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Eosinophilic activation in cystic fibrosis

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Abstract

Background **– The neutrophil is a potent contributor to pulmonary destruction in cystic fibrosis. Since eosinophils also possess destructive potential the involvement of eosinophils in cystic fibrosis has been investigated.**

Methods **– Eosinophil numbers and levels of eosinophil cationic protein (ECP), a marker of eosinophil activation, were determined in the serum of 42 patients with cystic fibrosis and in the sputum of 10 of them. To determine neutrophil activation levels of myeloperoxidase (MPO) were also measured.**

Results **– In cystic fibrosis increased serum levels of ECP were detected compared with healthy non-atopic subjects. Serum ECP levels were not related to the peripheral blood eosinophil count. A strong correlation with ECP concentrations in sputum indicated that the level of ECP in serum was representative of its pulmonary level. Levels of MPO were also increased in cystic fibrosis. A strong correlation was found between MPO and pulmonary function. In addition, ECP was related to arterial oxygen and carbon dioxide tensions. Antibiotic treatment reduced neutrophil activation without effect on ECP levels.**

Conclusions **– Until now *Pseudomonas aeruginosa* and neutrophils were held to be primarily responsible for progressive tissue damage in cystic fibrosis. The results of this study suggest that eosinophils might also participate in such pulmonary destruction.**

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Neutrophils contribute to pulmonary destruction in cystic fibrosis by production and release of cytotoxic enzymes (elastase, myeloperoxidase) and toxic oxygen metabolites.[1,2] Eosinophils are also potent producers of toxic oxygen metabolites as well as producing highly toxic proteins.[3,4] Of these proteins, eosinophil cationic protein (ECP) is well established as a marker of eosinophil activation.[5] In asthma increased eosinophil activity has been assessed by measurement of ECP.[6,7] It is considered that activated eosinophils may be responsible for damage to bronchial epithelium, decreased ciliary function, and increased non-specific bronchial hyperreactivity.[3,8,9] In cystic fibrosis, besides lung destruction, diminished ciliary clearance, loss of cilia, and bronchial hyperreactivity also occur.[10] We therefore hypothesised that these effects could be partially caused by eosinophils. The role of eosinophils in cystic fibrosis has not been investigated extensively, which may be because of the presence of normal eosinophil numbers in the blood and the lung in most subjects with cystic fibrosis.[11] However, recent work has shown that eosinophils in lung biopsies appear to be activated.[12]

We determined peripheral blood eosinophil numbers and assessed their activation by measuring ECP levels in serum from patients with cystic fibrosis. In addition, neutrophil numbers and myeloperoxidase (MPO), a marker of neutrophil activation, were determined because of the role neutrophil products play in damaging lung tissue in cystic fibrosis.[13]

Methods

PATIENTS AND CONTROLS

Forty two consecutive patients (20 men and 22 women) with cystic fibrosis from the Vienna University Cystic Fibrosis Care Centre were studied (mean age 14·5 (range 0·75–28) years). In 33 patients chronic *Pseudomonas aeruginosa* infection was demonstrated by sputum culture. There was no clinical evidence of viral infection at the time of blood sampling. Of the patients, 18 were atopic based on total IgE antibodies (atopic patients with cystic fibrosis: range 180–1999 kU/l, median 449 kU/l; non-atopic patients with cystic fibrosis: range 3–90 kU/l, median 26 kU/l) and specific IgE antibodies (positive results RAST class >2), skin prick tests (positive when weal sizes induced by allergens were equal to the positive control), and clinical symptomatology. None of the patients received steroids or antibiotics within the month before blood sampling.

To determine the effect of antibiotic treatment on granulocyte activation, eight patients were studied before and after 2–3 weeks of treatment with aminoglycoside and ceftazidime. As a control group 30 healthy non-atopic subjects (16 men and 14 women) of mean age 13·4 (range 4–32) years were also studied.

Parental or the patient's agreement to blood sampling was obtained in all cases and blood was obtained at routine sampling for clinical evaluation.

