

Blood eosinophils, eosinophil-derived proteins, and leukotriene C₄ generation in relation to bronchial hyperreactivity in children with atopic dermatitis

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To assess the relation among eosinophil-related variables in the peripheral blood, bronchial hyperreactivity, and the presence of atopic dermatitis in children aged 5–14 years, we studied 11 patients with atopic dermatitis alone, six with asthma and atopic dermatitis, 12 with asthma alone, and 12 healthy controls. Eosinophil counts, levels of eosinophil cationic protein, and the capacity of eosinophils to generate leukotriene (LT) C₄, as well as bronchial hyperreactivity and a severity score for atopic dermatitis, were determined. Eosinophil variables were significantly higher in both patient groups with atopic dermatitis than in normal controls. In particular, ionophore A 23187 LTC₄ generation was higher in patients with atopic dermatitis alone (median 82, range 25–273 ng/10⁶ cells) and patients with combined asthma and atopic dermatitis (median 68, range 32–583 ng/10⁶ cells) than in normal controls (median 9, range 1–67 ng/10⁶ cells). However, there was no difference between the group of atopic dermatitis patients with asthma and without asthma. We conclude that eosinophil variables in the peripheral blood are mainly influenced by the presence of atopic dermatitis, and not the presence and the severity of asthma in patients with both asthma and atopic dermatitis.

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In recent years, it has become increasingly clear that eosinophils play an active part in the development of atopic diseases. Activated eosinophils significantly contribute to tissue injury by generating high-energy oxygen species (24, 25) and releasing major basic protein (3). Thus, eosinophils which form a major component of the cellular infiltrate during the late asthmatic response (1) cause the damage to respiratory epithelium found in asthma (17) and contribute to symptoms of bronchial hyperresponsiveness.

On the other hand, eosinophils and eosinophil products are present in the blood and the skin of patients with atopic dermatitis (18). In asthma, the number (5, 11), and activity (7, 23), of blood eosinophils have been found to show strong correlation with the degree of bronchial hyperreactivity. Likewise, strong correlation between the degree of skin involvement and eosinophil-related variables has been found (13, 20). These observations have prompted attempts to use eosinophil-related variables to de-

termine the degree of atopic inflammation (28). However, especially in childhood, many patients suffer from both atopic dermatitis and asthma, and even patients with atopic dermatitis without respiratory symptoms display increased bronchial reactivity (8, 22). In this report, we investigated whether eosinophil number, serum concentration of eosinophil cationic protein (ECP), and the ability of purified eosinophils to generate leukotriene (LT) C₄ is related to bronchial hyperreactivity or atopic dermatitis.

Material and methods

Patients

Nonatopic controls. Twelve volunteers aged 6–12 years (median 8½ years) with no history of asthma, skin disease, or other allergic diseases were examined. The children had a negative personal and family history of atopy and a normal level of total serum IgE. In addition, no specific IgE against inhaled

Table 1. Characterization of study groups

	No.	Sex (M:F)	Age* at onset (months)	Age*	Skin prick tests positive*	IgE* (IU/ml)	FEV ₁ * (% pred)	Medication**	Skin severity score*
Nonatopic controls	12	7:5	—	8.5 6–12	—	29 3–58	100 87–125		0
Asthma	12	8:4	60 12–120	9.0 7–13	2.5 1–9	400 25–350 0	81 61–110	D:6 T:8 S:10	0
Atopic dermatitis alone	11	8:3	30 18–36	9.0 6–12	1.5 0–6	86 11–500	93 82–116	—	20.8 6–40
Asthma and atopic dermatitis	6	4:2	18 6–60	9.5 5–14	2.0 0–5	236 76–849	89 67–130	D:4 T:4 S:3	14.3 5–70

* Median values and range are shown.

** D=inhaled disodium cromoglycate, T=oral theophylline, S=inhaled salbutamol.

allergens could be detected by skin prick test (Table 1).

Patients with asthma. We studied 12 children in whom asthma has been diagnosed on the basis of a history of episodic wheezing and impaired lung function. These patients were free of lesions from atopic dermatitis.

Patients with atopic dermatitis alone. Atopic dermatitis was diagnosed according to the criteria of Hanifin (9). Eczema consisted of scaly, red, itchy skin lesions, particularly occurring in flexural areas, face, or trunk. Eleven children comprised this group (Table 1).

