

Virus, bacteria and lipopolysaccharide increase basophil cell response to histamine releasing stimulators and calcium

Examination of allergic and normal individuals

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Histamine release from human basophil leukocytes from allergic patients or controls was induced by specific antigens, anti-IgE or calcium ionophore A23187. Influenza A virus, *S. aureus* and lipopolysaccharide from *S. typhimurium* increased the maximum release of histamine and caused a shift to the left of the dose-response curves showing increased cell sensitivity and lowering of the threshold to these stimuli. The mechanism of action was elucidated by examining the mediator release as a function of increasing extracellular concentration of calcium. In these experiments the dose-response curves were changed by the microorganisms and lipopolysaccharide as before. This indicates that the microorganisms and lipopolysaccharide change the basophil cell response to IgE-dependent and non-immunological stimuli by causing a change in the subcellular handling of calcium.

Key words: asthma; antigen; basophils; calcium; calcium ionophore; histamine release; influenza A virus; lipopolysaccharides; *Staphylococcus aureus*.

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CLINICAL ASPECTS

Infections in the respiratory tract are known to precipitate or exacerbate attacks of bronchial asthma. We find that influenza A virus, *S. aureus* and lipopolysaccharides from *S. typhimurium* enhance the release of histamine and lower the histamine releasing threshold to both IgE-dependent and non-immunological histamine releasing stimuli. Such effects might play a role in obstructive airway diseases in connection with airway infections.

Respiratory tract infections often provoke or exacerbate attacks of bronchial asthma (1, 12, 25), and it is therefore possible that microorganisms play an important role in airway hyperreactivity.

We have previously shown that virus (3, 6), bacteria (19, 21, 22) and bacterial products (20, 21) enhance IgE-dependent and non-IgE-dependent histamine release from human basophil leukocytes. Microorganisms seem therefore to cause an increased cell reactivity. In the present study we examined whether cell reactivity, sen-

sitivity, and threshold were changed to these stimuli and, furthermore, to calcium in order to elucidate the mechanism behind the changed cell response.

MATERIAL AND METHODS

Twelve healthy individuals aged between 31 and 55 years served as donors. No one had any previous history of atopy and they received no medication. Eight patients suffering from bronchial asthma, some in connection with hay fe-

ver, and with a well-documented history of allergy to either house dust mite, grass pollen or birch pollen were also included. These patients had a positive skin prick test and a positive basophil histamine release to these allergens.

The following allergens and stimulators were used: house dust mite (*Dermatophagoides pteronyssinus*), grass pollen (*Phleum pratense*) and birch pollen (*Betula verrucosa*), all Soluprick-SQ 3HEP from ALK, Denmark. Rabbit-antihuman IgE (ϵ -chain), 400,000 IU/ml was from Behringwerke AG, Germany. Calcium ionophore A23187 was purchased from Calbiochem AG, Switzerland.

Human influenza A virus A/Caen/1/84 (H3N2) was obtained as previously described (4). Whole formalin-killed *Staphylococcus aureus* Wood 46 (protein A-deficient) was prepared according to Espersen et al. (8) and as endotoxin we used *Salmonella typhimurium* lipopolysaccharide (LPS) preparation of Westphal type (protein-free) from Difco Laboratories, USA; both were dialysed against isotonic NaCl. As *Haemophilus influenzae* LPS we used a preparation of the extracellular products (EP) of the bacterium (5). Besides LPS the EP also contained IgA-1 protease. EP was obtained from a non-capsulated (non-typeable) *Haemophilus influenzae* biotype II strain isolated from a patient with chronic bronchitis. If not otherwise stated, influenza A virus was used in a final concentration of 0.1 ng viral protein/ml, *S. aureus* 0.15 mg/ml (wet weight) *S. typhimurium* LPS 0.1 μ g/ml, and EP in a final dilution of 5%.

