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A computational approach to the description of individual immune responses

IgE and IgG-subclass allergen-specific antibodies formed during immunotherapy

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Detailed evaluation of the IgE and IgG-subclass immune response during immunotherapy can now be performed by crossed radio immunoelectrophoresis (CRIE). Some new concepts are introduced facilitating the handling of the vast amount of data obtained by quantitating the immune response. These concepts are "distance" between antibody responses and "immune response width". The 20 patients included in this study were pollen-allergic patients who underwent specific immunotherapy in a 3-year prospective study. It was found that the immune response during immunotherapy was restricted to IgG_1 and IgG_4 antibodies. The semi-quantitative CRIE analysis correlated with the RAST analysis for the IgE samples before start of immunotherapy, for the IgG_1 samples at week 12, and for all the IgG_4 samples. During immunotherapy the number of IgG_1 antibodies directed to the different antigens increased towards 11 antigens and decreased towards six. For the IgG_4 antibodies the number of reactions increased towards 15 antigens and decreased towards four. The increase is generally paralleled by an increase in the quantitative immune response as well. For some of the antigens a rise in the IgE antibodies is contrasted by a fall in the IgG_4 antibody response, and for other antigens the opposite was true, indicating a regulatory mechanism between the IgE and the IgG_4 synthesis. The IgE immune response to a number of antigens, including the major allergens before the start of immunotherapy, was quantitatively diminished during the period of immunotherapy when IgG_1 was present early (week 12) in the period, and for other antigens there was a rise in IgE without an early IgG_1 antibody response. This suggests that IgG_1 can have a regulating influence on the IgE synthesis. Finally, we have found that IgE antibodies with specificities not present in the samples taken before immunotherapy were formed during immunotherapy. These new IgE antibodies do not, however, seem to impair the outcome of treatment.

Key words: crossed radioimmunoelectrophoresis; IgE; IgG-subclass antibodies; immunotherapy; pollen allergy, timothy.

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A study of the IgG-subclass antibody response during allergen-specific immunotherapy of IgE-mediated allergic diseases has previously been performed using the RAST technique (5, 7). A modification of the crossed radioimmunoelectrophoresis (CRIE) technique (20) and the aid of monoclonal antibodies (21) has now made it possible to analyse the IgG-subclass antibody response in more detail. The RAST shows that the IgG-subclass antibody response during immunotherapy with grass pollen is restricted to IgG_1 and IgG_4 (4, 5) and that prolonged immunization with some antigens may lead to an IgG_4-dominated antibody response (1). In most patients an IgG_1 antibody response is seen at the beginning of the immunization period, later followed by an IgG_4 antibody response (5). It has been found in grass pollen-allergic patients (5) that a high amount of IgG_4 allergen-specific antibodies after 12 weeks of immunotherapy was significantly

correlated to bad outcome. Further, there was a tendency towards a positive correlation between a high preseasonal amount of IgG_4 and a high symptom score in the following season (5).

A detailed knowledge of the individual immune response during therapy could lead to development of new antigen preparations for immunotherapy (14, 18, 19). The aim of the present study was to devise a model for future CRIE studies and use it on actual serum samples. Two new concepts useful for handling the data obtained in this type of study are introduced: the immune response width and the distance between patterns. These methods can be used to see whether the CRIE patterns in the immune response before and after immunotherapy, i.e. the IgE and the IgG-subclass antibody responses, follow common rules in different patients.

MATERIAL AND METHODS

Patients and procedure

The study design, patients and immunotherapy procedure have been described in detail elsewhere (27). The 20 study patients were immunized with a partially purified timothy grass pollen extract (Alutard® SQ 225, ALK, Copenhagen, Denmark). This allergen preparation is a biologically standardized extract adsorbed to $Al(OH)_3$ consisting of 22 of the approximately 40 antigens in timothy (*Phleum pratense*) pollen. The immunotherapy started in January 1978 with weekly treatments until June 1978 and beginning with 2 biological units (BU). The maintenance dose, which was 10,000 BU or the highest tolerated, was reached in week 12. From the grass pollen season 1978 the interval between injections was increased to 4 weeks and in the season the dose was reduced to 1/3-1/4. The serum samples used for the present work were collected at week 0 (before treatment, January 1978, sample 1), after week 12 (preseason 1, 1978, maintenance dose reached, sample 3), and after week 116 (preseason 3, 1980, end of immunotherapy, sample 7).

