

Research

Open Access

Circulating immune complexes and complement C3 and C4 levels in a selected group of patients with rhinitis in Lebanon

Alexander M Abdelnoor*¹, Firas Kobeissy^{1,2}, Daad Farhat¹ and Usamah Hadi³

Address: ¹Department of Microbiology & Immunology, Faculty of Medicine, American University of Beirut, P. O. Box 11-0236, Riad el-Solh, Beirut, 11072020, Lebanon, ²Department of Neuroscience, University of Florida, Gainesville, FL, 32608, USA and ³Department of Otolaryngology, Faculty of Medicine, American University of Beirut, P. O. Box 11-0236, Riad el-Solh, Beirut, 11072020, Lebanon

Email: Alexander M Abdelnoor* - aanoor@aub.edu.lb; Firas Kobeissy - firasko@ufl.edu; Daad Farhat - df01@aub.edu.lb; Usamah Hadi - uhadi@dm.net.lb

* Corresponding author

Published: 22 April 2004

Received: 30 December 2003

Clinical and Molecular Allergy 2004, 2:6

Accepted: 22 April 2004

This article is available from: <http://www.clinicalmolecularallergy.com/content/2/1/6>

© 2004 Abdelnoor et al; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

Abstract

Background: A number of reports indicate that circulating immune complexes (CIC) and activation of the complement system contribute to the pathogenesis of Type I allergy. The aim of this study was to investigate the status of CIC in 113 patients with rhinitis in Lebanon and determine complement components C3 and C4 serum levels in the CIC-positive patients. Serum specific IgE antibodies were previously detected and reported in 74 of the 113 patients.

Methods: CIC were detected by polyethylene glycol precipitation and serum C3 and C4 levels quantified by radial immunodiffusion.

Results: CIC was positive in 20 of the specific IgE-positive and 13 of the specific IgE-negative patients. C3 and C4 levels were within the normal range in all the 33 CIC-positive patients.

Conclusions: The antibody class that constitutes the complexes does not seem to be IgG or IgM. Moreover, complement activation does not seem to be involved in the allergic reaction since both C3 and C4 levels were normal in all patients. The role of these complexes, if any, in the pathogenesis of rhinitis is yet to be determined.

Background

In atopic individuals, exposure to an allergen would result in the production of specific IgE antibodies that bind to Fc receptors (FcεRI) on tissue mast cells and basophils. On re-exposure, the allergen cross-links bound IgE molecules. This results in the immediate release of histamine and various enzymes from these granulated cells. Later, prostaglandins and leukotrienes are produced as a consequence of arachidonic acid metabolism, and released. Moreover, Th2 cytokines are generated and there is an influx of cells, in particular, eosinophils into the site of allergen entry. In

this Type I reaction, the mediators released from the cells (mast cells, basophils, Th2-lymphocytes, eosinophils) cause increases in blood flow and vascular permeability, contraction of smooth muscle and tissue damage leading to the signs and symptoms that are observed [1].

Some reports indicated that activation of the complement system by an allergen or immune complex would result in the generation of anaphylatoxins (C3a, C4a, and C5a). These anaphylatoxins would amplify mast cell degranulation leading to an increased Type I response [2-7].

Atopic rhinitis is caused by the inhalation of allergens (foreign proteins) that induce the above-described Type I, and possibly Type III reactions. Avoiding contact with the allergen, if possible, and desensitization usually improves the condition of the patient.

The aim of this study was to detect the presence of CIC and determine the serum levels of C3 and C4 in patients and to see if a correlation existed between these parameters and allergic rhinitis.

Methods

Subjects and blood specimens

One hundred and thirteen patients with rhinitis (65 females, 48 males, age range between 6 and 82 years) were included in the study. Criteria used to diagnose rhinitis included history, nasal obstruction, rhinorrhea and nasal itchiness. Blood was collected from each patient in a plain vacutainer, allowed to clot, the serum separated and stored at -20°C till performance of tests.