Patients with combined asthma and atopic dermatitis. Six patients fulfilled the criteria for both asthma and atopic dermatitis (Table 1).

Informed consent

The study was approved by the ethics committee of Philipps University (Marburg, Germany). Both parents and children gave their informed consent.

Clinical evaluation

All patients entering the study were assessed in the afternoon to avoid circadian variations in bronchial hyperreactivity and eosinophil functions. Medication was withheld for at least 12 h before venous puncture and pulmonary function testing. The patients and their parents were asked about the severity of respiratory symptoms, and the patients underwent a detailed physical examination and a skin test. Extracts of nine common allergens (*Dermatophagoides pteronyssinus*, timothy, birch, mugwort, cat,

dog, *Alternaria*, *Cladosporium*, and *Aspergillus*). A wheal larger than 2 mm was considered to be positive. Age at onset of asthmatic symptoms and atopic dermatitis, medication, and number of positive skin tests were recorded (Table 1). Blood was drawn for the determination of serum IgE and eosinophil function, and pulmonary function tests including histamine challenge were done.

Serum IgE

Serum IgE of each child was detected with a commercial ELISA kit (Behringwerke, Marburg, Germany).

Severity score for atopic dermatitis

The severity of atopic dermatitis was assessed by a semiquantitative score. The skin was divided into 1044 areas and body surface involvement was assessed. In addition, a skin lesion score (0–3) was recorded for each of the following features: redness, vesiculation, crusting, excoriation, and lichenification, giving a maximum of 15 points. The severity score was calculated as:

score

$$= \frac{\text{areas involved} \times 50}{1044} + \frac{\text{skin lesion score} \times 50}{15}$$

giving a range from 0 to 100.

Pulmonary function test

A maximum flow expiratory volume curve was recorded to determine FEV₁ (forced expiratory volume in 1 s). For this measurement, mouth flow was integrated to obtain volume. FEV₁ was given as per-

centage of the predicted value according to the height and weight of the patient (29).

The degree of bronchial hyperreactivity was determined by unspecific provocation testing with inhaled histamine, and a modified version of the protocol described before was used (23). Histamine dehydrochloride in buffered saline was nebulized in a 10-l reservoir by pressure nebulizer (Pari Provotest, Starnberg, Germany; particle size 0.5–5 μm , output 0.75 ml/min) with increasing concentrations (0.5, 1, 2, 4, 8 mg/ml). To fill the reservoir, 0.75 ml histamine solution and 120-s nebulization were necessary. When the reservoir was full, the nebulized aerosol was inhaled by tidal inhalation through a one-way valve. One minute after inhalation, the thoracic gas volume (TGV) and airway resistance (R_{aw}) were measured in a pressure-corrected flow plethysmograph with the patient in the sitting position. The specific conductance sG_{aw} was calculated from the reciprocal R_{aw}/TGV . Bronchial hyperreactivity was determined as the concentration of inhaled histamine that caused a fall of 60% in the specific conductance ($\text{PC}_{60}sG_{aw}$).

Duplicate reactivity tests within 1 week displayed a difference between two measurements within 1.1 doubling steps of histamine.

Cell preparation

Eosinophils and neutrophils were prepared according to the method of Klopogge et al. (16). Briefly, 3–10 ml blood was drawn directly into ethylenediaminetetraacetic acid (EDTA). Anticoagulated blood was sedimented in 0.5 V of 3% gelatin in saline solution for 45 min at 37°C. The plasma leukocyte suspension was washed twice in Ca^{2+} -free Tyrode buffer, pH 7.0, containing 2 mM EDTA. After resuspension in washing buffer, the cells ($2 \times 10^6/\text{ml}$) were layered onto a discontinuous isotonic Percoll (Pharmacia, Freiburg, Germany) gradient with densities of 1.070 and 1.085 g/ml and centrifuged for 30 min (1200 g, 25°C). The eosinophilic leukocytes at the bottom of the tubes were resuspended in washing buffer, counted, assessed for purity, and, in cases of purity lower than 75%, layered onto a second discontinuous isotonic Percoll gradient with densities of 1.095 and 1.105 g/ml in the ratio 1:0.5, respectively. The cells were separated by centrifugation for 20 min (1200 g, 25°C). The recovery of eosinophils collected from the fraction 1.105 g/ml Percoll was >90% in all subjects studied.

