

A. M. Witteman^{a,c}
S. O. Stapel^c
D. H. S. Sjamsoedin^b
H. M. Jansen^a
R. C. Aalberse^c
J. S. van der Zee^a

Departments of

^a Pulmonology and

^b Otorhinolaryngology, Academic Medical
Center, University of Amsterdam, and

^c Laboratory for Experimental and Clinical
Immunology, Central Laboratory of The
Netherlands Red Cross Bloodtransfusion
Service, Amsterdam, The Netherlands

Key Words

Skin test

IgG antibody

IgE antibody

Blocking antibody

Radioallergosorbent test

Fel d I

Fel d 1-Specific IgG Antibodies Induced by Natural Exposure Have Blocking Activity in Skin Tests

Abstract

Cat-allergic patients frequently have IgG antibodies directed against Fel d I. The aim of this study was to investigate whether these IgG antibodies influence the results of the skin test. Titrated skin tests were performed with Fel d I and IgE and IgG antibody levels were measured in 59 patients with cat allergy. Levels of specific IgG against Fel d I ranged from less than 0.25 to 3.5 µg/ml. By means of a multiple regression analysis it was shown that the amount of specific IgG antibodies contributes significantly to the results of the skin test. Presence of specific IgG against Fel d I was accompanied by higher skin thresholds for Fel d I. In conclusion, this study indicates that even low levels of specific IgG, induced by natural exposure to cat allergens, have a blocking effect on the early phase skin reaction.

Introduction

The role of IgG antibodies in skin tests still needs to be established. Already before 1940 it was shown that in sera of patients that had received a course of allergen-specific immunotherapy a heat-stable antibody had developed [1, 2]. This antibody was called blocking antibody because of its allergen-neutralizing capacity in skin tests [3–5]. Allergen extracts preincubated with serum of desensitized patients showed significantly decreased diameters of wheals and flares in the skin test compared to allergen extracts incubated with serum of the same individuals before immunotherapy or with saline. The question arises of whether allergen-specific IgG antibodies also act as blocking antibodies without preincubation, an approach that resembles more closely the physiological situation.

In vitro, the inhibitory effect of IgG antibodies on basophil histamine release was demonstrated by several investigators [6–10]. In several studies on immunotherapy a de-

crease in early phase skin reactivity was observed [11–15]. However, those studies frequently failed to show a correlation between the decrease in skin reactivity and the concentration of IgG antibodies. This might have been due to the fact that in these studies IgG antibodies against total allergen extracts were measured. The results may then be confounded by the inclusion of IgG antibodies with irrelevant specificities. IgG antibodies to irrelevant, i.e. non-allergic material in allergen extracts or to allergens against which no IgE antibodies are directed, will not contribute to the blocking of the binding of specific IgE antibodies. IgG antibodies that are directed against the same molecules as the IgE antibodies and probably even the same or adjacent epitopes should be measured. For these reasons purified major allergens offer a better opportunity to investigate the influence of specific IgG on the skin test.

IgG antibodies against allergens are commonly found in allergic patients. It has been shown that atopic patients have increased levels of IgG to the grass pollen allergen Lol p I

KARGER

E-Mail karger@karger.ch
Fax +41 61 306 12 34

© 1996 S. Karger AG, Basel
1018–2438/96/1094–0369 \$10.00/0

Correspondence to: Dr. J. S. van der Zee
Department of Pulmonology, F4-208
Academic Medical Center, University of Amsterdam
PO Box 22700
NL-1100 DE Amsterdam (The Netherlands)

Received:
March 14, 1995
Accepted after revision:
October 23, 1995

[16] and to the house dust mite allergen Der p 1 [17]. The amount of specific IgG is related to the exposure level [18, 19]. Higher levels of IgG antibodies are found to the cat allergen Fel d 1, when a cat is in the house [20]. In case of occupational exposure to experimental animals, with very high exposure levels, not only allergic but also nonallergic individuals develop IgG antibodies [21]. The aim of this study was to investigate whether IgG antibodies induced by natural exposure to cats have an inhibitory effect on the skin test results.

