

Endotoxin from *Haemophilus influenzae* enhances IgE-mediated and non-immunological histamine release

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Haemophilus influenza and its extracellular products (EP) did not release histamine from basophil leukocytes in cell suspensions from normal individuals, patients with chronic bronchitis or patients allergic to either house dust mite, grass pollen, cat dander or to their own bacteria. However, the EP was found to enhance their basophil histamine release. IgE-mediated histamine release was examined by stimulation of the cells with anti-IgE or the specific allergens, and non-immunological histamine release by stimulating the cells with the calcium ionophore A23187 or *Staphylococcus aureus*. In all the experiments EP caused a significant increase in the histamine release. When *H. influenzae* endotoxins were removed from the EP, the potentiating effect of EP was completely abolished, whereas heating (80°C, 30 min) or treatment of EP with proteinase did not influence the potentiating effect. These results indicate that *H. influenzae* endotoxin potentiates histamine release caused by IgE-mediated reactions or by non-immunological mechanisms.

Key words: allergens; bacteria; basophils; chronic bronchitis; endotoxin; *H. influenzae*; histamine release; Type I allergy.

Accepted for publication 12 July 1989

CLINICAL ASPECTS

H. influenzae is suspected of playing a role in acute exacerbations of chronic obstructive pulmonary disease (COPD). Since several bacteria have the capability to induce release of mediators or to enhance the histamine release, these effects have been suspected of being of importance for triggering or aggravating respiratory attacks in airway diseases. Our results indicate that *H. influenzae* endotoxin enhances mediator release and this effect might contribute to the acute exacerbations in COPD.

Haemophilus influenzae is the bacterial species most often isolated from the sputum of patients with chronic obstructive pulmonary disease, and the symptoms associated with the isolation of the bacterium include dyspnoea, increased sputum purulence and wheeze (16). Furthermore, inhalation of *H. influenzae* as well as its endotoxin causes bronchial obstruction (26).

Bacteria-induced mediator release is suggested to play a role in airway diseases, since bacteria have been found to trigger release of histamine from human lung mast cells and basophils or to enhance the mediator release (3, 5, 13) and bacterial products as endotoxins also share some of these properties (13). Therefore, in this study we examined the capability of *H. influen-*

zae and its extracellular products to trigger and enhance histamine release from human basophils.

MATERIAL AND METHODS

Subjects

The study comprised five patients (age 21 to 56 years) with bronchial asthma and with a well-documented history of allergy to either house dust mite, grass pollen or cat dander. All patients had a positive skin prick test and positive basophil histamine release to these allergens, and their asthma was controlled by local β_2 -agonists and/or local steroids. The study also included a 60-year-old woman suffering from sepsis with *Salmonella enteritidis* who was sensitized to this bacterium.

Further, six patients (age 54 to 78 years) with chronic bronchitis showing excessive sputum production for more than 10 years were included. All had impaired pulmonary function with a forced expiratory volume in 1 s below 50% of the expected value, showing no improvement after bronchodilators or steroids.

Also 11 normal individuals (age 22 to 65 years) were included.

Material

Non-capsulated (non-typeable) *H. influenzae* biotype II strain (HK 300) was isolated from a patient with chronic bronchitis. The bacteria were cultured on the surface of dialysis membranes placed on chocolate agar plates (12). The dialysis membranes were thereafter washed and the bacteria separated from their extracellular products by centrifugation (12). The viable, whole bacteria were washed and adjusted to 100 mg/ml wet weight. The extracellular products (EP) consisting of a crude IgA-1 protease preparation containing endotoxin were dialysed against isotonic NaCl. In some experiments the EP was heated to 80°C for 30 min to inactivate the protease activity. In other experiments the EP was shaken with Detoxi-Gel (De-

pyrogenation Support, cross-linked 6% beaded agarose from Pierce Chemical, USA) for 2 h and centrifuged, and the endotoxin-poor supernatant was used for the experiments. Removal of endotoxin from the EP was verified by a sensitive *Limulus* test combining the *Limulus amoebocyte* lysate test with rocket immunoelectrophoresis (1). Decomposition of protease and other proteins in the EP preparation was performed by incubating the preparation with proteinase K (Sigma Chemical Company, USA) for 1 h at 37°C. To remove the proteinase K by centrifugation the enzyme was immobilized to sepharose and a possible residue was inactivated by heating for 30 min at 70°C.