Quantitation of leukotriene

The radioimmunoassay (RIA) (10) for LTC_4 was done in 300- μl volumes that contained Tris Isogel buffer (0.1 M Tris-HCl, 0.14 M NaCl, 1.5 mM

CaCl_2^{+} , and 5 mM MgCl_2^{+} , pH 7.4), culture supernatant or synthetic ^3H -labeled LTC_4 (specific activity 34 Ci/mmol, New England Nuclear, Boston, MA, USA) and rabbit anti- LTC_4 antiserum (also specific for LTD_4 and LTE_4) in 1.5-ml polypropylene test tubes. A volume of 100 μl goat antirabbit IgG serum was added at slight antibody excess, and incubation was continued for 18 h at 4°C. The immune precipitate was sedimented by centrifugation at 2500 g for 25 min at 4°C, dissolved in 500 μl 0.1 N NaOH, mixed with 4 ml ReadySolve (Beckman), and assessed for radioactivity in a liquid scintillation counter. A calibration curve was established with synthetic LTC_4 in the range 0.4–10 ng. The data were expressed as the mean of duplicate samples. The method has been shown to be suitable for the detection of LTC_4 in supernatants of ionophore A 23187-stimulated cells (26).

Assay for eosinophil cationic protein

For the measurement of ECP, serum was collected after 30 min clotting time. ECP was measured by an RIA kit (Pharmacia). The range of the standard curve was 2–200 $\mu\text{g}/\text{ml}$. All assays were run in duplicate.

Statistical methods

The Kruskal-Wallis test and, where appropriate, the Mann-Whitney U-test were used to compare results of the different patient groups. Multiple regression analysis was used to assess the correlation between eosinophil variables on one hand and skin severity score and the degree of bronchial hyperreactivity on the other hand.

Results

Of the 17 children with atopic dermatitis, six suffered from symptoms of asthma. These six patients displayed a high bronchial hypersensitivity comparable with that measured in children with asthma alone. The 11 patients with atopic dermatitis alone without any history of asthma showed a bronchial reactivity significantly higher than bronchial reactivity in healthy controls (Fig. 1) but lower than in patients with asthmatic symptoms.

The individual values for blood eosinophil counts are illustrated in Fig. 2. Healthy controls displayed significantly lower eosinophil counts than did the three groups of atopic patients. Eosinophil counts in patients with atopic dermatitis were comparable with values obtained in asthmatic patients. Within the group of patients with atopic dermatitis, there was no difference in eosinophil counts regardless of the presence of asthma (Fig. 2). As shown in Fig. 3,