Materials and Methods

Patients

The studies were performed in 59 cat-allergic patients attending the Department of Otorhinolaryngology and the Department of Pulmonology. Patients with allergic rhinitis had a history of sneezing, itching in the nose, a running nose or a blocked nose. Asthma was defined according to the criteria of the American Thoracic Society [22]. Patients with asthma had a history of paroxysms of dyspnea, wheezing and coughing. The median age of the patients was 30 years (range 15–53 years). Patient characteristics are given in table 1. Patients were selected on basis of a positive radioallergosorbent test (RAST) for cat dander. Excluded from the study were patients with eczema in the test area, patients treated with oral steroids or antihistamines and patients treated with immunotherapy. The study was approved by the local medical ethical committee and informed consent was given by all patients.

Preparation of Purified Fel d 1

The isolation of a major allergen with monospecific polyclonal antibodies for application in vivo was performed as described previously [23]. Briefly, Fel d 1 for immunization of rabbits was isolated from cat dander extract (HAL Allergen Laboratories, Haarlem, The Netherlands) by affinity chromatography with monoclonal antibodies against Fel d 1 [24, 25]. Rabbit polyclonal antibodies were purified by affinity chromatography with cat dander extract immunosorbent; the cat extract was coupled to CNBr-activated Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden; 1 mg protein/100 mg Sepharose). This Sepharose was incubated with rabbit serum containing anti-Fel d 1 for 12 h at 4°C (500 µl serum/100 mg Sepharose). The Sepharose was washed 5 times with phosphate-buffered saline (PBS), containing 0.01% Tween 20, and 5 times with PBS. Rabbit anti-Fel d 1 was eluted with a buffer containing 10% dioxane (v/v) in 0.1 M glycine at pH 2.5. After dialysis, the eluate was coupled to CNBr-activated Sepharose (1 mg protein/100 mg Sepharose). Subsequently, this anti-Fel d 1 allergosorbent was incubated with cat extract for 12 h at 4°C (5 mg protein/100 mg Sepharose). The allergosorbent was washed 5 times with PBS-Tween and 5 times with PBS. Fel d 1 was eluted with a buffer containing 10% dioxane (v/v) in 0.1 M glycine at pH 2.5. The eluate was dialyzed against PBS. Specificity of the monoclonal and polyclonal anti-Fel d 1 and characterization of Fel d 1 purified by affinity chromatography were described previously [24, 25]. For the skin test the Fel d 1 was diluted in PBS, containing 0.03% human albumin and 0.5% phenol and stored at 2–8°C.

Fel d 1 Assay

An inhibition assay analogous to the assay described by Chapman and Platts-Mills [26] was applied to measure the Fel d 1 concentration in the extract. To 0.5 ml protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden; 1 mg/ml), 0.05 ml diluted rabbit antibodies, 0.05 ml ¹²⁵I-labeled Fel d 1 and 0.05 ml of the test extract were added. The mixture was incubated overnight under vertical rotation at room temperature. After centrifugation and washing of the Sepharose, radioactivity bound to the solid phase was counted. The Fel d 1 levels were read from the FDA (United States Food and Drug Administration) standard curve (1 FDA unit = 4 µg Fel d 1) [27].

Radioallergosorbent Test

RAST was performed as described previously [28]. Monoclonal antibody affinity-purified Fel d 1 was coupled to CNBr-activated Sepharose 4B (100 µg to 100 mg Sepharose). Per test 50 µl of serum were added to 0.5 mg Sepharose in a final volume of 300 µl [PBS-AT: PBS/0.3% (w/v) BSA/0.1% (v/v) Tween 20]. Each serum was tested in duplicate, in 3-fold dilutions of the Fel d 1-Sepharose. Immune detection of IgE was performed with ¹²⁵I-sheep antihuman IgE. Each serum was tested in such a dilution that the percentage binding of radioactivity was less than 20% radioactivity added. The amount of specific IgE was calculated as described by Aalberse et al. [29]. The results were read from a standard dilution curve with a reference serum with anti-IgE Sepharose. Therefore the results were expressed in international units per milliliter specific IgE. Total serum IgE was quantified by an inhibition assay as described earlier [30].

