

Urinary eosinophil-derived neurotoxin/protein X: A simple method for assessing eosinophil degranulation in vivo

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Background: Eosinophil-derived neurotoxin/protein X (EDN/EPX), one of the cationic granule proteins released by polymorphonuclear eosinophils, can be detected in human urine. **Objective:** We sought to evaluate whether the urinary release of EDN/EPX was dependent on the blood eosinophil cell count, the bronchoalveolar eosinophil cell count, or both and on the clinical diagnosis. We also attempted to determine the precise kinetics of decrease of EDN excretion and eosinophil counts after the onset of corticosteroid treatment.

Methods: Daily urinary release of EDN/EPX was measured by radioimmunoassay in 28 patients with high hypereosinophilia (group 1), 32 patients with moderate hypereosinophilia (group 2), 26 patients without hypereosinophilia at the time of the study but with a known pulmonary disease involving eosinophils (group 3), and 13 control patients (group 4).

Results: The urinary excretion of EDN/EPX was significantly higher in patients from groups 1 or 2 than in patients from groups 3 or 4. Particularly high levels of EDN/EPX excretion were observed in patients from groups 1 or 2 with chronic eosinophilic pneumonia (chronic eosinophilic pneumonia: 4.7 ± 8.1 mg/day, control subjects: 0.39 ± 0.33 mg/day, $p < 0.001$). Urinary excretion of EDN/EPX was significantly correlated with blood ($r = 0.66$, $p < 0.001$) and differential bronchoalveolar ($r = 0.62$, $p = 0.04$) eosinophil cell counts in patients from group 1 but not from the other groups. Corticosteroid treatment was followed by a significant decrease in EDN/EPX excretion. The kinetics of decrease in EDN/EPX were delayed as compared with the dramatic drop in peripheral eosinophil counts. Distinct kinetics between urinary EDN/EPX and eosinophil counts differentiated the recurrence of chronic eosinophilic pneumonia from an asthma attack in one patient.

Conclusion: Measurement of urinary EDN/EPX excretion may be a useful indicator of eosinophil degranulation in vivo. (*J Allergy Clin Immunol* 1998;101:116-23.)

Key words: Eosinophil, degranulation, cationic protein, eosinophil-derived neurotoxin, chronic eosinophilic pneumonia

Abbreviations used

ECP:	Eosinophil cationic protein
EDN/EPX:	Eosinophil-derived neurotoxin/protein X
EPO:	Eosinophil peroxidase
MBP:	Major basic protein

It is generally accepted that eosinophil leukocytes play a role in the pathogenesis of asthma^{1,2} and a variety of diseases, such as parasitic infestations, eosinophilic pneumonia, vasculitic granulomatous diseases, interstitial lung disorders, eosinophilic pleural effusion, and eosinophilia associated with neoplastic and myeloproliferative disease.^{3,4} Eosinophils possess a wide range of biologic properties that may participate in the pathogenesis of these diseases by releasing proteins contained in their cytoplasmic granules, oxygen-free radicals, eicosanoids, sulfido-peptide leukotrienes, platelet activating factors, cytokines, and growth factors.⁵⁻⁷ Granule-derived proteins released by activated eosinophils include major basic protein (MBP); eosinophil cationic protein (ECP); eosinophil peroxidase (EPO), which has been shown to be cytotoxic to airway epithelium;⁸ and eosinophil-derived neurotoxin/protein X (EDN/EPX), which has been less extensively studied, although its release by eosinophils in vitro is commonly used as a marker of eosinophil degranulation.⁹⁻¹¹

Small amounts of EDN/EPX can be detected in normal human urine.¹² We have previously found high levels of urinary EDN/EPX in the urine of patients with idiopathic chronic eosinophilic pneumonia¹³ and Churg-Strauss syndrome.¹⁴ Slightly increased urinary excretion of EDN/EPX has also been reported in children with atopic asthma.¹⁵ Because the measurement of urinary EDN/EPX may be a useful indicator of eosinophil degranulation in vivo,¹⁶ the aim of this study was to evaluate whether the urinary release of EDN/EPX was dependent on the blood eosinophil cell count, the bronchoalveolar eosinophil cell count, or both and on the clinical type of disorder associated with hypereosinophilia. We also attempted to determine the precise kinetics of decrease of EDN/EPX excretion after the onset of corticosteroid treatment.

