

The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and postchallenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein X

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The late asthmatic response to bronchial allergen challenge and the associated increase in nonspecific bronchial reactivity provides a model for studying the acute inflammatory mechanisms in asthmatic airways. In 12 asthmatic patients (aged 22-37 years) with known dual reaction to allergen challenge, salmeterol 50 µg, 100 µg, or placebo was administered as a single dose 10 min before allergen challenge in a double-blind, randomized order on three different study days 2 weeks apart. The bronchial reactivity (BH) to histamine was measured the day before and 24 and 48 h after allergen challenge. Salmeterol significantly inhibited the early ($P < 0.02$) and the late ($P < 0.05$) asthmatic reactions. After placebo, mean BH was significantly increased above base line at 24 and 48 h ($P < 0.02$). After 50 and 100 µg salmeterol, BH was less than base line at 24 h and returned to prechallenge values at 48 h. Blood eosinophils increased significantly ($P < 0.05$) 24 and 48 h after allergen challenge, and no difference was found between treatments. After pretreatment with placebo, serum eosinophil cationic protein (s-ECP) and serum eosinophil protein X (s-EPX) increased significantly ($P < 0.05$) 24 and 48 h after allergen challenge. After treatment with salmeterol 50 µg, s-EPX, but not s-ECP, increased significantly 24 h after challenge, but was normal at 48 h. After salmeterol 100 µg, no change in s-EPX or s-ECP was found. The results showed that salmeterol eliminated the allergen-induced dual asthmatic reaction and gave protection against increased BH. Measurement of blood eosinophils, s-ECP, and s-EPX indicated that salmeterol may modify this inflammatory cell activity, because raised s-levels of ECP and EPX may be linked to eosinophil activation and granule secretion. In addition to inducing a long-lasting bronchodilation, salmeterol may influence acute inflammation.

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Lung challenge with inhaled extracts of common aeroallergens in selected subjects results in the development of an early asthmatic reaction (EAR) followed by a late asthmatic reaction (LAR)(22). Development of the LAR is associated with an increase in bronchial reactivity (6) and accumulation of inflammatory cells in the bronchial mucosa (21). In

addition, after the LAR a rise occurs in the levels of blood eosinophils, serum eosinophil cationic protein (s-ECP), and serum eosinophil protein X (s-EPX) (29) at 24 h after challenge (8) and in eosinophil number in bronchial lavage fluid after 6 h (10). The lavage fluid eosinophilia is accompanied by an elevated ECP/albumin ratio (9).

The late asthmatic response to bronchial allergen challenge and the associated increase in nonspecific bronchial reactivity provides a model for studying the acute inflammatory mechanisms in asthmatic airways. The aim of this study was to investigate the influence of inhaled salmeterol, a long-acting β_2 -adrenoceptor agonist, on the allergen-induced EAR and LAR and the postchallenge variation in bronchial reactivity and levels of blood eosinophils, s-ECP, and s-EPX as a measure of eosinophil activity.

Methods

Patients

Twelve patients, three women and nine men, with a mean age of 28 years (range 22–37 years) were selected for the study. All patients had bronchial asthma as defined by the American Thoracic Society (1). The patients gave informed consent and the study was approved by the Ethics Committee of Aarhus and the National Board of Health and Welfare and was performed according to the Declaration of Helsinki.

Base-line lung function measured as forced expiratory volume in 1 s (FEV_1) with a dry spirometer (Vitalograph, Model S, Vitalograph Ltd, Buckingham, UK) was 96.5% of predicted normal value (range 78.0–114.3%) (12). All patients used inhaled bronchodilators when needed and had not been treated with corticosteroids, disodium cromoglycate, or oral bronchodilators for the previous 6 months. Inhaled bronchodilator therapy was withheld for 8 h before challenge.

All patients had an allergic component to their disease and had a positive skin prick test, RAST, and inhalation challenge test to the allergen used in the study. Table 1 gives the demographic details of the patients and the allergens used. Allergens were

purchased from ALK, Copenhagen, Denmark, and were standardized and partially purified extracts.

Study design

The study consisted of three periods: control, study, and base-line.

