

Variations of Blood Eosinophils and Eosinophil Cationic Protein in Serum in Patients with Bronchial Asthma

Studies during Inhalation Challenge Test

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Inhalation challenge test was performed in 12 patients with bronchial asthma. The subsequent variation in blood eosinophils and serum-eosinophil cationic protein was followed up. Uniform patterns in both parameters were seen suggesting active participation of the eosinophil leucocyte in allergic inflammation.

Key words: Bronchial asthma, eosinophil cationic protein, eosinophil leucocyte, inhalation challenge test.

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An increase of blood eosinophil leucocytes is common in patients with bronchial asthma. It has previously been shown that intravenous injection of allergen into allergic persons results in a decrease of eosinophils in the blood (6). Booij-Noord et al. found a substantial increase in the number of blood eosinophils 24 h after an inhalation challenge and only in asthmatic patients who developed a late asthmatic reaction (2).

A cationic protein, eosinophil cationic protein (ECP), which constitutes about 80% of the eosinophil granular proteins, has been purified (7). It can be estimated in serum by a sensitive radioimmunosorbent assay (9).

In normal controls the number of blood eosinophils are well correlated to serum-ECP (S-ECP), whereas in patients with bronchial asthma we have found lower levels of S-ECP than expected (10).

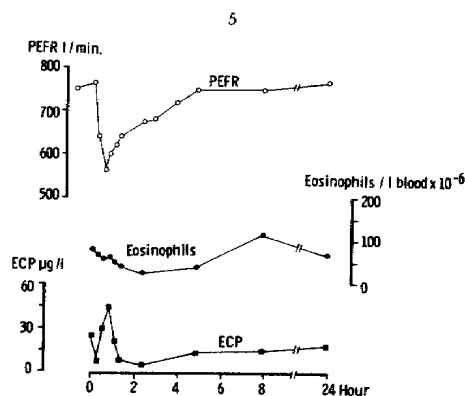
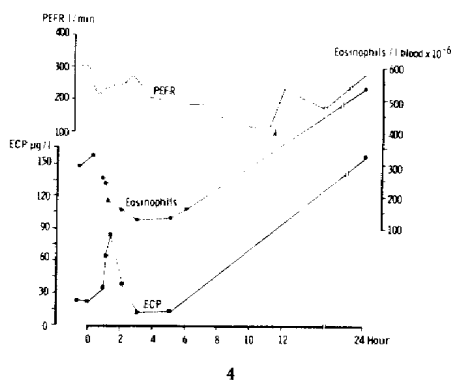
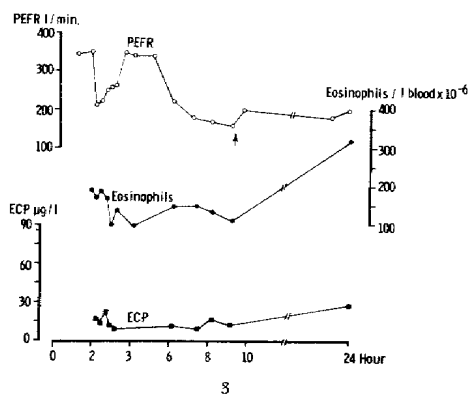
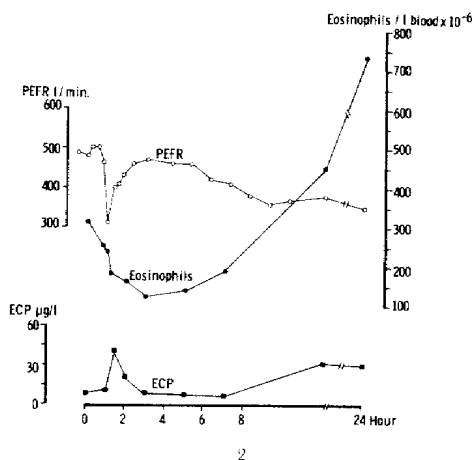
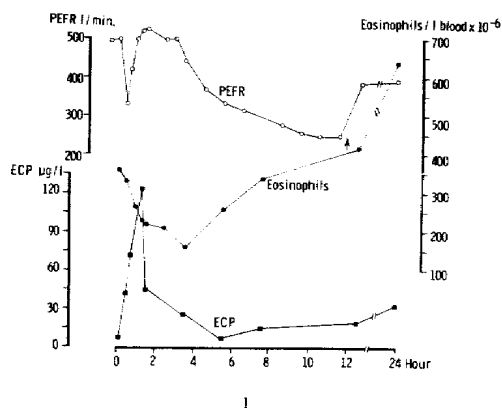
The present study was performed in an attempt to elucidate the variations of blood eosinophils during challenge tests and the

