

Seasonal variation in specific IgE antibodies of grass-pollen hypersensitive patients depends on the steady state IgE concentration and is not related to clinical symptoms

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To evaluate parameters that determine the serum titer of specific antiallergen IgE antibodies, we graded the clinical symptoms of 78 grass-pollen hypersensitive patients during two consecutive seasons, while serum total and specific antigass-pollen IgE antibodies were titrated every 2 weeks. Correlation studies of clinical symptoms, grass-pollen counts, and specific IgE antibodies demonstrated that (1) bronchial asthma and nasal symptoms cannot be predicted on the basis of preseasonal IgE titers, (2) clinical symptoms are not related to seasonal antigass-pollen IgE antibodies, (3) antigass pollen and total IgE antibodies are not directly dependent on the air pollen point concentration, (4) increase in specific IgE antibodies during the pollen season is strongly correlated to preseasonal specific IgE titers, and (5) individual fluctuations of specific IgE antibody titers during the pollen season are proportional to preseasonal specific IgE titers. These findings suggest that titration of serum-specific IgE antibodies is of little use in predicting or monitoring the clinical symptoms of grass-pollen hypersensitive patients, since IgE titers strongly depend on individual immune responsiveness. (J ALLERGY CLIN IMMUNOL 1989;83:486-94.)

Specific IgE antibody titration is a keystone in the clinical evaluation and diagnosis of immediate hypersensitivity. However, in early studies,^{1,2} the relationship between serum-specific IgE antibodies and clinical symptoms was found to be loose. This finding is perhaps not so surprising, since first, most IgE antibodies are bound to tissue,³ and second, the mean half-life of serum IgE is 2.7 days only.⁴

More recently, the validity of the relationship between IgE antibodies and symptoms has been questioned,⁵ even when the local production of IgE antibodies in target organs was evaluated.⁶ To examine further this matter, we measured serum IgE antibody titers every 2 weeks, before, and during two consecutive pollen seasons in 78 grass-pollen hypersensitive patients who kept a daily clinical symptom record. The presented results demonstrate that (1) preseasonal

Abbreviations used

BBS:	Borate-buffered saline, 20 mmol/L, pH 8.2
BSA:	Bovine serum albumin
GBS:	Glycine-buffered saline, 50 mmol/L, pH 9.2
GBS-BSA:	GBS containing 1% BSA
GBS-Tween:	GBS containing 0.1% Tween 20
Lx:	Latex particles
PBS:	Phosphate-buffered saline, 8 mmol/L, pH 7.4
TRIS:	Tris(hydroxymethyl)-aminomethane
VAS:	100 mm visual analogue scale
B2:	Mixture of 12 grass pollens

serum grass pollen-specific and total IgE antibodies have no predictive value as to severity of symptoms observed during the subsequent pollen season, (2) the seasonal increase in specific IgE antibodies does not precede nor follow the symptoms, (3) seasonal-specific and total IgE antibody levels are not directly related to pollen counts, and (4) the relative increase and variation in specific IgE antibodies during the season is proportional to the preseasonal titer.

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MATERIAL AND METHODS

Reagents and Buffers

The following reagents were purchased: glycerinated allergen extracts of timothy and ryegrass pollens and B2 from Bencard, Ltd. (Epsom, Surrey, U.K.); Lx of 0.8 μ m, 10% wt/vol, with carboxyl groups (Estapor K1.080) and Lx of 2.3 μ m (L200) from Rhône-Poulenc (Courbevoie, France); 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide HCl and pepsin from Sigma Chemical Co. (St. Louis, Mo.); TRIS from Merck-Schuchardt (Munich, West Germany); BSA and IgE standard serum from Calbiochem-Behring (La Jolla, Calif.); and Tween 20 from Technicon Instruments Corp. (Tarrytown, N.Y.).

Buffers were BBS, 20 mmol/L, containing 9 gm/L NaCl, pH 8.2; GBS, 50 mmol/L, containing 9 gm/L NaCl, pH 9.2; GBS-Tween; GBS-BSA; PBS, 8 mmol/L, containing 9 gm/L NaCl, pH 7.4; and 2 mol/L of TRIS NaOH, pH 11.

