

Clinical correlations between long-term (IgE) and short-term (IgG S-TS) anaphylactic antibodies in atopic and 'non-atopic' subjects with respiratory allergic disease

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Summary

The sera of atopic and non-atopic persons with allergic pulmonary disorders were examined for long-term sensitizing, IgE and short-term sensitizing heat-stable (S-TS) antibodies which were present separately or together in the sera of some patients sensitive to antigens such as budgerigar serum proteins and *Aspergillus fumigatus*.

In fourteen atopic patients with extrinsic asthma, six had both types of antibody to common allergens, and of nine non-atopic patients with cryptogenic (intrinsic) asthma, four had only heat-stable short-term sensitizing antibodies. The sera of atopic subjects with type I prick test reactions and positive RAST's, showed specific IgE antibody by baboon PCA tests to budgerigar serum proteins, *A. fumigatus*, Timothy grass pollen extract and hen egg extract, and not to *Dermatophagoides farinae*, possibly because of naturally occurring mite antibodies in the baboon.

The sera of non-atopic asthmatics, who had given negative prick test but positive immediate, dual or late intracutaneous tests, and only late asthmatic reactions, contained precipitins in most cases and gave little or no RAST reaction. On baboon PCA these sera contained either, S-TS antibody alone, or S-TS plus long-term sensitizing antibody, or long-term sensitizing antibody alone. Some of the sera with long-term sensitizing antibody contained blocking antibody which could diffuse away in the 24 hr delay for the baboon PCA test and could also be responsible for the negative RAST.

Tests with insoluble anti-IgE immuno-adsorbents on two sera from persons sensitive to aspergillus confirmed that the S-TS activity was not due to IgE, and on two sera with negative RAST and negative prick tests to budgerigar serum antigens confirmed that the 24 hr monkey PCA responses were due to IgE.

Introduction

Heat-labile, long-term sensitizing antibodies are the usual mediators of type I, immediate allergic reactions. Specific IgE antibodies present in very small amounts may be

detected by the radioallergosorbent, RAST, technique (Wide, Bennich & Johansson, 1967; Wide *et al.*, 1971; Wide, 1973; Johansson, Bennich & Berg, 1971). Good agreement, 90–100%, between the RAST, prick and provocation tests has been reported for several common allergens and between the size of the prick test weal and the RAST (Wide *et al.*, 1971; Aas & Lundkvist, 1973; Pepys *et al.*, 1975; Leegard & Roth, 1977; Stenius *et al.*, 1971; Pauli *et al.*, 1977). There are, however, many examples where these correlations are absent.

Another antibody capable of mediating type I reactions is found in some allergic subjects and so far designated IgG S-TS (short-term sensitizing). This sensitizes human or primate skin for 2 to 4 hr, occurs in low titre, is heat-stable, and resistant to 2-mercaptoethanol, and it appears to compete for a short period with IgE for receptor sites on mast cells (Parish, 1970, 1971, 1973). Short-term heat-stable sensitizing antibody has been reported by Bryant, Burns & Lazarus (1973, 1975); Longbottom *et al.* (1976); Berry & Brighton (1977); Warren, Cherniack & Tse (1977); Lakin *et al.* (1978).

We report here details of baboon PCA tests made between 1971 and 1975 and presented as a summary (Pepys *et al.*, 1975) for IgE and S-TS, presumably IgG antibodies in the sera of patients allergic to one or several common allergens.

We have looked in these studies for an association of IgE and IgG S-TS antibodies in atopic and non-atopic subjects with respiratory disease in relation to: common allergens in atopic subjects; the presence of precipitins in general and in non-atopic subjects with farmers' lung due to *M. faeni*; non-atopic subjects with asthma due to avian allergens, and a comparison between subjects with atopic, extrinsic asthma and cryptogenic (intrinsic) asthma.

Materials and methods

Patient and study groups

The term 'atopy' was used here to describe the production of IgE antibody by the exposures of ordinary daily life to common allergens and demonstrable by skin prick and RAST tests.

IgE and IgG S-TS antibodies to common allergens (Table 1). The sera of groups of asthmatics ranging from seventeen to twenty-five subjects were examined for antibodies to: *D. farinae* (twenty-five patients); *A. fumigatus* (twenty-five); Timothy grass pollen (twenty-six); milk (twenty-three) and egg (seventeen).

