

# Influence of Bacterial Endotoxins on Basophil Histamine Release

## Potentialiation of Antigen- and Bacteria-Induced Histamine Release

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The histamine-releasing capability of lipopolysaccharides (LPS) was examined in human leukocyte suspensions. LPS alone did not release histamine, but was found to enhance the histamine release caused by anti-IgE. Also the IgE-mediated histamine release caused by specific antigens (allergens or bacteria) in sensitized individuals was enhanced by LPS. The potentiating effect of LPS was observed in grass pollen and dog dander allergic patients as well as in patients sensitized to *E. coli* or *Staph. aureus* bacteria. No potentiation was obtained by exposure to unspecific allergens or bacteria to which the persons were not sensitized. Bacteria can release histamine by immunological or non-immunological mechanisms, and only the immunological histamine release was found to be potentiated by LPS. It is speculated that endotoxins reinforce release of histamine caused by allergens in allergic patients or by bacteria in persons sensitized to these microorganisms.

**Key words:** allergens; allergic; anti-IgE; asthma; bacteria; basophil leukocytes; endotoxins; histamine release; intrinsic asthma; lipopolysaccharides; non-immunological; potentiation.

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In our previous studies we found that several species of bacteria caused release of histamine from human basophil leukocytes *in vitro* and it is speculated that this release could be a pathogenic mechanism in intrinsic asthma (IA) and infectious diseases (4, 6, 11). These studies were based on both whole bacteria (2, 11) and bacterial ultrasonicates (6), and the histamine-releasing capability was more pronounced in preparations of ultrasonicates than in suspensions of whole formalin-killed bacteria. Liberation of bacterial endotoxins from gram-negative bacteria might explain why sonicated bacteria were more active than whole bacteria. In the present study, therefore, we have examined a histamine-liberating capability of endotoxins

and the influence of these bacterial products on histamine release induced by bacteria, allergens, and anti-IgE.

## MATERIAL AND METHODS

### Subjects

The study comprised three patients with *E. coli* bacteraemia, with fever and chill where *E. coli* was cultured from blood and urine, six normal individuals, and six patients with a well-documented history of symptoms of grass pollen or dog dander hay fever allergy showing a positive skin prick test and positive basophil histamine release to these allergens. Furthermore, six

children, aged 9 to 16 years, with moderate to severe intrinsic asthma (IA) were included according to the criteria previously described (6).

#### Endotoxins

*Salmonella* and *E. coli* lipopolysaccharide (LPS) preparations of the Boivin (B) and Westphal (W) type were supplied by Difco Laboratories, Detroit, Mich. *Salmonella* LPS contains a mixture of LPS isolated from *S. abortus equi*, *S. thyphimurium*, *S. thyphi* and *S. enteritidis*, and *E. coli* LPS from *E. coli* 0111: B4, 026: B6, 0128: B12, 0127: B8 and 055: B5. Purified LPS from the heptose-deficient mutant *Salmonella minnesota* R595 (*S. minnesota* R595 LPS), which represents a polysaccharide-poor LPS, was kindly donated by Morrison (16).

#### Bacteria

The bacterial species and strains included whole formalin-killed *Staph. aureus* Wood 46 (protein A-free), *Staph. epidermidis* A1271/76, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, and *E. coli* EPEC strains (a mixture of Stoke W 0111: H-; Aberdeen 1064 055: H6; F41 026: H-; 4932-53 0127: H-, and Cigleris 0128: H2).

All bacteria were cultured in a fluid Truche medium, killed by formalin (1.5%) in saline, washed six times, thereafter resuspended in saline and adjusted to 100 mg/ml wet weight as described by Espersen et al. (2).

#### Allergens and anti-IgE

Extracts of *Phleum pratense* and dog dander were supplied by Pharmacia, Sweden in stock solutions of 1000 BU/ml and anti-IgE (300,000 IU/ml) was from Behringwerke, Germany.

#### Histamine release

Human leukocyte suspensions containing approx. 2% basophilocytes obtained by Ficoll-Hypaque gradient centrifugation were washed twice and suspended in a glucose-free Tris-AMC buffer containing 25 mM Tris at pH 7.6,