Assay of IgG against Fel d 1

One microliter of serum was incubated with 250 µl protein A-Sepharose (2 mg/ml) and 50 µl radiolabeled Fel d 1. IgG4 antibodies were detected with anti-IgG4 Sepharose as described previously [31]. After overnight incubation under vertical rotation the Sepharose was washed with PBS containing 0.1% Tween, and bound radioactivity was measured. Results were expressed in arbitrary units (AU), read from a rabbit reference serum. The amount of specific IgG antibodies in serum was calculated according to Steward and Petty [32]. Binding of radiolabeled Fel d 1 to insolubilized rabbit IgG was measured after the addition of increasing amounts of unlabelled Fel d 1. The antigen binding capacity of the IgG was determined by extrapolation of the line resulting from the plot 1/bound antigen versus 1/free antigen. It was calculated that 1 AU is 0.35 µg IgG. Positive sera showed more than 2% binding of total radioactivity, corresponding to more than 0.25 µg/ml. After immunotherapy with cat dander extract patients on average have IgG levels against Fel d 1 of 35 µg/ml.

Skin Test

Standard intracutaneous skin tests were performed by injection of 0.02 ml of a 10-fold dilutions into the forearm. The highest concentration of Fel d 1 was 100 ng/ml, the lowest concentration was 0.001 ng/ml. After 15 min the longest (A) and orthogonal (B) erythema and wheal diameters were measured. Mean diameters were calculated as (A + B)/2. The Fel d 1 concentrations eliciting an erythema of 10 mm and a wheal of 5 mm were calculated by log linear interpolation. Histamine sensitivity was expressed as mean diameter of erythema (mm) for 10 µg/ml histamine phosphate. The negative control wheal with dilution buffer had to be smaller than 2 mm without erythema. All skin tests were performed by the same investigator. A high correlation between the allergen threshold as detected by a wheal (5 mm) and flare (10 mm) reaction ($r = 0.98$; $p < 0.001$) was found.

