

Failure to demonstrate complement activation during bronchial challenge test

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ABSTRACT: In order to demonstrate a possible complement activation during early bronchospastic reaction in asthma, we have measured plasmatic C3d (a split product of C3) and the C3d/C3 index, both of which are sensitive indices of complement activation. Twenty-nine allergenic bronchial challenge tests were accomplished, with an absence of response in six cases, an early reaction in sixteen cases and a dual reaction in seven cases. Changes in plasmatic C3d or C3d/C3 five min after an early reaction, or five min after the last dose of allergen (in the six cases without bronchial response) were insignificant. However, complement activation in the lungs during asthmatic reaction cannot be completely excluded without studies using the bronchoalveolar technique. *Eur Respir J*, 1988, 1, 168-170.

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Several studies have tried to demonstrate *in vivo* the participation of the complement in the early bronchospastic reaction. The results are controversial [1, 7].

Our purpose is to investigate a possible complement activation in early bronchospastic reaction during bronchial challenge by measuring plasmatic C3d and C3d/C3, which are very sensitive indices of complement activation. The C3d produced during the C3 activation is a low molecular weight molecule with a long half-life [2], so with a good diffusion from tissular to intravascular compartment. The C3d and the C3d/C3 were already used successfully to demonstrate a complement activation in many different clinical situations [4, 6]. Furthermore, in a previous study, we could demonstrate that the C3d/C3 ratio in plasma was increased, at the basal state, in asthmatic subjects [8].

Material and methods

Population

Twenty-three asthmatics (ten men and thirteen women) were studied (table I). All, except three, were submitted to a determination of the specific IgE level (RAST, Pharmacia). Total IgE level was measured (PRIST^R, Phadebas; normal values <200 U/ml) in eighteen patients. Immediate skin reactions (Bencard products) were performed in sixteen patients, using the prick test method and by measuring the skin reaction after 15 min.

Bronchial challenge tests

Twenty-nine tests have been carried out. A forced expiratory volume in one second (FEV₁) of at least

1.5 l and airway resistances (Raw) of less than 3 cmH₂O·l⁻¹·s were required. Allergens were aerosolized through a mistogen nebulizer (model EN 143), delivering a continuous aerosol (with an output of 0.35 ml/min), dispersed during 2 min at tidal volume. The allergen concentration was increased 10 times every 30 min, starting with a dilution of 1/100 000. The functional parameters were measured every 5 min. We considered the test as positive in the case of a FEV₁ drop of 20% or more and/or a doubling of the prechallenge Raw value. The functional parameters were then monitored every hour; a late reaction (4-6 h after allergen inhalation) was defined with the same characteristics as defined for the early reaction. The allergen products used were: DPT, house dust, pollen B2 (Bencard Ltd.), based on the results of RAST, skin tests and anamnestic factors. Six subjects were submitted to bronchial challenge test for both DPT and house dust.

Complement determination

Peripheral blood plasma was collected on EDTA (and stored at -20 °C until assayed) immediately before the bronchial challenge test and five min after the dose of allergen inducing a significant reaction, or five min after the last dose in unreactive patients.

The measurement of CH50, C3, C4 and C3d has been described previously [5, 8]. Briefly, C3d was measured by radial immunodiffusion in 2% agar gel containing human anti-C3d antibody (Red Cross Blood Bank, Amsterdam) after plasma precipitation in borate buffer with 22% polyethylene glycol (MW: 6000; 11% final dilution) and centrifugation (1500 rpm, 30 min at 4°C) according to the method of PERRIN *et al.* [9]. Fractions C3 and C4 were measured

Table I. - Clinical and allergological characteristics of the subjects.