CYSTIC FIBROSIS SCORE AND PULMONARY FUNCTION

The clinical severity of cystic fibrosis was assessed by the Shwachman-Kulczycki score.[14] Pulmonary function was determined by whole body plethysmography (Masterlab; Jaeger, Germany) and expressed in relation to predicted values.[15]

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BLOOD AND SPUTUM SAMPLES

Blood eosinophil and neutrophil counts were determined by automated counting (Sysmex NE-5500; Müller, Austria), with a coefficient of variation for eosinophils and neutrophils of 7·0% and 4·8% respectively. Sputum samples (1 g) were extracted immediately to avoid cell damage in 1 ml phosphate buffered saline and vortex mixed for 30 seconds. Extracts were centrifuged at 3000 rpm for 20 minutes at 4°C. Approximately 80% of sputum samples yielded clear supernatants after this procedure. In the remaining 20% the extremely viscous mucus did not separate initially into two phases and the process was repeated.

Concentrations of ECP and MPO in serum and sputum supernatant were determined by double antibody radioimmunoassays (Pharmacia Diagnostics AB, Sweden). ECP or MPO concentrations in the samples competed with ^{125}I-labelled ECP or MPO for binding to specific antibodies.[16 17] The coefficients of variation of the radioimmunoassays for ECP and MPO in serum were 7·2% and in sputum 8–9%.

Regression analysis of the dose-response curves of sputum samples showed no differences from the ECP/MPO standards or ECP/MPO in serum samples. Arterial P_{CO_2} and P_{O_2} values were determined in ear lobe capillary blood (Blood Gas Analyzer 1306 pH; Instrumentation Laboratory; USA).

STATISTICAL ANALYSIS

Results were expressed as mean (SD). Correlations were calculated by means of the Kendall-Tau b coefficient of correlation. The significance of differences between groups was tested by the Kruskal-Wallis test or the *t* test (for normal distribution). A p value $<0·05$ was considered to be significant.

Results

CHARACTERISTICS OF PATIENTS

One patient had a Shwachman-Kulczycki score <40 (severe), six 40–55 (moderate), 32 55–70 (mild), and three patients 70–85 (good). None had a score >85. Forced expiratory volume in one second (FEV_1) ranged from 17·1% to 125·5% predicted (mean 67·8 (32·35)%), maximum expiratory flow at 50% of vital capacity (MEF_{50}) from 4·4% to 130·2% (mean 52·0 (42·45)%), and intrathoracic gas volume from 70·8% to 265·8% (mean 134·4 (41·12)%). There was a correlation between FEV_1 and Shwachman score ($r=0·401$, $p<0·001$). Arterial P_{CO_2} ranged from 4·11 to 5·89 kPa (mean 5·02 (0·50) kPa) and P_{O_2} from 6·6 to 13·4 kPa (mean 10·0 (1·58) kPa), and both correlated with FEV_1 ($r=-0·275$, $p<0·05$; $r=0·495$, $p<0·0001$).

PERIPHERAL BLOOD EOSINOPHILS AND SERUM/SPUTUM LEVELS OF ECP

The blood eosinophil counts ranged from 45 to 1565 cells/μl (mean 336 (289·7) cells/μl) in patients with cystic fibrosis, and from 50 to 425 cells/μl (mean 225 (153·4) cells/μl) in control subjects. In the group with cystic fibrosis only six patients showed elevated eosinophil numbers and no differences occurred between groups. Increased serum levels of ECP were detected in the group with cystic fibrosis (range 13·2–141·8 μg/l; mean 59·7 (28·99) μg/l) compared with the control group (range 3·5–15·9 μg/l; mean 8·1 (2·85) μg/l) (fig 1).

In sputum from patients with cystic fibrosis ECP levels ranged from 1·0 to 1·45 mg/l (mean 1·2 (0·17) mg/l), and were strongly correlated with serum levels of ECP (fig 2). No relation occurred between ECP levels, the blood eosinophil count, and age in the patients with cystic fibrosis. Serum concentrations of ECP were related to arterial P_{CO_2} and P_{O_2}, and the severity of cystic fibrosis (table). Non-atopic patients with cystic fibrosis had similar serum levels of ECP (60·6 (32·78) μg/l) to atopic patients with cystic fibrosis (58·4 (23·99) μg/l). No differences in serum ECP concentrations were observed between patients with and without *Pseudomonas aeruginosa* colonisation or between patients with positive or negative skin prick test results to *Aspergillus* sp. Antibiotic treatment had no effect on peripheral eosinophil numbers (before treatment 497 (462·4) cells/μl; after treatment 554 (332·0) cells/μl) or ECP levels (before treatment 83·9 (28·63) μg/l; after treatment 72·7 (28·78) μg/l) (fig 3).

