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Activation of Blood Coagulation during Inhalation Challenge Tests

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Bronchial challenge tests were performed in 16 asthmatic subjects. Eight developed an immediate and eight a dual asthmatic response. In all patients serial blood samples were analysed for plasma recalcification time and Factor XII activation rate. In all instances a profound shortening of the recalcification time was found, but only in patients with a dual response could an increased activation rate of Factor XII be detected. A positive correlation, although weak, was found between the time for maximum shortening of recalcification time and the time of onset of the late reaction. The study suggests a relationship between activation of blood coagulation factors and the type of asthmatic response elicited after challenge test.

Key words: blood coagulation; bronchial asthma; Factor XII; inhalation challenge test; plasma recalcification time.

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Release of mast cell granule components is an important pathogenetic mechanism of allergic immediate type reactions (10). Kallikrein of anaphylaxis is one of the mast cell products that may be secreted, and activation of Factor XII and the coagulation cascade could result from mast cell mediator release. Eosinophil granulocytes also participate in allergic reactions and eosinophil cationic protein (ECP) is secreted during allergic reactions (2). As ECP has been shown to facilitate activation of Factor XII-dependent reactions (1,8) and an activation of blood coagulation could also be brought about by ECP, the present study was undertaken to find out if blood coagulation is activated during allergen-induced asthmatic reactions.

SUBJECTS AND METHODS

Patients

Sixteen patients with allergic bronchial asthma, seven women and nine men, were studied. The age range was 18–39 years. They all had positive skin prick tests to more than two common allergens and the clinical history, skin test, IgE – RAST test and inhalation challenge test were positive toward the allergen used in the present study to provoke an asthmatic reaction. None of the patients had received corticosteroids within the past 3 months and all medication was stopped 24 h before and during the test period.

Patients were informed of the nature and purpose of the study and all gave their consent to participate.

Challenge tests

The patients were hospitalized during the investigation and placed under constant supervision. Only quiet sedentary activity was allowed.

Each patient underwent an inhalation challenge test, using the smallest allergen dose necessary to provoke a fall in peak expiratory flow rate (PEFR) of more than 20%. The allergens used were house dust mite *(Dermatophagoides pteronyssinius)* in seven patients, house dust in two patients, cat dander in three patients, ti-

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mothy *(Phleum pratense)* in two patients, birch *(Betula pendula)* in one patient and *Cladosporium herbarum* 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 and every 15 min for the first 2 h after the challenge and subsequently every hour for 10–12 h, or until a late reaction was established. A late reaction was present if PEFR values fell more than 20% from the highest value reached after reversal from the immediate reaction, and clinical signs of increasing bronchospasm developed. Treatment was started when a late reaction had developed.

Blood samples

Blood was taken from a catheter placed in an antecubital vein. Samples were drawn before challenge, every 15 min after the challenge for 2 h, and 4 and 6 h after the challenge.

Platelet poor citrate plasma was prepared by centrifugation at 2000 *g* for 20 min and assays performed within 4 h.

Plasma coagulation was measured as plasma recalcification time at 37°C in glass tubes by determination of the time from addition of calcium to clot formation. The coefficient of variation was less than 4%.

Factor XII was determined at 37°C. Patient plasma was diluted to 10% in 0.05 mmol/l imidazole buffer, pH 7.3. 100 μl diluted patient plasma was mixed with 100 μl Factor XII-deficient plasma (Ortho Diagnostics, Ravidan, New Jersey, USA), and incubated for 300 sec before addition of calcium. The time required for clot formation was recorded. The rate with which Factor XII was activated – hereafter called the *initial activation rate* – was determined by incubating the diluted patient plasma with Factor XII-deficient plasma for 60 sec before addition of calcium. The coefficient of variation was less than 4%.

Controls

Blood was taken and analysed from six volunteers at similar time intervals as the patients.

RESULTS

In control experiments no variation was found in plasma recalcification time during 6 h. In all series of plasma samples taken from controls and during inhalation challenge no change was found in Factor XII measured by the conventional technique, with 300 sec preincubation of diluted patient plasma and Factor XII-deficient plasma before addition of calcium. In eight patients an immediate reaction occurred and in eight patients the immediate reaction was followed by a late reaction with reversal of PEFR in between. There was no difference between the two groups in the time course for development of the immediate reaction or in the measured maximum fall in PEFR (Figs. 1 and 2). The late reaction started 3–12 h, mean 6.5 h, after allergen inhalation.

