

Immunoglobulin E Antibodies against Budgerigar and Canary Feathers

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Abstract. In 212 sera from budgerigar and canary fanciers with symptoms of rhinitis and/or bronchial asthma, IgE antibodies against budgerigar feathers (Budf) or canary feathers (Canf) were determined. In 25 of 98 Canf-specific IgE antibody measurements, and in 28 of 154 Budf-specific IgE antibody measurements, a significant (6% or more binding of ¹²⁵I-anti-IgE) level of specific IgE was found. In 3 sera with the highest levels of Canf- or Budf-specific IgE, IgE antibodies against sera from both birds were present. It is concluded that IgE antibodies against canary and/or budgerigar feathers are present in about 20% of canary and budgerigar fanciers with symptoms of atopic disease. Canary and budgerigar feathers contain IgE-binding antigens that are not present in the corresponding bird sera and droppings.

Introduction

Hargreave et al. [1] showed that pigeon and budgerigar breeders may develop a pulmonary disease which resembles farmer's lung. They termed this disease 'bird breeder's (fancier's) lung'. In serum from the affected patients precipitins were present that were directed against antigens from avian droppings and sera. Fink et al. [2] and recently Banham et al. [3] showed that pigeon feathers are also an important source of antigen in bird fancier's lung. Faux et al. [4] found IgE antibodies against budgerigar serum proteins in atopic patients with asthma due to budgerigar allergen. The aim of the present study was to compare the IgE response against canary and budgerigar serum with the IgE response against the corresponding bird feathers and droppings.

Materials and Methods

Human Sera and Leukocytes

Sera were obtained from patients who all had symptoms of rhinitis and/or bronchial asthma and who all were frequently exposed to canaries and/or budgerigars. None of the patients showed the clinical features of hypersensitivity pneumonitis (i.e. progressive dyspnea, cough, malaise, weakness, weight loss or fever). Leukocytes were donated by patients with serum IgE antibodies against budgerigar (Budf) and canary feathers (Canf), and from control donors, with their informed consent.

Allergens

Lyophilized extracts of Budf and Canf and lyophilized extracts of budgerigar- and canary droppings were obtained from Diephuis, Groningen, The Netherlands. Budgerigar serum (Buds), canary serum (Cans) and house dust mite culture (*Dermatophagoides pteronyssinus*) were also obtained from Diephuis. Egg yolk allergen was prepared as follows: 14 g chicken egg yolk were mixed with 150 ml acetone for 3 h at room temperature. After filtering off the acetone, the remaining material was mixed with 150 ml acetone for 3 h; after filtering off the acetone, the remaining material was air-dried at room temperature. The yield (expressed as percentage of the starting material, w/w) was 54%. Chicken egg white was prepared as described before [5].

Estimation of Total IgE and Allergen-Specific IgE

Total IgE was determined by a noncompetitive immunoradiometric assay (Tandem IgE kit, Hybritech, Luik, Belgium) according to the manufacturers' instructions. Allergen-specific IgE was determined by a radioallergosorbent test (RAST), using agarose beads as allergen support [6]. Allergens were coupled to CNBr-activated Sepharose 4B according to the manufacturers' (Pharmacia) instructions. For the preparation of Sepharose-coupled extracts of feathers and droppings from budgerigars and canaries, 100 mg lyophilized feather or droppings extract was dissolved in 1-2 ml coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl) and coupled to 100 mg CNBr-activated Sepharose 4B. After 4 cycles of acid/base washings [6], the allergen-Sepharose preparations were washed once with RAST buffer [0.82% NaCl, 10 mM phosphate, 0.1% NaN₃, 0.2% Tween 20, 0.3% bovine serum albumin (BSA; Sigma), pH 7.4] and finally resuspended in RAST buffer. For the preparation of Buds- and Cans-Sepharose, 50 µl bird serum were coupled to 100 mg CNBr-activated Sepharose 4B. For the preparation of chicken egg yolk Sepharose, 5 mg acetone-treated chicken egg yolk was extracted for 1 h in 1-2 ml coupling buffer at room tempera-