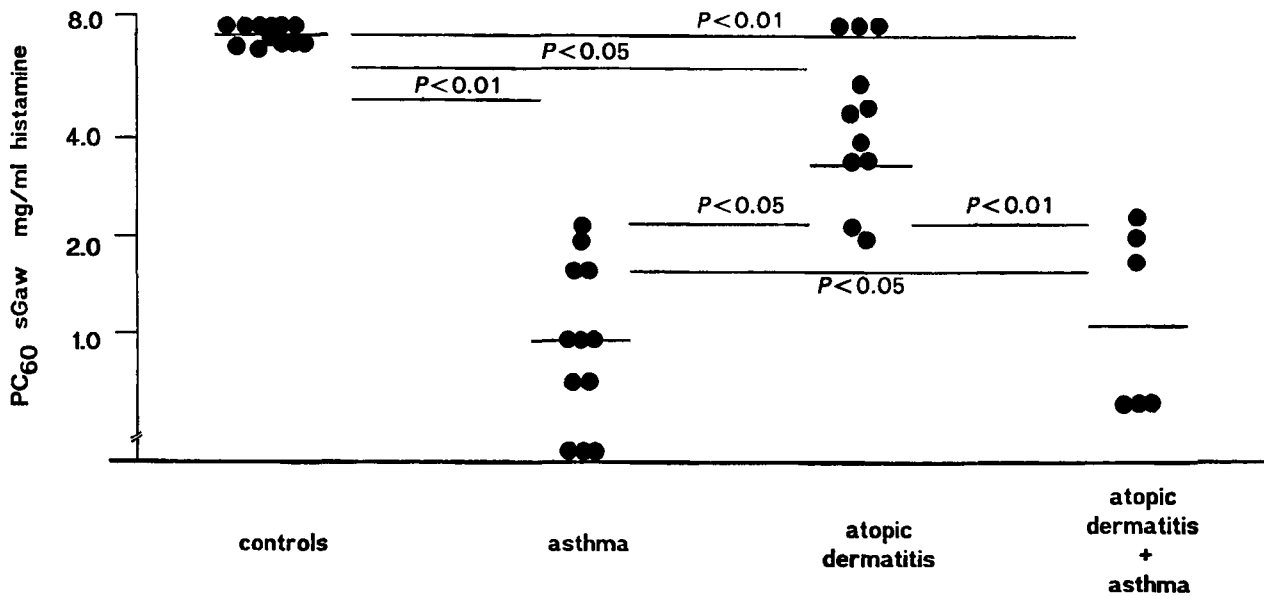


Fig. 1. Bronchial reactivity (PC₆₀sG_{aw}) measured in 12 healthy controls, 12 asthmatic children, 11 patients with atopic dermatitis alone, and six patients with atopic dermatitis and asthma. Median values are represented by lines. Levels of significance are shown.

ECP levels showed essentially the same pattern as eosinophil counts. Patients displayed significantly higher values for ECP than did controls, but among the different groups no differences were found (Fig. 3). In contrast to eosinophil count and ECP, LTC₄ generation was not significantly elevated in patients with only asthma, whereas eosinophils from patients with atopic dermatitis gave a comparably high yield of LTC₄ regardless of the presence of

asthma (Fig. 4). Fig. 5 shows the correlation between skin severity score and bronchial hyperreactivity, and LTC₄ generation. The skin severity score correlated significantly with LTC₄ generation ($P = 0.0038$; $r = 0.44$). The correlation was even more pronounced when the influence of the other variables was excluded by multiple regression analysis ($P < 0.01$; $r = 0.58$). In addition, multiple regression analysis revealed a correlation of eosinophil count

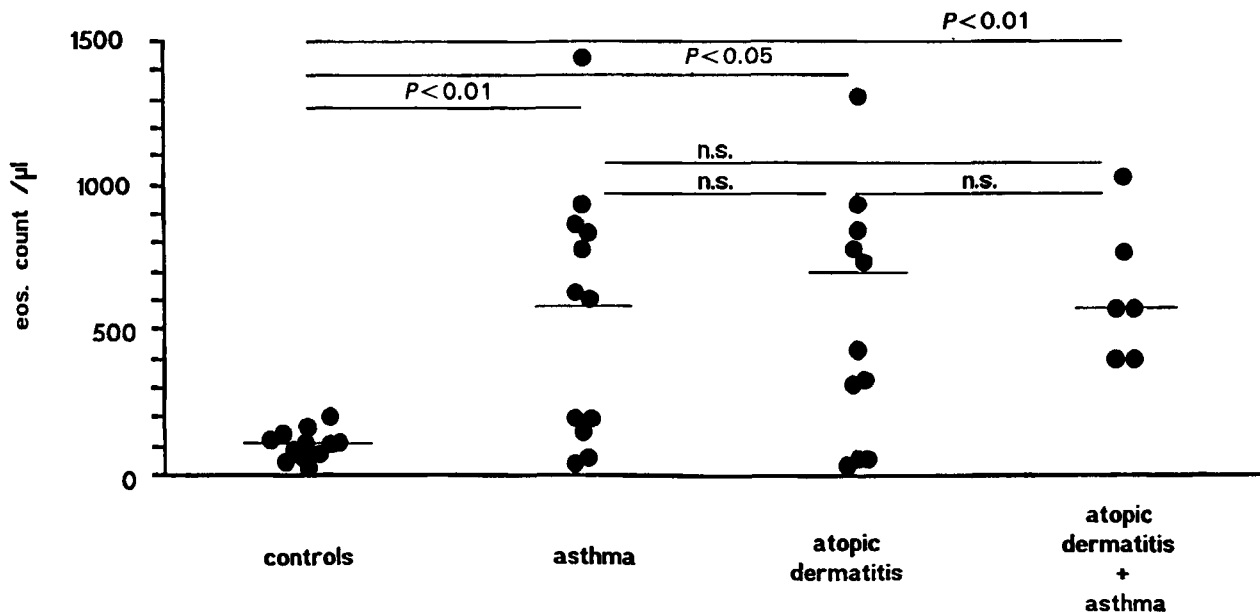


Fig. 2. Blood eosinophil counts measured in 12 healthy controls, 12 asthmatic children, 11 patients with atopic dermatitis alone, and six patients with atopic dermatitis and asthma. Median values are represented by lines. Levels of significance are shown.

