

Circulating eosinophils in asthma, allergic rhinitis, and atopic dermatitis lack morphological signs of degranulation

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Summary

Background In allergic diseases, eosinophils in affected tissues release granule proteins with cytotoxic, immunoregulatory, and remodelling-promoting properties. From recent observations, it may be assumed that eosinophils degranulate already in circulating blood. If degranulation occurs in the circulation, this could contribute to widespread systemic effects and provide an important marker of disease.

Objective To determine the degranulation status of circulating eosinophils in common allergic diseases.

Methods Using a novel approach of whole blood fixation and leucocyte preparation, the granule morphology of blood eosinophils from healthy subjects, non-symptomatic patients, symptomatic patients with asthma, asthma and Churg–Strauss syndrome, allergic rhinitis, and atopic dermatitis was evaluated by transmission electron microscopy (TEM) and eosinophil peroxidase (TEM) histochemistry. Plasma and serum levels of eosinophil cationic protein were measured by fluoroenzymeimmunoassay. Selected tissue biopsies were examined by TEM.

Results Regardless of symptoms, circulating eosinophils from allergic patients showed the same granule morphology as cells from healthy subjects. The majority of eosinophil-specific granules had preserved intact electron-density (96%; range: 89–98%), while the remaining granules typically exhibited marginal coarsening or mild lucency of the matrix structure. Abnormalities of the crystalline granule core were rarely detected. Furthermore, granule matrix alterations were not associated with any re-localization of intracellular EPO or increase in plasma eosinophil cationic protein. By contrast, eosinophils in diseased tissues exhibited cytolysis (granule release through membrane rupture) and piecemeal degranulation (loss of granule matrix and core structures).

Conclusion In symptomatic eosinophilic diseases, circulating blood eosinophils retain their granule contents until they have reached their target organ.

Keywords allergic asthma, allergic rhinitis, atopic dermatitis, blood, cell degranulation, Churg–Strauss syndrome, cytoplasmic granules, electron microscopy, eosinophils

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Introduction

Elevated levels of circulating blood eosinophils and tissue eosinophilia are prominent features of allergic diseases. The role of eosinophils in the pathogenesis of disease has been debated but is currently under reappraisal [1, 2]. From histological studies, it is clear that tissue-dwelling eosinophils

in asthma, allergic rhinitis (AR), and atopic dermatitis undergo extensive degranulation [3–8]. Among the released granule products are eosinophil-specific cytotoxic proteins [9] as well as several growth factors [10] and immunoregulatory cytokines [11, 12] all with the capacity to exert potent effector functions. The possibility that eosinophils may degranulate in the circulation would agree with the notion of allergic conditions as a systemic disease complex [13, 14], and has attracted interest in the search for indices to diagnose and predict development of allergic disease [15–17]. However, examination of the degranulation activity of circulating eosinophils is associated with significant difficulties. Extracellularly released eosinophil-specific granule proteins (e.g. eosinophil cationic protein (ECP) and eosinophil peroxidase

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(EPO) in plasma) are difficult to determine accurately, in part because of rapid and variable turnover rates of the circulating proteins in the blood stream [18, 19]. In addition, the frequently measured serum level of ECP is only an indirect measurement, reflecting the number of circulating eosinophils and the propensity of the eosinophils to release ECP during the clotting process *in vitro* [20]. Hence, quantitative measurements besides those of protein levels are warranted to assess whether eosinophils degranulate in the circulation.

Quantitative analysis of granule structure changes, using transmission electron microscopy (TEM), has emerged as an appropriate method to determine eosinophil degranulation in tissue [7, 21]. Morphological changes in the eosinophil such as loss in electron density of the granule core or matrix structures (i.e. piecemeal degranulation) correspond to granule protein relocation and release, as revealed by ultrastructural analysis of both tissue [22, 23] and *in vitro* stimulated [24, 25] eosinophils in combination with cytochemistry or immunocytochemistry. Interestingly, based on TEM observations in a few mild asthmatic subjects [26], it has recently been forwarded that circulating eosinophils exhibit clear morphological signs of piecemeal degranulation. However, the methods used in that study may not have been validated with regard to risks for artefactual granule alterations. This latter aspect is important because the processing of blood samples by the use of common fixation [27] or cell isolation procedures may induce marked signs of piecemeal degranulation of the sampled eosinophils [28]. So far, there is also a lack of quantitative TEM studies on circulating eosinophils from allergic patients. New studies are therefore required to determine the actual degranulation status of circulating eosinophils in allergic diseases.

