

Variations of Cationic Proteins from Eosinophil Leukocytes in Food Intolerance and Allergic Rhinitis

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Challenge tests were performed in patients with food intolerance and allergic rhinitis to evaluate the usefulness of measurement of the eosinophil cationic protein (ECP) of serum to distinguish different allergic reactions. In one group of patients with food intolerance symptom-induced challenge resulted in a marked decrease of serum-ECP. The number of blood eosinophils decreased simultaneously in some but not all of these patients. In another group of patients with food intolerance serum-ECP displayed peak-like increases followed by a decrease. The decrease in serum-ECP may reflect that consumption of ECP is a result of idiosyncrasy in the target organ. In allergic rhinitis some patients showed an initial peak-like increase of serum-ECP, which was abolished by pretreatment with disodium-cromoglycate but not by pretreatment with antihistamine. Similar results have previously been demonstrated for allergic asthma. The difference obtained in serum-ECP upon challenge in typical reagin-mediated allergy and food intolerance may indicate that the latter is not reagin-mediated. However, the interpretation of data is difficult because of lack of knowledge regarding the turnover in the circulation of ECP.

Key words: allergic rhinitis; eosinophils; eosinophil cationic protein; food intolerance.

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CLINICAL ASPECTS

Hypersensitivity reaction to food is well recognized but the diagnosis of food allergy or food intolerance is often difficult to establish. The challenge test with simultaneous measurement of ECP seems a valuable method, and the test results also may give information regarding the type of reaction involved. The test also makes it possible to evaluate the protective effect of different drugs.

The test may also be used for diagnosing and evaluating the effect of treatment in other kinds of hypersensitivity reactions like allergic rhinitis.

According to one prevailing concept the eosinophil leukocyte is regarded as a regulatory cell directed to limitation of reactions elaborated by mast cell-derived mediators of the immediate hypersensitivity reaction (4). This theory is based on the association between eosinophilia and allergy and the finding of inactivation of mast cell products by eosinophil agents (4). One agent of human eosinophils is the eosinophil cationic protein (ECP) (5), which can be detected in serum by a radioimmunoassay (6). Healthy individuals show a correlation between serum-ECP and eosinophil counts but, for in-

stance in bronchial asthma, a lower than expected level of serum-ECP is found most likely to indicate consumption of ECP (7). Treatment with beta-2-adrenergic drugs may, however, result in low levels of ECP (2). The present work was done to evaluate changes of serum-ECP during challenge test in patients with food intolerance and allergic rhinitis. Such studies were thought to give information on the possible release and consumption *in vivo* of ECP and, subsequently, the involvement of eosinophils in allergic reactions.

MATERIALS

Eighteen patients (four males and 16 females) with a history indicating intolerance against cow's milk were studied. The age range was 24–55 years. Patients were selected with regard to a history indicating intolerance to cow's milk. They had a normal lactose tolerance test but described discomfort when drinking cow's milk. Gastrointestinal symptoms – diarrhea, abdominal pain, distention – dominated, but some also described extraintestinal symptoms such as headache, arthralgia, eczema, etc. Regarding serum-IgE-concentration and RAST against cow's milk – see Results.

The controls consisted of two males and one female without any history indicating food intolerance.

Five patients with seasonal allergic rhinitis due to grass pollen were also studied. They all had positive skin and ophthalmic tests for the allergen.

METHODS

The patients with a history indicating food intolerance and the controls were free of milk for 6 days. A challenge test was then performed with 150 ml cow's milk. Blood was taken via a venous catheter before challenge and 15, 30, 60 min, 2, 4, 8 h after the challenge. The number of blood eosinophils were measured in a Fuchs-Rosenthal chamber using the method of Forsham et al. (3). Serum was collected within 2 h and stored at -17°C until assayed for ECP.

