Eosinophil Response to Toxoids in Actively and Passively Immunized Mice.* (22101)

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The recent(1) development of technics for quantitatively estimating the number of cells in body cavity fluids has made it possible to determine the cellular reactions outside the blood following administration of hormones, drugs or other physiologically active substances. In a study of such reactions in the peritoneal exudate marked changes were found in the number of cells per cubic millimeter of peritoneal fluid depending upon a) the material injected, b) the degree of sensitization of the animal to that material, and c) the level of adrenal cortical hormones. An accumulation of eosinophils in the peritoneal fluid between the 2nd and 10th days appeared to be the specific local cellular response of the sensitized mouse to intraperitoneal injection of sensitizing antigen(1-3). The antigens used included pollen extract, bovine albumin, horse serum, ascaris extract and foreign erythrocytes.

This paper reports further studies concerning the local and systemic cellular responses to antigens and the chemotactic factors involved. It was found that injections of tetanus or diphtheria toxoid produced a local accumulation of eosinophils in actively immunized mice, but not in non-immunized mice or mice passively immunized with heterologous antibody.

Materials and methods. The mice used in these experiments were BBF_1 hybrids and C 57 Br/a mice from the research colonies of the R. B. Jackson Memorial Laboratory. The animals were from 2½ to 4 months of age and both sexes were used. The mice were immunized to diphtheria toxoid (Parke Davis and Co.) by a series of 5 subcutaneous injections as follows: 0.1 ml on days 1 and 2, and 0.2 ml on days 3, 6 and 13. The final challenging injection was given intraperitoneally one week after the last immunizing injection. Two different procedures were used for immunizing mice to tetanus toxoid. The first consisted of a series of injections of fluid toxoid (Lederle) as given above for diphtheria toxoid. The second consisted of injecting subcutaneously 0.05 ml of an alum phosphate precipitated toxoid (Lederle) followed by 0.05 ml fluid toxoid 15, 22 and 29 days later. On the 36th day a challenging injection of 0.05 ml fluid toxoid was given intraperitoneally. Passive immunization was produced by injecting mice with tetanus antitoxin (Lederle) either 1 or 72 hours prior to the injection of fluid tetanus toxoid. Two different dosages were used; half the animals were injected subcutaneously and the remainder intraperitoneally.

The methods used for estimating number of blood and peritoneal cells have been published elsewhere(1,4-6). A modification of Randolph's solution(7) was found to be a satisfactory diluent for the cell determinations. Each animal was used only once for the determination of cells and then discarded.

Results. The numbers of eosinophils in the peritoneal fluids of immunized and non-immunized mice, 4 days after intraperitoneal injections of toxoid, are compared in Fig. 1. In non-immunized animals a single injection of tetanus toxoid did not increase the number significantly. The immunized animals tended to have slightly higher numbers of eosinophils in the peritoneal fluid prior to the challenging injection. The animals which received the intraperitoneal injection of toxoid, however, showed as much as a 50-fold increase in these

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IMMUNIZATION (SUBCUTANEOUS INJECTIONS)			FINAL INJECTION (INTRAPERITONEAL)		NO. OF MICE	EOSINOPHILS PER CUBIC MILLIMETER
MATERIAL	ML PER INJ.	NO. OF INJ.	MATERIAL	DOSE (ML)		20,000 40,000 60,000 80,000 100,000
NONE			NONE		9	
NONE			TETANUS TOXOID	0.05	3	
TETANUS TOXOID	0.05	4	NONE		6	
TETANUS TOXOID	0.0008	5	TETANUS TOXOID	0.0008	5	
	0.002	5		0.002	3	
	0.02	5		0.02	3	
	0.05	4		0.05	6	
DIPHTHERIA TOXOID	0.002	5	DIPHTHERIA TOXOID	0.002	3	
	0.02	5		0.02	3	
	0.2	5		0.2	3	

FIG. 1. No. of eosinophils in peritoneal fluid 4 days following intraperitoneal injection of toxoid in immunized and non-immunized mice.

cells. Similar data were obtained with diphtheria toxoid as the antigen.

The numbers of blood leucocytes and peritoneal cells observed following an injection of 0.05 ml of fluid tetanus toxoid in actively immunized and non-immunized mice is shown in Fig. 2. In the non-immunized mice, tetanus toxoid produced no marked changes in number of blood or peritoneal cells. However, in the actively immunized mice, there was a slight increase in blood eosinophils, and a marked increase in peritoneal eosinophils. A slight increase in the number of mononuclear cells and a transient neutrophilia also occurred.

The eosinophil responses in the peritoneal fluid on the 4th day following an intraperitoneal injection of 0.05 ml of tetanus toxoid in actively and passively immunized mice are compared in Table I. A marked eosinophilia

