

Influenza A virus potentiates bacteria-induced histamine release

Examination of normal individuals and patients allergic to bacteria

P. CLEMENTSEN, M. PEDERSEN,¹ H. PERMIN,¹ F. ESPERSEN² & S. NORN

Dept. of Pharmacology, University of Copenhagen, Copenhagen. ¹Laboratory of Immunology, University Clinic for Infectious Diseases M and ²Statens Seruminstitut, Dept. of Clinical Microbiology at Rigshospitalet, Copenhagen N. Denmark

Influenza A virus was found to enhance basophil histamine release induced by *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus sanguis*, but did not *per se* release histamine. This potentiating effect of the virus was seen both when the bacteria-induced mediator release was IgE-dependent (i.e. patient allergic to bacterium) and when the bacterium caused histamine release by a non-immunological mechanism independent of IgE (putative sugar-lectin mediated). Also histamine release induced by other immunological and non-immunological stimuli, such as anti-IgE, calcium ionophore or agarose beads was enhanced in the presence of the virus. The potentiating effect of the virus on bacteria-induced mediator release might be of importance for the conversion from latent to manifest asthma in upper respiratory tract infections.

Key words: bacteria; basophils; calcium ionophore; histamine release; influenza A virus; anti-IgE; asthma; potentiation.

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CLINICAL ASPECTS

Upper respiratory tract infections have often been shown to precipitate or exacerbate attacks of bronchial asthma. The finding that influenza A virus can enhance allergic and non-allergic histamine release induced by bacteria *in vitro* might play a role for the conversion from latent to manifest asthma in mixed bacterial and viral infections.

It has been shown that bacterial and viral infections in the upper respiratory tract can provoke or exacerbate attacks of bronchial asthma (1, 11, 14, 15, 20). Bacteria have been found to release histamine from human basophils (13, 16, 17), lung and tonsillar mast cells (4), and mast cells obtained by bronchoalveolar lavage (5). Viruses enhance basophil histamine release triggered by either IgE-dependent stimuli (2, 3, 6, 7, 12), endotoxin-induced complement activation (8) or bacteria (9). Since bacteria trigger histamine release by

two different mechanisms, an immunological (IgE-dependent) and a non-immunological mechanism (16), we have examined which of these two mechanisms (or both) was enhanced by the virus.

MATERIAL AND METHODS

Six patients infected with and sensitized to bacteria were included.

Patient No. 1: 99-year-old man with catheter *in situ* for 1 year because of hypertrophy of prostate; patient had septicaemia with *E. coli*

and was treated with antibiotics 1 week before inclusion in the study. This bacterium was also found in the urine. No atopic diseases.

Patient No. 2: 59-year-old woman with diabetes mellitus and collagenosis, the latter treated with immunosuppressives. Septicaemia with *S. enteritidis* (serotype dublin) was demonstrated 12 days before inclusion in the study, and this bacterium was also found in urine and faeces. The patient was treated with antibiotics. No atopic diseases.

Patient No. 3: 45-year-old woman with diabetes mellitus. Septicaemia with *E. coli* was demonstrated 13 days before her inclusion in the study and was treated with antibiotics. No atopic diseases.

Patient No. 4: 37-year-old man with increased total IgE and suspected collagenosis, which was treated with the immunosuppressives, azathioprine and prednisone. *S. aureus* phage type 6-47-54+ was isolated from abscesses in the skin, from which the patient had suffered for many years. No atopic diseases.

Patient No. 5: 57-year-old woman with *S. pneumoniae*-induced meningitis and septicaemia demonstrated 10 days before inclusion in the study. The patient was allergic to various animals and had asthma and bronchitis.

Patient No. 6: 38-year-old man, with heart failure since childhood, treated with antibiotics for mitral valve endocarditis. Septicaemia with *S. sanguis* demonstrated 37 days before participation in the study. No atopic diseases.

Seven healthy individuals (age 23-64 years) were also included.

Whole formalin-killed *S. aureus* strain Wood 46 (protein A-deficient) was prepared according to (10) and *E. coli*, *S. enteritidis* (serotype dublin), *S. pneumoniae* and *S. sanguis* isolated from the blood of the patients with sepsis from these bacteria, and *S. aureus* isolated from an abscess in the skin of patient No. 4, were prepared in a similar manner. Human influenza A virus A/Caen/1/84 (H3N2) was obtained as previously described (8). Rabbit-antihuman IgE (ϵ -chain), 400,000 IU/ml was from Behringwerke AG, FRG, calcium ionophore A23187 from Calbiochem AG, Switzerland, and agarose beads i.e. polymers of

D-galactose, from Kem En Tec, Copenhagen, Denmark.

