High Concentrations of Eosinophil Cationic Protein and Eosinophil Protein X in Eosinophilic Pleural Effusions*

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To analyze the association of the eosinophil granulocyte with pleural effusions, we measured the concentrations of two eosinophil proteins, eosinophil cationic protein (ECP) and eosinophil protein X (EPX), in pleural fluid and serum of 92 patients with pleural effusions of various causes. We observed significantly higher ECP and EPX concentrations in eosinophilic than in noneosinophilic pleural effusions (p < 0.001 and p < 0.05, respectively) and a positive correlation between the concentrations of both eosinophil proteins in pleural fluid and the total number of eosinophils in pleural fluid (ECP: r = 0.66, p < 0.0001; and EPX: r = 0.62, p < 0.0001). There was a positive correlation between the concentrations of ECP in pleural fluid and serum (r = 0.74, p < 0.0001) and between the concentrations of EPX in pleural fluid and serum (r = 0.41, p < 0.001). High ECP and EPX concentrations in pleural fluid indicated nonspecific etiology and not tuberculosis as the cause of the effusion. Our results suggest that eosinophils in pleural effusions release eosinophil proteins and probably actively participate in the local inflammatory reaction.

(Chest 1993; 103:475-78)

ECP = eosinophil cationic protein; EPX = eosinophil protein X

METHODS

The study population consisted of 92 adult patients admitted to Mjölbolsta Hospital for diagnostic evaluation of a pleural effusion. The patients were divided into five groups on the basis of the final diagnosis, which rested on clinical, radiologic, and laboratory findings. (1) Four patients had a parapneumonic effusion. (2) Six patients had tuberculous pleurisy diagnosed on the basis of a positive culture of Mycobacterium tuberculosis or histopathologic findings consistent with tuberculosis. (3) Thirty-three patients had pleural effusion due to a malignant tumor. Eighteen of these patients had lung cancer (five adenocarcinomas, five oat-cell carcinomas, two squamous cell carcinomas, and six poorly differentiated carcinomas of uncertain type) and 15 had extrapulmonary cancer, three of which were breast cancers and two were malignant mesotheliomas. (4) Twenty-one patients had exudative pleural effusion, the cause of which could not be established. (5) Ten patients had transudative pleural effusion due to congestive heart failure. Of the remaining 18 patients, four had bacterial empyema, two had rheumatoid arthritis, two had systemic lupus erythematosus, two had drug-induced (bromocriptine) pleural effusion, two had malignant neoplasms with no evidence of tumor cells in pleural fluid or on pleural biopsy specimen, one had sarcoidosis, one had tularemia, one had pulmonary embolism, one had pancreatitis, one had thoracic trauma, and one had suspected tuberculosis.

Pleural fluid was obtained by thoracentesis, and a sample of peripheral blood was collected by venipuncture on the same occasion. Blood specimens were allowed to clot for 1 h before serum was removed. All pleural fluids were cultured and stained for the presence of bacteria, including M tuberculosis, and analyzed cytologically for the presence of tumor cells. The total leukocyte count of pleural fluid was measured in a hemocytometer (Bürker). The leukocyte differential cell counts were determined from May-Grünwald-Giemsa-stained pleural fluid cytospin preparations (CytoTek, Miles Scientific, 2000 rpm for 5 min), in each of which 400 cells were differentiated. Pleural fluid protein, glucose, and lactate dehydrogenase (LDH) concentrations were measured using standard laboratory procedures. Pleural fluid lysozyme (LZM) concentration was determined with a turbidimetric method (Behring Institute, Germany). For the determination of ECP and EPX, the radioimmunoassays

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developed by Peterson et al. and Carlson et al., respectively (Pharmacia AB, Uppsala, Sweden) were used. The ECP was measured in fresh serum and pleural fluid, and the EPX was measured in samples that had been stored at -20°C. The reference 95 percent range for ECP in serum of healthy individuals is 2.3 to 16 μg/L and that for EPX is 8.2 to 38.5 μg/L.

