

Anti-IgG Antibodies and Antinuclear Antibodies in Allergic Patients

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With an indirect immunofluorescence technique 77% of 96 patients with type I allergy and 40% of 20 patients with intrinsic bronchial asthma showed positive reactions for IgG anti-IgG antibodies in serum. They were present partly in an aggregated state not directly detectable before treatment with dithiothreitol. The aggregates could be removed by precipitation with polyethylene glycol. The IgG anti-IgG in hyposensitized patients were directed against both F(ab')₂ and Fc fragments of rabbit IgG. Thirty of the type I allergic patients were examined once during hyposensitization as well. Before treatment 87% had IgG anti-IgG (titres 9–72). After ≥ 13 months of treatment 100% were positive (titres 36–288). Eight patients were also examined after hyposensitization had been discontinued for at least 12 months. The titres of IgG anti-IgG had then reverted to the levels obtained before hyposensitization. Of 116 controls matched for sex and age, 7% had IgG anti-IgG antibodies. It is suggested that the production of IgG anti-IgG may be stimulated by the presence of immune complexes and that purity, amount and/or combination of allergens administered during hyposensitization may influence the production of anti-IgG antibodies. Neither IgE anti-IgG nor antinuclear antibodies seem to be of particular significance in allergic patients.

Key words: anti-IgG antibodies; antinuclear antibodies; hyposensitization; immune complexes.

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Previous studies have demonstrated the presence of autoantibodies in patients with atopic diseases. The prevalence of rheumatoid factor (IgM anti-IgG) in bronchial asthma and other allergic diseases has varied between 0.8% (20) and 17% (16) and of antinuclear antibodies (ANA) between 0.41% (20) and 16% (8). Immune complexes have been demonstrated in hyposensitized patients (2, 25) and it has been suggested that histamine release from leucocytes of allergic donors may be caused by anti-IgG antibodies (7).

Studies concerning type I hypersensitivity phenomena in autoimmune diseases such as rheumatoid arthritis have demonstrated the presence of eosinophilia (13, 24), raised level of

IgE (9), IgE ANA (6, 14) and IgE anti-IgG (Permin & Egeskjold, unpublished data). Agents which release histamine from heart-lung preparations of normal guinea pigs have been found in serum and synovial fluid of rheumatoid patients (1) and basophils from patients with active rheumatoid arthritis may respond to ANA and aggregated IgG with histamine release, pointing to an involvement of autoimmune type I reactions in the pathogenesis (15).

The above mentioned findings have contributed to the interest in resemblances and possible mutual pathogenic characteristics between allergic and rheumatic diseases.

The present paper deals with the prevalence

Table 1
Thirty patients with type I allergy

Diagnosis	Allergic to	Hyposensitized with	Perennial treatment (p.t.)	Duration of treatment, months	Clinical effect	before treatment	Titres of IgG during treatment	after treatment
1. AB	animal dander, dust mite, mould fungi, pollen	allpyral, dog, dust mite, 5 grass mix, horse, cat, 4 mould	p.t.	36	good	36	144	36
2. AR	pollen	allpyral, 5 grass mix	preseasonal treatment		good	neg.	72	
3. AR + AB	pollen	allpyral, 5 grass mix	p.t.	34	good	neg.	288	36
4. AR + AB	pollen	allpyral, 5 grass mix	p.t.	36	good	18		18 (11 mos.)
6. AB + AR	pollen, dust mite, animal dander	allpyral, 5 grass mix, dust mite, cat, horse, dog	p.t.	29	improved	72	72	
8. AR	pollen	allpyral, 5 grass mix	preseasonal treatment		improved	36	72	
10. AR + AB + PB	grass pollen	allpyral, 5 grass mix	p.t.	34	good	36		144 (6 mos.) 72 (12 mos.)
12. AB + AR	grass pollen, mould fungi	aqueous extract, grass barley, Alternaria tenuis	p.t.	34	improved	18	72	
13. AB + AR	grass pollen	allpyral, 5 grass mix	p.t.	25	good	36	72	
17. AR + PB	grass pollen, herbs	allpyral, 5 grass mix, rape, mugwort	p.t.	24	good	36	36	
18. AB + AR	grass pollen, herbs	allpyral, 5 grass mix, oxeye	p.t.	37	improved	72		72 (5 mos.) 36 (12 mos.)
19. AR + AB	grass pollen, herbs, animal dander	allpyral, rye, wheat, 5 grass mix, 5 flower mix, cat	p.t.	37	improved	36	144	72 (12 mos.)
20. AB + AR	grass pollen	allpyral, 5 grass mix	p.t.	28	improved	18	72	
21. AR + AB	grass, corn	allpyral, 5 grass mix, wheat	p.t.	36	good	36		36 (6 mos.) neg. (12 mos.)

