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Increased Histamine Sensitivity in Mice After Administration of Endotoxins

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CFW mice given submicrogram doses of endotoxins intravenously became highly susceptible to the lethal effects of 0.5 mg of histamine given intraperitoneally 1 to 2 h later. The histamine-sensitizing effects of the endotoxins were transitory and disappeared within 6 to 8 h. L-Epinephrine administered intravenously immediately after histamine challenge protected mice from death, but arterenol and isoproterenol were ineffective. The histamine-sensitizing effect in endotoxins was precipitated by anti-endotoxin sera with a concomitant eightfold loss in activity. However, dissociation of the immune complex in 0.25 M acetic acid fully restored histamine-sensitizing activity. The transitory nature of the hypersensitivity produced by endotoxin and the high heat resistance of the active material prove that it is different from the histamine-sensitizing effects of pertussigen.

Previous investigations on the ability of endotoxins to produce histamine sensitivity in mice indicated that endotoxins were much inferior to the histamine-sensitizing factor from *Bordetella pertussis* in efficacy. Malkiel and Hargis (5) found that 100 to 200 μg of lipopolysaccharides from *B. pertussis*, *Salmonella typhosa*, or *Escherichia coli* was required to produce lethal sensitivity to a histamine challenge given intraperitoneally (i.p.) 4 days later (36 μg of histamine phosphate/g of body weight). Pieroni et al. (8) obtained similar results with *S. typhosa* endotoxin, but got only low-level sensitization with large doses (1 mg) of *B. pertussis* endotoxin. It has been our experience too that, when histamine sensitivity is tested 4 days after endotoxin administration, there is usually no sensitivity, although an erratic and low-level sensitivity does occur infrequently.

Semipurified preparations of the factor from *B. pertussis*, which we have named pertussigen, when given intravenously (i.v.) to mice, produced lethal sensitivity to 1 mg of histamine 90 min later (7). (A unified name, "pertussigen," has been proposed for the factor in *B. pertussis* cells that produces a variety of biological effects, i.e., for histamine-sensitizing factor, lymphocyte-promoting factor, adjuvant factor, factor that promotes development of hyperacute experimental allergic encephalomyelitis, etc. [J. J. Munoz, Fed. Proc. 35:813, 1976]. We believe that there is adequate proof that all of these effects are produced by a single substance from *B. pertussis* cells, and the name pertussigen will be used in this paper to refer to the substance from these cells that increases the sensitivity of mice to histamine.) It was assumed that the active principle was the same as that which produces histamine sensitivity 4 days after i.p. administration and that the i.v. administration merely provided a more rapid distribution of the factor to the reactive tissues in the mouse. Subsequent work revealed that those crude preparations of pertussigen which produced histamine sensitivity 90 min after i.v. injection were still active after heating at 80°C for 30 min (unpublished observations). This observation raised the question of whether the early development of histamine sensitivity after i.v. administration of pertussigen might be mainly due to endotoxin contamination. We report here our studies on the capability of endotoxins to induce histamine sensitivity in mice within 1 to 2 h after i.v. administration.

MATERIALS AND METHODS

Mice. CFW male and female mice reared in our laboratory were used at 6 to 7 weeks of age. Males from different hierarchical groups were not mixed. Mice were housed in glass jars in groups of five on wood shavings and allowed food (Purina Laboratory Chow) and water ad lib.

Endotoxin preparations. Endotoxin from *B. pertussis* 04965, agglutinogen type 1, was obtained by the trichloroacetic acid method as described by Kabat (3), and *B. pertussis* 3779 BL_2S_4, agglutinogen type 1, 3, 6, was extracted by either the trichloroacetic acid method (3) or the phenol-water method of Westphal (10). *E. coli* 180 and *Salmonella enteritidis* 389 endotoxins, supplied by K. Milner, had been prepared by the phenol-water (10) and aqueous

ether (9) methods, respectively. The endotoxin preparations were made up at the desired concentration in physiological saline, and doses were given i.v. in 0.2-ml volumes.

Pertussigen. An alkaline saline extract of acetone-dried *B. pertussis* cells was made as previously described (6). Doses were made up at the desired concentration in physiological saline and administered i.v.