Statistical calculations (Student's t test, cross tabulation with χ² test, multiple regression) were performed (using the Solo Statistical System, version 3.0, by BMDP Statistical Software, Inc., Los Angeles, Calif.) in a computer (Nixdorf 8810 M55).

**RESULTS**

In our series of 92 patients, 17 (19 percent) had pleural fluid eosinophilia (more than 10 percent eosinophils). Among these 17 patients, 14 had nonspecific exudative pleural effusion and three had pleural effusion due to a malignant tumor (two lung carcinomas and one breast carcinoma). The percentage of eosinophils in pleural fluid ranged from 13.0 to 85.5 among the 14 patients with nonspecific pleural effusion and from 10.5 to 48.5 among the three patients with a malignant tumor. In the whole group of patients with nonspecific pleural effusion (21 patients), pleural fluid eosinophilia was a common finding; it occurred in two thirds of the patients.

Patients with pleural fluid eosinophilia had significantly higher mean pleural fluid ECP and EPX concentrations than patients without pleural fluid eosinophilia (167.5 μg/L vs 23.4 μg/L and 592.7 μg/L vs 82.5 μg/L; p<0.001 and p<0.001, respectively, Table 1). In patients with pleural fluid eosinophilia, the mean pleural fluid to serum ECP and EPX ratios were significantly higher than in patients without pleural fluid eosinophilia (ECP: 7.4 vs 1.8; confidence intervals, 3.6 to 11.1 and 0.9 to 2.8, respectively, p<0.01; and EPX: 14.3 vs 2.9; confidence intervals, 4.2 to 25.5 and 0.8 to 4.9, respectively, p<0.05).

Table 2 shows the ECP and EPX concentrations in pleural fluid of patients with various diagnoses. The distribution of patients in the different diagnostic groups by the level of ECP and EPX concentration in pleural fluid is shown in Tables 3 and 4, respectively. There were significantly more patients with high values of ECP and EPX among those with nonspecific pleural effusion than in all the other groups (p<0.0001). These other patient groups did not differ significantly from each other either with respect to pleural fluid ECP or pleural fluid EPX concentrations. The concentration of ECP and EPX in serum did not differentiate between the various patient groups.

### Table 3—Distribution of Patients in Different Diagnostic Groups According to Concentration of Eosinophil Cationic Protein (ECP) in Pleural Fluid

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<td>&lt;30</td>
<td>3</td>
<td>4</td>
<td>30</td>
<td>6</td>
<td>10</td>
<td>53</td>
</tr>
<tr>
<td>30-90</td>
<td>1</td>
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<td>3</td>
<td>3</td>
<td>0</td>
<td>9</td>
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<tr>
<td>&gt;90</td>
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<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>6</td>
<td>33</td>
<td>21</td>
<td>10</td>
<td>74</td>
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</table>

*Significantly more patients with ECP values above 90 μg/L than in all other groups (p<0.0001, χ² test).
Table 4—Distribution of Patients in Different Diagnostic Groups According to the Concentration of Eosinophil Protein X (EPX) in Pleural Fluid

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<td>25</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>40-120</td>
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<td>3</td>
<td>5</td>
<td>2</td>
<td>2</td>
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<tr>
<td>&gt;120</td>
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<td>0</td>
<td>1</td>
<td>12</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>4</td>
<td>5</td>
<td>31</td>
<td>18</td>
<td>10</td>
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</tbody>
</table>

*Significantly more patients with EPX values above 120 µg/L than in all other groups (p<0.0001; χ² test).

Pleural fluid ECP and EPX concentrations correlated positively with the total number of eosinophils in pleural fluid (r = 0.66, p<0.0001 and r = 0.62, p<0.0001, Figs 1 and 2) but did not correlate with the total number of leukocytes or neutrophils in pleural fluid. However, the four patients with an abundance of neutrophils in pleural fluid due to empyema had high pleural fluid ECP (mean, 159.7 µg/L; range, 38.1 to 405.8) and EPX levels (mean, 330.5 µg/L; range, 53.7 to 677.0). In these patients, differential cell count of the pleural fluid cells revealed no eosinophils.

