

Immunologic and clinical correlates of bronchial challenge responses to Bermuda grass pollen extracts

Thomas R. Adler, M.D., Gildon N. Beall, M.D., Douglas C. Heiner, M.D.,
Umesh K. Sabharwal, M.D.* and Kent Swanson, B.S.

Torrance and Los Angeles, Calif.

We have studied 26 patients with asthma who had positive skin tests for Bermuda grass pollen antigen (BGP) with history, immunologic assays, and bronchial challenge with BGP. In these patients IgE anti-BGP RAST titers were higher in those with positive, $6.8 \pm 4.4\%$ (mean $\pm$ SEM) compared to those with negative bronchial challenges, $2.7 \pm 0.4\%$ ($p < 0.02$). Historical data did not predict bronchial challenge response. IgG₄ anti-BGP RAST was not different in patients with positive and negative challenges but was markedly increased, $15.3 \pm 11.9\%$, in patients who had previously had BGP immunotherapy when compared to untreated patients, $3.6 \pm 5.1\%$ ($p < 0.01$). IgE anti-Bermuda grass appears to be the major contributor to susceptibility to BGP bronchial challenge. IgG₄ anti-Bermuda grass is apparently unrelated to the sensitivity to challenge but appears to be a significant component of the immunologic response to immunotherapy. (J ALLERGY CLIN IMMUNOL 75:31-6, 1985.)

The relationship between asthma and allergic responses to pollen allergens needs further clarification.

In southern California and in most of the southwest United States, BGP appears to be the most common cause of allergic rhinitis. Although the plant pollinates 9 or 10 mo out of the year, pollination is heaviest and nasal symptoms are most prevalent in the spring months. Asthma, however, is not more prevalent in the spring. Most patients with asthma have perennial symptoms. Exacerbations occur without a marked seasonal pattern, although in southern California as in other parts of the world, hospital and emergency room visits for asthma peak in the fall.¹

Although a seasonal pattern is difficult to recog-

Abbreviations used

BG:	Bermuda grass
BGP:	Bermuda grass pollen
PD ₂₀ :	Provocative dose causing a 20% fall in FEV ₁

nize, many asthmatic patients have IgE antibodies to BGP, and many will develop reversible airway obstruction when they inhale extracts of BGP, indicating that the antigen can provoke asthma. In order to better characterize the patients whose asthma may be caused by BGP antigens, we have compared historical, immunologic, and bronchial sensitivity data in 26 patients with asthma and allergy to BGP.

METHODS

Patients

Twenty-six asthmatic patients with positive skin tests to BGP antigens were studied. Patients with these characteristics were recruited from the clinic at Harbor-University of California Los Angeles Medical Center and from the private practices of cooperating physicians in the local area. The study was approved by our institutional review board, and informed consent was obtained from all patients. Each patient was skin tested with serial dilutions of BGP extract,

From the Departments of Medicine and Pediatrics, Harbor-University of California Los Angeles Medical Center, Torrance, Calif., and University of California Los Angeles School of Medicine, Los Angeles, Calif.

Supported in part by grants from the Asthma and Allergy Foundation of America, Los Angeles Chapter, and the Thrasher Research Fund, Salt Lake City, Utah.

Received for publication Dec. 15, 1983.

Accepted for publication June 7, 1984.

Reprint requests: Gildon N. Beall, M.D., Harbor-UCLA Medical Center, 1000 W. Carson St., Torrance, CA 90509.

*Recipient of a Research Fellowship from the Arthritis Foundation.

