

Bacteria-induced histamine release from human bronchoalveolar cells and blood leukocytes

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Histamine release induced by *Staphylococcus aureus* was examined in cells obtained by bronchoalveolar lavage (BAL) in non-atopic individuals. Approximately half of the individuals responded with mediator release to the bacterium, and the release was found to be time- and concentration dependent. No difference was found between the patients who responded and those who did not respond in regard to age, sex, smoker/non-smoker, % recovery of BAL-fluid, total cell count, differential cell counts, histamine content per mast cell, or diagnoses. Also stimulation of the BAL-cells with the calcium-ionophore A23187 resulted in histamine release. *S. aureus*-induced histamine release from basophils was examined in leukocyte suspensions obtained from the same individuals, and in all experiments release was found. The dose-response curves were similar to those obtained with BAL cells. The bacteria-induced mediator release from superficially lying cells in the airways epithelium might be of importance for the precipitation or exacerbation of bronchial asthma in respiratory tract infections.

Key words: asthma; bacteria; basophils; bronchoalveolar lavage; calcium ionophore; histamine release.

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CLINICAL ASPECTS

Microorganisms are suspected of playing a role in acute exacerbations of asthma. The finding that *S. aureus* induces histamine release from cells obtained by BAL in non-atopic individuals might be of importance in bacterial infections for the conversion from latent to manifest asthma. Whether BAL cells from asthmatics will show an increased response to bacteria, has to be investigated.

Respiratory tract infections often precipitate or exacerbate attacks of bronchial asthma (4, 12, 20). We have previously shown that several species of bacteria cause release of histamine from human basophil leukocytes *in vitro* (22). In the present study we investigated whether bacteria could trigger histamine release from cells obtained by bronchoalveolar lavage (BAL). Further, we compared results obtained with leukocytes and BAL cells from the same donors.

MATERIAL AND METHODS

This study comprised 40 non-atopic patients without asthma (19 women, 23 smokers, median age 59 years, range 21-77) with clinical indication for fiberoptic bronchoscopy, i.e. bronchopulmonary symptoms and/or chest X-ray lesions. No patients received antibiotics or steroids. All gave informed consent for BAL.

The bacteria used were whole formalin-killed *Staphylococcus aureus* Wood 46 (protein A-deficient) prepared according to Espersen et al. (9). Calcium ionophore A23187 was from Calbiochem AG, Switzerland.

Cells were obtained by BAL using an Olympus BF-10 fiberoptic bronchoscope in the lung opposite to any localised bronchoscopic or radiological abnormality, using the segments of the right middle lobe or left lingula. Premedication consisted of diazepam, midazolam and atropine, and local anaesthesia was achieved with topical lidocaine. Prewarmed isotonic saline (5 × 60 ml) was instilled and approx. 60% was recovered in human serum albumin-coated glass bottles at room temperature. The BAL fluid was filtered through sterile gauze and the cells were recovered by centrifugation (1860 *g*, 10 min, 16°C) washed twice (830 *g*, 10 min, 4°C) and resuspended in glucose-free Tris-AMC containing 25 mM Tris at pH 7.6, 0.12 M NaCl, 5 mM KCl, 0.6 mM CaCl₂, 1.1 mM MgCl₂, and 0.3 mg human serum albumin/ml (28).

Total cell numbers were determined after resuspension of the cells in Tris-AMC using a Fuchs-Rosenthal counting chamber. Cytocentrifuge smears were made and dried in air. Differential cell counts were performed by counting a minimum of 400 cells on cytocentrifuge smears stained with hematoxylin-eosin. Esterase staining was used for determination of alveolar macrophages (13) and the number of mast cells was determined by counting a minimum of 4000 cells on cytocentrifuge smears fixed with Carnoy's fluid and stained in the alcian blue safranin reaction (11).

