



In vitro complement activation by ragweed allergen extract in the sera of ragweed allergic and non-allergic persons

Tünde Hidvégi^a, Béla Schmidt^a, Lilian Varga^a, Mária Dervaderics^c, Ákos Lantos^c,
Zsuzsanna Gönczi^e, József Barok^b, Miklós Otos^b, Michael Kirschfink^d, Peter Späth^f,
George Füst^{*a}

^aNational Institute of Haematology, Blood Transfusion and Immunology, Budapest POB 44, H-1502, Hungary

^bNuclear Power Station Ltd., Health Care Department, Paks, Hungary

^cInstitute of Pulmonology, Semmelweis University Medical School, Budapest, Hungary

^dInstitute of Immunology, Ruprecht-Karls Universität, Heidelberg, Germany

^e18th District Government Health Care Center Department of Allergology-Asthma, Budapest, Hungary

^fZLB, Bern, Switzerland

Received 13 July 1995; revision received 7 August 1995; accepted 8 August 1995

Abstract

Ragweed allergen (RWA)-induced complement activation in sera of 40 RW allergic patients and of 40 non-allergic controls was investigated. After treatment of the sera with RWA, levels of C3a, C5a, C3bBbP, C1rC1sC1inh, SC5b-9 and granulocyte-aggregating activity were determined. Concentration of RW-specific IgG was also measured. After RWA treatment dose-dependent complement activation was detected in sera of RW allergic and non-allergic persons. C3a generation was observed mostly in the sera of RW allergic individuals, while levels of C3bBbP and of RW-specific IgG were significantly higher in sera of allergics, and a strong correlation was found between these two parameters. In a prospective clinical study, a significant positive correlation was observed between the extent of RWA-induced alternative pathway (AP) activation and the severity of symptoms of allergic rhinoconjunctivitis that were developed in a 4-week period subsequent to blood sampling. These observations suggest that complement activation has a role in the development of the symptoms of RW allergy.

Keywords: Complement activation; Ragweed allergy; Rhinoconjunctivitis; Asthma bronchiale

1. Introduction

In recent years much evidences have been accumulated which indicates that different clinical manifestations of allergic diseases cannot be explained exclusively by IgE-mediated immediate-type hypersensitivity reactions [1,2]. Cell-mediated immune reactions are implicated in the pathomechanism of allergy [3,4]

and lymphokines also may be important, not only in the regulation of IgE synthesis but also in the development of certain symptoms of allergic diseases [5].

There are some manifestations of such diseases that can be best explained by type II or III (complement and/or immune complex mediated) hypersensitivity reactions. For example, after allergen-exposition, in some patients immediate-type reaction is followed by a delayed-type reaction [6]. Both diagnostic skin tests and hyposensibilization treatment can lead to type II or III skin reactions in some allergic individuals [7].

The role of complement in allergy has been assessed by several authors [8–11]. Different allergens were found to be able to effectively activate complement system [12–18]. However, neither the mechanism of the

Abbreviations: AP, alternative pathway of complement activation; CP, classical pathway of complement activation; RW, ragweed; RWA, ragweed allergen; AU, allergic unit; U, unit; NHS, pooled normal human serum; VBS, veronal-buffered saline (pH 7.4); SEM, standard error of mean.

* Corresponding author, Tel./Fax: +36 1 2092310.

allergen-induced complement activation nor its clinical relevance have been definitely clarified.

The aim of the present study was to investigate in more detail the role of complement in the pathomechanism of RW allergy.

RW allergy is one of the most serious environmental risks in many countries of the world. About 10% of the Hungarian population were found to be allergic for RW with clinical symptoms during the RW blooming season. RW rhinoconjunctivitis can be considered as a good model for pollen allergy since its symptoms are well characterized, easily recognizable and scorable by the patients and are limited to a relatively short period of the year.

In the present study it was demonstrated that RW extract is able to activate complement in vitro in the sera of most individuals tested. Activation of the alternative complement pathway was more pronounced in sera of persons allergic to RW. Moreover, in a prospective study, a significant positive correlation was found between the in vitro generation of alternative pathway activation products and the severeness of different symptoms of RW rhinoconjunctivitis which developed in 4 weeks after blood sampling during the RW season. These observations indicate that complement activation may play a role in the development of different manifestations of RW allergy.