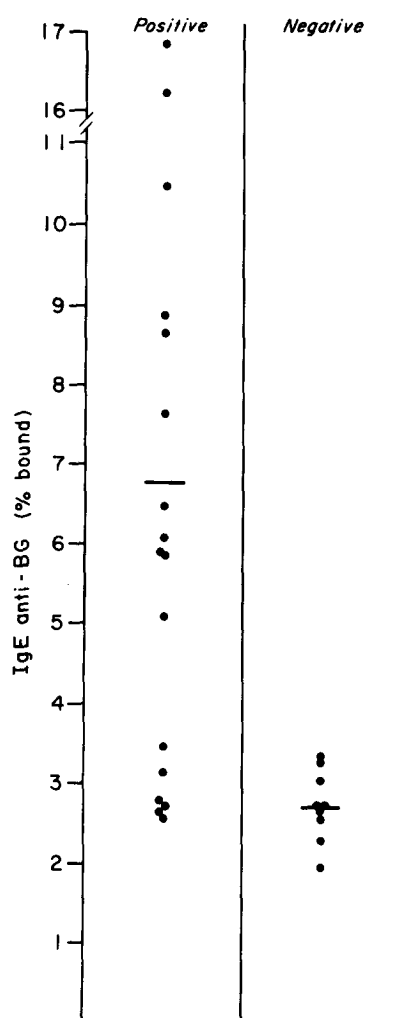


FIG. 1. IgE anti-BG RAST activity (percent bound) in patients with positive and negative challenges. Values were significantly higher in patients with positive challenges. Positive responders $6.78 \pm 4.39\%$ (mean $\pm$ SD) vs. $2.71 \pm 0.45\%$ ($p < 0.02$).

subjected to bronchial challenge, the patient's serum was assayed for IgG4 and IgE antibodies to BG, and the patient's history was obtained.

Clinical evaluation

A standardized history was obtained from each patient, specifically including questions about asthma, other allergic diseases, asthma treatment, and residence. Seasonal variation of asthma and allergic rhinitis was recorded. In addition, patients were questioned as to their symptoms when they were exposed to grass. On review of the questionnaires, the patient's disease was classified as minimal, moderate, severe, or severe and refractory. Patients with minimal disease (class 1) had attacks once or twice per month for less than 4 hr and seldom missed work or school. Class 2 (moderate) patients had attacks on the average twice a week for 4 to 10 hr and missed activities up to twice

TABLE I. Comparison of results of patients challenged with BGP extract

	Positive	Negative
No.	17	9
PD ₂₀ (breath units)		
Median	12	
Range	0.06 to 60	>83.3
Female	14	7
Age: mean	36 (19 to 56)	32 (23 to 38)

Range in parenthesis.

TABLE II. Historical and clinical characteristics related to bronchial challenge results

	Positive BG challenge	Negative BG challenge
No.	17	9
Class*	1.6 ± 0.9	1.8 ± 1.2
mean $\pm$ SD		
Asthma in the springtime	15	5†
Asthma when exposed to grass	13	7
Cutaneous symptoms when exposed to grass	10	7

*See Methods section for definitions.

† $\chi^2 = 1.9$, $0.2 > p > 0.1$

weekly. Severe (class 3) asthma occurred daily for many hours and caused alteration of activities more than two times per week. Class 4 asthma was continuous and almost totally disabling to the patient.

Antigens

BGP antigen extract was obtained from Hollister-Stier Laboratories, Spokane, Wash. One batch was used for all bronchial challenges, skin tests, and antibody assays.

Skin testing

Serial tenfold dilutions of BG were injected in 0.02 ml volumes intracutaneously. The concentration producing a 6 to 8 mm (2+) wheal was used as an index of cutaneous reactivity for comparison with other patients and for determining appropriate BG concentrations for bronchial challenge.

Bronchial challenge

The procedure of Chai et al. was used.² The cumulative dose of inhaled antigen required to produce PD₂₀ was obtained for each patient. Late asthmatic reactions were prevented by administration of 20 mg of prednisone orally after a positive bronchial challenge procedure.

TABLE III. Immunologic characteristics of patients with positive and negative bronchial challenges

	Positive BG challenge	Negative BG challenge
IgE anti-BG*	6.78 ± 4.39	2.71 ± 0.45 ($p < 0.02$)
IgG4 anti-BG*	12.11 ± 12.01	8.36 ± 8.39
Skin test response†	1:10 5.6 ± 0.8	1:10 4.4 ± 0.9 ($p < 0.01$)

*Percent radioactivity bound. Mean ± SD.

