

Short communication

The severity of clinical symptoms in ragweed-allergic patients is related to the extent of ragweed-induced complement activation in their sera

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We have previously reported a correlation between the extent of ragweed allergen (RWA)-induced *in vitro* serum complement activation and the symptom scores registered daily during the ragweed (RW)-blooming season in RW-allergic patients. The present study was performed in 22 15–17-year-old RW-allergic adolescents. Serum samples were incubated with 100 µg/ml RWA, and the generation of different complement activation products was measured by ELISA or RIA. Symptom scores were registered for 4 weeks during the RW-blooming season. The patients were divided according to the extent (low or high) of the generation of complement activation products, and symptom scores registered in the two groups were compared by two-way ANOVA. Significantly higher symptom scores were obtained in the high than in the low complement activation group (*P* values: 0.049 for C1rC1sC1inh, 0.022 for C3bBbP, 0.015 for C5b-9, 0.0001 for C3a, and 0.0008 for C5a). Similar results were obtained at the measurement performed in the sera obtained from the same patients half a year before the season (*P* values: 0.022 for C3bBbP, and 0.005 for C5b-9). These findings indicate that complement activation induced by the allergen may enhance the clinical symptoms of RW allergy.

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In a previous prospective study of 26 ragweed (RW)-allergic patients (1), we found a significant positive correlation between ragweed allergen (RWA)-induced alternative complement pathway activation and the severity of the symptoms of allergic rhinoconjunctivitis that developed in a 4-week period after blood sampling during the RW-blooming season. On the basis of this observation, the role of complement activation in the development of symptoms of RW allergy was assumed. In the present study, we reinvestigated in another group of RW-allergic patients a possible correlation between the extent of RWA-induced complement activation measured in the sera of RW-allergic patients (taken at the beginning of the RW-blooming season) and the frequency and severity of clinical symptoms that developed in the same

patients during a 4-week period of the season. In order to extend the study, the relationship between the reactivity of the complement to RWA and the symptoms in the season was also compared in the sera obtained from the same patients outside the blooming season. In addition, the relationship between the extent of RWA-induced complement activation and the level of RW-specific IgE in the sera of the RW-allergic subjects was also investigated.

Material and methods

Patients

At the outpatient department for respiratory allergic diseases of the 18th district of Budapest,

Hungary, random allergy screening with a skin prick test with four standard allergens (mite, mixed grass, common RW, and mugwort) was performed on 932 14–18-year-old high-school students. The tests were done outside the pollen seasons. Of the adolescents tested, 168/932 (18%) were found to be allergic to RW by prick test. Of them, 60 (36%) were allergic to RW only.

The present study was performed in 22 of the 168 RW-allergic (10 RW-monoallergic) adolescents (seven boys, 15 girls) who visited the outpatient department during the RW-blooming season. They gave informed consent to the investigation.

Allergen extract

RW extract purchased from ALK (Copenhagen, Denmark) was used. The extract contained only nondialyzable material with a molecular weight over 10 kDa.

Serum samples tested

Serum samples of the patients were separated within 5 h after blood sampling and stored at -70°C until used. Serum samples taken from 19 patients half a year before the blooming season and stored at -70°C were also available.

Measurement of complement activation

Serum samples (100 μl) were quickly thawed and incubated at 37°C for 60 min with 10 μl of RWA (final concentration: 100 $\mu\text{g}/\text{ml}$) or with 10 μl of VBS (control). The formation of five different complement activation fragments was measured as follows. Formation of the classical pathway (C1inhC1rC1s) complexes was measured by the method of Nielsson & Back (2). Generation of the alternative C3-convertase (C3bBbP) complexes was measured by the method of Zilow et al. (3). Formation of terminal complement activation complexes (SC5b-9 complexes) was measured by the method of Deppish et al. (4). Generation of C3a and C5a was determined by commercially available human complement C3a-des-arg and C5a-des-arg RIA kits (Amersham, UK), according to the instructions of the manufacturer. Levels of C3bBbP, C1rC1sC1inh, and SC5b-9 protein complexes, as well as C3a and C5a generated in the serum samples incubated with RWA, were given in percentage of the buffer control.

Measurement of RW-specific IgE

RW-specific IgE antibodies were measured by RAST (Pharmacia, Uppsala, Sweden), the level of antibodies being expressed in PRU units.

