

Complement activation on the nasal mucosal surface – a feature of the immediate allergic reaction in the nose

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Complement is a system of functionally linked serum proteins that interact to exert biologic effects in inflammatory and immunologic processes. As part of a larger study with a potential topical antiallergic drug, we measured C3a des Arg and C5a des Arg in 13 patients with seasonal allergic rhinitis and in five nonatopic controls after placebo treatment. After 1 week of placebo treatment, a nasal allergen challenge with increasing doses of pollens was performed in both allergic subjects and controls. A symptom score method was used, and in returned nasal lavage fluid, the activity of C3a des Arg and C5a des Arg was measured. We found that allergen challenge in the allergic subjects induced nasal symptoms concomitantly with increased levels of C3a des Arg and C5a des Arg ($P < 0.05$). No increases either in symptoms or in the very low base-line levels of C3a des Arg and C5a des Arg were observed in the nonallergic controls. We conclude that the activation of the complement cascade is one part of the vasculature exudative response during the immediate allergic reaction in the upper airways. Because of their biologic potency, these proteins may be an essential part of the exudative response which perpetuates the ongoing inflammatory reaction.

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Provocative agents such as allergens on the airway mucosal surface in sensitized subjects activate the mucosal defense systems including the exudation of plasma from the microvessels to the mucosal surface (6). Recent data from guinea pig and human nasal airways suggest that the exudation process is a non-injurious, fully reversible process of an almost unfiltered bulk flow of different-sized proteins (12). The plasma exudate may thereby provide a wide range of inflammatory enzymes and peptide mediators, immunoglobulins, and coagulation systems, which could operate on the mucosal surface by neutralizing the inflammatory stimuli.

Recently, it was demonstrated that allergen challenge of the nose induces a prompt exudative response of different-sized plasma proteins (2). The natural course of allergic rhinitis is further characterized by different-sized plasma proteins on the nasal mucosal surface, and the exudation of proteins is reduced by topical glucocorticoid treatment (14).

The complement system consists of a complex formation of proteins, which may play an important role in the local and systemic immunologic and inflammatory reactions. The central component of the complement system is a protein called C3, and the proteolytic cleavages of this protein are the biologically active forms. Further steps in the complement cascade include the cleavage of the C5 protein and the binding of more complement proteins to the activating surface. The final step leads to the formation of the membrane attack complex which causes cell lysis (1). Different functions of the complement system are mediated by different proteins during complement activation. The recruitment and activation of leukocytes are mediated by the cleavage products of C3a and C5a.

To further characterize the role of the exudation process, we measured C3a and C5a in nasal lavage fluid after allergen challenge in both allergic subjects and nonallergic controls.

Material and methods

Subjects

Thirteen men with ages ranging from 20 to 47 years (mean age 29) participated in the study. They all had a history of allergic rhinitis and a positive skin prick test to birch or timothy pollen extract. They had all previously shown a positive nasal response to an allergen challenge with birch or timothy pollen extract. Five nonatopic controls, with ages ranging from 24 to 40 years, underwent the same allergen challenge procedure. They had no history of allergic disease and showed a negative skin prick test for birch and timothy pollen extract.

Study design

The study was performed as part of a double-blind, placebo-controlled, crossover study outside the pollen season. The placebo treatment was applied four times daily for 1 week in each nostril before the challenge procedure. On the challenge day, the doses were also given 1 h before and immediately before the first diluent challenge, and immediately before each allergen challenge. The placebo spray contained distilled water (98.7%), sodium chloride (0.8%), benzalkonchloride (0.01%), and phenylethanol (0.4%).

Nasal challenge procedure

An allergen challenge model was used according to Naclerio et al. (11). This protocol includes initial lavages to reduce the cell-free mediators on the nasal mucosal surface. Then 100 µg oxymethazoline (Nezeril®, Astra Draco AB, Lund, Sweden) was sprayed into each nasal cavity to maintain nasal patency during the challenge procedure. Two subsequent challenges with diluent were performed (the same as for the pollen extract) to establish a base line. Then a serial challenge with three increasing doses of pollen allergen was undertaken. The doses used were 100, 1000, and 10 000 BU (biologic units) of birch or timothy pollen extract (Pharmalgen®, Pharmacia, Uppsala, Sweden). Lavages with 10 ml of saline solution were performed 10 min after all challenges. The returned lavage fluid was kept cold (4°C) until the end of the experiment; then it was immediately cold-centrifuged at 210 g for 10 min. The supernatants of the initial lavage, second diluent lavage, and the following lavages after allergen challenge were separated and stored at -80°C until they were analyzed.