Histamine and serotonin challenges. The histamine sensitivity of endotoxin-treated mice was tested by administering 0.5 mg of histamine base i.p. (given as histamine diphosphate, Sigma) in 0.2 ml of physiological saline at various intervals after administration of endotoxin. Serotonin was given as 5-hydroxytryptamine creatinine sulfate (Sigma), but doses are expressed as the base and were given in 0.2 ml of saline i.p.

Catecholamine treatment. L-Epinephrine, DL-arterenol-hydrochloride (Sigma) and isoproterenol-hydrochloride (Winthrop) were dissolved at appropriate concentrations in a vitamin C-physiological saline solution (1 mg of ascorbic acid per ml of saline). Two or three drops of 2 N HCl were added to the L-epinephrine solution to promote solubility. Doses are expressed as the amine base and were given i.v. in 0.2-ml volumes.

Antisera. Two antisera were used to determine whether the histamine-sensitizing activity in the endotoxin preparations could be neutralized. One was a pool of rabbit sera obtained from rabbits that had been immunized with an extract from *B. pertussis* cells and known to contain precipitins against *B. pertussis* endotoxin. The other antiserum was a pool of sera from 20 rabbits that had been immunized with *S. enteritidis* and had a high titer of antibodies to *S. enteritidis* endotoxin.

RESULTS

Histamine sensitization. After preliminary experiments had shown that small i.v. doses of endotoxin induced a high degree of susceptibility to a histamine challenge given i.p. 90 min later, more extensive experiments were performed with different preparations and doses of endotoxin. The results are summarized in Table 1. The 50% sensitizing dose (SD_{50}) for the various endotoxins ranged from 0.007 μg for *B. pertussis* 3779 BL_2S_4 endotoxin (phenol-water extract) to 0.146 μg for *B. pertussis* 04965 endotoxin (trichloroacetic acid extract). Table 1 shows that there was frequently a poor dose-response relationship in the induction of increased histamine sensitivity by endotoxin. This was further exemplified by measuring histamine mean lethal dose (LD_{50}) values in four groups of mice that received fourfold increasing doses of *S. enteritidis* endotoxin. Table 2 shows that although the endotoxin dose increased 64-fold, there was little, if any, change in the histamine LD_{50} values.

Transitory nature of endotoxin-induced histamine hypersensitivity. An experiment was performed to examine the histamine sensitivity at various intervals after i.v. administration of endotoxin, heat-inactivated crude pertussigen (80°C for 0.5 h), and unheated crude pertussigen. As shown in Fig. 1, the enhanced sensitivity produced by 0.125 μg of *S. enteritidis* endotoxin or 20 μg of heated pertussigen was transitory and lasted only 2 to 4 h. Figure 1 also shows that the histamine hypersensitivity produced by 20 μg of crude unheated pertussigen is probably due to both the transitory effects of an endotoxin contaminant and the longer-lasting hypersensitivity induced by pertussigen. That pertussigen can produce its effects within 2 h is shown in Fig. 2. The dose of crude pertussigen was reduced to 2.5 μg; at this dose, the endotoxin contaminant was too weak to produce any significant histamine hypersensitivity, as shown by the response to the heated crude pertussigen, but the unheated material produced significant sensitivity 2 h after administration. However, at this low dose level the response became biphasic, and there was a decline to an unsensitized state for several hours and then a rise to increased sensitivity at 4 days (Fig. 2).

Age of mice and susceptibility. The development of endotoxin-induced histamine hypersensitivity was also influenced by the age of the mice (Fig. 3). Male mice of different ages received 0.125 μg of *S. enteritidis* endotoxin and were challenged i.p. 90 min later with 0.5 mg of histamine. It can be seen that 3- to 4-week-old mice did not develop a susceptibility to histamine, 5- to 6-week-old mice had an intermediate response, and 7-week-old mice were highly susceptible.