low levels of S-ECP in patients with bronchial asthma.

PATIENTS AND METHODS

Patients

Twelve patients with allergic bronchial asthma, two women and 10 men, were studied. The age range was 15–35 years. Their histories, prick tests, IgE-RAST tests and inhalation challenge tests were all positive towards the allergen used to provoke an asthmatic reaction by inhalation. At the time of study they were in a clinically optimal state with normal or near normal peak expiratory flow rates (PEFR). None of the patients had been treated with corticosteroids. They were hospitalized during the investigation and placed under the constant supervision of one of our team. They were informed of the purpose and nature of the study and all gave their verbal



Figs. 1-5. Variation of peak expiratory flow rate (PEFR), blood eosinophil count and serum eosinophil cationic protein concentration (ECP) after inhalation challenge test.

consent to participate. Drugs were not allowed 12 h before the challenge test or during the investigation. In three patients, however, it was necessary to give an inhalation of salbutamol.

Methods

An inhalation challenge was performed in each patient, using the minimum allergen dose necessary to produce an immediate asthmatic reaction, i.e. a fall in PEFR of at least 20%. The allergens used were house dust mite (*Dermatophagoides pteronyssinus*) in nine patients, timothy grass pollen in two patients, and birch pollen in one patient. The allergens were produced and standardised by Allergologisk Laboratorium, Copenhagen.

All challenges started at 9 a.m. PEFR was measured before the challenge, every 15 min the first hour after the challenge and thereafter every 1–3 h during the day and evening. The last measurement was taken the next morning at 9 a.m. On the same occasions blood was drawn from an antecubital vein. Eosinophils were counted using Rud's modification (8) of Dunger's method (3). Serum was collected within 1 h and kept frozen at -17°C until assayed for ECP by a radioimmunosorbent test (9).

RESULTS

The test elicited an immediate reaction only in four patients and a dual reaction in eight patients. A uniform pattern in the variation of blood eosinophils was found. Figs. 1–5 show the characteristic pattern. The number of blood eosinophils began to decrease immediately after the challenge and reached a minimum count 2–4 h later. Their number then started to increase and was 1 1/2–3 times the initial value after 24 h (Figs. 1–4). However, in one patient, who had only an immediate reaction, the initial decrease was followed by a rise in blood eosinophils which reached a maximum count 8 h after the challenge, and returned to the initial value at 24 h (Fig 5). One patient with a dual reaction

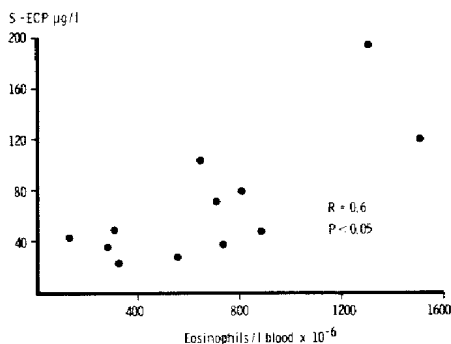


Fig. 6. Relation between serum peak values of eosinophil cationic protein (ECP) and blood eosinophil count 24 hours after inhalation challenge test.

showed no late increase in blood eosinophil count. The variation in S-ECP was also uniform. S-ECP concentration showed a rapid increase, just after the maximal fall in PEFR in the immediate asthmatic reaction, followed by a rapid decrease with the lowest values 3–5 h after the challenge. Thereafter a slow increase in S-ECP was seen (Figs. 1–5). The late rise in S-ECP concentration seemed to begin after the eosinophil count started to rise.