Whole formalin-killed *S. aureus* strain Wood 46 (protein A-deficient) was prepared according to (6), and *Salmonella enteritidis* isolated from the blood of a patient suffering from sepsis with this bacterium was prepared in a similar manner. The following allergens were used: house dust mite (*Dermatophagoides pteronyssinus*), grass pollen (*Phleum pratense*), and cat dander, all Soluprick-SQ 3HEP from ALK, Denmark. Rabbit-anti-human IgE (Σ -chain), 400,000 IU/ml, was from Behringwerke AG, FRG, the calcium ionophore A23187 was obtained from Calbiochem AG, Switzerland.

Preparation of cell suspension

From each person blood was drawn by venipuncture, anticoagulated with EDTA in a final concentration of 0.01 M and mixed with double volume of 0.9% NaCl. Leukocytes with approx. 2% basophilocytes were obtained by Ficoll-Hypaque gradient centrifugation (21), 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 CaCl_2 , 1.1 mM MgCl_2 , and 0.3 mg of human serum albumin/ml (21).

To examine whether the histamine release induced by *S. aureus* or *Salmonella enteritidis* was related to an immunological (IgE-dependent) or a non-immunological reaction, cell bound IgE was removed from the basophil leukocytes by exposure of the cell suspension to pH 3.8 for 5

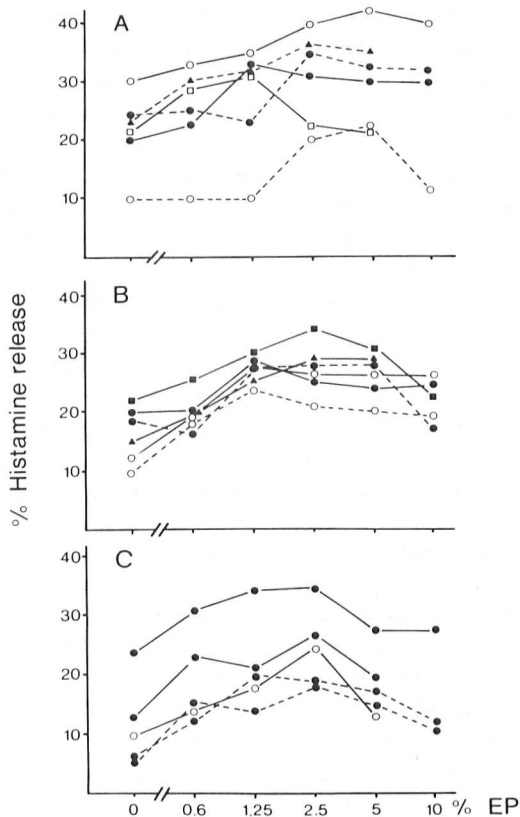


Fig. 1. Enhancement of IgE-mediated histamine release by the extracellular products (EP) from *H. influenzae* in leukocyte suspensions from (A) 6 normal individuals, (B) 6 patients with chronic bronchitis challenged with anti-IgE, and (C) 5 patients allergic to either house dust mite (●—●), grass pollen (○—○) or cat dander (●---●) challenged with specific antigens. Duplicate determinations.

min at 4°C (22) before challenge of the cells with these bacteria. The removal of IgE-immunoglobulin was controlled functionally by the absence of histamine release after challenge of the cells with anti-IgE.

Histamine release

Histamine release experiments were performed by incubation of 40 µl leukocyte suspension for 40 min at 37°C with µl Tris-AMC buffer and 5 µl of diluted or undiluted preparations of *S. aureus*, *Salmonella enteritidis*, *H. influenzae* or its

EP, anti-IgE, house dust mite, grass pollen, cat dander or the calcium ionophore A23187. The histamine release was assayed spectrofluorometrically by determination of the residual histamine in the washed cell sediment (21). In experiments including *H. influenzae* the residual histamine content of the samples was assayed according to the method described by Shore (20), since this bacterium interfered with the assay first mentioned. A positive response was defined as a release of more than 10% of the total histamine content in the sample, since the spontaneous release of histamine amounted to up to 10%.

