

Reaginic and non-reaginic antibodies against mammalian danders

OUTI KUULIALA, K. AHO, K. A. J. JÄRVINEN and T. PALOSUO

National Public Health Institute, Helsinki, and Hospital for Allergic Diseases, Helsinki University Central Hospital, Helsinki, Finland

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Summary

Solid phase enzyme immunoassay (EIA) and passive haemagglutination techniques were used to study the occurrence of IgG-type antibodies against feline and bovine epithelial extracts among RAST-positive patients, senior veterinary students, cat fanciers (representing exposed subjects) and healthy controls. In most groups both techniques exhibited equal sensitivity being positive in about half of the RAST-positive cases. The levels of both EIA-reactive IgG-class and IgA-class antibodies were increased in most of the sera ($> \text{mean} + 2 \text{ s.d. of controls}$), while only a few sera showed increased IgM values.

Introduction

There is increasing evidence that IgG antibodies are involved in various types of asthma (Turner-Warwick, 1978). Firstly, certain IgG antibodies may exhibit reagin-like activity promoting immediate hypersensitivity reactions. Secondly, immunotherapy (hyposensitization) with allergen extracts has been used in the treatment of atopic asthma, and its success is commonly attributed to the development of a blocking IgG-type antibody. Thirdly, IgG antibodies are thought to provoke late asthmatic reactions by means of local immune complex formation.

The late (non-immediate) asthmatic reaction takes the form of an airways obstruction which appears slowly several hours after the exposure and is only poorly reversed by bronchodilators (Pepys & Turner-Warwick, 1975). It is occasionally associated with constitutional signs such as fever and malaise. There may thus be a considerable physiological overlap between patients exhibiting late asthmatic reactions and those with allergic alveolitis (Schatz, Patterson & Fink, 1979). The late reaction as an isolated phenomenon is typically elicited in non-atopic subjects, whereas patients with atopic asthma in most instances exhibit either the early reaction alone or a dual response.

Correspondence: Dr T. Palosuo, National Public Health Institute, Mannerheimintie 166, 00280 Helsinki 28, Finland

The late reactions are probably heterogeneous in mechanism (Schatz *et al.*, 1979). Evidence for the role of immune complexes is mainly indirect and is based on analogy with late cutaneous reactions which in certain instances have been described as compatible in histological picture with experimental Arthus immune complex reaction (Pepys *et al.*, 1968). On the other hand, late cutaneous reactions caused by other antigens, e.g. pollens, obviously can be mediated by IgE (Solley *et al.*, 1976). Non-immunological factors, such as altered bronchial reactivity, may be involved in some instances.

Late asthmatic reactions are fairly common in mammalian dander allergies. Figures given for the occurrence of IgG-type antibodies in these conditions are conflicting (Kuuliala *et al.*, 1979; Cockcroft *et al.*, 1981). In the present paper we provide further evidence of specific IgG-type antibody response, while its pathogenetic significance remains unresolved.

Material and methods

Patients and controls

Sera from twenty-four patients allergic to cows and from 109 patients allergic to cats, as shown by positive RAST results (see below), were obtained from the Hospital for Allergic Diseases, University Central Hospital, Helsinki and from the Department of Immunobiology, National Public Health Institute, Helsinki, Finland. Serum samples from sixty-seven senior veterinary students, together with details of any allergy symptoms obtained through a questionnaire, were collected in 1978 by courtesy of Dr Markus Sandholm of the College of Veterinary Medicine, Helsinki. Sera and clinical information from 103 cat fanciers were available to us from a previous study (Kuuliala *et al.*, 1979). Control specimens were obtained from fifty healthy blood donors from the Finnish Red Cross Blood Transfusion Service, and from 106 healthy pregnant women, taken primarily for antenatal Rh-testing.

Serological tests

Specific IgE antibodies. The determination of specific IgE antibodies to bovine and feline epithelial antigens (RAST) was performed by radioimmunoassay with commercial kits (Pharmacia Diagnostics, Sweden).

