

Short communication

Urine eosinophil protein X (EPX) is an *in vitro* parameter of inflammation in atopic dermatitis of the adult age

Background: Eosinophils are important effector cells in several atopic diseases. The levels of eosinophil granule-derived mediators (ECP, EPX) in serum and body fluids have been proven to be correlated with disease activity in atopic respiratory diseases and atopic dermatitis. The study aimed to demonstrate an interrelationship between urine EPX and disease activity in adult patients with atopic dermatitis.

Methods: We determined urine EPX concentration, serum ECP concentration, and peripheral blood eosinophil count in 40 adult patients with mild to severe atopic dermatitis and compared it with the disease activity as assessed with the SCORAD index.

Results: Urine EPX and serum ECP concentrations were significantly higher in patients with severe atopic dermatitis than in patients with mild or moderate disease (median values 123.5 vs 78.3 µg/mmol creatinine, $P < 0.0001$; 25.4 vs 14.9 µg/l, $P < 0.0001$, respectively). We found a significant correlation between urine EPX levels, serum ECP levels, and the SCORAD ($r = 0.36$, $P < 0.0001$ and 0.34, $P < 0.0001$, respectively).

Conclusions: Urine EPX is a useful *in vitro* parameter of inflammation in atopic dermatitis of the adult age.

K. Breuer, A. Kapp, T. Werfel

Department of Dermatology and Allergology, Hannover
Medical University, Hannover, Germany

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Kristine Breuer, MD
Department of Dermatology and Allergology
Hannover Medical University
Ricklinger Straße 5
D-30449 Hannover
Germany

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Atopic dermatitis (AD), a chronic inflammatory skin disease that often begins in early infancy, is characterized clinically by pruritus, typical form and distribution of skin lesions, and a personal or familial history of atopic diseases such as allergic asthma, allergic rhinoconjunctivitis, and AD (1). Various types of inflammatory cells have been shown to be involved in the pathogenesis of atopic diseases. Patients suffering from atopic diseases often show high blood eosinophil counts. Various cytokines and chemokines, such as RANTES, eotaxin, IL-5, and IL-3, produced mainly by antigen-presenting cells and T cells, attract eosinophils to the site of the inflammation and activate them, leading to the release of several proinflammatory mediators (2–4).

Among them, the highly toxic, granule-derived mediators major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil derived neurotoxin/eosinophil protein X (EDN/EPX) can cause severe tissue damage and therefore may contribute to the maintenance and exacerbation of atopic diseases. Deposits of these proteins have been found not only in the bronchial mucosa of asthma patients (5) but also in the biopsies of chronic eczematous skin lesions and atopy patch test lesions of patients suffering from AD by immunohistologic staining (6).

Elevated levels of ECP have been detected in the serum, sputum, nasal secretion, and BAL of patients with atopic diseases (7–9). Several studies have shown that the level of serum ECP is correlated with the disease activity of asthma and AD (8, 10). EPX shares many properties with ECP; however, it is not cytotoxic to epithelial cells. In contrast to ECP, EPX is excreted in significant amounts in the urine (11). Serum and urine EPX levels are elevated in patients with allergic asthma (12), and significant concentrations can be found in nasal secretions and BAL fluid. Furthermore, urine EPX levels are correlated with the pulmonary function (13). Recently, an interrelationship between disease activity and urinary EPX levels could be demonstrated in children with AD (14). In this study, we show that urine EPX levels are also significantly higher in adults with severe AD than in patients with mild or moderate disease.

Material and methods

Patients

Forty adult patients with mild to severe AD were recruited from the Department of Dermatology and Allergology of the Hannover Medical University (median age 31 years; range: 18–63 years). AD was diagnosed by the criteria of Hanifin & Rajka (15). The clinical

severity of AD was assessed by the SCORAD index, which is a composite score combining subjective (pruritus, sleeplessness) and objective (extent, severity) criteria of AD (16).