Table 1. Patient characteristics

	Age years	Gender	Disease	Skin test ng/ml	Specific IgE IU/ml	Specific IgG µg/ml	Total IgE IU/ml	Cat at home
1	33	M	A, R	4.46	1.39	<0.26	1,894	no
2	38	F	A	0.04	89.35	0.46	455	no
3	35	F	R	4.33	0.86	<0.26	130	no
4	29	F	A, R	0.46	22.21	3.46	181	yes
5	37	F	R	7.00	0.62	0.39	718	no
6	31	M	A	0.55	0.95	0.38	56	yes
7	31	M	R	0.04	25.51	0.39	130	no
8	34	M	A	7.43	21.31	2.12	2,587	yes
9	52	M	A, R	2.29	2.77	<0.26	171	no
10	36	M	A, R	0.55	6.40	<0.26	1,323	no
11	46	M	A	0.51	0.69	<0.26	340	no
12	43	M	A	0.10	1.05	<0.26	52	no
13	18	F	A, R	4.33	5.25	0.91	1,467	yes
14	22	F	A, R	0.57	1.90	<0.26	288	no
15	36	M	A, R	3.90	16.54	2.84	140	yes
16	33	M	A	0.21	17.75	3.22	232	yes
17	29	F	A	0.09	9.98	1.44	106	yes
18	36	M	R	2.29	0.73	<0.26	34	no
19	18	M	R	5.09	0.82	<0.26	188	no
20	25	F	R	5.29	0.30	0.29	699	yes
21	25	F	R	0.05	29.60	0.65	106	no
22	36	M	A, R	0.07	35.78	0.54	506	no
23	40	M	A	4.00	2.01	<0.26	1,200	no
24	28	M	A, R	0.39	4.51	<0.26	3,400	no
25	20	F	A, R	0.55	0.65	0.31	1,100	no
26	29	F	R	4.60	0.99	<0.26	456	no
27	32	M	R	5.09	0.36	<0.26	355	no
28	22	F	A, R	0.41	59.87	3.31	571	yes
29	53	M	A	0.31	12.71	0.34	1,600	no
30	15	F	R	0.53	0.65	<0.26	257	no
31	23	F	R	6.00	0.26	0.52	694	no
32	28	M	A, R	0.14	23.88	1.13	824	no
33	21	M	R	0.05	3.56	<0.26	84	no
34	44	F	A, R	0.55	1.78	0.56	293	no
35	41	F	A, R	0.009	49.09	<0.26	1,300	no
36	37	M	A, R	0.79	0.10	<0.26	2,200	no
37	42	M	R	0.04	13.71	<0.26	300	no
38	29	M	A, R	0.006	13.23	0.81	215	no
39	21	M	A, R	0.05	359.69	0.74	3,000	no
40	24	F	A	4.0	1.16	<0.26	1,500	no
41	25	F	A, R	0.04	2.11	<0.26	461	no
42	30	F	A, R	0.15	15.70	1.48	100	yes
43	26	F	A	0.06	11.07	<0.26	35	no
44	24	M	R	6.62	0.14	0.83	344	no
45	22	F	A, R	0.18	2.42	<0.26	308	no
46	44	M	A, R	5.09	1.68	<0.26	607	no
47	35	M	A, R	0.63	1.52	<0.26	761	no
48	29	M	R	0.06	15.32	<0.26	421	no
49	26	F	R	4.21	0.41	<0.26	627	no
50	25	F	R	0.06	2.01	0.97	78	no
51	27	M	A, R	5.50	0.62	<0.26	171	no
52	40	M	R	0.09	1.86	0.48	145	no
53	35	F	R	0.10	0.31	0.27	63	no
54	31	M	A, R	0.41	0.84	0.32	836	no
55	24	F	R	13.91	0.22	0.89	125	yes
56	23	F	R	4.21	0.21	<0.26	75	no
57	18	M	R	4.46	0.60	0.60	57	yes
58	26	F	R	0.66	0.52	<0.26	96	no
59	43	F	A	1.00	0.52	0.68	383	yes

A = Asthma; R = rhinitis.

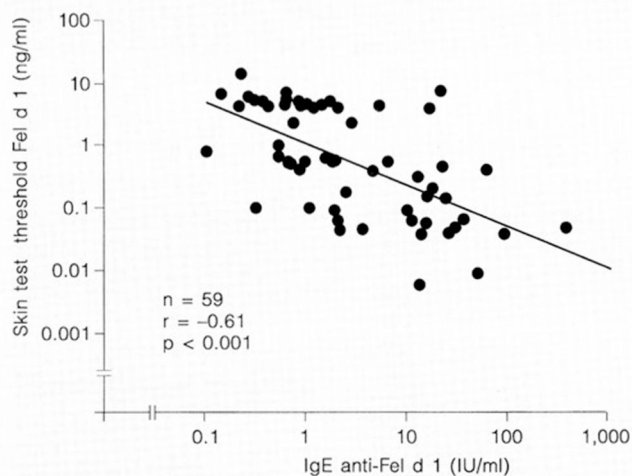


Fig. 1. Relation between skin test threshold and specific IgE.