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TABLE I. Study population: Patients with a very high blood ($>1 \times 10^9/L$), bronchoalveolar ($>40\%$), or both types of eosinophil cell counts (group 1)

Diagnosis	No. of patients (corticosteroid-treated)	Sex	Age	Blood eosinophil count ($\times 10^9/L$)	Bronchoalveolar eosinophil count (%)
Chronic eosinophilic pneumonia	7 (5)	3M, 4F	50.3 $\pm$ 17.1 (22-70)	3.13 $\pm$ 2.3 (0.65-7.62)	70 $\pm$ 21 ($n = 6$) (40-95)
Churg-Strauss syndrome	2 (0)	2M	39.0 $\pm$ 14.1 (29-49)	3.87 $\pm$ 4.2 (0.9-9.84)	47 $\pm$ 4 ($n = 2$) (44-50)
Idiopathic hyper-eosinophilic syndrome	1 (1)	1M	16	1.83	NA
Asthma	4 (3)	4M	57.3 $\pm$ 19.3 (34-76)	1.09 $\pm$ 0.1 (1.0-1.17)	11 ($n = 1$)
Paraneoplastic eosinophilia	4 (3)	4M	70.8 $\pm$ 9.1	2.44 $\pm$ 1.4 (1.71-4.58)	NA
Non-Hodgkin's lymphoma	2 (0)	1M, 1F	40.5 $\pm$ 3.5 (38-43)	1.19 $\pm$ 0.9 (0.50-1.87)	69 ($n = 1$)
Cryptogenic organizing pneumonia	1 (0)	1F	67	0.34	47 ($n = 1$)
Diffuse interstitial pneumonitis	1 (0)	1M	33	3.22	NA
Chronic heroin-induced eosinophilic pneumonia	1 (0)	1M	36	1.2	NA
Chronic eosinophilic dermatitis	1 (0)	1F	23	1.0	NA
Tuberculosis	1 (0)	1M	28	1.44	NA
Bacterial pneumonia	1 (0)	1M	78	1.29	NA
Chronic obstructive pulmonary disease	1 (1)	1M	73	1.68	NA
Intestinal parasitosis	1 (0)	1F	75	1.86	NA
TOTAL	28 (13)	20M, 8F	51.9 $\pm$ 19.9 (16-79)	2.14 $\pm$ 1.8 (0.34-7.62)	58 $\pm$ 24 ($n = 11$) (11-95)

Results are mean $\pm$ SD (range).

NA, Not available.

METHODS

Study population

The patient population is described in Tables I, II, and III. Eighty-six patients entered the study. They were distributed among one of the following four groups according to their initial blood and bronchoalveolar cell counts and their underlying diseases. Group 1 consisted of patients with high hypereosinophilia ($n = 28$) defined by a blood eosinophil cell count greater than $1 \times 10^9/L$ ($n = 24$), a bronchoalveolar eosinophil cell count greater than 40% ($n = 10$), or both. Group 2 consisted of patients with moderate hypereosinophilia ($n = 32$) defined by a blood eosinophil cell count greater than $0.5 \times 10^9/L$ (but $<1 \times 10^9/L$), a bronchoalveolar eosinophil cell count greater than 5% (but $<40\%$), or both. Group 3 consisted of patients with a pulmonary disease known to involve eosinophils ($n = 26$) such as chronic eosinophilic pneumonia (Carrington's disease, $n = 3$), Churg-Strauss syndrome ($n = 2$), pulmonary parasitosis ($n = 2$), eosinophilic pleuritis ($n = 3$), and allergic asthma ($n = 16$), but without hypereosinophilia at the time of the study because of treatment, disease remission, or both. Group 4 consisted of control patients without eosinophilia and without a known eosinophil-related lung disease ($n = 13$).

In a small subgroup of patients, serial investigations of urinary EDN/EPX levels were done during follow up of the treated patients. Detailed clinical data on one patient with chronic eosinophilic pneumonia, one with Churg-Strauss syn-

drome, and one with idiopathic hypereosinophilic syndrome were previously published.^{13, 14, 17}

Design of the study

For each patient, 24-hour total urine samples were collected, and daily urinary excretion of EDN/EPX was measured. Eosinophil blood counts were determined at the same time with an automatic counter (H1, Technicon) and visual verification with Wright's staining. Bronchoalveolar lavage with flexible bronchoscopy was done for some of the patients, and the percentage of eosinophils at the time of bronchoalveolar lavage was evaluated by visual count with Wright's staining. Serum creatinine levels were also determined to detect any renal insufficiency.

