

Original Article

Validation of short-term skin-sensitizing IgG antibodies to egg in atopic dermatitis by Prausnitz-Küstner (P-K) and oral P-K tests

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ABSTRACT

We tried to evaluate the role of IgG antibodies in the wheal and flare reaction of infantile atopic dermatitis by *in vivo* skin-sensitizing methods. Sera from 49 patients under 6 years of age were heated at 56°C for 30 min to inactivate IgE antibodies, according to previous reports. Two-fold serial dilutions of the heated serum were injected intracutaneously in both forearms of a non-allergic recipient. One hour later, the whole egg antigen (1:1000) was injected in the serum transfer sites of an arm and the reaction was read at 15 min (IgG Prausnitz-Küstner (P-K) test). Immediately afterwards, a raw hen's egg was swallowed by the recipient and the serum transfer sites of the other arm were observed for 2 h (oral IgG P-K test). Three patients under 1 year of age showed an immediate wheal and flare reaction in the IgG P-K test, but none of the patients showed a positive reaction in the oral IgG P-K test. The patients with a positive IgG P-K test had an IgE radioallergosorbent test (RAST) score >4 to egg white and a positive IgE P-K titer of >2⁸ to whole egg antigen. Two patients showed a positive reaction to the oral IgE P-K test to raw egg ingestion. Ovalbumin-specific IgG₁, IgG₃ and IgG₄ antibodies were assayed in 20 patients. IgG₁ and IgG₃ antibodies were significantly increased in two patients with a positive IgG P-K test, while levels of the IgG₄ antibody in positive patients were not significantly increased in comparison with levels in negative patients. In

conclusion, these results suggest that the IgG antibody may play a role in the immediate type allergic reaction in infantile atopic dermatitis.

Key words: atopic dermatitis, egg allergy, IgG antibody, Prausnitz-Küstner test.

INTRODUCTION

The IgG antibody in human serum was first reported to sensitize the skin, as did the IgE antibody, by WE Parish in 1970.¹ He reported that when some human sera containing heat-stable antibodies were transferred into the skin of a recipient, an immediate wheal and flare reaction was produced by antigen injection 1–4 h after serum transfer. The function of this short-term skin-sensitizing IgG antibody has not yet been well understood. High levels of various types of specific IgE antibodies are detected in atopic dermatitis (AD), suggesting an intimate relationship between the specific IgE antibodies and AD, but it has often been observed that the wheal and flare reaction in AD does not always parallel the IgE antibody titer. There is an idea, therefore, that IgG antibodies, particularly short-term skin-sensitizing IgG antibodies, play a role in the pathogenesis of AD by a mechanism that is independent of the IgE allergic reaction. In the present paper we report results of a study that evaluated the skin sensitizing activity and subclass nature of AD patients, following the method described by Parish.¹

METHODS

Patients

The subjects in the present study were 49 patients with AD under the age of 6 (<1 year of age $n = 23$; 1 year of age

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$n = 14$; 2 years of age $n = 7$; 3–5 years of age $n = 5$). For the purpose of the present investigation, patients were selected mainly from a group with positive IgE antibodies to foods. All subjects satisfied the diagnostic criteria of Hanifin and Rajka for AD.²

Blood samples were obtained by venipuncture and separated sera were stored at -80°C until use.

Radioallergosorbent and skin tests

The radioallergosorbent test (RAST) to egg white was performed using a Phadebas IgE-RAST kit (Pharmacia, Tokyo, Japan) and a RAST score of 2 or more was defined as a positive reaction. The intradermal test was conducted by conventional methods using a commercial egg white allergen (Torii, Tokyo, Japan).³ The immediate reaction was read at 15 min and a wheal size larger than 10 mm in diameter was regarded as positive. The late-phase reaction was read at home by each patient between several and 24 h after the injection. Erythematous induration was regarded as positive.

IgE Prausnitz-Küstner (P-K) test and oral IgE P-K test

An ordinary P-K test (termed 'IgE P-K test' in contrast with the following IgG P-K test) and a P-K-mimicking reaction induced by oral challenge with a raw egg (known as the Walzer reaction and termed 'oral IgE P-K test' in the present paper) were performed as previously described.³

IgG P-K and oral IgG P-K tests

These tests were performed to detect immediate reactions caused by anti-egg IgG antibodies and egg antigens *in vivo*, as proposed by Parish.¹ The procedures were conducted in a similar manner to those for the IgE P-K and oral IgE P-K tests mentioned above. The differences between the IgG and IgE tests were as follows: (i) in the IgG tests, patients' serum was inactivated by heat treatment at 56°C for 30 min; and (ii) antigen challenge was performed 1 h after serum transfer in the IgG tests in contrast to 2 days after serum transfer in the IgE tests. These procedures are termed IgG P-K and oral IgG P-K tests in the present paper.