Serotonin and serotonin-histamine sensitivity. Although the ability of endotoxin to induce histamine sensitivity in mice was striking, it did not have the same enhancing effect on serotonin sensitivity or on sensitivity to a combination of histamine and serotonin. We found no increase in sensitivity to serotonin given alone after endotoxin treatment and only about a twofold increase when a combination of serotonin and histamine was given. The LD_{50} decreased from about 0.05 mg of serotonin plus 0.2 mg of histamine for normal mice to about 0.027 mg of serotonin plus 0.11 mg of histamine for endotoxin-treated mice. In another strain of mice reared in our laboratory (RML strain), endotoxin had little, if any, ability to induce either histamine or histamine-serotonin sensitivity.

Protection afforded by catecholamines. As shown in Table 3, i.v. administration of 2.5 to 5.0 μg of L-epinephrine immediately after the

TABLE 1. *Histamine sensitivity in mice 90 min after receiving various doses of endotoxin*

Sex	Source of endotoxin	Dose of endotoxin (μg)	D/T[a]	SD_{50}[b] (μg)
F	*B. pertussis* 04965, trichloroacetic acid and ethanol extract	0.25	5/10	0.146
		0.125	6/10	
		0.0625	1/10	
		None	0/10	
F	*B. pertussis* 3779 BL_2S_4, trichloroacetic acid and ethanol extract	0.125	7/10	0.042
		0.0625	13/20	
		0.0311	4/10	
		0.0156	4/10	
		0.0078	0/10	
		None	1/20	
M	*B. pertussis* 3779 BL_2S_4, trichloroacetic acid and ethanol extract	0.0625	6/10	0.027
		0.0311	5/10	
		0.0156	5/10	
		0.0078	1/10	
		None	0/10	
F	*B. pertussis* 3779 BL_2S_4, phenol-water extract	0.0311	10/10	0.007
		0.0156	14/15	
		0.0078	5/10	
		0.0039	3/10	
		None	0/10	
M	*B. pertussis*, 3779 BL_2S_4, phenol-water extract	0.5	9/10	0.035
		0.125	8/10	
		0.0311	5/10	
		0.0078	2/10	
		None	0/10	
M	*S. enteritidis* 389	0.0625	7/10	0.011
		0.0311	6/10	
		0.0156	7/10	
		0.0078	7/10	
		None	1/10	
M	*E. coli* 180	0.0625	8/10	0.018
		0.0311	4/10	
		0.0156	5/10	
		0.0078	5/10	
		0.0039	1/10	
		None	0/10	

[a] Deaths per total number of animals tested after a challenge with 0.5 mg of histamine.
[b] Calculated dose of endotoxin that would sensitize 50% of the mice to the lethal effects of 0.5 mg of histamine.

TABLE 2. *Histamine LD_{50} values in mice 90 min after receiving different doses of S. enteritidis endotoxin*

Dose of endotoxin (μg)	Histamine LD_{50} (mg)
None	>20
0.0078	0.74
0.0312	0.75
0.1250	0.41
0.5000	0.61

histamine challenge produced significant protection of endotoxin-treated mice. Administration of DL-arterenol or isoproterenol did not produce any significant protection.

Attempts to neutralize histamine-sensitizing activity in endotoxins with antisera. Antisera and their corresponding endotoxins were mixed at equivalent ratios and incubated in the cold overnight. On the following day, the immune precipitate was collected by centrifugation. Half of the precipitate was resuspended in saline and the other half was dissociated in 0.25 M acetic acid. After the immune precipitate was dissociated, a series of fivefold dilutions was made in saline and administered immediately i.p. to mice to obviate reassociation of endotoxin and antibody. As shown in Table 4, the supernatant fluid had very little ability to induce histamine hypersensitivity and the immune precipitate had about an eightfold decrease in its activity, but full activity was restored to the immune precipitate when the antigen-antibody complex was dissociated in 0.25 M acetic acid.