Relationship of IgE and IgG S-TS antibody and precipitins to A. fumigatus (Table 2). Sera from groups of patients with (a) pulmonary aspergilloma (twenty-two patients), with strong precipitin tests in all cases, and with positive prick tests in a small number, to *A. fumigatus*; (b) allergic bronchopulmonary aspergillosis (thirty-four), that is, asthma and pulmonary infiltrations with blood eosinophilia and type I reactions to *A. fumigatus* in prick tests (McCarthy & Pepys, 1971; Malo *et al.*, 1977), with weak precipitin tests in most cases and (c) uncomplicated asthma (ten patients) with positive prick tests to *A. fumigatus* and negative precipitin tests.

IgG S-TS and precipitins to M. faeni (Table 1). Sera from a group of twenty-five patients with farmers' lung, all of whom had positive precipitin tests to *M. faeni* were examined.

IgE and IgG S-TS antibodies to avian antigens in non-atopic asthmatic patients

Table 1. Summary of results of monkey PCA for IgE and S-TS antibodies in sera of persons allergic to various antigens

Donors allergic to	Number examined	Number prick test positive	Number with precipitins	Monkey PCA		
				S-TS		
				24 hr	2 hr	4 hr
<i>D. farinae</i>	25	14	na	0	0	0
Grass pollen	25	17	na	8	2	na
Milk	23	8	na	0	—	2
Egg	17	12	na	1	—	2
<i>M. faeni</i> (farmers' lung)	25	na	25	0	0	0

na = not available.

Table 2. Allergy to *Aspergillus fumigatus*. Prick test responses, precipitin tests and monkey PCA for IgE and S-TS antibodies

Disorder	Number examined	Prick test response	Number with precipitins	Monkey PCA			
				24 hr	2 hr	4 hr	Total
Aspergilloma	22	8 Positive	22	3	0	0	
		4 Negative		0	0	0	
		10 n.a.		5	0	0	8
<i>A. fumigatus</i> allergy	10	10 Positive	0	3	na	1	4
Allergic bronchopulmonary aspergillosis	34	34 Positive	34	31	10	6	31

na = not available.

(Tables 3, 4, 5). Sera from a group of eleven non-atopic budgerigar fanciers with asthma, and negative prick tests and only weakly positive RAST's in two cases.

Comparison of IgE and IgG S-TS in atopic, extrinsic asthma and cryptogenic asthma (Table 6). A group of fourteen atopic asthmatics, all of whom had had infantile eczema and early onset of asthma and gave positive prick test reactions to several common allergens were compared with a group of nine patients defined as cryptogenic (intrinsic) by virtue of the 'late' onset of their asthma and negative prick test reactions to a battery of twenty-two routine common allergens. Tests were made by baboon PCA for 24 hr sensitization by unheated and 2 and 4 hr sensitization by heated serum.

Absorption studies for IgE and IgG S-TS antibodies. The effects of absorption of the sera with an anti-IgE immunosorbent on the S-TS activity was studied with two sera containing IgE and IgG S-TS to *A. fumigatus*. One control serum of a grass pollen-sensitive subject with IgE antibodies and two sera from avian-sensitive subjects were similarly studied.

Materials

Tests in patients. The allergens used for prick-testing were prepared from freeze

Table 3. Results of tests on eleven non-atopic patients with asthma induced by sensitivity to budgerigar serum antigens

Patient	Years exposed to budgerigars	Avian Ag		Bronchial challenge-budgerigar serum	PCA no. + /3 baboons				RAST budgerigar sorbent units	IgE total units	PPTNS budgerigar serum	Blocking antibody
		Routine prick test	Intra-cutaneous		24 hr Unheated	2.5 hr						
						Unheated	Heated					
B.A.	10	—	Late	Late	3	0	0	0	0.54	488	+	Yes
B.U.L.	8	—	Dual	Na	0	0	0	0	Nil	147	+	
B.U.R.	1	—	Dual	Late	0	1	1	1	0.64	9.5	+	
B.U.S.	13	—	Dual	Late	0	1	1	1	1.30	10.5	+	
C.A.	25	—	Dual	Na	3	2	2	2	Nil	16.4	+	
C.O.	1	—	Late	Late	1	0	0	0	Nil	34.6	+	Yes
D.	15	—	Dual	Late	1	0	0	0	0.70	290	+	
G.	4	—	Late	Late	0	0	0	0	0.49	50	+	
M.A.	29	—	Imm	Na	3	2	2	2	0.80	650	—	No
M.I.	2	—	Na	Na	0	0	0	0	0.57	1.6	—	
P.	13	—	Imm	Na	3	2	1	1	Nil	33.5	+	Yes