2. Materials and methods

2.1. Ragweed allergen (RWA)

Short ragweed (*Ambrosia elatior*) allergic extract, 100 000 allergic units/ml (AU/ml, Pharmacia; Uppsala, Sweden) was used throughout. The stock solution of the allergen was diluted in VBS, at 80 mg/ml final concentration (1 ml of VBS was added to the 100 000 AU).

2.2. Patients and samples

Blood samples containing 0.01 M EDTA were taken from 24 non-allergic and 80 RW allergic patients, in RW blooming season, 1991, to determine in vivo complement activation.

Sera of 40 RW allergic and 40 non-allergic persons were taken for in vitro RWA treatment in February, 1992 (i.e., out of RW blooming season).

RW allergy was diagnosed by IgE Quick Test (Dexall Biomed. Labs., Gaithersburg, MD, USA), and by prick skin-test. Samples were aliquoted to 1 ml and stored at -70°C .

C2 deficient sera were obtained from three homozygous C2 deficient patients studied by Dr. Peter Späth, Berne, Switzerland [19]. C2-depleted serum were from Sigma (St. Louis, MO).

2.3. Treatment of samples

Three hundred μl of sera were incubated (37°C , 30

min) with 30 μl of VBS, as control, and/or with 30 μl of RWA, at 2, 20 or 100 AU/ml doses (1.6, 16 and 80 $\mu\text{g/ml}$ final concentrations).

2.4. Activation of the classical complement pathway (CP ELISA)

During CP activation, amounts of C1inhC1rC1s complexes were measured by the method of Nielsson et al. [20]. Plates were coated by goat anti-C1 inhibitor antibodies (ATAB, Stillwater, MN). NHS treated with heat-aggregated human IgG was used as standard. Samples were incubated with PEG 6000 (Fluka) in 6% final concentration after treatment with RWA or VBS, and supernatant was used in 1:20 dilution. The amount of bound C1inh-C1s complexes were determined by goat anti-human C1s antibodies (ATAB, Stillwater, MN) and TMB substrate (Sigma, St. Louis, MO).

2.5. Activation of the alternative complement pathway (AP ELISA)

Amounts of the alternative C3-convertase (C3bBbP complexes) were measured by the method of Zilow et al. [21]. Plates were coated by goat anti-human properdin antibodies (ATAB, Stillwater, MN), and blocked by PBS containing 2% low-fat cows milk. Samples were applied 1:20 in PBS-Tween containing 0.01 M EDTA to coated microtiter plates after treatment with RWA or VBS. The amounts of C3bBbP complexes were determined by rabbit anti-C3c (DAKO, Denmark) biotin and streptavidine peroxidase (Sigma, St. Louis MO), using TMB substrate at the final step.

2.6. Formation of terminal complement activation complexes (TCC ELISA)

SC5b-9 complexes were measured by the method of Deppish et al. [22]. Plates were coated with mouse anti-human C9 monoclonal antibodies (Lea, Oslo, Norway). Samples were applied 1:30 in PBS-Tween containing 1% BSA and 10 mM EDTA to the microtiter plates. Zymosan-treated NHS was used as standard. Then rabbit anti-human C5 antibodies (DAKO, Denmark) were 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.

2.7. Calculations for complement ELISAs

Levels of C3bBbP, C1rC1sC1inh and SC5b-9 protein complexes were given as a percentage of the buffer control.

