

Eosinophil cationic protein relates to sputum neutrophil counts in healthy subjects

Richard Leigh, MBChB,^a Jose Belda, PhD,^a Margaret M. Kelly, MBChB,^a Gerard Cox, MB,^a Diane L. Squillace, BA,^b Gerald J. Gleich, MD,^b and Frederick E. Hargreave, MD^a *^a Hamilton, Ontario, Canada, and Rochester, NY*

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Eosinophil cationic protein (ECP) has been regarded as an eosinophil-specific protein. Sputum ECP levels have been shown to be elevated (median [interquartile range (IQR)], 1040 [3008] $\mu\text{g/L}$) in stable asthmatic subjects with sputum eosinophilia (median [IQR], 5.2% [19.4%] eosinophils).¹ However, it has recently been shown that proteins immunochemically indistinguishable from ECP are also present in peripheral blood neutrophils.² High levels of sputum ECP have also been observed in subjects without associated sputum eosinophilia.³ These data raise the possibility that ECP may be produced by other cells in addition to eosinophils. One situation to investigate this possibility is in healthy subjects in whom sputum eosinophils are absent or few in number. We have therefore examined the normal range of ECP in sputum and assessed its relationship to cell counts in a healthy nonsmoking, nonasthmatic population of adults.

SUBJECTS AND METHODS

Subjects

Sixty-eight (male, 42) healthy adult subjects, mean age of 35.7 ± 8.3 years, were recruited through media advertising. They were part of a larger cohort in whom normal reference values for induced sputum cell counts have been reported.⁴ All 68 subjects were life-long nonsmokers, had no nasal or chest symptoms, and had no history of asthma or other chronic respiratory disease. They had normal airway spirometry (FEV_1 mean, 3.8 ± 0.7 L; percent predicted FEV_1 , 102.3 ± 12.2 ; $\text{FEV}_1/\text{slow vital capacity}$ ratio, $82.2\% \pm 6.9\%$), PC_{20} methacholine level of more than 16 mg/mL, and normal sputum cell counts⁴ (Table I). Twenty-nine subjects were atopic, as indicated by 1 or more positive allergy skin tests to 16 common allergen extracts. The study protocol was approved by the research committee of St Joseph's Hospital.

Abbreviations used

ECP: Eosinophil cationic protein
IQR: Interquartile range

Study design

This was a prospective cross-sectional study in which the laboratory measurements were performed blind to clinical data. Relevant details were collected by a standard questionnaire; spirometry, methacholine inhalation tests, and sputum inductions were performed.

Procedures

Spirometry was performed with the use of a pneumotachograph system (Koko-Trek, Louisville, Colo), and the measurements were performed according to American Thoracic Society criteria. The PC_{20} methacholine level was measured by the tidal breathing method (normal PC_{20} methacholine level, >16 mg/mL).

Sputum induction and processing were performed as described by Pizzichini and others.¹ Briefly, after pretreatment with inhaled salbutamol, an aerosol of hypertonic saline solution was inhaled from a Medix ultrasonic nebulizer (Clement Clarke, Harlow Essex, UK). Concentrations of 3%, 4%, and 5% saline solution were each inhaled for 7-minute intervals. At the end of each inhalation, subjects were asked to cough and expectorate into a container. Sputum was processed within 2 hours and selected from the expectorate; a weighed aliquot was dispersed with 4 volumes of 6.5 mmol/L (0.1%) dithiothreitol. Four volumes of phosphate-buffered saline solution were added to the dispersed suspension, which was then filtered. Total cell counts were performed in a hemocytometer, and cell viability was determined by the trypan blue exclusion method. Cytospins were prepared (Cytospin 3 Cytocentrifuge; Shandon Inc, Pittsburgh, Pa) and stained with Wright's stain, and a 400 nonsquamous differential cell count was performed. Sputum supernatant was collected and stored at -70°C . It was later thawed, and the ECP was measured by radioimmunoassay (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) with a detection limit of less than 2 $\mu\text{g/L}$ and low specificity ($<0.5\%$) for other eosinophil and neutrophil proteins. In addition, sputum supernatant eosinophil peroxidase was measured with the use of an immunoradiometric assay that used 2 monoclonal antibodies with a detection limit of 0.125 $\mu\text{g/L}$.⁵ ECP and eosinophil peroxidase measurements were adjusted for the dilution of sputum during the processing.