The antigen used was a standardized aqueous extract of timothy pollen *Phleum pratense* obtained from Allergologisk Laboratorium (ALK) A/S (Copenhagen, Denmark). The extract was lyophilised and dissolved in 0.15 M saline prior to use.

Antisera

Antibodies against the allergen extract were raised in rabbits by established procedures, and the immunoglobulin fraction was isolated as described (9). Monoclonal antibodies to human IgG-subclasses were purchased from Unipath (London, U.K.) as ascitic fluid containing 1 mg/ml sodium azide (code Nos. BAM 15(anti-IgG_1), BAM 10 (anti-IgG_2), BAM 08 (anti-IgG_3) and BAM 11 (anti-IgG_4)). Except for the addition of aprotinin (100 KU/ml) to inhibit proteolysis, the ascitic fluids were used as received. The specificity of the monoclonal IgG subclass antibodies was tested as previously described (7). Normal rabbit serum (NRS) (Gibco Europe Ltd., Paisley, Scotland) was heat-inactivated at 56°C for 30 min and sodium azide (1 mg/ml) was added. Bovine gammaglobulin was from Sigma (St Louis, U.S.A.). ^{125}I-labelled anti-human IgE (RAST Isotop®) was from Pharmacia, Uppsala, Sweden. Rabbit anti-mouse IgG was obtained from DAKO, Copenhagen, Denmark, and was purified and labelled as described (7, 8).

IgE and IgG subclass CRIE procedures

CIE was done according to (24) and the IgE and IgG subclass CRIE were subsequently performed using these CIE plates as described previously (21, 26) with buffers and washing solutions originating from (6).

Quantitation of reactions in CRIE

The radiostaining was quantitated using a grey scale made by applying different amounts of labelled IgE to filter paper discs. The discs were numbered from 1 to 6 and double-up doses of labelled IgE were applied so that disc No. 1 had the lowest amount of radioactivity. After autoradiography of the discs the precipitates in the CRIE were visually compared with this arbitrary grey scale.

RAST for determination of IgE antibodies to timothy antigens

Serum IgE antibodies to timothy pollen extract were measured using the Al-RAST procedure (13, 25, 28). The timothy pollen antigens were coupled to $Al(OH)_3$ (Alhydrogel®, Superfos, Vedbæk, Denmark) and used as allergosorbent. The bound IgE antibodies were detected with ^{125}I-labelled anti-human IgE. Finally, the results were expressed as sorbent units/ml undiluted serum (SU/ml) (27, 28).

RAST for determination of IgG subclass antibodies to timothy allergens

The serum IgG subclass antibodies to timothy pollen antigens were measured using the three-layer immunoradiometric assay (TIRA) as described in detail elsewhere (7). The antibody concentrations were expressed in units/ml serum (U/ml) after calibration of the assay with a serum sample arbitrarily ascribed 100 U/ml IgG_4 antibody to timothy pollen antigens in undiluted serum. The assay was found to be specific for antibodies to grass pollen as well as being subclass and isotype specific (7).

Concepts in evaluation of antibody responses with CRIE

CRIE results can be evaluated from two main view points. The antibody response in a given isotype can be seen statistically as patients' reactions to given antigens. Such terms as major allergens and allergogram have been previously defined (11), and here we define the terms *major immunogen* and *immunogram*. Major allergens and major immunogens are antigens which bind IgE and IgG subclass antibodies from at least 50 % of the tested patient sera. Allergograms and immunograms are graphical representations of patients' reactions to each antigen by IgE and IgG antibodies. The term antigen will be reserved for the antigens reacting with the antibodies that form the basis of the CRIE analysis, *in casu* the rabbit antibodies.

The other view point allows us to study the development of the immune response in the different isotypes in individual patients. This way we study a given patient's antibody responses to all possible antigens in the extract. This view is connected to the concepts allergoprint (11) and immunoprint, which are graphical representations of the individual patient's reactions to all the antigens in IgE and IgG subclasses. For further analysis of the problem we define two new concepts, the distance between antibody responses and the immune response width. The distance is a single figure relating to the matching between two antibody responses, i.e. a large distance means few reactions to the same antigens and vice versa. The immune response width is the number of antibody responses in a given sample for a given patient and a difference in immune response width is the diffence between the immune response width for two samples. These new concepts are not restricted to the analysis of CRIE patterns but could equally well be used for evaluation of other types of patterns, i.e. from immunoblotting of isoelectric focusing and SDS PAGE gels. These methods can also be combined with digital image processing (22) in order to analyse and evaluate a larger number of patterns.

