

Short communication

Urinary eosinophil protein X in children with atopic dermatitis: relation to atopy and disease activity

Background: Parameters of eosinophil inflammation have been suggested as markers of disease activity in atopic dermatitis (AD), but the value of urinary eosinophil protein X (U-EPX) in children with AD, as well as the influence of allergic sensitization, is not known.

Methods: We measured U-EPX in 59 atopic and 29 nonatopic children with mild ($n=32$), moderate ($n=34$), and severe ($n=22$) AD, as well as in 64 controls.

Results: U-EPX was increased in children with AD (110; 67–164 $\mu\text{g}/\text{mmol}$ creatinine, median; quartiles) compared to controls (62; 41–95, $P<0.001$). Children with mild (97; 63–164, $P<0.01$), moderate (108; 67–157, $P<0.01$), and severe disease (152; 99–202, $P<0.001$) had levels of U-EPX higher than controls. U-EPX was significantly higher in children with severe AD than in mild and moderate disease ($P<0.05$ for both). Children with AD and a positive skin prick test (120; 81–176) had higher levels of U-EPX than children with a negative skin prick test (87; 56–155, $P<0.05$).

Conclusions: U-EPX is significantly increased in children with AD and may reflect disease activity. U-EPX may also reflect differences in eosinophil activation between those sensitized and those not sensitized to common allergens.

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The pathogenesis of atopic dermatitis (AD) is not fully understood, but the major immunopathogenic abnormality is thought to involve T cells with hyperstimulatory activity (1, 2). Furthermore, there is strong evidence of an active role for eosinophils in the pathogenesis of AD (2–4). Infiltration of eosinophils has been demonstrated in the skin of patients with AD, and eosinophil counts and serum eosinophil cationic protein (ECP) seem to correlate with disease activity (2, 4–6).

Eosinophil protein X (EPX) is one of the major granule proteins released by activated eosinophils (7). Deposition of EPX, as well as ECP, has been demonstrated in sections of AD lesions (8), whereas EPX in urine (U-EPX) has been measured in only a few patients with AD (8, 9). EPX is the only basic eosinophil granule protein that can be accurately measured in urine (10). Urine sampling is simple, noninvasive, and painless, and measurements of U-EPX may therefore be especially valuable to monitor disease activity in children.

The role of allergy in the pathogenesis of AD is controversial (1). The concentration of IgE is raised in some, but not all, patients with AD, and differences in immunologic mechanisms and eosinophil involvement between allergic and nonallergic AD have been reported (1–3). However, the influence of allergic sensitization on markers of eosinophil inflammation is not known.

The aim of the study was to determine U-EPX in

children with AD without clinical signs of asthma. Furthermore, U-EPX was measured in both children with and without a positive skin prick test to evaluate the influence of atopy.

Material and methods

Study subjects

Eighty-nine children with AD diagnosed by the criteria of Hanifin & Rajka (11) were included in the study (Table 1). The children had no history of cough and wheeze indicative of asthma, and no history of allergic rhinitis or conjunctivitis. Assessment of disease severity was measured by the simple grading system suggested by Rajka & Langeland (12). The grading includes the extent of skin lesions, the course of the disease, and the intensity of itching. Each parameter was graded from 1 to 3. By this grading, 32 children were found to have mild disease, 34 moderate disease, and 22 severe disease. With the exception of three children, the extent of skin lesions was grade 1 in children with mild disease, grade 2 in children with moderate disease, and grade 3 in children with severe disease.

Fifty-nine of the children were atopic and 29 non-atopic. Atopic status was defined as having at least one positive skin prick test (SPT) for common allergens

(including egg, milk, pea, hazelnut, peanut, cod, shrimp, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, cat, dog, grass pollen, and tree pollen [Soluprick, ALK, Denmark]). We used histamine as the positive control and a 0.9% saline solution as the negative control. A wheal of at least 3 mm in diameter was defined as a positive result.