PERIPHERAL BLOOD NEUTROPHILS AND SERUM/SPUTUM LEVELS OF MPO

The blood neutrophil counts ranged from 2050 to 12 400 cells/μl (mean 5029 (2642·7) cells/μl) in patients with cystic fibrosis and from 3025 to 7120 cells/μl (mean 4427 (1619·5) cells/μl) in control subjects. The concentration of MPO was increased in serum (range 190·0–1887·3 μg/l; mean 837·4 (432·62) μg/l) and sputum (range 90–140 mg/l; mean 110 (49) mg/l) from patients with cystic fibrosis (fig 1), and serum results were related to FEV_1 and Shwachman score (table). Serum concentrations of ECP and MPO were related ($r=0·241$, $p<0·05$), and the serum concentration of MPO, similar to ECP, was related to its concentration in sputum ($r=0·972$, $p<0·0005$). Antibiotic treatment was associated with a reduction in neutrophil numbers (before treatment 7020 (1895·7) cells/μl; after treatment 4420 (1978·2) μg/l; $p<0·01$) and serum MPO concentration (fig 3).

Discussion

The increased serum and high sputum ECP levels in patients with cystic fibrosis suggest activation of eosinophils.[5] These findings are in accord with the suggestion that eosinophils are a component of injury in cystic fibrosis.[12] This possibility has previously received little attention.

The role of eosinophils in inflammation remains obscure. The hypothesis that eosinophils may act as a homeostatic immune modulator with an anti-inflammatory role is

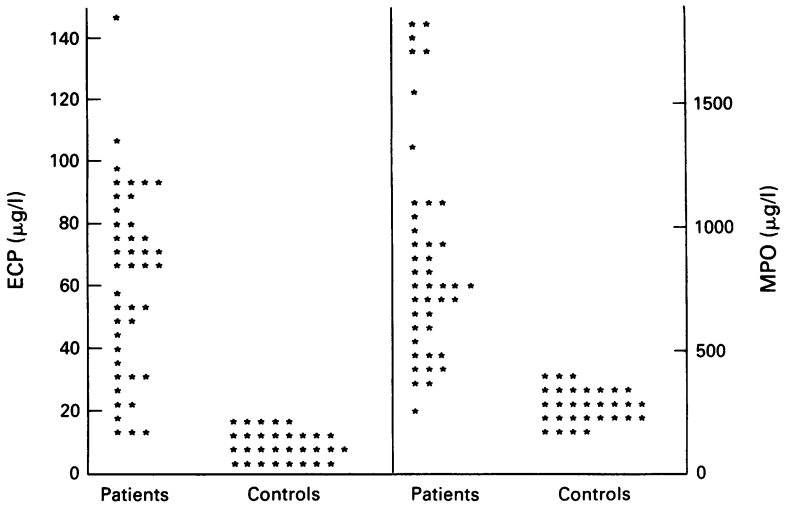

Figure 1 Serum eosinophil cationic protein (ECP) and myeloperoxidase (MPO) concentrations in 42 patients with cystic fibrosis and in 30 control subjects.

Correlations of serum variables in patients with cystic fibrosis

	FEV_1	Shwachman score	Po_2	Pco_2
Eosinophils	NS	NS	NS	NS
ECP	$r=-0.249$ $p<0.05$	$r=-0.282$ $p<0.01$	$r=-0.394$ $p<0.0005$	$r=0.661$ $p<0.0001$
Neutrophils	$r=-0.426$ $p<0.0005$	NS	NS	NS
MPO	$r=-0.404$ $p<0.001$	$r=-0.343$ $p<0.005$	$r=-0.284$ $p<0.01$	$r=0.236$ $p<0.05$

FEV_1 = forced expiratory volume in one second; Po_2 and Pco_2 = arterial blood oxygen and carbon dioxide tensions; NS = not significant; ECP = eosinophil cationic protein; MPO = myeloperoxidase.

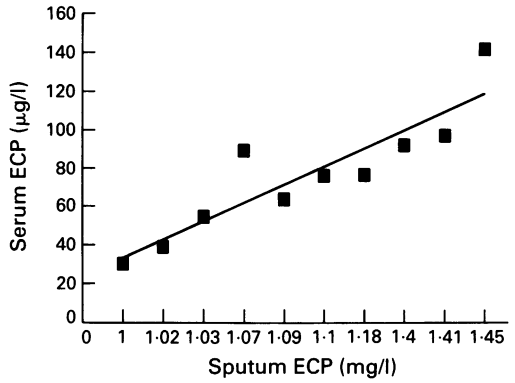

Figure 2 Correlation of levels of eosinophil cationic protein (ECP) in serum and sputum samples from 10 patients with cystic fibrosis ($r=0.847$, $p<0.005$).

