

Local release of eosinophil peroxidase following segmental allergen provocation in asthma

V. J. Erpenbeck*†, J. M. Hohlfeld*†, J. Petschallies†, E. Eklund‡, C. G. B. Peterson‡, H. Fabel† and N. Krug*

*Fraunhofer Institute of Toxicology and Aerosol Research, Hannover, Germany, †Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany and ‡Pharmacia Diagnostics AB, Uppsala, Sweden

Summary

Background Eosinophil peroxidase (EPO) is an eosinophilic basic protein, which leads to increased permeability and damage of bronchial epithelial cells in asthma.

Objective As little is known about its local expression and release in humans the intracellular expression in lung and peripheral eosinophils and the concentrations of EPO in bronchoalveolar lavage (BAL) fluid and serum was investigated in patients with asthma.

Methods Twelve mild atopic asthmatic and nine control subjects underwent segmental sham and allergen challenge. EPO concentrations in BAL fluid and serum were determined by immunoassay and flow cytometry was used to determine the intracellular expression of EPO in BAL-derived and peripheral eosinophils.

Results In asthmatic patients a large increase in BAL eosinophils – total cells: median 9.5×10^6 (range: 0.5 to 455.0×10^6); relative: 38% (1 to 91%) – was detectable 24 h following allergen challenge, but peripheral blood eosinophil counts did not change. Concentrations of EPO in BAL fluid increased from 1 µg/L (1.0 to 6.8 µg/L) to 42 µg/L (5.6 to 379.6 µg/L; $P < 0.01$) after allergen but not after saline challenge (1.5 µg/L; 1.0 to 21.9 µg/L), whereas in control subjects all measurements were below the detection limit. Serum concentrations of EPO increased slightly from 18.3 µg/L (3.0 to 56.8 µg/L) to 27 µg/L (3.8 to 133.9 µg/L; $P < 0.05$) 24 h after allergen challenge in asthmatic patients. Furthermore, the intracellular expression of EPO (measured as mean fluorescence intensity) was decreased in BAL eosinophils compared with blood eosinophils (mean fluorescence intensity 29 (7 to 71) vs. 48 (20 to 85); $P < 0.01$) after allergen challenge.

Conclusion The finding of increased EPO concentrations in the BAL fluid and decreased intracellular EPO expression in pulmonary eosinophils of asthmatic patients reflects the allergen-triggered release of EPO into the bronchial space.

Keywords asthma, eosinophil, eosinophil peroxidase

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Introduction

Eosinophil granulocytes are important effector cells in allergic inflammatory disorders. During the allergic response eosinophils are present at the site of inflammation, where they produce a variety of inflammatory mediators and reactive oxidant species, generate free radicals and release toxic granules upon activation. They contain large amounts of cytotoxic and basic proteins, which are known as major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophilic neurotoxin (EDN/EPX) and eosinophil peroxidase (EPO) [1, 2]. Large amounts of these eosinophilic products have been identified in tissues and fluids affected by allergic diseases such as asthma and rhinitis [3, 4].

EPO is one of the most abundant proteins in eosinophils, comprising approximately 25% of the total protein mass of

specific granule protein [5]. It is solely produced by eosinophils and used as a cell specific marker for these cells [6]. Hydrogen peroxide and halide acids, which are generated by EPO, are effective in the defence of helminthic infections but they also cause damage of the epithelium in the asthmatic airways [2]. It has been shown that oxidative damage of proteins through bromination of tyrosine residues is caused by catalytic-acting EPO [7, 8]. As a selective marker of EPO activity 3-bromotyrosine has been found to be markedly increased in BAL fluid of asthmatics [9, 10].

Using transmission electron microscopy and histochemical staining Erjefält et al. have shown increased levels of EPO-stained mucosal eosinophils and free EPO-stained granules in nasal biopsies of allergen-challenged patients with allergic rhinitis [11]. Sedgwick et al. showed increased levels of EPO in bronchoalveolar lavage (BAL) fluid 48 h after segmental allergen challenge of allergic rhinitis patients [12]. Increased serum levels of EPO have been detected in patients with allergic asthma [4, 13, 14]. However, so far no quantitative data exist on the release of EPO in the asthmatic airways during the allergic response.