Table 4. Presence or absence of blocking antibody for RAST (for IgE antibody to avian antigen) in four non-atopic asthmatic sera related to other findings, and compared to results from two atopic sera

Atopic status	Patient	Prick test		Intracutaneous injection avian	Bronchial challenge	PPTNS Budgerigar	PCA 24 hr No. pos/3	RAST IgE		Blocking antibody
		Routine	Avian					Budgerigar	Total	
Atopic	H.	* + + +	† + + +	Dual	Imm	+	2	1.9	466	
Non-atopic	S.	+	+	Late	nd	—	3	44	78	
	B.	—	—	Late	Late	+	3	0.5	488	Yes
	P.	—	—	Imm	nd	+	3	0	33.5	Yes
	C.O.	—	—	Late	Late	+	1	0	34.6	Yes
	N.A.	—	—	Imm	nd	—	3	0.8	650	No

* + + + + +, Positive reactions to common allergens.

† + + + + + + +, Intensity of reaction to prick test with avian allergen.

Table 5. Summary of the results of tests made on eleven non-atopic persons with asthma due to sensitivity to budgerigar serum antigen

Test	No. pos/No. tested
Prick test responses to routine and to avian antigens	0/11
Intracutaneous injection of avian antigens responses:	
immediate two, late three, dual five persons	10/10
Bronchial challenge to budgerigar antigen (all late responses)	6/6
Precipitins to budgerigar antigen	9/11
RAST 'sorbent units 1 to budgerigar antigen	1/11
Rast 'sorbent units 1 to one of five other avian antigens (one antigen positive in each of two persons)	2/11
Blocking antibody for RAST antibody to budgerigar	3/4
Monkey PCA tests: 24 hr alone, two; S-TS alone, two; 24 hr + S-TS, four persons	8/11

Table 6. Heat-stable short term antibody and 24 hr IgE-like antibody from patients with extrinsic or cryptogenic asthma mediating PCA in baboons

Antigens two pools of three antigens	Number of prick tests positive	Baboon PCA: no. positive after hours sensitisation				
		Short		Long 24 hr	Total Short + Long	Total positive
		1 hr	4 hr			
Extrinsic asthma (infantile eczema) total 14						
Milk	2	n.d.	6	6		
Pollen	13		6	6	6	6
Hen egg	4					
<i>Candida albicans</i>	1	0/8	0/8	0/8	0/8	0/8
<i>Aspergillus fumigatus</i>	4					
<i>D. farinae</i>	13					6/14
Cryptogenic (intrinsic) asthma total 9						
Milk/pollen/egg	0	3	1	0	0	3
<i>C. albicans</i> / <i>A. fumigatus</i> & <i>D. farinae</i>	0	3	2	0	0	3
						4/9*

* In the cryptogenic asthma group the total positive reactions for S-TS IgG antibody for four out of nine was made up by two sera reacting to the first pool of antigens and another two to the second pool of antigens.

dried extracts at 10 mg/ml, except for sterile budgerigar serum which was used undiluted and diluted after heating at 56°C for 2 hr in order to inactivate any virus contamination. Intracutaneous tests were made with budgerigar serum diluted 1/10 up to undiluted serum and the same materials were used for inhalation provocation tests.

Tests on baboons. In the comparison of atopic extrinsic, and cryptogenic asthmatics, two mixtures of antigen were used, the first containing equal parts of milk, Timothy grass pollen and egg extracts and the second equal parts of *C. albicans*, *A.*

fumigatus and *D. farinæ*, as below. In the other studies, the individual specific relevant antigens were used.

Methods

The total IgE level of the sera was determined with a solid phase sandwich technique (System 3 in Wide, 1971) as described previously (Stenius *et al.*, 1971).

The radioallergosorbent test (RAST) (Wide *et al.*, 1967) was used to assay allergen specific IgE in the sera. The activities were expressed in sorbent units (SU) (Stenius & Wide, 1969) and the reaction was regarded as positive when the activity was one SU or more.

Blocking antibodies were measured by a RAST neutralization test (Wide, 1976). A serum with a strong positive RAST reaction was used as a reagent and incubated with the test sera and control sera. The capacity of antibodies in the test serum to prevent the reaginic IgE antibodies from binding to the allergen was then measured.