Preparation of cell suspension

Blood was drawn by venipuncture, anticoagulated with EDTA in a final concentration of 0.01 M and mixed with the two-fold volume of 0.9% NaCl. Leukocytes with approx. 2% basophils were isolated by the Ficoll-Hypaque gradient method (24). The leukocytes were washed twice with and suspended in a glucose-free Tris-AMC containing 25 mM Tris at pH 7.6, 120 mM NaCl, 5 mM KCl, 0.6 mM CaCl_2 , 1.1 MgCl_2 , and 0.3 mg of human serum albumin/ml (24). In some experiments the Tris-AMC

(albumin, magnesium, calcium) was replaced by Tris-A containing 1 mM EGTA for the first wash and Tris-AM for the second, and the cells were then resuspended in Tris-AMC with calcium in concentrations varying between 0.006 and 18.0 mM.

Histamine release

Histamine release experiments were performed by incubation of 40 μ l leukocyte suspension for 40 min at 37°C with 5 μ l of diluted anti-IgE, antigen or calcium ionophore A23187 and 5 μ l Tris-AMC. The release of histamine was assayed spectrofluorometrically by determination of the residual histamine in the washed cell sediment (24). Only release of more than 10% of the total histamine content in the sample was considered significant, since the spontaneous release of histamine amounted up to 10%. A change in the cell response to anti-IgE, antigen, A23187 or extracellular calcium was examined by replacing the 5 μ l Tris-AMC by diluted influenza A virus, *S. aureus*, LPS or EP.

Statistics

Student's t-test was used when comparing samples with and without LPS or EP.

RESULTS

The influence of influenza A virus on histamine release induced by specific antigens was examined by incubating cells from patients allergic to birch pollen, house dust mite or grass pollen with the respective antigens in increasing concentrations. Fig. 1A shows a dose-response curve obtained by birch pollen. Inclusion of the virus caused a shift to the left of the dose-response curve of one decade, indicating that virus lowered the threshold to the antigen. This shift of the dose-response curve also means enhanced cell sensitivity to the antigen, which expressed by the change in EC_{50} (the concentration of cell stimulator giving 50% of the maximum release) constituted a $1/4$ decade (Fig. 1A).

In the absence of virus the maximum release of histamine was 40 % and by inclusion of influenza A virus it was enhanced to 55 %, indicating that virus caused an enhanced cell reactivity to the antigen (Fig. 1A). When house dust mite or grass pollen was used as specific antigen, a similar pattern was seen when influenza A virus was included in the experiments (Fig. 1B).

In experiments with cells from normal individuals IgE-mediated histamine release was obtained by anti-IgE (Fig. 2). Influenza A virus caused a shift to the left of the dose-response curve corresponding to $1/2$ a decade and the maximum release of histamine was enhanced from 35 to 43 %. The change in cell sensitivity was a $1/4$ decade. When virus was used in 100-fold higher or lower concentrations (10 and 10^{-3} ng viral protein/ml), only a small or no change in the dose-response curve was seen (data not shown), indicating that in these concentrations of virus no change was obtained in cell reactivity, cell sensitivity or threshold to anti-IgE.

A similar pattern was obtained by *S. aureus*, i.e. an enhancement of cell sensitivity and lowering of the threshold to anti-IgE by approx. $1/2$ decade and an increase of the maximum release by 20 %. *S. typhimurium* LPS caused similar changes except for the cell sensitivity which was enhanced by only $1/4$ decade. However, no

change in the dose-response curves was seen when the bacterium was used in two-fold higher and lower concentrations (0.3 and 0.075 mg/ml) (data not shown) or when the LPS was used in 10-fold higher or lower concentrations (1 and $0.01 \mu\text{g/ml}$) (Fig. 5A).