Distance between different CRIE patterns

To compare the CRIE antibody responses in different antibody classes or different samples in the same antibody class, an antibody response to a certain antigen was allocated a value of 1 (one) if present and a value of 0 (zero) if absent. The distance (d) between the reaction in different samples or different isotypes to a given antigen is then 0 if a reaction is present in both cases, and 1 if there is a reaction in one of the cases and no reaction in the other. Then when comparing two antibody responses the total normalized distance D can be calculated by summation of all distances as follows:

$$D = 1/n \quad \overset{n}{*\Sigma}_{i=1} \quad \text{abs}\,(d1_i - d2_i)$$

where n is the total number of precipitates.

This index has a value of 0 if the CRIE reactions match totally and a value of 1 if the CRIE reactions do not match at all. The concept can be seen as a key and lock concept, i.e. the smaller the

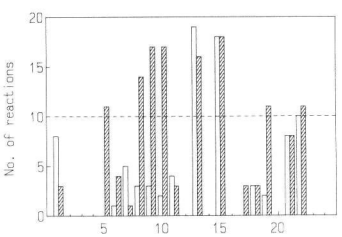

Fig. 1. Allergogram showing the number of reactions in the IgE (sample before start, empty columns) and IgE (week 116, hatched columns) samples from the 20 patients to each of the antigens used for immunotherapy. Columns exceeding the dotted line represent the major allergens.

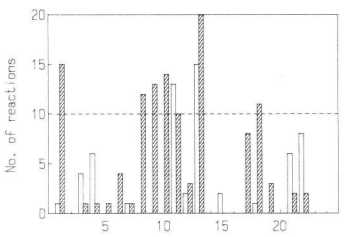

Fig. 2a. Immunogram showing the number of IgE_1 reactions to each of the antigens in week Nos. 12 (empty columns) and 116 (hatched columns) from the 20 patients. Columns exceeding the dotted line represent the major immunogens.

distance the better the fit. However, equal distance does not indicate that the reaction patterns are equal, but rather that there is a match between two reaction patterns.

Table 1.

The quantitated immune response measured by CRIE to all antigens from all patients.

Precipitate No.	Week no.	IgE 0	IgE 116	IgG_1 12	IgG_1 116	IgG_4 12	IgG_4 116
1		15	3	2	27	3	4
2		0	0	0	0	0	2
3		0	0	8	2	2	4
4		0	0	12	2	4	6
5		0	13	0	1	0	38
6		3	6	0	8	8	39
7		15	1	2	2	0	5
8		8	18	0	26	11	8
9		7	22	0	18	13	13
10		5	22	0	32	10	8
11		8	3	14	10	14	57
12		0	0	4	6	2	8
13		48	28	20	49	21	50
14		0	0	0	0	0	0
15		30	20	2	0	10	37
16		0	0	0	0	2	10
17		0	4	0	17	23	5
18		3	3	1	11	4	51
19		6	15	0	3	27	15
20		0	0	0	0	2	2
21		31	15	12	4	11	47
22		40	21	17	4	22	51
	Total	219	294	94	222	189	460

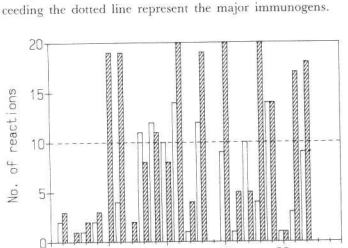

Fig. 2b. Immunogram showing the number of IgE_4 reactions to each of the antigens in week Nos. 12 (empty columns) and 116 (hatched columns) from the 20 patients. Columns exceeding teh dotted line represent the major immunogens.

Calculation of immune response width

The number of radiostained precipitates for a given patient in a sample is defined as the immune response width (W), and the difference in the number in two different samples is defined as delta W. This value indicates the development of the immune response over a period of time, or for different patients at the same time, in terms of the number of reactive precipitates. As an example of the nomenclature used the difference in width between the IgE response in the sample at start (sample 1) and the IgE response in the sample at week 116 (sample 7) will be written delta W IgE(1)/

Table 2.