Correspondence: V. J. Erpenbeck, Fraunhofer Institute of Toxicology and Aerosol Research, Nikolai-Fuchs-Straße 1, 30625 Hannover, Germany. E-mail: Erpenbeck@ita.fraunhofer.de

Using a previously described method for the intracellular detection of EPO as well as measuring the levels of EPO in BAL fluid and serum we examined the effect of local allergen challenge on EPO release by eosinophils [14]. Segmental allergen and saline challenge was performed in asthmatics as well as in healthy controls and material was obtained before and 24 h after provocation. Subsequent intracellular staining and flow cytometric measurement allowed the detection of EPO at the single cell level in BAL- and blood-derived eosinophils. We hypothesized that the allergen challenge in allergic asthmatics leads to a release of EPO from local eosinophils with an increase of EPO in BAL fluid and a decreased amount of intracellular EPO in local eosinophils.

Materials and methods

Study subjects

Twelve patients with mild asthma and nine normal controls participated in the study (for patient characteristics see Table 1). All patients had mild allergic asthma as defined in the Consensus Report [15]. They had a history of intermittent wheeze with reversible airflow obstruction, and asthma had previously been diagnosed by an independent physician. Each patient had a positive skin prick test, defined as > 4 mm diameter skin weal response to one or more of nine common

allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, mixed grass pollen, mixed tree pollen, mixed herb pollen, dog hair, cat fur, *Cladosporia* and *Alternaria*; allergen extraction from Abelló, Bornheim, Germany). The allergen extract used for segmental allergen challenge (mixed grass pollen or *D. pteronyssinus*; Abelló) was that which produced the largest weal response on skin prick testing and the chosen concentration was one-tenth the dilution in saline that elicited a 3-mm diameter skin weal response. The instilled amount of antigen was 0.01 to 0.1 µg for mixed grass pollen (mixture of the major allergens Dac g5, Fes p5, Lol p5, Phl p5, Poa p5) and 0.6 µg for *D. pteronyssinus* (mixture of the major allergens Der p 1 and Der p 2). Bronchial hyper-responsiveness and PC₂₀FEV₁ were determined as described [16].

Patients were only using salbutamol when required for relief of symptoms. None were treated with corticosteroids, sodium cromoglycate or theophylline. The normal controls had no history of allergic or other diseases, negative skin-prick tests, normal IgE (≤ 100 kU/L), normal lung function tests and no bronchial hyper-responsiveness (PC₂₀ > 8 mg/mL). All study subjects were non-smokers and no subject had an acute bronchitis 4 weeks prior to the investigations. All subjects were volunteers and gave their written consent after being fully informed about the purpose and nature of the studies, which were approved by the Ethical Committee of Hannover Medical School.