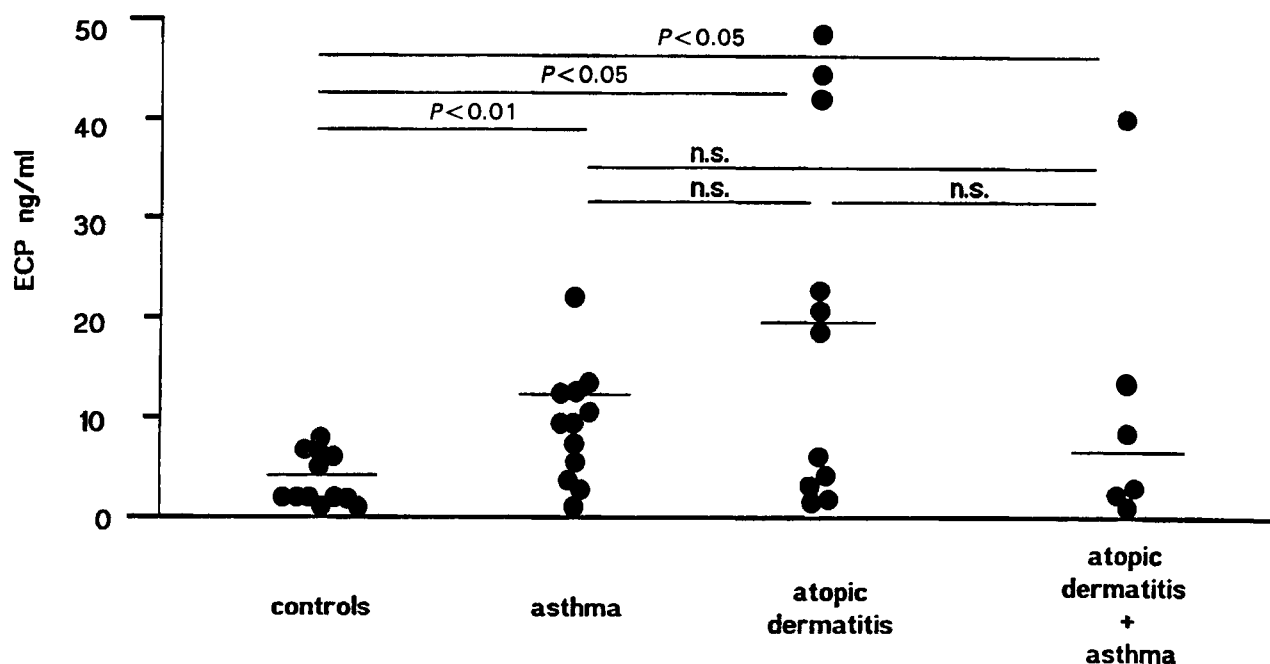


Fig. 3. ECP concentrations measured in 12 healthy controls, 12 asthmatic children, 11 patients with atopic dermatitis alone, and six patients with atopic dermatitis and asthma. Median values are represented by lines. Levels of significance are shown.

($P < 0.05$; $r = 0.36$) with skin severity score, but not with degree of bronchial hyperreactivity.