Histamine release experiments were performed by incubation of 45 µl cell suspension containing 1.3×10^6 BAL cells (range 0.6 – 2.5×10^6) for 20 min at 37°C with either 5 µl of the diluted preparation of *S. aureus*, 5 µl A23187 or 5 µl Tris AMC. Thereafter 200 µl ice-cold isotonic saline containing 0.3 mg human serum albumin/ml was added and the samples were centrifuged at 2100 *g* for 10 min at 4°C. The supernatants containing released histamine were discarded and the residual histamine was determined in the cell sediment according to the

method described by Shore (27). To the cell sediment (50 µl) was added 40 µl 0.6 M HClO₄ and 1050 µl H₂O, and the samples were centrifuged at 4300 *g* for 10 min at 16°C. 1000 µl of the supernatant was transferred to polypropylene tubes containing 500 mg of solid NaCl, 100 µl of 5 N NaOH, and 3.3 ml of *n*-butanol. The tubes were shaken for 5 min to extract the histamine into the butanol, and after centrifugation (910 *g*, 5 min, 20°C), 3 ml of the butanol-phase was shaken with 1.7 ml NaCl-saturated 0.1 N NaOH for 2 min. After centrifugation (910 *g*, 5 min, 20°C) 2.6 ml of the butanol phase was shaken with 850 µl 0.1 N HCl and 4 ml *n*-heptan for 2 min. The tubes were centrifuged (550 *g*, 5 min, 20°C), and the histamine in the aqueous phase (750 µl) was assayed spectrofluorometrically by addition of 150 µl 1 N NaOH, 40 µl of a *o*-phthal-dialdehyde solution (10 mg OPT/ml methanol), and after 4 min 75 µl 3 N H₃PO₄, whereafter the samples were centrifuged (2700 *g*, 10 min, 20°C) and read at 355/455 nm (excitation/emission). The release of histamine was expressed as a percentage of the total histamine content of the sample. All values were corrected for the spontaneous release, which amounted to up to 5%. Standard histamine curves were run in parallel.

For preparation of leukocytes, blood anticoagulated with EDTA in a final concentration of 0.01 M was obtained from each patient. The blood was mixed with double volume of 0.9% NaCl and the leukocytes with approx. 2% basophils were obtained by Ficoll-Hypaque gradient centrifugation (28). The leukocytes were washed twice and suspended in a glucose-free Tris-AMC buffer as mentioned above (28).

Histamine release experiments were performed by incubation of 45 µl leukocyte suspension for 40 min at 37°C with 5 µl of the diluted preparation of *S. aureus* or 5 µl Tris-AMC. The bacteria-induced release of histamine was assayed spectrofluorometrically by determination of the residual histamine in the washed cell sediment (28). All values were corrected for the spontaneous release, which amounted to up to 10%. Leukocytes and BAL cells were run in parallel.

Table 1.

Percentage recovery of BAL fluid, total cell numbers and cellular composition in 40 non-atopic patients without asthma

Fluid recovery		60.8±1.7
Total cells (x10 ⁷)		2.6±0.3
Macrophages	%	83.7±1.8
Mast cells	%	1.0±0.2
Lymphocytes	%	5.2±1.3
Neutrophils	%	5.4±1.1
Other cells	%	4.6±1.0

(Mean±SEM is given)

Statistics. Student's t-test was used.

RESULTS

The cellular composition of the fluid recovered by BAL in 40 non-atopic subjects is given in Table 1. The histamine content per mast cell (MC) was calculated in 20 subjects on the basis

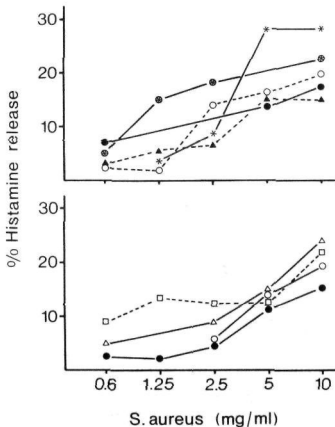


Fig. 1. Histamine release induced by *S. aureus* in BAL cells from 9 non-atopic individuals. Mean of duplicate determinations is given.

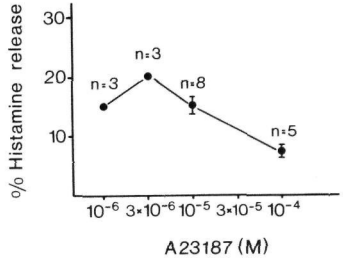


Fig. 2. Calcium ionophore A23187-induced histamine release in BAL cells. Mean of *n* experiments in duplicate ± SEM.

of the histamine content of 10⁶ BAL cells and the percentage of mast cells in this population, and was found to be 7.5±2.8 pg histamine/MC (mean ± SEM).