Table 1. Clinical characteristics of the study subjects

Subjects	Age	Sex	FEV ₁ (%)	PC ₂₀ FEV ₁ Histamine (mg/mL)	IgE kU/L	Allergen sensitivity†	Allergen provocation‡
Control	33	F	103	10.2	62	–	–
	32	M	95	11.9	5	–	–
	23	M	103	10.5	52	–	–
	27	M	101	20.0	7	–	–
	23	M	104	25.4	7	–	0.1 µg gp
	25	M	108	11.9	3	–	0.1 µg gp
	24	M	103	11.3	9	–	0.1 µg gp
	28	M	96	16.1	8	–	0.6 µg Dp
	33	F	104	20.1	8	–	0.6 µg Dp
Median (range)	27 (23–33)	2F/7M	103 (95–108)	11.9 (10.2–25.4)	8 (3–62)		
Asthmatic	24	F	106	11.2	50	gp, tp, hp, c	0.01 µg gp
	22	M	91	12.4	310	gp, hp, Al, c	0.1 µg gp
	29	M	101	5.2	390	gp, Dp, Df, d	0.1 µg gp
	22	F	100	5.6	415	gp, tp, Al, Cl, c	0.01 µg gp
	27	M	109	2.9	323	gp, hp, tp, Al	0.1 µg gp
	24	F	90	2.6	728	gp, hp, tp, Dp, Df, c	0.6 µg Dp
	28	M	99	8.4	110	gp, tp, Al	0.1 µg gp
	26	M	96	3.6	73	gp, Al, c	0.1 µg gp
	24	M	90	8.5	168	gp, tp, Dp, Al, c	0.1 µg gp
	26	M	119	4.1	194	gp, hp, Dp, Df, c	0.1 µg gp
	23	F	79	0.5	96	gp, Dp, Df, c	0.1 µg gp
	22	M	115	13.0	40	tp, Dp, Df, c	0.6 µg Dp
Median (range)	24 (22–29)	4F/8M	99.5 (79–119)	5.4* (0.5–13.0)	181* (40–728)		

†Allergen sensitivity was determined by skin prick test (gp, mixed grass pollen; hp, mixed herb pollen; tp, mixed tree pollen; Dp, *D. pteronyssinus*; Df, *D. farinae*; Al, *Alternaria*; Cl, *Cladosporia*; c, cat fur; d, dog hair). ‡Allergen provocation was done with either grass pollen mix (gp) (Dac g5; Fes p5, Lol p5, Phl p5, Poa p5) or *Dermatophagoides pteronyssinus* mix (Dp) (Der p 1, Der p 2). **P* < 0.01 compared with controls.

Segmental allergen challenge

Segmental allergen challenge was performed as previously described [16]. A commercially available endotoxin detection assay (E-toxate, Limulus amoebocyte lysate; Sigma, St Louis, USA) was used to demonstrate that the instilled saline and allergen solutions were free of endotoxin. Briefly, all subjects received nebulized salbutamol (1.25 mg), atropine (0.5 mg s.c.) and midazolam (mean 4.8 mg i.v., range 0 to 8 mg) prior to the bronchoscopy. Lidocaine was used to achieve local anaesthesia of upper and lower airways. The bronchoscope (P30, Olympus Optical, Tokyo, Japan) was wedged into the inferior lingular bronchus and BAL was performed with 5×20 mL sterile saline. The instrument was passed into the superior lingular bronchus and 10 mL saline solution was instilled as a control challenge. Finally, the bronchoscope was passed to the medial segment of the middle lobe and 10 mL allergen solution was instilled. After 24 h, subjects (all asthmatics and five controls) were re-bronchoscoped with the same pre-medication, and the superior lingular bronchus and the medial middle bronchus were lavaged with 100 mL saline.

Processing of blood and BAL cells

Blood was taken on the first and the second day before bronchoscopy and serum was obtained in a strictly standardized manner (clotting time 1 h at room temperature, 10 min centrifugation at 2500 g, storage at -20°C until analysis). Furthermore, 5 mL blood was drawn in heparin-containing tubes for intracellular analysis using flow cytometry.

BAL fluid samples were processed as described [16]. Briefly, cells were filtered through a 100- μm filter, centrifuged and the supernatant was stored at -20°C . The total count of nucleated cells was performed by using a Neubauer haemocytometer. Differential cell counts were performed from cytopspin slides with 300 cells per slide being counted. An aliquot of the cells was separated for intracellular flow cytometric analysis.

Immunoassay for EPO

The concentration of EPO in serum and BAL fluid was measured as described, using a prototype CAP System EPO FEIA (Pharmacia Diagnostics AB) [17]. The principle of the test is as follows: anti-EPO coupled to ImmunoCAP reacts with EPO in the sample. After washing, B-galactosidase-anti-EPO reacts with bound EPO. After another wash bound enzyme-anti-EPO reacts with 4-methylumbelliferyl-B-D-galactosidase forming a fluorescent product. Highly purified EPO (415/280 nm ratio of 1.14) served as a standard. Monoclonal antibodies were produced by the hybridoma technique. One was covalently coupled to the ImmunoCAP and another was enzyme labelled. The intra- and interassay coefficients of variation are under 9% and levels under 0.5 $\mu\text{g/L}$ are undetectable.

