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Lymphoid Cell Dependence of Eosinophil Response to Antigen. IV. Effects of *in Vitro* X-Irradiation on Adoptive Transfer of Anamnestic Cellular and Humoral Responses[1] (37976)

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Recently, we have reported the detection of two types of memory cells induced by a priming injection of tetanus toxoid—one memory cell mediating the secondary eosinophil response and a separate memory cell associated with antitoxin production. It was further demonstrated that formation of both memory cells was dependent upon the presence of thymic cells at the time of priming. Memory cells involved in eosinophil responses, however, are formed earlier and become more widely distributed in lymphatic tissues than do memory cells involved in antitoxin responses. Consequently, adoptive transfer of specific lymphoid tissues at selected intervals after priming can demonstrate the presence of one type of memory without the other (1, 2). For example, at 10 days after priming, cells present in the spleen were capable of inducing a secondary rise in eosinophils, but not antitoxin. However, at 30 days, splenic cells were capable of inducing both responses. In the experiments to be described, we have demonstrated differing sensitivities of these memory cells to *in vitro* X-irradiation.

Materials and Methods. The experimental procedure is similar to that utilized in previous studies (1–3). Briefly, adult BAF_1 (C57BL × A/J) female donor mice were primed with a single subcutaneous injection of 0.2 ml aluminum phosphate-adsorbed tetanus toxoid (National Drug Co., Philadelphia, PA) in an adjuvant of 0.2 ml pertussis vaccine (Eli Lilly Drug Co., Indianapolis, IN) diluted 1:10 with saline (PVTT). Thirty days after priming, the mice were sacrificed and cell suspensions made of the spleen. These suspensions were exposed *in vitro* to various doses of X-irradiation using a Maxitron unit (General Electric Co., Waterford, NY) at 250 kVp and 15 mA. The exposure rate was 32 R/min with 0.25-mm copper and 1.0-mm aluminum filters at a distance of 77 cm. The irradiated spleen cells were adjusted to give a final concentration of 10^7 cells/ml, and 1.0 ml was injected intraperitoneally into recipient mice which had been previously lethally irradiated (900 R) and reconstituted by an intravenous injection of 10^7 fetal liver cells/0.5 ml. Approximately 1 hr later, recipient mice were injected intraperitoneally with 0.4 ml aluminum phosphate-adsorbed tetanus toxoid (TT) or 0.4 ml aluminum phosphate-adsorbed diphtheria toxoid (DT). These mice were then sacrificed at 18 days after adoptive transfer and challenge, a time previously shown to be optimal for demonstrating both eosinophil and antitoxin responses (1). At the time of sacrifice, blood was taken from the retro-orbital plexus for antitoxin assay using a modification of the bioassay technique of Ipsen (4). Brush smears were made of the peritoneal exudate for May–Greenwald–Giemsa staining and differential counting. The remaining cells in the peritoneal cavity were flushed into collecting vials with a

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total of 25 ml cold Isoton (Coulter Electronics, Hialeah, FL). Quantitative cell counts were performed on a Coulter Counter Model B (Coulter Electronics, Hialeah, FL), and the absolute number of each cell type was estimated on the basis of total counts and differential percentages.

Results. The results have been tabulated in Table I. Spleen cells from primed mice were capable of adoptively transferring secondary type eosinophil responses after doses of from 0 to 400 R. An irradiation dose of 450 R, however, significantly reduced the capacity to respond ($P < 0.10$). Doses greater than 450 R essentially abolished the eosinophil-inducing capacity of primed cells.

On the other hand, much lower doses of irradiation reduced the capacity of the same population of cells to transfer anamnestic antitoxin responses. Primed spleen cells receiving no irradiation induced high antitoxin titers on adoptive transfer and challenge (37,400 units). A dose of 100 R significantly lowered these titers to 18,300 units ($P < 0.05$), while increasing doses of X-irradiation reduced these levels even further ($P < 0.01$). Three types of control mice received either (a) normal spleen cells and were challenged with TT, (b) DT-primed spleen cells and were challenged with TT, or (c) TT-primed spleen cells and were challenged with DT. The results obtained in these groups have been combined, since the eosinophil responses were all very low (0.21×10^6 cells) and no measurable antitoxin was detected.