Forty-two of the atopic children had a positive SPT to egg; of these, 24 were positive to egg alone, whereas 18 also had a positive SPT to cow's milk or another food allergen. Twenty-three of the children had a positive SPT to at least one aeroallergen, and 17 of these had a positive SPT to at least one food allergen.

Sixty-four children without a history of AD or any other inflammatory disease, and without a history of infection in the last 2 weeks served as controls (Table 1). These children had neither parental history of allergy, AD, or asthma nor a history of any allergic disease such as asthma or wheezing, food allergy, or allergic rhinoconjunctivitis.

Study design

Children with AD were consecutively included, as they were examined at the outpatient clinic as part of their routine examination. The control children were recruited at the outpatient clinic among children attending for noninflammatory conditions, or from a baby clinic. The parents of all children answered a questionnaire about atopy and smoking habits in the family, and a history including infections in the last 2 weeks was taken.

Ethics

The study was approved by the regional ethics committee, and signed statements of informed consent were obtained from all parents.

Laboratory analyses

Urine was collected at home in the morning (13) and kept in a refrigerator until aliquoting and freezing (-20°C) within 6 h.

U-EPX was analyzed by a specific radioimmunoassay (Pharmacia, Sweden). The detection limit in the assay was $<3\text{ }\mu\text{g/l}$ and the coefficient of variation was $<5\%$ within the assay and $<10\%$ between the assays. Urine creatinine levels were measured by the Vitros 250 system (Ortho Clinical Diagnostics, USA). All measurements were done in duplicate. U-EPX concentrations are presented as $\mu\text{g}/\text{mmol}$ creatinine.

Statistical methods

Nonparametric tests were used in comparative analysis. The Kruskal–Wallis analysis of variance was first applied to the groups. When this was significant, each pairing was analyzed by the Mann–Whitney U-test. Results are presented as median and quartiles.

For investigation of the influence of covariates on U-EPX, the determinants of interest were estimated in linear regression models after logarithmic transformation of U-EPX (normally distributed, $P=0.2$). The covariates of disease severity and atopy were fitted into the model, and adjusted for age and sex as potential confounders.

Data were analyzed by the SPSS 9.0 statistical package (SPSS, Inc, Chicago, IL, USA). Tests were two-tailed and probabilities of less than 5% were considered significant.

Results

Differences between groups and controls are presented in Table 1. The levels of U-EPX in children with AD were higher than in controls ($P<0.001$). Children with mild, moderate, and severe disease had levels of U-EPX higher than controls (Fig. 1). U-EPX was higher in children with severe disease than in those with moderate

Table 1. Study subjects and levels of U-EPX in children with atopic dermatitis¹

Study subjects	No.	Atopy (yes/no)	Sex (M/F)	Age in months (mean; range)	U-EPX ($\mu\text{g}/\text{mmol}$ creatinine)
Atopic dermatitis	88	59/29	51/37	44; 3–135	110; 67–164 ²
Mild	32	20/12	16/16	48; 6–122	97; 63–164 ³
Moderate	34	23/11	22/12	41; 4–135	108; 67–157 ³
Severe	22	16/6	13/9	42; 3–110	152; 99–202 ^{2,4}
Atopics	59		33/26	50; 3–99	120; 81–176 ^{2,5}
Nonatopics	29		15/14	41; 4–135	87; 56–155 ⁶
Controls	64	0/64	32/32	45; 2–152	62; 41–95

¹ Results are expressed as median and quartiles.

² $P<0.001$ vs controls.

³ $P<0.01$ vs controls.

⁴ $P<0.05$ vs mild and moderate disease.

⁵ $P<0.05$ vs nonatopics.

⁶ $P<0.05$ vs controls.

and mild disease ($P < 0.05$ for both) (Fig. 1). There was considerable overlap between groups and controls (Fig. 1).