The haemagglutination test. This test for antibodies to bovine and feline epithelial extracts was performed using chromic chloride as the coupling agent as previously described (Kuuliala *et al.*, 1979). The feline epithelial extract was purchased from Bencard (U.K.), and the bovine epithelial antigen was prepared by extracting 10 g cow dander in 100 ml of 7 mM phosphate buffer containing 0.5% NaCl and 0.4% phenol, pH 7.0, by continuous shaking for 2 days at room temperature.

Solid-phase enzyme immunoassay (EIA). For the demonstration of antibodies to bovine epithelial extract, a macro-EIA employing disposable 12 × 50 mm polystyrene tubes (Mekalasi Ltd, Finland) was used. EIA-reactive antibodies to cat epithelial extract were measured by micro-EIA using ninety-six-well microtitre plates (Lindbro®, Flow Laboratories, U.K.).

IgG-class antibodies to bovine epithelial extract. These were demonstrated as follows. The antigen, which was the same as in the HA assay, was diluted in 0.05 M carbonate buffer, pH 9.2, to a protein concentration of 3–5 µg/ml, placed in 0.4-ml volumes into polystyrene tubes, and incubated at 37°C for 16 hr. The tubes were

emptied and 0.4 ml of 0.05 M carbonate buffer, pH 9.2, containing 0.5% bovine serum albumin (BSA) was added and the tubes incubated at 37°C for 2 hr. After three washes with phosphate-buffered saline (PBS), pH 7.3 containing 0.2% BSA and 0.05% Tween 20 (PBS-BSA-Tween), 0.3 ml serum dilutions (1:10 and 1:100), made in the same buffer, were pipetted into the tubes, which were then incubated at 4°C overnight. The tubes were washed three times with PBS-BSA-Tween and 0.3 ml alkaline phosphatase-conjugated swine anti-human IgG (Orion Diagnostica, Finland), diluted 1:100, was added. Incubation was at room temperature for 5 hr, followed by three washes, after which 0.3 ml of the substrate solution (p-nitrophenyl-phosphate, Sigma Chemical Co., U.S.A., 1 mg/ml in 0.05 M carbonate buffer, pH 9.8, containing 0.001 M MgCl_2) was added. The tubes were incubated for 30 min at 37°C and the reaction was stopped by the addition of 75 μl of 3 M NaOH and 0.6 ml distilled water. The absorbance was measured at 405 nm on a Perkin-Elmer spectrophotometer.

IgG-class, IgA-class and IgM-class antibodies to feline epithelial extract (Allergologisk Laboratorium A/S, Denmark). These were demonstrated using micro-EIA. Bencard's antigen preparation used in HA tests was no longer available. The tests were performed as follows: optimally diluted antigen (protein concentration 4–8 $\mu\text{g}/\text{ml}$) in 0.05 M carbonate buffer, pH 9.6, was incubated on microtitre plates (250 $\mu\text{l}/\text{well}$) at 4°C overnight. The plates were emptied, shaken dry, and 250 μl of 0.05 M carbonate buffer, pH 9.6, containing 0.5% human serum albumin, was added followed by incubation for 3 hr at room temperature. The plates were emptied and washed three times with PBS containing 0.05% Tween 20 (PBS-Tween). Serum dilutions (1:20 and 1:100) in 200 μl volumes, were then pipetted onto the plates and incubated overnight at 4°C. After three washes with PBS-Tween, 200 μl of 1:100 diluted alkaline phosphatase-conjugated swine anti-human IgG, anti-human IgA or anti-human IgM (Orion Diagnostica) was added and the plates were incubated for 5 hr at room temperature. After three washes, 200 μl p-nitrophenyl-phosphate (1 mg/ml in 0.05 M carbonate buffer, pH 9.8, containing 0.001 M MgCl_2) was added and the plates incubated for 30 min at room temperature. The reaction was stopped by adding 50 μl of 3 M NaOH per well and the absorbances were measured at 405 nm on a Titertek Multiskan® photometer (Labsystems Oy, Finland). All measurements were carried out in duplicates.