Symptom registration

Immediately before each lavage, the symptoms of blockage, secretion, and itching were rated on a five-point scale, where 0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe, and 4 = intolerable symptoms. In addition, the number of sneezes was counted. A composite nasal symptom score was calculated; it included the sum of the blockage, secretion, and itching scores and a sneezing score (0 sneezes = 0, 1–4 sneezes = 1, 5–9 sneezes = 2, ≥ 10 sneezes = 3).

Measurement of C3a and C5a

C3a des Arg and C5a des Arg were measured according to the method described by Chenoweth & Hugli (5). This method utilizes a competition between the complement fragments and a certain fixed amount of complement labeled with ¹²⁵I against a specific antibody. In brief, it includes the following steps: centrifugation at 210 g for 15 min to precipitate the proteins, addition of a fixed amount of C3a or C5a labeled with ¹²⁵I, and incubation at room temperature with an antiserum (anti-C3a or anti-C5a) for 30 min or 3 h, respectively. Then followed a second incubation for 30 min with a second anti-antiserum and centrifugation at 210 g for 10 min. Finally, the unbound fractions were eliminated, and the united complexes were counted for 2 min in a gamma scintillator. The radioactivity measured in the tubes is inversely related to the concentration of the complements.

Statistics

The statistical evaluation was performed on a microcomputer (Macintosh, Apple, Cupertino, CA, USA) with the aid of a statistical software package (Statview 512+, by Brainpower, Inc, Calabassas, CA, USA). A Wilcoxon signed-rank test was used for comparisons between the different challenges with the diluent challenge as base line. Values of $P < 0.05$ were considered to be significant. All values were given as mean ± SEM.

Results

We found as in other challenge studies that allergen challenge caused an immediate nasal response with an increase of nasal symptoms in the allergic patients, but not in the nonallergic controls. The total nasal symptoms for the allergic patients are shown in Fig. 1 (upper graph).

In the allergic subjects, one patient had extremely high yet not detectable levels of C3a in all the measured lavages. The other 12 patients responded with

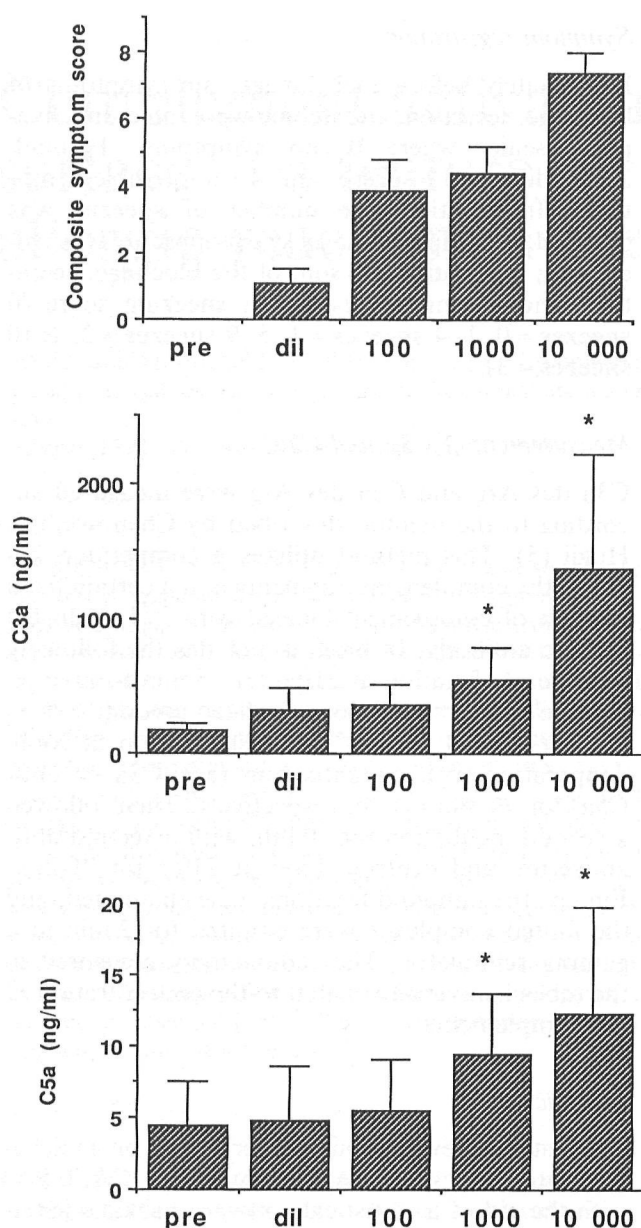


Fig. 1. Upper graph: total nasal symptoms scores in 13 allergic patients. Middle graph: levels of C3a des Arg in nasal lavage fluid ($n = 13$). Lower graph: levels of C5a des Arg ($n = 12$). Values are given as mean $\pm$ SEM. * indicates significant ($P < 0.05$) increase, as compared with levels after diluent challenge. pre = the initial lavage; dil = lavage after diluent challenge; 100, 1000, 10000 BU, lavage after increasing doses of allergen.