Patients

During 2 consecutive years, we studied 26 and 52 patients, respectively, who had seasonal rhinitis for at least 3 years caused by grass-pollen hypersensitivity. This group of patients consisted of 38 men and 40 women with an average age of 30 years (range, 11 to 57 years). Acute conjunctivitis was frequently associated with rhinitis, and two thirds of the patients had a former history of seasonal bronchial asthma. Specific serum IgE evaluated by RAST and positive skin prick tests to grass pollens were present in every case. Half the patients had been desensitized in the past with grass-pollen extracts by conventional methods.

Clinical evaluation

Bronchial asthma- and nasal-symptom diaries were kept by every patient during the grass-pollen season, which, in Belgium (western Europe), lasts from late May to mid-July. Nasal symptoms (obstruction, rhinorrhea, and pruritus) and asthma symptoms (shortness of breath, cough, and wheezing) were graded for severity on a 0 to 4 discontinuous intensity scale.⁷ A daily score, with a maximum value of 12, was calculated for both asthma and rhinitis. In addition, a VAS was used for an overall self-evaluation during the second grass-pollen season. Drug intake, including antihistaminics (terfenadine), inhaled bronchodilator (salbutamol), and oral methylprednisolone, was recorded. Data of pollen rain, measured with a Burkard volumetric trap (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, England), were obtained from Institut d'Hygiène et d'Epidémiologie, Brussels, Belgium (courtesy of Dr. N. Noland).

Preparation of Lx

Lx of 0.8 μ m diameter were coated with F(ab')₂ fragments obtained by pepsin digestion of antihuman Fc, rabbit IgG.⁸

Glycerinated allergen extracts of timothy and ryegrass pollens and of B2 were extensively dialyzed against PBS before use.

Lx of 2.3 μ m diameter were used as allergosorbent. One

hundred microliters of a 10% (wt/vol) suspension was washed in BBS and resuspended in 200 μ l of the same buffer; 100 μ l was retrieved and mixed with 100 μ l of a 10 mg/ml carbodiimide solution in water. After 5 minutes of incubation at 21° C, 10 μ l of a 10 mg/ml allergen solution in BBS was added.

The final volume was adjusted to 1 ml with water, and the suspension was further incubated at 21° C for 4 hours while it was being vortexed.

After centrifugation at 10,000 g for 5 minutes, Lx 2.3 were resuspended by short sonication (model B12 sonifier, Branson Sonic Power Co., Danbury, Conn.) followed by successive centrifugation in GBS-BSA, GBS-Tween, and twice in GBS-BSA. The pellet was finally resuspended in 1 ml of GBS-BSA, sonicated, and kept at 4° C.

Assay of total IgE antibodies

Blood was obtained before and every other week throughout the grass-pollen season (five to seven bleedings for each patient). Serum samples were stored at -20° C for analysis. Total serum IgE was titrated after pepsin digestion as previously described.⁹

Thirty microliters of serum was digested for 5 minutes by adding 160 μ l of 5 mg/ml pepsin in 0.4 N HCl and 70 μ l of water. Sixty microliters of 2 mol/L TRIS-NaOH, pH 11, was then added to increase the pH. Thirty microliters of the solution was mixed with 30 μ l of antihuman Fc, Lx 0.8 (0.04% suspension in GBS) and incubated for 40 minutes at 37° C with continuous shaking.

Fifty microliters of twofold dilutions of a standard IgE solution in GBS containing 5% BSA was digested with pepsin and used to construct a reference curve. Lx agglutination, which was proportional to the concentration of IgE fragments in the solution, was measured by instrumental counting of the residual nonagglutinated particles in the IMPACT system (Acade Diagnostic Systems, s.a., Brussels, Belgium).

Assay of grass pollen-specific IgE antibodies

As described elsewhere,¹⁰ in this assay, 100 μ l of serum was incubated in a microtest tube 3811 (Eppendorf, Hamburg, West Germany) with 50 μ l of allergen-coated Lx 2.3 (0.5% suspension in GBS) for 210 minutes at 37° C with continuous shaking. The suspension was then centrifuged for 2 minutes at 9000 g in a Biofuge A (Heraeus Christ, Osterode, West Germany); the pellet was washed with 1 ml of 9 gm/L of NaCl and resuspended in 50 μ l of GBS containing 5% BSA. Bound IgE antibodies were then digested for 30 minutes at 21° C by adding 1 mg/ml of pepsin in 80 μ l of 0.15 N HCl; 20 μ l of 2 mol/L of TRIS-NaOH, pH 11, was then added to restore the physiologic pH. Thirty microliters of the suspension was retrieved to calculate IgE concentration as above.