Assay of urinary EDN/EPX

Each urine sample was run on an agarose gel electrophoresis (Hydrogel, Sebia, France) and stained with Coomassie Blue to detect a far-cathodic band suggestive of EDN/EPX. Quantitative evaluation of urinary EDN/EPX was performed as previously described.¹⁴ Briefly, an immunoassay was performed on microplates coated with anti-EDN/EPX polyclonal rabbit antibodies. These specific antibodies raised in our laboratory with EDN/EPX purified from the urine of a patient with chronic eosinophilic pneumonia detected only one band in urinary proteins separated by agarose gel electrophoresis.¹⁸ These antibodies did not cross-react with ECP. The detection threshold was 8 ng/ml, and the interassay variability coefficient was 11.3% for a control urine sample.

TABLE II. Study population: Patients with moderate hypereosinophilia (blood eosinophilia 0.5 to $1 \times 10^9/L$, bronchoalveolar eosinophilia 5% to 40% , or both (group 2))

Diagnosis	No. of patients (corticosteroid-treated)	Sex	Age	Blood eosinophil count ($\times 10^9/L$)	Bronchoalveolar eosinophil count (%)
Chronic eosinophilic pneumonia	3 (2)	2M, 1F	64.0 ± 14.1 (54-74)	0.24 ± 0.23 (0.01-0.48)	17 ± 19 ($n = 3$) (5-39)
Churg-Strauss syndrome	1 (1)	1M	65	0.68	NA
Idiopathic hyper-eosinophilic syndrome ¹⁷	1 (1)	1M	38	0.65	NA
Asthma	7 (7)	5M, 2F	49.4 ± 18.6 (28-76)	0.78 ± 0.15 (0.59-0.97)	38 ($n = 1$)
Paraneoplastic disease	3 (1)	3M	65.7 ± 11.2 (56-78)	0.66 ± 0.10 (0.55-0.75)	3 ($n = 1$)
Lymphoma	2 (2)	1M, 1F	44.5 ± 16.3 (33-56)	0.68 ± 0.23 (0.52-0.84)	2 ($n = 1$)
Cryptogenic organizing pneumonia	2 (1)	2F	63.0 ± 1.4 (32-64)	0.56 ± 0.26 (0.38-0.75)	6 ± 6 ($n = 2$) (2-11)
Chronic obstructive pulmonary disease	3 (0)	3M	66.0 ± 5.6 (60-71)	0.85 ± 0.10 (0.73-0.91)	NA
Parasitosis (pulmonary tropical eosinophilia, anguillulosis)	2 (0)	1M, 1F	46.5 ± 13.4 (37-56)	0.67 ± 0.02 (0.65-0.68)	5 ($n = 1$)
Idiopathic pulmonary fibrosis	2 (2)	2M	58 ± 15.6 (47-69)	0.53 ± 0.20 (0.38-0.67)	13 ($n = 1$)
Diffuse interstitial pneumonitis	4 (0)	1M, 3F	53.8 ± 20.8 (26-75)	0.38 ± 0.16 (0.18-0.53)	15 ± 11 ($n = 4$) (2-26)
Pleural diseases	2 (0)	1M, 1F	31 ± 7.1 (26-36)	0.93 ± 0.06 (0.89-0.97)	NA
TOTAL	32 (17)	21M, 11F	54.0 ± 16.1 (26-78)	0.63 ± 0.24 (0.01-0.97)	13 ± 13 ($n = 14$) (2-39)

Results are mean $\pm$ SD (range).

NA, Not available.

