Modification of the Hemorrhagic Action of Endotoxin by *Bordetella pertussis* Vaccine.* (28407)

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The nature of the biological activities of bacterial endotoxins continues to puzzle investigators. One view postulates that endotoxin produces its effects through a direct pharmacological action; a second hypothesis maintains that the reaction to endotoxin is related to immunological and hypersensitivity phenomena(1).

In the present study two different effects of endotoxin were related—namely, its lethal effect and its ability to induce necrosis in transplanted tumors. Both effects are considered by some investigators to be the result of a hypersensitivity reaction. In experiments described here mice were made immunologically hyperreactive by injections of *Bordetella pertussis* vaccine(2) and the animals were tested for possible alteration in their responses to endotoxin.

Materials and methods. Endotoxin. The somatic polysaccharide isolated from *Salmonella enteritidis* by the Boivin method was obtained from the Difco Co., Detroit.

Animals. CAF_1 mice, each weighing approximately 20 g, were obtained from the NIH breeding colony.

Lethality in mice. Groups of animals were injected intraperitoneally with 0.5 ml of endotoxin preparations. Deaths in each group were recorded during a period of 72 hours.

Induction of hemorrhagic necrosis in tumors. CAF_1 mice with sarcoma-37 tumors implanted in the thigh 6 days earlier were injected intraperitoneally with dilutions of endotoxin. The following day the animals were sacrificed, and the tumors were examined for presence of hemorrhagic necrosis.

Sensitization of mice by pertussis vaccine. *B. pertussis* vaccine, lot 2076, was obtained from Lederle Laboratories, Pearl River, N. Y. Mice were sensitized by one intraperitoneal injection of 0.4 ml of the vaccine suspension. The vaccine was administered to the animals 12 days prior to injection of endotoxin.

Active sensitization of mice to anaphylaxis. Animals were sensitized by 4 intraperitoneal injections of 0.1 ml of normal horse serum on alternate days. The shock inducing dose was given intravenously 10 days later. When pertussis vaccine was also given, this was injected 10 days before the shock inducing dose of horse serum. Anaphylaxis was characterized by prostration and ruffled fur, which persisted for several hours. Death usually occurred in 1 to 2 hours, but animals that survived were apparently normal the following day.

Results. It has been reported previously that pretreatment of mice with pertussis vaccine as well as the bearing of transplantable tumors will increase the susceptibility of these animals to the toxic effects of endotoxin (3,4). To explore the possibility that both methods of increasing susceptibility to endotoxin are due to common factors, it seemed of interest to determine whether the effects were additive. For this purpose tumors were implanted in 2 groups of mice, of which one group had been pretreated with pertussis vaccine. A third group was given pertussis vaccine alone, and a fourth group served as controls. Subsequently all mice were given endotoxin in varying doses. Animals pretreated with pertussis vaccine were about 15 times more susceptible to the lethal effects of endotoxin than were the control animals (Table I). Likewise, tumor bearing animals were found to be more susceptible to death from endotoxin than were animals without tumors. The animals which were injected with vaccine and also implanted with tumors, however, did not become more affected by endotoxin than those that were only given

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TABLE I. Lethal Effect of Endotoxin for Mice.

Dose per mouse (μg)	Treatment prior to endotoxin injected*			
	Controls (untreated)	Pertussis vaccine	Implanted tumors	Impl. tumors + pertussis v.
3	0/10	0/10	0/10	0/10
6	0/10	3/10	0/10	2/10
12.5	0/10	7/10	2/10	8/10
25	0/10	10/10	4/10	9/10
50	0/10	9/10	6/10	10/10
100	2/10	10/10	9/10	10/10
200	7/10	10/10	10/10	10/10

* Fractions = deaths/total No. tested.

vaccine. In other words, while each of these treatments increased the susceptibility of the animals to endotoxin, both treatments together did not result in an additive effect.

Since it was found that pertussis vaccine increases susceptibility of the mice to the lethal effect of endotoxin, we tested whether this treatment also altered the reactivity of implanted tumors to the same endotoxin. Animals were given pertussis vaccine and 6 days later were implanted with sarcoma-37 tumors. These mice and a control group of animals which were not injected with pertussis vaccine were challenged with sublethal amounts of endotoxin, and the tumors were examined for the appearance of hemorrhagic necrosis. The results are shown in Table II.