Statistical methods

Serum IgE data were transformed to their logarithmic values for analysis. Statistical parametric methods were used

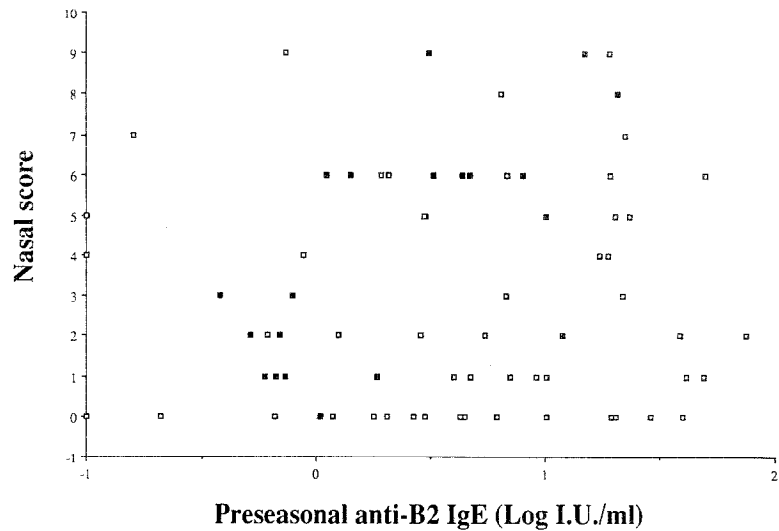


FIG. 1. Mean nasal scores (maximum value, 12) were calculated for each patient during 7 days during the peak of pollen rain and related to the preseasonal anti-B2 IgE antibody titers; first year (■); second year (□).

TABLE I. Preseasonal IgE antibodies and clinical symptoms

IgE	Clinical scores		
	Bronchial asthma (<i>r</i>)	Rhinitis (<i>r</i>)	VAS (<i>r</i>)
First year (N = 26)			
Total	0.10	0.08	ND
Anti-B2	0.47*	0.65†	ND
Antitimothy	0.61†	0.47*	ND
Antiryegrass	0.33	0.48*	ND
Second year (N = 52)			
Total	0.19	0.17	0.07
Anti-B2	0.05	0.02	0.02
Antitimothy	0.01	0.01	0.09
Antiryegrass	0.03	0.10	0.18

ND, Not done.

Correlations between preseasonal total and specific IgE antibody titers and clinical scores evaluated during the peak of pollen rain.

Correlation coefficients (*r*) were not significant except:

**p* < 0.05.

†*p* < 0.01.

for analysis of serum IgE titers, clinical scores, and pollen counts.

RESULTS

Grass-pollen seasons

Patients studied during the second grass-pollen season did not differ from patients studied during the first season as to age, sex, disease duration, or mean symptom score (previous years). The proportion of patients previously desensitized by conventional methods was

similar in both groups. We found no direct relationship between medication intake and symptom scores, probably because the patients were instructed to take the medication only when it was necessary and not for symptom prevention. Mean drug intake was similar during both years, and all patients were symptom free when the study began.

Mean pollen counts measured in grains per cubic meter per day was 34.9 ± 12.6 (SEM) and 26.6 ± 9.4 (SEM) for the first and second year, respectively.

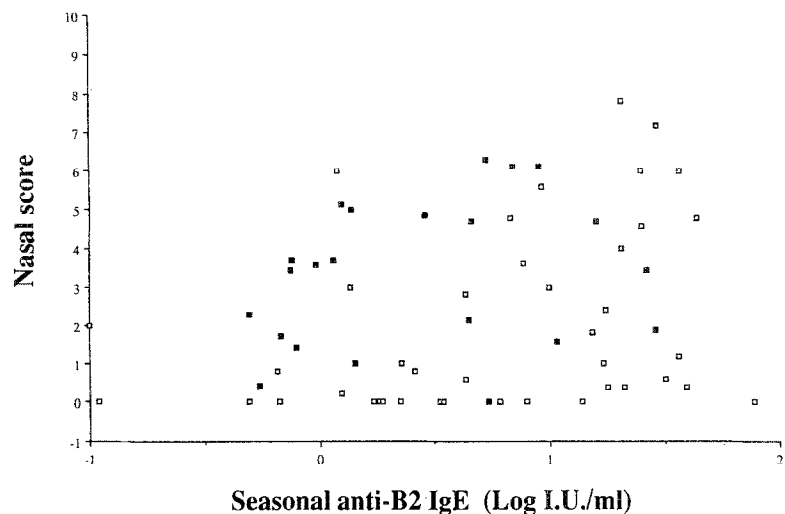


FIG. 2. During the peak of pollen rain, mean nasal scores (maximum value, 12) were calculated for each patient during 5 days that followed anti-B2 IgE titration; first year (■); second year (□).