2.8. ELISA for determination of RW specific IgG

One part of the ELISA plate was coated with PBS (pH 7.4), the other part with RWA (10 $\mu\text{g/ml}$ in PBS) at 4°C , overnight. 5% cows milk in PBS (containing 15 mM Na-azide) was used for blocking (1 h, RT). Samples

Table 1

Complement activation in sera of persons non-allergic and allergic to RW incubated with RWA at 37°C for 30 min as compared to activation measured in the same sera incubated with VBS^a

Parameters of complement activation	100 AU/ml RWA (mean $\pm$ S.E.M. (<i>P</i> values) ^b		20 AU/ml RWA (mean $\pm$ S.E.M. (<i>P</i> values) ^b	
	Non-allergic persons (<i>n</i> = 40)	RW-allergic persons (<i>n</i> = 40)	Non-allergic persons (<i>n</i> = 40)	RW-allergic persons (<i>n</i> = 40)
ClrClsC1inh	105 $\pm$ 5 (N.S.)	113 $\pm$ 5 (6×10^{-3})	103 $\pm$ 5 (N.S.)	106 $\pm$ 5 (N.S.)
C3a	109 $\pm$ 5 (N.S.)	129 $\pm$ 10 (7×10^{-3})	104 $\pm$ 7 (N.S.)	142 $\pm$ 22 (4×10^{-2})
C3bBbP	127 $\pm$ 5 (3×10^{-6})	155 $\pm$ 14 (4×10^{-4})	100 $\pm$ 5 (N.S.)	130 $\pm$ 12 (1×10^{-2})
C5a	135 $\pm$ 7 (3×10^{-4})	113 $\pm$ 5 (8×10^{-3})	114 $\pm$ 10 (N.S.)	120 $\pm$ 14 (N.S.)
sC5b-9	124 $\pm$ 14 (3×10^{-6})	118 $\pm$ 7 (1×10^{-2})	N.D.	N.D.
GAA ^c	14 $\pm$ 2 (1×10^{-7})	17 $\pm$ 4 (5×10^{-4})	11 $\pm$ 3 (1×10^{-4})	8.5 $\pm$ 4 (3×10^{-2})

N.S., not significant. N.D., not done.

^aValues in VBS-treated samples were taken as 100%.

^bCalculated by the Mann-Whitney-Wilcoxon test.

^cIn zymosan equivalent units; values measured in VBS-treated samples were subtracted from those measured in RWA-treated samples.

were diluted 1:100 in PBS (containing 0.05% Tween 20) and applied to both RWA-coated and PBS-incubated parts of the plate. Serum mixtures of known high (as positive) and/or low (as negative) RW-specific IgG concentrations were used as standards (1:100). After washing, rabbit anti-human IgG (DAKO) was applied as first antibody (1:2000 in PBS containing 5% cows milk and 15 mM Na-azide, 37°C, 1 h). Goat anti-rabbit IgG-peroxidase conjugate (Dianova) was used as second antibody (1:5000 in PBS-Tween, 37°C, 1 h). OPD/H₂O₂ was used as substrate, and the reaction was stopped by 2 M H₂SO₄. OD values of PBS-coated wells were subtracted from those of RWA-coated ones. IgG contents in the positive and negative standard mixtures were arbitrarily taken as 10 and 1 unit (U), respectively.

2.9. Radioimmunoassay for C3a and C5a determination

Commercially available human complement C3a-desarg and C5a-desarg RIA kits were used (Amersham, Buckinghamshire, UK), according to the instructions of the manufacturer.

2.10. Granulocyte aggregating activity (GAA)

Peripheral blood granulocytes were separated from fresh normal human anti-coagulated blood by dextran sedimentation, osmotic lysis of erythrocytes, and density-gradient centrifugation as previously described [23]. The cells were suspended in HBSS containing 0.5% salt-poor human serum albumin at a concentration of 2×10^7 cells/ml. PMN aggregometry was performed in a standard platelet aggregometer (Chrono-log aggregometer 430, Chrono-log Corp., Havertown, USA). We added 25 μ l of serum to 225 μ l of stirred, Cytochalasin-B treated cell suspension, and the increase in light transmission was measured. The calibration curve of activated serum and granulocyte aggregating activity was given in micrograms zymosan equivalents per ml [24].

2.11. Statistical evaluation of the results

Statistics were calculated by Mann-Whitney-Wilcoxon and Kendal non-parametric methods.