0.12 M NaCl, 5 mM KCl, 0.6 mM  $\text{CaCl}_2$ , 1.1 mM  $\text{MgCl}_2$ , and 0.3 mg of human serum albumin/ml (14). The use of washed cells in the absence of serum excludes the possibility of histamine release by complement activation. Histamine release caused by LPS (final concentrations from 1 pg to 100  $\mu\text{g/ml}$ ), anti-IgE (3–30 IU/ml), grass pollen (1–3 BU/ml), dog dander (0.1 BU/ml) or bacteria (*Staph. aureus*, *Staph. epidermidis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *E. coli* bacteria, all in 20 mg/ml wet weight) was performed by incubation of 60  $\mu\text{l}$  of cell suspension for 40 min at 37°C with 20  $\mu\text{l}$  of the undiluted or diluted preparations and 20  $\mu\text{l}$  Tris-AMC. In the potentiation experiments the cells (60  $\mu\text{l}$ ) were incubated with LPS (20  $\mu\text{l}$ ) for 10 min at 37°C. Twenty  $\mu\text{l}$  of anti-IgE, allergen or bacteria was thereafter added to trigger histamine release, and the mixture was incubated for 40 min at 37°C. The agents were omitted for spontaneous histamine release and for determination of the total histamine content; in the latter case the samples were left at 4°C. Thereafter, 3 ml of ice-cold Tris-AMC was added to all of the samples, and they were centrifuged at  $2,000 \times g$  for 10 min. In addition to released histamine, the supernatant also contains protein, the test agents and other substances which interfere with histamine analysis. The supernatant was therefore discarded, and the residual histamine was determined in the cell sediment by the spectrofluorometrical OPT method (14). The release of histamine was expressed as the percentage of the total histamine content of the sample. In the absence of agents the spontaneous histamine release amounted to 3 to 10% in all experiments. Therefore, it should be noted that only release of >10% is considered significant.

To examine whether the histamine release was caused by type I allergic reactions or non-immunologic mechanisms, or to examine the importance of IgE-mediated reactions, cell-bound IgE (and IgG if present) were removed from the basophil leukocytes by exposure of the cell suspension to pH 3.8 for 5 min at 4°C (15) before challenge of the cells with allergen or bacteria. The removal of these immuno-

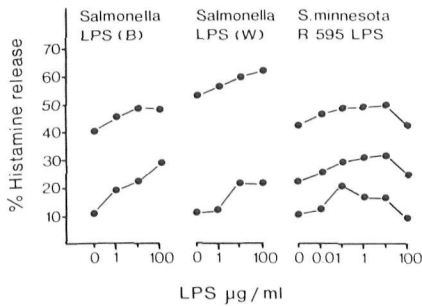


Fig. 1. Potentiation of anti-IgE-induced histamine release by *Salmonella* LPS of the Boivin (B) and Westphal (W) type and *S. minnesota* R595 LPS in normal individuals. LPS was examined in 10-fold increasing concentrations. Each experiment was performed with two or three different concentrations of anti-IgE (3, 10 and 30 IU/ml). Each point represents the mean of three determinations.

globulins was controlled functionally by the absence of histamine release induced by anti-IgE and anti-IgG. It should be noted that no IgG was found on the intact cells in these experiments.

## RESULTS

### Direct effect of endotoxin

A direct histamine-liberating capability of LPS was examined on human leukocytes. Incubation of the leukocytes for 40 min at 37°C with *Salmonella* LPS (B and W) in 10-fold increasing concentrations from 1 pg to 100 µg/ml did not release histamine from the basophil leukocytes in the six normal individuals and the five grass pollen-allergic patients examined, and the same was true for *S. minnesota* R595 LPS (data not shown).

### Potentiation of anti-IgE-induced histamine release

To examine whether these compounds might change histamine release caused by anti-IgE, the leukocytes were preincubated with LPS (10 min at 37°C) and then incubated with anti-IgE (40 min at 37°C). Fig. 1 shows representative examples from five normal individuals. Anti-IgE-induced histamine release was enhanced by *Salmonella* LPS (B and W) and *S. minnesota* R595 LPS in the range of approx.  $10^{-5}$  ng/ml to

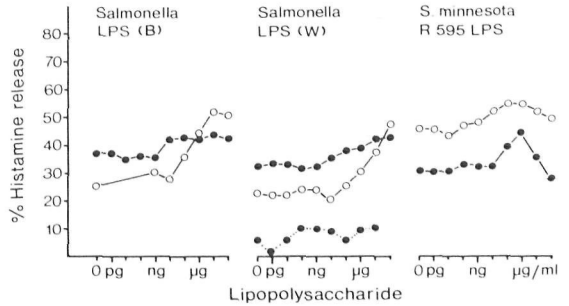


Fig. 2. Potentiation of antigen-induced histamine release by LPS in allergic patients. LPS was examined in 10-fold increasing concentrations from 1 pg – 100 µg/ml. Challenge with grass pollen (○—○) and dog dander (●—●). Control experiments with cells deprived of IgE (●—●).