Children with AD and atopy had higher levels of U-EPX than those nonatopic ($P < 0.05$). No differences in U-EPX were observed between children who had a positive SPT to one allergen and those with a positive SPT to more than one allergen, or between those who had positive SPT to aeroallergens and those with positive SPT only to food allergens. The nonatopic children with AD had higher levels of U-EPX than controls ($P < 0.05$), but nonatopic children with mild and moderate disease alone (83; 48–112, $P = 0.25$) had levels similar to controls.

When the covariates were studied in a linear regression model including all children, atopy corresponded to an increase in U-EPX, whereas having AD did not correspond significantly (Table 2). If only children with AD were studied, having severe disease corresponded significantly to increased U-EPX compared to those with mild and moderate disease, as did having atopy compared to the nonatopic children (Table 2). Adding age and sex as covariates did not change the significance of the regression models.

Discussion

In this study, we have demonstrated U-EPX to be higher in children with AD than in healthy controls, and that children with severe AD had higher levels than children with mild and moderate disease. Moreover, children with AD and a positive SPT had higher levels of U-EPX than those with a negative SPT.

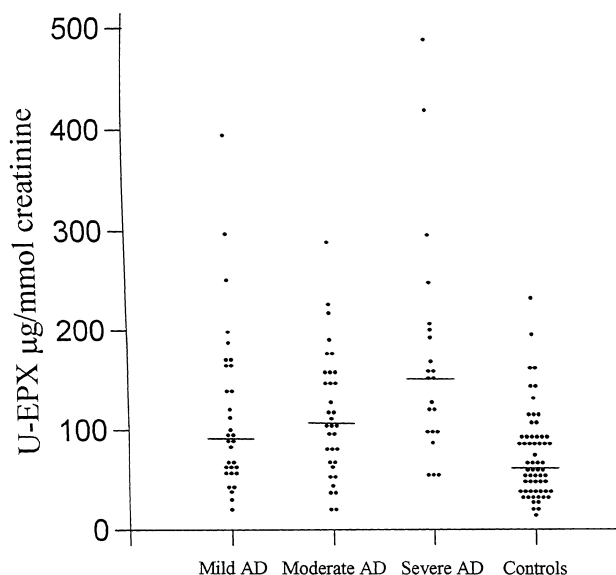


Figure 1. Urinary eosinophil protein X (U-EPX) in children with atopic dermatitis (AD) with mild disease ($n = 32$), moderate disease ($n = 34$), and severe disease ($n = 22$), and in controls ($n = 64$).

Table 2. Multiple regression analysis to examine effects on U-EPX of atopy, atopic dermatitis, and disease severity

Log U-EPX	P value	Beta coefficient	95% CI
All children			
Atopy	0.01	0.16	0.04 to 0.28
Atopic dermatitis	0.07	0.11	−0.08 to 0.23
Constant	0.00	1.81	1.74 to 1.9
Atopic dermatitis			
Atopy	0.02	0.15	0.03 to 0.27
Severe disease	0.01	0.17	0.04 to 0.30
Constant	0.00	1.71	1.52 to 1.9

Multiple linear regression table of beta coefficients and 95% confidence intervals (CI) for logarithm (log) U-EPX against atopy with simultaneous inclusion of atopic dermatitis (yes/no) or disease severity (severe disease vs mild and moderate disease [1/0]).

There is strong evidence that eosinophils play a role in the pathogenesis of AD, especially in chronic lesions (2–4). Deposition of extracellular eosinophil granule proteins, such as ECP and EPX, in affected skin suggests that degranulation of eosinophils has occurred. In addition, several studies have shown an association between serum ECP and disease activity in AD, indicating that granule proteins have escaped from the eosinophils as part of the disease process (3, 4, 6). The present report demonstrating increased levels of U-EPX correlating with disease activity provides further evidence for eosinophil involvement and activation in AD. However, in some children with high disease activity, levels of U-EPX were similar to controls, and it is possible that in this group mechanisms other than eosinophil activation caused the relapse of AD (9). Our results are consistent with a recent study by Pucci et al. that demonstrated higher levels of U-EPX in young children and infants with AD than controls (9). Moreover, in both studies, levels of U-EPX increased significantly only from moderate to severe forms of AD.