To examine whether the microorganisms and LPS caused a similar change in the cell response to extracellular calcium, cells from patients allergic to grass pollen or house dust mite were pretreated with EGTA to remove calcium and then stimulated with calcium in various concen-

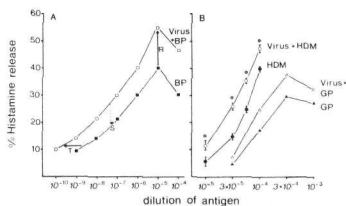


Fig. 1. Histamine release from basophils obtained by stimulating cells from allergic patients with increasing concentrations of specific antigen (closed symbols). The dose-response curves are moved to the left and the maximum histamine release is enhanced in the presence of influenza A virus (open symbols). Change in cell reactivity (R), cell sensitivity (S) and the threshold (T) to antigen. BP: birch pollen, GP: grass pollen (duplicate determinations), HDM: house dust mite (mean of 5 experiments in duplicate $\pm$ SEM; $*P < 0.01$).

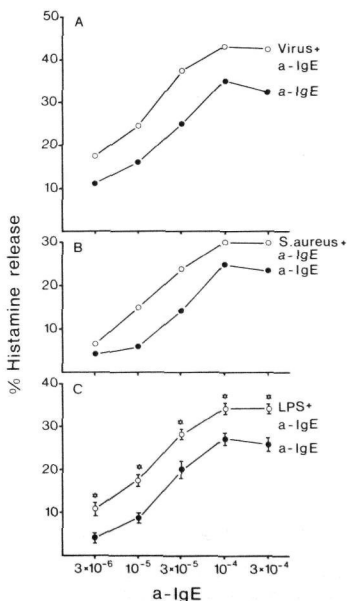


Fig. 2. Dose-response curves obtained by stimulating cells from normal individuals with anti-IgE (closed symbols) are moved to the left and the maximum mediator release is enhanced by inclusion of (open symbols) A: influenza A virus ($n = 4$), B: *S. aureus* ($n = 3$) and C: LPS from *S. typhimurium* ($n = 6$). Mean of n experiments in duplicate ($\pm$ SEM), $*P < 0.01$.

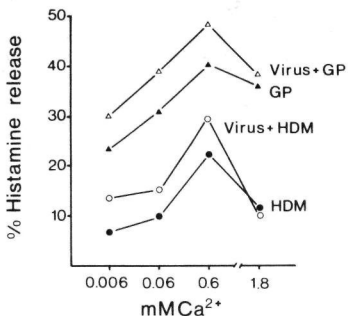


Fig. 3. Histamine release induced by specific antigens in cell suspensions from allergic patients is obtained as a function of increasing concentrations of extracellular calcium. The dose-response curves (closed symbols) are moved to the left and the maximum histamine release is enhanced in the presence of influenza A virus (open symbols). Duplicate determinations.

trations in the presence of a fixed dilution (10^{-4}) of specific antigen (Fig. 3). In the first experiment with grass pollen, influenza A virus caused a 1 decade shift of the curve to lower calcium concentrations, indicating a lowering of the threshold to calcium. The cell reactivity to calcium was enhanced, since the maximum histamine release, which was obtained at 0.6 mM calcium, was increased from 40% to 48%. A similar change in the cell response to calcium was obtained in the second experiment with house dust mite (Fig. 3).

When cells from normal individuals were exposed to a 10^{-4} dilution of anti-IgE, the calcium-dependent histamine release curve was changed in a similar way by influenza A virus, *S. aureus* and LPS from *S. typhimurium* and *H. influenzae* (EP) (Fig. 4). The changes included increased cell sensitivity (a shift of $1/4$ to $1/2$ decade) and reactivity (maximum release enhanced by 20 to 50%) and a reduced threshold (a shift of $1/2$ to 1 decade) to calcium. However, no change was found when *S. aureus* was used in a 2-fold higher or lower concentration, influenza A virus in a 100-fold higher or lower concentration, or when