100 µg/ml and the potentiating effect was not changed by washing of the cells after the treatment with LPS and before the incubation with anti-IgE. It seems that no important difference was obtained in the potentiating effect between the three types of LPS.

### Potentiation of antigen-induced histamine release

Potentiation of antigen-induced histamine release was examined in five patients allergic to grass pollen and/or dog dander, and representative examples are shown in Fig. 2. When the leukocytes were preincubated with LPS and thereafter incubated with specific antigen, a potentiating effect was observed in the range of approx. 10 ng to 100 µg/ml of LPS in each of the experiments. The effect was maximal at relatively high concentrations and was often decreased by higher concentrations. The preincubation time of 10 min ensured a maximal potentiating effect and the potentiation was not changed by washing of the cells after the treatment with LPS and before the challenge with antigen.

No potentiation was obtained when cells from dog dander-allergic persons were exposed to unspecific antigens as grass pollen or mite extract, when cells from normal individuals were challenged with allergens or when IgE was removed from the cell surface in the allergic patient before the experiment (Fig. 2).

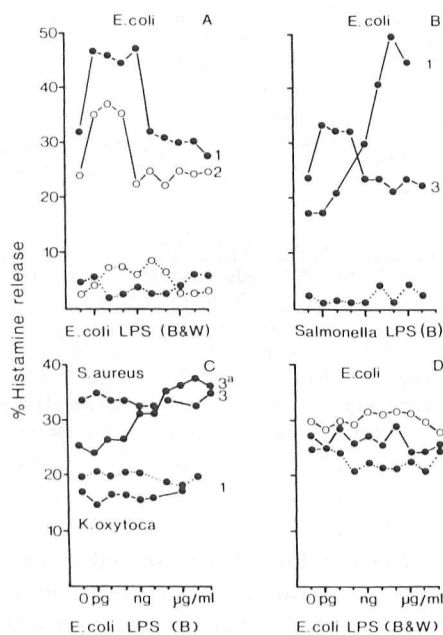


Fig. 3. Potentiation by LPS of *E. coli*-induced histamine release in three patients (1-3) sensitized to these bacteria. LPS was examined in 10-fold increasing concentrations from 1 pg - 100 µg/ml. *E. coli* or *Salmonella* LPS of the Boivin type (●—●) or Westphal (○—○). Control experiments with cells deprived of IgE (●—●; ○—○). Experiments with *E. coli*-infected patients (A-C) and with normal individuals (D). Note that patient No. 3 also became sensitized to *Staph. aureus* (C, 3<sup>a</sup>).

#### *Influence on the immunological and non-immunological histamine release caused by bacteria*

The influence of LPS on bacteria-induced histamine release was examined in patients sensitized to bacteria and in persons not sensitized to bacteria. Fig. 3A shows *E. coli*-infected patients who responded to *E. coli* with an IgE-dependent histamine release, since only intact cells but not cells deprived of IgE released histamine. Preincubation of the intact cells with *E. coli* LPS (B and W) caused an enhancement in the immunological histamine release induced by the subsequent incubation with *E. coli* (Fig. 3A). The potentiation was obtained in the range of approx. 1 to 100 pg/ml and disappeared at higher concentrations. A similar potentiation was obtained when *E. coli* LPS was replaced by *Salmonella* LPS (B) (Fig. 3B). The LPS preparations alone did not release his-

tamine. No potentiation was found in cells deprived of IgE (Fig. 3A and B).

Fig. 3C shows experiments where the cells were challenged with *Staph. aureus* and *Klebsiella oxytoca* instead of *E. coli*. These bacteria caused a similar release of histamine in intact cells and cells deprived of IgE, indicating a non-immunological release mechanism, i.e. that the *E. coli*-infected patients were not sensitized to these species. *E. coli* LPS (B) did not potentiate the non-immunological histamine release caused by these bacteria. However, 2 weeks later patient No. 3 became sensitized to *Staph. aureus*, since this species now released histamine in intact cells but not in IgE-deprived cells, and endotracheal suction showed purulent secretion with significant growth of the microorganisms. LPS was found to potentiate the immunological histamine release caused by *Staph. aureus* (Fig. 3C, 3<sup>a</sup>). When cells from normal individuals were examined no potentiation was found by *E. coli* LPS in *E. coli*-induced histamine release (Fig. 3D). The absence of potentiation in persons not sensitized to the relevant bacteria was also demonstrated in histamine release caused by other species such as *Staph. aureus*, *Staph. epidermidis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* (data not shown). These experiments included normal persons, IA patients and allergic patients, and *Salmonella* LPS did not potentiate the bacteria-induced histamine release, since no significant difference was obtained in the presence or absence of LPS ( $n = 6$ ;  $P < 0.01$  by Student's t-test).