	No reaction	Early reaction	Dual reaction
n	6	16	7
Mean age yr	31.8 (range 17-47)	31.6 (range 17-53)	31.0 (range 22-47)
Males/females	3/3	8/8	3/4
Corticosteroids +	0	1	0
RAST +	1/4	15/16	7/7
IgE 200 IU/ml	3/4	14/15	6/7

* on testing day

by standard nephelometry on a Technicon auto-analyser II, using goat human anti-C3 and C4 antibodies (Atlantic Antibodies). The CH50 was measured using the automated method of VARGUE and TAGNON [10] with Technicon auto-analyser I. The normal values are 69–195 mg/dl for C3, 13–41 mg/dl for C4, 245–600 U/ml for CH50 in our laboratory. The upper limits of normal values are 7.3% for C3d and 5% for the C3d/C3 ratio [8].

Statistical analysis

Paired values were compared with the Wilcoxon test and the medians were compared with the Mann-Whitney test. A $p < 0.05$ was considered as significant.

Results

The subjects characteristics are illustrated in table I. As expected, in patients with a positive bronchial reaction, an increased level of total and specific IgE was often found.

On the twenty-nine bronchial challenge tests, six negative and twenty-three positive (seven dual and sixteen early) reactions were observed. Among the six patients sensitive to house dust, five presented also an immediate associated sensitivity to DPT. The subjects with a dual response presented a more severe immediate reaction ($\Delta FEV_1 = -40\%$) than subjects with isolated immediate reaction ($\Delta FEV_1 = -20\%$) ($p < 0.05$).

The CH50 and C4 did not change significantly during the challenge test. No patient had modified plasmatic C3d or C3d/C3 after the early bronchial reaction or the last allergen dose. However, the basal value of C3d/C3 (before challenge tests) was significantly increased in eight cases.

Discussion

No change in plasmatic C3d/C3 suggests a lack of complement activation during a bronchospastic reaction (caused by antigen). Our results agree with others showing no change in blood levels of CH50,

C3, C4 during an allergenic asthmatic reaction [1] and with the only study that has measured the C3d during challenge tests in nine asthmatics [7].

However, the unchanged C3d during an asthmatic attack does not necessarily exclude a local bronchial complement activation, because the rate of the C3d local production may be too low, or the C3d may be locally consumed. This last point is supported by the existence of several mechanisms of regulation and kinetic properties of the complement system which limit its activation to the immediate micro-environment. Indeed, at basal state, an increase of the C3d/C3, in asthmatics, was reported [8] and could be due to the production of proteins of the acute inflammatory phase, such as C-reactive protein, inducing the complement activation, or to a chronic bronchial complement activation with diffusion of split products in the plasma. This was confirmed in the present study, showing a frequent increase of the plasmatic C3d/C3 at basal state in asthmatics. Complement depositions were reported in the bronchial basal membrane of patients who died of asthma [3]. In bronchoalveolar lavage (BAL) fluid of normal subjects, the release of neutrophil chemotactic factor (with neutrophilic infiltration) was correlated with the bronchoconstriction induced by an extract of cotton bract [5]. In this study, the BAL C5 des arg was unrelated to the bronchoconstriction, suggesting the presence of bronchial complement inactivator in subjects who did not react.

In conclusion, using a sensitive index of complement activation in plasma, we could not document its activation during an asthmatic attack. However, the bronchial complement activation could be a local process that could be better studied by a more local approach such as using bronchoalveolar lavage.

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RÉSUMÉ: Afin de démontrer une activation éventuelle du complément au cours de la réaction bronchospastique précoce dans

l'asthme, nous avons mesuré le taux plasmatique de C3d (un produit de dégradation du C3) et le rapport C3d/C3, considérés comme des indices sensibles d'activation du complément. Vingt neuf tests de provocation bronchique aux allergènes ont été pratiqués avec une absence de réponse dans six cas, une réaction précoce dans seize cas, une réaction mixte précoce et tardive dans sept cas. Cinq minutes après la réaction précoce ou après la dernière dose d'allergène (chez les 6 malades sans réaction) il n'y a pas de changement du taux plasmatique de C3d, ni du rapport C3d/C3. Cependant une activation du complément au niveau pulmonaire pendant les réactions asthmatiques ne peut être formellement exclue sans étude du liquide de lavage bronchoalvéolaire.