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Effect of X-Rays on Hemolysin Plaque Forming Cells.* (30460)

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The effect of X-irradiation on the production of serum hemolysin levels has been studied by many investigators and was recently reviewed by Taliaferro *et al*(1). These studies have demonstrated the enhancing and depressing effects of X-rays on serum antibody titers(1-3). They have shown that the changes brought about by radiation are related to timing of radiation, antigen dose, radiation dose and species involved. Some insight into the effects of radiation on antibody response might be gained from an analysis of the antibody-forming cells. A recent hemolysin agar plaque technic described by Jerne and coworkers(4) provides a method for enumerating the hemolysin plaque forming cells in a heterogeneous cell population, such as spleen tissue. This report deals with the dose effect of X-ray administered at various times before and after antigen injection. The number of plaque forming cells of the spleen was correlated with serum hemolysin titers and with spleen indices (mg of spleen/g of body weight).

Materials and methods. Male LAF (C57L $\times$ A/He) mice, approximately 3 to 4 months of age, were given a single intraperitoneal injection of 8×10^8 sheep red cells. Animals were sacrificed 5 days after antigen injection. Spleens were removed and immersed in Eagle's medium without serum(5). Cells were separated from connective tissue stroma by compressing the tissue against the sides of a Dounce homogenizer with the loose fitting pestle. The cell suspension was then passed through nylon mesh bolting. Cell density was adjusted so that 0.1 ml of suspension contained one million cells. The plating technique was done according to Jerne *et al*(4). Serum hemolysin titers were assayed as described by Kabat and Meyers(6).

Results and discussion. Our studies indicated that hemolysin plaque forming cells in LAF mice spleens reached their peak 5 days after the mice received a primary intraperitoneal injection of 8×10^8 sheep red cells. The schedule for whole body X-irradiation, administered either before or after sheep red cell injection is outlined in Fig. 1. Data from these experiments are summarized in Table I. Four to 9 animals were used in each group.

Animals which had been irradiated with 500 R of X-ray all showed a pronounced decrease in spleen indices, in hemolysin plaques per million spleen cells, and in hemolysin plaques per total spleen. Animals irradiated at 3 days post-antigen injection were the least sensitive at this X-ray dose.

Mice receiving 250 R showed a varied response. Animals irradiated one day prior to or 3 days after sheep red cell injection showed a statistically significant (the level

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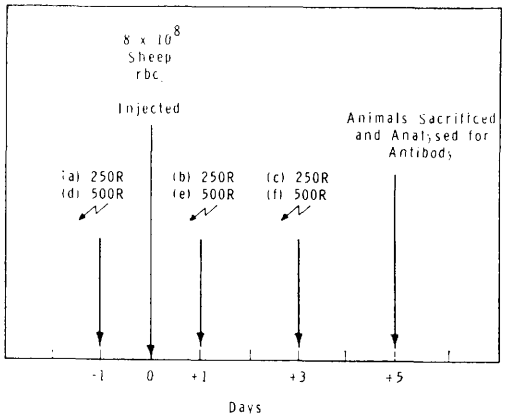

FIG. 1. Schedule for antigen injection and whole body X-irradiation.

of significance used for these studies was 0.05) decrease in all measured parameters (Table I, a, c). By contrast, mice irradiated one day after antigen injection (Table I, b) were not significantly affected.

Since antibody formation appeared to be least radiosensitive when radiation was administered one day after antigen stimulation, it was of interest to compare the effect of other doses of X-rays at this time interval. The results of such a comparison are shown in Fig. 2. No significant differences in the measured parameters were observed between non-irradiated controls and mice receiving 100 R of X-ray. At 250 R there was a slight but significant increase in the spleen index resulting from increased spleen weights and a significant decrease in serum hemolysin titer. Because of the considerable variation in numbers of spleen plaque forming cells within animals of each group, changes in this parameter were not statistically significant. With 350 R of X-ray, one day post-antigen injection, spleen index, serum hemolysin titer, and hemolysin plaque forming cells per spleen were significantly decreased below controls. By contrast, the hemolysin plaque forming cells per million spleen cells were significantly higher than in controls. Radiation at this level causes a general depletion of mitotically active cells resulting in significantly smaller spleens, which is reflected in a cor-

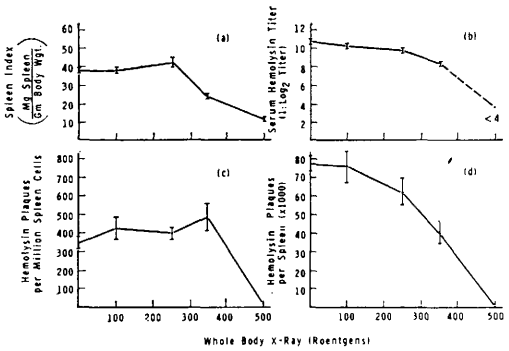

FIG. 2. Effect of whole body X-ray administered one day post-antigen injection.

responding fall in total spleen hemolysin plaque forming cells and serum hemolysin titer. However, the significantly higher number of hemolysin plaque forming cells per million spleen cells indicates that the cells remaining are more resistant to ionizing radiations at this time than is the general cell population of the spleen.

