

Haemagglutinating antibodies to cat dander in relation to exposure and respiratory allergy

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Summary

Cat epithelial extract was coupled with chromic chloride to red cells and used as an antigen in a haemagglutination assay. Agglutinins coexisted with IgE type antibodies in 44% of patients with respiratory allergy and sensitivity to cat dander but occurred in only 1% of asthmatic patients sensitized to other allergens or in controls. The agglutinins were detected in six out of 104 cat fanciers, and three of those with agglutinins had asthma. The agglutinins were of IgG type and were heterogeneous in specificity.

Introduction

Patients with asthma can exhibit two types of reaction to a bronchial challenge with allergen (Pepys & Turner-Warwick, 1975). The first (early or immediate) reaction appears rapidly and is readily reversed by bronchodilators. The second (late or non-immediate) reaction appears slowly several hours later and is only poorly reversed by these drugs. Patients with atopic asthma in most instances exhibit either the early reaction alone or a dual response. The late reaction as an isolated phenomenon is typically elicited in non-atopic subjects.

The early reaction is attributed to IgE antibodies. The mechanism of the late reaction is not known although it is commonly supposed to be mediated by immune complexes. Favouring this view is the time course of the reaction and the occurrence of precipitating serum antibodies against certain allergens capable of provoking late reactions. On the other hand, immunotherapy (hyposensitization) with allergen extracts has gained widespread acceptance in the treatment of atopic asthma, and success in its use is commonly accounted to the development of blocking IgG type antibody. Thus there exists an obvious controversy concerning the role of IgG antibodies in asthma.

To shed some light on this problem we have initiated a series of experiments on the occurrence and specificity of IgG antibodies in patients with respiratory allergy and

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their relation to exposure and clinical manifestations of the disease. We report here on our findings of antibodies against feline epithelial extract.

Patients and methods

Patients included in the study, most with bronchial asthma, presented themselves for an allergy evaluation at the Hospital for Allergic Diseases, University Central Hospital, Helsinki. Control specimens were from pregnant women, taken primarily for antenatal Rh-testing. Blood specimens from the members of the local cat pedigree association ('cat club') were collected in 1973. The sera were stored at -20°C until used for the present study. Most of the club members lived in relatively small flats in apartment houses in urban areas and many of them had several cats within their households. Further information on the length of exposure, closeness of daily contacts, etc. was obtained through a questionnaire.

The haemagglutination test was performed using chromic chloride as a coupling agent (Gold & Fudenberg, 1967). One volume of packed Rh-positive erythrocytes of group O was suspended in two volumes of appropriately diluted antigen (Bencard 3204 G 1857-1; protein content 2.6 mg/ml) dialyzed against saline. Then two volumes of chromic chloride in saline was added. After 5 min at room temperature the erythrocytes were washed three times with saline and used in the test as a 1% saline suspension containing 0.25% bovine serum albumin. The optimum coating was achieved using 0.075% chromic chloride and antigen diluted 1:8. Threefold dilution series of each serum, in 0.05 ml volumes, were prepared on microtitre plates with V-shaped bottom. Coated cells were then added in 0.05 ml volumes of suspension. The results were read after 4 h at room temperature.

The determination of IgE antibodies (RAST) was performed by radioimmunoassay with commercial kits (Pharmacia Diagnostics).

Results

Table 1 shows that antibodies were detected in forty-three out of ninety-eight (44%) patients allergic to cat as evidenced by history and a positive radio-allergosorbent test (RAST), whereas they occurred very seldom in controls and in asthmatic patients sensitized to other allergens. In the reactive sera titres up to 21,870 were recorded. The

Table 1. Occurrence of haemagglutinating antibodies to cat epithelial extract in patients with respiratory allergy, cat fanciers and in controls

	Number of subjects	Number of haemagglutination positive subjects	Positive (%)
Patients, positive RAST to the cat allergen	98	43	44
Patients, negative RAST to the cat allergen	128	2	2
Cat fanciers	104	6	6
Controls	206	2	1

same epithelial extract was used as antigen in agarose gel double diffusion test, but all sera proved to be nonreactive.

The occurrence of haemagglutinating antibodies in relation to the length of cat exposure was studied in 104 members of the 'cat club' (Table 2). Four subjects were positive in the RAST against the cat antigen, and of those the two with the strongest reactions also had haemagglutinating antibodies. Four subjects negative in the RAST were positive in the haemagglutination test. The average length of exposure to cat in the haemagglutination-positive subjects was 10 years as compared with 18 years in the whole series of the 'cat club'.