Antibodies capable of simultaneous binding both to a solid phase antigen and to the antigen labelled with ^{125}I (double binding antibodies) were measured according to Wide (System 4 in Wide, 1971).

Immuno-adsorbent to remove IgE from the sera. An immuno-adsorbent to remove IgE was prepared by coupling the IgG fraction of a rabbit anti-human IgE to cyanogen bromide activated Sepharose 4B (Fuchs & Sela, 1973). The washed suspension was used to absorb: sera of two aspergillus-sensitive patients, which contained both IgE and IgG S-TS antibodies; two sera of budgerigar-sensitive patients to confirm the presence of IgE, and one serum of a person sensitive to grass pollen. The sera were examined for antibody by monkey PCA and for total IgE by RIST.

Passive cutaneous anaphylaxis in baboons. Baboon PCA tests were done as before on the clipped skin of baboons (Parish, 1973). The baboons were tranquillized by i.m. injection of 1 ml of phencyclidine (Sernylan, Parke Davis, Middlesex) diluted 1/10.

Baboons were sensitized with unheated serum 24 hr before testing and again with the same heated sera at 4 hr or shorter intervals before challenge. Those injected at 4 hr or lesser intervals before challenge were heated at 56°C for 2 hr, though in some tests unheated samples were also injected for the shorter period of sensitization. Each serum was either injected at contra lateral sites at the different time intervals, or at adjacent sites. Sera were injected intradermally in 0.1 ml volumes undiluted, or if required, were diluted at 1/10 and 1/50 for the 24 hr tests, and 1/4, 1/8 and 1/16 for the shorter period tests.

Before challenge, the animals were examined for any change or blemish at the injected skin sites. Then 2 ml of 5% Evans blue dye was injected intravenously. The animals were observed for 10 min to detect any extravasation of the dye at the skin sites due to the activity of the injected sera. Antigen was then injected intravenously, and appropriate to the specificity of the antibody-containing sera. The amounts as indicated, were 0.25 ml of the mixed groups of antigens or of budgerigar, pigeon or chicken sera; 5 mg egg albumin; 4 mg *Micropolyspora faeni* antigen; 1 or 3 mg *Aspergillus fumigatus* antigen; 1 mg *Candida albicans* antigen; 1 mg *Dermatophagoides farinae* antigen. When available, a positive serum was always included in the test. The results were recorded after 15 to 20 min. Each reaction was measured, and the intensity matched against a standard colour card. An i.c. injection of histamine to give a reaction of about 10 × 10 mm was made after antigenic intravenous challenge to show that dye could still diffuse into such sites by analogy into anaphylactic sites.

Reactions were assessed as positive when of a 10 × 10 mm diameter. Some short-term reactions were designated positive when of an 8 × 10 mm diameter, and if there were small spots of dye or coloured hair papillae outside the main blue area. S-TS sites, i.e. heated serum tested 2 to 4 hr after skin sensitization presented sometimes as a ring of dye with a pale centre instead of the usual homogeneous blue response.

Results

IgE and IgG S-TS antibodies to common allergens (Table 1)

Positive PCA reactions at sites sensitized with both unheated and heated S-TS sera were obtained to grass pollen, with only two reactions to milk and to egg at 2 hr sensitized sites, with one positive to egg at the 24 hr sensitized site. The absence of IgE mediated reactions to *D. farinae* may have been due to natural antibodies in the baboons (see later).

*IgE and IgG S-TS antibodies to *A. fumigatus* (Table 2)*

Of the twenty-two aspergilloma group sera with strong precipins, eleven showed the presence of 24 hr unheated sensitizing antibodies and no S-TS antibodies. In the thirty-four with allergic bronchopulmonary aspergillosis, all had only weak precipitins, thirty-one gave positive reactions at the 24 hr sensitized sites and ten of these had S-TS antibody at 2 hr and six of the latter at 4 hr as well. In the ten with uncomplicated asthma three gave positive reactions at the 24 hr sites sensitized with unheated serum.

*IgG S-TS and precipitins to *M. faeni* (Table 1)*

None of the twenty-five farmers' lung sera with precipitins to *M. faeni* gave positive reactions at the 24 hr and 2 hr sensitized sites.