†Amount of antigen that produced 2+ (6 to 8 mm wheal) response expressed as mean ± SD log₁₀ of dilution.

IgG4 and IgE anti-BG assays

IgE and IgG4 antibodies to BGP extracts were assayed by use of BG antigens coupled to paper discs. Antisera consisted of rabbit antihuman IgE and monoclonal mouse anti-human IgG4. Detailed procedures have been previously published.³⁻⁵

Statistics

Correlations were done by Spearman rank correlation (r_s) or by calculation of the least squares regression (r). Means of groups were compared with Student's t test. Frequencies of observations were compared with the chi-squared method by use of Yates' correction.

RESULTS

Of the 26 patients with asthma who had positive skin tests to BGP extract, 17 developed 20% or higher decreases in their FEV₁ after inhalation of BG, whereas nine had no significant response to the largest dose used (five breaths of 1:500 w/v, 83.3 breath units). The age and sex distribution of patients with positive challenges was not different from those with negative challenges (Table I).

The historical data obtained from the patients did not predict whether or not bronchial challenge would be positive. The severity and chronicity of the asthma was similar in each of the groups (Table II). Fifteen out of 17 (88%) of those who had positive challenges had asthma during the spring months, whereas five of nine (55%) with negative challenges had springtime exacerbations. This difference in frequency, however, did not achieve statistical significance ($\chi^2 = 1.94$, $0.2 > p > 0.1$). All but one of the 26 patients had symptoms of allergic rhinitis in the spring, consistent with their positive skin tests.

Most patients had respiratory and cutaneous symptoms when they were exposed to grass. However, there was little difference between the groups with positive and negative bronchial challenges with regard to these symptoms.

IgE anti-BG RAST was significantly higher in the group with positive bronchial challenges, 6.78 ± 4.39% (mean ± SD), as compared to those with neg-

TABLE IV. Relationship of previous immunotherapy to bronchial challenge responses to BGP extracts

	Positive challenges	Negative challenges
No.	17	9
Previous immunotherapy	10	6
Mean duration (yr)	4.3	3.4

ative bronchial challenges, 2.71 ± 0.45% ($p < 0.02$) (Fig. 1). IgG4 anti-BG was slightly higher in patients with positive challenges than in those with negative challenges, 12.11 ± 12.01% versus 8.3 ± 8.39% (mean ± SD), a difference that was not statistically significant (Fig. 2). Skin test responses were significantly higher in the group with positive challenges (Table III). The amount of antigen capable of producing a 6 to 8 mm wheal also correlated strongly with IgE anti-BG RAST ($r_s = 0.78$, $p < 0.001$). Skin test reactivity did not correlate with IgG4 RAST ($r_s = 0.15$, $p =$ not significant). Despite the relationship between bronchial challenges and IgE RAST demonstrated in Fig. 1, no correlation was found between the bronchial sensitivity in breath units and IgE anti-BG RAST. Similarly, bronchial sensitivity did not correlate with IgG4 anti-BG RAST assays.

Since many of our subjects had had immunotherapy with multiple allergens, including BG antigens, we compared the history of immunotherapy with immunologic responses. Sixteen patients had received immunotherapy and 10 had not. Of the nine patients with negative bronchial challenges, six had had immunotherapy (66%), whereas 10 of the 17 with positive challenges had received immunotherapy (59%) (Table IV). The mean duration of immunotherapy was similar in each group. There was, however, a substantial difference in IgG4 anti-BG RAST in the patients who had had immunotherapy (Fig. 3). Mean for the treated group was 15.3 ± 11.9%, mean ± SD, compared to 3.62 ± 5.11% in those patients who had