We recently demonstrated a method by which whole blood can be immediately fixed at sampling, and the leucocytes can be separated and further processed for electron microscopy while retaining their ultrastructural morphology [28]. As a result, it was possible to analyse the baseline degranulation status of normal circulating blood eosinophils, which had not undergone any other physical strain than that caused by blood sampling and fixation [28]. Using this novel approach, we have now determined the granule ultrastructure of

circulating eosinophils in mild AR and asthma at baseline and in response to seasonal allergen exposure and single allergen challenge. We have also analysed circulating eosinophils from patients with asthma, atopic dermatitis, and Churg–Strauss syndrome seeking medical care for moderate to severe symptoms. In summary, our extensive and detailed analysis reveals that blood eosinophils from all these patient groups lack signs of any significant degranulation.

Methods

Human subjects and study design

This study comprises data from healthy individuals and from subjects suffering from different eosinophilic diseases (Table 1). There was an equal distribution of men and women within the study groups. Generally, the use of contraceptives and paracetamol was allowed besides specific disease treatments as specified below. Approval for the study was obtained from the local Ethics Committees in Lund/Malmö and Gothenburg, Sweden. All subjects gave their written informed consent before inclusion.

Seasonal birch-pollen exposure in asthma and allergic rhinitis The included subjects had a history of birch pollen-related AR or mild allergic asthma, and all were skin prick positive to birch allergen. Non-allergic healthy subjects were controls. In April 2001, prior to the pollen season, all subjects were non-symptomatic. Nasal glucocorticosteroids (GCS) or antihistamines were not allowed 2 weeks or 3 days, respectively, before the first visit and during the study. Asthmatic individuals were allowed inhaled GCS and short-acting β_2 -agonists were allowed for asthma patients throughout the study. Starting from their first visit, all patients scored their daily symptoms as 'runny nose', 'blocked nose', 'sneezing', or 'asthma symptoms' during the past 24 h. All symptoms were documented according to a 4-point scale, with 0 indicating no symptoms and three indicating severe symptoms. Blood was sampled for cell count and morphological analysis, before the season and at two time points

Table 1. Subjects characteristics and responses to allergen exposure

Subjects	N	Age	Allergen exposure	Before allergen exposure			During/after allergen exposure		
				Symptoms	Whole blood leucocytes ($\times 10^9$ cells/L)	Eosinophils (%)	Symptoms	Whole-blood leucocytes ($\times 10^9$ cells/L)	Eosinophils (%)
Healthy	9	24 (23–44)	None	None	6.0 (4.3–7.8)	2.4 (1.7–4.8)	–	–	–
Healthy	12	22 (21–29)	Birch pollen (seasonal)	None	5.0 (3.8–7.6)	2.5 (1.3–1.9)	None†	5.4 (4.0–9.2)	3.0 (1.2–10)
Rhinitis	11	24 (19–41)	Birch pollen (seasonal)	None	4.9 (4.4–7.6)	3.5 (2.7–8.9)	Mild†	5.1 (4.7–6.5)	5.0 (3.4–10)
Rhinitis	11	24 (18–54)	Birch pollen challenge	None	5.2 (3.3–8.3)	3.1 (1.4–5.7)	–	5.4 (4.2–6.9)	2.9 (1.9–6.8)
Asthma	5	46 (20–53)	Birch pollen (seasonal)	None	5.6 (4.1–7.5)	4.0 (1.8–7.3)	Mod†	6.2 (4.7–6.3)	6.3* (4.3–12.8)
Asthma	8	28 (23–45)	Cat allergen challenge	None	6.2 (5.8–6.3)	4.0 (1.6–10.3)	Mild	6.2 (5.5–8.7)	6.2*** (2.7–14.5)
Asthma	5	59 (39–78)	Not specified	–	–	–	Mod	5.8 (5.5–7.2)	6.1 (3.6–9.7)
Dermatitis	7	38 (25–54)	Not specified	–	–	–	Mod/Sev	8.2 (7.2–9.2)	6.3 (4.3–8.3)
Churg–Strauss Syndrome	1	62	Not specified	–	–	–	Mod/Sev	8.4	47.6

All values are expressed as medians (range) of *n* subjects.

