

Ragweed allergy: Correlation between skin reactivity and in vitro complement activation

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Abstract

Recently we have reported on several observations which indicate that allergen-induced complement activation contributes to the development of the symptoms of ragweed allergy. In the present paper a new finding that supports this assumption is summarized. In 48 ragweed-allergic patients individual skin reactivity to ragweed allergen extract (RWA) was assessed using dilution skin prick testing. Sera of these patients were incubated with 20, 100, and 400 U/ml RWA and generation of two complement activation products, alternative pathway C3-convertase (C3bBbP) and terminal pathway activation complex (C5b-9) was measured by ELISA methods. A strong positive correlation (Spearman correlation coefficient $r = 0.495$, $P = 0.0004$, and $r = 0.454$, $P = 0.0012$, respectively) was found between individual skin reactivity to RWA and C3bBbP generation induced by 20 and 100 A allergological units/ml (U/ml) RWA. This finding further supports the role of complement activation products in the aggravation of the basic IgE-mediated immunopathology of ragweed allergy. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

In recent years several advances have been made in understanding the human immune response to inhaled allergens. Pollen allergy basically is a type I hypersensitivity reaction which is initiated by development of specific IgE antibodies to different allergens. During the season, symptoms are triggered by cross-linking of IgE antibodies attached to IgE-Fc receptors on mucosal mast cells by allergens in the pollen grains. Cross-linking of the receptors leads to degranulation of mast cells

and release of histamine and other stored mediators so inducing acute inflammatory reactions. In recent years, however, it became clear that besides IgE-dependent mast cell and basophil degranulation other immunological factors also contribute to the development of symptoms of pollen allergy. The role of eosinophils, and neutrophil granulocytes is well known in the late phase reaction that follows the early events after allergen exposure [1]. Recently the significance of T cell mediated processes in the late consequences of type I hypersensitivity reactions was elucidated [2,3].

Early work of Berrens and his co-workers [4,5] pointed out the possible role of the allergen-induced complement activation in the development of allergic symptoms. Allergens were found to be very potent complement activators [6]. Recently we published several findings which indicate that complement activation occurs on the allergen-exposed mucosa of the sensitized

Abbreviations: AP, alternative pathway; AU, allergological units; CP, classical pathway; C3bBbP, properdin-stabilised alternative pathway C3 convertase; C5b-9, terminal pathway activation complex; RW, ragweed; RWA, ragweed allergen extract; SRU, skin reactivity unit; VBS, veronal-buffered saline.

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patients, contribute to the development of allergic inflammation and may determine the severity of symptoms. Ragweed allergy was selected as a model of pollen allergy since it is the most common inhalative allergen in the USA and in Hungary [7]. In adults allergic to ragweed the incidence and severity of symptoms registered during the blooming season were markedly higher in patients in which sera ragweed allergen extract [RWA] induced formation of significant amounts of C3bBbP (the activation product of the alternative complement pathway) as compared to those with no significant C3bBbP generation [8]. In adolescents allergic to ragweed, the same strong positive correlation with clinical symptoms was observed in the case of all five measured complement activation products but no such correlation with serum IgE levels was obtained. The same strong correlation between the activation of complement and clinical symptoms was observed when the serum samples tested were taken from the same patients 6 months prior the blooming season [9]. We could reproduce these observations in a third series of 23 RW-allergic patients in sera taken before immunotherapy (Varga et al., in preparation).

Similar results were obtained by Andersson et al. [10] who found a strong correlation between the severity of clinical symptoms and the amount of complement activation products generated when allergic patients underwent nasal provocation with different doses of the appropriate allergen.

In the present work we analyzed the relationship between the results of the usual allergologic tests (IgE estimation, titration skin prick test) and the extent of in vitro ragweed allergen extract (RWA)-induced complement activation in the sera of the same patients.

It seems most probable that complement activation by RWA is triggered through the classical pathway [CP] [8,11]. According to recent studies by Berrens and de la Caudra Lopez [12], complement activation is related to the peptide-bound flavonoids. Since CP-activation under our experimental conditions allows a positive feedback at the C3-amplification loop, we found it advantageous in the present work to measure complement activation with sensitive enzyme immunoassays for the C3bBbP complex and SC5b-9 complex assembled during activation of the alternative and common terminal complement pathways, respectively.