3. Results

3.1. In vitro complement activation by RWA in the sera of persons allergic or not allergic to RW

Sera were obtained from blood taken from 40 individuals with proven RW allergy and from 40 individuals not allergic to RW in February, 1992 (i.e., out of RW blooming season). The sera were incubated at 37°C for 60 min with different amounts (2, 20 or 100 AU/ml corresponding to 1.6, 16 and 80 μ g/ml final concentrations, respectively) of RWA and the generation of different complement activation products was measured. The levels of these products detected in the same serum samples incubated as control with VBS was taken as 100% (Table 1).

In the sera incubated with 2 AU/ml RWA, no formation of complement activation products exceeding the control values was observed (data not shown). In serum samples incubated with 20 AU/ml RWA, significant differences were observed in the formation of some complement activation products between the groups of RW allergic and non-allergic individuals. In samples from the former group incubated with RWA, significantly higher amounts of C3a, C3bBbP and GAA were generated than in the same samples incubated with VBS. By contrast, in the sera of non-allergic individuals, RWA did not induce C3a or C3bBbP formation exceeding the control values.

As for the formation of C3 activation products, similar differences between the RW allergic and non-allergic individuals were found in the samples incubated with 100 AU/ml RWA. C3a generation exceeding the control values was measured only in the sera of allergic

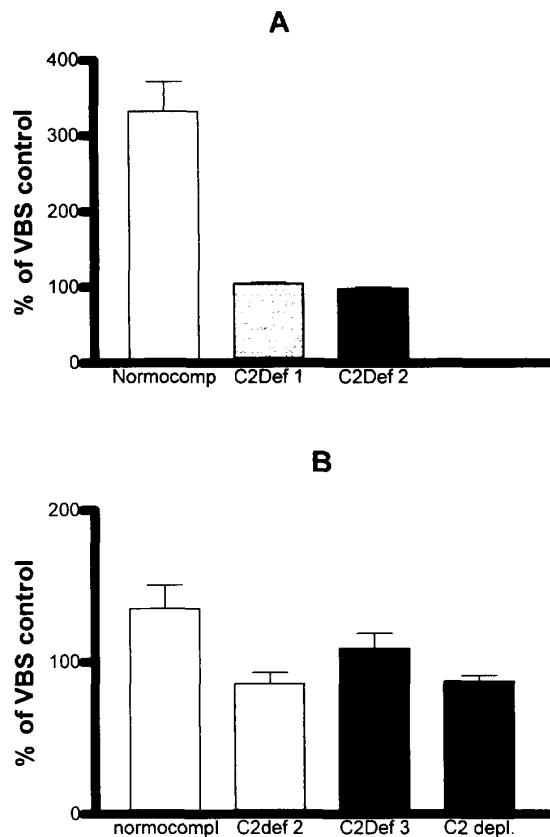


Fig. 1. C3bBbP generation in sera of three RW non-allergic normocomplementaemic and three homozygous C2-deficient individuals as well as in a C2-depleted human serum preparation (Sigma). Sera were incubated with VBS or RWA (100 AU/ml in VBS) and then C3bBbP (A) and C3a (B) generation were measured.

persons. Significant C3bBbP formation was observed in both groups, much more C3bBbP was generated, however, in the group of RW allergic than in the non-allergic individuals. A weak but significant formation of the CP activation product, C1rC1sC1inh was also observed but only in the group of RW allergic individuals. One hundred AU/ml RWA generated C5 activation products, C5a and C5b-9, in the sera of both groups. Interestingly enough, significantly ($P = 0.01$) more C5a formation was observed in the sera of control, non-allergic persons as compared to the allergic individuals, whereas no significant differences between the two groups were found in C5b-9 and GAA formation.

3.2. Mechanism of complement activation by RWA

Serum samples from three non-allergic normocomplementaemic individuals, as well as from three C2-deficient patients and a C2-depleted serum preparation were incubated with 100 AU/ml RWA and, as control,

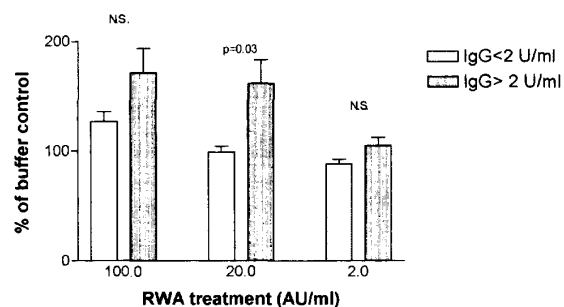


Fig. 2. RWA-specific IgG content and C3bBbP complex formation in sera of allergics. Sera of RW allergic patients were treated in vitro by 2, 20 and 100 AU/ml RWA and then C3bBbP content was measured. RW-specific IgG contents of the patients' sera were also determined.

