

Evidence of a common regulation of IgE and IgG-subclass antibodies in humans during immunotherapy

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Based on a 3-year prospective study of 20 pollen-allergic patients, where a detailed analysis of the IgE, IgG₁ and IgG₄ immune response was performed, we propose that a common regulatory mechanism exists between the IgE and IgG₁ synthesis and between IgE and IgG₄ synthesis during immunotherapy. It was found that the IgE immune response to a number of antigens was quantitatively diminished during the period of immunotherapy when IgG₁ was present early (week 12), and for other antigens there was a rise in IgE without an early IgG₁ antibody response. Additionally, it was found that for some antigens a rise in IgE antibodies was contrasted by a fall in the IgG₄ antibody response and for other antigens the opposite was true, indicating a regulatory mechanism between the IgE and the IgG₄ synthesis. A statistical analysis showed that these findings were statistically significant at the 0.01% level for the IgE/IgG₁ relationship and at the 0.05% level for the IgE/IgG₄ relationship. These findings could have implications for future immunotherapy regimens.

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During allergen-specific immunotherapy (hyposensitization) IgG subclass antibodies with a number of specificities are formed, the isotypes of which will depend on the allergen source (4). Previously, it has been shown by an IgG-subclass radioallergosorbent test (RAST) that for pollen-allergic patients the IgG-subclass response is restricted to IgG₁ and IgG₄ (5). It has been found by use of the RAST technique that the IgG-subclass antibody response during immunotherapy with grass pollen is restricted to IgG₁ and IgG₄ (4, 5) and that prolonged immunization with some antigens (grass pollen and others) may lead to an IgG₄-dominated antibody response (1, Poulsen, unpublished). In most patients an IgG₁ antibody response is seen at the beginning of the immunization period followed later by an IgG₄ antibody response (5). It has been found in grass pollen-allergics (5) that a high amount of IgG₄ allergen-specific antibodies after 12 weeks of immunotherapy was significantly correlated to a bad therapeutic outcome. Further, there was a tendency towards a positive correlation between a high preseasonal amount of IgG₄ and a high symptom score in the following season (5). These studies of the IgG-subclass antibody response during allergen-specific immunotherapy of IgE-mediated allergic diseases has until re-

cently solely been performed using the IgG radioallergosorbent test (RAST) technique (5, 7). We have developed crossed radioimmuno-electrophoretic (CRIE) methods (18, 19, 23) capable of measuring the specificities of IgE and IgG-subclass antibodies against the individual antigens in grass pollen (20), thus making it possible to study the immune response in far more detail than before. This knowledge would make it possible to further develop individual antigen preparations for immunotherapy (12, 16, 17). The sera used for the present study have previously been used to study the IgE response towards a total extract of *Phleum pratense* pollen antigens and towards only 2 of the major allergens (24, 25, 26).

The aim of the present study was to find whether there were common patterns in the patients' IgE and IgG-subclass responses to immunotherapy and any indirect evidence of common regulating mechanisms in antibody synthesis. For this purpose we studied 20 pollen-allergic patients during 2 years of immunotherapy (20). Based on a further analysis of the data obtained, we propose that a common regulatory mechanism exists between the IgE and IgG₁ synthesis during immunotherapy.

Material and methods

The study design, patients and immunotherapy procedure have been described in detail elsewhere (24).

CIE was done according to (22) and the IgE and IgG subclass CRIE were subsequently performed using these CIE plates as described previously (19, 23) with buffers and washing solutions originating from (6).

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 such that disc No. 1 had the lowest amount of radioactivity. After autoradiography of the discs the precipitates in the CRIE were then visually compared to this arbitrary grey scale.