Distance between the IgE immune response before immunotherapy (week 0) and the IgE and IgG-subclass response during immunotherapy (weeks 12 and 116) and between the IgG subclass responses during immunotherapy (weeks 12 and 116)

Week No. 0/116	IgE/IgE 0/116	IgE/IgG_1 0/12	IgE/IgG_1 0/116	IgE/IgG_4 0/12	IgE/IgG_4 0/116	IgG_1/IgG_1 12/116	IgG_4/IgG_4 12/116
Pt. No.							
4	.23	.50	.32	.50	.27	.36	.59
6	.27	.32	.41	.18	.27	.45	.18
8	.18	.23	.45	.09	.36	.50	.36
9	.14	.05	.45	.14	.23	.41	.36
10	.23	.27	.55	.36	.41	.45	.50
12	.27	.05	.23	.14	.41	.18	.45
13	.18	.05	.27	.27	.59	.23	.68
15	.36	.14	.32	.41	.23	.36	.55
18	.41	.18	.36	.41	.32	.27	.45
20	.14	.23	.32	.23	.27	.36	.32
21	.27	.09	.32	.27	.36	.23	.45
23	.23	.14	.32	.45	.50	.36	.23
26	.27	.36	.23	.36	.50	.32	.23
27	.05	.23	.09	.27	.59	.23	.32
28	.23	.27	.27	.27	.36	.09	.36
29	.41	.23	.27	.41	.23	.23	.45
30	.14	.23	.14	.36	.14	.27	.41
34	.18	.23	.36	.32	.45	.41	.50
37	.23	.23	.27	.23	.41	.23	.36
40	.32	.05	.18	.50	.50	.14	.27
Median	.23	.23	.32	.30	.36	.30	.39

IgE(7) and is equal to (W IgE(7)-W IgE(1)). The sign obtained when calculating the difference is kept to indicate the development in width. When looking for regulating factors the presence or not of a reaction is more important than the quantitative response. The quantitative response might be important because there could be a threshold above which an antibody could exert its influence and under which it has none. As we do not know this threshold it is important to record all the reactions and then seek for patterns. This is in accordance with (4).

Statistics

The relations between the IgE and IgG subclass antibody concentrations in the different samples were tested by calculation of Spearman's rank correlation coefficient, r_s (17). The significance of the rank correlation coefficients was tested by calculation of a t-value as described (17). Correlations with a two-sided stochastic probablility ≤ 0.05 were considered statistically significant. All the statistic calculations were performed using the Minitab® statistics software (release 82.1) on an IBM AT3 compatible microcomputer.

RESULTS

In the CRIE analyses only antibodies to timothy pollen antigens of the IgG_1 and IgG_4 subclasses could be detected, and the same applied throughout the immunization period with the IgG subclass RAST (5).

Major allergens and major immunogens

Fig. 1 shows an allergogram with the number of IgE reactions for each patient to timothy pollen antigens at start and at week 116. The corresponding quantitated antibody responses to each antigen by the patients are shown in Table 1. The major allergens, i.e. antigens which bind IgE from at least 50% of the tested patients, are the antigens above the dotted line in Fig. 1. Thus, there are three major allergens before immunotherapy and eight major allergens after. Fig. 2a and b shows in a similar manner the immuno-

Table 3.

The immune response width, i.e. the number of antigens as seen in the CRIE to which each patient reacts with IgE and IgG subclass antibodies. In parentheses are the respective summed quantitated antibody responses for each patient

Pt. No. / Week No.	IgE 0	IgE 116	IgG_1 12	IgG_1 116	IgG_4 12	IgG_4 116
4	11(34)	14(21)	nd	8(16)	nd	13(25)
6	8(22)	12(20)	1(1)	11(22)	8(11)	12(25)
8	2(3)	6(7)	5(8)	10(20)	2(2)	10(21)
9	3(4)	nd	2(3)	11(22)	0(0)	8(19)
10	5(15)	nd	1(1)	11(25)	3(4)	12(22)
12	3(4)	9(13)	2(4)	6(11)	2(3)	12(27)
13	2(2)	4(4)	1(1)	6(9)	4(6)	15(28)
15	5(10)	7(10)	4(6)	6(10)	10(15)	8(20)
18	3(4)	12(18)	1(1)	7(10)	6(8)	6(11)
20	5(15)	8(10)	nd	8(15)	nd	7(14)
21	4(7)	10(11)	2(3)	7(13)	6(8)	10(19)
23	2(3)	7(8)	3(4)	7(13)	12(20)	13(25)
26	4(9)	6(7)	6(10)	5(7)	8(12)	13(26)
27	0(0)	1(1)	5(9)	2(3)	6(11)	13(25)
28	6(26)	7(15)	nd	2(3)	nd	8(20)
29	7(22)	10(19)	6(10)	1(2)	12(19)	12(27)
30	4(7)	5(5)	7(12)	1(2)	10(14)	7(18)
34	4(7)	6(6)	5(8)	6(10)	9(11)	12(28)
37	7(24)	8(11)	6(11)	1(2)	10(23)	16(39)
40	1(1)	8(8)	2(2)	5(7)	12(22)	12(21)
Total	86	140	59	121	120	219