#### DISCUSSION

Previously we found that several species of bacteria caused release of histamine from basophil leukocytes *in vitro*. In the case of gram-negative bacteria, it is possible that bacterial endotoxins could be involved in the bacteria-induced histamine release, since the histamine-releasing capability was more pronounced in bacterial ultrasonicates (6) than in suspensions of whole bacteria (2, 11). Endotoxins constitute an integral component of the outer membrane of gram-negative bacteria. They exert multiple biologic effects on coagulation and complement,

on platelets, polymorphonuclear leukocytes and endothelial cells, and especially on macrophages/monocytes (8). Chemically, endotoxins consist of lipopolysaccharide-protein complexes composed of polysaccharide, lipid A, and lipid A-associated protein (8, 12, 18). Most of the endotoxic effects are due to LPS and, in most instances, also to lipid A (8, 18). We therefore examined LPS from *Salmonella* and *E. coli* of both the Boivin and Westphal type (8), and in substitution of lipid A we used a polysaccharide-poor LPS (*S. minnesota* R595 LPS). These compounds were tested in a wide range from 1 pg/ml to 100 µg/ml, since pyrogenic effect is observed in man in the range of pg to ng/kg body weight (19) and *in vitro* effects on cell functions have been found in the range of pg to 1000 µg/ml (1, 3, 8, 17).

LPS alone did not release histamine, either in normal individuals or in allergic patients or patients with intrinsic asthma. The absence of a direct effect is in accordance with Smith et al. (16) in their study of the polysaccharide-poor LPS in normal persons. However, LPS enhanced the histamine release caused by anti-IgE in normal individuals and by specific antigen in allergic patients. The potentiation was observed by all three types of *Salmonella* LPS, which confirms the study of the polysaccharide-poor LPS (16). It was more pronounced when the histamine release was low and occurred in the range of 10 ng/ml to 100 µg/ml.

We have previously found that bacteria can trigger histamine release by two different mechanisms, an immunological (IgE-dependent) and a non-immunological mechanism (4, 10). A differentiation must therefore be made between persons who are sensitized and who are *not* sensitized to bacteria. In the first category the bacterium (antigen) induces histamine release in the intact leukocytes containing basophilocytes armed with specific IgE, but no release is observed in cells deprived of IgE (4, 10). In the second category intact and IgE-deprived cells show a similar release of histamine (4, 10).

LPS enhanced the immunological histamine release caused by *E. coli* and *Staph. aureus* in persons sensitized to these bacteria (Fig. 3).

The potentiating effect of LPS was not species-specific, since *Salmonella* LPS like *E. coli* LPS enhanced the histamine release caused by *E. coli* bacteria. The enhancement of *Staph. aureus*-induced histamine release by *E. coli* LPS indicates that LPS also potentiates the immunological histamine release by gram-positive bacteria.

In persons not sensitized to the relevant bacteria we found no potentiation by LPS in the non-immunological histamine release caused by bacteria. These persons included normal individuals challenged with *E. coli* bacteria (Fig. 3D), or with other species (data not shown) and allergic patients and patients with intrinsic asthma who were not sensitized to these species (data not shown). The lack of potentiation was also observed when the *E. coli*-infected patients were challenged with *Staph. aureus* or *K. oxytoca* to which species they were not sensitized (Fig. 3C, 1 and 3). A non-immunological release of histamine is also triggered by other events than bacteria stimulation, for instance, by Concanavalin A and the calcium ionophore A23187 (5, 13), and neither was this release potentiated by LPS (unpublished results).

Our experiments suggest that immunological histamine release is enhanced by LPS, whereas this does not seem to be the case in histamine release caused by non-immunological reactions. That only specific IgE-mediated histamine release is potentiated by LPS is supported by the lack of potentiation in cells challenged with unspecific allergens or bacteria to which the person was not sensitized. Furthermore, cells deprived of IgE and intact cells from normal individuals showed no potentiation. On the basis of our findings it is tempting to speculate that endotoxins reinforce release of histamine caused by allergens in allergic patients or by bacteria in persons sensitized to these micro-organisms, since it is well known that allergic symptoms are increased during infections.

The mechanism of the potentiation is unknown. However, the enhancement in histamine release was not changed by washing of the cells after the treatment with LPS and before the challenge with antigens, which indi-

cates a binding of LPS to the cell membrane, an insertion known in B lymphocytes and platelets (7, 9).

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