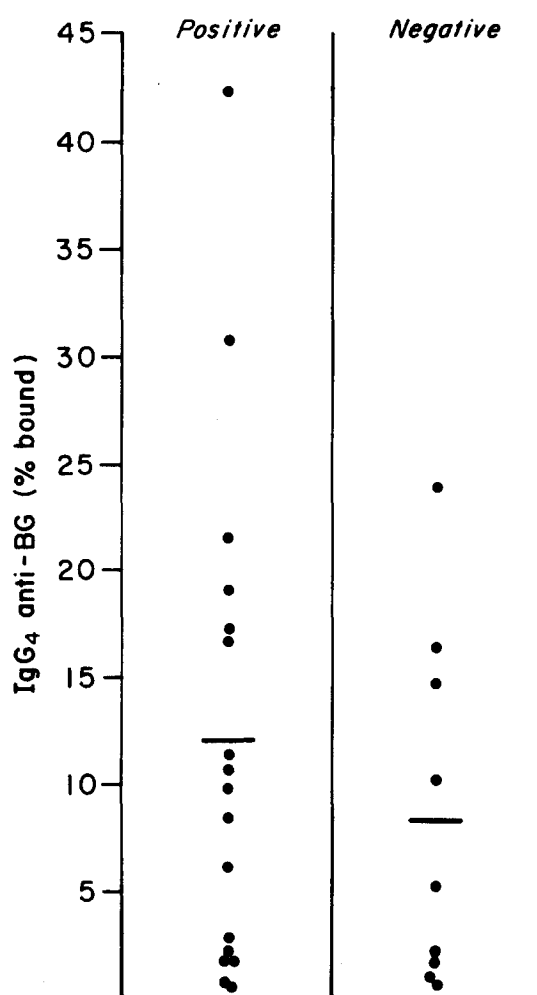


FIG. 2. IgG4 anti-BG RAST activity (percent bound) in patients with positive and negative bronchial challenges to BG extract. There is no significant difference between the two groups in amounts of IgG4 antibody activity. Positive responders $12.11 \pm 12.01\%$ (mean $\pm$ SD) vs. $8.3 \pm 8.39\%$.

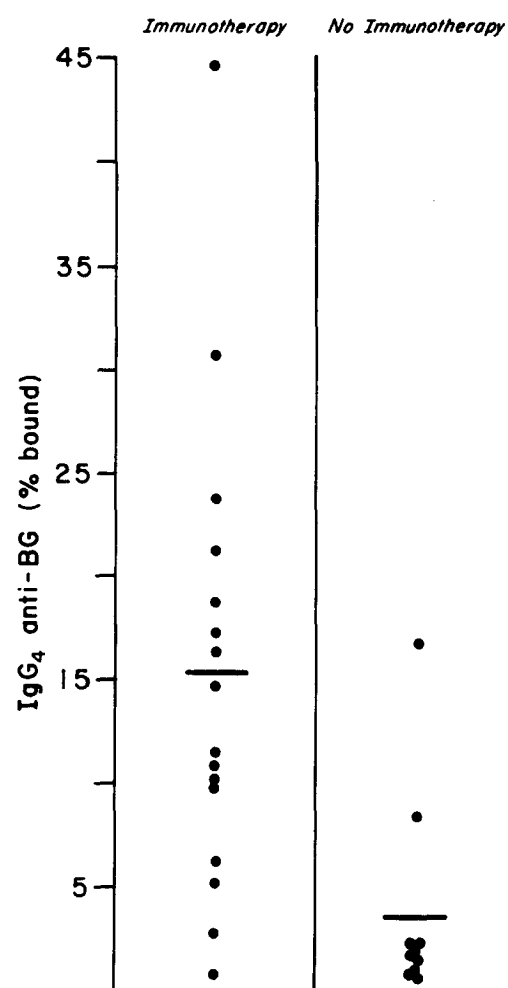


FIG. 3. IgG4 anti-BG RAST binding activity in patients with and without history of immunotherapy. IgG4 activity was distinctly higher in patients who had received immunotherapy in the past, $15.3 \pm 11.9\%$ (mean $\pm$ SD), compared to those who had not received immunotherapy, $3.61 \pm 5.11\%$ ($p < 0.01$).

not received immunotherapy ($p < 0.01$). In contrast, IgE anti-BG RAST binding was similar in both those who had immunotherapy and those who had not, mean $\pm$ SD, $4.12 \pm 2.5\%$ versus $6.15 \pm 4.66\%$. The duration of immunotherapy did not correlate with either IgE or IgG4 anti-BG RAST.