Assay of IgG subclass antibodies

Ovalbumin-specific IgG₁, IgG₃ and IgG₄ antibodies were assayed in the serum of 20 patients by enzyme-linked

immunosorbent assay (ELISA) using human IgG subclass-specific monoclonal antibodies (Yamasa, Tokyo, Japan) following the method illustrated in Fig. 1, which has been reported previously.⁴

The present study needed a passive transfer of the serum of patients to other subjects. Therefore, the study subjects were limited to parents who were keen to know whether their children had a food allergy and who affirmed that the patients had not been affected by recent infectious diseases and had no history of hepatitis and had normal liver function tests with negative hepatitis B surface antigen. Informed consent was obtained from the recipient.

RESULTS

RAST and skin test

Table 1 shows the results of the RAST and skin test with egg and their relationship with age. Test results with milk, wheat and soy bean are also summarized to show the allergic background of the patients. Of 49 patients, RAST

96-well microtiter plate (Dinach)
 — Ovalbumin 1 $\mu\text{g}/\text{mL}$ in 0.05 mol/L PBS (pH 7.2)
 4°C overnight
 — Washing
 — 1% BSA-PBS overnight
 37°C 1 h
 — Washing
 — Patient's diluted serum
 37°C 2 h
 — Washing
 — Human IgG subclass-specific antibody (IgG₁, IgG₃, IgG₄) diluted in 1:200, 1:500, 1:500, respectively (Yamasa)
 4°C overnight
 — Washing
 — Peroxidase-anti-mouse IgG (1:10 000)
 37°C 1 h
 — Washing
 — O-phenylenediamine
 Room temperature, 1 h
 — 4 mol/L H₂SO₄
 OD 500 nm measurement

Fig. 1 Assay procedure of IgG subclass antibodies (ELISA). PBS, phosphate-buffered saline; BSA, bovine serum albumin; OD, optical density.

with egg white was positive in 43 (88%) patients and the skin test with whole egg antigen was positive in 46 (94%) patients. Three patients were negative to both RAST and the skin test with egg. Twenty-two (96%) of 23 patients under 1 year of age were RAST- and skin test-positive to egg antigen and most were also positive to other antigens. Therefore, our patients belonged to a select group with multi-allergy to various foods.

P-K and oral P-K tests

The results of the P-K and oral P-K-tests in each age group are given in Table 2.

The IgE P-K test was positive in 40 patients (82%). Younger patients were more frequently positive than were older patients; namely, all 23 children under 1 year of age, 10 of 14 (91%) patients who were 1 year of age, four of seven (57%) patients who were 2 years of age and three of five (60%) patients who were between 3 and 5 years of age showed positive reactions. As for the oral IgE P-K test, 17 patients (42%) showed positive results. Of

these, 12 were under 1 year of age, four were 1 year of age and one patient was 2 years of age.

However, in IgG P-K test, only three patients (6%) under 1 year of age were positive and no patient was positive in the oral IgG P-K test. The late-phase reaction was not observed in either the IgE or the IgG P-K test. The relationship between the RAST score and the results of the P-K and oral P-K tests is shown in Table 3. Eleven of 12 patients (92%) with a RAST score of 4 and 23 of 24 patients (96%) with a score of 3 were positive in the IgE P-K test. Four of seven (57%) patients with a score of 2

Table 3. Relationship between RAST score and egg white and oral P-K tests

	RAST score to egg white				Total
	4	3	2	2>	
No. positives					
IgE P-K test	11	23	4	2	40
IgG P-K test	3	0	0	0	3
Oral IgE P-K test	6	10	1	0	17
Oral IgG P-K test	0	0	0	0	0

Table 1. Results of the IgE allergy tests

	Age (years)				Total
	0	1	2	3-5	
No. patients	23	14	7	5	49
No. RAST-positive patients to:					
Egg	22	13	4	4	43
Milk	10	3	2	1	16
Wheat	9	3	5	2	19
Soy bean	2	3	2	3	10
No. skin test-positive patients to:					
Egg	22	14	5	5	46
Milk	7	4	1	1	13
Wheat	1	1	0	0	2
Soy bean	5	3	1	2	11

Table 2. Results of P-K and oral P-K tests to egg white

	Age (years)				Total
	0	1	2	3-5	
No. positives					
IgE P-K test	23	10	4	3	40
IgG P-K test	3	0	0	0	3
Oral IgE P-K test	12	4	1	0	17
Oral IgG P-K test	0	0	0	0	0