All 40 patients were investigated on their first visit as outpatients. Blood and urine samples were collected. Fifteen of the 40 patients were also investigated at 8–13 time points over a period of 6 months. Peripheral blood eosinophil counts were determined in 19 patients by staining with eosin-methylene blue solution and light microscopy of blood smears. Leukocytes were identified and a differential count was made.

Eosinophil mediators

Urine EPX. Urine samples of the patients and six healthy control persons were frozen immediately and stored at -20°C . EPX was measured by competitive RIA, according to the manufacturer's instructions (Pharmacia and Upjohn, Freiburg, Germany). Considering the patient's renal function, the EPX concentrations were referred to the urine creatinine concentration and expressed as $\mu\text{g}/\text{mmol}$ creatinine.

Serum ECP. Blood samples were coagulated for 60 min at room temperature, followed by a 30-min incubation at 4°C . Samples were centrifuged for 10 min (4°C , 850 g). Sera were frozen immediately and stored at -20°C . ECP was determined by the CAP System ECP FEIA (Pharmacia and Upjohn, Freiburg, Germany) after the completion of the study (17).

Statistical analysis

The comparison of the medians of nonparametric distributed results of different patient groups was performed with the Mann–Whitney rank sum test. Different parameters (SCORAD, ECP/EPX concentrations, and peripheral blood eosinophil count) were correlated with the Spearman rank order correlation.

Results

Urine EPX levels and serum ECP levels were determined in 40 adult patients with AD. The ECP concentrations exhibited a wide range from 2.3 to $471.9 \mu\text{g}/\text{l}$ (median value: $17.5 \mu\text{g}/\text{l}$). The EPX concentration ranged from 3.2 to $520.3 \mu\text{g}/\text{mmol}$ creatinine (median value $100.2 \mu\text{g}/\text{mmol}$ creatinine). The clinical severity of AD was assessed with the SCORAD index (range: 1.2–77.5; median value: 36.2). Peripheral eosinophil counts were determined in 19 patients (range: 0.9–27.6% of blood leukocytes; median value: 6.8%).

The median urine EPX and serum ECP levels were compared between patients with severe AD (SCORAD ≥ 40) and patients with mild/moderate disease (SCORAD < 40). Urine EPX concentrations were significantly higher in severe AD than in mild/moderate AD (median values 123.5 and $78.3 \mu\text{g}/\text{mmol}$ creatinine, respectively, $P < 0.0001$) (Fig. 1B). Furthermore, there was a statistically significant difference in serum ECP levels between severe and mild/moderate cases (median values: 25.4 and $14.9 \mu\text{g}/\text{l}$, respectively; $P < 0.0001$) (Fig. 1A).

We also found a significant correlation between urine EPX/serum ECP levels and the SCORAD

($r = 0.36$, $P < 0.0001$; $r = 0.34$, $P < 0.0001$, respectively). When the ECP and EPX concentrations were correlated, we found a clear interrelationship between ECP and EPX levels ($r = 0.48$, $P < 0.0001$).

Urine EPX was also determined in six healthy controls (median value: $26.0 \mu\text{g}/\text{mmol}$ creatinine; range: 23.1 – $52.1 \mu\text{g}/\text{mmol}$ creatinine). Urine EPX concentrations were significantly lower in healthy subjects than in patients with mild/moderate AD and severe AD ($P < 0.001$).

Peripheral eosinophil counts were obtained from 19 patients. In 15 of these patients, eosinophil counts were determined at 8–13 time points. Both urine EPX levels and serum ECP levels were correlated with the number of eosinophils in the peripheral blood ($r = 0.63$, $P < 0.0001$; $r = 0.60$, $P < 0.000$, respectively).

Discussion

Taken together, our data demonstrate an interrelationship between urine EPX levels and disease activity in adult patients with AD as assessed by the SCORAD index and serum ECP levels. Our results indicate that eosinophils are important effector cells not only of allergic respiratory diseases but also of AD.