Table I. Results of 98 Canf IgE and 154 Budf IgE antibody measurements, arranged according to binding percentage of ^{125} I-anti-IgE

Binding ^{125} I-anti-IgE, %	Number of specific IgE antibody measurements	
	Canf	Budf
0-5	73	126
6-10	16	15
11-20	8	11
21-30	1	2

Table III. IgE against avian antigens

Patient	Canary			Budgerigar			Chicken		House dust mite	Total IgE IU/ml
	feathers	serum	droppings	feathers	serum	droppings	egg yolk	egg white		
-	1.7	0.4	0.1	1.3	0.4	0.2	0.5	0.3	0.7	
A	10	1	0	14	1	2	1	0	32	650
B	2	0	0	12	0	1	1	0	34	500
C	7	1	0	16	1	1	1	1	35	1,300
D	15	3	1	3	1	3	4	4	6	4,250
E	8	1	0	0	0	1	1	1	2	105
F	27	38	4	5	17	10	18	3	0	650
G	18	1	2	4	2	5	3	1	9	1,450
H	12	27	9	29	35	27	20	25	1	640
I	9	16	8	23	28	25	12	10	2	430

Nine sera in which IgE against Canf and/or Budf was present were tested for IgE against sera and droppings from these birds and for IgE against chicken egg yolk and egg white. The results are expressed as binding percentage of ^{125} I-anti-IgE.

ture. After centrifugation, protein in the supernatant was coupled to 100 mg CNBr-activated Sepharose 4B. Per test 1 mg of the budgerigar feather-, serum and droppings-Sepharose preparations, 1 mg of the canary feather-, serum- and droppings-Sepharose preparations and 1 mg egg-yolk Sepharose was used. An extract of chicken egg white was coupled to Sepharose 4B as described before [5]; 0.75 mg egg white Sepharose was used per test. An extract of house dust mite culture (*D. pteronyssinus*) was coupled to Sepharose 4B as described before [7]; 2 mg of mite-Sepharose was used per test. In the RAST, 50 μ l of patient serum were incubated for at least 4 h at room temperature with the indicated amounts of allergen-sepharose with horizontal shaking (300 cycles/min). After 3 washes with physiological saline, 0.2% Tween 20 and 1 wash with RAST buffer, radioiodinated anti-human IgE antibodies (Pharmacia) were added. After an overnight incubation, the agarose beads were washed 4 times with physiological saline, 0.2% Tween 20. The percentage of bound radioactivity was counted in an LKB-Wallace gamma-counter.

Histamine Release from Washed Leukocytes

The isolation of human leukocytes and the incubation of leuko-

Table II. Test for nonspecific IgE binding to bird feathers

Control serum	Total IgE IU/ml	Budf IgE RAST ¹	Control serum	Total IgE IU/ml	Canf IgE RAST ¹
1	3,500	2	6	3,600	1
2	1,700	2	7	2,500	5
3	1,300	2	8	1,500	3
4	1,050	2	9	750	2
5	850	1	10	380	0

Sera with various total IgE levels were incubated with Sepharose-coupled Budf and Canf extract.

¹ Binding of ^{125} I-anti-IgE, %.

cytes with allergen were performed according to Siraganian and Hook [8]. In brief, 10 ml blood was mixed with 1 ml 0.1 M EDTA pH 7.4 and 2.5 ml physiological saline that contained 6% clinical dextran (Sigma) and 3% D-glucose. After 1 h sedimentation the yellow buffy coat layer was taken off. Leukocytes from the buffy coat were washed twice with PIPES-A [25 mM 1,4-piperazinediethane sulfonic acid, 120 mM NaCl, 5 mM KCl, 0.03% human serum albumin (Behring Werke, Marburg, FRG), pH 7.4] and finally resuspended in PIPES-ACM (PIPES-A, 1 mM CaCl₂, 0.5 mM MgCl₂). The leukocytes were incubated with allergen for 45 min at 37°C. The leukocytes were challenged with the same allergen preparations that were used in the RAST. Histamine in samples was determined by an automated fluorometric method [8]. The amount of histamine release was expressed as percentage of the total amount of histamine present in the cell suspension.