IgE and IgG S-TS antibodies to avian antigens in non-atopic asthmatic patients (Tables 3, 4, 5)

Eleven non-atopic patients with asthma induced by budgerigar serum proteins were examined for clinical responses in provocation tests and for specific IgE and IgG S-TS antibodies. The tests were done 'blind' in three laboratories without knowledge of the results obtained elsewhere.

The patients and their clinical responses

The asthma started in adult life in all cases and the age of onset was between 56 to 63 years. Most had kept budgerigars for many years, and there is some relation between the number of years of exposure to antigen and the ability of the patient's serum to mediate PCA in baboons (Table 3).

None of the patients reacted to prick tests with a battery of twenty common allergens. They also gave negative reactions to prick tests with undiluted and diluted budgerigar, pigeon and hen sera. Intracutaneous tests with the avian antigens in ten cases gave immediate reactions in two, 'late' reactions in three and dual, immediate and 'late' in five cases. More antigen was needed to elicit reactions than in the routine prick test.

Bronchial provocation tests with budgerigar serum proteins were made on six patients. All responded with 'late' asthma starting 2–4 hr after the challenge. All six had also given late (three) or dual (three) reactions to i.c. tests (Table 3).

In vitro tests for antibody

Nine of the eleven patients had precipitins to budgerigar antigen (Table 3). There was no relationship between the total amount of IgE or the IgE specific anti-budgerigar antibodies measured by RAST and the skin or bronchial challenge test results (Table 3). The total amount of IgE varied from 1.60 to 650 units and though the greatest amount, 650 units, was found in a patient giving an immediate response, the next highest concentration, 488 units, was found in a patient (B.A.) with late skin and bronchial challenge results. Moreover one (C.O.) with a late skin test reaction had almost the same amount of total IgE, 34.6 units, as a person (P) with an immediate skin test result, and having 33.5 units IgE (Table 3).

If a RAST result of 1.0 'sorbent unit or greater, is considered to be a significant amount of IgE specific antibody, only one patient, B.U.S., had a significant amount of IgE antibody to budgerigar serum. The other patients had only small amounts of IgE antibody, four patients having none, as measured by RAST, including patient P. who showed an immediate skin test reaction. The eleven sera were also examined by RAST to pigeon and hen sera, hen egg white and yolk, and to hen skin. Nine patients had no significant amount of IgE antibody (above 1 'sorbent unit) and several had no detectable antibody to these antigens in this test. The patients in Table 5 were examined by RAST to budgerigar, pigeon and hen serum and to hen skin and egg white. The only positive RAST findings for the patients reported in Table 3 were B.A. 2.2 units for egg white, and B.U.L. 1.15 units for hen skin. Of particular interest, when considering the results of monkey PCA and blocking antibody tests (Tables 3 and 4), is that no antibody at all was found by RAST to any of these antigens in two serum samples of P., and 0.9 units to hen skin only, in C.O.

Baboon PCA tests

Sera were tested unheated for PCA on three baboons for 24 hr sensitization, and unheated and heated for 2.5 hr sensitization. Sensitizing antibody was found in eight of the eleven patients (Table 3). Six of the sera had antibody sensitizing skin for 24 hr; four of the samples sensitized all three baboons and the other two sera sensitized one baboon only which appeared to be particularly discriminating in this test, and showed larger reactions at the positive sites than those seen in the other two animals.

There was no relationship between the ability of sera to sensitize the skin of baboons for 24 hr and the presence of specific antibody detected by RAST or the total amount of IgE in the serum (Table 3).

There was no significant relationship between the nature of the skin test response in the patient and the ability of the sera to mediate PCA. Of the four sera sensitizing all three baboons, two were obtained from patients with immediate, one a dual and one a late skin response. Of the two sera sensitizing one baboon only, one was obtained from a patient showing a late and one a dual response.

Heat-stable short-term sensitizing (2.5 hr) antibody was found in six patients. In four it was present together with the IgE antibody, but two sera (B.U.R., B.U.S.) had short-term sensitizing antibody only, though this was detected in only one of the three baboons (Table 3). The results showed that sera with IgE antibody activity did not always sensitize baboon skin when injected unheated and challenged in 2.5 hr as in sample B.A. This could reflect relatively small amounts of IgE or of IgE of relatively weak affinity for mast cells, or the presence of a blocking antibody in the serum that has not had time to diffuse away in 2.5 hours.

It is noteworthy that of the two sera without precipitins (M.A. and M.L.) only one had short-term sensitizing antibody.