Circulating Immune Complexes [8]

Nine parts of 4.166% polyethylene glycol in borate buffer were added to 1 part of a 1:3 dilution of serum to be tested. The mixture was allowed to stand at room temperature for 1 hour, after which the absorbance at a wavelength of 450 nm was read using the patients' serum as a blank. Absorbance greater than 0.23 were considered positive.

Complement C3 and C4 Levels

Five µl of serum were loaded into wells of ready to use C3 and C4 radial immunodiffusion plates (The Binding Site LTD., Birmingham, UK). Forty-eight hours later, the diameters of the white rings of precipitation formed were measured and concentrations of C3 and C4 were determined using the provided conversion table.

Experimental research on humans

Approval for this research project was obtained from the Institutional Research Board and is in compliance with the Helsinki Declaration.

Results and discussion

Earlier we reported on the frequency of causative allergens in groups of perennial asthmatics and rhinitics [9,10]. The most common causative allergen in these patients was house dust mite. Specific anti-allergen IgE antibodies were detected in 74 of the 113 patients with rhinitis (65.5%). *Dermatophagoides pteronyssinus* (Dpt) was the causative allergen in 62, *Dermatophagoides farinae* (Df) in 58, cat hair dander in 23 and dog hair dander in 9 patients (some patients were allergic to more than one allergen).

The presence of CIC in sera of patients was detected by a method reported by Riha et al. [8]. It involves the precipitation of CIC by polyethylene glycol and determining the absorbance of the turbidity produced at a wavelength of 450 nm using patients' serum as a blank. The results obtained paralleled those obtained when the C_{1q} assay was used. The absorbance values they obtained when sera obtained from apparently normal individuals were all less than 0.200. Their analysis of the precipitates formed using sera from 20 patients with diseases other than allergy indicated that IgM was present in all of them, IgG in 18 and IgA in 4. In the present study, CIC were detected in 33 of the 113 (29%) rhinitics, 20 (17.7%) of which also had IgE specific anti-dust mite antibodies (Table 1). In studies done earlier in our laboratory 4 of 61 (6.6%) apparently normal individuals were CIC-positive using polyethylene glycol precipitation to detect them (unpublished data). This value is significantly less than that obtained in the rhinitis patient group.

CIC can cause tissue injury by several ways [11]. IgG (excluding IgG₄) and IgM class antibodies in the form of complexes with antigen activate the classical pathway of the complement system resulting in products that lead to inflammation. Independent of complement, CIC-containing IgG could bind to F_c receptors expressed by various cell types including macrophages, neutrophils, eosinophils and platelets inducing them to release a number of mediators that contribute to the signs and symptoms observed in rhinitis.

Table 1: Specific IgE (sIgE) antibodies, circulating immune complexes (CIC) and mean complement C3 and C4 levels in selected groups of patients with rhinitis.

Group/number of patients	Number of CIC-positive patients	Mean C3 level (mg/L) ¹	Mean C4 level (mg/L) ¹
sIgE-positive (74)	20	1457 +/- 309	290 +/- 121
sIgE-negative (39)	13	1470 +/- 334	319 +/- 139

¹the mean C3 and C4 levels of the CIC-positive patients. C3 and C4 levels fell within the normal range for all CIC-positive patients (C3 normal range: 910–1500 mg/L; C4 normal range: 140–435 mg/L). C3 and C4 levels were not determined in CIC-negative patients. Four of 61 apparently normal individuals were CIC-positive

In addition to the IgE mediated type I reaction underlying the pathogenesis of atopic allergy, a number of studies have indicated that CIC and activation of the complement system might be involved. Brostoff et al. [12,13], Pagenilli et al. [14] and Carini et al. [15] reported on the presence of complexed IgE in the serum of patients with atopic allergy. Lukacs et al. [7] reported that in an acute lung injury model in mice, IgG immune complex deposition elicited severe airway hyperactivity. Moreover, it has been suggested that the complement system is activated by the allergen or the immune complex and the anaphylatoxins, C3a, C4a and C5a produced contributes to the severity of the disease [2-7].