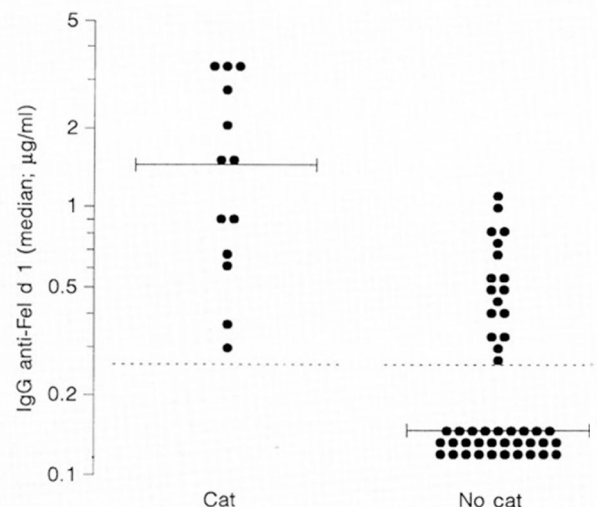


Fig. 2. Difference in the level of specific IgG between patients with and without a cat at home. Patients with a cat at home had higher levels (Mann-Whitney, $p < 0.001$) of specific IgG than patients without a cat at home.

To investigate the reproducibility of the endpoint concentration, we performed quadruplicate skin tests with serial dilutions in 4 persons. The coefficient of variation of all erythema diameters was 14.2%. The within-patient reproducibility of the 10 mm erythema threshold was ± 0.065 of a 10-fold dilution. Working dilutions were prepared on the day of the test and the testing was completed within a period of 4 months. During the study no significant change in the Fel d 1 concentration of the skin test material was detected with the Fel d 1 assay. Moreover, repeated skin tests in 1 patient before and after the study did not show a loss of biologic activity.

Statistical Methods

Parametric correlations between variables were expressed as Pearson's r . Differences between groups were analyzed with the Mann-Whitney U test. Stepwise multiple linear regression was performed with p value limits of < 0.05 (in) and > 0.1 (out). The regression equation was used to calculate the predicted skin test thresholds. Sera with IgG levels below the detection limit were allotted half the detection limit. The analyses were performed with the SPSS/PC+ statistical package (version 5.0). p values of less than 0.05 were considered significant.

Results

The relation between specific IgE anti-Fel d 1 and skin test threshold for Fel d 1 (expressed as Fel d 1 concentration eliciting an erythema of 10 mm) is shown in figure 1. There is a significant correlation (Pearson $r = -0.61$; $p < 0.001$) between log skin test threshold and log-specific IgE. However,

there are striking differences (sometimes $>$ factor 1,000) in skin test threshold between patients with a similar amount of specific IgE.

Thirty patients had detectable amounts of specific IgG antibodies. In only 20 patients IgG4 anti-Fel d 1 was detected. The average contribution of IgG4 anti-Fel d 1 to the protein A-binding isotypes in these patients was 55% (SD 33). To estimate exposure, patients were asked whether they had a cat at home. The maximum number of cats at home was three. Patients with a cat at home had higher levels (Mann-Whitney, $p < 0.001$) of specific IgG than patients without a cat at home (fig. 2). There was no significant difference in levels of specific IgE between patients with or without a cat.

To investigate the influence of IgG antibodies on the skin test a stepwise multiple regression analysis was performed including other independent variables known or suspected to act upon the skin reaction. In the multiple linear regression analysis with the log allergen threshold as a dependent variable only specific IgE, specific IgG, total IgE and histamine erythema contribute significantly to the prediction of the results of the skin test (table 2). When the same procedure was used only for the patients with detectable amounts of IgG the influence of specific IgG on the skin test result was similar to the result obtained in the whole group. The type of disease (asthma or rhinitis), time between isolation of Fel d 1 and skin testing, subclass distribution of the IgG

Table 2. Multiple linear regression analysis

	B	SEB	Beta	p value
Intercept	-0.59	0.53		0.27
Specific IgE (log IU/ml)	-0.88	0.11	-0.82	<0.001
Specific IgG (AU/ml)	0.13	0.04	0.37	<0.001
Total IgE (log IU/ml)	0.51	0.15	0.30	0.001
Histamine erythema (mm)	-0.03	0.01	-0.19	0.03

Regression coefficients and p values for predicting the skin test threshold (log ng/ml). Complete model: $r^2 = 0.57$, $p < 0.0001$.

B = Regression coefficient; SEB = standard error of B; beta = standardized regression coefficient.

antibodies, age and gender of the patients did not influence the results of the skin test. Figure 3 shows the relation between specific IgG and ratio of observed skin test threshold and threshold predicted on the basis of specific IgE, total IgE and histamine sensitivity.