In all patients a substantial fall in plasma recalcification time was found (Figs. 1 and 2). The fall was evident in the first sample taken after 15 min and the maximum fall was usually found 60–90 min after challenge, i.e. at a time when PEFR values had improved after the immediate fall. No difference in the time course or in the degree of decrease in recalcification time was found between patients with immediate or dual reaction.

A positive correlation was found in patients with dual reaction, between the time of the lowest recorded recalcification time and the time when the late asthmatic reaction started (Fig. 3); ($r = 0.67$ $P < 0.08$. Spearman's test).

In patients with only immediate reaction no changes could be detected in the initial activation rate of Factor XII (Fig. 1) during the first 4 h and a slight decrease was present 6 h after challenge.

In patients with a dual asthmatic response an increase in the initial activation rate of Factor XII was present during the whole observation period (Fig. 2).

DISCUSSION

In all patients studied both with immediate and dual asthmatic reaction, the blood coagulation system measured as recalcification time of plas-

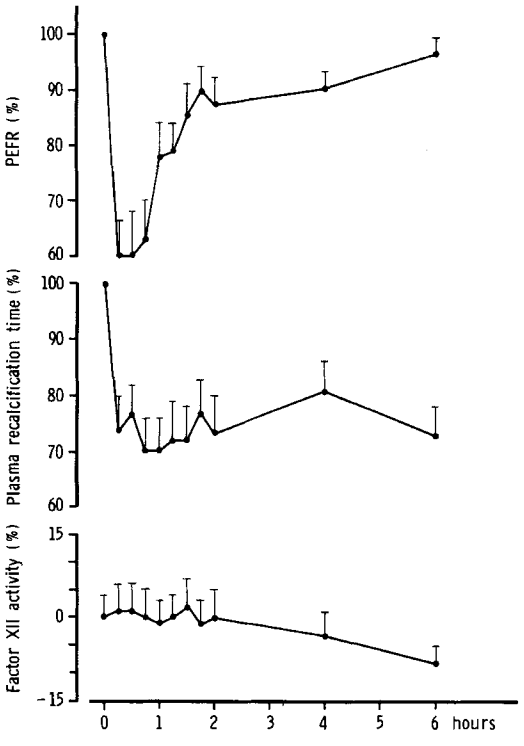

Fig. 1. Mean percentage change from initial: in recalcification time, peak expiratory flow rate (PEFR), and initial activation rate of Factor XII (Factor XII activity) in patients with immediate type reaction. Bars indicate s.e.m.

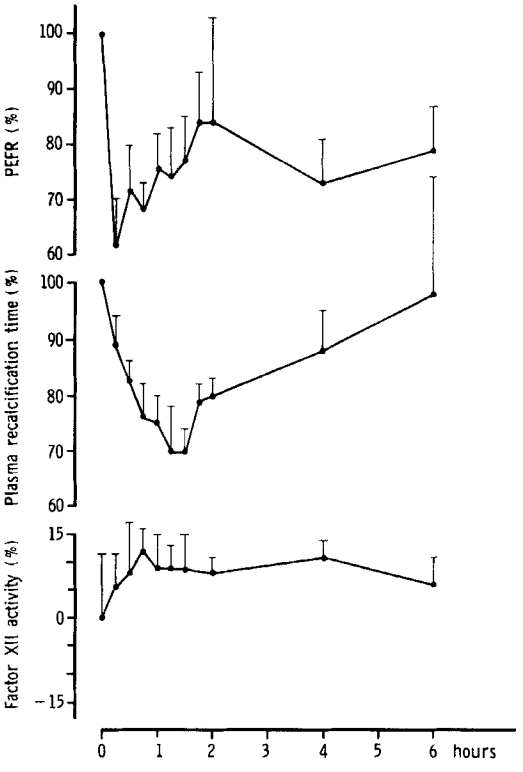

Fig. 2. Mean percentage change from initial: in recalcification time, peak expiratory flow rate (PEFR) and initial activation rate of Factor XII (Factor XII activity) in patients with dual asthmatic reaction. At 6 h the data represent the remaining five patients who had not been treated for a late reaction. Bars indicate s.e.m.

