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Evidence for marked eosinophil degranulation in a case of eosinophilic pneumonia

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Introduction

Eosinophils are inflammatory cells which are believed to play a key role in a variety of inflammatory conditions, including parasitic and allergic diseases. These cells are cytotoxic to parasites and mammalian cells, and can modulate the function of other cells. After activation, they are able to produce and release lipid-derivatives, oxygen free radicals and cytotoxic-granule-derived proteins. The presence of these eosinophilic cationic proteins, including eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase and eosinophilic protein X (EPX, also called eosinophil-derived neurotoxin), in biological fluids or inflammatory tissues is accepted as a marker of eosinophilic activation (1).

Although a high number of eosinophils are found both in the peripheral circulation and the bronchoalveolar lavage (BAL) fluid of patients with eosinophilic pneumonia, little is known about the extent of eosinophil degranulation encountered in this disorder. The present case report describes a patient with eosinophilic pneumonia in whom high serum levels of both ECP and EPX were observed, as well as a high level in the BAL fluid, indicating that marked eosinophil degranulation had taken place.

Materials and Methods

CASE REPORT

A 63-year-old woman presented with a 6-month history of productive cough, night sweats, lethargy and dyspnoea with wheezing. The illness had started a few weeks after returning from a safari in Kenya, Africa, and did not improve after symptomatic treatment and administration of antibiotics. Family history for asthma and atopic disorders was negative. Past medical history and environmental or medication exposure were not contributory. The patient did not take L-tryptophan-containing substances. She had no fever. Physical examination was normal apart from widespread inspiratory crackels.

A chest radiograph revealed dense infiltrates in both lower lobes. A computed tomographic (CT) scan of the chest showed patchy alveolar bilateral opacities. Laboratory values on admission included an elevated erythrocyte sedimentation rate of 54 mm h^{-1} (normal values 0–20 mm h^{-1}). The white blood cell number was 17 000 mm^{-3} (normal values 4300–10 000 mm^{-3}) with a 55% increase of eosinophils (absolute eosinophil count of 9420 mm^{-3}; normal <600 mm^{-3}). Pulmonary function tests revealed a normal airway resistance, with a decreased vital capacity (50% of predicted) and total lung capacity (74% of predicted). An arterial blood gas analysis showed hypoxaemia (PaO_2: 63 mmHg) with normocapnia.

There was no serologic evidence for recent exposure to fungi, mycoplasma, viruses, parasites or *Chlamydia psittaci*. Cultures of blood, urine, sputum and BAL fluid (see below) for viruses, bacteria, fungi and mycobacteria were negative. Antinuclear antibodies were normal. Stool cultures for ova and parasites were negative. Precipitating antibodies against fungi or avian serum were not present.

BRONCHOALVEOLAR LAVAGE

Fibre-optic bronchoscopy with BAL was performed to rule out infection before treatment was initiated. After local anaesthesia, the fibre-optic bronchoscope was inserted through the nose and wedged in a distal portion of a segmental bronchus at the site of maximal infiltrate. Three 50 ml aliquots of

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Table 1 Sequential serum levels of eosinophilic cationic protein (ECP) and eosinophil-derived neurotoxin (EPX) in a case of eosinophilic pneumonia before and after treatment with methylprednisolone

Day	Hours after treatment	ECP* ($\mu g\,l^{-1}$)	EPX† ($\mu g\,l^{-1}$)	Eosinophils (%)	Eosinophils (abs. number mm^{-3})
1	—	170	949	53	7420
5	—	281	1383	65	8515
7	—	245	2116	50	6484
7	3	47	199	21	1734
7	6	43	161	8	512
7	9	30	127	3	204
8	24	71	319	22	2376
11	96	40	181	17	1802
12	120	30	117	6	679
360	—	17	21	8	200

Normal values: *<20 $\mu g\,l^{-1}$; †8–40 $\mu g\,l^{-1}$.

0·9% saline were instilled through the bronchoscope. Eosinophils made up 96% of the cells recovered. Numerous eosinophils were present around bronchioles in a transbronchial biopsy. No granulomas were detected.