A potentiating effect of EP on histamine release was examined by replacing the 5 µl Tris-AMC buffer by the EP preparation used in final concentrations of 0.6, 1.25, 2.5, 5.0, and 10%.

Statistics

Student's t-test was used.

RESULTS

Examination of histamine release by *H. influenzae* and its EP

The capability of *H. influenzae* and its EP to stimulate human basophil leukocytes to release histamine was examined by incubating leukocyte suspensions for 40 min at 37°C with the whole bacterium or its EP. Both preparations were used in final concentrations from 0.04 to 10% vol/vol of the preparations. No release of histamine (< 10%) was obtained either by the whole bacterium or by its EP. The lack of response was observed in cell suspensions from the normal individuals, the patients with chronic bronchitis and the allergic patients.

A combination of *H. influenzae* and its EP was examined by using equal parts of both preparations and the mixture was used in final concentrations from 0.04 to 10%. No release of histamine was obtained in cell suspensions from the categories mentioned above.

Potential of IgE-mediated histamine release by EP

To examine whether EP might change histamine release caused by anti-IgE, cells from six normal individuals were incubated with anti-IgE. Final dilutions of 10^{-6} to 10^{-4} were used to obtain a histamine release between 10 and 30%. When EP was included in the samples the mediator release was enhanced by 40 to 140% and this enhancement was significant ($P < 0.01$) when comparing samples with and without EP. These experiments are shown in Fig. 1A and it is seen that maximal enhancement of anti-IgE-induced histamine release was obtained in final concentrations of the EP preparations corresponding to the range of 0.6 to 5%.

When the experiments were performed with cells from the six patients with chronic bronchitis, EP caused a similar enhancement (45 to

133%, $P < 0.01$) in anti-IgE-induced histamine release (Fig. 1B). In the five patients with bronchial asthma, allergic to either house dust mite, grass pollen or cat dander the cells responded with histamine release to the specific antigens and this release was enhanced in the presence of EP (Fig. 1C). Maximal enhancement of 43 to 130% was obtained when using the EP preparation in final concentrations of 1.25 or 2.5% and the enhancement was significant ($P < 0.01$).

An effect on IgE-mediated histamine release caused by bacteria was examined in cell suspensions from a patient suffering from sepsis with *Salmonella enteritidis*. In final concentrations of 4, 16 and 63 µg/ml wet weight the isolated bacterium caused a histamine release of 10, 15 and 17%, respectively. The histamine release was IgE-mediated, since no release was obtained when cell-bound IgE was removed before the challenge (Fig. 2B). When EP was included in the samples, the histamine release was en-

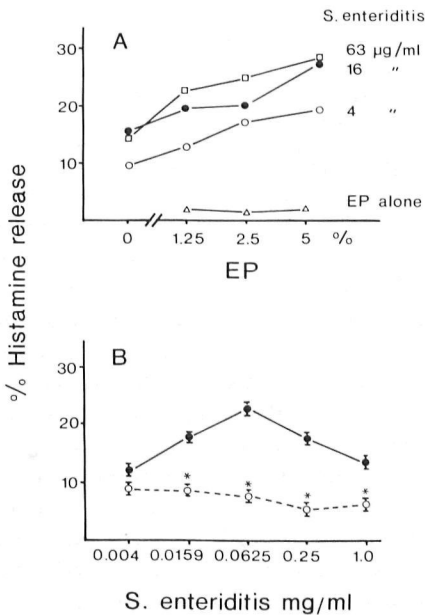


Fig. 2. (A) Enhancement of *S. enteritidis*-induced histamine release by EP from *H. influenzae* in cell suspensions from a patient suffering from sepsis with *S. enteritidis*. Duplicate determinations. (B) Challenge of intact cells (●—●) and cells deprived of IgE (○---○) with *S. enteritidis* to verify its IgE-mediated histamine release. Mean of 5 experiments $\pm$ SEM. * $P < 0.01$.