Intracellular detection of EPO in eosinophils

We have previously described a flow cytometric method for the detection of intracellular EPO in unseparated eosinophils [14, 18]. Briefly, blood or BAL samples (150 μL) were lysed by 2 mL lysing solution (Becton Dickinson, Heidelberg, Germany) for 10 min and washed in phosphate-buffered saline (PBS) (0.15 M). Cells were fixed in 0.4% parabenzoquinone (Sigma) in 0.01 M PBS for 10 min, washed in PBS and permeabilized in 0.74% n-octyl- β -D-glucopyranoside (Sigma) in PBS for 6 min.

Permeabilized cells were incubated for 30 min with monoclonal antibodies against EPO (2 μL , mouse IgG_{2a}, Oncogene Science, Uniondale, New York, USA) and isotype-matched non-specific control antibodies (2 μL , Coulter, Krefeld, Germany). Cells were washed in PBS and incubated with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG_{2a} (0.5 μL , Southern Biotechnology Associates, Birmingham, AL, USA) as a secondary antibody for 30 min. After a final wash in PBS, the cells were resuspended in PBS and kept at 4°C in the dark. The flow cytometric analysis (Calibur, Becton Dickinson) was done with 15 000 cells computed in list mode and analysed using Cellquest software (Becton Dickinson). For intracellular detection of EPO a gate was set on eosinophils (cells with preserved scatter properties in the forward (FCS) and side (SSC) scatter plot, for details see Krug et al. [14]). The expression of EPO was quantified as mean fluorescence intensity (MFI) of fluorescence 1 (FL-1) (Fig. 1). The MFI with control mAb was subtracted from the MFI for each specific antibody. In order to ensure standardized conditions, FITC-fluorescent microspheres with a definite fluorescence intensity (Fluoresbrite, Polyscience, Eppelheim, Germany) were used before each experiment to calibrate the flow cytometer.

Statistical analysis

The Mann–Whitney *U*-test was used for intergroup comparison between asthmatics and control subjects. For comparison of paired data within the major study groups (baseline, saline and allergen) the Wilcoxon test was used for the individual comparisons. The Bonferroni correction was used throughout. Probability values of $P < 0.05$ were accepted as significant.

Results

Clinical course and basic BAL data

The allergen provocation was well tolerated by all asthmatic patients. Forced expiratory volume in 1 s (FEV₁) % predicted dropped from 104% (median, range 86 to 120%) before the allergen challenge to 95% (76 to 123%; $P < 0.05$) 1 h after the procedure. At the second day FEV₁% predicted dropped from 103% (86 to 124%) before the bronchoscopy to 94% (61 to 109%; $P < 0.05$) 1 h after the bronchoscopy. Peak flow measurements performed hourly on the day of the allergen challenge did not show a late response in any asthmatic patient.

BAL recovery did not differ between baseline BAL, sham-challenged BAL and allergen-challenged BAL in both groups (range of medians: 72.0 to 78.0 mL). There was a statistically significant increased total cell count after saline and allergen challenge in asthmatic but not in control subjects (Table 2). The percentage of macrophages was decreased and the percentage of neutrophils increased in both asthmatics and controls, while the percentage of eosinophils was elevated after allergen challenge only in asthmatics. The percentage of lymphocytes was decreased in asthmatics after allergen challenge and in controls after saline challenge, but the total number did not change. In peripheral blood there was an increased number of neutrophils 24 h after provocation (asthmatics: median $2.5 \times 10^3/\mu\text{L}$ (range $1.2\text{--}5.9 \times 10^3/\mu\text{L}$) before and $3.5 \times 10^3/\mu\text{L}$ ($2.5\text{--}11.2 \times 10^3/\mu\text{L}$) after provocation ($P < 0.05$); controls: $2.3 \times 10^3/\mu\text{L}$ ($1.9\text{--}4.3 \times 10^3/\mu\text{L}$) before and $3.5 \times 10^3/\mu\text{L}$ ($3.2\text{--}7.2 \times 10^3/\mu\text{L}$) after