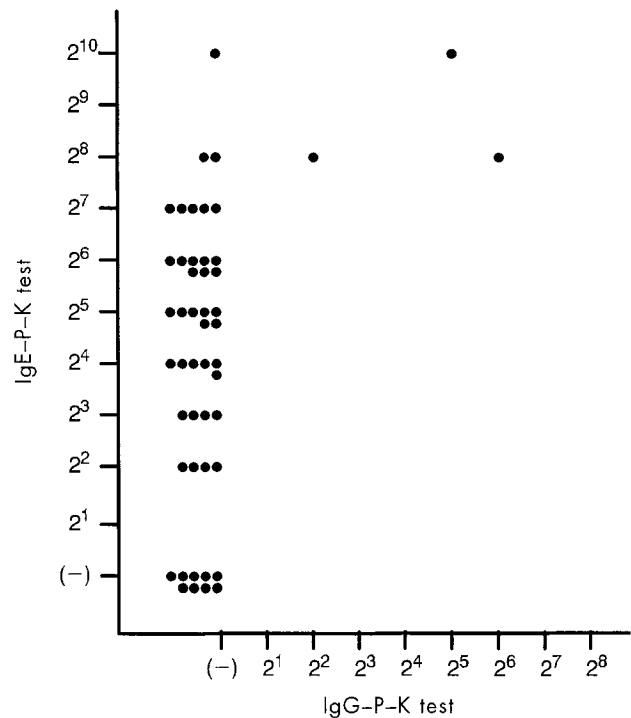


Fig. 2 Relationship between IgG-Prausnitz-Küstner (P-K) and IgE P-K tests. Forty-three patients with less than 2⁷ positive titers in the IgE P-K test were negative in the IgG P-K test. Three IgG P-K-positive patients belonged to IgE P-K highest positive group

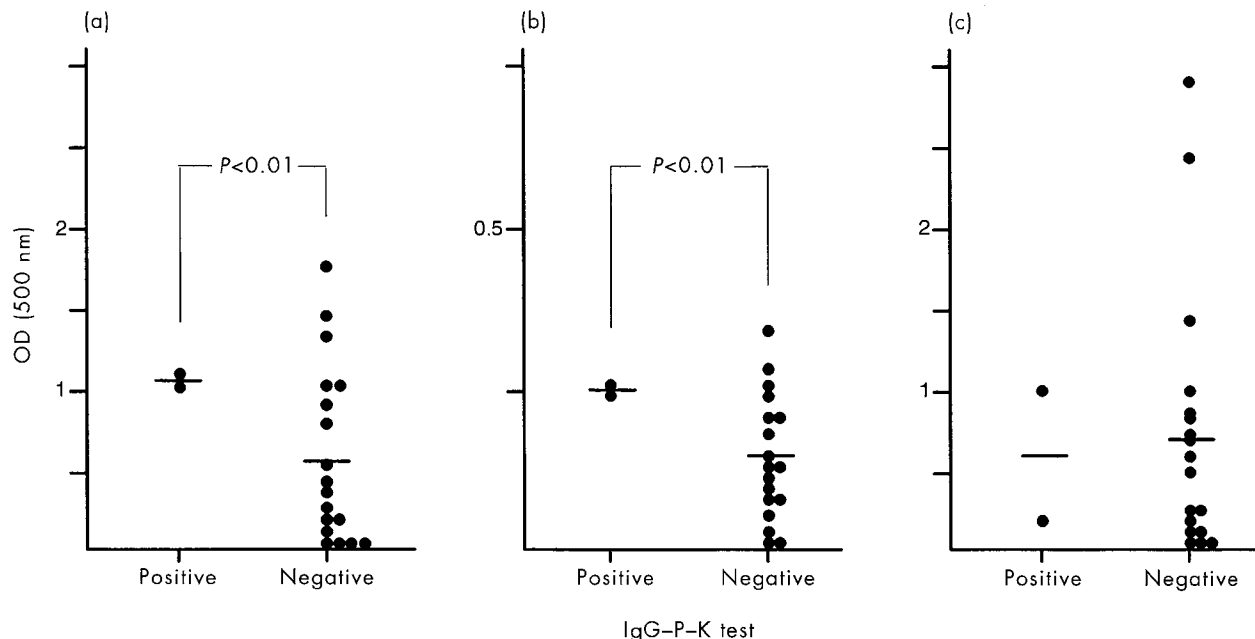


Fig. 3 Relationship between ovalbumin-specific serum (a) IgG₁, (b) IgG₃ and (c) IgG₄ antibody titers with the IgG-Prausnitz-Küstner (P-K) reaction to egg. The IgG₁ and IgG₃ levels were significantly ($P < 0.01$ by Fisher's exact test) higher in the IgG P-K positives than in the negatives, but the IgG₄ level was not different between the two groups.

were also positive. The oral IgE P-K test was positive in six of 12 (50%) patients with a score of 4, in 10 of 24 (42%) with a score of 3 and in one of seven (14%) patients with a score of 2. Similar results were obtained with regard to the relationship between the skin test and the P-K and oral P-K tests (data not shown).