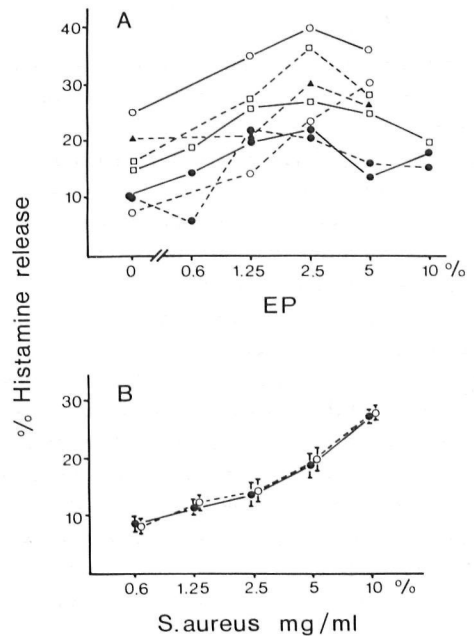


Fig. 3. (A) Enhancement of *Staph. aureus*-induced histamine release by EP from *H. influenzae* in cell suspensions from 7 normal individuals. Duplicate determinations. (B) Challenge of intact cells (●—●) and cells deprived of IgE (○---○) with *Staph. aureus* to verify its non-immunological histamine release. Mean of 7 experiments $\pm$ SEM.

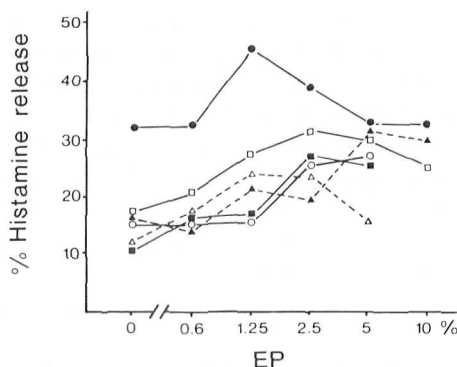


Fig. 4. Enhancement of calcium ionophore-induced histamine release by EP in cell suspensions from 6 normal individuals. Duplicate determinations.

hanced by 75 to 100 % (Fig. 2A). The enhancement was synergistic, since no release was obtained by EP itself.

Potentiation of non-immunological histamine release by EP

To elucidate whether EP could enhance non-IgE-dependent bacteria-induced histamine release, cells from seven normal individuals were stimulated with *S. aureus* to release histamine. The bacterium was used in final concentrations of 0.6 to 10 mg/ml wet weight and a similar release of histamine was obtained in intact cells and cells deprived of IgE (Fig. 3B), indicating a non-immunological release mechanism. When EP was included in the samples, the mediator release was enhanced by 50 to 200 % ($P < 0.01$) (Fig. 3A). In other experiments, cells from six normal individuals were stimulated with the calcium ionophore A23187 in final concentrations corresponding to the range of 3×10^{-7} to 3×10^{-6} M. The non-IgE-dependent mediator release was enhanced by 41 to 180 % when EP was included in the samples ($P < 0.01$) (Fig. 4).

Examination of the potentiating factor in EP

To verify that the factor in EP responsible for the potentiating effect might be endotoxin, en-

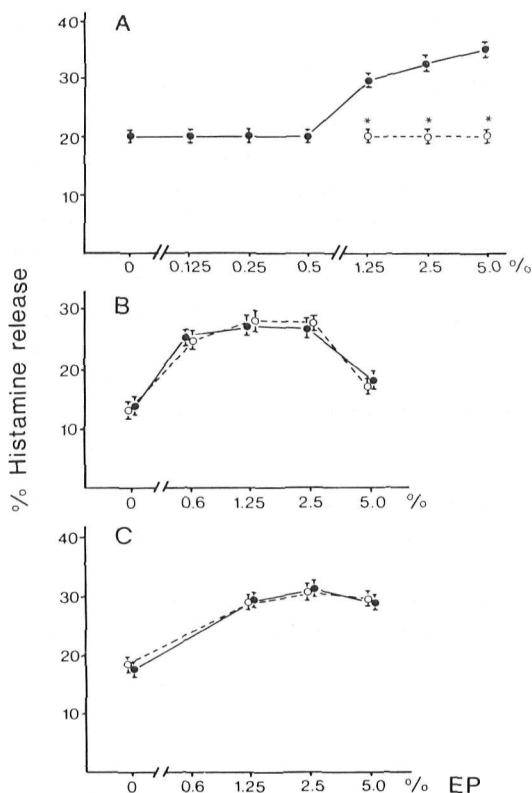


Fig. 5. Enhancement of anti-IgE-induced histamine release by EP before (●—●) and after (○---○) treatment of EP. The treatment included removal of *H. influenzae* endotoxin by Detoxi particles (A) or inactivation of protease and other proteins in EP by denaturation at 80°C (B) or disintegration by proteinase K (C). Mean of 5–6 experiments $\pm$ SEM, * $P < 0.01$.