Unlike the stimulation observed by others (1-3) on serum antibody titers, X-irradiation in our studies did not stimulate hemolysin

TABLE I. Effect of 250 and 500 R Whole Body X-Irradiation Administered at Various Times Before and After Antigen Injection.

	Spleen index (mg spleen/g body wt)	Hemolysin plaques per 10^6 spleen cells	Hemolysin plaques per spleen	Serum hemolysin $1:\log_2$ titer
Non-irradiated controls (AG given)	37.9 ± 1.77 (13)*	334 ± 28 (9)*	76,850 ± 4,750 (9)*	10.7 ± .2 (9)*
a—250 R 1 day before	21.9 ± 3.05 (8)	159 ± 38 (8)	11,400 ± 1,500 (8)	7.0 ± .5 (3)
b—250 R 1 day after	41.7 ± 2.53 (9)	385 ± 31 (9)	61,770 ± 7,060 (9)	9.1 ± .3 (4)
c—250 R 3 days after	18.2 ± .5 (5)	111 ± 20 (5)	6,205 ± 1,010 (5)	
d—500 R 1 day before	11.0 ± .7 (5)	5.3 ± 2.0 (5)	105 ± 50 (5)	
e—500 R 1 day after	11.9 ± .82 (4)	14.3 ± 4.6 (4)	209 ± 57 (4)	<4 (4)
f—500 R 3 days after	12.2 ± .34 (5)	54.8 ± 7.4 (5)	736 ± 305 (5)	

* Values represent mean ± standard error of mean. Number of animals used for each value is in parentheses.

plaque forming cells. It is possible that the peak response in the production of hemolysin plaque forming cells may have been delayed by radiation and may not have coincided with the assay time.

Doses of 500 R uniformly depressed all the measured parameters of the primary antibody response, indicating that with adequate X-ray doses the production of antibody forming cells is radiosensitive regardless of the interval betwen X-ray and antigenic stimulation. At lower X-ray doses the numbers of hemolysin plaque forming cells of the spleen are clearly dependent on this interval. A bi-phasic response to ionizing radiations in the production of antibody forming cells has thus been demonstrated, *i.e.*, a relatively radio-insensitive period between two radiosensitive periods.

Recent concepts suggest that immunization may be dependent temporally on a prior "processing"(7) of the antigen by the reticuloendothelial system. There thus exists the possibility that more than one cellular system is affected by ionizing radiations and that these different systems may display different radiosensitivities.

An interpretation of the bi-phasic response which is consistent with radiation effects on dividing cells is the following: If radiation (250 R) is administered before antigenic stimulus, both the reticulo-endothelial and lymphoid (plasmacytic) systems would be effectively suppressed; consequently, when the measured parameters are assessed 5 days post-antigen injection an inhibition is observed. Comparable doses of X-ray administered one day after antigenic stimulation result in less inhibition. This phase of the induction period may be associated with the maturation of antibody forming cells (lymphoid system) and would not be as radiosensitive as the "processing" period. When radiation was administered at 3 days post-antigenic stimulation, however, a significant decrease in the measured parameters was again observed. This might be explained by assuming that cells which received radiation at one day post-antigenic stimulation had sufficiently recovered by the time the assays were performed (4 days post-irradiation), whereas those irradiated at 3 days post-antigenic stimulation had not sufficiently recovered in the 2 days between irradiation and assay. The differences in spleen indices for 1- and 3-day groups give some support to this interpretation (Table 1 b and c). Further experiments are obviously required to clarify the mechanisms involved.

Summary. All measured parameters of the primary antibody response of C57L × A/He mice injected with sheep red cells were uniformly depressed by 500 R of X-ray. With adequate X-ray doses, the production of antibody forming cells was radiosensitive, irrespective of the several time intervals employed between X-ray and antigenic stimulation. At lower X-ray doses the numbers of hemolysin plaque forming cells are clearly influenced by this time interval. A bi-phasic response to X-rays in the production of antibody forming cells was demonstrated, *i.e.*, a relatively insensitive period in between two sensitive periods.

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