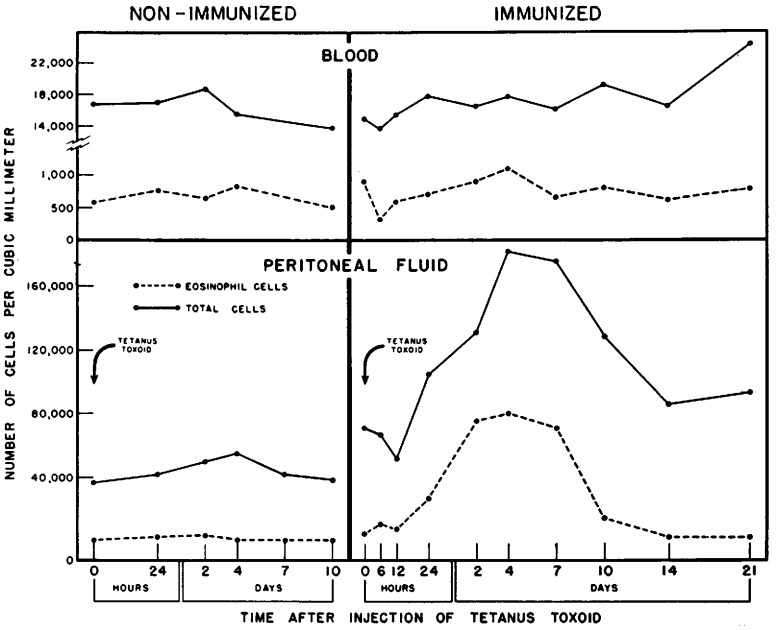

FIG. 2. Cellular responses following an intraperitoneal injection of tetanus toxoid in immunized and non-immunized mice. A total of 80 mice were used and each point represents the mean of 3 to 6 mice.

TABLE I. Comparison of Eosinophil Responses in Actively and Passively Immunized Mice 4 Days following an I.P. Injection of Tetanus Toxoid. 6 mice in each series.

Procedure for immunization				Response to tetanus toxoid eosinophils/mm³ peritoneal fluid ± S.E.
Active	4 S. Q. inj. of 0.05 ml toxoid during 4 wk period			80660 ± 7817
Passive	A. 550 units antitoxin*	1 hr prior to toxoid†		1208 ± 166
	B. 2780	1	†	783 ± 271
	C. 550	72	†	1117 ± 127
	D. 2780	72	†	1158 ± 417

* Antitoxin—Lederle Globulin Tetanus Antitoxin (1 ml ≅ 5560 units).
† Half of the mice injected IP, the remainder S. Q.

occurred in every animal which was actively immunized and then reinjected with the antigen. However, there was no evidence for an increase in the number of these cells in any of the mice passively immunized with antitoxin from a heterologous species.

Discussion. These experiments demonstrate that eosinophils accumulate in the peritoneal fluid following intraperitoneal injections of either tetanus or diphtheria toxoid into mice actively immunized to the respective toxoid. This local eosinophilia did not occur in control or passively immunized animals. The number of mononuclear cells also increased following the challenging injection of toxoid but the degree of increase was less.

It is well documented that antigenic material injected for the first time is rapidly removed from the injection site. However, in the actively immunized animal the antigen becomes fixed at the site of injection. This fixation is accompanied by antigen-antibody reactions, marked stimulation of antibody production, both locally, and in the draining lymph nodes, as well as an increase in the amount of local inflammation(8-10). It is shown in this paper that these reactions are also accompanied by an accumulation of eosinophils.

In the passively immunized animal also, there is fixation of antigen, antigen-antibody reactions, and local inflammation(9,10). However, an accumulation of eosinophils does not occur in these animals. It would therefore appear that the eosinophils are related to some phase of antibody production, since by the 4th day antibody production occurs in the actively immunized, but not in the passively immunized animal.

There has been a great deal of speculation as to which cells produce antibodies, but the eosinophil has not been considered by current workers or reviewers in this field despite earlier work suggesting this possibility(11-14). Eosinophils have been found to respond not only following the reinjection of toxoid, but also following the reinjection of a wide variety of particulate and soluble antigens, such as isoantigens, pollen extracts, albumins, horse serum, foreign red blood cells, parasitic extracts, etc.(1-3,15,16). The accumulation of these cells has been observed around the antigen whether it is introduced intraperitoneally, subcutaneously, intravenously, or via the digestive or respiratory tracts(3,17-20). In addition to the site of antigen injection, these cells also accumulate in lymph nodes draining an area of injection or infection and in the spleen following an intravenous injection of foreign protein(17,21). Furthermore, the time of local accumulation of eosinophils appears to precede slightly or to coincide with that of antibody production (22-24). It is also of interest to note that recent technics developed for staining antibody in plasma cells, also stain eosinophils in a similar manner(25,26). Finally, doses of cortisone and X-ray which inhibit antibody production also prevent the accumulation of eosinophils(27).

Summary. 1. Intraperitoneal injections of tetanus and diphtheria toxoid into actively immunized mice produce an accumulation of eosinophil cells in the peritoneal fluid which

reaches a peak at 4 days and lasts for at least 10 days. The degree of increase depends upon the dose of toxoid used. The local eosinophilia does not occur in non-immunized mice or in mice passively immunized with heterologous antibody. 2. The experiments suggest that the local eosinophilia is due neither to the antigen-antibody reaction nor to the accompanying inflammatory syndrome, but to an early stage of antibody formation during the secondary response.

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Thyroidal I^{131} Collection in Normal and Adrenalectomized Rats, Effect of Desoxycorticosterone Acetate and Physiological Saline.* (22102)

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Although it has often been suggested that changes in extrathyroidal distribution of iodine such as would be caused by body fluid shifts or variations in extracellular fluid volume will alter thyroidal I^{131} collection, little specific information on this point is available. Boatman *et al.*(1) reported an increase in thyroidal I^{131} concentration ratio 3 hours after injecting I^{131} in rats treated chronically with desoxycorticosterone acetate (DCA). It was postulated that this may have been due to the observed increase in blood radioactivity. In contrast to this, chronic treatment of rats with DCA was reported to cause no change(2) or a decrease(3) in 24 hour thyroidal I^{131} collection. In man(4) a decrease was also observed. Except for the report(5) that an increased amount of NaCl in the diet

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