Discussion

Our study indicates that levels of specific IgG (between 0.25 and 3.5 $\mu\text{g/ml}$), induced by natural exposure, have an inhibitory effect on the early phase skin reaction. We have no indication that these low IgG levels had an inhibitory effect on the detection of IgE antibodies. Previously we have confirmed that when an excessive amount of the solid phase-coupled allergen is used, the RAST score is independent of the amount of IgG in the serum [29, 33]. Even if there was some inhibitory effect of specific IgG in the RAST, the IgE levels of the patients with specific IgG would have been underestimated. In this case the inhibitory effect of IgG in the skin test would have been underestimated as well.

We expressed the skin test threshold as the allergen concentration eliciting an erythema because the dose-response curve of the erythema is steeper than that of the wheal [34]. However, we found a high correlation between the allergen

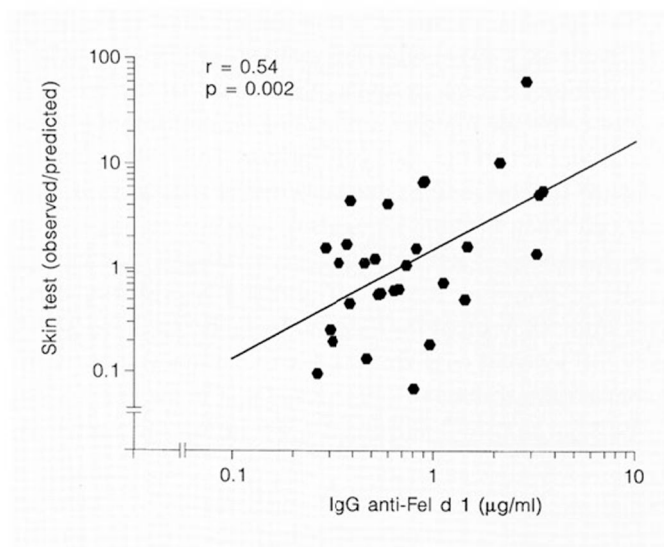


Fig. 3. Relation between specific IgG and the ratio of observed skin test threshold and predicted skin test threshold. The predicted skin test threshold was calculated from the regression equation with log allergen threshold as dependent variable and log-specific IgE, log total IgE and histamine erythema as independent variables.

threshold as detected by wheal and by flare reaction. Similar results with regard to the effect of IgG were found with wheal diameters.

In our assay of IgG against Fel d 1 we measured protein A binding isotypes (IgG1, IgG2 and IgG4). IgG3 antibodies, not measured in this study, are less likely to be relevant as allergen-binding antibodies. It has been shown that the IgG response against allergens is dominated by IgG1 and IgG4 [4, 35].

In sixteen patients in vitro histamine was released from blood basophils by Fel d 1. In these patients no effect of IgG was detected on the histamine release with Fel d 1. The histamine release experiments were performed with washed basophils; therefore, no serum factors were able to interfere with the results of this assay. This finding supports a causal relationship between specific IgG in serum and the decreased skin reactivity.

The results of the stepwise multiple regression analysis confirm that different factors, besides specific IgE and IgG, influence the results of the skin test. In another study we also found that a high total IgE is accompanied by a lower allergen sensitivity in the skin test [36]. The finding that inclusion of the data of the subclass distribution of the IgG anti-Fel d 1 did not contribute to the prediction of the skin test results makes it unlikely that significant differences in skin test-blocking activity exist between the IgG isotypes.

The question of how relevant the influence of specific IgG on the skin test results is, is interesting. The idea behind the inhibitory effects of IgG antibodies is that the IgG antibodies catch the allergen molecules before binding to the IgE antibodies on the mast cell surface. This means that the effect of IgG antibodies on the skin test will be most pronounced when limiting amounts of allergen are present. In most instances of natural exposure of allergic patients the model of limiting amounts of allergen is probably valid. This study shows that for these endpoint titrations there is a clear influence of specific IgG. Using the regression coefficients of table 2, it can be calculated that in the presence of 1.7 or 3.5 µg/ml IgG anti-Fel d 1, 4.5 or 20 times more al-

lergen is required for the same reactions as in the absence of specific IgG.