The statistics are based on a sign test. To show that a given antibody class (class 1) is present only when another (class 2) is absent, an estimate of the probability of the presence of the former antibody class given the presence or absence of the latter antibody class is calculated for each patient. The test parameter is then assigned 1 if the probability for presence of class 1 given absence of class 2 is larger than the probability for presence of class 1 given presence of class 2. Otherwise the test parameter is assigned a value of -1. The values are then tested by a sign test. This can be illustrated by an actual example concerning the IgE and IgG₁ relationship. For each patient the number of antigens to which

there is either a) a decline or no increase in IgE and no early IgG₁, b) an increase in IgE and no early IgG₁, c) a decline or no increase in IgE and early presence of IgG₁, and d) an increase in IgE and presence of early IgG₁ was calculated. The estimates of the probabilities for the presence of IgE, given that no IgG₁ is present ($P(\text{IgE} = 1 | \text{IgG}_1 = 0)$, left side in the inequality), and for the presence of IgE given the presence of IgG₁ ($P(\text{IgE} = 1 | \text{IgG}_1 = 1)$, right side in the inequality) are calculated; then a value of 1 is given to each patient if

$$\frac{b_i}{a_i + b_i} > \frac{d_i}{c_i + d_i}$$

a value of 0 if the 2 estimates are equal, and a value of -1 otherwise.

Results

The overall number of reactions from the 15 patients to the 22 antigens are shown in Table I. The IgE antibody responses to a number of antigens, including the major allergens before the start of immunotherapy, were shown to be quantitatively diminished during the period of immunotherapy when IgG₁ is present early (week 12). This and the fact that for other antigens there is a rise in the amount of IgE formed without an early IgG₁ antibody response, point to a common regulatory mechanism for IgE and IgG₁. Further, it was shown that a rise in the amount of IgE is connected with a fall in the amount

Table 1. The quantitated response to each antigen of 22 antigens from IgE and IgG-subclass antibodies at different times during immunotherapy. An extract containing these 22 antigens from *Phleum pratense* was used for immunotherapy of the 15 patients

Antigen 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

Table 2. Results of CRIE analysis of the IgE-IgG₁ relationship showing the number of antigens to which the reactions show either a) a fall or no increase in IgE and no early IgG₁, b) an increase in IgE and no early IgG₁, c) a fall or no increase in IgE and early IgG₁, or d) an increase in IgE and early IgG₁

Patient no.	a	b	c	d	T
6	7	5	1	0	1
8	1	4	5	0	1
12	1	6	2	0	1
13	1	3	1	0	1
15	2	5	4	0	1
18	3	8	0	1	-1
21	2	6	2	0	1
23	1	5	3	0	1
26	3	4	6	0	1
27	0	1	5	0	1
29	3	6	6	0	1
30	1	2	7	0	1
34	2	3	5	0	1
37	3	3	6	0	1
40	0	7	2	0	1

$$T_i = 1 \text{ if } \frac{b_i}{a_i + b_i} > \frac{d_i}{c_i + d_i} \text{ else } T_i = -1$$

Number of negative T values 1; number of positive T values 14. Result of sign test $p < 0.01$.

Table 3. Results of CRIE analysis of the IgE-IgG₄ relationship showing the number of antigens to which the reactions show either a) a fall or no increase in IgE and a fall or increase in IgG₄, b) an increase in IgE and a fall or no increase in IgG₄, c) a fall or no increase in IgE and an increase in IgG₄, or d) an increase in IgE and an increase in IgG₄

Patient no.	a	b	c	d	T
6	4	1	4	4	-1
8	0	2	8	2	1
12	1	2	7	4	1
13	3	0	11	3	-1
15	4	4	7	1	1
18	2	6	3	3	1
21	2	3	5	3	1
23	5	3	5	2	1
26	2	3	8	1	1
27	4	0	8	1	-1
29	4	4	7	2	1
30	5	2	6	0	1
34	2	3	12	0	1
37	6	2	9	1	1
40	4	6	4	1	1

$$T_i = 1 \text{ if } \frac{b_i}{a_i + b_i} > \frac{d_i}{c_i + d_i} \text{ else } T_i = -1$$

Number of negative T values 3; number of positive T values 12. Result of sign test $p < 0.05$.

of IgG₄ for certain antigens and for others a fall in IgE is connected with a rise in IgG₄. For antigens to which no IgE antibodies are formed there is a rise in the amount of IgG₄ antibodies. Table 2 shows for each patient the number of antigens to which there is either a) a decline or no increase in IgE and no early IgG₁, b) an increase in IgE and no early IgG₁, c) a decline or no increase in IgE and early presence of IgG₁, or d) an increase in IgE and presence of early IgG₁. It was shown ($p < 0.01$) that IgE and IgG₁ showed the following interrelation: a rise in the IgE level and no early IgG₁, and a decline or no rise in IgE and early IgG₁.