Control period. On control day 1, base-line FEV_1 was measured and base-line bronchial reactivity to histamine was determined. On the next day, bronchial allergen challenge was performed and the dose of allergen was determined that resulted in an EAR followed by an LAR. On the first 2 days in each subsequent challenge sequence, base-line FEV_1 had to be within 15% of the value measured on control day 1. On the first day in each subsequent challenge sequence, bronchial reactivity had to be within two doubling histamine concentrations of the base-line reactivity.

Study period. The study period consisted of three allergen challenges with 2 weeks' interval between each challenge. Salmeterol 50 μ g, 100 μ g, or placebo was inhaled from a metered dose aerosol 10 min before allergen challenge in a double-blind, randomized, three-way, cross-over design. Allergen challenge was performed with the predetermined allergen dose. FEV_1 was measured as described (see bronchial allergen challenge procedure). After the last measurements of FEV_1 at 12 h, the patients inhaled 0.2 mg of salbutamol and abstained from inhaled β_2 -agonists until the next morning. Bronchial reactivity to histamine was measured 24 h before and 24 and 48 h after allergen challenge. A blood sample was collected on each study day before the histamine and allergen challenges to measure blood eosinophil count and s-ECP, and s-EPX concentrations.

Table 1. Characteristics of the patients and the allergen-challenge dose

Patient	Age (years)	Sex	Duration of asthma (years)	FEV_1 % predicted	S-IgE IU/ml	Allergen	RAST PRU/ml	No. of inhalations	Concentration (SQ-U/ml)
B.H.	37	F	25	94.8	186	Timothy	30.3	2	100 000
H.S.	30	M	26	110.7	71	<i>D. pter.</i>	17.4	25	100 000
B.H.	31	M	4	94.9	79	Timothy	21.7	5	10 000
K.N.	22	M	1	82.2	452	Timothy	28.2	2	75 000
J.T.	26	M	12	96.7	502	<i>D. pter.</i>	24.3	5	100 000
S.K.	26	F	4	114.3	114	<i>D. pter.</i>	20.8	8	10 000
P.B.	37	M	8	88.9	107	<i>D. pter.</i>	16.7	4	100 000
E.J.	29	M	7	78.0	113	<i>D. pter.</i>	10.8	8	100 000
J.P.	28	M	22	100.2	474	<i>D. pter.</i>	17.5	30	100 000
L.H.	25	M	1	93.4	186	<i>D. pter.</i>	16.7	4	100 000
T.R.	22	F	4	102.4	253	<i>D. pter.</i>	24.7	12	100 000
K.G.	23	M	10	101.4	519	<i>D. pter.</i>	26.6	24	100 000

S-IgE = serum IgE; SQ-U/ml = standardized quality units per milliliter; *D. pter.* = *Dermatophagoides pteronyssinus*; PRU/ml = Phadebas RAST units per ml.

Base-line period. Diluent challenge was performed on 2 days with 2 weeks' interval. Salmeterol 50 µg or placebo was inhaled from a metered dose aerosol 10 min before diluent challenge in a double-blind, randomized, cross-over design. FEV₁ was measured as described for bronchial allergen challenge.

An interval of 2 weeks separated the control period, the study period, and the base-line period.

Bronchial allergen challenge

The allergen solutions were prepared from freeze-dried allergen in diluent (saline containing 0.5% albumin) to the final concentrations and stored at 4°C. Immediately before challenge the allergen solution was warmed to room temperature. The challenge was performed with a Wright nebulizer with a small reservoir which makes it possible to operate the nebulizer with 0.2–1.10 ml solution in the chamber (19). The nebulizer was calibrated to give a constant output of 0.13–0.15 ml/min. The mist was inhaled during tidal breathing through a mouthpiece and the nose was clipped. FEV₁ was measured before the start of the allergen challenge procedure, and FEV₁ after inhalation of diluent was used as base line for the allergen challenge. The allergen dose was established that resulted in an EAR defined as a fall in FEV₁ of 20% or more within 60 min and an LAR defined by a fall of 20% or more in FEV₁ between 2 and 9 h after challenge. FEV₁ was measured at 5, 10, 15, 20, 30, 45, and 60 min and every hour between 2 and 12 h.