TABLE III. Study population: Patients from group 3 without current hypereosinophilia

Diagnosis	No. of patients (corticosteroid-treated)	Sex	Age	Blood eosinophil count ($\times 10^9/L$)	Bronchoalveolar eosinophil count (%)
Chronic eosinophilic pneumonia	3 (3)	1M, 2F	60.3 ± 2.1 (58-62)	0.10 ± 0.06 (0.04-0.15)	NA
Churg-Strauss syndrome	2 (2)	2F	62.0 ± 7.1 (57-67)	0.17 ± 0.03 (0.15-0.17)	2 ± 2.8 ($n = 2$) (0-4)
Asthma	16 (15)	8M, 8F	53.4 ± 14.6 (26-75)	0.15 ± 0.15 (0-0.49)	NA
Parasitosis	2 (0)	1M, 1F	56.0	0.34 ± 0.13 (0.25-0.43)	NA
Eosinophilic pleuritis	3 (0)	3M	46.0 ± 2.8 (44-48)	0.29 ± 0.17 (0.16-0.49)	NA
TOTAL	26 (20)	13M, 13F	54.5 ± 12.5 (26-75)	0.18 ± 0.15 (0-0.49)	2 ± 2.8 ($n = 2$) (0-4)

Results are mean $\pm$ SD (range).

NA, Not available.

Statistical analysis

Results are presented as means $\pm$ SD (range). Because of the small size of the samples, differences between groups were tested by the nonparametric Mann-Whitney U test or Wilcoxon test for paired values with software (Statworks; Data Metrics, Philadelphia, Pa.) run on a Macintosh computer. The chi-squared test was used to compare frequencies. Correlations were calculated with the linear correlation test. Differences were considered significant for p values less than 0.05.

RESULTS

Study population

The main characteristics of the study population are shown on Tables I, II, and III. Serum creatinine levels were $104.1 \pm 21.6 \mu\text{mol/L}^{-1}$ (range, 83 to $200 \mu\text{mol/L}^{-1}$) in group 1, $105.1 \pm 19.9 \mu\text{mol/L}^{-1}$ (range, 73 to $155 \mu\text{mol/L}^{-1}$) in group 2, and $102.1 \pm 14.8 \mu\text{mol/L}^{-1}$ (range, 76 to $132 \mu\text{mol/L}^{-1}$) in group 3. A mild renal insufficiency, as indicated by the finding of high levels of serum

creatinine (from 120 to 200 $\mu\text{mol/L}^{-1}$), was found in one patient from group 1, six patients from group 2, and three patients from group 3 and may have caused a diminished urinary excretion of EDN/EPX.

Urinary EDN/EPX excretion in control subjects

Control subjects without any known hypereosinophilic disorder had low urinary EDN/EPX excretion (0.39 ± 0.33 mg/day [range, 0.08 to 0.9 mg/day]).

Urinary EDN/EPX excretion in patients with high or moderate hypereosinophilia

Fifty-three of 86 patients (62%) had a very basic protein band on urine agarose gel electrophoresis suggestive of EDN/EPX excretion, which represented less than 1% of total urinary protein in 40 patients, 1% in five patients, 2% in four patients, and 3% or more in four patients.

The urinary excretion of EDN/EPX depending on the patient groups and treatment status is shown on Fig. 1. The urinary excretion of EDN/EPX was significantly higher in patients with high hypereosinophilia (group 1) (2.04 ± 5.11 mg/day; range, 0.08 to 27.2 mg/day) than in patients from group 3 (0.33 ± 0.26 mg/day; range, 0.01 to 0.97 mg/day; $p = 0.01$) or control patients (0.39 ± 0.33 ; range, 0.08 to 0.98 mg/day; $p = 0.03$). The excretion of EDN/EPX was significantly higher in group 2 (0.93 ± 1.13 mg/day; range, 0.07 to 5.65 mg/day) than in group 3 ($p = 0.003$) or the control subjects ($p = 0.003$). Statistical differences could not be found between groups 1 and 2 or between group 3 and the control subjects.