Discussion

A number of reports suggest that there is a correlation between bronchial hyperreactivity and eosino-

phil activity in the peripheral blood (5, 23, 30), and that these variables may be useful in the management of asthma. In the present study, we show that eosinophil count, serum ECP concentration, and LTC_4 generation by eosinophils in patients with asthma and atopic dermatitis are mainly influenced by the degree of skin involvement rather than the

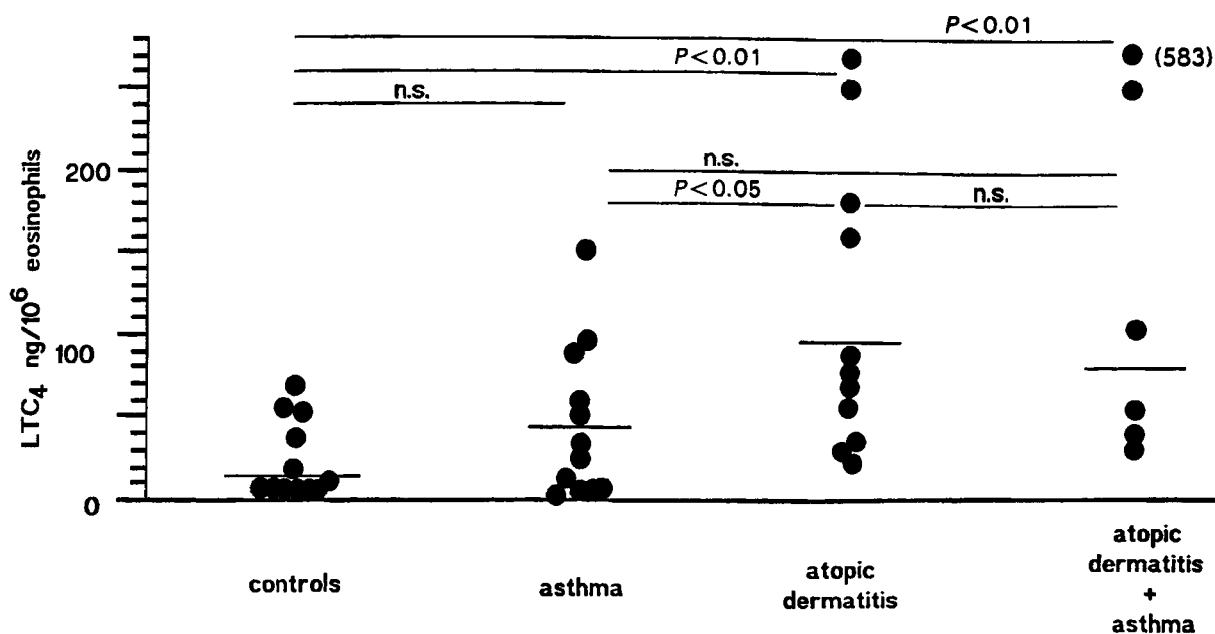


Fig. 4. LTC_4 generation of eosinophils measured in 12 healthy controls, 12 asthmatic children, 11 patients with atopic dermatitis alone, and six patients with atopic dermatitis and asthma. Median values are represented by lines. Levels of significance are shown.

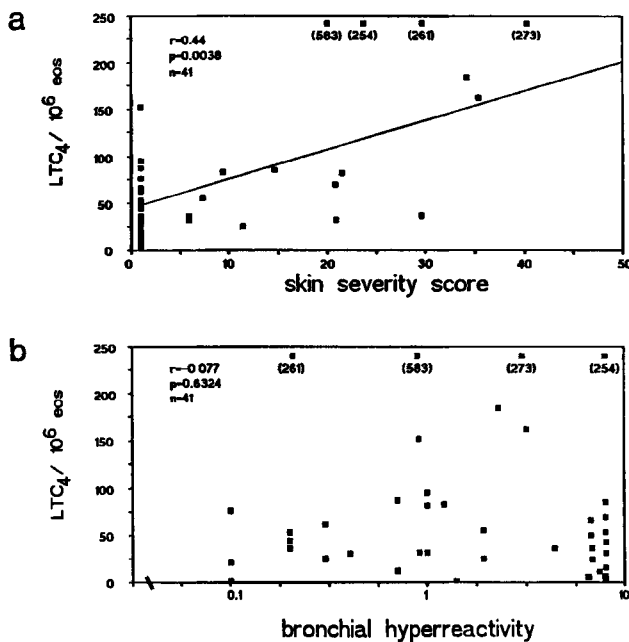


Fig. 5. Correlation between LTC₄ generation of eosinophils and skin severity score (a) or bronchial hyperreactivity (b) measured in all subjects ($n = 41$) included in study.

degree of bronchial hyperreactivity. Because atopic dermatitis has a prevalence in asthmatic children of 30–60% (14), these considerations are of practical importance.