grams with the IgG_1 and IgG_4 subclass reactions to antigens at weeks 12 and 116. Like major allergens we here define the term major immunogens. These are antigens to which at least 50 % of the patients react with IgG subclass antibodies and are, as mentioned above, the antigens above the dotted line in the immunograms. It can be seen that for IgG_1 and IgG_4 there are two and seven major immunogens, respectively, before immunotherapy and seven and 10, respectively, after. The concept major allergen/immunogen should be seen as a dynamic entity (see Discussion) and therefore the major allergens/immunogens are given for each sample throughout the immunization period.

Distance between IgE and IgG subclass antibody responses

The calculated distances between the IgE antibody response before immunization and the IgE and IgG subclass antibody responses in the samples taken at different times throughout the immunization period are shown in Table 2. Although no IgG_1 and IgG_4 subclass antibodies could be detected in the first samples, i.e. before the start of the immunization, the distance between the IgE and IgG subclass antibodies would not necessarily be 1 because no patients have an IgE antibody response covering all the antigens. Development towards a larger distance can often be seen when comparing one isotype at a given time with another isotype at a different time. This can mean (but not always) that the immune response in the second isotype has become broader with time.

Immune response width

Table 3 shows the number of antigens as seen in the CRIE to which each patient reacts with IgE and IgG subclass antibodies. Thus the immune response width is the number of antibody responses in a given isotype as "seen" by the patient. Table 3 also shows that for the IgE antibody response during immunotherapy there is always an increase with time in the number of antigens to which antibodies are formed. This is not paralleled by an increase in the quantitated antibody response also

Table 4.

Correlation between IgE, IgG_1 and IgG_4 antibody concentrations in CRIE at different times during immunotherapy

	Week No.	IgE 0	IgE 116	IgG_1 12	IgG_1 116	IgG_4 12
IgE	116	0.680				
IgG_1	12	0.219	-0.297			
IgG_1	116	0.011	0.280	-0.655		
IgG_4	12	0.273	0.072	0.441	-0.661	
IgG_4	116	0.059	-0.098	0.248	-0.248	0.231

The correlations are Spearman's rank correlation coefficient.

t-Values and sample size:	Sample No.	IgE 1	IgE 7	IgG_1 3	IgG_1 7	IgG_4 3
IgE	7	3.710, 18*				
IgG_1	3	0.869, 17	-1.121, 15			
IgG_1	7	0.047, 20	1.167, 18	-3.357, 17*		
IgG_4	3	1.099, 17	0.260, 15	1.903, 17	-3.412, 17*	
IgG_4	7	0.251, 20	-0.394, 18	0.991, 17	-0.991, 20	0.920,17

t: t-value, calculated as described in (15), for the null hypothesis that r_s is zero.

* Two-sided probability for the observed t-value $< = 0.05$

seen in Tables 3 and 1. In general, the width of the IgG_1 antibody response increases during immunotherapy but some patients show a decrease in the immune response width for IgG_1. These changes are all paralleled by corresponding changes in the quantitative antibody responses. For IgG_4 there is a general increase in the immune response width. The corresponding quantitative antibody response is generally increasing. The width can also be compared for different isotypes simultaneouely, showing that for approx. 60 % of the patients the IgE (start) antibody responses are wider than the IgG_1 (week 12) antibody responses, while this figure falls to about 30 % when compared with the IgG_1 (week 116). Concerning the IgG_4 responses 30 % of the patients had wider IgE responses (start) than IgG_4 (week 12) responses and no patients had wider IgE responses than IgG_4 (week 116). Generally, the IgG-subclass responses at week 116 are wider than those at week 12.