Blood from each person was drawn by venipuncture, anti-coagulated 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 (18), washed twice, and suspended in Tris-AMC 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 (18).

To examine whether the histamine release induced by bacteria was related to an immunological (IgE-dependent) or a non-immunological reaction, stimulation of the cells without surface IgE was also performed. By exposing the cells to pH 3.8 at 4°C followed by wash and resuspension in Tris-AMC, the IgE molecules were dissociated from the cells (19). The removal of IgE-immunoglobulin was controlled functionally by the absence of histamine release induced by anti-IgE.

Histamine release experiments were performed by incubation of 40 μl leukocyte suspension for 40 min at 37°C with 5 μl Tris-AMC and 5 μl of diluted or undiluted preparations of bacteria (*E. coli*, *S. enteritidis*, *S. aureus*, *S. pneumoniae* and *S. sanguis*), anti-IgE, the calcium ionophore A 23187, or agarose beads. Histamine release was assayed spectrofluorometrically by determination of the residual histamine in the washed cell sediment (18). 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 influenza A virus on histamine release was examined by replacing the 5 μl Tris-AMC buffer with influenza A virus.

Statistics

Student's t-test was used when comparing histamine release from intact cells and cells deprived

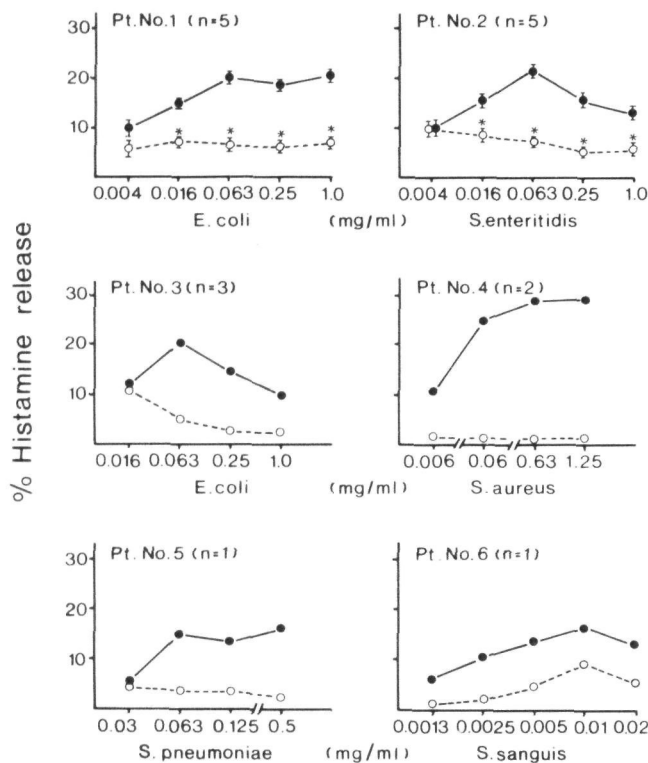


Fig. 1. Verification of IgE-mediated histamine release in cell suspensions from 6 patients infected with *E. coli*, *Salmonella enteritidis*, *Staph. aureus*, *Streptococcus pneumoniae* or *Streptococcus sanguis*. Intact cells (●—●) and cells deprived of IgE (○—○) were challenged with the patients' own bacterium. Mean $\pm$ SEM for n experiments in duplicate is given, * $P < 0.01$.

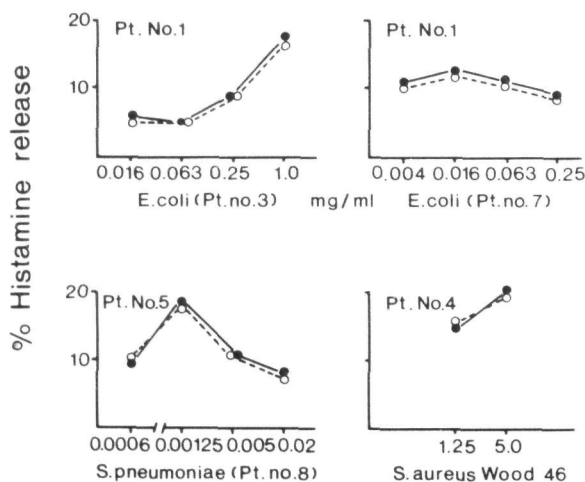


Fig. 2. Histamine release induced by bacteria strains to which the patients were not sensitized. Cells from Patients 1, 4 and 5 were stimulated with bacteria, with which the patients were not infected. Some of the bacteria were obtained from patients not included in the study (Pts. 7 and 8). Intact cells (●—●) and cells deprived of IgE (○—○). Duplicate determinations.