The patients with allergic rhinitis were challenged by application of a pollen extract in the conjunctival sac. In order to reduce symptoms some were challenged after oral pretreatment with antihistamine. Dexchlorpheniraminum (Polaramin) was given – 2 mg 12 h and 2 mg 2 h before the challenge. One patient was challenged after oral pretreatment with disodium cromoglycate (Lomudal®) 200 mg $\times$ 4 for 2 days. Cromoglycate was not applied locally in the conjunctival sac. Blood was taken via a venous catheter before and every 15 min after the challenge.

S-ECP was measured by a radioimmunoassay as previously described (6).

RESULTS

Seven of the 18 patients with a history of intolerance to cow's milk showed a decrease in serum-ECP and to some extent also in eosinophil counts upon challenge with milk (Fig. 1). The lowest levels for serum-ECP (approx. 30% of initial value) were seen after 2 h. All these patients showed gastrointestinal symptoms within 2–3 h of the challenge.

In five of the 18 patients with milk intolerance the challenge produced a decrease in the eosinophil count, but serum-ECP displayed peak-like increases within 2 h followed by a decrease (Fig. 2). These patients showed late symptoms (after 4 h or later) often from extraintestinal organs, e.g. arthralgia.

Only one of these 12 patients had an elevated serum-IgE-concentration. Eight of the patients were tested for IgE antibodies against cow's milk (RAST) and were negative.

Six of the 18 patients with a history of intolerance to milk, as well as three healthy individuals, showed no significant changes of serum-ECP or eosinophil counts (data not shown), nor any symptoms upon challenge.

Four of five patients with allergic rhinitis showed a more or less profound peak-like increase of serum-ECP approximately 1 h after challenge with grass pollen (Fig. 3). Data for eosinophil counts showed no consistent variations. Pretreatment with disodium cromoglycate abolished the peak-like increase of serum-ECP upon challenge (Fig. 4). Pretreatment with antihistamine did not abolish the increase of serum-ECP upon challenge, but it eventually seemed to be delayed (Fig. 4).

DISCUSSION

Recent work has produced some new information on the properties of eosinophil leukocytes with regard to granule content, cell surface receptors and molecular interactions *in vitro* (4). There is a great need for methods to study eosinophil behaviour *in vivo*. One possibility

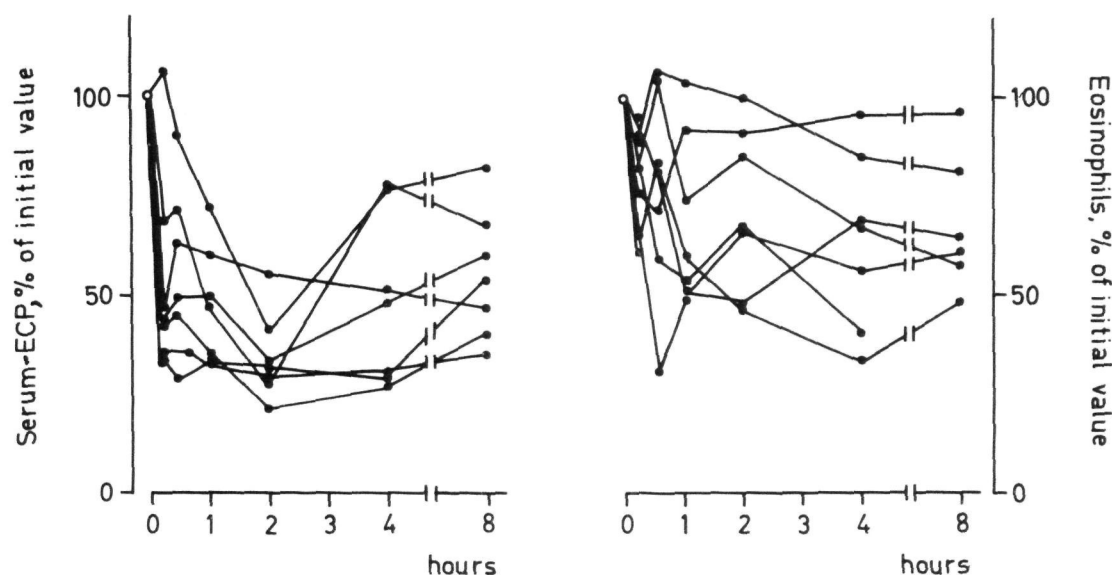


Fig. 1. Serum-ECP and eosinophil counts during challenge tests with milk in seven patients who showed a decrease of serum-ECP upon challenge. Initial levels of serum ECP varied between 8–59 $\mu\text{g/l}$. Initial eosinophil counts varied between 62–424 cells/ μl blood.