Table 5

Correlation betwen CRIE and RAST values obtained during the immunotherapy

Week No.	CRIE	RAST	*n*	r_s	t
0	IgE	IgE	20	0.708	4.253**
116	IgE	IgE	18	0.278	1.158
12	IgG_1	IgG_1	17	0.570	2.687*
116	IgG_1	IgG_1	20	0.112	0.478
12	IgG_4	IgG_4	17	0.837	5.924**
116	IgG_4	IgG_4	20	0.484	2.347*

n: Sample size

r_s: Spearman's rank correlation coefficient.

t: t-value, calculated as described in (15), for the null hypothesis that r_s is zero

* Two-sided probability for the observed t-value ≤ 0.05

** Two-sided probability for the observed t-value ≤ 0.001

Correlations between quantitative CRIE antibody responses

Table 4 shows the correlations between the quantitated CRIE antibody responses at different times during immunotherapy. There is a significant positive correlation between the IgE antibody response before start and after 116 weeks of immunotherapy. A significant negative correlation is seen between the IgG_1 antibody response at week 12 and the IgG_1 antibody response at week 116. Also there is a significant negative correlation between the IgG_4 antibody response at week 12 and the IgG_1 antibody response at week 116.

Regulation of antibody response

Table 1 shows the quantitated immune response to every antigen in every antibody class by all the

patients. A rise in IgE antibody response against single antigens from the sample at start to the sample at week 116 is contrasted by a fall in the IgG_4 antibody response and vice versa. The cases in which this is true account for the reactions to 17 of 22 antigens. For IgE and IgG_1 the following relationship is seen. With eight antigens there was a fall or no increase in IgE antibody responses during immunotherapy only if IgG_1 was present early in the period (week 12). To an additional three antigens there were no IgE responses but there were early IgG_1 responses. With seven antigens there was a rise in the IgE antibody responses and no early IgG_1 antibody responses. Finally, to four antigens there were no IgE and no IgG_1 antibody responses.

Comparison and correlation between RAST and CRIE

Table 5 shows a correlation between IgE (CRIE) and IgE (RAST) values for the sample at start, between IgG_1 (CRIE) and IgG_1 (RAST) for the sample at week 12 and between IgG_4 (CRIE) and IgG_4 (RAST) for both samples at start and at week 116.

DISCUSSION

Using our IgG subclass RAST method we have previously shown that the nature of the IgG-subclass antibody response to timothy pollen antigens (4, 5) is restricted to IgG_1 and IgG_4. However, to rule out the possibility that certain antigens might not have been bound to the solid phase in our RAST system we performed a number of CRIE experiments to see if this completely different solid phase, i.e. antigen/antibody complexes in a gel-matrix, might behave differently. These experiments confirmed our RAST results, namely that no IgG_2 and IgG_3 antibodies against timothy pollen antigens could be detected. The serum samples used in the present study have been frozen and thawed repeatedly and could have led to an underestimation of the more labile IgG_3. However, as experiments on fresh serum samples from grass pollen-allergic patients also failed to reveal any IgG_3 antibody response, we are convinced that no IgG_3 antibodies to these antigens are formed. The conclusion of these experiments is that the IgG-subclass antibody responses to timothy pollen antigens are restricted to IgG_1 and IgG_4.

As seen from Table 1 the term major allergen must be a dynamic entity because the major allergens in the IgE sample before treatment start are not the same as those in the last IgE sample. It is obvious, however, that although the number of antigens called major allergens have increased during the immunization period the overall quantity of IgE to these antigens has decreased. Furthermore, the formation of IgE antibodies to new antigens does not necessarily mean that the patient will experience an increase in symptoms. That immunotherapy introduces the antigens by another route, could lead to the formation of IgE antibodies to antigens to which the patient is not normally exposed through penetration of mucosal membranes. Earlier studies of the same sera have failed to reveal any new IgE specificities (29) and this apparent discrepancy could be because in this study we have been using another antibody preparation. This preparation has the same specificity as measured by CIE but the rabbit antibodies might have a different specificity on the antigenic determinant level. Thus in the previous series of experiments the antibodies could have been covering the determinants "known" by the IgE antibodies. The present results have been confirmed by analysis performed using the same sera on blottings of SDS-PAGE separations of the antigens (Jarolim, E. et al. to be published). Another explanation could be the induction of IgE specificities during the season due to natural exposure to the pollen antigens. This has been seen (30) in the comparison of two extracts, one with only two antigens and the presently used extract. The patients in the two groups developed weak IgE antibody responses towards some antigens and the patients treated with the two-component extract developed IgE antibodies to antigens not present in the extract used for immunotherapy (30). The presence of these IgE antibodies was therefore considered as a background noise in the detection system.