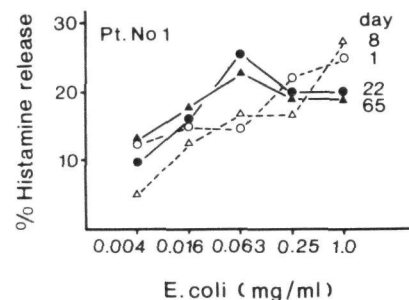


Fig. 3. IgE-mediated histamine release induced by *E. coli* in cell suspensions from Patient 1 suffering from sepsis with and sensitized to this bacterium. The histamine release was followed-up for a period of 2 months. Note a shift to the left of the curves. No release of histamine was obtained from cells deprived of IgE.

of IgE, or comparing samples with and without virus.

RESULTS

Patients sensitized to bacteria

Histamine release was examined in cell suspension from six patients infected with either *E. coli*, *S. enteritidis*, *S. aureus*, *S. pneumonia* or *S. sanguis*. Stimulation of the cells with the patients own bacterium in final concentrations of 0.0013 to 1.25 mg/ml resulted in release of histamine, and this release was IgE-mediated, since no significant release was obtained when cell-bound IgE was removed before challenging the cells with the bacterium in question (Fig. 1). In contrast, non-immunological histamine release appeared when the cells were stimulated with strains with which the patient was not infected and to which he was not sensitized, since similar dose-response curves were obtained using intact cells and cells deprived of IgE (Fig. 2).

The duration of the IgE-mediated reaction was examined in two of the patients. When cells from Patient 1 were stimulated with the patient's own *E. coli* bacterium the IgE-mediated histamine release lasted for at least 2 months, since within this period intact cells released histamine, whereas no release was obtained from cells deprived of IgE (Fig. 3). A shift to the left of the dose-response curve was obtained after 3 weeks, indicating an increased

cell sensitivity to the bacterium. Patient 2 was followed up for 1 month and showed IgE-mediated histamine release throughout this period when tested with the patient's own *S. enteritidis* and no change was obtained in cell sensitivity (data not shown).

A potentiation by influenza A virus of the IgE-mediated histamine release was examined by including the virus in the samples. When cells from the six patients were stimulated by a combination of the bacterium to which the patient was sensitized and influenza A virus, the bacteria-induced histamine release was enhanced by 40 to 200 % ($P < 0.01$) (Fig. 4) and peak was obtained in final concentrations of the virus in the range 10^{-5} ng to 10 ng viral protein/ml. The potentiating effect of the virus was due to synergism, since the virus did not release histamine *per se*.