Analysis

Descriptive statistics were used to summarize the baseline characteristics of the study subjects. The results were expressed as the mean (SD) unless otherwise stated. Spearman's rank correlation was used to determine the correlation between the sputum ECP levels and the sputum cell counts; multiple linear regression (with sputum cell types as independent variables and ECP as a dependent variable) was used to determine the effect of sputum cell types on ECP measurements. Measurements were log-transformed for

From the ^aAsthma Research Group, Department of Medicine, St Joseph's Hospital-McMaster University, Hamilton, Ontario Canada, and the ^bDepartments of Immunology and Medicine, Mayo Clinic and Foundation, Rochester, NY.

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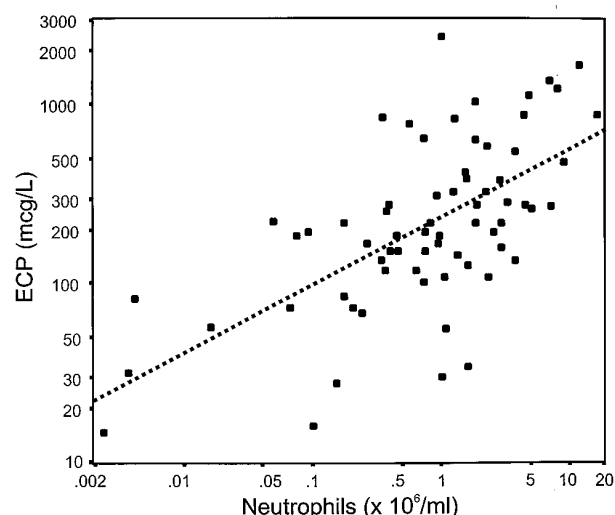


FIG 1. Correlation between sputum ECP levels and sputum neutrophil counts; $r = 0.59$; $P < .001$.

TABLE I. Sputum cell counts

	Median*	IQR	Normal values ⁴
Total cell count $\times 10^6/\text{mL}$	2.5	3.8	<9.7
Cell viability (%)	78.7	19.1	>40.0
Neutrophils (%)	39.2	27.0	<64.4
Eosinophils (%)	0.0	0.5	<1.1
Macrophages (%)	56.3	25.9	<86.1

*Median squamous cell contamination was 4.5% (normal, <20%), which indicates that selecting sputum from expectorate was successful in minimizing salivary contamination.

graphic display. A 2-tailed probability value of less than .05 was considered to be significant.

RESULTS

The mean (2 SD) level of ECP was 351 (1173) $\mu\text{g/L}$, and the median (IQR) level was 196 (284) $\mu\text{g/L}$. There was a significant relationship between sputum ECP and sputum neutrophils ($r = 0.59$; $P < .001$; Fig 1). There was also a significant relationship between sputum ECP and sputum macrophages ($r = 0.43$; $P < .001$). Multiple linear regression confirmed the significant relationship between ECP and neutrophils ($P < .001$), but macrophages were not significant in the model ($P = .21$). There was no correlation with eosinophils or other cells. EPO measurements were below the limit of detection of the assay in 58 of 68 subjects. In the remaining 10 subjects, the mean (2 SD) level of EPO was 13.6 (49.6) $\mu\text{g/L}$, and the median (IQR) was 5.2 (8.8) $\mu\text{g/L}$. There was no correlation between sputum ECP and EPO.

DISCUSSION

We have established normal reference values for ECP in sputum selected from the expectorate in a large adult population in our laboratory. We have also shown that ECP is present in sputum of healthy nonsmoking, nonasthmatic subjects in the absence of sputum eosinophils. Although there are no published data that localizes ECP messenger

RNA to neutrophils, our data indicate that ECP may be produced by neutrophils, in addition to eosinophils. Alternatively, there may be neutrophil-derived proteins present in sputum that cross react with the ECP RIA.

The median (IQR) of the percent of sputum eosinophils was 0.0% (0.5%), making it unlikely that eosinophils were responsible for the measured ECP levels. Eosinophil peroxidase, considered to be an eosinophil-specific protein,⁵ was below the limit of detection in 58 of the 68 subjects, which indicated that significant numbers of eosinophils were unlikely to have been present in the sputum samples. However, in the absence of published spiking studies, it is possible that EPO measurements are affected by sputum processing methods, giving falsely low results.

In conclusion, our observations support previous data, which suggest that ECP is not a distinctive eosinophil protein³ and that caution should be exercised in interpreting the presence of sputum ECP as a specific marker of airway eosinophil activity.

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