Evaluation of the symptoms of the patients

Each patient kept a diary for 4 weeks during the RW-blooming season. The following subjective scores were applied: 0=no symptoms, 1=mild symptoms, 2=symptom of medium severity, 3=severe symptoms, disturbing daily life. The following symptoms were scored by the patients:

- 1) *symptoms of rhinitis*: nose obstruction, nose-running, nose-itching, sneezing attacks, impaired sense of smell
- 2) *symptoms of conjunctivitis*: tear-shedding, flush of conjunctiva, aching eyes, swollen eyes
- 3) *symptoms of pharyngitis*: throat-itching, hoarseness, coughing
- 4) *symptoms of laryngitis and asthma*: dyspnea, suffocation, thoracic wheezing, viscous sputum
- 5) *ear symptoms*: ear-itching, impaired hearing.

Scores of symptom groups 1–5 were separately summed up in the case of each patient, and these values were used at the final evaluation.

Statistical evaluation of the results: study of a possible association between the extent of the formation of complement activation products and the symptom scores

The same evaluation strategy was applied to each complement activation product and the IgE antibodies.

- 1) The median value of the extent of generation of the parameter was determined.
- 2) The patients were divided into two groups according to the median value or a value close to median: those with a low (below this value) or high (above this value) extent of formation of the complement activation product or level of specific IgE.
- 3) The sum of symptom groups 1–5 in the two groups was jointly compared by two-way analysis of variance (ANOVA) (GraphPad PRISM, Version 2.0). In some cases, Spearman correlation coefficients were also calculated.

Results

Development of RW-allergy symptoms during the RW-blooming season in patients with high and low extent of generation of different complement activation products in serum samples taken at the beginning of the same season

In the last week of August 1995, serum samples were taken from 22 adolescent patients with proven RW allergy according to skin test and the IgE Quick test. The sera of the patients were

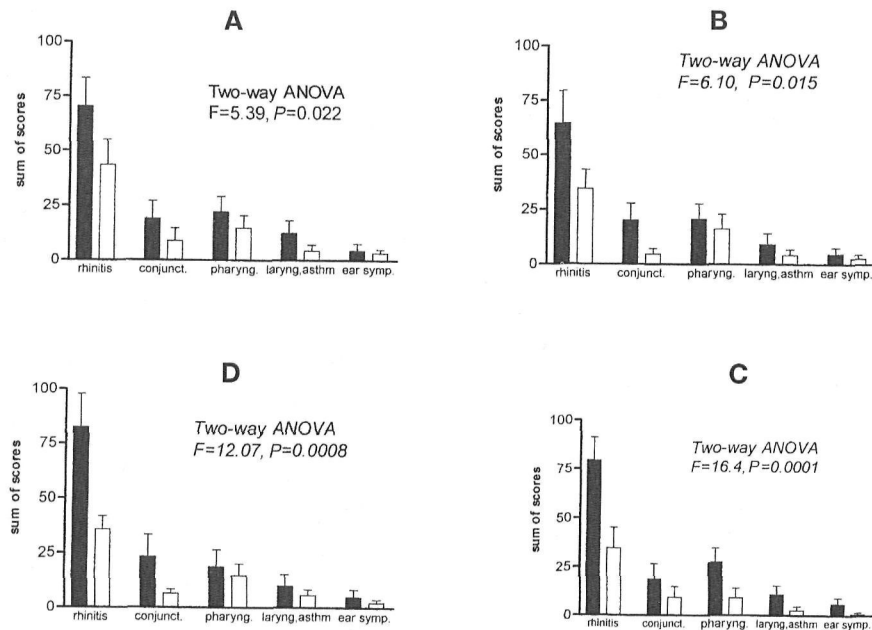


Fig. 1. Sums (mean $\pm$ SEM) of different symptoms of RW allergy that developed in 4-week period of RW-blooming season (1–29 September 1995) in patients with high ($>$ median of whole group, open columns) or low ($\leq$ median of whole group, closed columns) extent of RWA-induced generation of C3bBbP (A), C5b-9 (B), C3a (C), and C5a (D) complement activation products.

divided into aliquots and stored at -70°C . Samples thawed immediately before the measurement were incubated with $100\text{ }\mu\text{g/ml}$ of RWA, and the generation of different complement activation products was measured. The results were evaluated as described in Material and methods (Fig. 1).