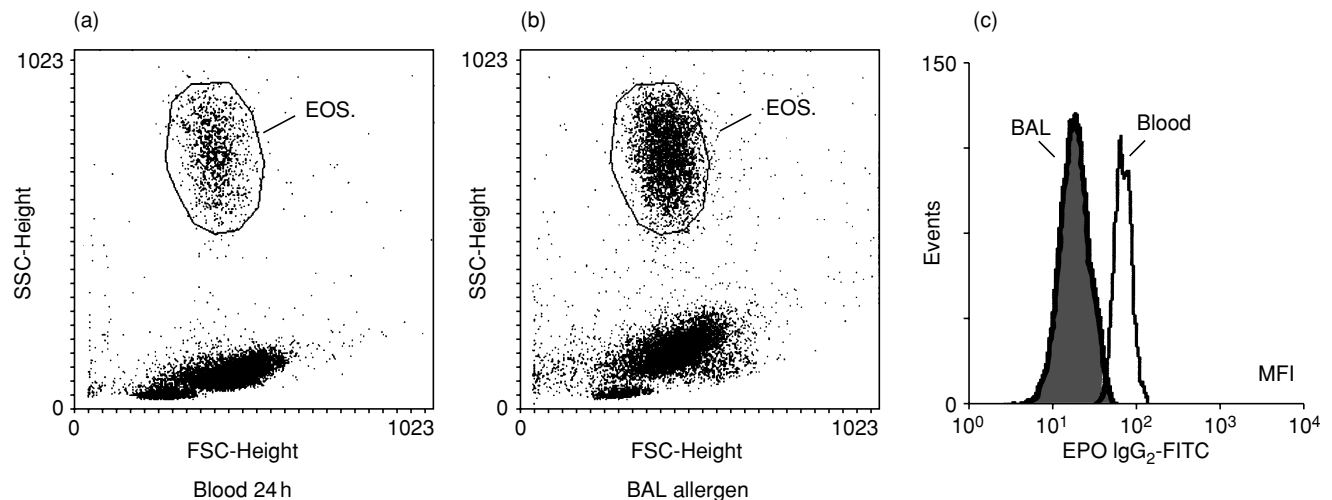


Fig. 1. Flow cytometric measurement of intracellular EPO in eosinophils derived from peripheral blood and BAL 24 h after allergen challenge. After fixation and permeabilization, eosinophils are easily distinguished from other cells by their side scatter properties (a and b). Histogram overlay from gated eosinophils. Staining with fluorochrome-labelled anti-EPO shows decreased mean fluorescence intensity (MFI) from eosinophils derived from BAL compared to eosinophils from peripheral blood (c).

