

The Content of Eosinophil Cationic Protein in Eosinophil Leucocytes

Study on Normal Controls and Patients with Bronchial Asthma

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Eosinophil cationic protein constitutes a major part of eosinophil leucocyte granule protein. Low serum concentrations have previously been found in patients with bronchial asthma. As this might reflect a low intracellular content, eosinophils were isolated from normal controls and patients with bronchial asthma. Eosinophil cationic protein was estimated after extraction of the cells and a similar content was found in cells from both groups.

Key words: bronchial asthma; eosinophil cationic protein; eosinophil leucocyte.

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Highly cationic proteins have been isolated from human myeloid cells (5). The fifth component, eosinophil cationic protein (ECP), is an arginine-rich cationic protein with a molecular weight of 21,000. It derives primarily from the eosinophil granulocytes in which it constitutes about 30% of the granule protein (6). It can be estimated in serum with a sensitive radioimmunosorbent assay (8).

In normals the serum ECP (S-ECP) concentration is correlated to the blood eosinophil count. In patients with bronchial asthma, however, the S-ECP levels were lower than would be expected from the blood eosinophil count (10).

In patients with allergic disorders Connell has described vacuolised blood eosinophils (1), possibly representing partly degranulated cells or cells with less granule content than normal (7).

If eosinophils in patients with bronchial asthma contain less granules this might account for the lower levels of S-ECP found in asthmatics. We therefore decided to

estimate the intraeosinophilic content of ECP in normal controls and patients with bronchial asthma.

MATERIAL AND METHOD

Eighteen normals and 14 patients with bronchial asthma were studied. 20 ml heparinised venous blood was collected from each person. Eosinophil leucocytes were isolated according to Day (3). The blood was allowed to sediment in 2% dextran-500 (Pharmacia Fine Chemicals, Uppsala, Sweden) for 30 min at room temperature. The leucocyte-rich supernatants were carefully layered over sodium-metrizoate (Nyegaard & Co., Oslo, Norway), density 1, 146 at 4°C, and the eosinophil leucocytes collected by centrifugation at 400 g for 40 min at room temperature. The cells were washed twice in Ringerdext® (Pharmacia, Uppsala, Sweden) and the suspensions contained 64-90% eosinophils. The eosinophils were counted according to

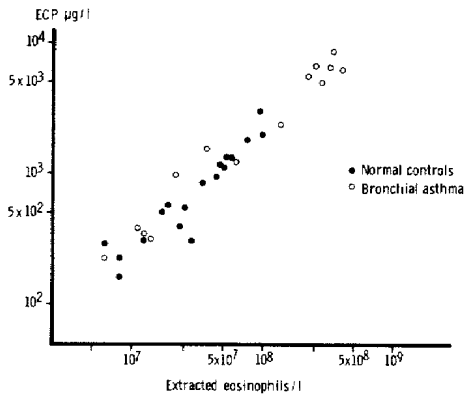


Fig. 1. Eosinophil leucocyte content of eosinophil cationic protein (ECP) in normal controls and patients with bronchial asthma.

Dunger (4) and extracted with 0.3% cetyltrimethylammonium bromide (E. Merck, Darmstadt, Germany). ECP was estimated by a radioimmunosorbent assay (8). The sample was incubated with Sephadex-bound anti-ECP for 2 h before addition of ¹²⁵I-ECP and incubation continued for 20 h during vertical rotation. After washing the sample four times the radioactivity bound in the pellet was determined in a scintillation counter.

RESULT

The result is shown in Fig. 1. There was a high correlation between the number of isolated eosinophils and the corresponding extractable ECP ($P=0.91$) for both normal controls and patients with bronchial asthma. One million eosinophils contain 26 µg ECP and similar amounts were found in both groups.

DISCUSSION

This study shows that eosinophils from normal controls and patients with bronchial asthma contain similar amounts of ECP. The

low serum ECP levels found in patients with bronchial asthma must therefore result from other causes. Possibly ECP is consumed during allergic inflammations by binding to proteins generated. Venge et al. (9) found that ECP would bind to substance in plasma. One of these substances had a molecular weight of 2000–7000 and was generated by immune complex activation of plasma.

In vivo studies of asthmatic reactions have also indicated that a consumption of ECP takes place during the induced reaction (2, 10). ECP has not been found to possess enzymatic activity (6), but its influence on other biological systems is at present under investigation in our laboratories.

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