BAL cells from 12 patients were used for preliminary histamine release studies (results not shown). Histamine release induced by *S. aureus* was examined by stimulating BAL cells from 20 individuals with the bacterium in final concentrations from 0.6 to 10.0 mg/ml wet weight. A concentration-related release of histamine was found in experiments with BAL cells from nine subjects (*S. aureus*-responders) and the maximal release amounted from 15 to 30% with a mean of 20% (Fig. 1). However, no release was found with BAL cells from 11 individuals (*S. aureus*-non-responders).

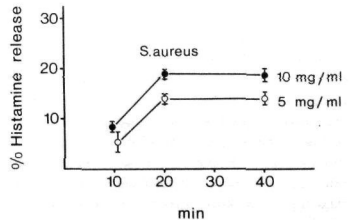


Fig. 3. Time course for *S. aureus*-induced histamine release from BAL cells. Mean of 5 experiments in duplicate ± SEM.

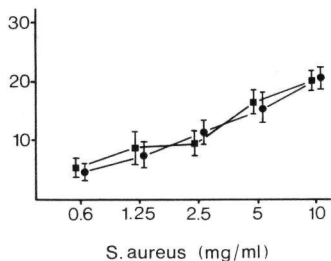


Fig. 4. Histamine release from basophil leukocytes induced by *S. aureus*. The leukocytes were obtained from individuals whose BAL cells responded (closed symbols) and did not respond (open symbols) with histamine release to the bacterium. Mean of 9 and 11 experiments in duplicate $\pm$ SEM.

Calcium ionophore-induced histamine release was assessed in BAL cells from eight individuals. A23187 was used in a final concentration of 10^{-5} M, but in some experiments also lower and higher concentrations were used (Fig. 2). Maximal histamine release was obtained at 3×10^{-6} M.

To investigate the time course of the bacteria-induced histamine release, BAL cells were stimulated with the two concentrations of *S. aureus* giving maximal release (i.e. 5 and 10 mg/ml) for 10, 20 and 40 min. Fig. 3 shows that the release was completed within 20 min, since a plateau was reached at this time.

The basophil cell response to *S. aureus* was examined by stimulating blood leukocytes obtained from the BAL-examined *S. aureus*-responders and *S. aureus*-non-responders with the bacterium. Basophil histamine release was obtained in all experiments, and no significant difference was found between the dose-response curves from the two groups (Fig. 4). Fig. 5 demonstrates that *S. aureus* induced a similar release of histamine from BAL cells and leukocytes from the responding individuals; the two cell types were obtained from the nine *S. aureus*-responders and stimulated with the bacterium in parallel.

No significant difference was found between the BAL-examined patients who responded and

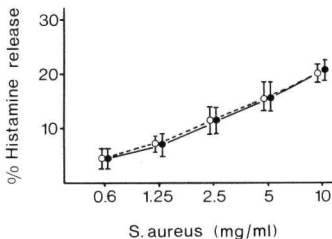


Fig. 5. Comparison of *S. aureus*-induced histamine release from BAL cells (■) and basophils (●). The two cell types were obtained from the same 9 individuals and were run in parallel. Mean $\pm$ SEM of duplicate determinations.

those who did not respond with histamine release to *S. aureus* according to age, sex, smoker/non-smoker, % recovery of BAL fluid, total cell counts, differential cell counts, and histamine content per MC (data not shown). Further, responders and non-responders were not related to a certain kind of diagnosis, since 12 had normal chest X-ray and bronchoscopy and the ratio responders/non-responders was 7/5, 5 lung cancer (ratio 1/4), 1 sarcoidosis (1/0), 1 systemic lupus erythematosus (0/1) and 1 Hodgkin's disease (0/1). There was no correlation between the number of mast cells and either smoking, age or sex. No difference in BAL-fluid recovery, total cell count or cellular composition was found between smokers and non-smokers (data not shown).

DISCUSSION

Various gram-positive and gram-negative bacteria have been found to release histamine from human cells including basophil leukocytes (22), tonsillar mast cells (6), nasal mucosa (2) and pulmonary mast cells obtained by enzymatic dispersion of lung tissue (6). Protein A from *S. aureus* release histamine from basophils (5, 19, 25), but not from parenchymal lung mast cells (5), whereas bacterial antigens (manufactured by Bencard) have been demonstrated to release histamine from BAL cells (3). Further, virus,

bacteria and endotoxins potentiate IgE-dependent and non-immunological histamine release from human basophil leukocytes (24).