DISCUSSION

The characteristics of the endotoxin-induced histamine hypersensitivity reported here are

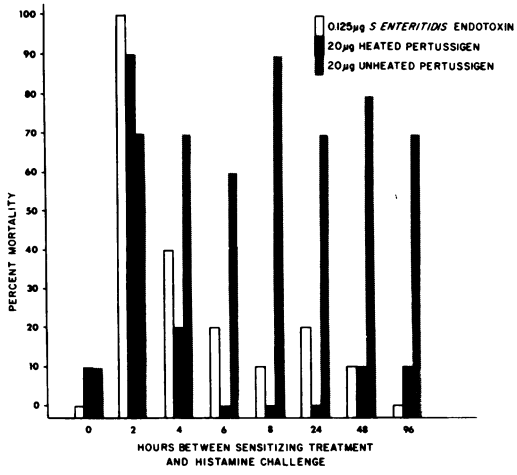

FIG. 1. *Enhanced histamine sensitivity in mice at various intervals after i.v. administration of 0.125 μg of S. enteritidis endotoxin, 20 μg of heated crude pertussigen, or 20 μg of unheated crude pertussigen. Histamine challenge (0.5 mg) was given i.p.*

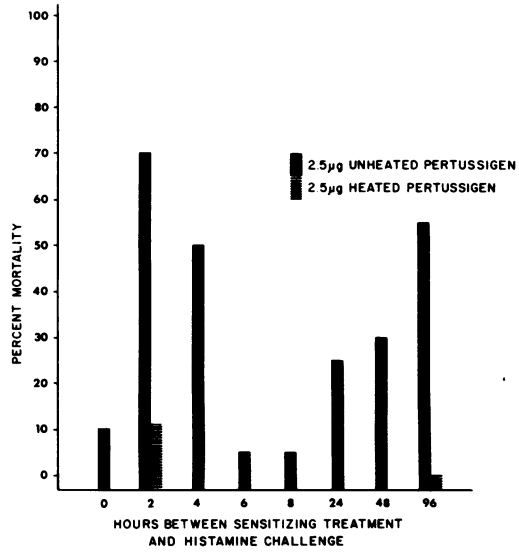

FIG. 2. *Biphasic development of increased histamine sensitivity in mice receiving 2.5 μg of crude pertussigen i.v. Histamine challenge (0.5 mg) was given i.p.*

different from those previously reported by other workers (5, 8). This may primarily be due to the fact that we found histamine hypersensitivity much sooner after giving endotoxin than reported previously (5, 8). Whereas the previous studies required 100 to 1,000 μg of endotoxin to produce moderate sensitivity (sensitivity tested 4 days after giving endotoxin) (5, 8), our results were obtained with doses of tenths and even hundredths of a microgram (sensitivity tested 90 min after giving endotoxin). The elevated sensitivity was transitory and after it reached its maximum level in 1 to 2 h, it disappeared a few hours thereafter. At the time the sensitivity to histamine was measured by Malkiel and Hargis (5) and Pieroni et al. (8), our preparations of endotoxin in the doses given had no detectable activity.

Although treatment with either pertussigen or endotoxin induces a striking hypersensitivity to histamine in mice, there are some distinct differences in the character of these two induced hypersensitivities. Pieroni et al. (8) observed the following difference in sensitivity produced by endotoxin as compared with that

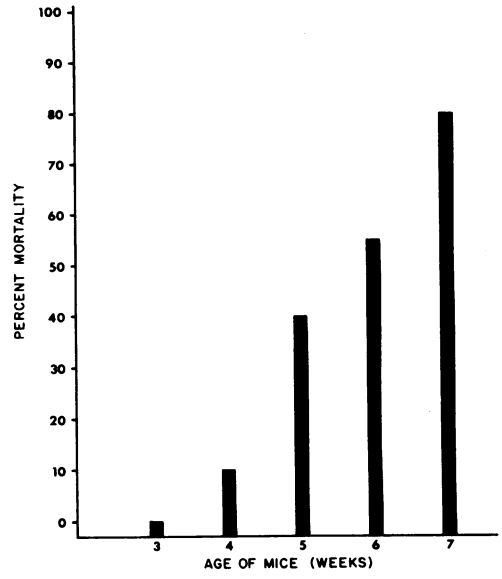

FIG. 3. *Effect of age on susceptibility of mice to endotoxin-induced histamine sensitivity. Mice received i.v. 0.125 μg of endotoxin from S. enteritidis 389 and were challenged 90 min later with 0.5 mg of histamine i.p. (Data from two experiments, 20 mice for each age group.)*

TABLE 3. *Catecholamine protection against histamine toxicity in mice treated with endotoxin (0.125 μg per mouse) from B. pertussis 3779 BL_2S_4*[a]

Catecholamine	Amt given (μg)	D/T[b]
L-Epinephrine	2.5	2/10
L-Epinephrine	5.0	0/10
DL-Arterenol	5.0	7/10
Isoproterenol	2.5	8/10
Isoproterenol	5.0	6/10
Isoproterenol	10.0	13/20
None		20/30

[a] Data from three separate experiments.
[b] Deaths per number of mice tested.