There was an indication that the longer the patients had been exposed to budgerigars, the most likely they were to have formed skin-sensitizing antibody, particularly those exposed for 10 or more years.

Blocking antibody for RAST

Tests for antibody blocking the RAST for IgE anti-budgerigar antibodies were made by L.W. independently, without knowledge of the monkey PCA results.

Serum S., from an atopic person with 44 'sorber' units of IgE anti-budgerigar antigen (Table 4) was tested in the presence of sera B.P., C.O. and M.A. which sensitized baboons for 24 hr PCA reactions but had little or no antibody detectable by RAST (Tables 3 and 4).

Sera B, P and C.O. contained an antibody, probably IgG, which inhibited the RAST for serum S. This antibody is the probable explanation for the failure to detect IgE antibody to budgerigar serum proteins in sera B, P and C.O. These sera contained precipitins to budgerigar serum proteins (Tables 3, 4, 5) and bivalent antibody as shown in another test by L.W.

However, the presence of precipitins is not the whole explanation for the blocking activity. Two sera from atopic patients, H. and S., were examined as controls to the tests on the non-atopic persons, and serum H. had precipitins to budgerigar antigen, yet had a significant 1.9 'sorber' units in RAST, and sensitized two of three baboons for 24 hr PCA. Another inconsistency in attributing the failure to detect IgE antibody by RAST to blocking antibody is serum M.A. This serum had little specific IgE to budgerigar antigen, 0.8 RAST 'sorber' units, but sensitized three of three baboons for 24 hr PCA, yet it contained no precipitins to the antigen (Table 4) and no bivalent antibody in the tests by L.W. Thus the absence of blocking antibody is consistent with the absence of precipitins, but the failure to detect IgE by RAST in this serum cannot be attributed to blocking antibody.

Blocking antibody may have been responsible for the negative prick-tests on the patients. The small amount of antigen penetrating with a prick-test needle may be insufficient to overcome the inhibition by blocking antibody, whereas larger amounts of antigen entering by injection or by inhalation may induce an allergic response (Tables 3, 4, 5) because some penetrates to react with the sensitized mast cells.

Overall results

The results of these tests on eleven non-atopic patients with asthma and sensitivity to budgerigar antigens are summarized in Table 5. None of the patients responded with prick tests to avian antigens, but all of the ten tested by i.c. injection showed immediate and/or late reactions. Only one of eleven patients had a significant amount of IgE antibody to budgerigar antigen when examined by RAST. Despite the negative RAST results, six sera sensitized baboons for PCA for 24 hr, and 8/11 sensitized baboons for either 24 hr (unheated samples) or 2.5 hours (heated samples). It was confirmed that IgE and S-TS antibodies could co-exist in the same sample. One serum without precipitins had S-TS activity. Of four RAST negative sera so tested, three had bivalent blocking antibodies.

IgE and S-TS antibodies in extrinsic and cryptogenic asthma (Table 6)

Of the fourteen atopic subjects with extrinsic asthma, all gave positive prick test reactions to several of the battery of six allergens tested, particularly the Timothy grass pollen (thirteen patients), and *D. farinae* (thirteen). Six of the fourteen sera examined by monkey PCA gave positive reactions at the 24 hr sites sensitized with unheated serum and at the 4 hr sites sensitized with heated serum to the pool of milk, pollen and egg extracts. None of the eight sera tested with the pool of *C. albicans*, *A. fumigatus* and *D. farinae* gave positive PCA reactions at sites sensitized with unheated or heated serum.

Of the sera of the nine subjects with cryptogenic asthma, none gave PCA reactions at sites sensitized with unheated serum, and three each gave positive reactions to the two pools of antigen after 1 hr sensitization, at sites sensitized with heated serum. Two sera reacted to both pools of antigen, making a total of four out of the nine cryptogenic asthmatics with S-TS antibodies. Of these, two gave histories suggestive of pollen sensitivity, but negative skin prick tests.

Absorption studies for IgE and S-TS antibodies

Sera from two persons allergic to *A. fumigatus* and containing both IgE and S-TS antibodies were absorbed with the insoluble immuno-adsorbent of anti-IgE antibodies bound to Sepharose. A serum of a hay fever patient with IgE antibody to Timothy grass pollen was absorbed as a control for the efficacy of the removal of IgE from the serum.