Results

Antibodies to bovine epithelial extract

The results concerning HA-reactive and EIA-reactive (IgG-class) antibodies among allergic patients, RAST-negative senior veterinary students (representing subjects exposed regularly to cows) and controls (healthy pregnant women) are shown in Table 1. In the sera of the patients and the controls both tests exhibited equal sensitivity, whereas the HA test was superior to EIA in detecting antibodies to bovine epithelial antigens among the veterinary students. The results of HA, EIA and RAST were then compared among the whole group of sixty-seven veterinary students, six of whom were RAST-positive. The results, shown in Table 2, indicate that positive RAST results coincided in five out of six cases with elevated values in both the HA test and EIA.

Antibodies to feline epithelial extract

The results concerning HA-reactive and EIA-reactive (IgG-class) antibodies among RAST-positive patients allergic to cat, cat fanciers (representing regularly exposed

Table 1. Haemagglutinating (HA) and EIA-reactive antibodies to bovine epithelial antigen

Subjects	Number of sera tested	HA-positive*		EIA-positive†	
		<i>n</i>	%	<i>n</i>	%
RAST-positive patients with allergic symptoms to cow	24	14	58	15	63
RAST-negative veterinary students (exposed subjects)	61	32	53	15	25
Healthy pregnant women	106	5	5	3	3

* Ha-titre ≥ 30

† EIA-result > mean of controls + 2 s.d.

Table 2. Haemagglutinating (HA), EIA-reactive, and RAST-reactive antibodies to bovine epithelial antigen among sixty-seven senior veterinary students

EIA result	Number of subjects with HA-titre: (number in parentheses indicate the number of RAST-positive individuals)		
	0-10	30-270	≥ 810
$\bar{x} + > 4$ s.d.*	0	4 (1)	4 (3)
$\bar{x} + 2-4$ s.d.	1	6 (1)	0
$\bar{x} + < 2$ s.d.	34 (1)	18	0

* $\bar{x}$ = mean of control subjects; s.d. = standard deviation

individuals) and controls (blood donors) are shown in Table 3. The results resemble those obtained with the bovine antigen, with the exception that fewer positive reactions were found among the exposed subjects (cat fanciers compared with veterinary students). The results of HA, EIA and RAST were then compared among 103 cat fanciers. Table 4 shows that the two positive results in RAST coincided with high values in both the HA test and EIA.

IgG-class, IgA-class and IgM-class antibodies to feline epithelial extract demonstrated by EIA

Sera from nineteen RAST-positive patients allergic to cat and from nineteen blood donors were tested by EIA at a serum dilution of 1:20. The results, depicted in Fig. 1, show that antibodies belonging to all the major immunoglobulin classes were present. In the majority of the patient sera, the levels of both IgG-class and IgA-class antibodies

Table 3 haemagglutinating (HA) and EIA-reactive antibodies to feline epithelial antigen

Subjects	Number of sera tested	HA-positive*		EIA-positive†	
		<i>n</i>	%	<i>n</i>	%
RAST-positive patients with allergic symptoms to cat	109	51	47	45	41
Cat fanciers (exposed subjects)	103	5	5	15	15
Blood donors	50	NT‡		2	4

* HA titre ≥ 30 † EIA result $>$ mean of controls $+ 2$ s.d.

‡ NT = not tested. In our previous study, two of 206 (1%) control subjects were positive in the the HA assay with feline antigen

Table 4. Haemagglutinating (HA), EIA-reactive, and RAST-reactive antibodies of feline epithelial antigen among 103 cat fanciers

EIA result	Number of subjects with HA-titre: (numbers in parentheses indicate the number of RAST-positive individuals)		
	0-10	30-270	≥ 810
$\bar{x} + > 4$ s.d.*	1	1	3 (2)
$\bar{x} + 2-4$ s.d.	10	0	0
$\bar{x} + < 2$ s.d.	87	1	0

* $\bar{x}$ = mean of control subjects; s.d. = standard deviation

were elevated ($>$ mean $+ 2$ s.d. of controls), but only a few sera showed elevated IgM values.