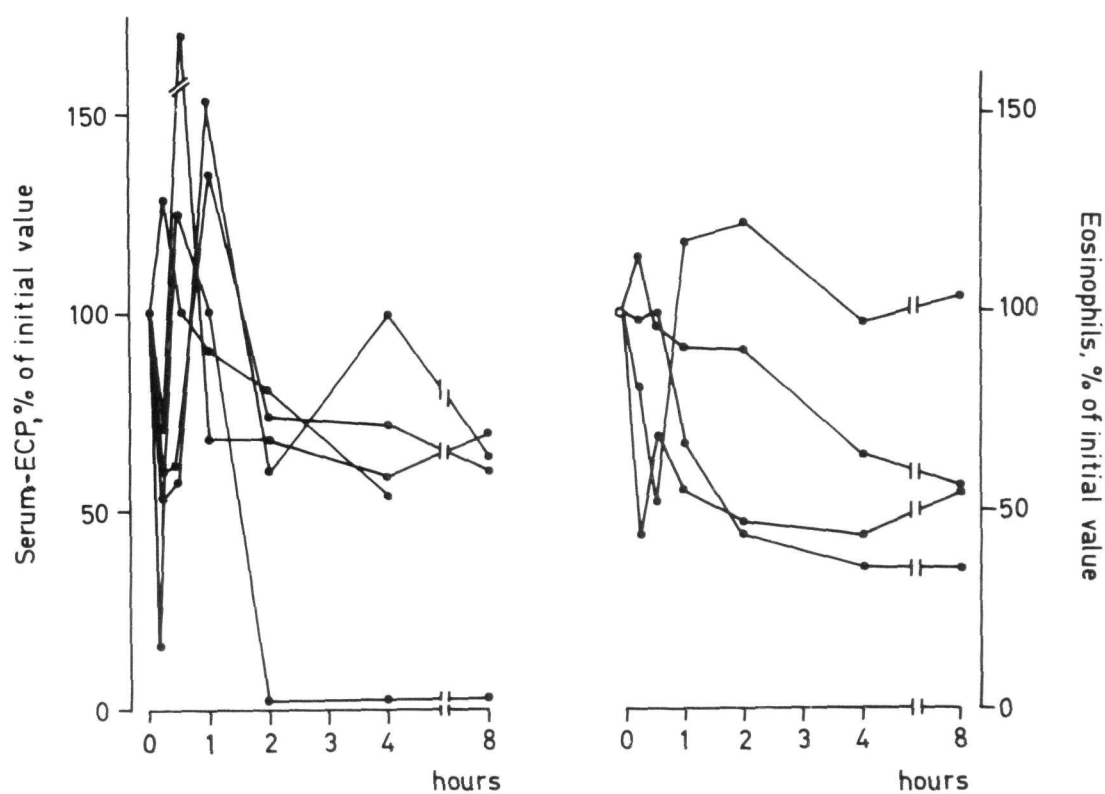


Fig. 2. Serum-ECP and eosinophil counts during challenge tests with milk in five patients who showed an initial increase of serum-ECP followed by a decrease upon challenge. Initial levels of serum-ECP varied between 4–12.5 $\mu\text{g/l}$. Initial eosinophil counts varied between 53–184 cells/ μl blood.

available is to monitor variations of the serum content of the specific eosinophil product, ECP. This study has shown that changes occur in serum-ECP and eosinophil counts during symptom-inducing challenge in food allergy. One consistent pattern was a marked decrease of serum-ECP which did not correlate directly

with the number of blood eosinophils. In addition, some patients demonstrated an early peak-like rise of serum-ECP similar to our findings in challenge of patients with allergic rhinitis.