The strong influence of atopic dermatitis on eosinophil function is most clearly seen with LTC₄ generation. Eosinophils from patients with atopic dermatitis displayed a significantly higher LTC₄ generation than controls and patients with only asthma. Moreover, multiple regression analysis, which allows separate analysis of the influence of bronchial hyperreactivity and severity of atopic dermatitis, shows a clear correlation between the degree of skin involvement and LTC₄ generation. The difference is less clear with ECP and eosinophil count.

LTC₄ is not a stored mediator like ECP but has to be newly generated upon activation (23). The ability to generate LTC₄ is enhanced by various lymphokines and monokines (12, 27), as well as lipid mediators such as platelet-activating factor (23). Thus, LTC₄ generation might be a more sensitive variable than ECP levels or eosinophil numbers for detection of the actual degree of eosinophil activation.

Our observations are consistent with several other aspects of the relation of asthma and atopic dermatitis. Usually, atopic dermatitis precedes asthma in the development of atopic diseases. After early onset of atopic dermatitis, there is a high risk of allergic airway disease (2). Even if a patient suffers from atopic dermatitis alone without any respiratory

symptoms, there is mild bronchial hyperreactivity as shown in our study as well as several other studies in adults (4) and children (8, 22).

These findings suggest a latent predisposition to asthma in atopic dermatitis. Pathophysiologically, this predisposition might be explained by the strong activation of inflammatory cells in atopic dermatitis. In our study, as well as in several other studies, eosinophils in atopic dermatitis displayed a high degree of activation. Experimental studies in adults showed that eosinophils enter the skin readily upon allergen exposure (6). However, in chronically inflamed lesions of atopic dermatitis, there is a marked dermal deposition of eosinophil-granule major basic protein, while eosinophils are scarcely found (19). This suggests that eosinophils had been present but had degranulated and so were no longer identifiable. Complete degranulation is consistent with a high degree of activation of eosinophils (15). It is conceivable that these highly activated eosinophils also enter bronchial tissue.

However, for the development of overt asthma, additional factors have to be present. These theoretic considerations require further confirmation. The results of the present study are of practical importance as they indicate that eosinophil variables in the peripheral blood are of limited value in monitoring the extent of bronchial eosinophilic inflammation and the severity of asthma in patients with concomitant atopic dermatitis. Material from other sources, such as sputum induced by inhalation of hypertonic saline (21), seems to be more suitable to assess airway inflammation, but this has to be evaluated for practical application in children.

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References

1. AALBERS R, KAUFFMAN HF, VRUGT B, et al. Bronchial lavage and bronchoalveolar lavage in allergen-induced single early and dual asthmatic responders. *Am Rev Respir Dis* 1992; 147: 76–81.
2. ÅBERG N, ENGSTRÖM I. Natural history of allergic diseases in children. *Acta Paediatr Scand* 1990; 79: 206–11.
3. AYARS GH, ALTMAN LC, MCMANUS MM, et al. Injurious effect of the eosinophil peroxidase-hydrogen peroxide-halide system and major basic protein on human nasal epithelium *in vitro*. *Am Rev Respir Dis* 1989; 140: 125–31.
4. BARKER AF, HIRSHMAN CA, D'SILVA R, HANIFIN JM. Airway responsiveness in atopic dermatitis. *J Allergy Clin Immunol* 1991; 87: 780–3.
5. BOUSQUET J, CHANEZ P, LACOSTE JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323: 1033–9.
6. BRUYNZEEL KOOMEN CA, VAN WICHEN DF, SPRY CJ, VENGE P, BRUYNZEEL PL. Active participation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis. *Br J Dermatol* 1988; 118: 229–38.