* $P < 0.05$, *** $P < 0.001$ compared with values before allergen exposure. †At visit 3.

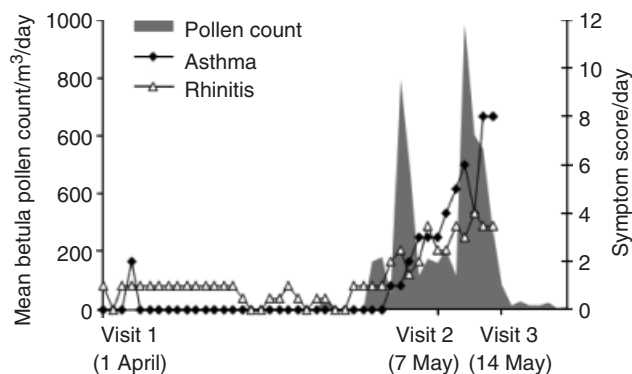


Fig. 1. Mean daily symptom score in asthma and allergic rhinitis patients in relation to the pollen curve during the 2001 season. Blood was sampled for morphological analysis before the season (visit 1) and during the season (visits 2 and 3).

(visits 2 and 3; see Fig. 1) in May 2001 during the peak of the birch-pollen season in south Sweden.

Laboratory birch-pollen challenge in allergic rhinitis The included patients had a history of pollen-related AR and were skin prick positive to birch allergen. Prior to challenge, all subjects were non-symptomatic. Systemic or nasal GCS was disallowed 8 and 4 weeks, respectively, before the study. There was no concomitant medication during the study. The patients were exposed to nasal spray of a birch pollen extract (10,000 SQ/U per nostril; ALK, Copenhagen, Denmark). Blood and nasal biopsies were sampled for cell count and morphological analysis before and 24 h after the allergen challenge. Control blood and nasal biopsies were taken from non-allergic healthy subjects.

Laboratory cat-allergen challenge in asthma The patients had a history of cat allergy-related mild bronchial asthma, were skin prick positive to cat allergen, and demonstrated increased airway responsiveness to metacholine ($PC_{20} < 8$ mg/mL). The patients were also required to demonstrate a positive response to inhaled cat allergen extract (ALK-Abelló, Hørsholm, Denmark), at least 20% fall in forced expiratory volume in 1 s (FEV_1) at $< 64,000$ SQ/mL of allergen concentration. The provocation tests were carried out as described previously [29, 30]. Subjects in this study were not allowed any GCS within 4 weeks of the first visit and during the study. None of the patients were treated with theophyllines or long-acting β_2 -agonists. Treatment with inhaled short-acting β_2 -agonists was not allowed 6 h prior to challenge. Lung function was measured, and blood and bronchial biopsies were sampled before and at 24 h after the allergen challenge.

Asthmatics with moderate to severe exacerbation or Churg–Strauss syndrome Subjects with allergic asthma seeking medical care because of moderate or severe exacerbation of asthma presented with rhonchi, an FEV_1 between 60% and 80% of that predicted, more than 12% reversibility of airway obstruction after inhalation of bronchodilator, and blood eosinophilia (Table 1). Notably, they were either steroid-naïve or conceded no compliance on their prescribed GCS treatment. In addition, one female patient diagnosed

with the Churg–Strauss syndrome was included. She suffered from moderate allergic asthma and had received daily treatment with anti-leukotrienes since 5 years. At the time point of blood sampling, she presented with reddened papulous lesions with a blue hue, systemic leucocytoclastic vasculitis with extravascular eosinophil infiltration, blood eosinophilia (Table 1), and a serum immunoglobulin E (IgE) level of 239 kU/L. Venous blood was sampled from all asthmatic patients for cell count and morphological analysis. Blood plasma and serum from the asthmatic patients with exacerbations were sampled for ECP analysis. From the Churg–Strauss patient, a skin biopsy was taken from the site of a lesion and processed for light microscopy and TEM.

Subjects with atopic dermatitis The patients with atopic dermatitis, but without symptoms of rhinitis or asthma, showed blood eosinophilia (Table 1) and a range of symptoms from nummular patches on the extremities to eczema of the whole body. Previously measured serum IgE levels were between 77 and 239 kU/L (median: 167 kU/L). At blood sampling, most patients were treated with serial phototherapy for a few months. No systemic drugs were used, but two of the patients were treated with topical GCS. Venous blood was sampled for cell count and morphological analysis.