with VBS. Formation of C3bBbP and C3a were measured (Fig. 1). RWA induced a strong C3bBbP formation (332%, $P < 0.001$) and a significant C3a generation (135%, $P < 0.05$) as compared to VBS in the complement-sufficient sera. By contrast, significant C3bBbP and/or C3a generation was not observed in either of the three homozygous C2-deficient patients tested. Similarly, no activation was found in the C2-depleted human serum preparation.

3.3. RWA-specific IgG content and complement activation

Serum levels of RW-specific IgG were significantly ($P = 10^{-3}$) higher (mean = 3.28 ± 0.45 U/ml vs. 1.35 ± 0.22) in the group of RW allergics. Twenty-five percent of the control persons, but 71% of the allergics, had RW-specific IgG levels higher than 2 U/ml. In the patient's group, significantly more ($P = 3 \times 10^{-2}$) C3bBbP formation was observed in the sera with RW-specific IgG higher than 2 U/ml as compared to those with lower than 2 U/ml IgG if incubated with 20 AU/ml RWA (Fig. 2). A similar tendency in difference was found in samples incubated with 100 AU/ml RWA, this difference, however, was not significant.

Table 2
RWA (20 AU/ml serum)-induced complement activation in the group of RW allergic patients with ($n = 34$) or without ($n = 22$) bronchial asthma (Wilcoxon-test)

Parameter	Non-asthmatic (mean + S.E.M.)	Asthmatic (mean + S.E.M.)	P
C3bBbP	128 ± 7.7	148 ± 11.7	3×10^{-2}
SC5b-9	151 ± 26.3	125 ± 17.7	5×10^{-2}
C1rC1sC1inh	148 ± 31.8	105 ± 5.7	NS
IgG	6.2 ± 1.4	6.9 ± 1.3	NS

3.4. Correlation between the symptoms of RW allergy and the extent of *in vitro* complement activation by RWA

Thirty-four of the 56 allergics developed bronchial asthma. We found that *in vitro* RWA treatment (20 AU/ml) caused significantly higher ($P = 3 \times 10^{-2}$) AP activation in the sera of asthmatics than in those of non-asthmatics. Formation of terminal complement complexes, however, was higher in sera of non-asthmatics. The difference was of marginal significance (Table 2). No significant differences were found between the two allergic groups in CP activation and specific IgG concentration.

In early August 1992 (that is during the RW blooming season) blood samples were taken from 40 RW allergic patients. These sera were incubated with RWA (20 AU/ml) and the formation of complement activation products was determined. After taking blood samples the allergic patients were asked to fill diaries with regard to their symptoms during the subsequent 4 weeks. This diary included subjective scores to be given to nasal, eye, throat, pulmonary and ear symptoms (0 = no symptom, 1 = slight symptom, 2 = medium symptom, 3 = severe symptom, disturbing daily life). Symptoms' scores were summed. Twenty-six of the diaries could be evaluated, and compared with RWA-induced AP activation (C3bBbP formation). We found a significant positive correlation between eye and throat symptoms, and the activation of AP ($P = 3 \times 10^{-2}$ and $P = 6 \times 10^{-3}$, respectively). The correlation between nasal symptoms and C3bBbP formation was of marginal significance ($P = 7 \times 10^{-2}$). There was no significant correlation with RW-specific IgG levels.

When the 26 allergics were divided into two groups according to the extent of C3bBbP formation (>120%,

$n = 15$ or $\leq 120\%$, $n = 11$, compared to buffer control) upon incubation with 20 AU/ml RWA, we found that the sum of eye and throat symptom scores obtained in different patients were significantly higher in patients with more pronounced allergen-induced AP activation (Fig. 3).

