7	10	13
A	1	479	378	73		80	80	320	640	320	640
	2	336	301	"		80	80	320	640	1,280	640
	3	245	210	"		10	20	320	640	160	160
B	4	427	378	none		160	640	40,000	40,000	20,000	20,000
	5	336	301	"		40	640	40,000	40,000	20,000	20,000
	6	245	210	"		40	160	20,000	20,000	20,000	5,000
C	7	525	476	171		80	40	80	320	Died	
	8	434	399	"		80	40	40	40	40	320
	9	343	308	"		20	20	640	320	160	320
	10	"	"	"		40	40	80	80	320	160
D	11	525	476	none		160	320	40,000	10,000	20,000	40,000
	12	434	399	"		10	160	20,000	40,000	20,000	10,000
	13	343	308	"		±	40	5,000	5,000	5,000	5,000
	14	"	"	"		20	320	40,000	40,000	20,000	10,000
A*	1	577	476	171	98	160	1,280	20,000	10,000	5,000	10,000
	2	434	399	"	"	80	320	10,000	10,000	2,560	10,000
	3	343	308	"	"	20	320	10,000	10,000	2,560	2,560

* Group A reinjected with Groups C and D. Third BGG injection was the previous test injection in this group.

fact that animals tested 24 weeks after irradiation exhibited, if anything, poorer responses than those tested at 10 weeks. The readaptation for a vigorous secondary response at 24 weeks in those animals (Group A) which had exhibited X-ray suppression at 10 weeks indicates that the observed depression was not attributable to residual general effects of irradiation.

X-radiation appeared to have destroyed all memory of the animals' previous antigenic experience and to have returned them to the status of normals. The most obvious explanation for the effect is that cells adapted for a secondary response suffered X-ray damage resulting in eventual death or inability to proliferate.

The data have important implications also concerning the immune status of persons severely exposed to ionizing radiation. It remains to be seen whether or not X-radiation can result in similar destruction of capacity for a booster response to antigens of infectious agents. Such destruction could eliminate the chief protective value of immunity acquired by natural infection or artificial immunization.

Summary. Rabbits adapted by previous exposure for vigorous secondary antibody responses to BGG lost this adaptation after sublethal X-radiation and were able to give only primary responses to reinjection of the antigen 24 weeks after irradiation. Data are presented indicating that the loss of secondary adaptation was not a result of general damage to the antibody-forming system. These results have implications both for understanding of the immune response and for practical problems of immunization.

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