The results from the analysis of the IgE-IgG₄ relationship is shown in Table 3. The results show ($p < 0.05$) that a rise in IgE and a decline (or no rise) in IgG₄ or a fall (or no rise) in IgE and rise in IgG₄ is connected.

Discussion

It is obvious that studies of this type will not reveal the exact nature of common regulating principles underlying antibody synthesis. However, they can support theories obtained on the basis of cellular studies and they can give impetus to new studies on the cellular level. T cells and T-cell products are important factors in the regulation of immunoglobulin selection and synthesis, and several investigators have pinpointed the different requirements for an *in vivo* IgE and IgG synthesis (3, 11, 21). This suggests that IgG₁, which forms the major part of the

IgG subclasses, in our patients seems to be regulated to IgE. Evidence has been found in animal model experiments that the IgG antibodies of the subclasses capable of binding to Fc-receptors on lymphocytes can exert an inhibiting influence on the IgE synthesis (2, 9).

The findings concerning the IgE/IgG₄ dichotomy are somewhat more surprising as contradictory results have been obtained *in vitro*, where it was found that interleukin 4 (IL-4) selectively stimulates B lymphocytes to synthesize IgE and IgG₄ but not the 3 other IgG subclasses (8, 10). These results parallel the *in vivo* findings that atopic dermatitis patients have increased IgE concomitant with IgG₄ (14). It should be emphasized, however, that these results concern a polyclonal response, whereas ours describe the individual allergens in a complex extract. It is possible that a given combination of allergen and individual might lead to different selection of isotypes in the immune response. In addition, it is likely that IgG₄ subclass antibodies, which neither activate complement via the classic pathway nor bind to cells (15), 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 antigens to which patients frequently react. Further, IgG₄ does not seem able to sensitize normal basophils (13).

The possible implication for future therapy could be as follows: if there is a connection between the down regulation of the IgE synthesis to certain antigens and the outcome of immunotherapy, therapeutic protocols should be aimed at producing an early IgG₁ response or maybe a late IgG₄ response.

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References

1. AALBERSE RC, VAN DER GAAG R, VAN LEEUWEN J. Serological aspects of IgG₄ antibodies. I. Prolonged immunization results in a IgG₄-restricted response. *J Immunol* 1983; 130: 722-6.
2. BOREL Y. Isologous IgG-induced immunologic tolerance to haptens: A model of self versus non-self recognition. *Transplant Rev* 1976; 31: 3-21.
3. DEFRAANCE T, VANBERVLIET B, PENE J, BANCHEREAU J. Human recombinant IL-4 induces activated B lymphocytes to produce IgG and IgM. *J Immunol* 1988; 141: 2000-5.
4. DJURUP R. The subclass nature and clinical significance of