Significantly more symptoms developed in patients with a high generation of C3bBbP or C5b-9 (protein–protein complexes formed at the activation of the alternative pathway [Fig. 1A] and common terminal pathway [Fig. 1B], respectively) than in patients with low generation of the same complement activation products. Similar, albeit less significant ($F=3.98, P=0.049$), differences were registered in the case of C1rC1sC1inh generation (protein–protein complexes formed at the activation of the classical pathway). The differences became even more significant when the generation of the low-molecular-weight complement activation products, C3a (Fig. 1C) and C5a (Fig. 1D) were measured. Similar but less significant associations between the complement activation and the symptom severity were obtained when the Spearman correlation coefficients between the sums of scores and the generation of complement activation products were calculated. For example, for C5b-9 and C3a generation, correlation coefficients of 0.518 ($P=0.033$) and 0.465 ($P=0.039$), respectively, were obtained.

Development of RW-allergy symptoms during the RW-blooming season in patients with high and low generation of complement activation products in serum samples taken half a year before the beginning of the same season

From 19 of the 22 patients, serum samples taken half a year (February–March 1995) before the RW-blooming season and stored at -70°C were also available. These serum samples were incubated again with $100\text{ }\mu\text{g/ml}$ of RWA, and the formation of two high-molecular-weight complement activation products was determined. As in the results obtained in sera taken at the beginning of the RW-blooming season, significantly more symptoms developed in patients with a high extent of RWA-induced C3bBbP ($F=5.59, P=0.022$) or C5b-9 ($F=8.30, P=0.005$) generation than patients with a low extent of C3bBbP or C5b-9 generation in their sera (data not shown).

Development of RW-allergy symptoms during the RW-blooming season in patients with high and low specific IgE levels in serum samples taken at the beginning of the same season

In contrast to the results for complement activation, no significant differences in the development of different symptoms were observed between patients with high (above the median of the whole group) and low (equal to or less than

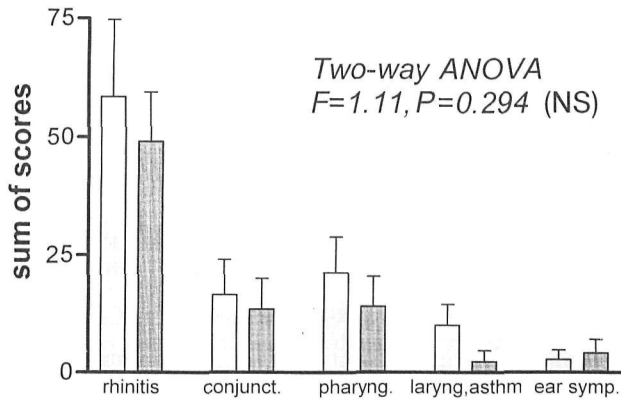


Fig. 2. Sums (mean±SEM) of different symptoms of RW allergy that developed in 4-week period of RW-blooming season (1–29 September 1995) in patients with high (> median of whole group, open columns) or low (≤ median of whole group, closed columns) RW-specific IgE level in sera taken at beginning of same season.

the median of the whole group) levels of RW-specific IgE antibodies as measured by RAST (Fig. 2).

Discussion

Our findings indicate a positive correlation between the extent of RWA-induced complement activation measured in the sera obtained from RW-allergic patients at the beginning of the RW-blooming season and the frequency and/or severity of the allergic symptoms developed in the same patients during that season. Considering the relatively low number of patients tested and the low frequency of some symptoms (pharyngitis, asthma, and ear symptoms), for the evaluation of the results, we used a statistical method (two-way ANOVA) suitable for joint evaluation of all symptoms together. By this method, significantly higher symptom scores were measured in patients with a high RWA-induced generation of all the five complement activation products tested than in patients where the same complement activation products were generated in a low quantity. These findings completely agree with our previous observations (1) obtained with 26 adult RW-allergic patients. In these subjects, we also found a significant positive correlation between the extent of RWA-induced C3bBbP formation and the symptoms of rhinitis, conjunctivitis, and laryngitis. Recently, we have found identical results in a third series of 23 RW-allergic patients (two-way ANOVA for C3bBbP generation, $P=0.0003$; for C5b-9, $P<0.0001$) (Varga et al., unpublished results).