Discussion

We reported previously that haemagglutinating IgG-type antibodies to cat dander co-existed with IgE antibodies in over 40% of patients with respiratory allergy and sensitivity to cat dander, whereas they were detected in only 3% of exposed but asymptomatic subjects and in 1% of asthmatic patients sensitized to other allergens (Kuuliala *et al.*, 1979). In the present communication we extended the study on the occurrence of IgG-type antibody in mammalian dander allergies to cover another (bovine) test antigen and another (solid phase EIA) technique.

Haemagglutinating antibodies against bovine epithelial extract were detected in exposed subjects more frequently than those against cat dander. Yet the RAST-positive cases accumulated among those with the highest haemagglutination titres. The

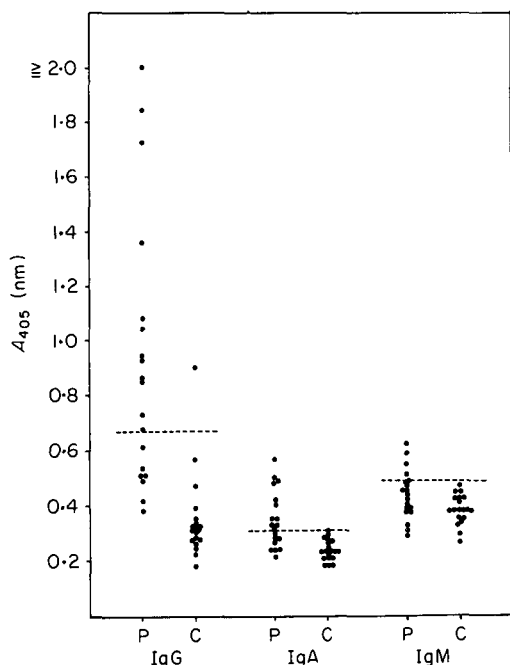


Fig. 1. Distribution of EIA-reactive IgG-class, IgA-class and IgM-class antibodies to cat dander in patients and controls. P, patients allergic to cat shown by positive RAST ($n = 19$); C, controls (blood donors, $n = 19$). Horizontal broken lines indicate the mean EIA value of the controls + 2 s.d. for each antibody class. A_{405} (nm) = absorbance at 405 nm.

occurrence of a specific IgG antibody response to mammalian danders was confirmed using EIA, although the distinction between positives and negatives was somewhat less clear than when using the haemagglutination technique. A likely reason for this is that only avid antibodies against major antigenic components are detected with the haemagglutination technique. Our observations are in contradiction with those of Cockcroft *et al.* (1981), who did not find any difference in IgG antibody levels between non-exposed and exposed subjects, or between those with and without animal-related symptoms. One possible reason for the discrepancy is that their antigens were prepared from serum and urine, whereas we used epithelial extracts.

It has been suggested that the inability to form specific IgA antibodies is one determinant predisposing to allergic clinical disorders (Stokes, Taylor & Turner, 1974). This view was disputed by Platts-Mills *et al.* (1978), who found a close correlation between the individual IgE, IgA and IgG antibody responses to pollen allergens in atopic individuals. Our findings are essentially in agreement with the latter work, although the correlations were not as clear-cut. This may be due to heterogeneity of the danders with respect to their antigenic properties and particle size. This, in turn, results in variation in the deposition of the antigenic material in the airways and may be important in determining the type of antibody response (Mygind, 1978).

The role of the IgG antibodies in extrinsic allergic alveolitis is well established (Pepys & Turner-Warwick, 1975), while their significance in asthma is not known. They may be harmful by provoking late asthmatic reactions, beneficial by blocking IgE-mediated hypersensitivity reactions, or only indirect markers of sensitization. The problem can be approached either on the basis of findings in bronchial provocation

tests or of the history given by the patients. In this respect it should be noted that patients with hay fever without any evidence of asthma may exhibit positive reactions in bronchial challenge (Lichtenstein, 1978).

We chose senior veterinary students in order to obtain a uniform study group capable of giving an accurate history. Yet it proved difficult to extract any clear-cut information. Several of the students gave a history of late symptoms, but their connection with animal exposure was by no means obvious, and the subgroup included RAST-positive individuals negative in HA tests and EIA. It thus appears that not all late reactions due to animal exposure are immune-complex-mediated responses.

Acknowledgments

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