Effect of Endotoxin on the Delayed Hypersensitivity Reaction.* (31814)

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Delayed hypersensitivity reactions can be produced on mice that have been sensitized by epicutaneous application of 1-chloro-2,4-dinitro-benzene(3). Two somewhat divergent theories have been used to explain the mechanism of reaction. One theory(8) suggests that the reaction of antigen with a low concentration of high affinity antibody is responsible while the second theory(1) suggests that cell-associated immune factors are important. The severity of the delayed hypersensitivity reaction can be assessed by measurement of the area of the reaction or by the percent of animals that respond(2). Several methods of modifying the development or the expression of existing hypersensitivity have been used and include use of: immuno-suppressants (12), oral administration of haptene(9) tween 80 or a phospholipid derived from arachis oil(3,6), lipids(7), humoral antibody (4), stress(11), and pertussis vaccine(5). The last observation was particularly interesting in view of the similarity between pertussis vaccine and bacterial endotoxin, a material known to modify humoral antibody responses. These investigations were undertaken to determine if administration of endotoxin to an animal could modify the development or the expression of hypersensitivity to 1-chloro-2,4-dinitro-benzene in a system that has been shown, by others(2), to form cell-associated immune factors, almost exclusively.

Materials and methods. Male Swiss mice‡ weighing 20-25 g were housed in wire mesh cages suspended over adsorbant bedding and fed laboratory animal pellets§ and water *ad lib.*

The mice were sensitized by two 50 μg epicutaneous applications of 1-chloro-2,4-dinitro-benzene (CDNB)‖ in freshly prepared acetone solution. The applications were made 14 days apart on the abdominal skin surface which was shaved with a safety razor 2 days before and wiped with an acetone saturated cotton pledget immediately prior to the application. A challenge dose of 5 μg of CDNB was similarly applied 14 days after the second sensitizing dose.

The endotoxin used was prepared from *Escherichia coli* 0127:B8;¶ suspended in sterile pyrogen free physiological saline** so that one dose was contained in a volume of 0.20 ml; and heated at 100°C for 30 minutes. The endotoxin was injected into the tail-vein without prewarming the mice(11). The injections were made 48 hours before one of the 3 applications of CDNB.

The reactions were measured with a millimeter rule to the nearest 0.5 mm in two directions 90° apart and the resulting numbers multiplied together in order to obtain the reaction area. The 3 parameters measured were, 1) percent of mice that produced a visible reaction, 2) mean area of the reaction on mice that responded, and 3) mean area of reaction on all mice within a treatment group. The last parameter was included so that one number could represent the findings in those cases where both the percent response and the area of reaction varied. The results obtained were subjected to an analysis using established statistical tests.

Results. A total of 989 mice were used in these studies; 556 were treated before challenge, 227 were treated before the anamnestic (second) sensitization, and 206 mice were treated before sensitization. Administration of endotoxin prior to challenge did alter the delayed hypersensitivity reactions that developed. The area of the reactions on both the

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TABLE I. Effect of Administration of Bacterial Endotoxin Prior to Challenge on the Delayed Hypersensitivity Reaction that Developed After Challenge.

Endotoxin MCG/ mouse	% mice +	Significance level*	Mean reaction area MM^2 + mice	Significance level†
0	100	Standard	45.2	Standard
25	92.4	N.S.	31.4	.01
50	100	"	35.4	.02
75	90	"	32.9	.05
100	100	"	24.6	.01
125	100	"	24.9	.01
150	100	"	28.8	.01
175	100	"	28.7	.01
200	100	"	27.7	.01
225	100	"	27.4	.01
250	100	"	24.0	.01

* p value using Chi Squared Test.
† p value using Student's "T" Test.
N.S. = not significant at p = 0.30.

responding and on all of the mice within a treatment group was depressed with a certainty of up to 1%. These data formed a hyperbolic shaped curve when graphed (Table I, Fig. 1). In experiments that are not shown, administration of doses of endotoxin ranging from 0.1 to 100 μg also produced a hyperbolic shaped dose-response relationship. The percent of the mice that responded was sometimes depressed.

Administration of endotoxin prior to the anamnestic sensitization produced a minimal change in the delayed hypersensitivity reactions that developed after challenge. The area of the reactions and the percent of the mice that responded were not different from those of the control mice when 0.01 to 100 μg of endotoxin were injected 48 hours before the second application of CDNB.