The interpretation of data on serum-ECP is complicated because of the lack of knowledge

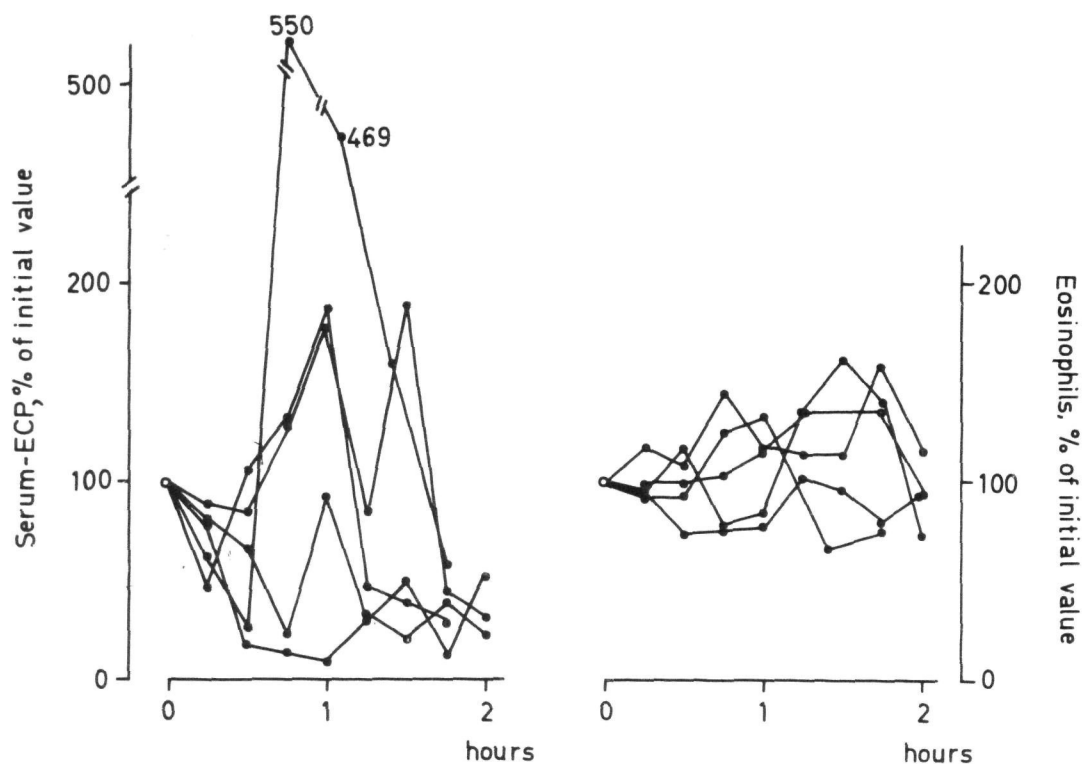


Fig. 3. Serum-ECP and eosinophil counts during challenge with local administration of grass pollen in five patients with allergic rhinitis. Initial levels of serum-ECP varied between 11–70 µg/l. Initial eosinophil counts varied between 69–1020 cells/µl blood.