Different types of inflammatory cells can be found in the lesional skin of AD and eczematous reactions after patch testing. Lymphocytes of the CD4-positive T-helper phenotype dominate the cellular infiltrate, but there are also significant numbers of Langerhans cells and eosinophils. A model for the pathogenesis of delayed hypersensitivity to aeroallergens has been proposed, connecting these different cell types. Upon stimulation by Langerhans cells bearing allergen bound to surface IgE, allergen-specific T cells secrete several proinflammatory mediators, which lead to the recruitment of other cell types to the site of inflammation. Among them, IL-4 and IL-5 have been shown to induce eosinophil differentiation and activation, and to prevent eosinophil apoptosis (18). After eosinophil degranulation, toxic granule proteins such as MBP, ECP, and EPX are involved in the process of inflammation and tissue damage. Apart from its cytotoxic capacity, ECP is able to induce histamine release from mast cells (19). Eosinophil mediators, released at the site of inflammation, have been shown to pass over into the peripheral blood and body fluids. Elevated levels of ECP and EPX have been detected in the serum, sputum, nasal secretion, and BAL fluid of patients with atopic respiratory diseases.

The correlation of serum ECP levels with the disease activity of asthma and, as in our study, with AD, was demonstrated in several studies (10, 14). Serum ECP has been proven to be a useful *in vitro*

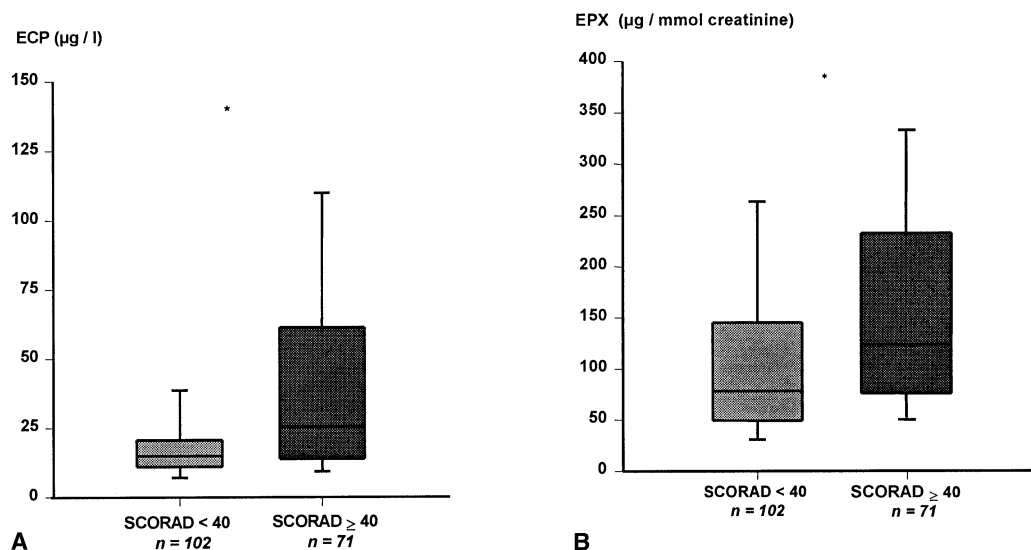


Figure 1. A) Serum ECP levels were compared in patients with severe AD (SCORAD ≥ 40) and patients with mild and moderate AD (SCORAD < 40). Horizontal lines indicate median values of EPX concentrations. Boxes show 25th and 75th percentiles. *Mann-Whitney rank sum test, $P < 0.0001$. B) Urine EPX levels were compared in patients with severe AD (SCORAD ≥ 40) and patients with mild and moderate AD (SCORAD < 40). Horizontal lines indicate median values of EPX concentrations. Boxes show 25th and 75th percentiles. *Mann-Whitney rank sum test, $P < 0.0001$.

parameter for the control of therapeutic efficacy and the outcome of challenge tests. Serum ECP levels decrease after successful therapy, independently of the therapeutic modus (20, 21). Furthermore, serum ECP and fecal ECP have been found to be elevated after oral challenge tests in children with AD (22, 23), and the fecal concentrations of ECP showed a decrease as a result of an elimination diet in patients with AD and food allergy (24).