Because of this lack of significant difference in EDN/EPX excretion between the whole groups 1 and 2, the results of these groups were pooled together and the results were analyzed according to diagnosis. As compared with control subjects, the urinary excretion of EDN/EPX was significantly higher in patients from both groups 1 and 2 with chronic eosinophilic pneumonia (4.69 ± 8.13 mg/day; range, 0.12 to 27.2 mg/day; $p < 0.001$), Churg-Strauss syndrome (1.17 ± 0.83 mg/day; range, 0.5 to 2.1 mg/day; $p = 0.03$), idiopathic hypereosinophilic syndrome (1.56 ± 1.12 mg/day; range, 0.77 to 2.35 mg/day; $p = 0.04$), paraneoplastic eosinophilia (1.4 ± 1.96 mg/day; range, 0.24 to 5.65 mg/day; $p = 0.03$), and eosinophilic pleuritis (1.01 ± 0.42 mg/day; range, 0.71 to 1.30 mg/day; $p = 0.04$). High levels of EDN/EPX excretion were observed in isolated cases of asthma, non-Hodgkin's malignant lymphoma, chronic obstructive lung disease, and eosinophilic dermatitis, but the difference in EDN/EPX excretion between these groups of patients and control subjects was not statistically significant: 0.54 ± 0.46 mg/day (range, 0 to 1.44 mg/day; $p = 0.15$), 0.61 ± 0.62 mg/day (range, 0.08 to 1.5 mg/day; $p = 0.32$), 0.80 ± 0.77 mg/day (range, 0.16 to 1.8 mg/day; $p = 0.09$), and 2.1 mg/day ($n = 1$), respectively. Levels of EDN/EPX excretion were not elevated in patients with interstitial lung disease (0.61 ± 0.48 mg/day; range, 0.07 to 1.65 mg/day; $p = 0.17$) such as idiopathic pulmonary fibrosis except in one patient (1.65

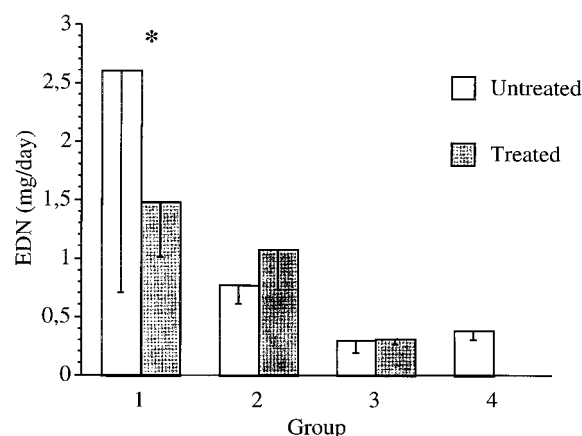


FIG. 1. Urinary EDN/EPX excretion in treated and untreated patients with high (group 1), moderate (group 2), or no eosinophilia (group 3) or control patients (group 4).

mg/day) who had both amiodarone-induced interstitial lung disease and lung carcinoma. Levels of EDN/EPX excretion were not significantly elevated in patients with parasitosis (0.41 ± 0.29 mg/day; range, 0.23 to 0.74 mg/day; $p = 0.27$), cryptogenic organizing pneumonia (0.22 ± 0.09 mg/day; range, 0.11 to 0.29 mg/day; $p = 0.42$), and bacterial pneumonia (0.69 ± 0.23 mg/day; range, 0.52 to 0.85 mg/day; $p = 0.11$).

To further determine the precise diagnosis-based differences in EDN/EPX excretion, we divided daily EDN/EPX excretion (milligrams per day) by the peripheral eosinophil cell count ($\times 10^9/\text{L}$) in patients from both groups 1 and 2. The amount of EDN/EPX excretion as related to eosinophil count ($\times 10^9/\text{L}$) was found to be markedly higher in patients with chronic eosinophilic pneumonia ($31.4 \pm 94.4 \times 10^{-9} \times \text{mg} \times \text{L/day}$, $n = 10$) than in patients with any of the other diagnoses (mean, $0.98 \pm 1.3 \times 10^{-9} \times \text{mg} \times \text{L/day}$ in the 50 patients with a diagnosis different from chronic eosinophilic pneumonia, $p = 0.005$).

Correlation of peripheral and bronchoalveolar eosinophil counts with urinary EDN/EPX

Urinary excretion of EDN/EPX was correlated with both blood ($r = 0.66$, $p < 0.001$) and differential bronchoalveolar ($r = 0.62$, $p = 0.04$) eosinophil cell counts in patients with a high eosinophilia (group 1). On the other hand, urinary excretion of EDN/EPX was not correlated with blood and bronchoalveolar eosinophil cell counts in patients with a moderate eosinophilia (group 2) ($r = 0.07$, $p = 0.7$ and $r = 0.24$, $p = 0.6$, respectively) or in patients without eosinophilia ($r = 0.28$, $p = 0.16$ for blood eosinophilia, unavailable for bronchoalveolar eosinophilia).