In routine diagnostic skin tests, the extent of the skin reaction to a full strength allergen extract is usually determined. We also investigated the influence of specific IgG antibodies on the extent of the skin reaction to a fixed allergen concentration above threshold. By means of a stepwise multiple linear regression, we found a significant effect of specific IgG on the size of the erythema as well as on the size of the wheal for a Fel d 1 concentration of 0.1 ng/ml.

In conclusion, even low levels of specific IgG have a blocking effect on the early phase skin reaction.

References

- Cooke RA, Bernard JH, Hebal S, Stull A: Serological evidence of immunity with co-existing sensitization in hay fever type of human allergy. *J Exp Med* 1935;62:733-750.
- Loveless MH: Immunological studies of pollinosis: I. The presence of 2 antibodies related to the same pollen-antigen in the serum of treated hay fever patients. *J Immunol* 1940;38:25-50.
- Maunsell K: Direct test for blocking antibody in treated hay-fever. *Lancet* 1946;ii:199-201.
- Van der Giessen M, Homan WL, van Kernebeek G, Aalberse RC, Dieges PH: Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int Arch Allergy Appl Immunol* 1976;50:625-640.
- Pecquet C, Murrieta M, Michelen V, Leynadier F, Dry J, Allary M, Fabre G, Saint-Blancard J: Blocking activity of mite-specific IgG antibodies studied by skin tests. *Allergy* 1989;44:427-431.
- Lichtenstein LM, Holtzman NA, Burnett LS: A quantitative in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *J Immunol* 1968;101:317-324.
- May CD, Lyman MS, Alberto R, Aduna N: On the measurement of blocking antibody. *J Allergy Clin Immunol* 1972;49:54-58.
- Schumacher MJ, Jeffery SE: Effect of quantity and quality of IgG antibodies on blocking of allergenic histamine release in vitro. *Int Arch Allergy Appl Immunol* 1979;58:38-43.
- Clinton PM, Kemeny DM, Youlten LJF, Lessof MH: Histamine release from peripheral blood leukocytes with purified bee venom allergens: Effect of hyperimmune beekeeper plasma. *Int Arch Allergy Appl Immunol* 1989;89:43-48.
- Van Ree R, Aalberse RC: Rabbit IgG directed to a synthetic c-terminal peptide of the major grass pollen allergen *Lol p I* inhibits human basophil histamine release induced by natural *Lol p I*. *Int Arch Allergy Immunol* 1995;106:250-257.
- Osterballe O: Nasal and skin sensitivity during immunotherapy with two major allergens 19, 25 and partially purified extract of timothy grass pollen. *Allergy* 1982;37:169-177.
- Bousquet J, Calvayrac P, Guerin B, Hejjaoui A, Dhivert H, Hewitt B, Michel FB: Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. *J Allergy Clin Immunol* 1985;76:734-744.
- Hedlin GH, Silber G, Nacleiro R, Proud D, Eggleston P, Adkinson NF: Attenuation of allergen sensitivity early in the course of ragweed immunotherapy. *J Allergy Clin Immunol* 1989;84:390-399.
- Van Metre TE, Marsh DG, Adkinson NF, Kagey-Sobotka A, Khattignavong A, Norman PS, Rosenberg GL: Immunotherapy decreases skin sensitivity to cat extract. *J Allergy Clin Immunol* 1989;83:888-899.
- Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, Kay AB, Durham SR: Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous response. *J Clin Invest* 1993;92:644-651.
- Platts-Mills TAE: Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979;122:2218-2225.
- Chapman MD, Platts-Mills TAE: Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F4P1). *Clin Exp Immunol* 1978;34:126-136.
- Homburger HA, Mauer K, Sachs MI, O'Connell EJ, Jacob GL, Caron J: Serum IgG4 concentrations and allergen-specific IgG4 antibodies compared in adults and children with asthma and nonallergic subjects. *J Allergy Clin Immunol* 1986;77:427-434.
- Van der Zee JS, Aalberse RC: The role of IgG; in Lessof MH, Lee TH, Kemeny DM (eds): *Allergy: An International Textbook*. London, Wiley, 1987, pp 49-67.
- Aalberse RC, Van Milligen F, Tan KY, Stapel SO: Allergen-specific IgG4 in atopic disease. *Allergy* 1993;48:559-569.
- Lee D, Edwards RG, Dewdney JM: Measurement of IgG subclasses in the sera of laboratory workers exposed to rats. *Int Arch Allergy Appl Immunol* 1988;87:222-224.
- American Thoracic Society: Medical section of the American Lung Association: Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;137:225-244.
- Van der Zee JS, Van Swieten P, Jansen HM, Aalberse RC: Skin tests and histamine release with P1-depleted *D. pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988;81:884-896.
- De Groot H, Van Swieten P, Van Leeuwen J, Lind P, Aalberse RC: Monoclonal antibodies to the major feline allergen *Fel d 1*. I. Serologic and biologic activity of affinity-purified *Fel d 1* and of *Fel d 1*-depleted extract. *J Allergy Clin Immunol* 1988;82:778-786.
- Van Milligen FJ, Van Swieten P, Aalberse RC: Structure of the major cat allergen *Fel d 1* in different allergen sources: An immunoblotting analysis with monoclonal antibodies against denatured *Fel d 1* and human IgE. *Int Arch Allergy Appl Immunol* 1993;99:63-73.
- Chapman MD, Platts-Mills TAE: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980;125:587-592.