TREATMENT

The patient was started on a course of intravenously administered methylprednisolone (SOLUMEDROL®, Upjohn) at a dose of 1 mg kg^{-1} for 5 days. This resulted in a dramatic improvement of the clinical condition of the patient, clearing of the chest X-ray and a rapid drop of the blood eosinophil count (Table 1). She was discharged from the hospital in good clinical condition after 5 days of treatment, when the intravenous route was changed into oral methylprednisolone (32 mg). She has been followed in another hospital for more than 1 yr with no evidence of relapse, after tapering the corticosteroids over a period of 8 weeks. A blood smear taken exactly 1 yr after the initial admission showed a number of neutrophils within the normal range and 8% eosinophils (normal 0–5%). Pulmonary function tests 1 yr after treatment were normal.

MEASUREMENT OF MARKERS OF EOSINOPHIL DEGRANULATION

Levels of ECP and EPX in serum and BAL fluid were determined using a double-antibody radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden), with results expressed in $\mu g\,l^{-1}$. To obtain serum, venous blood was collected in SST tubes and allowed to clot at room temperature for 60 min, centrifuged at 1350 *g* for 10 min and then stored at −70°C. Assay sensitivity for ECP and EPX was 2 $\mu g\,l^{-1}$ and 3 $\mu g\,l^{-1}$, respectively. Cross-reactions for both proteins between the two tests used was <0·03%. Serum from three healthy volunteers and sequential samples taken from an asthmatic patient were used as control serum samples, and processed in an identical way to that described above. Bronchoalveolar lavage fluid obtained from two healthy control patients, two patients with bacterial pneumonia (intubated on intensive care, isolation of *Eschericha coli* in both patients), two patients with bronchial asthma (stable on inhalation glucocorticoids) and two patients with full-blown adult respiratory distress syndrome (ARDS, intubated and ventilated on intensive care) were also assessed for the presence of these eosinophil markers. The same BAL procedure of 3 × 50 ml was used for all patients and controls. The first sample was discarded to avoid bronchial contamination. For all patients, ECP and EPX were measured in the pooled volumes recovered after instillation of the second and third 50 ml aliquot. All samples were analysed in duplicate.

Results

SERUM

In the three healthy volunteers studied, levels of ECP (median 11·0 $\mu g\,l^{-1}$, range 4·7–16·5 $\mu g\,l^{-1}$) and EPX (median 37·4 $\mu g\,l^{-1}$, range 14·5–37·6 $\mu g\,l^{-1}$) were observed well within the range reported for healthy volunteers. Very high serum levels for both EPX and ECP were observed in the patient with hypereosinophilia before treatment was initiated (Table 1). Some samples had to be diluted to fall within the range of detection of the assay. Serum levels of both ECP and EPX declined rapidly after initiation of treatment with corticosteroids, accompanied by a rapid decline in the number of eosinophils in the peripheral circulation (Table 1). When all serum samples of this patient were pooled, there was an excellent correlation between the number of circulating eosinophils and both the ECP levels (r=0·883, P=0·001) and the EPX levels (r=0·867, P=0·002), as well as between the ECP and EPX levels (r=0·967, P<0·0001).

BRONCHOALVEOLAR LAVAGE

The BAL sample of the patient with eosinophilic pneumonia had to be diluted (1/10 and 1/100 with the buffer supplied by the manufacturer) to fall within the range of detection for both the ECP (2–200 $\mu g\,l^{-1}$) and EPX (3–400 $\mu g\,l^{-1}$) assay (Table 2). Parallelism between the two different steps

Table 2 Bronchoalveolar lavage (BAL) fluid levels of eosinophilic cationic protein (ECP) and eosinophil-derived neurotoxin (EPX) in individuals from different patient groups

Diagnosis	Eosinophils in BAL fluid (%)	ECP ($\mu g\,l^{-1}$)	EPX ($\mu g\ l^{-1}$)
Eosinophilic pneumonia	96	856·6	677·1
Normal volunteer	1	<2	<3
Normal volunteer	0	<2	11·0
Bacterial pneumonia	1	4·7	11·8
Bacterial pneumonia	0	<2	5·0
Asthma	2	<2	<3
Asthma	2	<2	<3
ARDS	1	17·5	29·5
ARDS	1	6·5	9·0

ARDS=adult (acute) respiratory distress syndrome.

of dilution was observed. Lower levels were observed for the BAL fluid of the two ARDS patients investigated. No ECP was detected in the BAL fluid recovered from the control subjects and asthmatic patients (Table 2).