Blood and biopsy processing and determination of eosinophil morphology

Venous blood was collected in a vacutainer containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (Becton Dickinson, Stockholm, Sweden). Immediately after sampling, the whole blood was primary fixed in a 1 : 2 ratio of blood to 4% formaldehyde (EM grade; TAAB Laboratories Equipment, Berkshire, UK) in 0.1 M phosphate-buffered saline (PBS; 230–240 mOsm/kg), yielding a 2.7% total concentration of fixative. In selecting the present fixation method, we noted that deviations in the total concentration of fixative and osmolarity of the vehicle may result in artefactual granule morphology changes [28]. After 1 h fixation at room temperature, the leucocyte population was separated from the erythrocytes by dextran sedimentation as described previously [28]. Secondary fixation was carried out overnight at 4 °C in a mixture of 1% glutaraldehyde and 3% formaldehyde in phosphate-buffered saline (PBS) before embedding in agarose (type VII; Sigma Chemical Co., St Louis, MO, USA), post-fixation in 1% osmium tetroxide for 1 h at room temperature, and further processing for TEM [28]. In parallel, leucocytes in separate anticoagulated blood samples were routinely counted and differentiated. Biopsies were processed for TEM as described previously [31]. For each specimen, at least 250 eosinophil-specific secondary granules were examined, and the morphology was classified into the following categories (Fig. 2): intact core and matrix (type 0), marginal coarsening or mild loss of the matrix (type I), moderate to severe loss of matrix (type II), mild-to-severe loss of core (type III), or all grades of both matrix and core losses (type IV). In addition, the percentage of altered secondary granules in the individual eosinophils was calculated [7, 31]. The

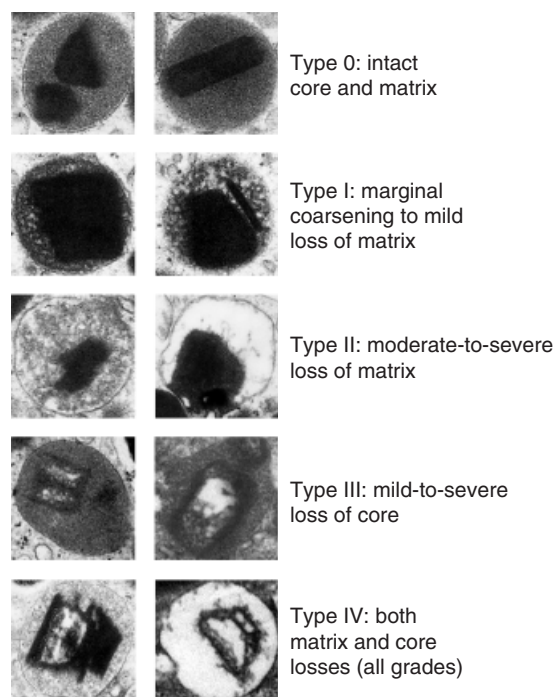


Fig. 2. High-power electron micrographs illustrating morphology-based classification of degranulation of human eosinophil secondary granules.

granules in a cross section of an analysed eosinophil were considered representative of the whole cell.

Intracellular distribution of eosinophil peroxidase

The intracellular distribution of cyanide-resistant EPO in blood eosinophils from patients with atopic dermatitis and asthmatic exacerbations was assessed using histochemical staining with 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co.) [22, 31]. Briefly, after secondary fixation, leucocytes were embedded in agarose, washed in PBS for 30 min, and then incubated with DAB (0.6 mg/mL) and hydrogen peroxide (1 μ L/mL) for 12 min (Sigma Chemical Co.). After repeated washes in PBS for 30 min, the gels were placed in a secondary fixative and prepared for TEM analysis. During the TEM analysis the DAB reaction product appears as a dark, electron-dense precipitate.

Serum and plasma eosinophil cationic protein

Serum was collected from venous blood that was left to clot at room temperature for 60 min and then centrifuged for 10 min at 1200 *g*. Plasma was collected from EDTA-anticoagulated venous blood after centrifugation at 1750 *g* for 10 min at room temperature. Serum and plasma aliquots were maintained at -70°C until analysis. EGP was analysed using the Pharmacia CAP system ECP fluoroenzymeimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden).