2. Materials and methods

2.1. Patients and samples

Forty eight patients were studied who suffered from late-summer allergy. The diagnosis of ragweed (RW) allergy was based upon the case history, the results of skin prick tests (Soluprick®, Copenhagen, Denmark),

specific IgE estimation (IgE Quick Test®, Dexall Biomedical, Gaithersburg, MD) and air-functional measurements (Medicor, Budapest, Hungary). Skin prick testing and blood sampling for complement and IgE measurements were performed outside the RW blooming season. Serum samples from the patients were stored in aliquots at -70°C .

2.2. Quantification of the skin prick test results

To obtain an estimate of skin reactivity of a patient to RWA, skin prick testing was performed by titration. The aqueous extract of RWA was applied in three different concentrations (10^3 , 10^4 , and 10^5 biological units [BU]/ml). The results were evaluated after 15 min. The diameters of wheals were determined and compared to the histamine reference. The lowest concentration which gave rise to a histamine-equivalent positive reaction was considered as the measure of skin reactivity and was expressed in arbitrary units. Skin reactivity was designated as one arbitrary skin reactivity unit (SRU) if 10^5 BU/ml RWA produced a wheal with a diameter equal to the histamine control in a given patient, while 10^3 and 10^4 BU/ml RWA induced a lower or no reaction. Skin reactivity was designated as 10 or 100 SRU if a histamine-equivalent positive reaction was obtained with 10^4 and 10^3 BU/ml RWA, respectively.

2.3. Measurement of complement activation

Serum samples (100 μl) were quickly thawed and incubated at 37°C for 60 min with 10 μl of RWA (ALK, Copenhagen, Denmark, final concentration: 20, 100 or 400 allergological units/ml (AU/ml)) or with 10 μl of VBS (control). Generation of the alternative C3-convertase (C3bBbP) complexes was measured by the method of Zilow et al. [13]. Briefly, microtiter plates were coated by goat anti-human properdin antibodies (ATAB, Stillwater, MN) and blocked by PBS containing 2% low-fat cow milk. Samples were applied 1:20 in PBS-Tween containing 0.01 M EDTA to coated plates after treatment with RWA or VBS. The amounts of C3bBbP complexes were determined by rabbit anti-C3c (DAKO, St. Louis, MO), using TMB substrate in the final step. Formation of terminal complement activation complexes (SC5b-9) was measured by the method of Deppish et al. [14]. Briefly, plates were coated with a mouse anti-human C9 monoclonal antibody (Lea, Oslo, Norway). Samples were applied 1:30 in PBS-Tween containing 1% BSA and 10 mM EDTA to the microtiter plates. Then rabbit anti-human C5 antibody (DAKO, Denmark) was applied, containing 2% mouse serum to avoid cross-reaction. Then goat anti-rabbit IgG-peroxidase conjugate (Dianova) and TMB (Sigma, St. Louis, MO) substrate were used. Zymosan-treated

Table 1
RWA-induced generation of the alternative complement pathway activation product C3bBbP^a (mean $\pm$ SEM) in the sera of patients with different skin reactivity to RWA

Sera incubated with RWA (U/ml)	Skin reactivity to RWA ^b			P-values (Mann–Whitney test)		
	1 arbitrary skin reactivity unit (SRU) (n = 12)	10 arbitrary skin reactivity units (SRU) (n = 17)	100 arbitrary skin reactivity units (SRU) (n = 19)	1 SRU vs 10 SRU	1 SRU vs 100 SRU	10 SRU vs 100 SRU
20	108.7 $\pm$ 4.7	118.9 $\pm$ 4.7	161.4 $\pm$ 13.7	n.s.	0.0035	0.0113
100	115.1 $\pm$ 4.9	126.6 $\pm$ 4.5	163.9 $\pm$ 12.7	n.s.	0.0075	0.0325
400	154.1 $\pm$ 7.7	169.9 $\pm$ 10.7	186.4 $\pm$ 15.7	n.s.	n.s.	n.s.

^a In % of control (C3bBbP generation in the same sera incubated with buffer).

^b Skin test with serial dilutions of RWA, skin reactivity: 1 arbitrary SRU was defined as histamine equivalent skin reaction with 10⁵ BU/ml RWA only, 10 or 100 SRU, histamine equivalent skin reaction with 10⁴ BU/ml RWA and 10³ BU/ml RWA, respectively.