Discussion

This case report describes a patient with marked peripheral and bronchoalveolar eosinophilia. As no cause of the eosinophilia was found, the diagnosis of idiopathic eosinophilic pneumonia was made based on the profile of the BAL cells, radiographic pattern of the chest and the clinical presentation (2). Other causes of an increased percentage of eosinophils in BAL were excluded (3), including interstitial lung disease, AIDS-associated pneumonia and drug-induced lung disorders. Moreover, the authors report on the very high levels of eosinophil granular proteins in both the serum and BAL fluid of this patient. Given the paucity of other inflammatory cells in the BAL fluid and the fact that most other inflammatory cells are known not to contain these proteins, the authors postulate that these cytotoxic compounds were released by the eosinophils.

The recognition of so-called hypodense eosinophils in eosinophilic pneumonia suggests that eosinophils are at least partially degranulated, and appear to be activated in this condition (4). However, only limited data are available regarding to what extent eosinophils release these proteins in patients with eosinophilic pneumonia. To the authors' knowledge, there have been only four prior case reports on the determination of ECP in either serum or BAL fluid in eosinophilic pneumonia and no quantitative data on EPX levels in BAL fluid of these patients. Janin *et al.* (5) have reported serum ECP concentrations of 28–33 $\mu g\,l^{-1}$ in four patients with chronic eosinophilic pneumonia. In the same study, it was shown that the eosinophil proteins (including ECP and MBP) were distributed in different macrophage compartments, and that numerous eosinophilic granules were present in the BAL fluid. Moreover, these features were not observed in four control patients with an eosinophilic infiltrate of new origin. Deviller *et al.* (6) have described a patient with an eosinophilic pneumonia and a serum ECP level of 36 $\mu g\,l^{-1}$ which declined to 14 $\mu g\,l^{-1}$ after 2 months of treatment. In this patient, a high amount of EPX was detected in the urine. Recently, Yoshida *et al.* (7) described a patient with eosinophilic pneumonia with a marked ECP concentration (in the circulation (318 $\mu g\,l^{-1}$) and BAL fluid (2·7 $\mu g\,l^{-1}$). Shijubo *et al.* (8) reported levels of 108·5 ± 42·4 $\mu g\,l^{-1}$ in six patients with eosinophilic pneumonia. The observed serum ECP and EPX levels in the present patient were very high as compared to those reported by Deviller *et al.* (1994), and in the same range as reported by Yoshida *et al.* (6) and Shijubo *et al.* (8). The levels in BAL fluid were extremely high as compared to most other reports. One other report has described high levels of major basic protein in pleural fluid recovered from a patient with chronic eosinophilic pneumonia (9).

Since comparable serum and BAL ECP levels were observed in the different control populations as reported in the literature, it is unlikely that a technical problem with either the preservation of the samples or the detection method can explain the high serum and BAL levels in the present patient. Indeed, mean serum ECP levels have been reported to range from 5·5 to 32·7 $\mu g\,l^{-1}$ in healthy volunteers (10–12), and mean ECP levels of 21 $\mu g\,l^{-1}$ in systemic sclerosis (11) and 32·7 $\mu g\,l^{-1}$ in healthy smokers (13) have been reported. In asthmatic patients, where increased numbers of peripheral eosinophils are also observed, there is much discussion about whether ECP levels in the serum are consistently elevated. Some authors believe that serum ECP is not increased in patients with asthma (14), while others have observed mean levels as high as 49·6 $\mu g\,l^{-1}$ (12).

The levels of ECP in BAL fluid have been reported as ranging from non-detectable to 19 $\mu g\,l^{-1}$ in non-smoking control patients (12,15–17), and from 2·4 to 15·6 $\mu g\,l^{-1}$ in asthmatic patients (12,15,18). A mean of 7·6 $\mu g\,l^{-1}$ has been reported in patients with bacterial pneumonia (19), and a mean of 163 $\mu g\,l^{-1}$

in patients with ARDS (16), although lavage in this latter group was performed with only one 60 ml aliquot.

It is certain that a marked inflammatory reaction occurs in eosinophilic pneumonia; Ogushi *et al.* (20) have demonstrated increased contents of PGE_2 in BAL fluid from patients with eosinophilic pneumonia as compared to those of normal volunteers, returning to a normal range after treatment with corticosteroids. Morphological and functional studies of eosinophils of patients with eosinophilic pneumonia have shown that they differ from normal peripheral eosinophils. The alveolar eosinophil population found in the BAL fluid is hypodense (21), which might be related to the state of activation (22). Surface expression of major histocompatibility complex (MHC) class II surface antigen HLA-Dr (23) and surface CD69 antigen (24) have been reported in eosinophilic pneumonia.