3.5. Complement activation products in the plasma samples of allergic and non-allergic individuals at the peak of RW season

Complement activation products were measured in EDTA plasma samples from 80 RW allergic and 24 RW non-allergic individuals in August–September, 1991. No significant differences were found in the levels of any complement protein cleavage products (C3a, C5a), or the protein-protein complex (C3bBbP) (data not shown).

4. Discussion

Our present findings demonstrate that RW pollen extract at a concentration range of 16–80 $\mu\text{g/ml}$ activates human complement. The same allergen preparation was used in the whole study. Activation of complement was reflected by the appearance of C3a and C5a, activation-specific protein-protein complexes, as well as a granulocyte-aggregating activity. The concentration range at which RW extract was found to be able to activate complement is comparable to amounts of other allergens (house dust, mite, *Aspergillus*, rye grass) which generated C3a, C4a and C5a, as well as neutrophil aggregating activity in pooled normal human serum [25].

Alternative pathway activation was detected even after incubation with low amounts of allergen, whereas the classical pathway seemed to be affected only in the sera of allergic patients and only if incubated with the highest allergen dose. However, since RW extract did not generate C3bBbP in C2-deficient and C2-depleted human sera, it seems that complement activation by RW extract was triggered through the classical pathway. The same mechanism for complement activation was found for house dust allergens by Nagata and Glovsky [25] and by Berrens [26]. The observed dominance of C3bBbP generation over the formation of C1rC1sC1inh complexes can be explained by the amplification of the CP activation by the AP amplification loop.

Several observations described in the present paper indicate that complement activation may be involved in the development of some clinical symptoms of RW allergy:

(A) C3a was generated by different doses of RW extract in the sera taken from individuals allergic to RW while no significant C3a formation was observed in the sera of most non-allergic persons incubated with the same amounts of RW. In addition, significantly higher

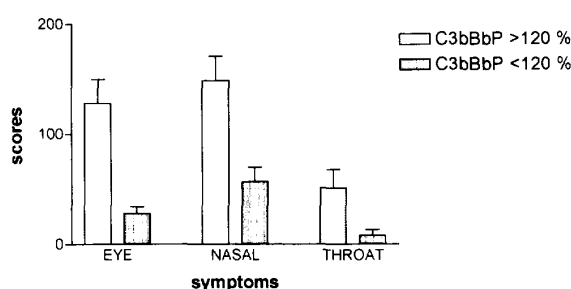


Fig. 3. Development of different symptoms during RW season in allergic patients with high or low AP activability. Twenty-six allergics were divided into two groups according to C3bBbP (>120% or $\leq 120\%$) formation upon incubation with 20 AU/ml RWA. Subjective scores were collected from the patients by means of a diary for nasal, eye, throat, pulmonary and ear symptoms (0 = no symptom, 1 = slight symptom, 2 = medium symptom, 3 = severe symptom, disturbing daily life). Symptoms' scores were summed. Eye ($P = 5 \times 10^{-2}$), nasal ($P = 3 \times 10^{-3}$) and throat symptom scores ($P = 7 \times 10^{-3}$) were much higher in patients with a more pronounced allergen-induced activation of the AP.

amounts of C3bBbP complexes were formed in the sera of the RW allergic individuals.

(B) When RW allergic patients with or without bronchial asthma were compared, markedly higher C3bBbP formation was observed in the sera of patients with asthma. This observation is at variance with the findings obtained by one of us (M.K.) in the reactivity of serum complement to house dust in patients with bronchial asthma [27].

(C) In a prospective study performed in 26 patients, a strong positive correlation was observed between the amounts of C3bBbP generated in the sera from blood taken at the beginning of RW blooming season and the extent of symptoms of RW allergic rhinoconjunctivitis observed in the same patients during a 4-week period after blood sampling. These findings indicate that complement activation has a major role in the pathomechanism of RW allergy. Since in vivo most pathological events leading to development of the symptoms of RW allergy occur on the surface of the mucosa of airways, most probably our findings on the association of an increased in vitro activability of complement and clinical symptoms of RW allergy also reflect complement activation that takes place on the mucosal surface. The lack of elevated levels of complement activation products in the sera of RW allergic patients during the RW blooming season is most probably due to the well-known fast turn-over of these products [28]. Complement activation products that were formed in vitro in the sera of most allergic patients tested may directly or indirectly contribute to the development of the clinical symptoms. C3a was found to be generated mostly in the sera of allergic persons. After inhalation, RW pollen interacts with the complement system at the mucosal surface of the respiratory tract. C3a, which is generated during complement activation can lead to increased vascular permeability [28], markedly facilitates mucus formation in the lower parts of the respiratory tract [29] and induces production and release of interleukin 1 by cultured human monocytes [30].