TABLE II. Seasonal IgE antibodies and clinical symptoms

IgE	Clinical scores		
	Bronchial asthma (<i>r</i>)	Rhinitis (<i>r</i>)	VAS (<i>r</i>)
First year (N = 26)			
Total	0.19	0.09	ND
Anti-B2	0.13	0.22	ND
Antitimothy	0.22	0.27*	ND
Antiryegrass	0.33*	0.39*	ND
Second year (N = 52)			
Total	0.08	0.07	0.08
Anti-B2	0.08	0.34*	0.06
Antitimothy	0.06	0.26*	0.13
Antiryegrass	0.02	0.23*	0.20

ND, Not done.

Correlations between total and specific IgE antibody titers measured during the peak of pollen rain and clinical scores calculated during 5 days after IgE titration.

Correlation coefficients (*r*) were not significant except:

**p* < 0.05.

As for the week when the highest pollen-grain concentrations were observed, mean values were of 85 and 67 grains per cubic meter per day for the first and second season, respectively. Since we considered that any allergic individual will experience symptoms when the pollen count rises above 50 grains per cubic meter, this difference was not judged as significant.

IgE antibodies and clinical symptoms

First, we considered whether the titer of preseasonal specific IgE antibodies could be used to predict the development and the severity of symptoms during the

next pollen season. For this purpose, we used either B2 or single pollen species, timothy and ryegrass, most frequently encountered in grass-pollen hypersensitivity. We calculated the mean nasal score during 7 days during which the peak of pollen rain occurred. No significant relationship was observed between this score and the preseasonal anti-B2 IgE antibodies, as illustrated in Fig. 1. Similarly, the mean bronchial asthma score calculated during the same period was inconstantly related to IgE antibodies (Table I). Analogous results were obtained when the IgE immune response to single pollen species was evaluated or

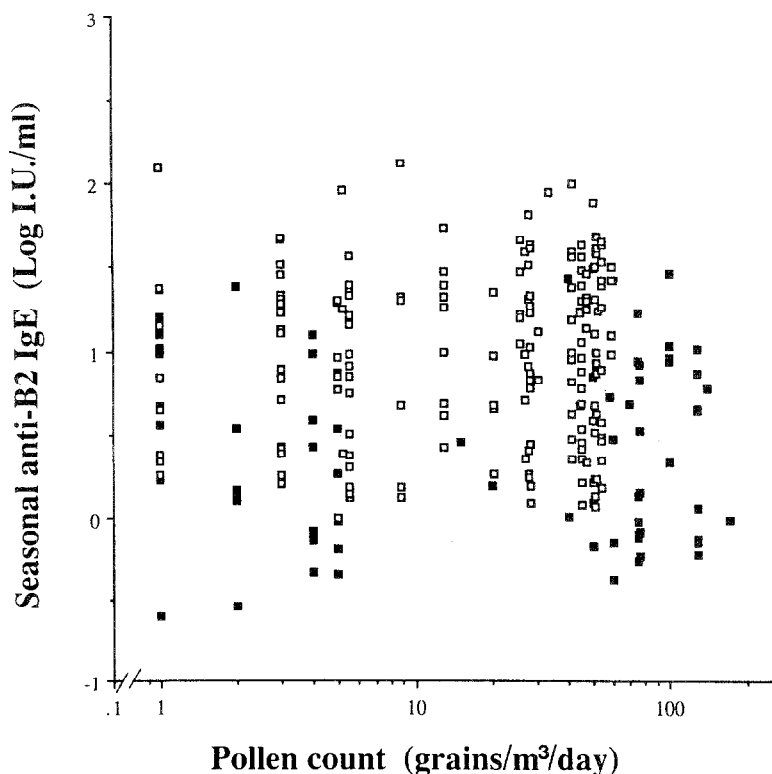


FIG. 3. Seasonal anti-B2 IgE antibodies were titrated for each patient and related to pollen density throughout pollen seasons. IgE titers were plotted with respect to the pollen counts during 5 days preceding serum sampling; first year (■); second year (□).

when the patients were classified according to whether or not they had been desensitized in the past.