In this study the release of histamine was examined from superficially lying mast cells in the airways epithelium obtained by bronchoalveolar lavage in non-atopic individuals. Whole formalin-killed *S. aureus* caused histamine release in BAL cells from half of the subjects. It was not possible to demonstrate a difference between responders and non-responders as regards of the cellular composition of the BAL cells, age, sex or smokers/non-smokers. A general diminished cell response to the bacterium was excluded, since in all experiments the basophil leukocytes were found to release histamine by stimulation with *S. aureus* and this release did not differ between responders and non-responders. However, it is possible that cellular events in the mast cells not appearing in the basophils could be responsible for the response/no response in BAL cells.

The time course of the *S. aureus*-induced histamine release from BAL cells showed that the mediator release was completed after 20 min. When the kinetics were examined in connection with anti-IgE, maximal release was achieved already within 2 min and almost one-third of this within 30 s (11). A similar dissociation was obtained in human tonsillar mast cells and, furthermore, the characteristics of mediator release with bacteria were quite different from that of IgE-dependent release in respect to temperature and pH profiles and lack of desensitization to anti-IgE (6). It is therefore suggested that the bacteria-induced histamine release from the BAL cells in analogy with that from the tonsillar mast cells is triggered by a non-immunological mechanism and not by crosslinking of IgE. Examinations of basophil histamine release triggered by bacteria suggest that the non-immunological mechanism depends on a sugar-lectin mediated reaction in which sugars on the bacterial cell wall interact with lectins on the target cell membrane leading to histamine release (15, 22). Other non-immunological mechanisms might also co-operate, since lectin binding sugars mostly caused only a partial inhibition of the histamine release induced by

bacteria (6, 16). However, bacteria-induced histamine release caused by an IgE-dependent mechanism might occur in different patient categories since this mechanism has been demonstrated in studies of basophil histamine release in patients suffering from asthma (22), sepsis (8, 22) or Job's syndrome of immune deficiency (15, 22).

Whether BAL cells from patients with extrinsic asthma will show an increased response to bacteria is now being investigated. However, enhanced cell response to anti-IgE has already been demonstrated, resulting in a greater release of histamine at all effective dilutions of the antiserum (5, 10, 17), a finding which was not confirmed by Wardlaw et al. (30). Enhanced release of histamine by the calcium ionophore A23187 could not be demonstrated in BAL cells from asthmatic subjects (5). Aggravation of mediator release in the asthmatics could also be obtained by an increased number of mast cells. Indeed, in asthmatics, increased number of mast cells was observed in the bronchial mucous membrane by biopsy (18) and a 3- to 6-fold increase in the percentage of mast cells was found in the lavage fluid (10, 29, 30), and this increase is reflected in a parallel elevation of the histamine content of the lavage cells (10, 17, 30).

When comparing the mediator release from BAL cells and leukocytes we found no difference in the bacteria-induced histamine release in the responding subjects. A similarity between these cell types is also observed in calcium ionophore-induced release, since maximal release is obtained by 10^{-6} to 3×10^{-6} M A23187 ((5, 7) and Fig. 2), but differences exist in the response to anti-IgE (5, 17). In BAL cells the histamine content was 7.5 pg mast cell which is within the range (1 to 15 pg/cell) found by others in non-asthmatics (1, 5, 11, 29, 30).

The recovery of BAL fluid and total cell count were higher (approx. 50%) than found by others (1, 10, 11, 17, 26, 29, 30), whereas the cellular composition (Table 1) corresponds with that for non-asthmatic individuals (10, 11, 17, 26, 29). Our finding of 4.6% "other cells" is comparable with data for eosinophils and epithelial cells in subjects without asthma (17, 29). Smoking increases the number of macrophages and neutro-

phils in BAL fluid (14, 21), but we did not find such an increase. No correlation was found between the number of mast cells and smoking, age or sex, which is in accordance with Tomioka (29).

The initial step in the pathophysiological mechanism of asthma takes place on the surface of the airways epithelium, where release of histamine from mast cells may increase mucosal permeability by opening of tight junctions (23). That bacteria can trigger histamine release from these superficially lying mast cells, might be of importance for the precipitation or exacerbation of bronchial asthma by infections.

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