There was a marginally significant relationship between binding activity for IgE and IgG4 anti-BG of patients in the two different immune systems as demonstrated in Fig. 4, which compares IgG4 and IgE anti-BG RAST assays ($r = 0.4$, $p < 0.05$).

DISCUSSION

A major goal of this study was to attempt to define the characteristics of patients who develop asthma on exposure to BGP antigens. All patients studied had asthma, and all had IgE anti-BGP antibodies detected

with skin tests. Negative bronchial challenges with BGP would of course be inconsistent with the possibility that BGP was causing asthma. Positive bronchial challenges can be found in patients without asthma, if they possess sufficient IgE antibodies to ragweed.⁶ Patients with allergic rhinitis and IgE antibodies may also have positive bronchial responses to BG, although we have not studied such patients. Asthmatic patients with negative BG bronchial challenges are unlikely to have BGP-induced asthma, whereas those patients who have BGP-induced asthma must be among the patients with positive bronchial challenges to BG. Comparisons between these two groups then can reveal useful information about the characteristics that might be, or are definitely not, correlated with BGP-induced asthma.

None of the usual historical markers widely held to

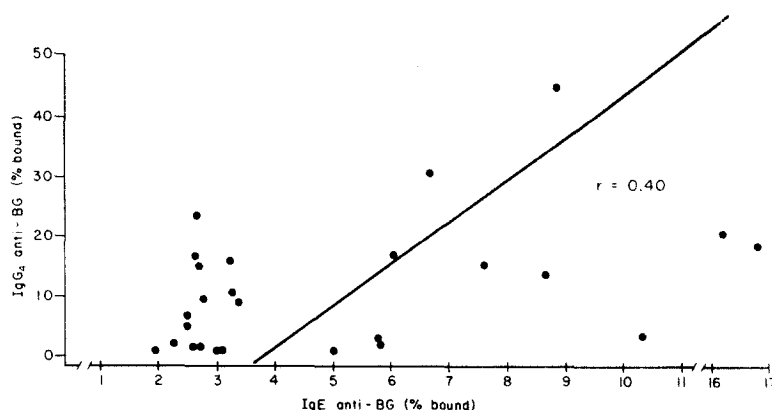


FIG. 4. IgG4 anti-BG RAST activity is demonstrated on the ordinate and IgE anti-BG activity on the abscissa for each of the 26 patients. There is a weak relationship between the two phenomena, $r = 0.4$, $p < 0.05$.

be important in the diagnosis of allergic diseases were helpful in predicting challenge responses. Most patients with positive challenges had respiratory and cutaneous symptoms when they were exposed to BG, but these phenomena were not much more frequent in those who had positive challenges than in asthmatic patients whose challenges were negative. A history of asthma in the spring was somewhat more common among patients who reacted positively to the inhalation of the antigen, but this difference was not statistically significant.

IgE anti-BG RAST activity in the serum, however, was discriminatory. Patients with large amounts of IgE anti-BG all had positive challenges, but approximately one third of those with positive challenges had IgE-BG binding activity in the same range as those with negative challenges. Skin test sensitivity to BG antigen was similarly discriminatory. When there are large amounts of IgE antibody to BGP, a correlation is found with bronchial sensitivity and thus a presumed correlation with asthma on exposure to the pollen antigen.