Urinary EDN/EPX in serial investigations in patients with treated hypereosinophilic disorders

Urinary EDN/EPX excretion was significantly higher in untreated than treated patients from group 1, and no statistical difference was found between untreated and

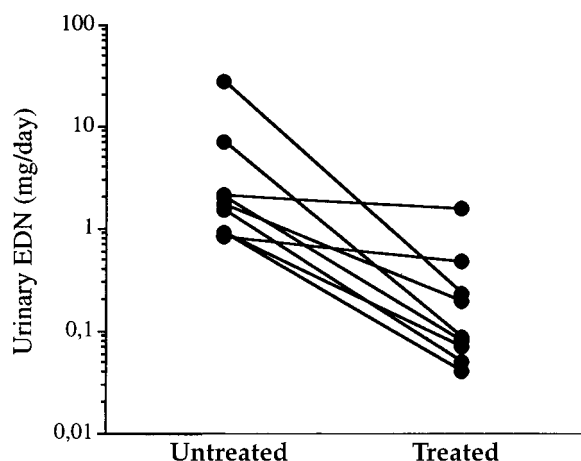


FIG. 2. Effect of corticosteroid treatment on EDN/EPX excretion. In seven patients EDN/EPX excretion was measured while patients did not receive any corticosteroid treatment or were still symptomatic (*Untreated*) and compared with the lowest measurement during treatment thereafter (*Treated*).

treated patients from groups 2 or 3 (Fig. 1). EDN/EPX measurements were serially repeated in seven patients with a high level of EDN/EPX excretion at first measurement. In all patients, longitudinal assessment of EDN/EPX excretion showed a decrease in EDN/EPX levels during corticosteroid treatment (Fig. 2). EDN/EPX excretion was significantly higher on first measurement compared with the lowest EDN/EPX level during the treatment ($p < 0.001$). Four representative cases are illustrated on Fig. 3. In all of these, we observed dissociated kinetics between blood eosinophil counts and EDN/EPX excretion, the decrease of which was delayed for several days in all patients. Blood eosinophil counts dropped to a low level within 24 hours or a few days after the onset of corticosteroid treatment in patients A and B with chronic eosinophilic pneumonia and in patient C with Churg-Strauss syndrome. Patient D, with idiopathic hypereosinophilic syndrome, already received corticosteroid treatment, and only a moderate decrease in eosinophil cell count could be obtained while continuing the treatment.

Interestingly, different kinetics were observed between patients A and B, both with chronic eosinophilic pneumonia (Fig. 3). In patient A the decrease in EDN/EPX excretion was delayed as opposed to the peripheral eosinophil count, which rapidly dropped to a normal level after the onset of corticosteroid treatment. No increase in eosinophil count or EDN/EPX excretion could be observed thereafter. This patient had a highly favorable clinical course under treatment, and corticosteroids could be withdrawn after 320 days. In patient B EDN/EPX excretion and eosinophil count similarly decreased after the onset of treatment, but an increase in blood eosinophil count was observed on two occasions thereafter. The first was in month 11, when EDN/EPX excretion remained at a low level while the patient