Table 2. Basic BAL data

	BAL	n	Total cells	Eosinophils		Neutrophils		Lymphocytes		Macrophages	
			$\times 10^6$	$\times 10^6$	%	$\times 10^6$	%	$\times 10^6$	%	$\times 10^6$	%
Control	B	9	7.3 (3.0–25.5)	0.0 (0.0–0.1)	0.0 (0.0–0.5)	0.1 (0.0–0.1)	1.0 (0.0–2.0)	0.3 (0.2–0.7)	5.0 (1.0–10.0)	7.0 (2.7–25.0)	93.0 (89.0–98.0)
	S	5	12.0 (3.0–75.0)	0	0	2.8 (0.1–42.8)*	23.0 (2.0–57.0)*	0.4 (0.2–1.5)	3.0 (2.0–6.0)*	8.6 (2.8–30.8)	72.0 (41.0–92.0)*
	A	5	17.4 (6.0–126.0)	0	0	4.7 (0.2–65.5)*	30.0 (4.0–52.0)*	0.9 (0.3–5.0)	4.0 (2.0–7.0)	11.8 (5.3–55.4)	68.0 (44.0–89.0)*
Asthma	B	12	10.5 (5.4–16.1)	0.1 (0.0–0.7)#	0.7 (0.0–4.1)#	0.1 (0.0–1.4)	0.6 (0.0–9.0)	0.6 (0.1–4.5)	6.0 (1.0–39.0)	9.4 (5.1–14.4)	91.0 (59.0–96.0)
	S	12	17.0 (3.9–80.5)*	0.1 (0.0–2.4)#	0.2 (0.0–3.0)#	4.1 (0.2–44.3)**	6.5 (2.0–46.0)**	1.0 (0.0–5.6)	6.5 (0.0–17.0)	12.2 (3.7–28.2)	71.5 (21.0–95.4)**
	A	12	35.5 (5.5–500.0)*	9.5 (0.5–455.0)##**	38.0 (1.0–91.0)##**	2.6 (0.5–262.0)**	23.5 (4.3–70.0)**	1.7 (0.2–18.6)	3.5 (1.0–7.3)*	13.5 (2.1–80.1)	36.0 (5.0–86.0)**

Cell counts and percentage of cells in bronchoalveolar lavage fluid from healthy control subjects and asthmatics. BAL was obtained at baseline (B) and 24 h after saline challenge (S) or allergen challenge (A). Values are given as median and range. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively, compared with baseline values; # and ## indicate $P < 0.01$ compared with controls.

provocation ($P < 0.05$). No change was observed in numbers of blood eosinophils (asthmatics: median $0.3 \times 10^3/\mu\text{L}$ (range $0.2\text{--}1.1 \times 10^3/\mu\text{L}$) before and $0.4 \times 10^3/\mu\text{L}$ ($0.2\text{--}0.7 \times 10^3/\mu\text{L}$) after provocation; controls: $0.1 \times 10^3/\mu\text{L}$ ($0.1\text{--}0.3 \times 10^3/\mu\text{L}$) before and $0.1 \times 10^3/\mu\text{L}$ ($0.1\text{--}0.3 \times 10^3/\mu\text{L}$) after provocation) and lymphocytes (asthmatics: median $2.2 \times 10^3/\mu\text{L}$ (range $1.8\text{--}3.2 \times 10^3/\mu\text{L}$) before and $2.0 \times 10^3/\mu\text{L}$ ($1.5\text{--}3.5 \times 10^3/\mu\text{L}$) after provocation; controls: $2.0 \times 10^3/\mu\text{L}$ ($1.9\text{--}3.1 \times 10^3/\mu\text{L}$) before and $1.9 \times 10^3/\mu\text{L}$ ($1.3\text{--}3.2 \times 10^3/\mu\text{L}$) after provocation).

BAL fluid levels, serum levels and intracellular expression of EPO

Concentrations of EPO in BAL fluid increased from $1 \mu\text{g/L}$ (median, range 1.0 to $6.8 \mu\text{g/L}$) to $42 \mu\text{g/L}$ (5.6 to $379.6 \mu\text{g/L}$;

$P < 0.01$) 24 h after allergen but not after saline challenge ($1.5 \mu\text{g/L}$, 1.0 to $21.9 \mu\text{g/L}$) in asthmatics (Fig. 2), whereas in control subjects all measurements were below the detection limit. Serum concentrations of EPO increased slightly from $18.3 \mu\text{g/L}$ (3.0 to $56.8 \mu\text{g/L}$) to $27 \mu\text{g/L}$ (3.8 to $133.9 \mu\text{g/L}$; $P < 0.05$) 24 h after segmental allergen challenge in asthmatics, whereas there were no changes in control subjects (Fig. 3).