As expected, patients with immediate-type positive challenges had larger amounts of serum-IgE anti-BG RAST activity and more skin test responsiveness than patients with no responses. The amount of IgE antibody present in the serum is probably not the only determinant of the bronchial challenge response to BG antigen, however, as evidenced by our inability to correlate the magnitude of the bronchial response with the level of specific-IgE antibody.

Studies with other allergens, particularly with ragweed, have revealed evidence that nonspecific bronchial hyperreactivity as detected by methacholine or histamine inhalation is another significant determinant of response to antigenic bronchial challenge.⁷⁻¹¹ It has been proposed that the nonspecific bronchial re-

sponse and the IgE-serum antibody titer can be used to predict the antigenic bronchial response. The two variables, however, are not independent. Nonspecific bronchial responses increase together with IgE antibody titers during a pollen season, and nonspecific bronchial responses decrease with allergen avoidance.^{11, 12} Additionally, some patients with positive challenge responses to antigen have little or no response to methacholine or histamine.¹³

IgG antibodies of the G4 subclass comprise about 5% of total IgG and have unique electrophoretic properties.¹⁴ They lack the ability to activate complement.¹⁵ They have been reported to bind to mast cells, monocytes, and macrophages. Clinical allergic diseases are occasionally observed in patients with low serum concentrations of IgE, negative skin tests, and no evidence of IgE antibodies with RAST assays. Therefore, specific immunologic mechanisms independent of IgE are being sought. Consequently, the proposal that IgG4 may play a role in allergic disease is of considerable interest.

A possible role for IgG4 antibodies in allergic disease has been a subject of controversy and conflicting experimental findings. Some investigators have found that IgG4 can release histamine from mast cells in the presence of anti-IgG4. Passive sensitization of primate skin with IgE has been blocked by IgG4, also suggesting that IgG4 may in some way modulate IgE-mediated mast cell mediator release.¹⁶ Others have been unable to release histamine from IgG4-sensitized human cells.^{17, 18} A role for IgG4 in late asthmatic responses has been proposed in one study.¹⁹

In this study we failed to provide support for the idea that IgG4 is involved in antigen-induced asthma. There was no relationship between the amount of IgG4 antibody present and the likelihood or degree of bronchial response to challenge. There was no rela-

tionship between IgG4 binding activity and skin tests for BGP antigens. The strong correlation of skin test reactivity with IgE anti-BG indicates that IgE is the major determinant of cutaneous reactivity.

Many of these patients had previously received immunotherapy with pollen antigens. Unfortunately, it was not possible to determine the quantity of antigen administered. Nevertheless, there appeared to be no relationship between the history of immunotherapy and the presence or absence of current challenge sensitivity to BGP antigens. Immunotherapy did result in increased IgG4 anti-BG RAST activity, confirming studies by others suggesting that IgG4 antibodies may be products of immunotherapy and possibly protective.²⁰⁻²²

Since we intentionally inhibited late asthmatic responses, none was observed in this study. Late asthmatic responses to challenge had been held to be important correlates of allergen-induced asthma.¹¹ The frequency of these responses has varied with different antigens and different laboratories. As noted, a correlation with IgG4 antibodies to the house dust mite has also been reported.¹⁹ Thus, late asthmatic reactions should be an appropriate subject for further studies of BGP-induced asthma.

In conclusion, IgG4 anti-BG is found in similar quantities in patients with and without immediate bronchial responses to BG inhalation challenge and, therefore, does not appear to be an important determinant of susceptibility to this type BG challenge.

Patients with positive immediate bronchial response to BG have significantly more IgE anti-BG than patients with negative challenges. IgE anti-BG thus may contribute to susceptibility to BG challenge. IgG4 anti-BG is found in significantly larger amounts in patients who have received immunotherapy than in untreated patients.

We wish to thank Aileen Myers for her excellent technical assistance and Hilda Dornfest for typing the manuscript. We are grateful to the physicians who helped us find patients for the study: Stephen Kolodny, Kenneth Landis, Harold Novey, Harold Ochsner, Maurice Rotbart, Richard Shapiro, Judson Schoendorf, Herbert Webb, and Ray Williams. Finally, we wish to thank our volunteer subjects whose contribution was truly indispensable.