dotoxin was removed from EP by Detoxi particles, and in experiments with cells from six normal individuals treated and untreated EP was tested in parallel for potentiating effect on anti-IgE-induced histamine release. Fig. 5A shows that the potentiating effect of EP was 75 % and that the potentiation was completely abolished by removal of endotoxin. When 0.9 % NaCl instead of EP was treated with the Detoxi particles, it did not change the potentiating effect of the untreated EP, indicating that the Detoxi treatment did not interfere. By the sensitive *Limulus* test, intact EP showed a content of 10 μ g endotoxin/ml and Detoxi-treated EP showed 1 μ g/ml. This reduction in endotoxin concentration to 1/10 fits with the cessation

of the potentiating effect of EP by dilution 1:10 (Fig. 5A).

In other experiments, EP was exposed to 80°C for 30 min and this treatment of EP did not change its potentiating effect on anti-IgE-induced histamine release. This is shown in experiments with cells from five normal individuals (Fig. 5B). By the sensitive *Limulus* test it was verified that heating to 80°C did not change the activity of endotoxin in the EP preparation. Decomposition of protease and other proteins in the EP preparation by treating EP with proteinase K at 37°C for 1 h followed by a complete removal of the proteinase K did not change the potentiating effect of EP on anti-IgE-induced histamine release. Fig. 5C shows the experiments including cells from five normals. 0.9% NaCl treated with proteinase K did not enhance anti-IgE-induced histamine release, indicating that the proteinase treatment did not interfere.

DISCUSSION

It is conceivable that *H. influenzae* plays an important role in acute exacerbations of chronic obstructive pulmonary disease (COPD). It is the bacterial species most often isolated from the sputum of patients with COPD and the non-capsulated (non-typeable) strains are often found predominantly during exacerbations (7, 8, 25). The most frequently recorded symptoms associated with isolation of *H. influenzae* were found to be cough, dyspnoea, increased sputum purulence and wheeze (16). In patients with chronic, non-specific lung disease, inhalation of *H. influenzae* caused a biphasic bronchial obstruction when bronchial hyperreactivity was evident in the patients, and the dual response was mimicked by *H. influenzae* endotoxin (26). Further, it has been suggested that bacterial endotoxins might be responsible for the symptoms including subjective breathing difficulties in humidifier disease (17, 18) and for the decrease in respiratory function in the byssinosis syndrome (2, 10, 19).

Mediator release triggered from various cells by bacteria and their extracellular products

could be responsible for the adverse respiratory effects. Several species of bacteria frequently encountered in the flora of the respiratory tract have thus been shown to cause release of histamine from human lung mast cells (3, 5) and basophil leukocytes (13), and endotoxins have been found to trigger basophil histamine release by complement activation (15) and to enhance IgE-dependent basophil histamine release (14, 23).

In this study we examined whether the non-capsulated *H. influenzae* was able to trigger histamine release from human basophil leukocytes or to change the histamine release. Both viable, whole bacteria and their extracellular products (EP) were used and neither of them were found to cause histamine release in patients or controls. The same was the case when the cells were stimulated by a combination of bacteria and EP. These results are in accordance with those of Clarke (4) who only found a marginal release induced by *H. influenzae* cell wall antigen. However, in some patients with intrinsic asthma our non-capsulated *H. influenzae* strain was found to cause an IgE-mediated histamine release, which indicates that these patients were sensitized to the type of *H. influenzae* used in this study (13).

The capability to enhance basophil histamine release was examined for the extracellular products. IgE-mediated histamine release was obtained by stimulating the cells with anti-IgE. When EP was included, the anti-IgE-induced histamine release was enhanced. The potentiating effect of EP was due to synergism, since EP *per se* did not release histamine. Similar results were obtained when the cells were derived from normal individuals or from patients with chronic bronchitis. This was also the case when IgE-mediated histamine release was caused by specific allergens in cell suspensions from allergic patients.