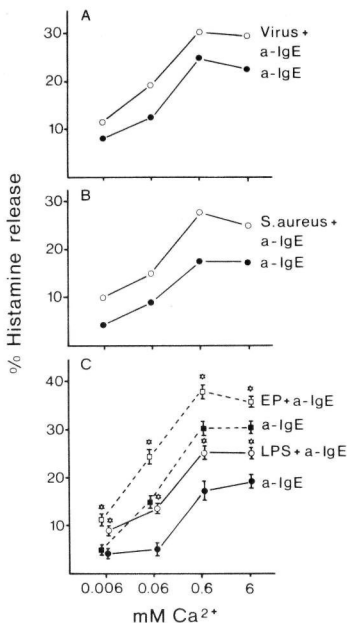


Fig. 4. Histamine release induced by anti-IgE in cells from normal individuals is obtained as a function of increasing concentrations of extracellular calcium. The dose-response curves (closed symbols) are moved to the left and the maximum histamine release is enhanced (open symbols) by inclusion of A: influenza A virus ($n = 4$), B: *S. aureus* ($n = 3$), C: EP from *H. influenzae* ($n = 7$) and LPS from *S. typhimurium* ($n = 6$). Mean of n experiments in duplicate ($\pm$ SEM), $*P < 0.01$.

S. typhimurium LPS (Fig. 5B) was used in a 10-fold higher or lower concentration.

The relationship between the response to a histamine releasing stimulator and the response to extracellular calcium is shown in Fig. 5. As mentioned, *S. typhimurium* LPS increased cell reactivity and sensitivity and lowered the threshold to both anti-IgE and calcium ($P < 0.01$) when used in a final

concentration of 0.1 $\mu\text{g/ml}$, whereas a 10-fold higher or lower concentration caused no significant change either to the stimulator or to calcium, indicating a relationship between the response to anti-IgE and calcium.

Fig. 6 shows similar experiments in which non-immunological histamine release was examined by incubating cells from normal individuals with the calcium ionophore A23187. In analogy with IgE-dependent histamine release lowering of the threshold, enhancement of cell reactivity and sensitivity to A23187 and extracellular calcium was obtained by influ-

enza A virus, *S. aureus*, and *S. typhimurium* LPS.

In all experiments a synergistic effect was obtained by influenza A virus, *S. aureus*, *S. typhimurium* LPS and the extracellular products (EP) from *H. influenzae* since these agents caused no histamine release *per se*.

DISCUSSION

In the present study the influence of microorganisms and endotoxin on histamine release from human basophil leukocytes was examined. Influenza A virus, *S. aureus*, and LPS

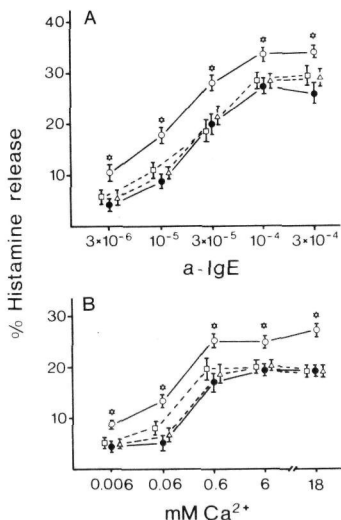


Fig. 5. Anti-IgE-induced histamine release in cell suspensions from normal individuals is given as a function of A: increasing concentrations of anti-IgE and B: increasing concentrations of extracellular calcium. Under both experimental conditions the dose-response curves (closed symbols) are moved to the left and the maximum mediator release is enhanced in the presence of LPS from *S. typhimurium* in a concentration of 0.1 $\mu\text{g/ml}$ ($\circ$), but not 0.01 (Δ) or 1.0 $\mu\text{g/ml}$ ($\square$). Each point represents mean $\pm$ SEM of 6 experiments in duplicate, * $P < 0.01$.

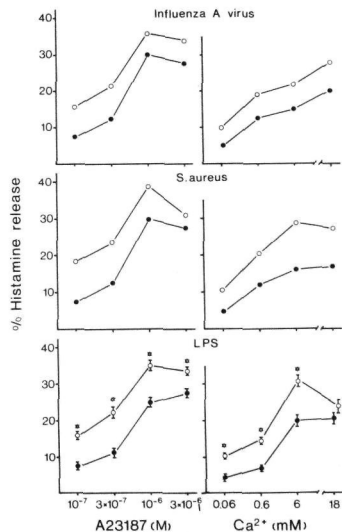


Fig. 6. A23187-induced histamine release given as a function of increasing concentrations of A23187 and increasing concentrations of extracellular calcium. The dose-response curves (closed symbols) are moved to the left and the maximum histamine release is enhanced (open symbols) in the presence of influenza A virus, *S. aureus*, and LPS from *S. typhimurium*. Each point represents the mean of 3 or 5 experiments in duplicate $\pm$ SEM, $P < 0.01$.