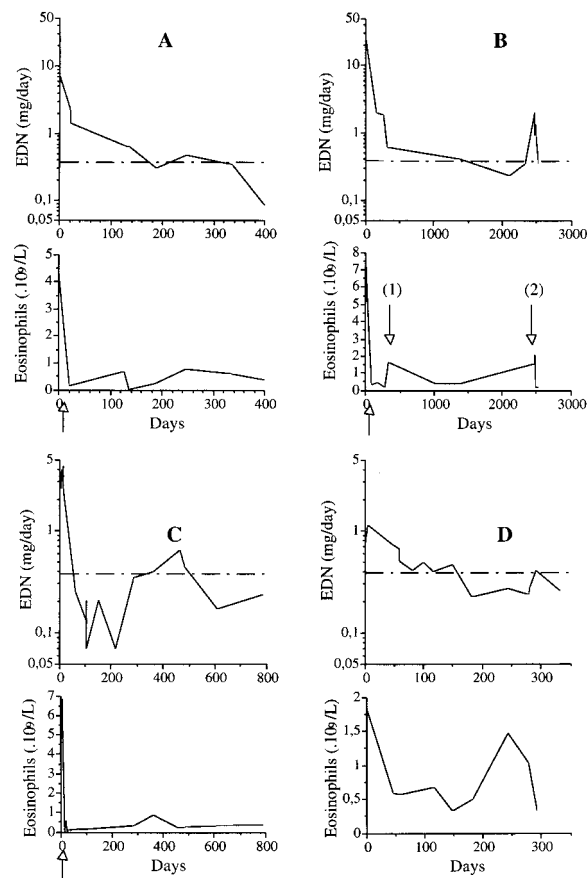


FIG. 3. Longitudinal assessment of urinary EDN/EPX excretion and blood eosinophil cell counts in four representative patients with chronic eosinophilic pneumonia (A and B), Churg-Strauss syndrome (C), and idiopathic hypereosinophilic syndrome (D) showing dissociate kinetics between EDN/EPX excretion and blood eosinophil counts. Arrows indicate onset of corticosteroid treatment; patient D already received corticosteroid treatment. (1), Asthma attack; (2), recurrence of chronic eosinophilic pneumonia.

presented with asthma exacerbation but without chronic eosinophilic pneumonia as judged by clinical and radiologic examination. The second occasion occurred in month 80, when both urinary EDN/EPX excretion and eosinophil count markedly increased, while clinicoradiologic examination revealed recurrence of chronic eosinophilic pneumonia without asthma. Isolated and slight increases in eosinophil counts (patient D) or EDN/EPX excretion (patient C) were observed in the other two patients, but these were not concomitant with any evident change in clinical state.

DISCUSSION

In this study we showed that the urinary release of EDN/EPX was correlated with blood and bronchoalveolar eosinophil cell counts before the onset of the treatment in patients with a high hypereosinophilia. The level of EDN/EPX excretion was further dependent on the clinical diagnosis, with very high levels being found in

chronic eosinophilic pneumonia. In all patients with serial investigations, corticosteroid treatment was followed by a significant decrease in EDN/EPX excretion. However, the kinetics of decrease in EDN/EPX levels were markedly delayed as compared with the dramatic drop in peripheral eosinophil count.

Despite isolated reports of high levels of urinary EDN/EPX excretion in patients with chronic eosinophilic pneumonia,¹³ Churg-Strauss syndrome,¹⁴ eosinophilia-myalgia syndrome associated with L-tryptophan ingestion,^{19, 20} and asthma,¹⁵ urinary EDN/EPX excretion has not been systematically studied in a spectrum of hypereosinophilic disorders, and diagnosis-based differences could not be evaluated. Moreover, discrepancies between studies might be increased because of differences in sample processing.²¹ This study clearly indicates that high EDN/EPX levels are found mainly in untreated patients with chronic eosinophilic pneumonia and to a lesser extent in patients with other diseases, such as Churg-Strauss syndrome, idiopathic hypereosinophilic syndrome, or bronchial carcinomas. Moreover, the amount of EDN/EPX excretion as related to eosinophil counts was found to be higher in patients with chronic eosinophilic pneumonia than in patients with any other diagnosis, suggesting that an especially high eosinophil degranulation occurs in chronic eosinophilic pneumonia. This result is consistent with previous studies that used density gradient centrifugation and showed a large proportion of "hypodense" eosinophils in the bronchoalveolar lavage fluid of patients with chronic eosinophilic pneumonia.²² These hypodense cells have been shown to be degranulated on ultrastructural analysis,²³ and may release specific granule proteins, including EDN/EPX. In patients with chronic eosinophilic pneumonia, eosinophil degranulation has been demonstrated on the basis of ultrastructural studies²⁴⁻²⁷; eosinophils are lysed,²² leading to eosinophilic microabscesses,²⁸ and deposits of MBP are found in parenchymal lesions.^{24, 28} Eosinophil degranulation has also been demonstrated in Churg-Strauss syndrome by ultrastructural studies¹⁴ and the discovery of large deposits of ECP and EDN/EPX within granulomas and necrotic lesions.²⁹ Hypodense eosinophils have also been described in patients with idiopathic hypereosinophilic syndrome²³ and neoplasia.⁵ Taken together, these data indicate that high levels of urinary EDN/EPX are found in patients with various disorders in which eosinophil degranulation has been demonstrated.