Recently a functional active receptor for C3a was found in chronic myelogenous leukaemia-derived basophil granulocytes [31] and it was demonstrated that C3a activates reactive oxygen radical species production in human polymorphonuclear neutrophil leukocytes [32], and human eosinophils [33]. In addition, prestimulation of human basophil granulocytes with IL-3 rendered them sensitive to nanomolar doses of C3a [31,33].

These findings indicate that C3a may play a part in the pathophysiology of inflammatory diseases mediated by eosinophils and PMN leukocytes such as allergic diseases [34]. Our present observation on a positive correlation between RWA-induced C3a generation in vitro and certain clinical symptoms of RW allergy suggest that C3a may participate in the development of these symptoms as well.

In contrast to C3 cleavage fragments, products of C5 activation, C5a and SC5b-9, as well as granulocyte aggregating activity, were found to be generated at the same extent in the sera of allergic and non-allergic persons, or even more such products were formed in the sera of control individuals. Therefore, the direct contribution of these products in the development of symptoms of RW allergic rhinoconjunctivitis does not seem to be probable. It was recently described, however, that TNF- α can efficiently prime neutrophils to the response to C5a, while TNF- α alone appeared to have minor direct stimulatory effect [36]. Recently increased TNF- α generation was described in allergic patients [37], therefore an additive effect of TNF- α and other inflammatory cytokines with C5a can be partly responsible for the generation of some symptoms of allergic inflammation.

As for the mechanism of complement activation by RW extract, a role of IgG cannot be excluded, since alternative pathway activation is most probably triggered by the activation of the classical pathway (Fig. 1). Moreover, significantly more C3bBbP was generated by RW extract in the sera from RW allergic individuals containing higher amounts of RW-specific IgG than in the sera with lower specific IgG levels. On the other hand, in a prospective study, no such correlation between IgG levels and the extent of C3bBbP generation was observed and no correlation between RW-specific IgG levels and symptoms of RW allergic rhinoconjunctivitis was found. These findings suggest that, besides an immune complex-triggered activation of the classical pathway, antibody-independent complement activation by RW may also have a role in the development of clinical symptoms of RW allergy.

Acknowledgements

This project was sponsored by the National Committee for Technical Development of Hungary (project N: 3160/910607), National Ministry of Welfare (project N: T-51), and EUREKA (project N: 190).

References

- [1] Geha, R.S. (1992) *Curr. Opin. Immunol.* 6, 779–781.
- [2] Serafin, W.E. and Austen, K.F. (1987) *New Engl. J. Med.* 317, 30–34.
- [3] Kapsenberg, M.L., Jansen, H.M., Bos, J.D. and Wierenga, E.A. (1992) *Curr. Opin. Immunol.* 6, 788–793.
- [4] Kay, A.B. (1986) *Eur. J. Respir.* 69, 38–43.
- [5] Weller, P. (1992) *Curr. Opin. Immunol.* 6, 781–785.
- [6] Étiauvant, M., Leluc, B. and David, B. (1988) *Agents Actions* 24, 137–144.
- [7] Chapel, H. and Haeney, M. (1984). In: *Essentials of Clinical Immunology*, Blackwell Scientific Publ., Oxford.
- [8] Moro, E. (1932). In: *Klinik und Pathogenese*, Springer-Verlag, Berlin.
- [9] Clock, R.O. (1917) *J. Infect. Dis.* 21, 387–391.
- [10] Armstrong, C. and Harrison, W.T. (1925) *US Publ. Health Rep.* 40(2), 1466–1472.