from *S. typhimurium* increased the basophil cell response to specific antigens (house dust mite, grass pollen and birch pollen), anti-IgE and to the calcium ionophore A23187. The changes included increased maximum release of histamine, i.e. enhanced cell reactivity. This potentiation of mediator release confirms earlier results obtained by influenza A virus, *S. aureus* and LPS from *S. typhimurium* and *H. influenzae* (5, 6, 20, 22). Further, a shift to the left of the dose-response curves was obtained, showing increased cell sensitivity and lowering of the threshold to the cell stimuli. An increased sensitivity to allergen might be more important in asthma than an increased maximum mediator release, since the former and not the maximum release reflects the severity of the disease as measured by bronchial provocation with the relevant allergen (18). Similar changes in cell reactivity, cell sensitivity and threshold were obtained to antigen, anti-IgE and the calcium ionophore. These results show that the microorganisms and the LPS change the basophil cell response to both IgE-dependent and non-immunological stimuli.

The mechanism of action was elucidated by examining the cell response to extracellular calcium. In the presence of a fixed concentration of antigen, anti-IgE or A23187, increasing concentrations of calcium caused calcium-dependent histamine release curves. Influenza A virus, *S. aureus* and *S. typhimurium* LPS caused similar changes in cell reactivity, cell sensitivity and threshold as those mentioned before. Further, a dose-dependent relationship was found between the changes to histamine releasing stimulators and to extracellular calcium. This relationship indicates that calcium might play a role in the change in cell response. As mentioned before the microorganisms and LPS caused similar changes in the cellular response to specific antigens and anti-IgE (both open the calcium channels by crosslinking IgE (9, 13, 14) as well as to A23187, which acts as a calcium carrier, bypassing the calcium channels (10, 16, 17). Rather than enhancing the cellular influx of calcium, the microorganisms and LPS might therefore interfere with the next steps such as calcium sequestering or

some calcium-dependent step leading to mediator release.

Influenza A virus appears to act by its neuraminidase located on the surface, since the potentiating effect of the virus is mimicked by isolated neuraminidase (3) and abolished by either monoclonal antibodies directed against the viral neuraminidase or by the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (2). Isolated neuraminidase enhances basophil histamine release to anti-IgE, A23187 and to extracellular calcium (15).

It appears that carbohydrate residues in the microorganisms and LPS are responsible for the potentiation by interacting with binding sites on the basophil cell membrane. *Firstly*, the neuraminidase portion of influenza A virus contains carbohydrates such as galactose and N-acetylglucosamine (7). Endotoxins as *S. typhimurium* LPS also contain these sugars (11) and N-acetylglucosamine is found in the peptidoglycan structure of bacteria (23), and peptidoglycan isolated from *S. aureus* shows a potentiating effect (22). *Secondly*, the potentiation by influenza A virus, *S. aureus* and *S. typhimurium* LPS is inhibited or abolished by low concentrations (10^{-7} – 10^{-6} M) of the free sugars, galactose or N-acetylglucosamine (21). It is therefore possible that the microorganisms and LPS enhance the basophil cell response to IgE-dependent and non-immunological stimuli by such an interaction between microbial sugars and binding sites on the basophilocytes, leading to a change in the subcellular handling of calcium rather than to its cellular influx.

It is tempting to speculate that these effects of virus, bacteria and endotoxin, which include enhancement of mediator release and lowering of the threshold to allergens and other histamine releasing stimuli, might play a role in obstructive airway diseases in connection with airway infections by triggering or aggravating asthmatic attacks.