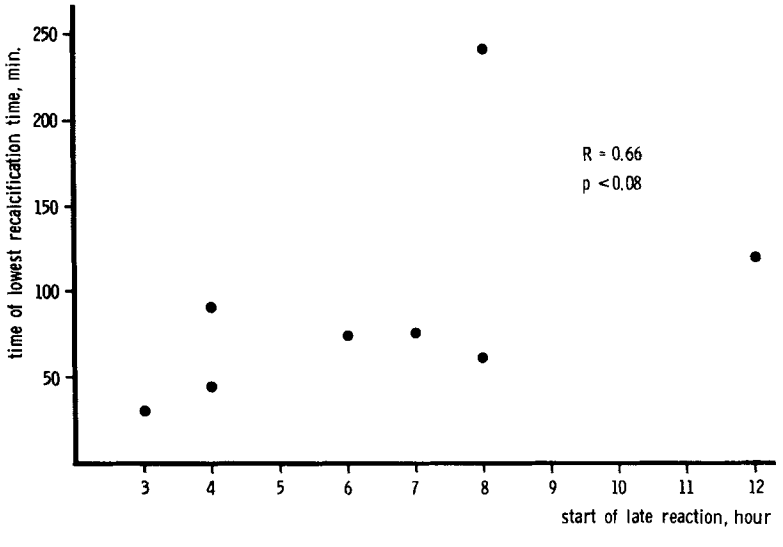

Fig. 3. Correlation between the time of observed maximum fall in plasma recalcification time and the start of a late asthmatic reaction.

ma was found to be activated and to a similar degree in the two groups. This indicates that activation of blood coagulation was primarily dependent on the immediate allergic reaction.

This finding is in agreement with the observation of Halonen & Pinckard (3) and Pinckard et al. (7) who studied reagin-mediated reactions in sensitized rabbits after intravenous challenge, and found an initial shortening time followed by an increase, in part due to consumption of Factor XII. This animal model involved, however, more profound and anaphylactic reactions, but the studies show that IgE-mediated reactions result in activation of blood coagulation and that activated coagulation factors may be consumed.

Only patients who developed a late reaction were found to have a measureable increased initial activation rate of Factor XII. This indicates the appearance of substances with influence on Factor XII or Factor XII-dependent factors (tentatively prekallikrein/kallikrein and high molecular weight kininogen), and may express that a more profound IgE-mediated reaction may have occurred in these patients. An expression of the relationship between the activation of blood coagulation and the clinical reaction was the positive correlation found between the time of maximum shortening in recalcification time and the onset of the late reaction.

These observations indicate a relationship between the type of asthmatic reaction and factors in blood coagulation. The blood coagulation system is closely interrelated with the pathways of kinin generation, fibrinolysis, and the complement system (9), and the common factor between the systems is Factor XII and/or the Factor XII-related factors. Both kinins and activated complement factors can induce bronchospasm and are involved in the inflammatory process. The late asthmatic reaction may therefore in part result from a facilitated activation of Factor XII-dependent pathways.

The initiation phase in Factor XII activation is complex and the conditions for optimal activation rate and maximum Factor XII activity are dependent on high molecular weight kininogen, prekallikrein and other factors (4, 5). An increased activation rate of Factor XII in allergen-induced asthma may be facilitated by eosinophil cationic protein (ECP), a highly basic protein derived from eosinophil granulocytes' granule protein (6). *In vitro* studies have shown that very small amounts of ECP, shorten plasma coagulation time by activating Factor XII or Factor XII-dependent pathways (8). During allergen-induced asthma serum ECP may have a peak-like increase in connection with the immediate reaction (2). ECP could therefore contribute to the facilitated activation of Factor XII in allergen-induced asthma measured as the initial activation rate of Factor XII. Kallikrein of anaphylaxis is an enzyme localised in granules of basophils and is released from human lung tissue and basophils by IgE-dependent reactions (11). Kallikrein is involved in Factor XII activation and secretion of kallikrein from mast cells or basophils after allergen challenge may also contribute to an increased Factor XII activation-rate.

The present *in vivo* study in humans with bronchial asthma shows that allergen challenge activates blood coagulation. In patients with a late asthmatic reaction this included an influence on Factor XII activation rate. As the total amount of measureable Factor XII was not altered this must have been caused by an increase in substances with ability to facilitate Factor XII. Blood coagulation is one of the body's inflammatory cascade systems and activation of blood coagulation can be regarded as a defence mechanism, but may at the same time lead to the generation of substances which aggravate the inflammation, e.g. in the bronchial mucosa.

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