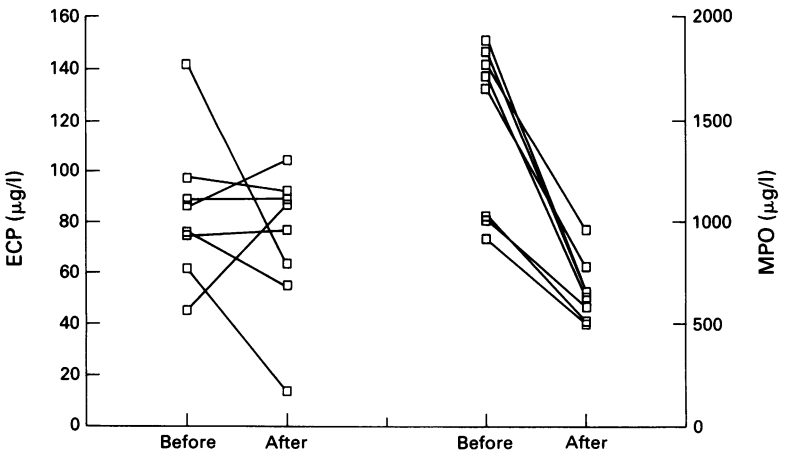

Figure 3 Changes in levels of eosinophil cationic protein (ECP) (not significant) and myeloperoxidase (MPO) ($p<0.0001$) in serum after antibiotic treatment for 2–3 weeks in eight patients with cystic fibrosis.

supported by eosinophil mediated inhibition of mast cell and basophil activity,[18] although eosinophils damage tissue releasing cytotoxic products such as ECP.[4] Eosinophils are also involved in promoting fibrosis, in increasing bronchial hyperreactivity and mucus secretion, and in affecting ciliary function.[19–21] The possible role of eosinophils in lung injury in cystic fibrosis is suggested by relationships between ECP levels and severity of the disease, FEV_1, $Paco_2$ and Pao_2. These findings are analogous to results in patients with asthma and with respiratory syncytial virus infection[6 22] in which the destructive role for eosinophils is assumed. Although these associations do not prove a causal relation between eosinophils and pulmonary destruction in cystic fibrosis, they suggest that eosinophils may be doing more harm than good.

The high concentrations of ECP in sputum support this hypothesis since concentrations are higher than those needed for tissue injury in vitro. In contrast to asthma, increased serum ECP levels occurred with a normal peripheral blood eosinophil count in most of the patients with cystic fibrosis. Eosinophils in cystic fibrosis may have an increased propensity to release their granule proteins. They can be activated by several exogenous and endogenous stimuli,[3] but *Ps aeruginosa* infection is probably not a stimulus as patients with and without colonisation had similar serum ECP concentrations. Endogenous factors may therefore be responsible for eosinophil activation. Such factors include interleukin 3 and 5, and tumour necrosis factor α, which is increased in cystic fibrosis,[23] and possibly by IgE and IgG mediated mechanisms acting with complement via CR1 and CR3 receptors.

The importance of neutrophils in tissue damage in cystic fibrosis has been demonstrated.[24] The neutrophil MPO-H_2O_2-halide enzyme system produce hypochlorous acid and chlorinated amine compounds capable of killing various target cells.[1] The increased serum and sputum levels of MPO in our patients are analogous to previous findings,[12] and support a role in lung injury. It is unknown to what extent neutrophils and eosinophils are beneficial in *Ps aeruginosa* infection in cystic fibrosis. The failure of antibiotic treatment to reduce ECP and MPO to normal levels, although the latter decreased significantly, indicates a potential for injury.

Additional forms of treatment in cystic fibrosis need to be evaluated with the aim of reducing eosinophil and neutrophil activation.

Previously *Ps aeruginosa* and neutrophil products were considered to be responsible for progressive lung damage in cystic fibrosis. Our results suggest the participation also by eosinophils in continuing pulmonary destruction in cystic fibrosis.

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