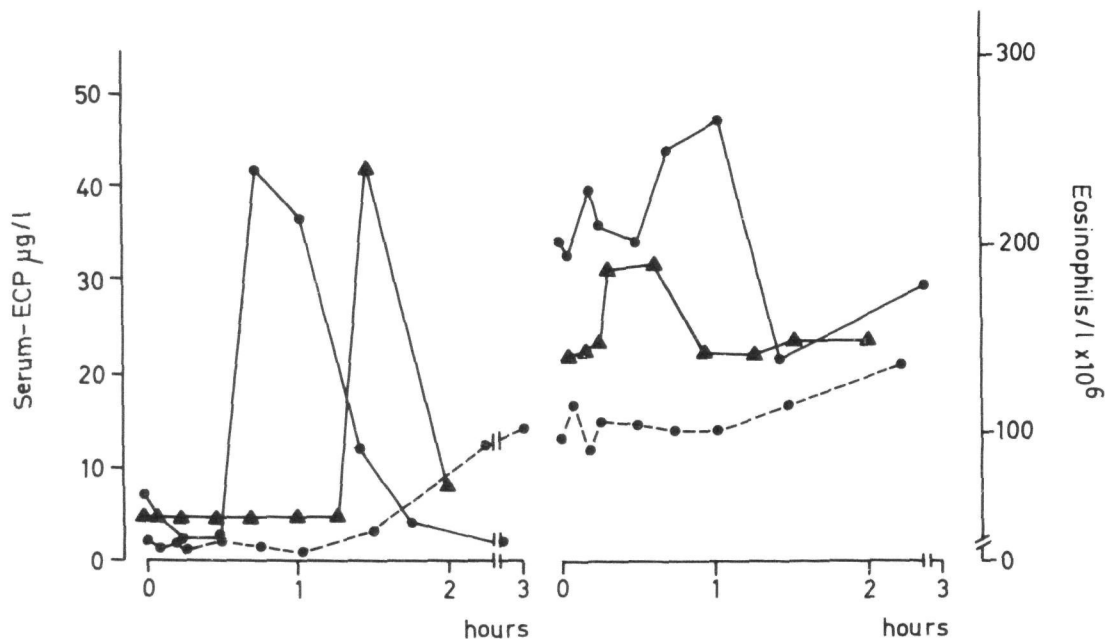


Fig. 4. Serum-ECP and eosinophil counts during challenge with grass pollen (●—●) in a patient with allergic rhinitis who had been pretreated with disodium cromoglycate (●---●) or antihistamine (▲—▲).

about the normal rate of turnover in the circulation of ECP. It is not known if all serum-ECP derives from circulating eosinophils as these represent only a minor part of the total eosinophil mass. Changes in binding proteins with consequences for the elimination could occur during pathological conditions. Nevertheless,

the decrease of serum-ECP found during challenge in food allergy most likely depends on an increased rate of elimination and perhaps consumption in the target organ. The decrease in blood eosinophils occurring simultaneously may also be partly responsible for a decreased serum-ECP. Challenge in asthma patients by

inhalation of specific allergen often resulted in an early peak-like increase of serum-ECP (1). Such a rapid change of serum-ECP must result from release from circulating eosinophils or eosinophils activated in the lung; it occurred in spite of a simultaneous decrease of blood eosinophil counts. Initial peak-like increases of serum-ECP were seen only in a few patients with food intolerance and these usually had no immediate symptoms upon challenge but late symptoms instead. Food intolerance with early symptoms was seen only in patients with a decrease in serum-ECP. It is possible that these early symptoms do not depend on reagin immediate type hypersensitivity but on some type of reaction of idiosyncrasy. It should be mentioned that patients with symptoms from intrinsic asthma (aspirin idiosyncrasy) have very low levels of serum-ECP (7). It is possible that there is no early release of ECP from eosinophils in idiosyncrasy but only consumption of ECP. Peak-like increases of ECP in reagin-mediated allergic reactions may depend on the formation of immune complexes which either locally or in the circulation induce exocytosis of ECP.

Allergic rhinitis is a reagin-mediated immediate type hypersensitivity reaction. In spite of the small size of the target organ some patients showed a clear peak-like increase of serum-ECP upon challenge. Both the symptoms and the peak-like increase of serum-ECP were abolished by pretreatment with disodium cromoglycate, indicating that changes of serum-ECP are somehow dependent on mast cell degranulation. On the other hand, pretreatment with antihistamine only abolished the symptoms and not the peak-like increase of serum-ECP, even if it was perhaps delayed (because of the absence of a histamine-induced increase of permeability?).

These studies have suggested that serum-ECP may be useful for distinguishing certain types of allergic reactions, but interpretation of the data is complicated due to the lack of knowledge regarding the turnover of serum-ECP.

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