Next, we examined the relationship between specific or total IgE antibodies corresponding to the peak of pollen rain and mean symptom scores measured on three occasions: 5 days before IgE titration and 1 day and 5 days after IgE titration. Results presented in Fig. 2 illustrate that, during the peak of pollen rain, anti-B2 IgE antibody titers were not correlated with nasal symptoms averaged during 5 days after IgE titration. These results are extended in Table II to bronchial asthma and to the VAS and demonstrate in addition that IgE antibody titers to single pollen species are not related to symptoms either.

We made a similar observation when the change in specific IgE concentration, corresponding to the difference between highest and initial values, was compared to the symptom scores. Evaluations performed on individual data, on data combined for the two seasons, or when a mean symptom score was calculated 5 days before or 1 day after IgE titration, yielded the same results (data not presented).

Specific IgE antibodies and pollen counts

Since clinical symptoms and specific IgE antibodies did not influence each other directly, we posed a ques-

tion as to whether IgE antibodies depended on pollen air concentration. Pollen counts 5 or 10 days before titration of antigress-pollen IgE were chosen for this analysis. The results depicted in Fig. 3 demonstrate the absence of a direct relationship between seasonal anti-B2 IgE antibodies and pollen counts. A similar result was obtained for the IgE immune response to timothy and ryegrass pollens, or when individual data were analyzed (data not presented).

Preseasonal and seasonal IgE antibodies

Finally, we tried to determine whether the variation of specific IgE antibodies throughout the pollen season was related to the level of specific IgE measured before the season. In Fig. 4, the relationship between pre-seasonal anti-B2 IgE antibodies and the highest and lowest anti-B2 IgE concentrations evaluated during the season for every patient is illustrated. That the increase in specific IgE antibodies is proportional to the preseasonal IgE concentration is also illustrated in Fig. 4. Moreover, individual specific IgE concentrations fluctuate considerably during the season; these variations are themselves in proportion with the pre-seasonal IgE antibody concentration. A similar conclusion was reached with antitimothy or antirye grass IgE antibodies (data not presented).

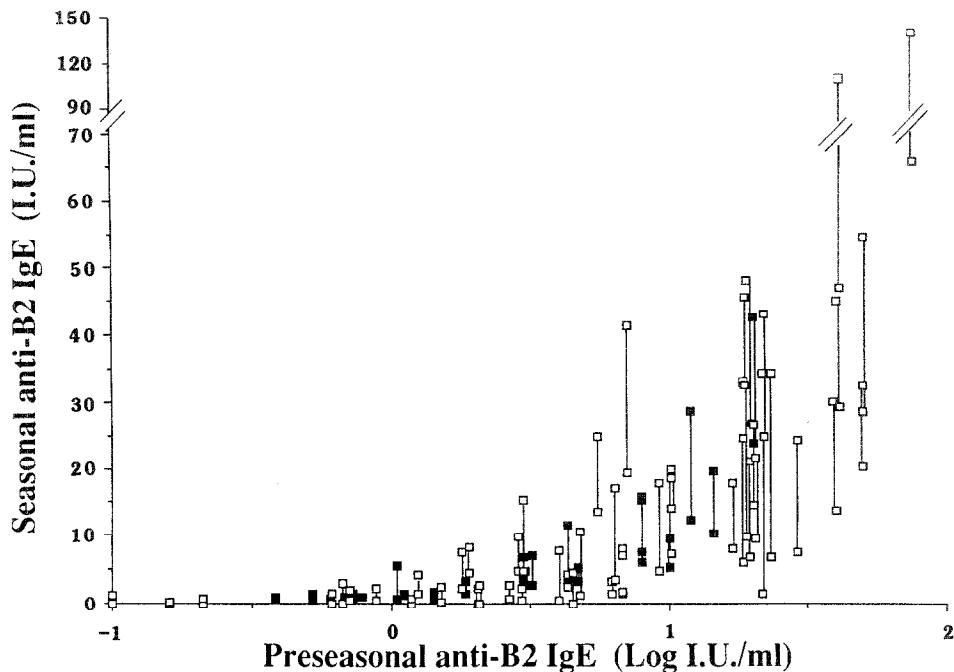


FIG. 4. Anti-B2 IgE antibodies were measured for each patient before the pollen season and compared to seasonal anti-B2 IgE titers. Vertical lines connect the lowest and the highest individual IgE concentrations; first year (■); second year (□).