The subjects listed in Table 2 were invited to a clinical examination at the Hospital for Allergic Diseases. Four of them accepted (Nos. 1, 2, 3 and 5), and they all proved to have a bronchial asthma.

IgG was purified from three sera positive in the haemagglutination test. As seen in Table 3, haemagglutinating activity in high titres was recovered in the IgG fraction.

Immunoelectrophoresis of the epithelial extract carried out against anti-feline serum showed that it contained albumin, several unidentified components in the α - and

Table 2. Cat fanciers with antibodies to cat epithelial extract

Club member	Age and sex	Length of exposure to cats (years)	RAST	Haemagglutination titre
1. (Ko)	18 M	4	3	2430
2. (Ri)	33 F	7	2	810
3. (Pe)	40 F	27	0.5	< 30
4. (Ma)	45 M	n.k.	0.5	< 30
5. (Ka)	47 M	25	0	810
6. (Vi)	35 F	n.k.	0	270
7. (Ha)	10 F	8	0	30
8. (Fr)	41 M	7	0	30

n.k. = not known.

Table 3. Antibody titres in sera and their respective IgG fractions* from three patients

Patient	IgG concentration mg/100 ml	Haemagglutination titre
Nu, serum	1.33	21870
Nu, IgG	0.61	10935
Kä, serum	1.26	7290
Kä, IgG	0.44	1215
Ka, serum	1.45	7290
Ka, IgG	0.45	405

* IgG was purified with DEAE cellulose column chromatography, its purity was checked by immunoelectrophoresis and concentration determined by single radial immunodiffusion.

β -globulin area and traces of IgG. To obtain information on the specificity of the haemagglutinating antibodies, cat serum was insolubilized with glutaraldehyde (Avrameas & Ternynck, 1969) and used for absorption. Table 4 shows that the antibodies could be absorbed with cat serum proteins in certain sera but not in others.

Table 4. Effect of absorption by glutaraldehyde-polymerized cat serum on haemagglutinating antibody titres in eight sera

Serum number	Haemagglutination titre	
	Before absorption	After absorption
1	270	30
2	21870	30
3	810	90
4	90	< 30
5	810	810
6	270	270
7	90	90
8	30	30

Discussion

In atopic subjects, precipitating antibodies may be present together with IgE antibody, e.g. in asthmatic reactions to enzymes of *Bacillus subtilis* and in certain avian allergies (Pepys & Turner-Warwick, 1975). Since the above agents frequently provoke late asthmatic reactions, this has been taken as evidence that these reactions are mediated by immune complexes. On the other hand, the existence of precipitating serum antibodies against many typical inhalant allergens such as tree or grass pollen or mammalian dander has not been documented.

In the present work we used a haemagglutination technique in which the antigen is coupled to red cells with chromic chloride. Antibodies could be demonstrated against cat epithelia in over 40% of patients with positive RAST to the cat antigen, whereas they were not detected in patients allergic to epithelial antigens of other animals or to tree or grass pollen. The antibodies were heterogeneous in specificity resembling in this respect the IgE antibodies (Brandt, Ponterius & Yman, 1973; Ohman, Kendall & Lowell, 1977). In some sera they were directed against serum protein components present in the extracts and in others against epithelial antigens. The role of rheumatoid factors was ruled out since the extract was shown to contain only traces of gamma globulin, not capable of sensitizing the cells. Moreover, the sera were negative in the sensitized sheep cell (Waalser-Rose) test.

The significance of the haemagglutinating antibodies remains to be clarified. Our observation that IgG type antibodies are frequently present in the sera of asthmatic patients sensitized to cat provides some further evidence for the immune-complex nature of the late asthmatic reactions. On the other hand, the antibodies may have a beneficial role. It is a common belief that immunotherapy is most successful in the treatment of asthma and hay fever associated with hypersensitivity to pollen (Reeves, 1977). If the success depends on the development of blocking antibodies of IgG class,

the meagre effect in animal dander allergies may be due to the existence of pretreatment IgG antibodies of the type studied by us.

It is also possible that the haemagglutinating antibodies are only an indirect marker of sensitization. In this respect it is interesting to observe that only 6% of the members of the 'cat club' were positive and a half of those with haemagglutinating antibodies had asthma. Thus a close contact and long exposure are not sufficient for their production.

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