Precipitating Antibodies

Precipitating antibodies against feathers, serum and droppings from budgerigars and canaries were determined by double diffusion (DD) according to Ouchterlony [9]. The DD was performed with the same allergen preparations that were used in the RAST.

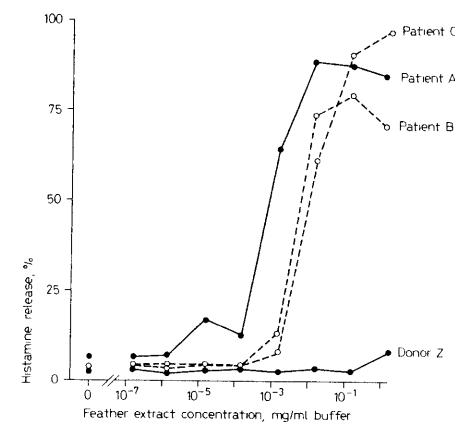


Fig. 1. Histamine release from leukocytes of 3 patients allergic to Budf and from leukocytes of a nonallergic donor on incubation with an extract of Budf.

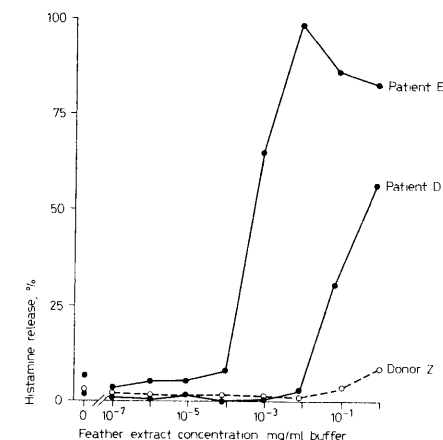


Fig. 2. Histamine release from leukocytes of 2 patients allergic to Canf and from leukocytes of a nonallergic donor on incubation with an extract of Canf.

Results

IgE against Bird Feathers

In 212 sera from budgerigar or canary fanciers with symptoms of rhinitis and/or bronchial asthma, IgE antibodies against Budf and/or Canf were determined. In 26% of the Canf IgE RASTs, and in 18% of the Budf IgE RASTs, 6% or more binding of ^{125} I-anti IgE was observed (table I). In order to test the specificity of the Canf- and Budf IgE RAST, sera with various total IgE levels were incubated with these immunosorbents. At a serum total IgE level of 1,500 IU/ml, the nonspecific binding was 2-3% (table II).

IgE against Bird Sera and Droppings

9 sera from table I with various IgE antibody levels against Canf and Budf were also tested for IgE antibodies against sera and droppings from these birds. Table III shows that IgE antibodies against the bird sera and droppings are only present in patient sera with high levels of IgE against bird feathers (sera F, H and I in table III). These 3 sera also contained IgE antibodies against chicken egg yolk; in sera H and I IgE antibodies against chicken egg white were present (table III).

Release of Histamine by Canary and Budgerigar Feathers

Leukocytes from 5 patients of table III were investigated in the histamine release test. Leukocytes from patients A, B and C (see table III) were incubated with Budf extract. With leukocytes from all 3 patients, more than 75% histamine release was observed (fig. 1). Leukocytes from patients D and E (see table III) released histamine upon challenge with Canf-extract (fig. 2). In three experiments with leukocytes from nonallergic control donors, no histamine release was observed on incubation of these leukocytes with Budf and Canf extract. A representative result of an experiment with leukocytes from a control donor (Z) is shown in figure 1 and 2.