- [11] Walker, I.C. (1917) *J. Med. Res.* 36, 243–266.
- [12] Weemaes, C.M.R., Stoop, J.W. and Berrens, L. (1977) *Clin. Allergy* 7, 75–82.
- [13] Van der Zee, J.S., van Swieteln, P. and Aalberse, R.C. (1988) *Mol. Immunol.* 25, 345–354.
- [14] Edwards, J.H. (1974) *Clin. Allergy* 4, 379–388.
- [15] Edwards, J.H. (1976) *Clin. Allergy* 6, 19–25.
- [16] Étivant, M., Chevance, L.G., Lesourd, M. and Ohayon, H. (1980) *Ann. Immunol.* 1310, 13–42.
- [17] Olenchok, S.A., Mull, J.C. and Major, P.C. (1980) *Ann. Allergy* 44, 23–28.
- [18] Srivastava, N., Grupta, S.P. and Srivastava, L.M. (1983) *Clin. Allergy* 13, 54–60.
- [19] Zimmerli, W., Schaffner, A., Scheidegger, R., Scherz, R. and Spath, P. (1991) *J. Infect.* 22, 59–69.
- [20] Nielsson, T. and Back, O. (1985) *Clin. Exp. Immunol.* 60, 178.
- [21] Zilow, G., Sturm, A., Rother, U. and Kirschfink, M. (1990) *Clin. Exp. Immunol.* 79, 151–157.
- [22] Deppish, R., Schmitt, V., Bommer, J., Hannsch, M., Ritz, E. and Rautenberg, E.W. (1990) *Kidney Int.* 37, 696–706.
- [23] Craddock, P.R., Hammerschmidt, D.E., White, D.g., Dalmaso, A.P. and Jacob, H.S. (1977) *J. Clin. Invest.* 60, 260.
- [24] Varga, L., Dervaderics, M., Zsiray, M., Kárpáthy, J. and Füst, G. (1986) *Diagn. Immunol.* 4, 140–144.
- [25] Nagata, S. and Glovsky, M.M. (1987) *J. Allergy Clin. Immunol.* 80(1), 24–32.
- [26] Berrens, L., Schoonenwolf, D.A. and Brujinzeel, P.L.B. (1978) *Allergol. Immunopathol.* 6, 45–54.
- [27] Castro, F.F.M., Schmitz-Schumann, M., Rother, U. and Kirschfink, M. (1991) *Int. Arch. Allergy Appl. Immunol.* 96, 305–310.
- [28] Morgan, B.P. (1978). In: *Complement — Clinical Aspects and Relevance to Disease*. Academic Press, London.
- [29] Marom, Z., Shelhamer, J., Berger, M. and Frank, M. (1985) *J. Exp. Med.* 161, 657–668.
- [30] Haeflner-Cavaillon, N., Cavaillon, J/M., Laude, M. and Kazatchkine, M.D. (1987) *J. Immunol.* 139, 794–799.
- [31] Kretzschmar, T., Jeromin, A., Gietz, C., Batusch, W., Klos, A., Kohl, J., Rechkemmer, G. and Bitter-Suermann, D. (1993) *Eur. J. Immunol.* 23, 558–561.
- [32] Elsner, J., Oppermann, M., Czech, W. and Kapp, A. (1994) *Blood* 83, 3324–3331.
- [33] Elsner, J., Oppermann, M., Czech, W., Dobos, G., Schöpf, E., Norgauer, J. and Kapp, A. (1994) *Eur. J. Immunol.* 24, 518–522.
- [34] Bischoff, S.C., de Weck, A.L. and Dahinden, D. (1990) *Proc. Natl. Acad. Sci. USA* 87, 6813.
- [35] Weller, P.F. (1992) *Curr. Opin. Immunol.* 4, 782–787.
- [36] Crouch, S. and Fletcher, J. (1991) *Br. J. Haematol.* 77, 158–164.
- [37] Cembrzynska-Nowak, M., Szklarz, E., Inglot, A.D. and Teodorczyk-Injeyan, J.A. (1993) *Am. Rev. Resp. Dis.* 147(2), 291–295.