The intracellular expression of EPO (measured as mean fluorescence intensity, MFI, Fig. 1c) was unchanged in peripheral blood eosinophils before (median MFI 43, range 18 to 98) and after allergen challenge (48, range 20 to 85). However, BAL eosinophils showed significantly decreased intracellular EPO content 24 h after allergen challenge (29, range 7 to 71; $P < 0.01$) compared with eosinophils from peripheral blood (Fig. 4).

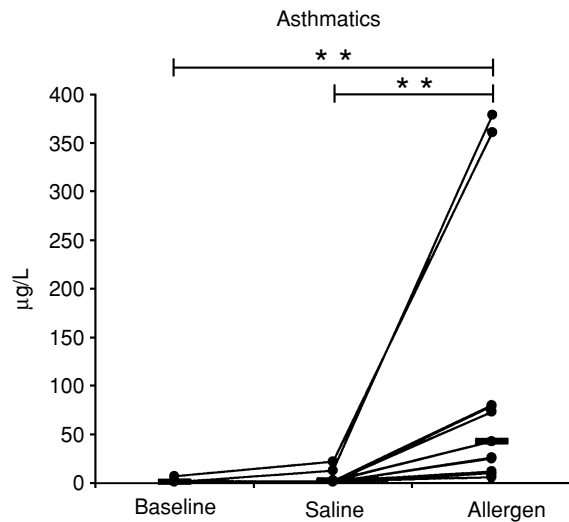


Fig. 2. Concentration of EPO in BAL fluid before and 24 h after segmental sham and allergen challenge in asthmatics. ** $P < 0.01$.

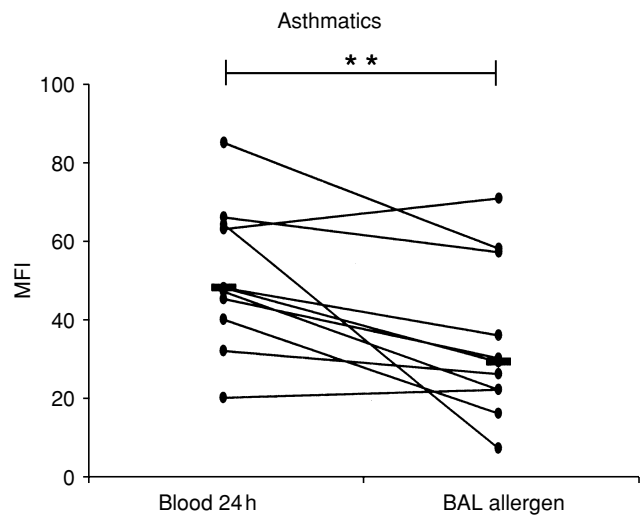


Fig. 4. Intracellular detection of EPO in eosinophils from peripheral blood and BAL 24 h after segmental allergen challenge. ** $P < 0.01$.

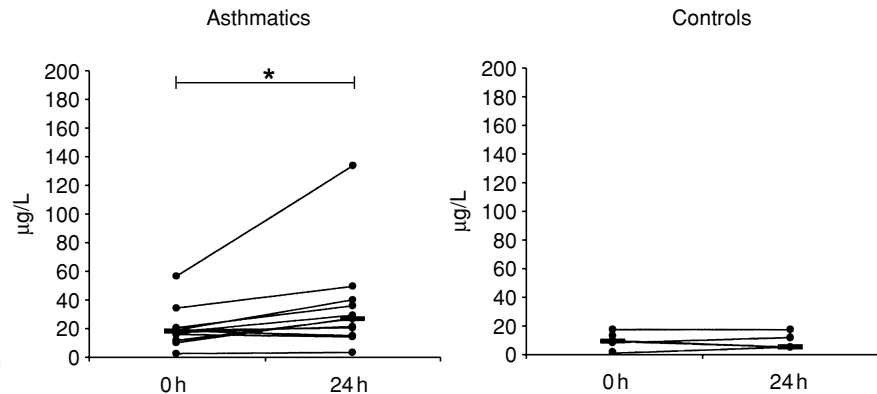


Fig. 3. Concentration of EPO in serum before and 24 h after segmental allergen challenge in asthmatics and controls. * $P < 0.05$.