Histamine challenge

Bronchial reactivity to histamine was performed in accordance with a method described by Cockcroft et al. (5). The aerosol was generated by a Wright nebulizer with 2 ml solution calibrated to give a constant output of 0.13–0.15 ml/min. The mist was inhaled through a mouthpiece and the nose was clipped. Histamine was inhaled for 2 min during tidal breathing. FEV₁ was measured before the start of the procedures and 30 and 90 s after termination of the inhalation. FEV₁ after isotonic saline was used as base line. Histamine dihydrochloride was inhaled in doubling concentrations from 0.03 mg/ml to 32.0 mg/ml. The result was expressed as the provocation concentration giving a 20% fall in FEV₁ (PC₂₀), obtained from the log dose-response curve by linear interpolation of the two last points.

Bronchial diluent challenge

Bronchial diluent challenge (isotonic saline containing 0.5% albumin) was performed on two occasions

with the Wright nebulizer with a small reservoir as previously described. Eight inhalations of diluent were given two times at 5-min intervals. FEV₁ was measured before the challenge and then at 5, 10, 15, 20, 30, 45, and 60 min and every hour between 2 and 12 h. The last measurement was performed 24 h after the challenge.

Laboratory analyses

A venous blood sample was collected, and the eosinophil number was counted in a Fuchs-Rosenthal chamber after staining with eosinophil staining solution (sodium citrate 0.3%, acetone 15%, eosin 0.1% w/v). The blood samples were stored 1 h at room temperature before centrifugation at 1600 g at 4°C for 10 min. Serum was collected and recentrifuged at 1600 g, 4°C for 10 min. The collected samples were stored in 1-ml aliquots at –70°C until analyzed. The concentrations of ECP and EPX were measured by radioimmunoassay (23, 30).

Statistics

The randomization for treatment allocation was made by a computer program. All individual FEV₁ values measured during allergen and diluent challenge days were expressed as percentages of predicted normal values. Changes in FEV₁ on allergen and diluent challenge days were expressed by the FEV₁ value measured after diluent inhalation as base line.

Twelve patients were challenged with allergens and in 10 cases diluent challenges were performed. In these 10 patients the corresponding FEV₁ values expressed as percentages of predicted normal value were subtracted to make a correction for the spontaneous and drug-induced variation in FEV₁ during the day. Results are given as mean values, and statistical analyses were made by Student's paired t-tests.

PC₂₀ histamine values were analyzed after logarithmic transformation. The changes in histamine reactivity (Δ PC₂₀) were expressed in doubling dilutions of histamine, the value 24 h before allergen challenge being used as base line (Δ PC₂₀ = log₂ [PC₂₀ base line] – log₂ [PC₂₀ test]). Statistical analyses were made by Student's paired t-tests.

Levels of blood eosinophils, s-ECP, and s-EPX are given as median values. Changes were expressed with the value on the allergen challenge day as base line. Statistical analyses were made by two-tailed Wilcoxon signed rank tests. *P* values equal to or less than 0.05 were considered to be statistically significant.

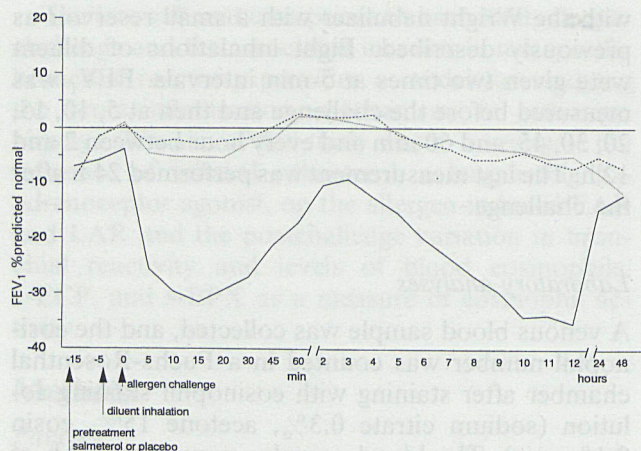


Fig. 1. Mean values of FEV₁ (% predicted normal) after allergen challenge and pretreatment with placebo —, salmeterol 50 µg ----, or 100 µg

Results

After placebo, the patients developed an EAR followed by an LAR, and the responses were similar to the base-line challenge. FEV₁ increased significantly ($P < 0.001$) after 50 and 100 µg salmeterol. There was a significant inhibition throughout ($P < 0.02$) the EAR and the LAR by salmeterol, and no difference was found between 50 and 100 µg (Fig. 1). FEV₁ increased significantly ($P < 0.05$) after diluent challenge on both diluent days (Fig. 2) with no difference between pretreatments of inhaled salmeterol or placebo.

