Suppression by Endotoxin of the Immune Response to Actinophage in the Mouse.* (29640)

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It has been repeatedly demonstrated that bacterial endotoxin enhances the immune response to protein antigens by a variety of animals(1,2). This is rather surprising in view of the known deleterious effect of endotoxin on the reticuloendothelial system(3). Actually, sensitivity to endotoxin seems to be a prerequisite for its adjuvant action(4). Moreover, time of administration of endotoxin and the intensity of the antigenic stimulation constitute determinative variables for demonstrating an enhanced immune response (5). This report is concerned with the effects of endotoxin on the immune response of mice to actinophage. Repeated administration of endotoxin suppressed production of both early and late phage neutralizing antibody.

Methods. Actinophage MSP8 was purified by differential centrifugation, gel filtration and elution chromatography(6). The purified actinophage was suspended in and diluted with 0.15 M NaCl. Early antibody was elicited by injecting 10^{10} actinophage intraperitoneally into adult Bagg albino mice (a subline of BALB/sy). Hyperimmune serum was obtained from mice given 5 weekly injections of 10^{10} phage. Blood was collected from the retro-orbital plexus and allowed to clot. Clotted blood was centrifuged to facilitate collection of the serum. Undiluted sera were stored at 4°C until their phage neutralizing activities were measured.

For determination of phage neutralizing activity, sera were heated at 56°C for 30 minutes, diluted appropriately in peptone-yeast extract broth(7), and mixed with actinophage MSP8 to give 10^5 particles/ml. The number of viable phage remaining after incubating a phage-serum mixture for 30, 90, 180 and 360 minutes at 30°C was determined (8). Neutralizing activities were expressed as K values(9).

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Results. Single, graded doses of bacterial endotoxin (Difco STO901) when injected concurrently with 10^{10} phage MSP8 did not significantly enhance early antibody production. In fact, 4 mg endotoxin/kg actually retarded the immune response (Table I). Decreased phage neutralizing activity at a given interval after stimulation resulted from a delay rather than permanent suppression of the immune response. Mice given 4 mg endotoxin/kg daily after antigenic stimulation produced little phage neutralizing antibody. When endotoxin was discontinued or given prior to antigenic stimulation, antibody production proceeded normally after a delay of 1 to 2 days (Table II). Similarly, daily doses of 4 mg endotoxin/kg retarded the anamnestic response to actinophage. After cessation of endotoxin administration, phage neutralizing activity did increase (Table III). Endotoxin not only suppressed the immune response to antigenic stimulation, it also accelerated loss of phage neutralizing antibody. Mice stimulated by one or more injections of actinophage retained significant antiphage titers after many months. Continued daily administrations of 4 mg endotoxin/kg resulted in a loss of neutralizing activity (Fig. 1, 2). The rate of decrease in antiphage titer

TABLE I. Effect of a Single Injection of Endotoxin on Production of Actinophage Neutralizing Antibody by the Mouse.

mg Endotoxin/kg	Neutralizing activity*	
	Endotoxin with antigen	Endotoxin 3 days after antigen
0	3.0	3.0
.2	3.8	3.0
.4	3.7	3.0
1.0	3.4	2.5
2.0	3.3	2.1
4.0	1.4 (3.3)†	1.3

* Sera were harvested 7 days after stimulation; phage neutralizing activity is expressed as the K-value.

† K-value of sera harvested 10 days after stimulation.

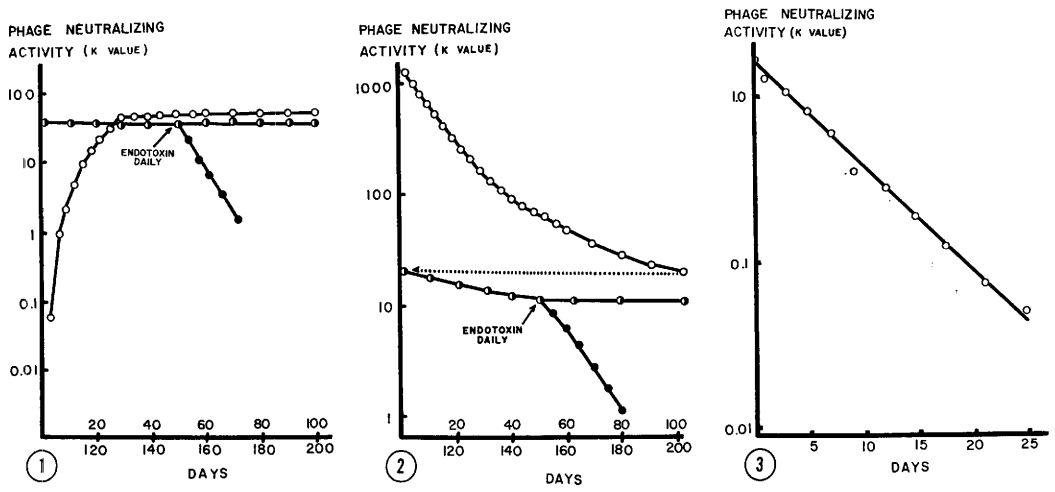

FIG. 1. Persistence of antibody in mice. Mice were immunized by one intraperitoneal injection of 10^{10} MSP8. Thereafter for one year serum (day 0 to 100, ○——○; day 100-200, ◒——◒) samples were collected and assayed for phage neutralizing activity. Starting on day 150, some of the mice were given 4 mg endotoxin/kg daily. Sera were collected and assayed (●——●).