Statistical analysis

All calculated values are expressed as medians with a min and max range. The statistical analyses, paired or equal variance

Student's *t*-test and one-way analysis of variance, were carried out using Astute 1.5, a statistics Add-in for Microsoft Excel (DDU Software, Leeds, UK). Probability values of (P) <0.05 were considered statistically significant.

Results

Clinical data

Effect of seasonal birch-pollen exposure During the birch-pollen season, rhinitis, and asthma patients scored mild symptoms, which increased for the asthma patients to moderate symptoms at the end of the season (Fig. 1). Only asthmatic patients had significantly increased levels of circulating eosinophils (Table 1) compared with pre-season measurements at visit 1. There was no difference in circulating levels of eosinophils for any study group between visit 2 (data not shown) and visit 3 (Table 1) during the season.

Effect of laboratory allergen challenge Following allergen challenge with cat extract, asthmatic patients showed a 20% fall in FEV₁ at the cumulative allergen concentration of 471 SQ/mL (range: 20–1231 SQ/mL) during the early phase and a median fall in FEV₁ of 10% (range: 1.3–24.5%) 24 h after exposure. At this time-point, this group of asthmatic patients also demonstrated increased levels of circulating eosinophils (Table 1). The patients with allergic rhinitis given a single dose of birch pollen suffered from acute nasal congestion in response to challenge but had no elevated levels of circulating eosinophils at 24 h after the allergen challenge (Table 1).

Clinical status of the patients seeking medical care All subjects seeking medical care for moderate to severe symptoms had blood eosinophilia (see Methods and Table 1).

Granule morphology of blood eosinophils

The morphology of blood eosinophils was, depending on the study group, examined before and after the pollen season, before and 24 h after allergen challenge, or during moderate to severe symptoms (Table 1). To examine the occurrence of piecemeal degranulation (i.e. loss of granule matrix and core structures), we analysed the morphology of the eosinophil secondary granules in detail. The appearance of blood eosinophil granules from any patient irrespective of disease or symptom did not differ from healthy control subjects (Fig. 3a). Thus, circulating eosinophils of patients with moderate-to-severe allergic airway and skin symptoms showed the same granule morphology as subjects without symptoms or disease (Figs 4a–c). For all blood samples, including those from healthy controls, the majority of eosinophil granules showed intact morphology (96%; range: 89–98%), while the remaining granules for the most part exhibited only a marginal coarsening or a mild loss of matrix contents (Fig. 3b). Only on rare occasions were core alterations detected (Fig. 3b).

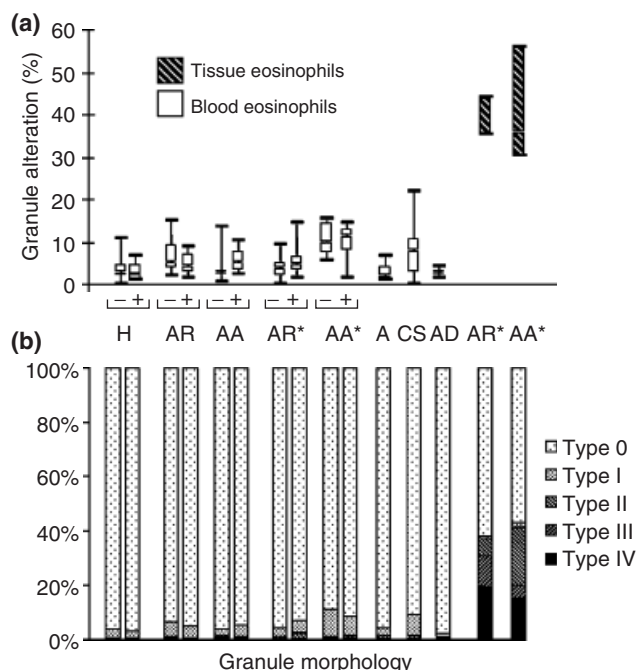


Fig. 3. (a) Occurrence of morphologic changes of secondary granules of blood eosinophils in healthy controls (H), in patients with allergic rhinitis (AR) or asthma (AA) before (–) and after (+) allergen challenge, and in patients with moderate to severe exacerbations of asthma (A), symptoms of atopic dermatitis (AD), and Churg–Strauss syndrome (CS). Comparison is made with tissue eosinophils in nasal biopsies from AR* and in bronchial biopsies from AA* patients. Granule changes are presented as the percentage of structurally altered secondary granules of total calculated granules. All values are medians (min and max range) of $n \geq 250$ granules for each sample. (b) Sub-classification of the morphology of all analysed eosinophil secondary granules (see Fig. 1).