Precipitating Antibodies

In Ouchterlony tests [9] with sera from patients A-I (see table III) no or only weak precipitates were seen after incubation of the sera with extracts of feathers, serum or droppings from canaries or budgerigars. With these extracts, precipitates were clearly demonstrable in sera from patients with extrinsic allergic alveolitis, induced by exposure to canaries and budgerigars.

Discussion

Respiratory disease induced by contact with birds like pigeons, budgerigars and canaries is generally thought to be an example of extrinsic allergic alveolitis. The pathogenesis of this disease involves both humoral factors (precipitins) and cellular factors [10]. In serum from many asymptomatic individuals exposed to organic dusts from pigeons, precipitins occur. Tests that demonstrate cellular rather than humoral mechanisms are reported to discriminate more accurately than the precipitin tests between ill and asymptomatic but exposed individuals [10].

There is evidence from the literature that IgE-mediated mechanisms may also be involved in allergy towards bird-derived materials. Barr and Sherman [11] showed in 1961 by passive sensitization studies that serum from patients with asthma to parakeet and canary feathers could passively sensitize the skin of a nonallergic recipient. Ebner et al. [12] recently described the occurrence of IgE antibodies against pigeon-droppings and -serum in patients with perennial asthma and a long-term exposure to bird antigens. The results of the present study (table I) show that IgE antibodies against bird feathers are present in about 20% of budgerigar and canary fanciers with symptoms of atopic disease. Many patients had developed an IgE response against both Budf and Canf. Table III however shows that there is no correlation between the Canf IgE RAST and the Budf IgE RAST. With the exception of serum A, the IgE response in the patients was mainly directed against feathers of only one bird. This suggests that the major allergen from Budf is different from the major allergen of Canf. Table II shows that the Budf- and Canf-Sepharose preparations used had a negligible IgE-binding with serum total IgE levels up to 1,500 IU/ml. This demonstrates that the IgE-binding of sera A-I (table III) to Budf- and Canf-Sepharose was not caused by nonspecific, nonimmune IgE-binding.

The house dust mite *D. pteronyssinus* grows on human and animal epithelial components [13]; theoretically, a positive RAST for bird feathers could in fact be caused by a positive RAST for (contaminating) house dust mite. Sera F, H and I were found definitely positive in the feather IgE RAST (table III); these sera were negative in the house dust mite IgE RAST. This demonstrates that the IgE binding to bird feather extracts was not caused by contamination of the bird feather extracts with house dust mite allergen.

The results of the histamine release test (fig. 1, 2) further confirm the presence of an IgE-mediated allergy towards Budf (patients A-C) and Canf (patients D and E). Leukocytes from 3 normal nonallergic donors did not release histamine on incubation with Budf or Canf extracts: this demonstrates that the release of histamine from leukocytes of patients A-E was not caused by a toxic effect of the feather extracts on the leukocytes. Only the 3 sera with the highest levels of IgE against bird feathers were found positive in the Cans or Buds IgE RAST (table III). This suggests that Canf and Budf contain more different IgE-binding antigens than the corresponding bird sera. This finding is in line with the results of Banham et al. [3], who recently showed that pigeon bloom, the waxy powder that coats the feathers of racing pigeons, is a highly antigenic material. These authors found a higher incidence (60%) of precipitins against bloom extract than against pigeon serum in pigeon fanciers (38%).

Sera with IgE antibodies against Cans and Buds also contained IgE antibodies against chicken egg yolk. Similar observations were made before by Aalberse (personal communication Dr. R.C. Aalberse, 1978). There was no clinical evidence of allergy to eggs in patients F, H and I. In summary, in about 20% of budgerigar and canary fanciers with symptoms of atopic disease, IgE antibodies against the corresponding bird feathers were present. More patients were found positive in the bird feather IgE RAST than in the corresponding bird serum or droppings IgE RAST.

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