Administration of endotoxin prior to sensitization of the mice also produced a minimal change in the delayed hypersensitivity reactions that developed after challenge. The mean area of the reactions that developed in those mice that responded or in all of the mice within the treatment group were not different from those of the control mice with a certainty of greater than 20% when the mice had been injected with 0.01 to 250 μg of endotoxin 48 hours before the first sensitizing application of CDNB. The percentage of the mice that responded was apparently

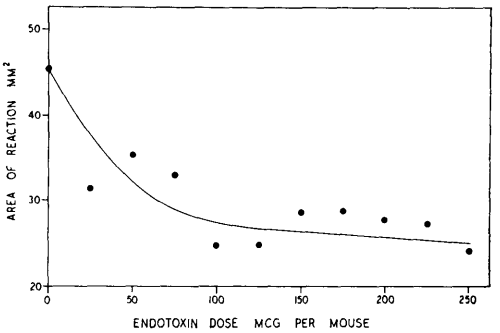

FIG. 1. Effect of endotoxin dose, administered before challenge, on delayed hypersensitivity response of mice.

decreased by the largest endotoxin dose used, 250 μg per mouse. In the control group, 94.3% of the mice responded with visible reactions, while only 66.6% of the mice treated with 250 μg of endotoxin responded.

Discussion and conclusions. The results presented here strongly suggest that the effects of endotoxin administration on the form of delayed hypersensitivity known as contact sensitivity are dependent on both the timing of the treatment and dose administered. Administration of endotoxin 48 hours prior to challenge resulted in a marked depression of the hypersensitivity response. This effect was noticed in the reduction of the areas of reaction and sometimes in the percentage of the animals that responded. The hyperbolic shape of the dose-response curve obtained when the data were plotted suggested that active site(s), possibly on the lymphoid series of cells, became saturated with endotoxin thereby either decreasing the number of sites available for interaction with the sensitizer modified skin proteins by a process analagous to competitive enzyme inhibition or perhaps resulting in the immune lysis of cells hypersensitive toward both the modified skin proteins and also the endotoxin. That sensitive cells can lyse in the presence of specific antigen is documented(13).

In contrast to the results obtained when the endotoxin was given prior to challenge, administration of endotoxin prior to or during active sensitization did not appear to increase the magnitude of the immune response as it has been reported in systems measuring humoral antibodies(10), and, in fact, the re-

sponse appeared to be diminished.

Additional study is required for a complete understanding of the mechanism by which endotoxin influences the delayed hypersensitivity reaction called contact sensitivity.

Summary. The data presented show that intravenous administration of bacterial endotoxin modify the delayed hypersensitivity reaction commonly called contact sensitivity. The effect on the reaction was dependent both upon timing of the injection and dose of endotoxin used.

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Influence of Stress on Granuloma Formation.* (31815)

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Studies in our laboratory have shown stress to decrease susceptibility of mice to viral infections(1,2), to retard viral clearance(3) and to suppress interferon production(4). Christian and Williamson(5) showed that stress of crowding decreased significantly foreign body granuloma formation in mice. The present study was undertaken to determine the influence on foreign body granuloma formation(6) by the previously employed forms of stress(1,2,3,4).

Materials and methods. Nine-week-old male Swiss, Webster BRVS strain, mice were used. Each foreign body granuloma was produced by subcutaneous implantation of a single commercial dental cotton pellet (Richmond #4) between the scapulae. This procedure was done under ether anesthesia, and the incision was closed with a wound clip which was removed after 48 hours. Two forms of stress were employed: high intensity sound (123 db) and avoidance-learning in a shuttle box apparatus. These have been described(2,7).

The mice were divided into 3 groups: Group I was given sound stress for 5 days before implantation and avoidance-learning stress for 14 days after implantation; Group II was given sound stress for 5 days before and 14 days after; Group III received no stress. On the 14th day after implantation, the granulomas were excised, trimmed of their tissue adhesions, and weighed. They were then fixed in 10% formalin, imbedded, sectioned at 5μ thickness, stained with hematoxylin and eosin, and graded on an arbitrary scale of 1-5 on the basis of macro- and microscopic appearance of the capsule and on the degree of cellular infiltration into the cotton pellet. The following scores were used for the capsules: 1 = none, 2 = some cellular adhesion, 3 = partial thin, 4= partial thick,

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