TABLE 4. *Effect of antiserum on histamine-sensitizing activity in SE-389 endotoxin*

Fraction tested	Calculated SD_{50} per ml	% of activity in starting material
Endotoxin in saline (1,000 μg/ml)	12,500	100
Supernatant fluid after centrifuging endotoxin-antiserum complex	<8	<0.1
Resuspended precipitate after centrifuging endotoxin-antiserum complex	1,495	12
Disassociated precipitate in 0.25 M acetic acid[a]	17,482	140

[a] Control mice given 0.2 ml of 0.25 M acetic acid in saline did not develop any histamine sensitivity.

produced by histamine-sensitizing factor from *B. pertussis*: (i) there was a poor dose response with endotoxin, (ii) it was never possible to achieve 100% mortality, and (iii) the factor in endotoxin was heat stable. Our work reported here showed that endotoxin-induced histamine hypersensitivity is transitory, whereas previous studies showed that histamine hypersensitivity induced by crude pertussigen lasts several weeks (7). Although 3- to 4-week-old CFW mice did not become more susceptible to histamine after endotoxin was administered, we have previously found that these mice, at this age, do become more sensitive when pertussigen is given (R. K. Bergman and J. J. Munoz, unpublished observations).

The experiment on the effects of specific antiserum on endotoxin showed that the histamine-sensitizing activity was precipitated, but it was not completely inactivated, by being complexed with antibody. Activity was completely restored by dissociating the complex in 0.25 M acetic acid (Table 4). Since all the endotoxin was precipitated by antibody, the remaining activity must have been due either to complexed endotoxin or to endotoxin that dissociated in vivo from the antibody. Another possibility for the reduction in activity could have been due to an artifact introduced while comparing activities by making dilutions of endotoxin trapped in large aggregates (antigen-antibody complexes) and endotoxin in molecular dispersion.

Mice made hypersensitive to histamine by administration of endotoxin were protected by small doses of L-epinephrine. The protection afforded by this catecholamine against the lethal effects of histamine in the endotoxin-treated mice was similar to that reported previously in histamine-sensitizing factor-treated mice (1, 2).

The early onset of histamine hypersensitivity after administration of high doses of pertussigen (20 μg) is, no doubt, partially due to an endotoxin contaminant. It is also obvious that the sensitizing effects of a high dose of pertussigen develop in a very short time and then remain for a long time. The time course of histamine hypersensitivity after administration of a low dose of pertussigen (2.5 μg) showed that sensitivity was induced at 2 h, became undetectable at 6 to 8 h, and then became high again at 96 h. This sensitivity was not induced by an endotoxin contaminant, since the hypersensitizing effect was destroyed by heating the pertussigen at 80°C for 30 min.

The mechanism by which low doses of endotoxin produce the transitory histamine sensitivity in these studies is not known. Whether it acts by affecting a specific locus in the hypothalamus as suggested by Kass et al. (4) or perhaps is due to a more generalized toxicity on the microvasculature (11) is not certain. The fact that epinephrine was beneficial in protecting the mice from the lethal effects of histamine would argue against death being caused by a hyper-reactivity of the vascular bed to endogenously released epinephrine. We investigated the possibility that the administration of endotoxin might result in adrenal depletion of epinephrine and make the mouse unable to compensate for the hypotensive effects of histamine given an hour or two later. However, adrenal catecholamines levels in mice, measured 2 h after administering 0.5 μg of endotoxin, were not lower than in control mice. Whatever the effect of the endotoxin was in these experiments, it apparently did not produce any permanent damage and the mice were able to overcome the effects within 4 to 6 h.

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