NHS was applied as standard. Levels of C3bBbP, and SC5b-9 protein complexes in the serum samples incubated with RWA were given as percentage of the buffer control.

2.4. Specific IgE estimation

The amount of RW-specific IgE antibodies was estimated by the IgE Quick Test[®] (Dexall Biomedical, Gaithersburg, MD).

2.5. Statistical evaluation of the results

The differences between the means of patients groups were calculated by the non-parametric Mann–Whitney test, correlation was estimated by the non-parametric Spearman method. All calculations were performed using GraphPad PRISM, version 2.0 software.

3. Results

3.1. Relationship between individual reactivity of complement to ragweed and the extent of skin reactivity to ragweed in ragweed-allergic patients

Sera of 48 ragweed-allergic patients were incubated with three different doses (20, 100, and 400 AU/ml) of RWA and the extent of two complement activation products, C3bBbP (alternative pathway activation) and C5b-9 (terminal pathway activation) were measured. The extent of skin reactivity of the patients was determined by titration skin prick test and expressed in SRUs as described above.

A strong positive correlation was found between individual skin reactivity to RWA and the extent of C3bBbP generation in serum samples of the patients incubated with 20 and 100 AU/ml RWA ($r = 0.495$, $P = 0.0004$, and $r = 0.454$, $P = 0.0012$, respectively). A

similar tendency but with no significant correlation was found with samples incubated with the highest RWA dose. Both 20 and 100 AU/ml RWA generated significantly more C3bBbP in the sera of patients with the highest skin reactivity as compared to those with the lowest or intermediate skin reactivity (Table 1).

In our previous study [8] significant AP activation (C3bBbP formation) was defined as more than 120%. When patients were divided into two groups according to this criterion, significantly higher skin reactivity to RWA was found in patients with a significant C3bBbP formation induced by RWA at two different concentrations as compared to those with no significant C3bBbP generation (Fig. 1).

In contrast to the C3bBbP generation, no significant correlation was found between individual skin reactivity to RWA and the extent of C5b-9 formation in serum samples of the patients incubated in either dose (20, 100, or 400 AU/ml) of RWA (data not shown).

3.2. Correlation between skin reactivity and the specific IgE levels in RW-allergic patients

IgE levels in the sera also correlated with skin reactivity but the significance of the correlation coefficient was only of borderline significance ($0.05 < P < 0.1$). When the IgE level in the sera of patients with high skin reactivity (2.87 ± 1.36) was compared to that of patients with low or intermediate skin reactivity (2.04 ± 1.17) a weak but significant ($P = 0.036$) difference was found.

3.3. Relationship between serum IgE levels and the RWA-induced complement activation

We found a weak positive correlation between IgE levels and the extent of C5b-9 generation in sera incubated with 100 AU/ml RWA ($P = 0.045$) and 400 AU/ml RWA ($P = 0.04$). When sera of patients with