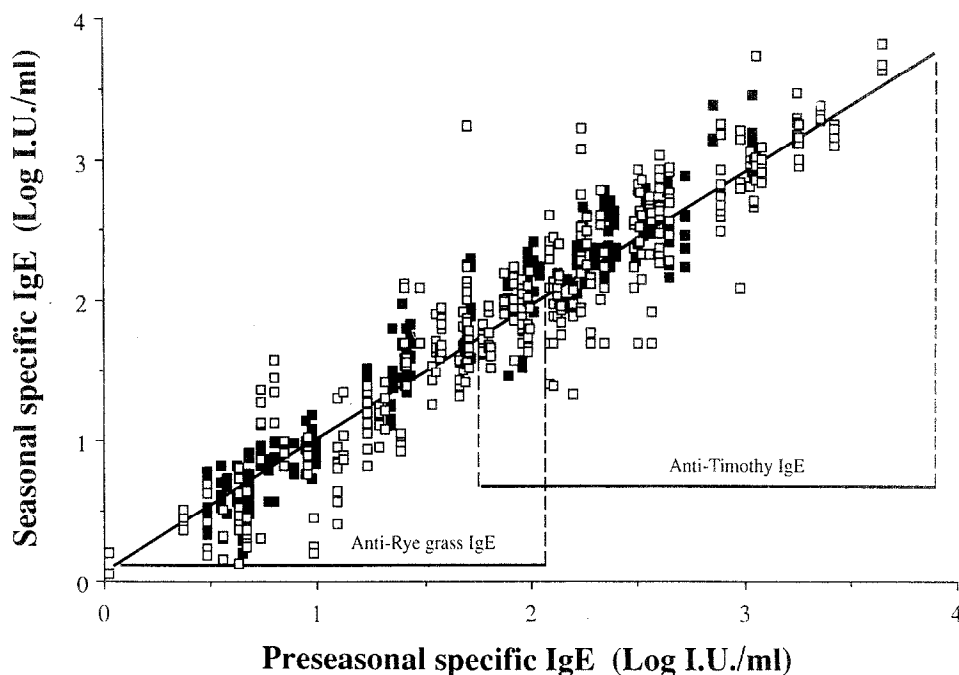


FIG. 5. Linear regression of preseasonal against seasonal specific IgE to ryegrass and timothy pollens. A total of 836 data are included. Log-Log regression equation is $y = 0.06 + 0.98 x$; $r = 0.94$; $p < 0.01$; first year (■); second year (□).

The variations in IgE antibody titers can be easily described by use of a simple linear regression model that expresses the log concentration of seasonal specific IgE antibodies as a function of the log concen-

tration of preseasonal specific IgE. The linear regression between log concentration of antitimothy- and antiryegrass-pollen IgE antibodies evaluated before and during the two pollen seasons is illustrated in Fig.

TABLE III. Preseasonal and seasonal IgE antibodies

IgE	n	Origin	Slope	r
First year				
Total	146	0.35	0.84	0.95
Anti-B2	174	0.09	0.96	0.94
Antitimothy	174	0.47	0.83	0.80
Antirycgrass	174	0.01	1.04	0.91
Second year				
Total	239	0.47	0.81	0.85
Anti-B2	244	0.23	0.80	0.80
Antitimothy	244	0.47	0.81	0.84
Antirycgrass	244	-0.02	0.98	0.91

n, Number of data.

Log-log correlations between preseasonal serum total or specific IgE throughout pollen seasons. Correlation coefficients (*r*) were highly significant in each case (*p* < 0.01).

5. Both the correlation coefficient (*r* = 0.94; *p* < 0.01) and regression equation (*y* = 0.06 + 0.98 *x*) indicate that the serum titer of specific IgE during the pollen season is strongly dependent on the serum titer of specific IgE before the season. Separate analysis of data obtained during the two pollen seasons yielded similar origins, slopes, and correlation coefficients (Table III).