The FEV₁ values measured after diluent inhalation and pretreatment with salmeterol 50 µg and placebo were subtracted from the corresponding values after allergen challenges and respective pretreatment. In this way a correction was obtained for the

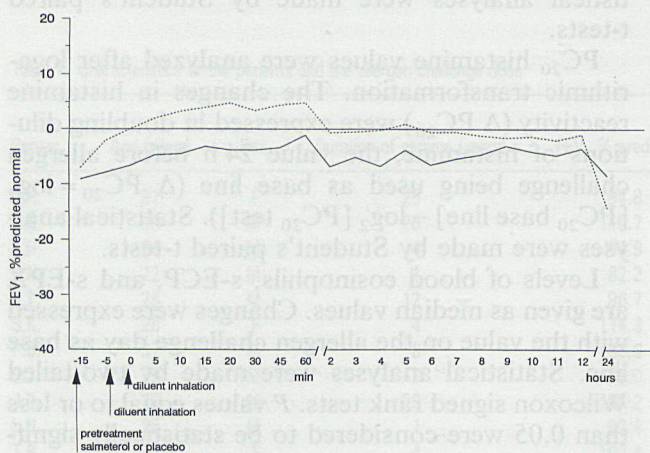


Fig. 2. Mean values of FEV₁ (% predicted normal) after diluent challenge and pretreatment with placebo —, and salmeterol 50 µg ----.

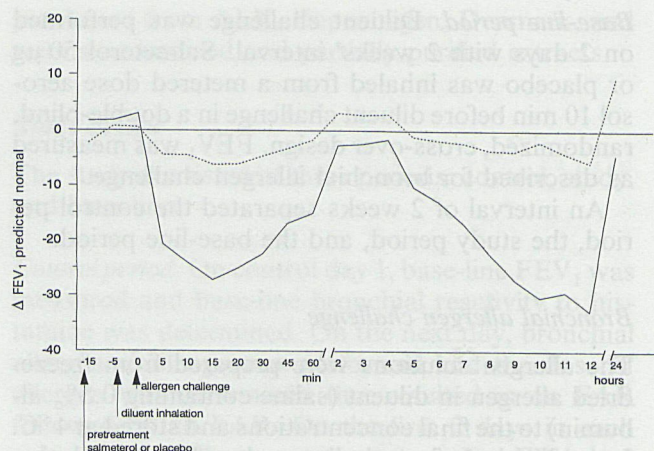


Fig. 3. Mean values of ΔFEV₁ (% predicted normal) after diluent challenge values were subtracted from values after allergen challenges, after treatment with placebo —, or salmeterol 50 µg ----.

spontaneous and drug-induced FEV₁ variation during the day. Even with this correction salmeterol eliminated the EAR and the LAR ($P < 0.05$) (Fig. 3).

After placebo, PC₂₀ histamine decreased significantly ($P < 0.02$) 24 and 48 h after allergen challenge (Fig. 4). After 50 and 100 µg salmeterol, PC₂₀ histamine increased at 24 h and returned to prechallenge values after 48 h.

Blood eosinophils increased significantly ($P < 0.05$) 24 and 48 h after allergen challenge, and no difference was seen between the pretreatments with placebo and salmeterol (Fig. 5). After placebo, s-ECP increased significantly ($P < 0.05$) 24 and 48 h after challenge (Fig. 6). However, after 50 and 100 µg salmeterol, s-ECP did not increase. S-EPX increased significantly 24 and 48 h after allergen challenge and placebo treatments (Fig. 7). After salmeterol 50 µg, s-EPX increased significantly 24 h after challenge and returned to prechallenge values after 48 h. No changes were found in s-EPX concentration after allergen challenge and pretreatment with 100 µg salmeterol.