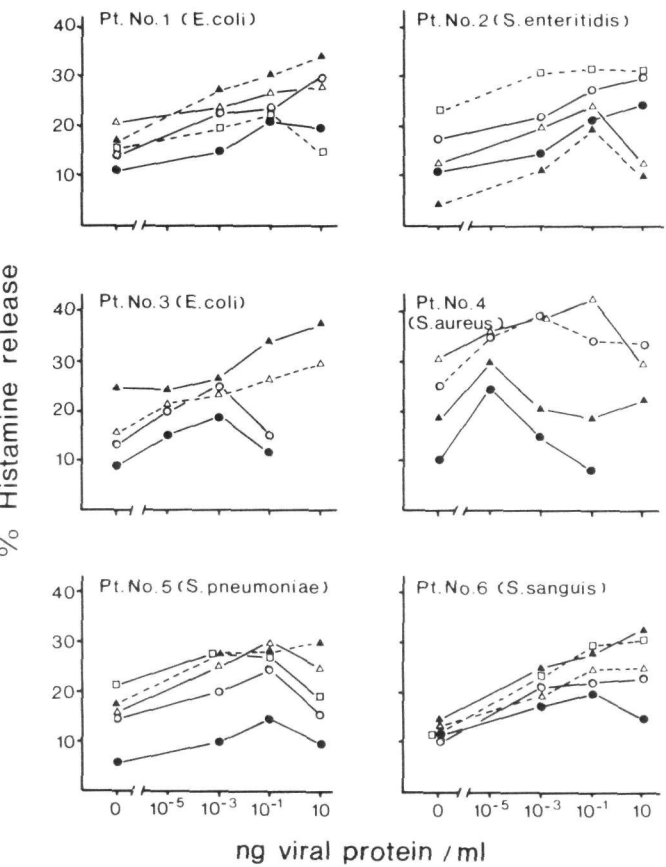


Fig. 4. IgE-mediated histamine release induced by bacteria is enhanced in the presence of influenza A virus. Experiments with cells from the 6 patients sensitized to *E. coli*, *Salmonella enteritidis*, *Staph. aureus*, *Streptococcus pneumoniae* and *Streptococcus sanguis*. The bacteria were used in different concentrations. Duplicate determinations. Note that influenza A virus - caused no histamine release *per se*.

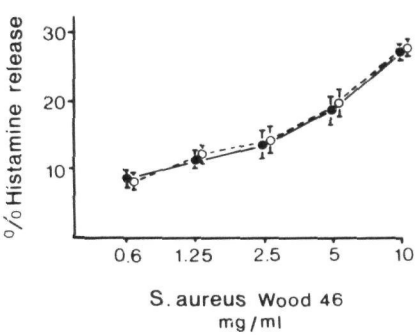


Fig. 5. Verification of non-immunological histamine release induced by *Staph. aureus* strain Wood 46 in cell suspensions from 7 normal individuals. Challenge of intact cells (●—●) and cells deprived of IgE (○-----○). Mean of 7 experiments in duplicate $\pm$ SEM is given.

Subjects not sensitized to bacteria

Cells from seven healthy individuals were stimulated with *S. aureus* strain Wood 46 in final concentrations of 0.6 to 10 mg/ml wet weight, and significant histamine release was obtained

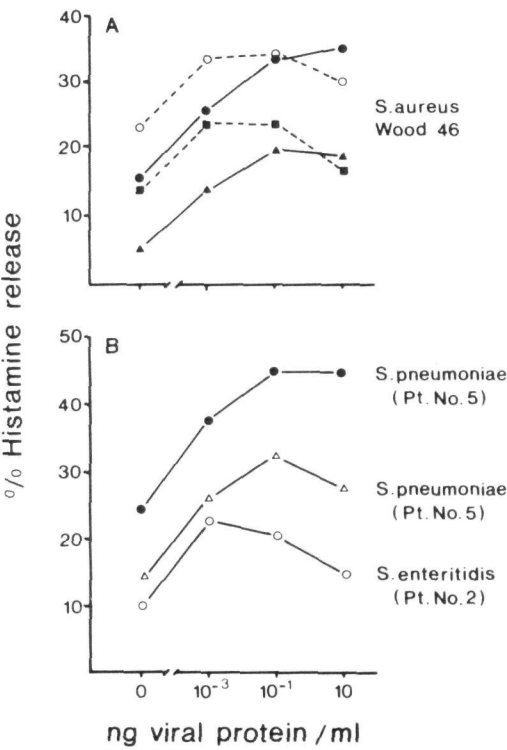


Fig. 6. Non-immunological histamine release induced by bacteria is enhanced in the presence of influenza A virus. Cells from 7 normal individuals were stimulated with *Staph. aureus* strain Wood 46 (A) and with *S. pneumoniae* and *S. enteritidis* (B). Duplicate determinations. Note that influenza A virus caused no histamine release *per se*.

in the range of 1.25 to 10 mg/ml bacterium (Fig. 5). Removal of cell-bound IgE before challenge of the cells with the bacterium did not change the histamine release, indicating a non-immunological release mechanism (Fig. 5). When the cells were stimulated with the bacteria isolated from the sensitized patients mentioned above no release of histamine was obtained in final bacteria concentrations from 0.0013 to 0.63 mg/ml; however higher concentrations (1.25 to 10 mg/ml) caused a non-immunological histamine release (data not shown).