- the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 1985; 40: 469-86.
5. DJURUP R, ØSTERBALLE O. IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. *Allergy* 1984; 39: 433-41.
6. DJURUP R, SØNDERGAARD I, MINUVA U, WEEKE B. A nylon ball solid-phase radioimmunoassay for specific antibodies in human sera. Application to measurement of IgG antibodies to pollen allergens. *J Immunol Methods* 1983; 62: 283-96.
7. DJURUP R, SØNDERGAARD I, MAGNUSSON CGM, WEEKE B. A three-layer immunoradiometric assay (TIRA) for determination of IgG subclass antibodies in human sera ("IgG subclass RAST"). Validation of subclass specificity and establishment of equipotency. *Allergy* 1984; 34: 51-63.
8. ISHIZAKA A, SAKIYAMA Y, NAKANISHI M, et al. The inductive effect of interleukin-4 on IgG₄ and IgE synthesis in human peripheral blood lymphocytes. *Clin Exp Immunol* 1990; 79: 392-6.
9. LEE WY, SEHON AH. Suppression of reaginic antibodies. *Immunol Rev* 1978; 41: 200-47.
10. LUNDGREN M, PERSSON U, LARSSON P, et al. Interleukin 4 induces synthesis of IgE and IgG₄ in human B cells. *Eur J Immunol* 1989; 19: 1311.
11. PENE J, ROUSSET F, BRIERE F, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferon gamma and alpha and prostaglandin E₂. *Proc Natl Acad Sci USA* 1988; 85: 6880-4.
12. POULSEN LK, SØNDERGAARD I, WEEKE B. Preparation of patient-related allergens for hyposensitization. I. Qualitative aspects. *Allergy* 1988; 43: 552-64.
13. POULSEN LK, SKOV PS, MOSBECH H, WEEKE B. The role of IgG₄ in histamine release from human basophil leucocytes. *Int Arch Allergy Appl Immunol* 1988; 86: 383-90.
14. REINHOLD U, PAWELEC G, WEHRMAN W, HEROLD M, WERNET P, KREYSEL HW. Immunoglobulin E and immunoglobulin G subclass distribution *in vivo* and relationship to *in vitro* generation of interferon-gamma and neopterin in patients with severe atopic dermatitis. *Int Arch Allergy Appl Immunol* 1988; 87: 120-6.
15. SCHANFIELD MS. Genetic markers of immunoglobulins. In: FUDENBERG HH, SITES DP, CALDWELL JL, WELLS JW, eds. *Basic and clinical immunology*, 2nd ed. Los Altos: Lange Medical Publications, 1978: 59-65.
16. SØNDERGAARD I, WEEKE B. Purification of patient-related allergens by means of bioaffinity chromatography on a sepharose anti-IgE immunosorbent. *Allergy* 1984; 39: 473-9.
17. SØNDERGAARD I, WEEKE B. Isolation of patient-related antigens from allergen extracts by means of protein A-sepharose-patient IgG_{1,2,4} bio-affinity chromatography. *Allergy* 1984; 39: 551-9.
18. SØNDERGAARD I, DJURUP R, WEEKE B. A modified crossed radioimmuno-electrophoretic method for determination of allergen specific IgG antibodies (IgG-CRIE). Elimination of non-specific binding by use of F(ab')₂-fragments of rabbit antibodies and ¹²⁵I-labelled protein A. *Allergy* 1984; 39: 135-40.
19. SØNDERGAARD I, DJURUP R, WEEKE B. Characterization of IgG-subclass antibodies to individual allergens by crossed radioimmuno-electrophoresis. *Allergy* 1984; 39: 622-9.
20. SØNDERGAARD I, POULSEN LK, ØSTERBALLE O, WEEKE B. A computational approach to the description of individual immune responses. IgE and IgG-subclass allergen specific antibodies formed during immunotherapy. *Allergy* 1991; 46: 10-9.
21. VITETTA ES, FERNANDEZ-BOTRAN R, MYERS CD, SANDERS VM. Cellular interactions in the humoral immune response. *Adv Immunol* 1989; 45: 1-105.
22. WEEKE B. Crossed immunoelectrophoresis. *Scand J Immunol* 1973; 2: Suppl 1: 47-56.
23. WEEKE B, SØNDERGAARD I, LIND P, AUKRUST L, LØWENSTEIN H. Crossed radioimmuno-electrophoresis (CRIE) for identification of allergens and determination of antigenic specificities of patients' IgE. *Scand J Immunol* 1983; 17: Suppl 10: 265-72.
24. ØSTERBALLE O. Immunotherapy in hay fever with two major allergens, 19,25 and partially purified extract of timothy grass pollen. A controlled double-blind study. *In vivo* variables, season I. *Allergy* 1980; 35: 473-89.
25. ØSTERBALLE O, LØWENSTEIN H, PRAHL P, SKOV P, WEEKE B. Immunotherapy in hay fever with two major allergens 19,25 and partially purified extract of timothy grass pollen. A controlled double blind study. *In vitro* variables, season I. *Allergy* 1981; 36: 183-99.
26. ØSTERBALLE O, LØWENSTEIN H, NORN S, et al. Immunotherapy with grass pollen major allergens. Immunological results from a prospective 3-year double blind study. *Allergy* 1982; 37: 491-501.

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