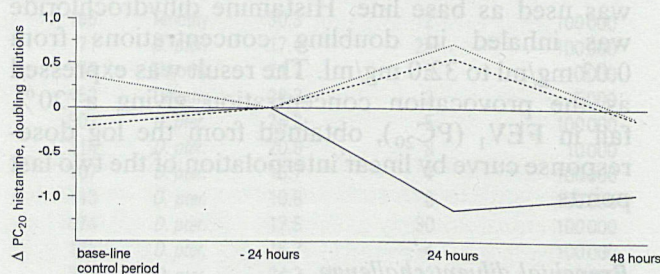


Fig. 4. ΔPC₂₀ histamine (doubling dilutions) before and 24 and 48 h after allergen challenge, after pretreatment with placebo —, salmeterol 50 µg ----, or 100 µg

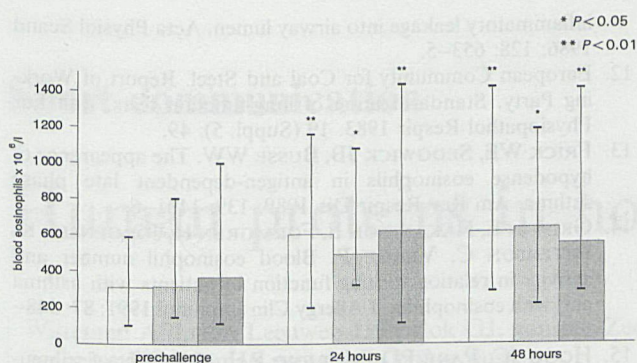


Fig. 5. Values of blood eosinophils before (prechallenge) and 24 and 48 h after allergen challenge, after treatment with placebo □, salmeterol 50 µg □, or 100 µg ■. Median and range.

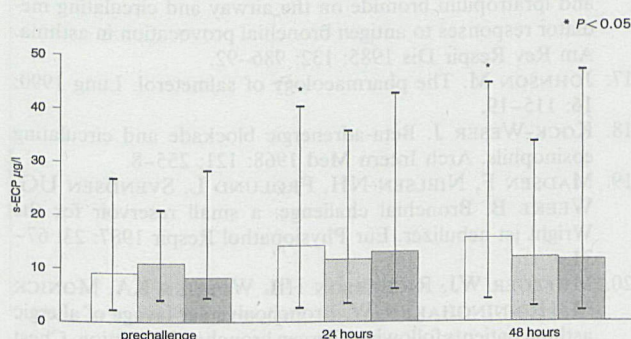


Fig. 6. Values of s-eosinophil cationic protein (ECP) before (prechallenge) and 24 and 48 h after allergen challenge, after treatment with placebo □, salmeterol 50 µg □, or 100 µg ■. Median and range.

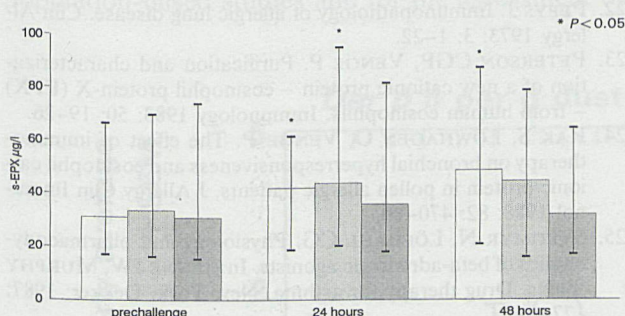


Fig. 7. Values of s-eosinophil protein X (EPX) before (prechallenge) and 24 and 48 h after allergen challenge, after treatment with placebo □, salmeterol 50 µg □, or 100 µg ■. Median and range.

Discussion

The present study confirmed the results of Twentyman et al. (26) who showed protection against the EAR, the LAR, and the postchallenge increase in histamine reactivity. We compared 50 and 100 µg salmeterol but found that both doses were equally effective at eliminating the allergen-induced early- and late-phase reactions. In the present study the increased bronchial histamine reactivity after 24 and 48 h was also eliminated. It is established that in-

haled β_2 -agonists effectively inhibit the EAR. This may be caused by a combination of inhibition of mast cell mediator release (16) and smooth-muscle relaxation (25). Likewise, the inhibition of the LAR may be caused both by long-lasting bronchodilator activity and by inhibition of the pathogenetic mechanisms operating in the evolution of the LAR and associated increase in bronchial reactivity. The exact mechanisms of the LAR are unknown; however, it is associated with an influx of cells into the bronchial wall (20) and plasma exudation. This creates an increase in airway wall thickness, with increased airway resistance after challenge as the explanation of the increased airways reactivity after an LAR (15). β_2 -adrenoceptor agonists can inhibit plasma exudation (11); indeed, a long-lasting inhibition or histamine-induced plasma leakage has been observed with salmeterol in experimental models (2, 4, 17).