The two sera with antibodies to *A. fumigatus* gave 24 hr baboon PCA tests with unheated serum at titres of 128 and 64 respectively and with serum heated at 56°C for 2 hr when tested at 2.5 hr in titres of 8. The two sera contained 110 and 72 units of total IgE respectively. The serum with antibodies to the grass pollen gave a 24 hr PCA test at a titre of 32 and had no S-TS activity. It contained 48 units of total IgE.

Treatment of the unheated serum samples with the anti-IgE immunosorbent completely removed their 24 hr PCA activity and also all of their content of IgE. The S-TS titres to *A. fumigatus* with the unheated and heated absorbed sera were 8 and 4 respectively, one of the sera having lost some activity.

The two sera (P. and C.O.) from subjects sensitive to budgerigar serum proteins gave negative RAST's for specific IgE antibody (Tables 3, 4) and were tested again on a single baboon. Samples of the sera were absorbed with the anti-IgE immunosorbent and were also heated at 56°C for 1 hr. The IgE was completely removed from the samples of serum. Sites sensitized with the unabsorbed sera, unheated and heated, and after absorption and unheated were tested after 24 hr by i.c. injection of 0.05 ml of budgerigar serum diluted so as to contain 50 µg protein. Positive reactions were elicited only at the sites sensitized with unheated serum, the blueing reactions measuring 20 × 23 mm (serum P.) and 12 × 14 mm (serum C.O.). The skin sensitizing activity in these sera were therefore mediated by IgE antibody.

Discussion

The clinical relevance of the heat and 2-mercaptoethanol stable antibody of the short-term sensitizing (S-TS) type against common allergens has yet to be determined. IgG S-TS antibody and antibody with the same properties, though not necessarily characterized as IgG antibody, has been detected by passive transfer (PCA) to non-

human primates and to man. Parish (1970, 1971, 1973) found IgG S-TS antibody mainly to tetanus toxoid and horse globulin by PCA tests in the monkey. Bryant *et al.* (1973) regarded IgG S-TS antibody to *Dermatophagoides* spp. extracts as the mechanism for asthmatic reactions to provocation tests in the absence of demonstrable specific IgE antibody. Longbottom *et al.* (1976) reported on S-TS antibody to the polysaccharide cell-wall antigen of *C. albicans*, a mannan, by PCA tests in the monkey.

Berry & Brighton (1977) describe a family with IgG S-TS antibody to grass pollens and in this instance giving vigorous reactions to prick tests. Warren *et al.* (1977) found S-TS antibody in non-atopic subjects with clinical avian sensitivity and giving positive dual i.c. test reactions and with negative prick tests and negative RAST for specific IgE. In subjects with sensitivity to moulds, particularly *Alternaria*, as shown by i.c. but not by scratch tests, histamine release was induced from the white cells by an antiserum to IgG₄ but not by anti-IgE (Vijay, Perlmutter & Bernstein, 1978). S-TS antibody has been attributed to IgG₄ on the evidence of blocking sensitization by IgE for 4 hr (Stanworth & Smith, 1973). In his early tests, Parish (1970) considered it to be complement-dependent as the skin reaction was much reduced in monkeys depleted of complement, though later (Parish, 1973), using different sera containing S-TS antibodies and purer preparations of the cobra venom reagent, found that depletion of complement did not influence the S-TS passive sensitization reactions. More recently, Lakin *et al.* (1978) reported on S-TS antibody against protamine sulphate in subjects who had become allergic to it, and it appeared to be complement-dependent, as reactions to it were prevented by deplementing the monkey recipient. The latter had no effect on IgE mediated reactions.

Our series of studies, showing the presence of IgE alone, S-TS antibodies alone and both together against common allergens, is of interest in terms of extrinsic asthma in which both antibodies were present together and of particular interest in cryptogenic asthma where only S-TS antibody was found in four out of nine patients. These antibodies are capable of mediating immediate reactions as shown by the baboon PCA, and may have some relevance to the aetiology of the cryptogenic asthma. IgG S-TS antibodies are less potent than IgE antibody in mediating release of histamine and other mediators. This could explain the absence of immediate reactions to the usual prick tests we employ and on a basis of which they are classified as non-atopics. Our tests in this study were made in three different laboratories without knowledge of each other's results. Intracutaneous tests can elicit immediate reactions, for example, in non-atopic subjects with avian sensitivity and Warren *et al.* (1977), have shown the presence of heat-stable S-TS antibody in them by passive transfer in man. These subjects required i.c. tests to elicit skin reactions, and it is postulated that this immediate reaction acts as an introductory mechanism, as does IgE antibody for the development of type III reactions. Of interest in the opposite direction is the demonstration of heat-labile long-term sensitizing antibody, presumably IgE, in non-atopic subjects with avian sensitivity and giving negative prick and RAST tests. Bivalent blocking antibodies were present in the sera of three out of four such subjects. The blocking antibody may diffuse away from the test site in the 24 hr sensitization period, making it possible for the IgE antibody sensitization to be shown.