Retention of granule matrix eosinophil peroxidase in blood eosinophils

To determine whether the marginal coarsening and mild loss of eosinophil granule matrix was a result of EPO translocation, we combined peroxidase histochemistry with TEM analysis. In circulating eosinophils of patients with moderate to severe asthma or atopic dermatitis, this analysis showed that EPO was restricted to the matrix compartment of the eosinophil secondary granules (Fig. 4d), as was EPO in eosinophils from healthy individuals. Furthermore, there was no evidence for intracellular mobilization of EPO such as positively stained intracellular vesicles.

As a further support for negligible degranulation of blood eosinophils, we demonstrated that plasma ECP levels in patients with moderate-to-severe asthma exacerbations ($2.2 \mu\text{g/L}$, range: 2.0 – $4.1 \mu\text{g/L}$) were not different from previous levels observed in health [32], while serum ECP, as expected, was high ($16.1 \mu\text{g/L}$, range: 8.8 – $49.3 \mu\text{g/L}$).

Degranulation of secondary granules of tissue eosinophils

Eosinophils in nasal and bronchial biopsies from the allergen-challenged subjects and a skin biopsy from the Churg–Strauss patient were evaluated for their degranulation status. There was a dramatic discrepancy between granule morphology of blood and tissue granulocytes within the same patient. This was particularly seen in the patient with the Churg–Strauss

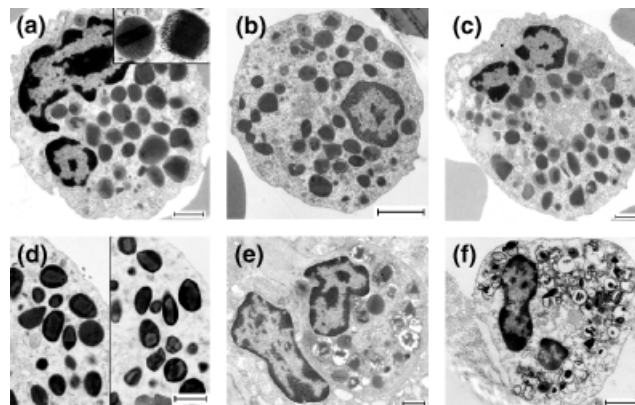


Fig. 4. Electron micrographs illustrating the morphology of eosinophils in immediately fixed whole blood (a–d) and in disease tissues (e,f). Healthy subject (a), patients with symptomatic allergic asthma (b), or asthma, and Churg–Strauss syndrome (c). Micrographs additionally illustrating that the EPO activity was retained within the specific granules of circulating eosinophils in patients with asthma exacerbations (d, left) and atopic dermatitis (d, right). There is a discrepancy in granule morphology between blood and tissue eosinophils as illustrated by degranulated tissue eosinophils in patients with allergic rhinitis (e), and Churg–Strauss syndrome (f). Bars: a and c–e, $1 \mu\text{m}$; b and f, $2 \mu\text{m}$.

syndrome where most granules of tissue eosinophils exhibited extensive losses of both core and matrix structures (Fig. 4f). Confirming previous findings in asthma and allergic rhinitis [7, 8], tissue eosinophils in these diseases (Fig. 4e) showed marked loss in granule core, matrix, and both core and matrix contents while the granule morphology of blood eosinophils remained intact (Figs 3 and 4). A similar difference in granule morphology was also observed for circulating and tissue neutrophils in these subjects (data not shown).

Discussion

In the present patients, with different eosinophilic diseases and with symptoms ranging from mild to severe, the circulating eosinophils lacked granule abnormalities. The retained granule ultrastructure as well as the confinement of EPO reactivity to the eosinophil granule matrix strongly suggest that no significant eosinophil degranulation occurred in the circulation. Moreover, as a contrast, the present study demonstrated a marked eosinophil degranulation in diseased tissues. Hence, our data strongly suggest that in eosinophilic allergic conditions, significant degranulation does not occur until the eosinophils have reached the local tissue site of inflammation.