A potentiation by influenza A virus of the non-immunological histamine release was examined by stimulating the cells with *S. aureus* strain Wood 46. When influenza A virus was included, virus enhanced the bacteria-induced histamine release by 47 to 167 % ($P < 0.01$) and peak was obtained in final concentrations of the virus from 10^{-3} ng to 10 ng viral protein/ml (Fig. 6A). When the cells were stimulated with the bacteria from the sensitized patients, influenza A virus caused a similar enhancement of the bacteria-induced histamine release (Fig. 6B). In all experiments enhancement was due to synergism since the virus itself caused no release of histamine.

Enhancement of histamine release induced by other stimulators

To examine whether influenza A virus could enhance histamine release caused by anti-IgE,

cells from healthy individuals were stimulated with anti-IgE in final dilutions from 10^{-5} to 10^{-4} , and in these experiments inclusion of the virus resulted in enhancement of mediator release by 73 % (Table 1).

In other experiments cells from normal individuals were stimulated with the calcium ionophore A 23187 in final concentrations from 3×10^{-7} to 10^{-6} M, or agarose beads in final dilutions of the preparation from 0.06 % to 0.3 %. The non-IgE-dependent histamine release was enhanced by 56 and 200 %, respectively, when influenza A virus was included in the samples (Table 1).

In all these experiments no significant release of histamine was obtained with the virus *per se*, indicating that the enhancement was due to synergism.

DISCUSSION

We have previously shown that influenza A virus enhances basophil histamine release induced by *S. aureus*, and it was suggested that this effect was due to viral neuraminidase on the surface of the virus, since a similar potentiating effect was caused by a purified neuraminidase preparation, and the effect of virus as well as of the purified neuraminidase was completely abolished by a potent neuraminidase inhibitor (9). Bacteria can trigger histamine release by two different mechanisms, an immunological (IgE-

Table 1
Potentiation by influenza A virus of histamine release induced by the stimulators: anti-IgE, the calciumionophore A 23187 and agarose beads

Stimulator	Histamine release caused by:			
	stimulator alone	stimulator and virus (ng viral prot./ml)		
		10^{-3}	10^{-1}	10
Anti-IgE	19.6	29.7	33.9	28.4
	±1.4	±1.7	±2.0	±2.4
A23187	19.8	29.3	30.8	26.3
	±2.0	±1.8	±1.8	±2.0
Agarose beads	5.0	15.3	13.5	8.5
	±0.2	±1.0	±1.0	±1.4

The histamine release is given independently of the concentration of stimulator in the experiments. Mean of 8-10 experiments ± SEM is given. In all experiments virus enhanced the histamine release significantly ($P < 0.01$).

dependent) and a non-immunological (16). The latter is regarded as a sugar-lectin-mediated reaction in which sugars on the bacterial cell wall interact with lectins on the basophil cell membrane leading to histamine release (13, 16, 17). The non-immunological mechanism might be of importance for our understanding of the role of bacteria in asthma, since a subject does not need to be sensitized to bacteria in order to induce histamine release.

In this study we examined the effect of influenza A virus on bacteria-induced histamine release to decide whether it was the IgE-dependent or the non-immunological reaction which was enhanced by the virus. IgE-mediated histamine release was obtained by stimulating cells from patients infected with and allergic to *E. coli*, *S. enteritidis*, *S. aureus*, *S. pneumoniae* and *S. sanguis* with their own bacteria. The mediator release was enhanced in the presence of influenza A virus. These results show that the IgE-dependent reaction caused by gram-positive and gram-negative bacteria is reinforced by the virus in analogy with the histamine release triggered by specific antigens (7) and anti-IgE (Table 1). When the non-immunological reaction was examined in cells from normal individuals by challenge with gram-positive and gram-negative bacteria a similar potentiation was obtained by the virus, showing that it also enhanced the non-immunological release mechanism induced by bacteria. Also other types of non-immunological histamine release were enhanced in the presence of virus. These included calcium ionophore A 23187 and agarose beads (polymers of D-galactose) which were used as a model for the putative sugar-lectin-mediated reaction mentioned above.

The enhancement by influenza A virus of bacteria-induced histamine release may play a role in mixed bacterial and viral infections when the patient is sensitized to the bacterium in question. It is especially tempting to speculate that this synergistic effect on mediator release could be of importance for the conversion from latent to manifest asthma by upper respiratory tract infections. Earlier, we found that the IgE-mediated histamine release triggered by bacteria lasted for only 2 to 4 weeks (16), but in this study exam-

ination of Patient 1 showed that the sensitization to the patients own bacterium was maintained for at least 2 months (Fig. 3). However, the other type of bacteria-induced histamine release (non-immunological mediator release), which does not need sensitization to the bacterium, was also enhanced by the virus. This finding presented the question of whether this reinforcement of mediator release might also influence the aggravation of asthma during infections.