The maximal concentration of S-ECP during the immediate reaction did not seem to be correlated to the appearance or severity of a late asthmatic reaction, but was correlated to the rise in blood eosinophils at 24 h (Fig. 6) ($R=0.6$, $P<0.05$). The ratio between blood eosinophil count and S-ECP concentration at the time of the lowest S-ECP concentration was significantly higher ($P<0.05$) compared with the ratio calculated for both the prechallenge values and the 24 h values.

DISCUSSION

The initial fall in blood eosinophils is possibly due to chemotactically active factors attracting eosinophils to the tissues where the allergic reaction takes place. This initial fall was not noted in the investigation of Booijs-Noord et al. (2) because in their study no

blood samples were taken during that time interval. The initial decrease observed in blood in this study cannot be a result of disintegration of eosinophils because this would be followed by a more prolonged and much more pronounced rise in S-ECP. The late increase in blood eosinophils may be a result of liberation of eosinophils from the bone marrow. It is possible that an immediate type reaction after the initial decrease may show a faster increase in blood eosinophils with a return to prechallenge values 24 h later, and therefore Booij-Noord et al. (2) found an apparent lack of blood eosinophil variation in immediate type reactions.

The variations in S-ECP may reflect similar variations in the reacting tissues. The highly cationic ECP, however, binds very easily to negatively charged structures which might reduce the liberation of ECP from the tissue to the blood and therefore more moderate tissue variations would be expected to be seen in the blood. That S-ECP variations are expressions of the tissue variations is supported by the correlation between the maximum peak values of S-ECP and the maximum eosinophil count at 24 h and not between the maximum peak of S-ECP and the actual blood eosinophil count. This means that the peak values of S-ECP may express the total reactive eosinophil mass of the bodies present.

The peak in S-ECP may be caused by the tissue eosinophils expelling their granular content. This may happen in response to chemotactically active factors as it is known that chemotactically stimulated leucocytes secrete part of their granular content (1). An eosinophil chemotactic factor is known to be secreted by mast cells during anaphylaxis (5), and other factors with leucocyte-stimulating properties are possibly generated during the allergic reaction. Another possible mechanism for granule release could be direct allergen interaction with eosinophil-bound allergen-specific IgE, as electron microscopic investigations have indicated the presence of IgE on eosinophils (4).

Following the initial peak the fall and sub-

sequent rise in S-ECP followed much the same variation in blood eosinophils. Blood eosinophils, however, were beginning to increase in number before the rise in S-ECP, and the appearance of the ECP peak indicates that S-ECP concentration is not merely a reflection of the actual blood eosinophil count.

The asthmatic disease state in allergic patients' everyday life is the result of a constant allergen load. The asthmatic reaction patterns which can be evoked in the laboratory by a single allergen inhalation may consist of an immediate, a late, or a combined dual reaction. Of these reactions the late phase is the disease state which clinically resembles asthma in everyday life. We have previously found low levels of S-ECP in asthmatics, especially those patients with a chronic disease (10) and the significantly lower S-ECP level compared with the actual blood eosinophil count during the late asthmatic reaction may be equivalent to clinical asthma. This may indicate a consumption of ECP possibly through its binding to other proteins generated during allergic inflammation.

The variations in blood eosinophils and S-ECP indicate that the eosinophils take an active part in allergic reactions and ECP should be added to the list of participants in allergic inflammation. The function of ECP is at present under investigation in our laboratory.

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