A non-IgE-dependent histamine release was obtained by stimulating cells from normal individuals with the calcium ionophore A23187 or by *S. aureus*. That the latter reaction was independent of IgE was verified by a similar release of histamine from intact and nude (IgE-deprived) cells. This reaction is regarded as a

sugar-lectin-mediated reaction where sugars on the bacterial cell wall interact with lectins on the basophil cell membrane leading to histamine release (13). Both types of non-IgE-mediated mediator release were enhanced by EP and the potentiating effect of EP was similar to that obtained in the IgE-mediated histamine release.

The enhancement of histamine release by the EP seems to be caused by its content of endotoxins, since removal of endotoxin from the EP by Detoxi particles (removal verified by a sensitive *Limulus* test) completely abolished the effect of the EP and since heat-treatment (80°C for 30 min) of the EP or decomposition of its protease and other proteins by proteinase K did not change the potentiating effect of the EP.

Our experiments with extracellular products from *H. influenzae* indicate that endotoxin is responsible for the potentiating effect on histamine release triggered by IgE or non-IgE-dependent mechanisms. It has previously been shown that *Salmonella* and *E. coli* endotoxins, including the Boivin and Westphal types, and LPS from a heptose-deficient mutant were able to enhance basophil histamine release (14, 23). Our results with *H. influenzae* endotoxin are in accordance with these findings of *Salmonella* and *E. coli* endotoxin. However, *H. influenzae* endotoxin also enhanced bacteria-induced histamine release caused by the non-IgE-dependent mechanism, i.e. the putative sugar-lectin-mediated reaction mentioned above, and this was not the case with *E. coli* endotoxin of the Boivin and Westphal types (14). Whether a more genuine preparation of *H. influenzae* endotoxin or the lack of 0-antigen (9) can explain this effect is not known.

On the basis of our findings it is tempting to speculate that a reinforcement of histamine release by *H. influenzae* endotoxin might play a pathophysiological role in chronic obstructive pulmonary disease. There are several possibilities for a penetration of *H. influenzae* and its endotoxin into the patients: a defect in the epithelium, failure of mucociliary clearance, stimulation of epithelial mast cells leading to histamine release, and opening of the tight junctions (13), or an impairment of the mucosal

immune barrier by cleavage of secretory IgA (11, 24).

ACKNOWLEDGEMENTS

This study was supported by grants from Thorvald Madsens Legat, Hafnia-Haand-I-Haand Fonden, Danmarks Sundhedsfond, Lundbeck Fonden, Nationalforeningen til Bekæmpelse af Lungesygdomme, P. Carl Petersens Fond and Fonden af 1870. The authors wish to thank Marianne Storgaard, Heidi Foldal, Lise Kolster and Karen Friis for excellent technical assistance.

REFERENCES

1. Baek, L.: New, sensitive rocket immunoelectrophoretic assay for measurement of the reaction between endotoxin and *Limulus* amoebocyte lysate. *J. Clin. Microbiol.* **17**, 1013-1020, 1983.
2. Castellán, R. M., Olenchock, S. A., Kinsley, K. B. & Hankinson, J. L.: Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N. Engl. J. Med.* **317**, 605-610, 1987.
3. Church, M. K., Norn, S., Pao, G.J.-K. & Holgate, S. T.: Non-IgE-dependent bacteria-induced histamine release from human lung and tonsillar mast cells. *Clin. Allergy* **17**, 341-353, 1987.
4. Clarke, C. W.: In vivo and in vitro reactions to antigens of *Haemophilus influenzae* in bronchial obstruction. *Br. J. Dis. Chest* **73**, 373-381, 1979.
5. Clementsen, P., Bisgaard, H., Pedersen, M., et al.: *Staphylococcus aureus* and influenza A virus stimulate human bronchoalveolar cells to release histamine and leukotrienes. *Agents Actions* **27**, 107-109, 1989.
6. Espersen, F., Jarlov, J. O., Jensen, C., Skov, P. Stahl & Norn, S.: *Staphylococcus aureus* peptidoglycan induces histamine release from basophil human leukocytes in vitro. *Infect. Immun.* **46**, 710-714, 1984.
7. Groeneveld, K., Alphen, L. van, Eijk, P. P., Jansen, H. M. & Zanen, H. C.: Changes in outer membrane proteins of nontypable *Haemophilus influenzae* in patients with chronic obstructive pulmonary disease. *J. Infect. Dis.* **158**, 360-365, 1988.
8. Groeneveld, K., Alphen, L. van, Geelen-van den Broek, N. J., Eijk, P. P., Zanen, H. C. & Ketel, R. J. van: Detection of *Haemophilus influenzae* with monoclonal antibody. *Lancet* **i**, 441-442, 1987.
9. Hitchcock, P. J., Leive, L., Mäkelä, P. H., Rietschel, E. T., Strittmatter, W. & Morrison, D. C.: Lipopolysaccharide nomenclature. Past, present and future. *J. Bacteriol.* **166**, 699-705, 1986.
10. Kennedy, S. M., Christiani, D. C., Eisen, E. A., et al.: Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am. Rev. Respir. Dis.* **135**, 194-200, 1987.
11. Kilian, M., Mestecky, J. & Russell, M. W.: Defense mechanisms involving Fc-dependent functions of immunoglobulin A and their subversion by bacterial immunoglobulin A proteases. *Microbiol. Rev.* **52**, 296-303, 1988.