Blood eosinophils have surface β_2 -adrenoceptors (31). *In vivo* studies have found that blood eosinophil count (7, 17) and s-ECP (7) are reduced after systemic treatment with β_2 -agonists. *In vitro* studies on purified eosinophils found no influence on stimulated superoxide generation after incubation with a β_2 -agonist. This discrepancy between *in vivo* and *in vitro* findings on β_2 -agonist influence on eosinophil function needs further study. Different eosinophil functions have been studied in the above mentioned publications. It is possible that *in vivo* findings on eosinophil behavior reflect not only a direct influence of β_2 -agonists on eosinophils but also indirect mechanisms governing eosinophil distribution and secretory function.

During the LAR, eosinophil activation (9, 13, 28) and recruitment to the airways has been found. S-ECP and s-EPX are markers of eosinophil activity and several studies have verified an association between ECP and indices of asthma severity. A negative correlation was found between lung function and s-ECP (14), and a positive correlation was evident between clinical asthma severity and the concentration of ECP in bronchoalveolar lavage (3). After natural exposure to birch pollen, allergic patients with asthma had an increased level of ECP in serum, and this correlated with PC₂₀ histamine (24). Finally, blood eosinophils and s-ECP increase after an LAR (8, 10). Systemic (7), but not inhaled (29), β_2 -adrenoceptor agonists can acutely reduce s-ECP, possibly through interaction with surface β_2 -adrenoceptors on the blood eosinophil granulocyte. However, these results suggest that sustained stimulation of lung β_2 -adrenoceptors not only masks an acute inflammatory response by bronchodilation but also interferes with the processes involved in this acute inflammatory process. In the present study an inhibition of the increase in s-ECP and s-EPX at 24 and

48 h postchallenge was found after salmeterol pretreatment, as compared with placebo, whereas salmeterol had no influence on the postchallenge rise in blood eosinophils.

Pretreatment with inhaled, short-acting β_2 -agonists such as salbutamol fails to inhibit the LAR and the postchallenge increase in s-ECP (28).

The inhibition of the postchallenge increase in PC₂₀ histamine at 24 h occurs at a time when the bronchodilator and bronchoconstrictor inhibition has ceased after single-dose salmeterol (26). This points to an influence of salmeterol on the events leading to and underlying the increase in bronchial hyperreactivity. This may be connected to the inhibition of eosinophil activation seen. Further investigations are needed to establish the influence of long-term treatment with salmeterol on mechanisms in the acute and chronic inflammatory processes in asthma.

In conclusion, the present results demonstrated that treatment with salmeterol 50 and 100 μ g eliminated the early and the late asthmatic responses and gave protection against the bronchial hyperreactivity 24 and 48 h after allergen challenge. In addition, salmeterol inhibited eosinophil activity after allergen challenge.