FIG. 2. Persistence of antibody in hyperimmunized mice. Mice were hyperimmunized by 5 weekly intraperitoneal injections of 10^{10} MSP8. Starting one week later and continuing for one year (day 0 to 100, ○——○; day 100-200, ◒——◒) serum samples were collected and assayed for phage neutralizing activity. Starting on day 150, some of the mice were given 4 mg endotoxin/kg daily. Sera were collected and assayed (●——●).

FIG. 3. Persistence of passively administered antibody in the mouse. Hyperimmune serum was injected intraperitoneally; thereafter serum samples were collected and assayed for phage neutralizing activity.

TABLE II. Suppression of Early Immune Response to Actinophage by Daily Administration of Endotoxin.

Endotoxin	Antigen	Neutralizing activity (K-value)
—	—	.02
daily	—	.11
—	1 I.P.	2.7
daily	1 I.P.	.18
daily*	1 I.P.	.52 (3.3)†

Mice were given a single I.P. injection of 10^{10} actinophage MSP8 and 4 mg endotoxin/kg daily for 6 days; mice bled on day 7.

* Endotoxin was given daily 1 wk prior to antigenic stimulation and not thereafter.

† Neutralizing activity on day 10.

resembled that of passively transferred neutralizing activity (Fig. 3).

Discussion. The mouse is relatively resistant to bacterial endotoxin, thereby permitting continued, intensive treatment of the animals. The power of endotoxin to suppress formation of phage neutralizing antibody can be reasonably attributed to its cytotoxicity, just as potent metabolic inhibitors are able to retard the immune response(10). Endotoxin, like other cytotoxic agents, has an adjuvant effect when administered with soluble protein antigens in properly adjusted doses and times(11). These diverse effects of endotoxin indicate that the immune response to particulate antigens and soluble antigens may be different at some stage. Possibly phagocytes capable of converting particulate antigens to soluble antigens are more sensitive to endotoxin than are the antibody producing cells. In cursory experiments, how-

TABLE III. Effect of Endotoxin on Anamnestic Response to Actinophage.

3 I.P. +	Endotoxin	Neutralizing activity (K-value)
no booster	none	28
" "	daily for 6 days	20
booster	none	187
"	daily for 6 days	44(132)*

Mice were given 3 I.P. injections of 10^{10} actinophage MSP8 at weekly intervals, followed by 90 days rest. Then a 4th injection of 10^{10} actinophage MSP8 was given; mice were bled 7 days later. Endotoxin was given daily at a dose of 4 mg/kg.

* Neutralizing activity on day 10; endotoxin was not administered days 7 through 10.

ever, the amount of phage in the blood and viscera following intraperitoneal injection was not significantly affected by treatment with endotoxin.

Ecologically, endotoxin may be an important controlling factor in antibody production. Endotoxin derived from the enteric bacteria, like orally administered actinophage (12) may be adsorbed in sufficient quantity to alter the antibody content of the sera(13).

Summary. Repeated injections of 4 mg endotoxin/kg, subsequent to antigenic stimulation with actinophage MSP8, retarded the early and late immune response in the mouse and resulted in an accelerated loss of preformed antibody. Cessation of endotoxin treatment permitted phage neutralizing activity in stimulated animals to increase.

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Effect of Guanethidine and Adrenal Demedullation on Hyperglycemic Responses to Diazoxide in Rats.* (29641)

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Diazoxide (7-chloro-3-methyl-1,2,4,-benzothiadiazine-1, 1-dioxide), a non-diuretic benzothiadiazine, is an effective hypotensive drug in animals(1-3) and in man(4,5). Unfortunately it produces significant hyperglycemia in normal, and especially, in diabetic subjects(6). The mechanism and the locus of action of this hyperglycemic effect are unknown. Diazoxide causes hyperglycemia in adrenalectomized, nephrectomized, or propylthiouracil-treated mice(7). Tolbutamide or insulin given to normal mice prevent or reverse the hyperglycemia(7,8). Adrenalectomy, alone or together with the administration of isopropyl methoxamine, a beta adrenergic blocker, or pretreatment with chlorisondamine, a ganglionic blocker, modify the response(7,9). These findings suggest that the metabolic effects of diazoxide are at least partly mediated by adrenergic hormones. The present studies were undertaken to see whether guanethidine, a peripheral depleter of catecholamines, and adrenal demedullation would suppress the hyperglycemic responses of rats to diazoxide.

Materials and methods. The experiments were done on 42 rats of the Long-Evans strain, weighing approximately 250 g each. The rats were fed Purina Laboratory Chow and water *ad libitum*. The effects of single injections of guanethidine, diazoxide, or both, on blood sugar and liver glycogen were measured in nonfasted normal rats and also in nonfasted rats which had surgical ablation of the adrenal medulla 2 weeks previously (Table I). Five normal rats and 5 demedul-

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