7. CARLSON M, HAKANSON L, KÄMPE M, STALENHEIM G, PETERSON C, VENGE P. Degranulation of eosinophils from pollen-atopic patients with asthma is increased during pollen season. *J Allergy Clin Immunol* 1992; 89: 131-9.
8. FABRIZI G, CORBO GM, FERRANTE E, et al. The relationship between allergy, clinical symptoms and bronchial responsiveness in atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1992; 176: 68-73.
9. HANIFIN JM. Basic and clinical aspects of atopic dermatitis. *Ann Allergy* 1984; 52: 386-95.
10. HAYES EC, LOMBARDO DL, GIRARD Y, et al. Measuring leukotrienes of slow reacting substance of anaphylaxis: development of a specific radioimmunoassay. *J Immunol* 1983; 131: 429-33.
11. HORN BR, ROBIN ED, THEODORE J, VAN KESSEL A. Total eosinophil count in the management of bronchial asthma. *N Engl J Med* 1975; 292: 1152-5.
12. HOWELL CJ, PUJOL JL, CREA AE, et al. Identification of an alveolar macrophage-derived activity in bronchial asthma that enhances leukotriene C₄ generation by human eosinophils stimulated by ionophore A23187 as a granulocyte macrophage colony stimulating factor. *Am Rev Respir Dis* 1989; 140: 1340-7.
13. JAKOB T, HERMAN K, RING J. Eosinophil cationic protein in atopic eczema. *Arch Dermatol Res* 1991; 283: 5-6.
14. KELLY WJW, HUDSON I, PHELAN PD, PAIN MCF, OLINSKY A. Atopy in subjects with asthma followed to the age of 28 years. *J Allergy Clin Immunol* 1990; 85: 548-57.
15. KITA H, WEILER DA, ABU GHAALEH R, SANDERSON CJ, GLEICH GJ. Release of granule proteins from eosinophils cultured with IL-5. *J Immunol* 1992; 149: 629-35.
16. KLOPROGGE E, DE LEEUW AJ, DE MONCHY JG, KAUFFMAN HF. Hypodense eosinophilic granulocytes in normal individuals and patients with asthma: generation of hypodense cell populations *in vitro*. *J Allergy Clin Immunol* 1989; 83: 393-400.
17. LAITINEN A, LAITINEN LA. Cellular infiltrates in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991; 143: 1159-61.
18. LEIFERMAN KM, ACKERMAN SJ, SAMPSON HA, HAUGEN HS, VENENCIE PY, GLEICH GJ. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis. Comparison with onchocerciasis. *N Engl J Med* 1985; 313: 282-5.
19. LEIFERMAN KM. Eosinophils in atopic dermatitis. *Allergy* 1989; 19: 20-6.
20. PAGANELLI R, FANALES-BELASIO E, CARMINI D, et al. Serum eosinophil cationic protein in patients with atopic dermatitis. *Int Arch Allergy Appl Immunol* 1991; 96: 175-8.
21. PIN I, FREITAG AP, O'BYRNE PM, et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. *Am Rev Respir Dis* 1992; 145: 1265-9.
22. SALOB SP, LAVERTY A, ATHERTON DJ. Bronchial hyperresponsiveness in children with atopic dermatitis. *Pediatrics* 1993; 91: 13-16.
23. SCHAUER U, DAUME U, MÜLLER R, RIEDEL F, GEMSA D, RIEGER CHL. Relationship between LTC₄ generation of hypodense eosinophils and bronchial hyperactivity in asthmatic children. *Int Arch Allergy Appl Immunol* 1990; 92: 82-7.
24. SCHAUER U, LEINHAAS C, JÄGER R, RIEGER CHL. Enhanced superoxide generation by eosinophils from asthmatic children. *Int Arch Allergy Immunol* 1991; 96: 317-21.
25. SEDGWICK JB, GEIGER KM, BUSSE WW. Superoxide generation by hypodense eosinophils from patients with asthma. *Am Rev Respir Dis* 1990; 142: 120-5.
26. SHAW RJ, CROMWELL O, KAY AB. Preferential generation of leukotriene C₄ by human eosinophils. *Clin Exp Immunol* 1984; 56: 716-22.
27. SILBERSTEIN DS, ALI MH, BAKER SL, DAVID JR. Human eosinophil cytotoxicity-enhancing factor. Purification, physical characteristics, and partial amino acid sequence of an active polypeptide. *J Immunol* 1989; 143: 979-83.
28. VENGE P. Serum measurements of eosinophil cationic protein (ECP) in bronchial asthma. *Clin Exp Allergy* 1993; 23: 3-7.
29. ZAPLETAL A, SAMANEK M, PAUL T. Lung function in children and adolescents; methods, reference values. Basel: Karger, 1987.
30. ZIMMERMAN B. Clinical experience with the measurement of ECP: usefulness in the management of children with asthma. *Clin Exp Allergy* 1993; 23: 8-12.