The correlation between IgE and IgG P-K tests is shown in Fig. 2. Three patients with the highest positive IgE P-K titer (2^8 or higher) showed a positive reaction to the IgG P-K test with a positive titer between 2^2 and 2^6 .

IgG subclass antibodies

Ovalbumin-specific IgG₁, IgG₃ and IgG₄ antibody titers in the serum of 20 patients are shown with regard to results of the IgG P-K test (Fig. 3). The number of positive cases were too few to allow a conclusion to be reached, but the IgG₁ and IgG₃ levels were significantly higher ($P < 0.01$ by Fisher's exact test) in positive patients than in negative patients, but the IgG₄ level was not different between the two groups.

DISCUSSION

The relationship between AD and IgE allergy has been disputed since the discovery of skin-sensitizing antibodies. The clinical features of bronchial asthma and

seasonal rhinitis depend largely on the IgE allergy, but this does not seem to be the case for AD as the IgE allergy can only cause an urticarial reaction in itself in the skin, while AD is pathologically similar to delayed-type contact dermatitis.

In addition, in recent concepts of AD, the pathological role of lymphocytes, particularly Th2 cells, is becoming more important in comparison with the role of Th1 cells in ordinary contact dermatitis⁵ and the role of immunoglobulins is becoming less important. There have been many studies that have evaluated the role of IgE and IgG allergy in AD.

IgG subclass antibodies have also been studied by many investigators. In particular, IgG₄ antibodies (one of the IgG subclasses), which are believed to modulate histamine release from mast cells as blocking antibodies,⁶ have attracted research interest.

In the present investigation we evaluated the short-term skin-sensitizing ability of sera of AD patients, as proposed by Parish in 1970, by using methods similar to the ordinary P-K and oral P-K tests after heat denaturation of the IgE antibody. If there was any IgG antibody that could sensitize the skin for a short while then a wheal and flare reaction would occur at serum-transferred sites after the injection of a food antigen or after ingestion of an allergenic food.

A few patients showed possible IgG skin sensitization (positive IgG P-K test). The positive patients, however, showed high IgE RAST scores and very high positive IgE P-K titers. These results indicate that although the IgG antibody may induce an immediate skin reaction, as does IgE, the possibility remains that IgE antibodies may not be inactivated completely by heat-treatment at 56°C for 30 min. Bryant *et al.* have reported that IgG antibodies may mediate the immediate allergic reaction, but at the same time there was a possibility that their reaginic activity may be due to contamination with small amounts of IgE antibodies.⁷ However, it was recently demonstrated that active antigen-specific anaphylaxis involving mast cell degranulation and histamine release could be induced in genetically IgE-deficient mice and it was proposed that the IgG antibody participates in this process.⁸

Significantly increased levels of IgG₁ and IgG₃ antibodies to ovalbumin were detected in two positive IgG P-K patients. The number of positive patients was too small to allow a definite conclusion to be drawn regarding the relationship between IgG₁ and IgG₃ with the P-K reaction. These IgG subclasses are known to possess the ability to form immune complexes to activate the complement system to release so-called anaphylatoxins.⁹ In the present study, IgG P-K-positive patients did not have higher IgG₄ levels than the negative patients, whose IgG₄ antibody levels ranged quite widely, as previously reported.⁴ In connection to this, Okahata *et al.* reported that ovalbumin-specific IgG, IgG₁ and IgG₄ in 54 children with AD under 1 year of age were higher than levels in healthy children or in children with bronchial asthma and/or allergic rhinitis.¹⁰ Shakib *et al.* have reported that raised egg-specific IgG₄ antibodies were detected in a large proportion of both atopic eczema (68.2%) and control (46.2%) groups and concluded that egg-specific IgG₄ antibodies had no clinical value in AD.¹¹ Wüthrich *et al.* also pointed out that IgG₄ levels in children with AD were within the normal range of healthy children, even in severe cases, but, in contrast, were elevated in AD associated with respiratory atopic manifestations.¹² Considering the opinion that IgG₄ levels in AD are raised by prolonged exposure to antigens,^{13,14} it seems important to analyze whether IgG₄ has a further blocking or promoting function in food

allergy. Thus, various explanations can be put forward for the relationship between the IgG antibody and AD, but our observations made by using biological methods seem to indicate that the IgG antibody participates, at least partially, in the immediate wheal and flare reaction in AD.

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