REFERENCES

- Booth S, DeGroot I, Marush R, Horton RJM: Detection of asthma epidemics in seven cities. *Arch Environ Health* 10:152, 1965
- Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL, Spector SL, Townley RG: Standardization of bronchial inhalation challenge procedures. *J ALLERGY CLIN IMMUNOL* 56:323, 1975
- Beck CS, Heiner DC: Selective immunoglobulin G₄ deficiency and recurrent infections of the respiratory tract. *Am Rev Respir Dis* 124:94, 1981
- Lee WY, Heiner DC: Preparation of rabbit anti-IgE for use in radioimmunoassays of total IgE and specific IgE antibodies. *J Immunol Methods* 20:185, 1978
- Shacks S, Conrad C, Heiner D: Antigen-specific immunoglobulin G subclass levels in Bermuda grass hypersensitive subjects. *Clin Res* 30:127, 1982
- Bruce CA, Rosenthal RR, Lichtenstein LM, Norman PS: Quantitative inhalation bronchial challenge in ragweed hay fever patients: a comparison with ragweed-allergic asthmatics. *J ALLERGY CLIN IMMUNOL* 56:331, 1975
- Bryant DH, Burns MW, Lazarus L: The correlation between skin tests, bronchial provocation tests, and the serum level of IgE specific for common allergens in patients with asthma. *Clin Allergy* 5:145, 1975
- Nathan RA, Kinsman RA, Spector SL, Horton DJ: Relationship between airways response to allergens and nonspecific bronchial reactivity. *J ALLERGY CLIN IMMUNOL* 64:491, 1979
- Cockcroft DW, Ruffin RE, Frith PA, Cartier A, Juniper EF, Dolovich J, Hargreave FE: Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration, and bronchial responsiveness to inhaled histamine. *Am Rev Respir Dis* 120:1053, 1979
- Neijens HJ, Degenhart HJ, Raatgeep HC, Kerrebijn KF: Study on the significance of bronchial hyperreactivity in the bronchus obstruction after inhalation of cat dander allergen. *J ALLERGY CLIN IMMUNOL* 64:507, 1979
- Boulet L-P, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE: Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J ALLERGY CLIN IMMUNOL* 71:399, 1983
- Platts-Mills TAE, Mitchell EB, Nock P, Tovey ER, Moszoro H, Wilkins SR: Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 2:675, 1982
- Klaustermeyer WB, Prescott EJ, Santiago SM, Kinney JL, Hale FC: The asthmatic airway response to inhaled antigen. *Ann Allergy* 45:338:1980
- Shakib F, Stanworth DR: Human IgG subclasses in health and disease. Part I. *Ric Clin Lab* 10:463, 1980
- Spiegelberg HL: Biological activities of immunoglobulins of different classes and subclasses. *Adv Immunol* 19:259, 1974
- Vijay HM, Perelmutter L: Inhibition of reagin-mediated PCA reactions in monkeys and histamine release from human leukocytes by human IgG4 subclass. *Int Arch Allergy Appl Immunol* 53:78, 1977
- van Toorenenbergen AW, Aalberse RC: IgG4 and passive sensitization of basophil leukocytes. *Int Arch Allergy Appl Immunol* 65:432, 1981
- van Toorenenbergen AW, Aalberse RC: IgG4 and release of histamine from human peripheral blood leukocytes. *Int Arch Allergy Appl Immunol* 67:117, 1982
- Gwynn CM, Ingram J, Almousawi T, Stanworth DR: Bronchial provocation tests in atopic patients with allergen-specific IgG4 antibodies. *Lancet* 1:254, 1982
- 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* 50:625, 1976
- Devey ME, Wilson DV, Wheeler AW: The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin Allergy* 6:227, 1976
- 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* 130:722, 1983