The antibody class composition of the CIC most probably was neither IgG (excluding IgG₄) nor IgM since only these classes in the form of complexes with antigen are capable of activating the complement system, and this did not seem to occur because C3 and C4 levels were within the normal range in all patients tested (Table 1). In support of the lack of involvement of the classical and lectin complement pathways is the study by Turner et al. [16] who reported C2 deficiencies in some patients with eczema or hay fever.

Both IgG₄ and secretory IgA in the form of complexes do not activate the complement system. Beauvais et al. [17] reported that IgG₄ mediated human basophil activation and histamine release and Iikura et al. [18] reported that immobilized secretory IgA on Sepharose beads was capable of inducing basophil degranulation and histamine release. The presence of IgA containing-immune complexes in nasal secretions was not determined in this investigation. If it is assumed that they were present, then their role in the pathogenesis of allergic rhinitis should be considered.

The antigenic composition of the CIC could have been house dust mite protein, which was identified as the causative allergen in most patients [10]. In one study [2] it was reported that extracts of *Aspergillus fumigatus* and house dust generated more anaphylotoxins than house dust mite and ryegrass. Whether the nature of the allergen influences the activation of the complement system is yet to be determined.

Some studies indicated that ragweed extract activated the complement system *in vitro* and suggest that this occurs *in vivo* as well [3-6]. They imply that the anaphylotoxins produced contribute to the severity of symptoms in allergy. Probably agents such as bacterial endotoxin present in the ragweed extract activated the complement system *in vitro*. Endotoxin is known to activate the alternate and classical pathways of the complement system [16]. Endotoxin is a lipopolysaccharide component of the cell wall of Gram

negative bacteria which is released by metabolically active cells, or when the cells die. Endotoxin has been detected in hay and straw dust, air and house dust [19-21]. Rather than contributing to the severity of symptoms due to its complement activating property, it has been reported that increased house-dust endotoxin concentrations were correlated with increased proportions of γ -interferon producing CD4 cells and prevention of allergen sensitization during infancy [22].

Conclusion

In conclusion, there did not seem to be a correlation between the presence of CIC and the activation of the complement system in patients with atopic rhinitis. Had the results indicated that complement was activated, blocking the complement pathway using agents suggested by Holers [23] might have been considered as a therapeutic approach. The role of CIC in the pathogenesis of allergic rhinitis independent of complement is yet to be determined in the patients that were studied.

Competing interests

None declared.

Authors' contributions

AMA was the principal investigator. He conceived of the study, and participated in its design and coordination. He supervised the bench work that was done (processing of blood specimens, CIC detection and determination of C3 & C4 levels. He drafted the manuscript.

FK was a research assistant. He participated in the bench work.

DF was a research assistant. She participated in the bench work.

UH was the otolaryngologist. He diagnosed the patients and referred them for inclusion in the study.

All authors read and approved the final manuscript.

References

1. Rosen F, Geha R: **Allergic asthma**. In *Case Studies in Immunology* 3rd edition. New York, Garland Publishing; 2001:127-134.
2. Nagata S, Glovsky M: **Activation of human serum complement with allergens**. *J Allergy Clin Immunol* 1987, **80**:24-32.
3. Hidvegi T, Schmidt B, Varga L, Dervaderics M, Lantos A, Gonczi Z, Barok J, Otos M, Kirschfink M, Spath P, Fust G: **In vitro complement activation by ragweed allergen extract in the sera of ragweed allergic and non-allergic persons**. *Immunol Lett* 1995, **48**(1):65-71.
4. Hidvegi T, Berrrens L, Vaqra L, Maranon F, Schmidt B, Kirschfink M, Fust G: **Comparative study of the complement-activating and specific IgE-binding properties of ragweed pollen allergen**. *Clin Exp Immunol* 1997, **108**(1):122-127.
5. Gonczi Z, Varga L, Hidvegi T, Schmidt B, Panya A, Kokai M, Fust G: **The severity of clinical symptoms in ragweed-allergic patients is related to the extent of ragweed-induced complement activation in their sera**. *Allergy* 1997, **52**(11):1110-1114.