There was a positive correlation between the concentrations of ECP and EPX in pleural fluid (r = 0.89, p<0.00000). There was also a positive correlation between the concentrations of ECP in pleural fluid and serum (r = 0.74, p<0.00000) and between the EPX concentrations in pleural fluid and serum (r = 0.41, p<0.001). No correlation was observed between pleural fluid total protein concentration and pleural fluid ECP or EPX concentrations. Positive correlations were observed between pleural fluid ECP and LzM concentrations (r = 0.21, p<0.05) and pleural fluid EPX and LDH concentrations (r = 0.32, p<0.01).

**DISCUSSION**

Identification of the highly cytotoxic cationic proteins in the granules of the eosinophil has changed our view of the role of the eosinophil in human disease.

The eosinophil must now be considered a cell that contributes to inflammatory reactions and that is capable of causing tissue injury. This tissue-injuring potential of the eosinophil has been best documented in asthma, but the eosinophil probably also plays a role in several other diseases. This is supported by the demonstration of major basic protein in the myocardium of patients with acute necrotizing myocarditis and the detection of high concentrations of ECP in various extravascular body fluids such as cerebrospinal fluid in meningitis and synovial fluid in arthritis.

The main new observation reported in our study is that eosinophilic pleural effusions contain high concentrations of the eosinophil proteins ECP and EPX. Patients with pleural fluid eosinophilia have higher concentrations of ECP and EPX in pleural fluid than in serum, and eosinophilic pleural effusions contain higher concentrations of these eosinophil proteins than noneosinophilic pleural effusions. The concentrations of ECP and EPX in pleural fluid correlate significantly with the number of eosinophils in pleural fluid. These findings indicate a local release of ECP and EPX by eosinophils in the effusion rather than a passive diffusion of the proteins from the blood into the pleural space. Our results corroborate the findings reported by Grantham et al who measured about...
100 times higher concentrations of major basic protein in pleural fluid of a patient with eosinophilic pneumonia than in pleural fluid of patients with other diseases.

Some of our patients who did not have pleural fluid eosinophilia still had high concentrations of ECP and EPX in pleural fluid, possibly as a result of previous degranulation of eosinophils. Interestingly, we found high concentrations of ECP and EPX in our patients with empyema, where enormous numbers of neutrophils but no eosinophils were detected. This finding conforms with the recent preliminary report by Sur et al.\(^{18}\) that ECP and EPX may not be found exclusively in the eosinophil but also to some extent in the neutrophil granulocytes. We observed positive correlations between pleural fluid ECP and LzM concentrations and pleural fluid EPX and LDH concentrations. This may be explained by simultaneous local activation of eosinophils and neutrophils in the pleural space. A parallel may be drawn to inflammatory joint effusions, where a correlation has been shown between ECP and lactoferrin concentrations.\(^{16}\)

Pleural fluid eosinophilia is a rather uncommon finding but it may be diagnostically and prognostically useful. Eosinophilic pleural effusions are generally considered benign, self-limiting diseases. The three most common causes seem to be infections, allergies, and traumas.\(^{19}\) Pulmonary embolism may be underestimated as the cause of pleural fluid eosinophilia. Pleural fluid eosinophilia discourages the likelihood of a tuberculous origin of the effusion but is occasionally seen when the effusion is caused by a malignant tumor.\(^{20}\) In our 17 patients with eosinophilic pleural effusion, cancer was the cause of the effusion in three cases, but in most cases no specific etiologic diagnosis could be made. In fact, pleural fluid eosinophilia was the common denominator for two thirds of our patients with nonspecific pleural effusion. Determination of ECP and EPX in pleural fluid did not add much to our present potential of determining the cause of pleural effusions. Most patients with high pleural fluid ECP and EPX concentrations were already categorized by their distinctive pleural fluid eosinophilia.

In conclusion, we have demonstrated high concentrations of ECP and EPX in eosinophilic pleural effusions. We hypothesize that the eosinophil granulocyte may actively participate in local inflammatory reactions in the pleura by releasing eosinophil proteins.

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**REFERENCES**