Several clinical and laboratory observations strongly suggest a role for allergy at least in some children with AD (2), and increased IgE production and expansion of IL-4- and IL-5-secreting TH2-cells are some of the immunologic features of this disorder (2). IL-5 is capable of both recruiting and activating eosinophils, resulting in degranulation and secretion of granule proteins such as EPX (14). Moreover, eosinophils from allergic patients with AD have increased migratory responses to certain chemotaxins and spontaneously migrate through endothelial cells, whereas eosinophils from nonallergic persons are unable to transmigrate until primed with cytokines (3). Recently, a distinction between two groups of patients with AD has been suggested. The term “intrinsic AD” is suggested for those 20% with low or moderate total serum IgE and with negative screening for specific IgE, whereas “extrinsic AD” refers to those with high total IgE levels, high specific IgE, or another coexisting atopic disease (15). Immunodermatologic investigations show differences in T-cell cytokine secretion and immuno-

histology between intrinsic and extrinsic AD (15). Therefore, it is possible that the differences in U-EPX between children with positive and negative SPT in this study reflect differences in eosinophil involvement and activation in the skin between these two types of AD.

In contrast to our results, Urisu et al. demonstrated no difference in serum ECP between patients with AD and positive IgE titers to specific allergens and those with negative titers (16). The conflicting results may be due to differences in patient populations. However, the mechanisms of eosinophil granule release in AD may be different for ECP and EPX (17), and it is possible that U-EPX distinguishes between allergic and nonallergic inflammation better than serum ECP.

Deposition of EPX and ECP has been demonstrated in AD skin lesions (8). Moreover, improvement of affected skin after treatment has been associated with normalization of both total eosinophil counts and circulating levels of eosinophil granule proteins (4, 18, 19). It is therefore probable that the increased levels of U-EPX demonstrated in this study reflect eosinophil activation in the skin lesions. However, atopic disorders are generally associated with blood eosinophilia (3); in a recent study, U-EPX was increased in children with a positive SPT without clinical allergic disease (20). We have consistently demonstrated U-EPX to be higher in asthmatic children with atopy than in nonatopics with the same severity of asthma (Øymar & Bjerknes, submitted for publication). Allergic stimulation in sensitive children induces both eosinophilopoiesis and activation of eosinophils in the periphery (3), and intestinal inflammation in children with AD and food allergy has been associated with increased concentrations of fecal EPX and ECP (21). It is therefore possible that nondermal mechanisms may have contributed to the increased levels of U-EPX in some of the children with a positive SPT.

In a recent study by Miyasato et al., serum ECP, but not plasma ECP, was increased in patients with AD (22). Hence, they suggested that the increased serum

ECP may have been a consequence of *in vitro* degranulation, and not of ECP release from eosinophils in lesional skin. Since U-EPX is an indicator of eosinophil activation *in vivo* (23), our results demonstrate that degranulation of eosinophils has actually occurred in the children with AD.

Whereas several studies have demonstrated that serum ECP may be used to monitor disease activity in AD (4–6, 19, 24), a similar role for U-EPX has not been well established. The present study suggests that U-EPX reflects disease activity in AD, but there was significant overlap between groups with different severity of disease. Therefore, in children with AD, levels of U-EPX alone are not sufficient to describe disease activity, and the clinical value may be limited. Moreover, the levels of EPX in nonsensitized children with AD was at the border of significance depending on whether the data were analyzed by a simple nonparametric test (Table 1) or by regression analysis (Table 2). However, one of the main findings in our study was the difference of U-EPX between children with positive and negative SPT. Therefore, a possible clinical role for U-EPX may be to assess the role of allergy in patients with AD. Moreover, intrinsic and extrinsic AD may represent subsets of AD with differences in prognosis and susceptibility from the subsequent development of other atopic diseases (15), and levels of U-EPX may possibly be employed in the distinction between these two groups of AD. However, further studies are needed to determine how the levels of U-EPX reflect the inflammation of affected skin in AD.

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