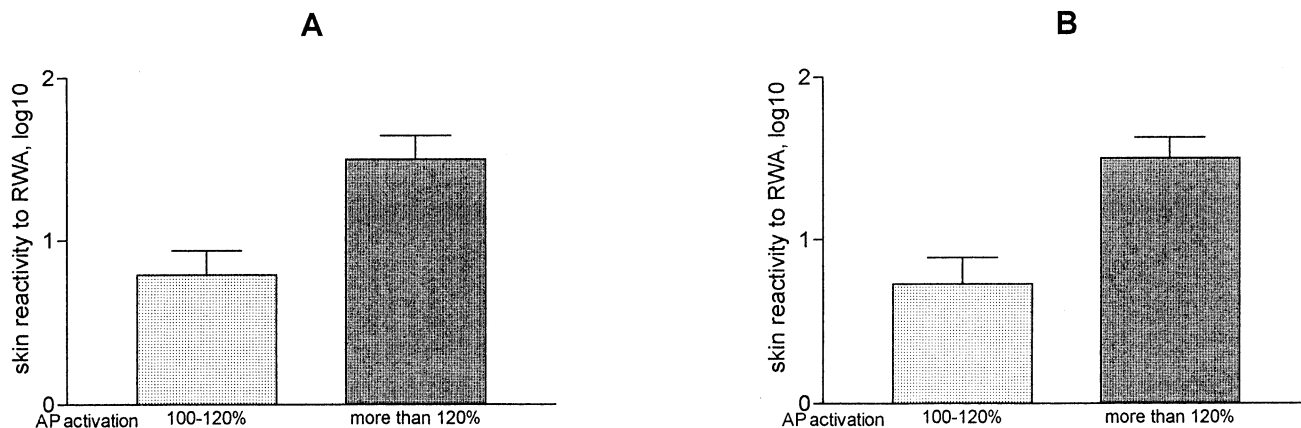


Fig. 1. Skin reactivity to RWA expressed in arbitrary skin reactivity units (SRU) in ragweed allergic patients in which sera RWA at a final concentration of 20 (A) and 100 (B) allergologic units (AU)/ml generated 100–120% or more than 120% C3bBbP as compared to C3bBbP generation in the same sera incubated with buffer. The difference between the medians of groups was calculated by the Mann–Whitney test: for A, $P = 0.0032$; for B, $P = 0.0018$.

IgE-Quick test of 0–2 and 3–4 were compared, a significant difference in the RWA-induced C3bBbP generation was found only in the case of 100 AU/ml RWA but no significant difference was observed between the two groups in the extent of C5b-9 formation at either RWA dose.

4. Discussion

Our present finding of a strong correlation between skin reactivity to RWA and the extent of RWA-induced C3bBbP generation confirms and extends our previous results on the contribution of complement activation in the development of allergen-induced inflammation in RW-allergic patients. Skin reactivity to RWA was determined by a threshold titration skin prick test using three different 10-fold dilutions of the allergen for each patient. Results of several studies indicated that the degree of sensitivity of an allergic patient can be characterized by the skin sensitivity titers. These titers strongly correlate to in vitro and in vivo mediator release, like histamine release from basophil leukocytes [15,16] in vitro, histamine release into skin chambers during allergen incubation in vivo [15,17], or bronchial sensitivity [17]. Norman et al. [15] have demonstrated a good correlation between patients' allergic sensitivities in terms of clinical symptom score and quantitative intradermal skin sensitivity. Threshold dilution prick tests are used to estimate the effect of immunotherapy, and clinically effective immunotherapy results in the decrease of skin sensitivity in patients allergic to peanut [18], *Parietaria judaica* [19], *Olea europaea* pollen [20] or grass pollen [21]. Thus the strong correlation between skin reactivity and complement reactivity to RWA in ragweed allergic patients lends credence to our working hypothesis on the role of

complement activation in the development of ragweed allergic inflammation [9]. According to this hypothesis, the allergen can activate the complement system both in RW-allergic and non-allergic individuals, that is, both in the absence and presence of IgE antibodies in the serum and/or on the IgE-receptors of basophils, mast cells and other cell types. Due to individual reactivity of serum complement to RWA, the allergen can generate low or high levels of complement activation products in different subjects. In persons not allergic to RW, complement activation products alone do not result in inflammatory symptoms. By contrast, on pollen-exposed mucosa of the RW-allergic individuals, complement activation products may enhance the basic, IgE-dependent early and/or late pathological mechanisms, i.e. degranulation of mast cells or the chemotaxis and activation of eosinophil leukocytes. Since the results of the skin prick tests were evaluated within 15 min, our present findings indicate that an early event, probably degranulation of mast cells and basophils is enhanced by the complement activation products. This assumption is also supported by the recent findings of Andersson et al. [10] who observed immediate 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 rhinoconjunctivitis. It is conceivable that the very potent inflammatory mediators that are formed from the complement proteins can amplify several processes triggered by the interaction of allergen with IgE attached to the IgE receptors of different cells. C5a has a well characterized receptor on leukocytes [22], and C3a was also found to bind strongly to basophils and mast cells and degranulate them [23]. C5a was observed to release histamine from monocytes, lymphocytes and mast cells [24].

Little is known about the mechanism of the allergen induced complement activation. Recent experiments from our group [25] suggest that complement activation and specific IgE binding are distinct molecular properties of ragweed pollen allergen. In accordance with this finding, in the present study only weak or no correlation was found between RW-specific IgE levels and generation of C3bBbP or C5b-9. This observation indicates that IgE antibodies have only a minor role in the allergen induced complement activation. Allergens trigger activation of the complement system most probably via the classical pathway [4–6,8]. Solid phase allergens were found by us to bind purified C1q and mannan binding lectin [11] indicating the possibility of an antibody-independent CP activation. Experiments studying this assumption are under way in our laboratory.

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