The factors responsible for eosinophilic degranulation have not been identified. *In vitro*, aggregated immunoglobulins, including IgG, IgA and especially secretory IgA, induce the eosinophil to release EPX (25). Various cytokines which are known to circulate in eosinophilic disorders (26) are able to modify Ig-induced eosinophil degranulation. The eosinophil chemo-attractants IL-5, IL-3 and GM-CSF all enhance Ig-induced EPX release, with IL-5 being the most potent (27). However, *in vivo* pathways of eosinophil secretion and degranulation might differ from the *in vitro* situation, depending on exposure to a particular local micro-environment.

Eosinophil-derived proteins have been implicated in tissue damage. These granular proteins appear to mediate damage to different organs including the respiratory epithelium in asthma (28) or the blood vessels (29). Although tissue-damaging effects of eosinophil granular products have been suggested, several anti-defence mechanisms might be present since clinical recovery is usually complete after appropriate treatment. Eosinophilic proteins have, for example, been detected in distinct cytoplasmatic structures in alveolar macrophages which may protect the lung from damage (5).

Although eosinophilic pneumonia is usually responsive to corticosteroids therapy, this responsiveness differs (2), which might be related to heterogenicity of the glucocorticoid receptor on human eosinophils (30). *In vitro*, glucocorticoids are known to inhibit chemotaxis of eosinophils (31) but to have only a limited effect on eosinophil degranulation (32). When the present patient was treated with corticosteroids, circulating ECP and EPX concentrations fell markedly and rapidly, and the patient showed a corresponding decline in the number of circulating peripheral eosinophils.

The present results support the theory of a high degree of activation of eosinophils in eosinophilic pneumonia, as has been suggested in the past by the presence of hypodense cells; the eosinophil actively degranulates its (cytotoxic) proteins. The present data also suggest that these eosinophils had released their different granular proteins, ECP and EPX, during their passage both towards the peripheral circulation and from the circulation into the lung. Treatment with glucocorticoids lead to a dramatic clinical improvement, accompanied by a rapid decrease in peripheral eosinophilia and serum levels of two of those proteins, ECP and EPX.

References

1. Gleich GJ, Adolphson CR. The eosinophil leukocyte: structure and function. *Adv Immunol* 1986; **39:** 177–253.
2. Umeki S. Reevaluation of eosinophilic pneumonia and its diagnostic criteria. *Arch Intern Med* 1992; **152:** 1913–1919.
3. Allen JN, Davis WB, Pacht ER. Diagnostic significance of increased bronchoalveolar lavage fluid eosinophils. *Am Rev Respir Dis* 1990; **142:** 642–647.
4. Durham SR, Loegering DA, Dinette S, Gleich GJ, Kay AB. Blood eosinophils and eosinophil-derived proteins in allergic asthma. *J Allergy Clin Immunol* 1988; **84:** 31–36.
5. Janin A, Torpier G, Courtin P *et al.* Segregation of eosinophil proteins in alveolar macrophage compartments in chronic eosinophilic pneumonia. *Thorax* 1993; **48:** 57–62.
6. Deviller P, Gruart V, Prin L *et al.* Detection of an eosinophil derived neurotoxin in the urine of a patient with idiopathic chronic eosinophilic pneumonia. *Clin Chim Acta* 1991; **201:** 105–112.
7. Yoshida K, Shijubo N, Koba H, Mori Y, Satoh M, Morikawa T, Abe S. Chronic eosinophilic pneumonia progressing to lung fibrosis. *Eur Respir J* 1994; **7:** 1541–1544.
8. Shijubo N, Shigehara K, Hirasawa M, Inuzuka M, Abe S. Eosinophilic cationic protein in chronic eosinophilic pneumonia and eosinophilic granuloma. *Chest* 1994; **106:** 1481–1486.
9. Grantham JG, Meadous JA, Gleich GJ. Chronic eosinophilic pneumonia. Evidence for eosinophil degranulation and release of major basic protein. *Am J Med* 1986; **80:** 89–94.
10. Peterson CGB, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover in vitro. *Clin Exp Allergy* 1991; **21:** 561–567.
11. Gustafsson R, Fredens K, Nettelbladt O, Hällgren R. Eosinophil activation in systemic sclerosis. *Arthritis Rheum* 1991; **34:** 414–422.
12. Ädelroth E, Rosenhall L, Johansson S, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. *Am Rev Respir Dis* 1990; **142:** 91–99.