Although several studies have demonstrated an interrelationship between urine EPX levels and disease activity in allergic asthma, only one study has established an interrelationship between urine EPX concentrations and the disease activity of AD. Ott et al. (25) first reported that serum and urine EPX levels are elevated in patients with AD, but no interrelationship could be established between EPX levels and disease activity in these patients. Pucci et al. showed that urine EPX levels are correlated to the severity of AD in children. A strong correlation could be specifically found with subjective symptoms such as pruritus and sleep loss; therefore, the authors concluded that eosinophil proteins may play a role in the pathogenesis of pruritus (14). Our present study demonstrates a relationship between eosinophil mediators and disease activity in adult patients with AD. In keeping with the results of Pucci et al., our data show that the urine levels of patients who are severely affected by AD are significantly higher than in patients with only mild or moderate AD. As a result of antiasthmatic therapy or elimination of aeroallergens, urine EPX levels decrease indepen-

dently of the treatment modality (12, 13, 26). As shown for ECP, fecal EPX decreases during an elimination diet in children with food allergy (24).

ECP and EPX exhibit different mechanisms and kinetics concerning the release from preactivated eosinophils, and different binding capacity to plasma proteins and clearance mechanisms, a fact which explains the different concentrations traceable in serum, plasma, and urine samples (27). Both ECP and EPX are released in significant amounts from preactivated eosinophil granulocytes during the process of coagulation in a time- and temperature-dependent manner. Low concentrations of both proteins can be detected in plasma samples, indicating a limited spontaneous release. Reimert et al. showed that the range of plasma ECP concentration is more limited than that of plasma EPX, a feature which is attributed by the authors to the different capacity to bind to plasma proteins and different renal clearance mechanisms (27). They conclude that the ECP and EPX concentrations reflect a combination of the state of activation and the number of eosinophils.

Certain properties make urine EPX a parameter of atopic inflammation that is superior to serum ECP: ECP is not detectable in urine samples. The determination of ECP in serum samples requires a highly standardized process concerning the handling of blood samples, since the release of ECP is dependent on the temperature and time of incubation before centrifugation of the blood sample. In contrast to ECP, EPX can be detected in significant amounts in the urine. Therefore, determination of EPX

requires no standardized protocol. Since blood sampling can be avoided, urine EPX is an appropriate measure of atopic disease activity in children.

Several substances secreted by other cell types such as activated endothelial cells and activated T cells have also been detected in the sera of patients with AD. The cytokine IL-2 is synthesized in the early phase of T-cell activation. A 42-kDa fragment of the IL-2-receptor α chain, which serves as a soluble IL-2 receptor, can also be traced in the serum of patients with AD. The concentration of the soluble IL-2 receptor (sIL-2R, CD23) was found to be correlated with the severity of cutaneous inflammation and the levels of total IgE (28). Karawajczyk et al. found an interrelationship between the clinical severity and the level of sIL-2R only in patients treated with corticosteroids, and not under other therapeutic modalities, a fact which points to a direct influence

of steroids on this parameter (29). The endothelial leukocyte adhesion molecule (ELAM-1, E-selectin) is expressed on the activated endothelium and was found to be elevated in inflammatory skin diseases such as AD and psoriasis, but a correlation between disease activity and sELAM-1 levels could be shown only in patients with AD (30). A recent study has correlated inflammatory activity to a higher extent to sELAM-1 than to serum ECP (31).

In conclusion, we suggest that the granule protein EPX is released from preactivated eosinophils that have been attracted to the site of cutaneous inflammation, where they may play a role in the pathogenesis of pruritus and consequently eczema. We also suggest that the urine EPX, which can be easily obtained by urine sampling, is therefore an appropriate parameter for the determination of disease activity in AD.

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