Surprisingly, urinary excretion of EDN/EPX was not significantly elevated in our asthmatic patients as compared with controls. This result contrasts with recent studies, relating elevated EDN/EPX levels in urine,^{15, 30} serum,^{31, 32} and bronchoalveolar lavage fluid³³ from patients with asthma. In one of these studies, urinary EDN/EPX excretion was found that was three times above the control values.¹⁵ Moreover, eosinophils have been shown to be degranulated during episodes of asthma,¹ and striking MBP depositions are observed by immunostaining bronchial mucosa from autopsy speci-

mens.³⁴ Discrepancies between our results and the above-mentioned reports of elevated EDN/EPX levels in various biologic fluids in asthmatic subjects may be explained by differences in treatment status. Our study was not specifically conducted to study EDN/EPX release in asthmatic subjects, a great majority of whom (25 of 27, see Tables I, II, and III) already received corticosteroids or were stabilized at the time of EDN/EPX measurement. On the other hand, only a few patients already received antiinflammatory treatment in the previous studies.^{15, 30, 32} This result is consistent with the demonstrated decrease in EDN/EPX release during corticosteroid treatment.^{15, 32}

Indeed, a major finding of this study is the striking relationship between EDN/EPX release and the clinical condition of the patients, particularly in patients with chronic eosinophilic pneumonia. Urinary EDN/EPX excretion was found at a higher level in patients with active disease compared with treated patients with a stabilized disease and decreased under treatment. This is consistent with previous reports in children with atopic asthma¹⁵ and adults with eosinophilia-myalgia syndrome.²⁰ Moreover, distinct kinetics between urinary EDN/EPX and eosinophil counts differentiated recurrence of chronic eosinophilic pneumonia requiring treatment adaptation from asthma attack. This observation suggests that although there is a correlation between eosinophilia and urinary EDN/EPX excretion, a high level of EDN/EPX excretion may reflect that the eosinophil leukocytes are activated and degranulate *in vivo* independently of the eosinophil cell count.

Interestingly, different kinetics were observed between the delayed decrease in the level of EDN/EPX excretion and the peripheral eosinophil count, which rapidly dropped to a normal value after the onset of corticosteroid treatment. The corticosteroid-induced decrease in EDN/EPX excretion is likely to be due to the suppression, by treatment, of cytokines capable of enhancing eosinophil degranulation, such as IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor.⁹ However, the explanation for a sustained release of EDN/EPX in patients who no longer have peripheral or bronchoalveolar hypereosinophilia is not clear. This may simply reflect continued release of cationic proteins by eosinophils that no longer circulate but still are present in the lung subepithelial mucosa, the parenchyma, or other tissues. Little is known about the kinetics and process of elimination of EDN/EPX after it has been released into the extracellular space. Nevertheless, the recently reported variation in EDN/EPX excretion from day to day and even within the day³⁰ excludes the hypothesis of a very long half-life for EDN/EPX. Because EDN/EPX is a basic protein with a marked adhesiveness for tissue constitence, it could be stored in tissue or associated with plasma proteins (as in the case of ECP³⁵) and then progressively released. EDN/EPX could also be released from macrophage granules after eosinophil phagocytosis by these cells or pas-

sively released by eosinophils undergoing corticosteroid-induced apoptosis.³⁶

EDN/EPX is clearly the predominant eosinophilic cationic protein present in urine (Philippe Deviller, personal communication, 1996), although ECP, MBP, and EPO are also contained in specific eosinophilic granules.⁵ Although this might be due to the selective release of EDN/EPX by eosinophils,³⁷ it is more probably related to the fact that molecules that are either aggregated (MBP) or have a higher molecular mass (EPO, 67 kd) fail to pass the glomerular filter.¹³ The observed low level of low molecular mass ECP (21 kd) in the urine of patients with a high level of urinary EDN/EPX may be consequent to a process of tubular reabsorption of ECP instead of the concentration of EDN/EPX in the urine,¹³ but this hypothesis remains speculative. Nevertheless, given the predominance of EDN/EPX in urine, we believe that the measurement of urinary EDN/EPX may be a method of choice for monitoring the release of granule proteins by eosinophils in vivo.

In summary, this study shows that measurement of urinary EDN/EPX excretion may be a useful indicator of eosinophil degranulation in vivo. Consequently, urinary EDN/EPX levels may reflect ongoing eosinophilic pulmonary inflammation. The simplicity of this noninvasive method has to be contrasted with the low feasibility of ultrastructural and density studies of eosinophils in clinical practice. However, the clinical usefulness of monitoring eosinophil degranulation in vivo remains to be confirmed.

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