The lack of morphological signs of degranulation of circulating eosinophils in symptomatic allergic disease, which we demonstrate in this study, is in contrast to a previous report of piecemeal degranulation of circulating eosinophils in asthma [26]. The discrepancy between our findings and this prior report is likely explained by differences in sample processing. We have recently demonstrated that the osmolarity and ion strength of solutions used at eosinophil processing are critical to prevent extensive artefactual loss in granule density [28]. The choice of primary fixative agent, its concentration, and the osmolarity of its vehicle is of particular importance for avoiding artefactual granule core

and matrix degranulation [27, 28]. This caveat may not apply to the same extent for fixation of tissue eosinophils, where the surrounding tissue apparently has a sufficient buffering capacity [33] to protect the dwelling eosinophils [7]. The magnitude and pattern of losses in granule density reported in the prior study claiming degranulation of circulating eosinophils [26] actually agree well with the granule abnormalities that are produced by routine eosinophil isolation or fixation in non-optimized fixative [28]. As artefactual structural changes by definition cannot explain the present negative findings, we submit that the previous observation in mild asthmatic patients [26] reflects artefactual activation of the blood eosinophils during the cell preparation procedure.

The patients in the present study were selected to represent a wide range of allergic conditions with minimal drug treatment in order to optimize the possibility of detecting eosinophil degranulation in the circulation. Yet, in none of a total of 120 blood samples from diseased patients could we detect any granule structures that differed from those present in healthy controls (45 samples). Even in a patient with the Churg–Strauss syndrome, where systemic eosinophil effector functions may be expected [34], there was no increase in granule alterations of blood eosinophils in this study. Although a low percentage of the eosinophil granules showed marginal matrix changes, this occurred equally frequently in healthy subjects and in allergic patients without symptoms. It is of note that such marginal granule matrix changes are also present in blood and bone marrow eosinophils from healthy mice [31]. Hence, granule matrix coarsening or mild loss in electron density may be a general phenomenon in a small fraction of granules of eosinophils. We speculate that this phenomenon may result from incomplete filling of a minority of granules during the eosinopoiesis. Alternatively, it cannot be completely excluded that such granule abnormalities may be minimal artefacts caused by the fixation or sample processing.

The present lack of degranulation agrees with the little discussed observations that plasma ECP, in contrast to serum ECP, is low in asthmatic patients [32] (this study) as well as in healthy controls [32]. Our data further agree with previous demonstrations of retained peroxidase content and cell shape of circulating eosinophils in asthmatic patients with mild to severe symptoms [35]. Previous ultrastructural studies of circulating eosinophils in patients with hypereosinophilic syndrome have not detected any piecemeal degranulation [36–38]. A study of patients with Löffler's cardiomyopathy claims to show degranulated eosinophils because of the presence of 'reversed' cores [39] (electron-light cores surrounded by an electron-dense matrix), but this morphological phenomenon is associated with a retained crystalline structure and has not been demonstrated to represent any true protein relocalization from the core [7, 31]. Taking the available data together, it appears that circulating granulocytes remain a non-degranulating phenotype irrespective of disease conditions. However, the present data do not exclude the possibility of a rise in cell activities other than degranulation including 'priming' of blood eosinophils in allergic disease [40, 41].

The absent eosinophil degranulation in the circulation in this study is in sharp contrast to findings obtained in the tissue of allergic subjects. Thus, in previous biopsy studies

extensive morphological granule alterations indicative of piecemeal degranulation and primary cytolysis have been demonstrated in the target tissue of patients with asthma [4, 7], AR [7], and atopic dermatitis [5]. By direct comparison in the same patient, a striking difference between blood and tissue eosinophils was also demonstrated in this study as regards degranulation patterns. Our data may tally with previous important findings on differences between circulating and airway luminal eosinophils in their content of EPO [42] or in their capacity to release reactive oxygen products upon *in vitro* stimulation [43]. The present data further underscore the general notion [21] that different body compartments give rise to distinct eosinophil phenotypes and disclose different information regarding the biological and pathogenic activity of these cells.

In conclusion, this study demonstrates that circulating eosinophils in symptomatic patients with different eosinophilic disease conditions show the same granule morphology and intracellular localization of EPO as eosinophils from non-symptomatic patients or healthy controls. From this finding and the striking eosinophil degranulation patterns in diseased tissues, as also demonstrated here in patients with asthma and allergic rhinitis, it can be hypothesized that circulating eosinophils retain their granule content until they have reached their target tissue.

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