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REFERENCES

- Busse WW. The relationship between viral infections and onset of allergic disease and asthma. *Clin Exp Allergy* 19, 1-9, 1989.
- Clements P, Douglas AR, Skehel JJ, 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.
- Clements P, Hannoun C, Norn S. Influenza A virus enhances allergic histamine release. Effect of neuraminidase. *Allergy* 44, 33-38, 1989.
- Clements P, Jensen CB, Hannoun C, Soborg 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.
- Clements P, Milman N, Kilian M, Fomsgaard A, Bæk L, Norn S. Endotoxin from *Haemophilus influenzae* enhances IgE-mediated and non-immunological histamine release. *Allergy* 45, 10-17, 1990.
- Clements P, Pedersen M, Permin H, Espersen F, Jarlov JO, Norn S. Virus enhances IgE- and non-IgE-dependent histamine release induced by bacteria and other stimulants. *Agents Actions* 30, 61-63, 1990.
- Colman PM, Ward CW. Structure and diversity of influenza virus neuraminidase. *Current Topics Microbiol. Immunol* 114, 177-255, 1985.
- Espersen F, Jarlov JO, 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.
- Foreman JC. Calcium and histamine secretion from mast cells and leukocytes. In: *Biochemistry of the acute allergic reactions*, pp. 315-328. Alan R. Liss, New York, 1981.
- Foreman JC, Mongar JL, Gomperts BD. Calcium ionophores and movement of calcium ions following the physiological stimulus to a secretory process. *Nature* 245, 249-251, 1973.
- Hitchcock PJ, Leive L, Mäkelä PH, Rietschel ET, Strittmatter W, Morrison DC. Lipopolysaccharide nomenclature - past, present and future. *J Bacteriol* 166, 699-705, 1986.
- Horn MEC, Reed SE, Taylor P. Role of viruses and bacteria in acute wheezy bronchitis in childhood: a study of sputum. *Arch Dis Child* 54, 587-589, 1979.
- Ishizaka T. Analysis of triggering events in mast cells for immunoglobulin E-mediated histamine release. *J Allergy Clin Immunol* 67, 90-96, 1981.
- Ishizaka T, Ishizaka K. Biology of immunoglobulin E. *Prog Allergy* 19, 60-121, 1975.
- Jensen C, Henriksen U, Dahl BT, Skov P Stahl, Norn S. Influence of neuraminidase and N-acetylneuraminic acid on basophil histamine release in vitro. *Allergy* 41, 151-156, 1986.
- Kazimierzczak W, Diamant B. Mechanisms of histamine release in anaphylactic and anaphylactoid reactions. *Prog Allergy* 24, 295-365, 1978.
- Lichtenstein LM. The mechanism of basophil histamine release induced by antigen and by the calcium ionophore A23187. *J Immunol* 114, 1692-1699, 1975.
- Mosbech H, Skov P Stahl. House dust mite-induced histamine release from washed blood cells. Evaluation of effect parameters. *Allergy* 42, 553-555, 1987.
- Norn S. A medical hypothesis. Bacteria-induced histamine release: possible relationship to asthma. *Rev Fr Allergol* 28, 199-203, 1988.
- Norn S, Bæk L, Jensen C, et al. Influence of bacterial endotoxins on basophil histamine release. Potentiation of antigen- and bacteria-induced histamine release. *Allergy* 41, 125-130, 1986.
- Norn S, Clements P, Kristensen KS, Hannoun C, Jarlov JO. Carbohydrates inhibit the potentiating effect of bacteria, endotoxin and virus on basophil histamine release. *Agents Actions* 30, 53-56, 1990.
- Norn S, Jarlov JO, Jensen CB, et al. Bacteria and their products peptidoglycan and teichoic acid potentiate antigen-induced histamine release in allergic patients. *Agents Actions* 20, 174-177, 1987.
- Schleifer KH, Kandler O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 36, 407-477, 1972.
- Skov P Stahl, 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.
- Welliver RC. Upper respiratory tract infections in asthma. *J Allergy Clin Immunol* 72, 341-346, 1983.

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