an increase in C3a from the initial lavage: 177.2 ± 54.3 ng/ml to 1378.3 ± 840.4 ng/ml (mean $\pm$ SEM) after the strongest dose of allergen. This increase was significant for the two strongest doses of allergen (both $P < 0.05$), as compared with the diluent challenge. Similarly, the levels of C5a increased from 4.4 ± 3.1 ng/ml in the initial lavage to 12.3 ± 7.5 ng/ml after the strongest allergen dose. This increase was also significant for the two strongest doses of allergen, as compared with the diluent

challenge (both $P < 0.05$). This is shown in Fig. 1 (middle and lower graphs).

Allergen challenge in the nonallergic controls did not cause any nasal symptoms, and there were almost no measurable levels of C5a, except in a very few lavages. C3a also remained very low in the lavages of the nonallergic patients throughout the challenge protocol, with no significant differences. C5a changed from an initial 47.72 ± 11.6 to 32.2 ± 1.83 (ng/ml) after the strongest allergen dose.

Discussion

In the present study we have, to the best of our knowledge, shown for the first time that it is possible to measure the signs of complement activation on the human nasal mucosa in direct response to an allergen challenge.

We used a well-established method of allergen provocation and collection of nasal surface liquids. This protocol is suitable for allergen challenge situations, and the final challenge is a fairly strong dose of allergen. A nasal decongestant is therefore needed to collect the returned lavage fluid. This dose of decongestant has previously been shown not to interfere with the measurements of released mediators (11). Although a placebo treatment for 1 week was initiated and also given before the challenges, it did not induce any increased levels of C3a and C5a by itself. This was shown by the levels of C3a and C5a, which were similar in the diluent and the initial lavages. Furthermore, the nonallergic subjects had very low levels of C3a and C5a in all the lavages measured. It therefore seems likely that the increase in the levels of complement was caused by the allergen itself and was not due to the placebo treatment. It is, however, extremely difficult to measure the biologic activity of C3a and C5a because of a very rapid enzymatic inactivation (4). We therefore measured the physiologically inactive products, C3a des Arg and C5a des Arg.

The activation of the complement system in serum plays an important role in host defense and in the pathophysiology of certain types of tissue injury. Complement may be activated by two different pathways: either the classical pathway via IgG- or IgM-containing immune complexes or through an alternative pathway (7). The alternative pathway can be activated by aggregated IgA or IgG, or by certain lipopolysaccharide Gram-negative organisms and the cell membrane of transformed cells (10). The activation of complement pathways leads to the generation of two important proteins, C3a and C5a, called anaphylatoxins (9). By measuring only C3a and C5a in the present study, however, we could not determine which one of the pathways had been activated. Recently, Inoue et al. demonstrated comple-

ment activation through the classical pathway in the sputa of asthmatic children (8).

When anaphylatoxins bind to mast cells or basophils, these cells may release vasoactive mediators, such as histamine, a potent exudative agent in the upper airways, which could then cause further plasma exudation. C5a has also been considered to have other direct effects on the vascular endothelium, which in turn may further increase the vascular permeability (1). The activation of the complement system during the allergic/inflammatory reaction may thus be one feature that may perpetuate the vascular exudative response. From a clinical point of view, it would be interesting to study drugs which may interfere with the complement activation system. It has previously been demonstrated that glucocorticosteroids inhibit the microvascular exudation of plasma proteins and the formation of peptides such as bradykinins (14). Thus, it could be that one of the beneficial clinical effects of topical glucocorticosteroids in the treatment of allergic airway diseases is an effect on complement activation.

Previous studies have shown that allergen inhalation induces complement activation in serum (13), and C3 anaphylatoxins have been measured in the tears of patients with conjunctivitis (3). This is, however, to our knowledge, the first time C3a des Arg and C5a des Arg have been measured on the mucosal surface in direct response to allergen provocation. It indicates that complement activation is one feature of the allergic/inflammatory exudative response in human upper airways.

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