In previous studies (Parish, 1973, 1974), IgG S-TS has usually but not always been found to be associated with precipitins to the same antigen. In the present study, no IgG S-TS was found in the sera of patients with farmers' lung and precipitins to *M. faeni*, nor in the sera of patients with aspergilloma who give very strong precipitin

reactions to *A. fumigatus* and some of which also contained IgE antibody. By contrast, one-third of the patients with allergic bronchopulmonary aspergillosis and giving weak precipitin reactions had S-TS antibody in addition to the IgE antibody demonstrable in all such subjects.

Tests on groups of subjects with uncomplicated extrinsic asthma with a battery of common allergens showed small numbers of sera with S-TS antibody, with eight out of twenty-five positive to grass pollen and two of these with S-TS antibody. Two of each out of test groups of seventeen to twenty-three subjects showed S-TS to milk and egg.

The S-TS antibodies shown here invariably gave more reactions when tested after one to two hr than later at about 4 hr, thus illustrating the short-term nature of their sensitization. The IgG S-TS is, however, less effective than IgE in releasing histamine from human lung (Parish, 1973) or human skin (Parish, 1974) on antigen challenge *in vitro*. Less histamine is released and sensitization by the S-TS antibody cannot always be achieved. On the assumption that IgG₄ acts as IgG S-TS, it seems possible from our studies of egg allergy (Davies & Pepys, 1976) that the presence together of IgE and IgG₄ may determine acute allergic reactions where IgE alone did not appear to do so. If this were so, it might explain why about 30% of the population show evidence of type I allergy to common allergens without clinical manifestations. A possible mechanism for the involvement of both classes of antibody could be the combination of allergen with long-term sensitizing IgE antibody on the surface of the mast or basophil cell, with production of a vascular reaction however strong and then being retained there so as to make it possible for S-TS antibody which sensitizes for only short periods of time to become available to react with it. Potentiation of the reaction might occur under these circumstances by mechanisms yet to be determined.

The failure to sensitize baboons for PCA with human IgE antibodies to the house dust allergy mite genus *Dermatophagoides* is attributed to sensitization of the baboons to pulmonary and other acari, resulting in the formation of blocking antibodies which could have reacted with the intravenously injected antigen. Evidence for this was obtained for baboons in the same laboratory, but their role was not examined in the current tests. Pulmonary acari (*Pneumonyssus simicola*) occur in several genera of monkeys, particularly the rhesus (Innes *et al.*, 1954; Lee *et al.*, 1954; Ogata *et al.*, 1971) and baboons, *Papio* species (Kim, Eugsten & Kalter, 1968). Baboons were used for the PCA tests in the present study, and one of us (W.E.P.) has failed in several studies to detect human IgE anti-house dust mite by monkey PCA tests, though the sera were RAST positive. This is attributed to a refractory state of the baboons sensitized previously to lung and possibly other mites. Agglutinins to mite antigens (Kim, Bang & Di Giacomo, 1972) and increased amounts of serum IgE, and IgE forming cells in mite-induced lesions (Ishizaka, Ishizaka & Tada, 1969) are reported in mite-infested monkeys.

Baboons used in our laboratory (W.E.P.) were shown to have IgG and IgM antibodies to mite antigens by haemagglutination antiglobulin tests, and by precipitation in gels confirmed by ¹²⁵I-conjugated rabbit anti-IgG and anti-IgM. IgE anti-mite was detected by RAST using reagents for the human test. Serum or the IgG fraction of serum, containing anti-mite precipitins could block the RAST for IgE. Intracutaneous injection of a 1:10,000 extract of the mite antigen elicited immediate reactions in three out of four baboons, and histological examination of the sites sampled four hours later showed numerous eosinophils.

The mite antigen used was prepared from *Dermatophagoides farinae*, which was

shown to be antigenically similar to *P. simicola* obtained from a rhesus monkey and to *D. pteronyssinus*.

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