Table 1 shows that a large number of antigens induce only a few reactions, one antigen (No. 16) shows only reactions from IgG_4 antibodies and one (No. 14) induces no reactions from any patients in any of the antibody classes. The reason for this is

not clear. The explanation could be the same as mentioned above that rabbit antibodies used for precipitating the antigen in the gel could cover or use the only available determinant(s) on this antigen thus rendering it impossible for the human antibodies to bind. Whether or not this is true can only be established by isolating the antigen and then using it for a solid phase immunoassay of the RAST type. Fortunately this is not a general problem in the CRIE analysis although it has been described previously for certain antigens (2).

A regulatory influence can be exerted by the presence of antibodies to a given antigen in minute amounts. Therefore to see whether certain antibodies have any regulatory influences we must consider the changes in the quantitative antibody responses as measured by the CRIE. We cannot expect a regulatory influence to be total in the sense that production of antibodies to a given antigen will cease, but rather that it would diminish. We must also expect differences in the way antibodies to different antigens are regulated because of differences in their physico-chemical properties. This can be seen from the concept of major allergens/immunogens that must be considered as a dynamic entity, as the route of administration is changed from inhalation to a combination of inhalation and injection throughout the immunotherapy period. We have shown that a rise in IgE is connected with a fall in IgG_1 for certain antigens and for other antigens a fall in IgE is connected with a rise in IgG_4. For other antigens to which no IgE antibodies are formed there is a rise in IgG_4 antibodies. This could mean that IgE is capable of regulating the formation of IgG_4 and vice versa. In addition, it is likely that IgG_4 subclass antibodies, which neither activate complement via the classical pathway nor bind to cells (16), except for mast cells and basophils, may exert their biological function by protecting the individual against inappropriate immunological reactions during continuing and persisting antigen exposure (5). This seems plausible because the antigens to which the IgE antibody responses are down-regulated are some of the major allergens and some antigens to which patients frequently react. Furthermore, IgG_4 antibodies do not appear able to sensitize normal basophils (15). The IgE antibody responses to a number of antigens, including the major allergens before start of immunotherapy, are quantitatively diminished during the period of immunotherapy when IgG_1 is present early (week 12) in the period. This and the fact that for other antigens there is a rise in the amount of IgE formed without an early IgG_1 antibody response point to a regulatory role of IgG_1. Evidence has been found in animal model experiments that the IgG antibodies of the subclasses capable of binding to F_c-receptors on lymphocytes can exert an inhibiting influence on the IgE synthesis (3, 10). Takatsu & Ishizaka (23) in an animal experiment using clustered injections of high allergen doses found a decline in the ongoing IgE synthesis and this fall was immediately preceded by a large increase in the synthesis of IgG_1 antibodies. This inhibition of the IgE synthesis could be accomplished through the formation of T-suppressor cells.

A correlation between the RAST and CRIE techniques would be assumed as the CRIE is a RAST test with quantitation on each antigen in the mixture used for the RAST. Such a correlation is seen for most samples with the exception of the IgE samples from week 116 and the IgG_1 samples from week 116. The lack of correlation between some RAST and CRIE results could be ascribed to a difference in sensitivity for measurement of antibodies to certain antigens.

The positive correlation shown between the quantitative IgE CRIE results at start and after 116 weeks, which was also found by the RAST, shows that patients starting with high levels of IgE will end with relatively high IgE levels at the conclusion of immunotherapy. However, these IgE levels are not correlated to the symptom score (5, Søndergaard, Poulsen et al. (in preparation)). The negative correlations found between IgG_1 (week 12) and IgG_1 (week 116) and between IgG_4 (week 12) and IgG_1 (week 116) could not be found by the RAST and could, as before, be ascribed to the difference in sensitivity between the two methods for certain antigens. Alternatively, it could be the lack of binding to the immunosorbent by some antigens in the RAST.

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