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REFERENCES

1. Busse, W. W.: The relationship between viral infections and onset of allergic disease and asthma. *Clin. Exp. Allergy* 19, 1-9, 1989.
2. Busse, W. W., Swenson, C. A., Borden, E. G., Treuhaft, M. W. & Dick, E. C.: Effect of influenza A virus on leukocyte histamine release. *J. Allergy Clin. Immunol.* 71, 382-388, 1983.
3. Chonmaitree, T., Lett-Brown, M. A., Tsong, Y., Goldman, A.S. & Baron, S.: Role of interferon in leukocyte histamine release caused by common respiratory viruses. *J. Infect. Dis.* 157, 127-132, 1988.
4. 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.
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. Clementsen, P., Douglas, A. R., Skehel, J. J., Hannoun, C., Bach-Mortensen, N. & Norn, S.: Influenza A virus enhances IgE-mediated histamine release from human basophil leukocytes. Examination of the effect of viral neuraminidase and haemagglutinin. *Agents Actions* 27, 58-61, 1989.
7. Clementsen, P., Hannoun, C. & Norn, S.: Influenza A virus enhances allergic histamine release. Effect of neuraminidase. *Allergy* 44, 33-38, 1989.
8. Clementsen, P., Jensen, C. B., Hannoun, C., Søborg, M. & Norn, S.: Influenza A virus potentiates basophil histamine release caused by endotoxin-induced complement activation. Examination of normal individuals and patients with intrinsic asthma. *Allergy* 43, 93-99, 1988.
9. Clementsen, P., Jensen, C. B., Jarløv, J. O., Hannoun, C., Søborg, M. & Norn, S.: Influenza A virus enhances *Staphylococcus aureus*-induced basophil histamine release in normal individuals and patients with intrinsic asthma. *Allergy* 44, 39-44, 1989.

10. Espersen, F., Jarløv, J. O., Jensen, C., Skov, P. S. & Norn, S.: *Staphylococcus aureus* peptidoglycan induces histamine release from basophil human leukocytes in vitro. *Infect. Immun.* 46, 710-714, 1984.
11. Horn, M. E. C., Reed, S. E. & Taylor, P.: Role of viruses and bacteria in acute wheezy bronchitis in childhood: a study of sputum. *Arch. Dis. Child.* 54, 587-589, 1979.
12. Ida, S., Hooks, J. J., Siraganian, R. P. & Notkins, A. L.: Enhancement of IgE-mediated histamine release from human basophils by viruses: role of interferon. *J. Exp. Med.* 145, 892-906, 1977.
13. Jensen, C., Norn, S., Skov, P. S., Espersen, F., Koch, C. & Permin, H.: Bacterial histamine release by immunological and non-immunological lectin-mediated reactions. *Allergy* 39, 371-377, 1984.
14. McIntosh, K., Elliot, F. F., Hoffman, L. S., Lybass, T. G., Eller, J. J. & Fulginiti, V. A.: The association of viral and bacterial respiratory infections with exacerbations of wheezing in young asthmatic children. *J. Pediatr.* 82, 578-590, 1973.
15. Minor, T. E., Dick, E. C., DeMeo, A. N., Quellette, J. J., Cohen, M. & Reed, C. E.: Viruses as precipitants of asthmatic attacks in children. *JAMA* 227, 292-298, 1974.
16. Norn, S.: A medical hypothesis. Bacteria-induced histamine release: possible relationship to asthma. *Rev. Fr. Allergol.* 28, 199-203, 1988.
17. Norn, S., Skov, P. S., Jensen, C., Jarløv, J. O. & Espersen F.: Histamine release induced by bacteria. A new mechanism in asthma? *Agents Actions* 20, 29-34, 1987.
18. 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.
19. 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.
20. Welliver, R. C.: Upper respiratory infections in asthma. *J. Allergy Clin. Immunol.* 72, 341-346, 1983.

Address:

P. Clementsen

Department of Pharmacology

University of Copenhagen

Juliane Maries Vej 20

DK-2100 Copenhagen Ø

Denmark

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