Discussion

The aim of the present study was to investigate the local release of EPO by eosinophils after segmental allergen provocation in allergic asthmatic patients.

Increased levels of EPO were found in the BAL fluid of asthmatics 24 h after allergen provocation but not after saline challenge, whereas in healthy subjects no EPO was detectable in the BAL fluid. Interestingly, serum levels of EPO, but not the number of peripheral eosinophils, increased significantly in asthmatics, although only one subsegment of the lung was challenged with allergen. Few studies are available that have determined EPO expression in asthma. While serum EPO has been shown to be elevated in asthmatics in correlation with asthma severity [4, 13, 14], no data regarding local expression have been obtained using BAL fluid samples. Sedgwick et al. have shown elevated concentrations of EPO in BAL fluid 48 h after segmental allergen challenge in patients with allergic rhinitis using a double antibody RIA technique [12]. Furthermore, indirect evidence of increased local EPO activity has been demonstrated in asthma: 3-bromotyrosine has been identified as a catalytic product of EPO activity when tyrosine residues of proteins are oxidatively damaged through bromination and it

was found to be markedly increased in BAL fluid of asthmatics [9, 10].

When the intracellular content of EPO in BAL-derived eosinophils was analysed after allergen challenge we demonstrated a significant decrease compared with peripheral eosinophils. Unfortunately, before allergen challenge there were not sufficient eosinophils present in the BAL for the intracellular detection method. Therefore, no comparison was possible between airway-derived eosinophils before and after allergen challenge. However, it is tempting to speculate that peripheral eosinophils are attracted into the airways during the allergic inflammatory process and that they locally release EPO, leading to increased concentrations in the BAL fluid. At the same time peripheral eosinophils did not change their content of EPO during the allergen challenge. Therefore, the increased serum concentration of EPO could be explained as a result of increased spill-over of EPO from the lung. On the other hand, serum EPO might mostly originate from eosinophils degranulated during coagulation as has been shown for ECP [19] and increased serum EPO levels might originate from primed eosinophils after blood has been drawn. Previous studies using peripheral blood showed hypodense eosinophils in asthmatics [20] and a decreased intracellular expression of

EPO in eosinophils of asthmatic children when compared with healthy controls [14]. Furthermore, an increase of hypodense peripheral blood eosinophils was found in allergic asthmatics during their pollen season that was related to the degree of eosinophil degranulation [21]. These studies suggest that not only in lung-derived but also in peripheral blood eosinophils degranulation with release of EPO occurs in asthma.

The mechanism that leads to the release of EPO is still insufficiently understood. In peripheral eosinophils a change of subcellular distribution of eosinophilic proteins was detectable in the pollen season, but no change in the total amount was found in the cells [21]. Both piecemeal degranulation as well as eosinophil cytolysis have been described and quantified as mechanisms leading to the release of eosinophil granules in the mucosa of patients with allergic rhinitis [11] and allergic asthma [3, 21]. Eosinophils undergoing piecemeal degranulation stay intact but they contain less granule proteins than eosinophils that undergo cytolysis [11]. As in our study BAL-derived eosinophils, despite their decreased amount of EPO, stayed intact and showed no changes in forward or side scatter properties compared with peripheral eosinophils, a possible explanation for EPO release could be piecemeal degranulation. However, since we have not performed electron microscopic investigations we can not answer this question definitively.

In vitro studies have shown that EPO release is driven by IgE, in contrast to ECP, which is released in an IgG-dependent way [22–24]. However, during the IgE-driven allergic response in asthmatics both EPO and ECP were found to be elevated in serum and BAL fluid. Further experiments are necessary to elucidate the release mechanism of EPO and whether it is different from other eosinophilic proteins.

We conclude that the finding of increased EPO concentrations in the BAL fluid and decreased intracellular EPO expression in pulmonary eosinophils reflects the allergen-triggered release of EPO into the bronchial space.

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