The tumors of the animals pretreated with pertussis vaccine, contrary to expectation, were much less affected by endotoxin than were the tumors of the control animals. Thus, tumors of pertussis treated animals were affected only by a dose of 25.0 μg of endotoxin whereas the hemorrhagic necrosis in 80% of control animals was detectable after injection of 1.0 μg of endotoxin.

TABLE II. Effect of Pertussis Treatment on Hemorrhagic Activity of Endotoxin.

Dose per mouse (μg)	Mice with induced tumor damage*	
	Untreated	Pertussis treated
.1	0/10	0/10
.5	4/10	0/10
1.0	8/10	0/10
2.5	10/10	0/10
5.0	10/10	1/10
10.0	10/10	3/10
25.0	10/10†	10/10

* Hemorrhagic necrosis in sarcoma 37 implants; fractions = mice with tumor damage/total No. of mice tested.

† 3 deaths.

It has been reported that hemorrhagic necrosis in transplantable tumors can also be induced by anaphylactic reaction(5). Since pertussis treatment seems to produce a protective effect against induction of hemorrhagic necrosis of tumors by endotoxin, it was of interest to determine if pertussis treatment would interfere with the hemorrhagic necrosis induced by anaphylaxis. The anaphylactic reaction in mice was induced by repeated injections of horse serum by the procedure described in *materials and methods*. The animals were sensitized with the horse serum with or without additional injections of pertussis vaccine. The results (Table III) show that in anaphylaxis, regardless of whether or not pertussis was given, the tumors of the animals became necrotic. It should be noted that the presence of the tumor had a sparing effect upon the animals since the control animals which did not bear tumors succumbed more readily to anaphylaxis than did the tumor bearing mice.

Discussion. In the foregoing experiments an attempt was made to relate 2 types of endotoxin effects which were considered by some investigators to have an immunological origin(1). It has been claimed that the lethal toxicity of endotoxin and its ability to induce hemorrhagic necrosis in tumors are both the result of a hypersensitive reaction. Our results do not seem to support this hypothesis. In pertussis-treated animals implanting of tumors did not increase susceptibility to lethal effects of endotoxin. Moreover, the tumors of pertussis-treated mice became much less susceptible to necrosis by endotoxin than did tumors of the non-treated animals. This pattern, however, was not ob-

TABLE III. Effect of Pertussis Vaccine on Hemorrhagic Necrosis of Tumors Induced by Anaphylactic Shock.

Anaphylaxis induced by horse serum (ml/animal)	Untreated	Treatment before induction of anaphylaxis*			
		Implant. tumor		Implant. tumor + pertussis v.	
	Survivors	Survivors	Tumor necrosis	Survivors	Tumor necrosis
.001	4	10	8	10	7
.005	2	8	7	7	7
.01	0	7	7	6	5
.05	0	5	5	4	4
.1	0	2	2	1	1

* Each group consisted of 10 mice.

served when the hemorrhagic necrosis was induced by anaphylaxis, since under the latter condition pertussis vaccine appeared to have no effect on the outcome of the reaction. It is to be noted that pertussis treatment affects the reactivity of the animals to a variety of substances such as histamine and serotonin as well as to endotoxin(6). Pertussis can also increase susceptibility of the animals to anaphylactic shock and can be used as an adjuvant for production of immune antibodies(2). Thus it is not clear which factors are responsible for the alterations in reactivity to endotoxin following pertussis treatment.

Summary. Pertussis-sensitized mice were tested for their reactivity to endotoxin as expressed by lethality and induction of hemorrhagic necrosis in implanted tumors. It was found that, whereas pertussis treatment increased the susceptibility of animals to the lethal effect of endotoxin, transplanted tumors became more resistant to its necrotizing effects. Induction of tumor necrosis by an anaphylactic reaction was not affected by pertussis treatment. Tumor-bearing mice injected with pertussis vaccine did not become more susceptible to the lethal effects of endotoxin than did animals not bearing tumors.

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Factors Which Affect Plasma Lactic Dehydrogenase in Tumor-Bearing Mice.* (28408)

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Increased activity of plasma lactic dehydrogenase (LDH) in neoplastic diseases has been observed in human subjects as well as in experimental animals(1,2,3,4,5). Although this phenomenon is encountered in a wide variety of disease states and is therefore by no means diagnostic of cancer, the possible significance of this manifestation in carcinogenesis has been of considerable interest. One or more of the following mechanisms has been suggested to explain the elevation of this enzyme during tumor development: 1. Tissue damage which accompanies tumor growth leads to release of LDH from cells (6). 2. Necrosis of neoplastic tissue results in liberation of LDH into intercellular fluid

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