Discussion. These experiments confirm previous reports that cells taken from lymphatic tissues of mice primed with PVTT are capable of adoptively transferring the capacity to induce secondary eosinophil accumulation at the site of antigen challenge and a rapid elevation of the humoral antitoxin titers. Furthermore, they support the finding that there are separate memory cells for each response. The data indicate that spleen cells taken 30 days after a priming injection of PVTT have the capacity to transfer both responses and that doses of *in vitro* X-irradiation greater than 500 R abolish this capacity. However, the two memory cells have different sensitivities to lower doses of irradiation. Thus, while the capacity for the humoral response is inhibited by doses greater than 100 R, the capacity for the eosinophil response remains intact after doses up to 400 R.

We have concluded from earlier experiments (1, 3) that the eosinophil response represents an expression of cell-mediated

TABLE I. Effect of *in Vitro* Irradiation on the Capacity of Primed Spleen Cells to Adoptively Transfer Eosinophil and Antitoxin Responses to Tetanus Toxoid.[a]

	X-ray dose (R)	No. of animals	Eosinophils $\times 10^6 \pm$ SE	Antitoxin $\times 10^3$
	0	10	3.16 ± 0.68	37.4
	100	3	2.53 ± 0.50	18.3
	300	4	3.62 ± 1.74	5.2*
	325	4	3.64 ± 1.48	5.2*
	375	7	3.81 ± 1.05	2.4*
Experimental	400	13	3.93 ± 0.80	1.1*
	450	3	1.16 ± 0.63	<0.3*
	500	8	0.32 ± 0.20*	<0.3*
	600	4	0.02 ± 0.005*	<0.3*
	800	4	0.14 ± 0.12*	<0.3*
	900	3	0.02 ± 0.02*	<0.3*
Controls[b]	0	15	0.21 ± 0.10	<0.3*

[a] Antitoxin titers of 1000 units may be obtained after priming. During a secondary response, titers of 10,000 units or more are generally obtained.

[b] Controls include: normal spleen cells challenged with TT; DT-primed spleen cells challenged with TT; TT-primed spleen cells challenged with DT.

* Significantly different from primed cells given no *in vitro* irradiation ($P < 0.01$).

immunity, with one of the chemical mediators released from sensitized cells on reexposure to specific antigen being chemotactic for eosinophils. Such eosinophilotactic factors have been described by others (5, 6) and there has been a recent report of a "lymphokine" released from sensitized cells which stimulates eosinophil migration (7). The association of the eosinophil response with cell-mediated immunity is strengthened by the present findings. In cases where the effects of irradiation on cell-mediated and humoral responses have been compared, the cell-mediated response is more radioresistant. Uhr and Scharff (8, 9) reported that delayed type hypersensitivity to various antigens may develop in irradiated animals, even under conditions where detectable antitoxin could not be produced. In these experiments, the discriminating level of irradiation (i.e., the dose at which *in vivo* delayed hypersensitivity reactions developed, but antitoxin production did not) was 400 R. In similar experiments, Kaplan (10), using an *in vitro* macrophage migration inhibition technique (cell-mediated) and serum antibody production, noted that 300 R inhibited the humoral response with no effect on the cell-mediated response.

The data of other investigators (11, 12) demonstrate a similar relationship between homograft reactivity and antibody production. Salvin and Nishio (13) observed that lymphocytes from the spleen or lymph nodes of sensitized guinea pigs retained their capacity to adoptively transfer delayed hypersensitivity responses to normal animals after *in vitro* irradiation with doses up to 480 R. The latter investigators did not, however, determine the radiosensitivity of humoral immunity in their system.

With respect to the cell types involved in the actual transfer of memory responses to tetanus toxoid, we have previously shown that their induction is dependent on the presence of thymic cells in the donor animal at the time of priming (1). Subsequent studies have shown them to be part of the nonadherent fraction of the primed spleen cell population (Ponzio and Speirs, unpublished observations). These data, taken with the present findings, suggest that the carriers of immunologic memory for both responses are lymphocytes.

Thus, we conclude that memory for the eosinophil and antitoxin responses to tetanus toxoid exhibits distinct sensitivities to *in vitro* X-irradiation. Memory cells mediating the antitoxin response are inhibited by doses greater than 100 R, whereas doses greater than 400 R are required to inactivate memory cells associated with the eosinophil response. The similarity in the radiosensitivity of cell-mediated and eosinophil responses further supports the association of these two expressions of immunity. We have described experiments in which both cell-mediated (eosinophil) and humoral (antitoxin) responses to a single antigen (tetanus toxoid) can be conveniently monitored in the same animal model.

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