13. Eklund A, Eriksson Ö, Hakansson L *et al.* Oral N-acetylcysteine reduces selected humoral markers of inflammatory cell activity in BAL fluid from healthy smokers: correlation to effects on cellular variables. *Eur Respir J* 1988; **1:** 832–838.
14. Venge P, Zetterström Y, Dahl R *et al.* Low levels of eosinophil cationic proteins in patients with asthma. *Lancet* 1977; **ii:** 373–375.
15. Bousquet J, Chanez P, Lacoste JY *et al.* Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of patients with asthma. *J Allergy Clin Immunol* 1991; **88:** 646–660.
16. Hällgren R, Samuelsson T, Venge P, Modig J. Eosinophil activation in the lung is related to lung damage in adult respiratory distress syndrome. *Am Rev Respir Dis* 1987; **135:** 639–642.
17. Schmekel B, Blom-Bülow B, Hörnblad Y *et al.* Granulocytes and their secretory products, myeloperoxidase and eosinophil cationic protein, in bronchoalveolar lavage fluids from two lung lobes in normal subjects. *Eur Respir J* 1991; **4:** 867–871.
18. Aalbers R, de Monchy JGR, Kauffman HF *et al.* Dynamics of eosinophil infiltration in the bronchial mucosa before and after the late asthmatic reaction. *Eur Respir J* 1993; **4:** 840–847.
19. Pohl WR, Schenk E, Umek H, Micksche M, Kummer P, Kohn H. Prognostic value of eosinophil cationic protein and myeloperoxidase assessment in bronchoalveolar lavage in patients with idiopathic pulmonary fibrosis. *Wien Klin Wochenschr* 1993; **105:** 387–392.
20. Ogushi F, Ozaki T, Kawano T, Yasuoka S. PGE2 and PGF2a content in bronchoalveolar lavage fluid obtained from patients with eosinophilic pneumonia. *Chest* 1987; **91:** 204–206.
21. Prin L, Capron M, Gosset P *et al.* Eosinophilic lung disease: immunological studies of blood and alveolar eosinophils. *Clin Exp Immunol* 1986; **63:** 249–257.
22. Kroegel C, Matthys H, Costabel U. Morphology and density features of eosinophil leukocytes in eosinophilic pneumonia; a case report. *Clin Investig* 1992; **70:** 447–453.
23. Beninati W, Derdak S, Dixon PF *et al.* Pulmonary eosinophils express HLA-DR in chronic eosinophilic pneumonia. *J Allergy Clin Immunol* 1993; **92:** 442–449.
24. Nishikawa K, Morii T, Ako H, Hamada K, Saito S, Narita N. In vivo expression of CD69 on lung eosinophils in eosinophilic pneumonia: CD69 as a possible activation marker for eosinophils. *J Allergy Clin Immunol* 1992; **90:** 169–174.
25. Abu-Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RS, Gleich GJ. Iga induced eosinophil degranulation. *J Immunol* 1989; **142:** 2393–2400.
26. Owen WF, Rothenberg ME, Petersen J *et al.* Interleukin-5 and phenotypically altered eosinophils in the blood of patients with the idiopathic hypereosinophilic syndrome. *J Exp Med* 1989; **170:** 343–348.
27. Fujisawa T, Abu-Ghazaleh RI, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* 1990; **144:** 642–646.
28. Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. *J Allergy Clin Immunol* 1986; **77:** 527–537.
29. Gleich GJ, Schroeter AL, Marcaux JP, Sachs NI, O'Connell EJ, Kohler PF. Episodic angioedema associated with eosinophilia. *New Engl J Med* 1984; **310:** 1621–1626.
30. Prin L, Lefebvre P, Gruart V *et al.* Heterogeneity of human eosinophil glucocorticoid receptor expression in hypereosinophilic patients: absence of detectable receptor correlates with resistance to corticotherapy. *Clin Exp Immunol* 1989; **78:** 383–389.
31. Altman LC, Hill JS, Hairfield M. Effects of corticosteroids on eosinophil chemotaxis and adherence. *J Clin Invest* 1981; **67:** 28–36.
32. Kita H, Abu-Ghazaleh R, Sanderson CJ, Gleich GJ. Effect of steroids on immunoglobin-induced eosinophil degranulation. *J Allergy Clin Immunol* 1991; **87:** 70–77.