Predicted values for seasonal IgE antibodies were obtained by solving multiple regression equations of the following form:

$$y = a + b_1 x_1 + b_2 x_2 + \dots + b_n x_n$$

where each regression coefficient (*b*₁ . . . *b*_{*n*}) denoted partial regression coefficient of *y* on all explanatory variables (*x*₁ . . . *x*_{*n*}). Stepwise calculations, including such variables as pollen counts, preseasonal IgE antibodies, VAS, and nasal and asthma symptoms, were performed. Partial regression coefficients were not statistically significant, except for preseasonal IgE antibodies (*p* < 0.01). Therefore, it was confirmed that the specific IgE concentration before a pollen season explained most of the observed seasonal IgE variations.

DISCUSSION

Exposure to grass pollen immediately triggers symptoms in hypersensitive patients¹¹⁻¹³ whose specific IgE antibodies increase toward the end of a pollination period.^{14, 15} Nevertheless, in spite of these observations, the relationship between specific IgE antibodies and the symptoms remains unclear.¹⁶ Indeed, very few investigators¹⁴ have carried out simultaneously the follow-up of variations in serum IgE antibody levels and the evaluation of symptoms during pollination periods.

To investigate further these relationships, we fol-

lowed 78 grass-pollen hypersensitive patients during two consecutive seasons. The study included a daily symptomatic evaluation and a titration of specific IgE antibodies every 2 weeks. We have observed that the main parameter that significantly influenced seasonal increase and variation of IgE antibodies is the pre-seasonal IgE antibody concentration.

The assay used for titration of serum-specific IgE differed in some respect from the RAST.¹⁰ Lx are activated with carbodiimide instead of cyanogen bromide. The surface accessible for antibody binding on Lx is 10 times larger and yet presents a lower degree of nonspecific adsorption. Comparative studies have demonstrated an increase in sensitivity and a strong correlation (*r* ≥ 0.92) between the two assays. It appears therefore unlikely that technical differences would have produced significantly different results.

We demonstrate that the titer of specific IgE (or total IgE) antibodies measured immediately before the pollen season is not helpful in anticipating the patient's clinical sensitivity. The type or amount of medication intake during the pollen season or before desensitization of patients has had no impact on the above relationship. These findings suggest that, for clinical purposes, a precise quantitative evaluation of serum-specific IgE is useless. For the same reasons IgE titers should not be taken into account when hyposensitization is considered.

Since symptoms did not coincide with specific IgE antibody variations during the pollen rain, it was difficult to attribute a direct responsibility for symptom triggering to IgE antibodies. However, as most of the latter are produced locally in target organs, specific antibodies in secretions could be more adequately related to symptoms.⁶ Secretory IgE was not measured in this study. Nevertheless, as serum IgE antibodies are known to be correlated with locally produced

IgE,¹⁷ it is unlikely that the latter would be more useful for symptom evaluation, as has already been pointed out by other authors.^{5, 18, 19}

When hypersensitive patients are exposed to allergens, inflammatory changes occur in the respiratory mucosa, and the resulting increase of permeability allows for an easier allergen access to the submucosal lymphoid tissue. Therefore, we wanted to find out whether clinical symptoms could trigger the increase in IgE antibodies. Although we have found no relationship between the symptoms and IgE antibodies measured immediately afterward, it is not excluded that changes in IgE titers during the pollen season are indirectly related to symptoms. Desensitization, when it is clinically successful, is characterized by disappearance of the seasonal increase in IgE antibodies. A recent study²⁰ mentioned, however, that changes in serum IgE antibodies occurring throughout the pollen season were not in accordance with symptom-medication scores.

Variations in specific IgE antibody titers during pollen exposure were unexpected. We were unable to disclose a direct relationship between the pollen point concentration and IgE titers. These variations resemble variations encountered in animal experiments²¹ in which antigenic stimulation is followed by an increase in antibodies both specific for the antigen and for the corresponding idiotype, with an inverse proportion between them. This hypothesis is currently under investigation.

Multiple regression analysis of biologic parameters with clinical symptoms and/or pollen counts confirmed that variation of specific IgE antibodies toward the end of the pollination period was strongly dependent on the preseasonal IgE concentration. Although the overall increase in specific IgE during the season remained moderate, it was still proportional to the initial level of specific IgE antibodies, an observation already made by other investigators.²² These findings should be considered with regard to the association between the IgE immune response to ragweed allergen Ra5 and HLA-Dw2.^{23, 24} High and low IgE responders will perhaps be identified in man,²⁵ and this would provide a new way for exploring the mechanisms underlying IgE production.

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