12. Mestecky, J. & Kilian, M.: Immunoglobulin A (IgA). *Methods Enzymology* 116, 37-75, 1985.
13. Norn, S.: A medical hypothesis. Bacteria-induced histamine release: possible relationship to asthma. *Rev. Fr. Allergol.* 28, 199-203, 1988.
14. Norn, S., Bæk, L., Jensen, C., et al.: Influence of bacterial endotoxins on basophil histamine release. *Allergy* 41, 125-130, 1986.
15. Norn, S., Jensen, C., Dahl, B. T., et al.: Endotoxins release histamine by complement activation and potentiate bacteria-induced histamine release. *Agents Actions* 18, 149-152, 1986.
16. Rhind, G., Gould, G. A., Ahmad, F., Croughan, M. J. & Calder, M. S.: *Haemophilus parainfluenzae* and *H. influenzae* respiratory infections: comparison of clinical features. *Br. Med. J.* 291, 707-708, 1985.
17. Rylander, R. & Haglind, P.: Airborne endotoxins and humidifier disease. *Clin. Allergy* 14, 109-112, 1984.
18. Rylander, R., Haglind, P., Lundholm, M., Mattsby, I. & Stenqvist, K.: Humidifier fever and endotoxin exposure. *Clin. Allergy* 8, 511-516, 1978.
19. Rylander, R., Haglind, P. & Lundholm, M.: Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. *Am. Rev. Respir. Dis.* 131, 209-213, 1985.
20. Shore, P. A., Burkhalter, A. & Cohn, V. A.: A method for fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* 127, 182-186, 1959.
21. Skov, P. S. & Norn, S.: A simplified method for measuring basophil histamine release and blocking antibodies in hay fever patients. Basophil histamine content and cell preservation. *Acta Allergol.* 32, 170-182, 1977.
22. Skov, P. S., Permin, H. & Malling, H. J.: Quantitative and qualitative estimations of IgE bound to basophil leukocytes from hay fever patients. *Scand. J. Immunol.* 6, 1021-1028, 1977.
23. Smith, T. F., Aelvoet, M. & Morrison, D. C.: The effect of bacterial lipopolysaccharide (LPS) on histamine release from human basophils. *Clin. Immunol. Immunopathol.* 34, 355-365, 1985.
24. Sørensen, C. H. & Kilian, M.: Bacterium-induced cleavage of IgA in nasopharyngeal secretions from atopic children. *Acta Pathol. Microbiol. Immunol. Scand. [C]* 92, 85-87, 1984.
25. Turk, D. C.: The pathogenicity of *Haemophilus influenzae*. *J. Med. Microbiol.* 18, 1-16, 1984.
26. Zwan, J. C., van der Orie, N. G. M., Kauffman, H. F., Wiers, P. W. J. & Vries, K. de: Bronchial obstructive reactions after inhalation with endotoxin and precipitinogens of *Haemophilus influenzae* in patients with chronic non-specific lung disease. *Clin. Allergy* 12, 547-559, 1982.

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