- 27 De Blay F, Heymann PW, Chapman MD, Platts-Mills TAE: Airborne dust mite allergens: Comparison of group II allergens with group I mite allergen and cat-allergen Fel d 1. *J Allergy Clin Immunol* 1991;88:919-926.
- 28 Aalberse RC, Koshte V, Clemens JGJ: Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. *J Allergy Clin Immunol* 1981;68:356-364.
- 29 Aalberse RC, Reerink-Brongers EE, Hoorweg E: Methods for the assay of allergen-neutralizing antibodies based on competition with IgE antibodies for allergen. in Peeters H (ed): *Protides of the Biological Fluids*. 24th Colloquium. Oxford, Pergamon Press, 1976, pp 725-729.
- 30 Stallman PJ, Aalberse RC: Estimation of basophil-bound IgE by quantitative immunofluorescence microscopy. *Int Arch Allergy Appl Immunol* 1977;54:9-18.
- 31 Van der Zee JS, Van Swieten P, Aalberse RC: Serologic aspects of IgG4 anti-bodies. II. IgG4 antibodies from small, non-precipitating immune complexes due to functional monovalency. *J Immunol* 1986;137:3566-3571.
- 32 Steward MW, Petty RE: The use of ammonium sulphate globulin precipitation for determination of affinity of anti-protein antibodies in mouse serum. *Immunology* 1972;22:747-756.
- 33 Aalberse RC, Reerink-Brongers EE, Vermeulen E: Rast-inhibiting factors in human serum. *Int Arch Allergy* 1973;45:46-49.
- 34 Turkeltaub PC, Rastogi SC, Baer H, Anderson MC, Norman PS: A standardized quantitative skin-test assay of allergen potency and stability: Studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results. *J Allergy Clin Immunol* 1982;70:343-352.
- 35 Aalberse RC, van der Gaag R, Van Leeuwen J: Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983;130:722-726.
- 36 Witterman AM, Stapel SO, Perdok GJ, Sjamsoedin DHS, Jansen HM, Aalberse RC, van der Zee JS: The relationship between RAST and skin test in patients with asthma and/or rhinitis: A quantitative study with purified major allergens. *J Allergy Clin Immunol*, in press.