References

1. American Thoracic Society. Medical section of the American Lung Association. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136: 225–43.
2. BALL DI, BRITTAIN RT, COLEMAN RA, et al. Salmeterol, a novel, long-acting beta-2-adrenoceptor agonist: characterization of pharmacological activity *in vitro* and *in vivo*. *Br J Pharmacol* 1991; 104: 665–71.
3. BOUSQUET J, CHANEZ P, LACOSTE JY, et al. Eosinophil inflammation in asthma. *New Engl J Med* 1990; 323: 1033–9.
4. BUTCHER PR, VARDEY CJ, JOHNSON M. Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. *Br J Pharmacol* 1991; 104: 672–6.
5. COCKCROFT DW, KILLIAN DN, MELLON JJA, HARGREAVE FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; 7: 235–43.
6. COCKCROFT DW, PUFFIN RE, DOLOVICH J, HARGREAVE FE. Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977; 7: 503–13.
7. DAHL R, VENGE P. Blood eosinophil count and eosinophil cationic protein. *In vivo* study of the influence of beta-2-adrenergic drugs and steroid medication. *Scand J Respir Dis* 1978; 59: 319–22.
8. DAHL R, VENGE P, OLSSON I. Variation of blood eosinophils and eosinophil cationic protein in serum in patients with bronchial asthma: studies during inhalation challenge test. *Allergy* 1978; 33: 211–15.
9. DE MONCHY JGR, KAUFFMAN HF, VENGE P, et al. Bronchoalveolar eosinophils during allergen-induced late asthmatic reaction. *Am Rev Respir Dis* 1985; 131: 373–6.
10. DURHAM SR, LOEGERING DA, DUNNETTE S, GLEICH GJ, KAY AB. Blood eosinophils and eosinophil derived proteins in allergic asthma. *J Allergy Clin Immunol* 1989; 84: 931–6.
11. ERJEFALT I, PERSSON CGA. Antiasthma drugs attenuate inflammatory leakage into airway lumen. *Acta Physiol Scand* 1986; 128: 653–5.
12. European Community for Coal and Steel. Report of Working Party. Standardization of lung-function tests. *Bull Eur Physiopathol Respir* 1983; 19 (Suppl. 5): 49.
13. FRICK WE, SEDGWICK JB, BUSSE WW. The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am Rev Respir Dis* 1989; 139: 1401–6.
14. GRIFFIN E, HÅKANSSON E, FORMGREN H, JÖRGENSEN K, PETERSON C, VENGE P. Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia. *J Allergy Clin Immunol* 1991; 87: 548–57.
15. HOGG JC, PARÉ PD, MORENO RH. The effect of submucosal edema on airways resistance. *Am Rev Respir Dis* 1987; 135: S54–6.
16. HOWARTH PH, DURHAM SR, LEE T, KAY AB, CHURCH MK, HOLGATE ST. Influence of albuterol, cromolyn sodium, and ipratropium bromide on the airway and circulating mediator responses to antigen bronchial provocation in asthma. *Am Rev Respir Dis* 1985; 132: 986–92.
17. JOHNSON M. The pharmacology of salmeterol. *Lung* 1990; 16: 115–19.
18. KOCK-WESER J. Beta-adrenergic blockade and circulating eosinophils. *Arch Intern Med* 1968; 121: 255–8.
19. MADSEN F, NIELSEN NH, FRØLUND L, SVENDSEN UG, WEEKE B. Bronchial challenge: a small reservoir for the Wright jet nebulizer. *Eur Physiopathol Respir* 1987; 23: 67–71.
20. METZGER WJ, RICHERSON HB, WORDEN KA, MONICK M, HUNNINGHAKE GW. Bronchoalveolar lavage of allergic asthma patients following allergen bronchoprovocation. *Chest* 1986; 89: 477–83.
21. METZGER WJ, ZAVALA D, RICHERSON HB, et al. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs: description of the model and local airway inflammation. *Am Rev Respir Dis* 1987; 135: 433–40.
22. PEPYS J. Immunopathology of allergic lung disease. *Clin Allergy* 1973; 3: 1–22.
23. PETERSON CGP, VENGE P. Purification and characterization of a new cationic protein – eosinophil protein-X (EPX) – from human eosinophils. *Immunology* 1983; 50: 19–26.
24. RAK S, LÖWHAGEN O, VENGE P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients. *J Allergy Clin Immunol* 1988; 82: 470–80.
25. SVEDMYR N, LÖFDAHL CG. Physiology and pharmacodynamics of beta-adrenergic agonists. In: JENNE JW, MURPHY S, eds. *Drug therapy for asthma*. New York: Dekker; 1987: 177–212.
26. TWENTYMAN OP, FINNERTY J, HARRIS A, PALMER J, HOLGATE S. Protection against allergen-induced asthma by salmeterol. *Lancet* 1990; 336: 1338–42.
27. VENGE P, DAHL R. Are blood eosinophil number and activity important for the development of the late asthmatic reaction after allergen challenge? *Eur Respir J* 1989; 2 (Suppl. 6): S430–4.
28. VENGE P, DAHL R, PETERSON CGB. Eosinophil granule proteins in serum after allergen challenge of asthmatic patients and the effect of anti-asthma medication. *Int Arch Allergy Appl Immunol* 1988; 87: 306–12.
29. VENGE P, HENRIKSEN J, DAHL R. Eosinophils in exercise-induced asthma. *J Allergy Clin Immunol* 1991; 88: 699–704.
30. VENGE P, ROXIN LE, OLSSON I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 1977; 37: 331–5.
31. YUKAWA T, UKENA D, KROEGEL C, et al. Beta₂-adrenergic receptors on eosinophils. Bindings and functional studies. *Am Rev Respir Dis* 1990; 141: 1446–52.

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