6. Dervadis M, Hidvegi T, Schmidt B, Fust G, Varga L: **Ragweed allergy: correlation between skin reactivity and in vitro complement activation.** *Immunol Lett* 1998, **64(2-3)**:119-123.
7. Lukacs NW, Glovsky MM, Ward PA: **Complement-dependent immune complex-induced bronchial inflammation and hyperreactivity.** *Am J Physiol Lung Cell Mol Physiol* 2001, **280**:L512-L518.
8. Riha I, Haskova V, Kaslik J, Maierova M, Stransky J: **The use of polyethylene glycol for immune complex detection in human sera.** *Mol Immunol* 1979, **16**:489-493.
9. Ramadan F, Hamadeh F, Abdelnoor AM: **Identification of allergens in a selected group of asthmatics in Lebanon.** *Eur J Epidemiol* 1998, **14**:687-691.
10. Abdelnoor AM, Kobeissy F, Farhat D, Hadi U: **Some immunological aspects of patients with rhinitis in Lebanon.** *Immunopharmacol Immunotoxicol* 2002, **24(2)**:289-301.
11. WHO Scientific Group: **The role of immune complexes in disease.** *World Health Organization Series* 1997, **606**:1-58.
12. Brostoff J, Johns P, Stanworth DR: **Complexed IgE in atopy.** *Lancet* 1977, **2(8041)**:741-742.
13. Brostoff J, Carini C, Wraith DG, Johns P: **Production of IgE complexes Allergen in atopic patients and the effect of sodium cromoglycate.** *Lancet* 1979, **1(8129)**:1268-1270.
14. Paganelli R, Levinsky RJ, Brostoff J, Wraith DG: **Immune complexes containing food proteins in normal and atopic subjects after oral challenge and effect of sodium cromoglycate on antigen absorption.** *Lancet* 1979, **1(8129)**:1270-1272.
15. Carini C, Brostoff J: **Evidence for circulating IgE complexes in food allergy.** *Ric Clin Lab* 1987, **17(4)**:309-322.
16. Turner MW, Mowbray JF, Harvey BA, Brostoff J, Wells RS, Soothill JF: **Defective yeast opsonization and C2 deficiency in atopic patients.** *Clin Exp Immunol* 1978, **34(2)**:253-259.
17. Beauvais F, Hieblot C, Burton C, Benveniste J: **Bimodal IgG₄-mediated human basophil activation. Role of eosinophils.** *J Immunol* 1990, **144(10)**:3881-3890.
18. Iikura M, Yamaguchi M, Fujisawa T, Miyamasu M, Morita Y, Iwase T, Moro I, Yamamoto K, Hirai K: **Secretory IgA induces degranulation of IL-3-primed basophils.** *J Immunol* 1998, **161(3)**:1510-1515.
19. Morrison DC, Kline LF: **Activation of the classical and properdin pathways by bacterial lipopolysaccharide.** *J Immunol* 1977, **118**:362-368.
20. Gereda JE, Leung DY, Thatayatikom A, Strieb JE, Price MR, Klinnert MD, Liu AH: **Relationship between house-dust endotoxin exposure, type I T-cell development, and allergen sensitization in infants at high risk of asthma.** *Lancet* 2000, **355(9216)**:1680-1683.
21. Andersson M, Laukkanen M, Salkinojasalonen M: **3-Hydroxy fatty acids: Stable indicators of endotoxin in hay and straw dust.** *Journ Endotoxin Res* 1994, **Suppl 1(1)**:45.
22. Laitinen SK: **Influence of filter media and their handling after sampling on airborne endotoxin concentrations.** *Journ Endotoxin Res* 1994, **Suppl 1(1)**:45.
23. Holers VM: **The complement system as a therapeutic target in autoimmunity.** *Clin Immunol* 2003, **107(3)**:140-151.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