Since, for two parameters, the same correlation could be observed in sera of the same patients obtained half a year before the RW-blooming

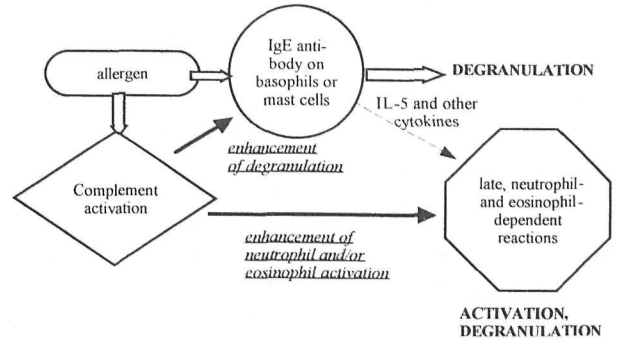


Fig. 3. Possible ways of amplification of IgE-mediated pathologic processes by complement activation (hypothesis). See text for details.

season, it seems that the low or high reactivity of complement to RWA is a long-lasting trait of allergic patients. In contrast, although IgE antibodies were found in the sera of all patients, no correlation between the level of these antibodies and the clinical symptoms or the extent of the RWA-induced complement activation was observed.

Other data from the literature also support the role of complement in the development of allergic symptoms. Allergens were found to be very effective complement activators (5, 6). Activation of the complement system was found to occur upon allergen exposure *in vivo* as well. Pryjma et al. (7) observed signs of complement activation after a house-dust-allergen bronchial provocation test. Similar results were obtained by us (8) with the blood of textile workers after textile dust inhalation. Ballou et al. (9) detected C3a anaphylatoxin in the tears of patients with allergic conjunctivitis. Recently, Andersson et al. (10) reported generation of two complement activation products (C3a-des-arg and C5a-des-arg) after nasal allergen challenge concomitantly with the development of nasal symptoms in patients with seasonal allergic rhinitis. In a guinea pig model, Fraser et al. (11), using trimellitic anhydride (TMA) (a low-molecular-weight industrial compound that can cause asthma-like symptoms in man), directly established the role of complement in the allergic response of the lung. Depletion of complement by cobra venom factor in animals before exposure to TMA prevented inflammatory cell infiltration in TMA-induced asthma.

The very potent inflammatory mediators formed from the complement proteins can amplify several processes triggered by the interaction of allergen with IgE antibodies attached to the IgE receptors of different cells. In addition, complement activation products were found to exert their effect in concert with some lymphokines. For example, presentation of human basophilic granulocytes with

IL-3 significantly increased the sensitivity of the cells to C3a (12). Furthermore, TNF- α was found to prime neutrophils readily to the response to C5a (13). According to Carlson et al. (14), IL-5 selectively primes human eosinophils and neutrophils for C3b-induced degranulation.

Considering these observations together, we think it probable that complement activation induced by the allergen *in vitro*, and probably *in vivo* (systemically or on the mucosal surface) as well, contributes to the development of allergic rhinoconjunctivitis and respiratory symptoms. This conclusion is summarized in Fig. 3 as a *working hypothesis*. According to this hypothesis, the allergen can activate the complement system both in RW-allergic and nonallergic subjects; that is, in both the absence and presence of IgE antibodies in the serum and/or on the IgE receptors of basophils, mast cells, and other cell types. The intensity of this activation, the individual reactivity of complement to RWA, is regulated by some still unidentified factors, and IgM antibodies may also be involved (15). Due to this individual reactivity, RWA can generate low or high levels of complement activation products in different subjects. In persons not allergic to RW, complement activation alone does not result in inflammatory symptoms. In contrast, on pollen-exposed mucosa of RW-allergic subjects, complement activation products may enhance the basic, IgE-dependent early and/or late pathologic mechanisms; e.g., degranulation of mast cells or chemotaxis and activation of eosinophil leukocytes. According to this hypothesis, the severity of the symptoms is related to the extent of complement activation. In RW-allergic subjects with high individual reactivity of complement to RWA, the symptoms will be more frequent and/or severe than in allergic subjects with low complement reactivity. Experiments to study the mechanism of the complement-dependent enhancement of the RWA-induced type I hypersensitivity reaction are in progress in our laboratory. Findings obtained by us recently (Dervaderics et al., submitted for publication) on a significant positive correlation between the reactivity of complement to RWA and the titer of a dilution skin test performed with RWA seem to support this hypothesis.

Complement is often even more activated by other pollen allergens than by RWA. Therefore, it is most important to determine whether the same correlation between the extent of complement activation and clinical symptoms can be demonstrated for other allergens as well. These studies are underway in our laboratories.

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