24. AB	house dust, animal dander	aqueous extracts, house dust, horse, goat, followed by allpyral, house dust mite	p.t.	37	good	18	72
27. AR + PB	grass, trees	allpyral, 5 grass mix, birch, alder	p.t.	22	improved	36	72 18 (12 mos.)
29. AR + PB	grass	allpyral, 5 grass mix	p.t.	13	improved	18	36
33. AR + AB	grass	allpyral, 5 grass mix	p.t.	25	good	36	72
34. AB + AR	grass	allpyral, 5 grass mix	p.t.	29	improved	18	72
35. AB + AR + PB	grass, animal dander, dust mite	allpyral, 5 grass mix, cow, horse, cat, dog, house dust mite	p.t.	32	good	18	72
39. AR + AB	house dust mite	allpyral, house dust mite	p.t.	13	improved	36	72
41. AB + AR	grass	allpyral, 5 grass mix	p.t.	25	good	36	72
42. AB + AR	grass, herbs	allpyral, 5 grass mix, oxeye	p.t.	27	improved	18	144
44. AR	grass, dust mite, animal dander	allpyral, 5 grass mix, horse, house dust mite	p.t.	33	good	36	144
48. AR	grass	allpyral, 5 grass mix	p.t.	25	uncertain	18	72
50. AR + AB	grass	allpyral, 5 grass mix	p.t.	27	uncertain	18	72
54. AR + AB	grass	allpyral, 5 grass mix	p.t.	26	improved	18	72
55. AR	grass, herbs	allpyral, 5 grass mix, 5 flower mix, mugwort, dandelion	p.t.	26	good	36	144 36 (12 mos.)
60. AR	grass, trees, herbs	allpyral, 5 grass mix, 5 flower mix, dandelion, nordic tree mix	p.t.	13	improved	neg.	72
66. AB + AR	dust mite	allpyral, house dust mite	p.t.	18	uncertain	neg.	144

AB = Asthma bronchiale.
AR = Allergic rhinitis.
PB = Prurigo Besnier.

of anti-IgG antibodies and ANA in allergic patients before, during and after hyposensitization.

MATERIAL AND METHODS

A total of 116 patients were studied. None had arthritis, connective tissue diseases or lymphoproliferative diseases. Ninety-six patients (58 males and 38 females, mean age 26 years (range 8–66)) were classified as type I allergic patients, and 20 (seven males and 13 females, mean age 48 years (range 15–66)) were classified as intrinsic asthmatic patients in whom the specific allergy examinations had failed to demonstrate reaction to any allergens. All patients were prick tested with at least 13 common inhalation allergens (grass, chrysanthemum, birch, horse, dog, cat, cow, sheep, house dust $\pm$ mite, alternaria, cladosporium, aspergillus). Supplementary prick tests were carried out when the anamnesis gave basis for suspicion of other allergens. If necessary, RAST analysis was carried out, and if allergy was still suspected but not verified, tissue provocation test was performed.

Thirty of these patients, nine females and 21 males (mean age 21 years (range 8–44 years)), two with type I bronchial asthma, nine with allergic rhinitis, and 19 with both diseases were hypersensitized. Two patients (No. 2 and 8) were treated preseasonally whereas everybody else received perennial treatment. Blood samples were obtained again after the patients had been treated continuously for at least 13 months with either allpyral (Dome Lab., USA) or aqueous extracts of allergens (Allergologisk Lab., Copenhagen). An outline of the diagnosis, relevant allergens, duration and effect of treatment can be seen from Table 1.

A total of 116 healthy persons matched for sex and age acted as controls for anti-IgG antibodies and 50 for ANA.

Assay for anti-IgG antibodies

The sera were tested for IgA- and IgE anti-IgG with an indirect immunofluorescence technique

(IIF). The specificity and sensitivity of the technique has been discussed in detail elsewhere (10).

Briefly the test was performed as follows: The sera were inactivated at 56°C for 30 min. Prior to examination for IgG anti-IgG the sera were treated with 0.01 M dithiothreitol (DTT) for 30 min at 37°C to destroy IgM anti-IgG, as the presence of these antibodies may lead to false positive reactions for IgG anti-IgG due to IgG bound to IgM anti-IgG (10). In the present study DTT was also employed for splitting bindings between IgG anti-IgG molecules so that anti-IgG antibodies were present as free antibodies only. Formalin-fixed sheep erythrocytes smeared on glass slides and coated with rabbit anti-sheep cell IgG (Nordic, Holland) were used as antigen. The serum samples were applied in twofold dilutions starting with 1:9, using PBS (pH 7.4) as diluent. Fluorescein isothiocyanate (FITC)-labelled monospecific anti-serum was added and the slides were finally washed as before and cover glasses were mounted in glycerol PBS. FITC-labelled monospecific rabbit anti-human IgG (Behringwerke; West Germany) and goat anti-human IgE (Miloy, USA) were used as antisera. The molar F/P ratio was 2.5 for IgG and 3.4 for IgE. Known IgG and IgE anti-IgG positive control sera were tested simultaneously and all slides were examined together with a control smear of non-sensitized sheep red cells. Sera from type I allergic patients and intrinsic asthmatic patients were coded, randomized and read without knowledge of the type of patients. Sera from hyposensitized patients during and after treatment were also coded and randomized before reading. The controls were tested separately. Titres ≥ 9 were considered elevated by this technique (10). The slides were examined in a fluorescence microscope using incident light illumination (Ortholux, Leitz).

Test for anti-F(ab')₂ antibodies

In a series of experiments the sheep erythrocytes were coated with rabbit anti-sheep cell IgG treated with pepsin 1:60000 twice crystallized (Sigma Chem. Co., St. Louis) at 37°C for

24 hours in 0.1 M acetate buffer, pH 4.0. The sedimentation coefficient of the digest was 4.9 consistent with that of $F(ab')_2$ tested with rate zonal ultracentrifugation (21).

Inhibition of antibodies to rabbit $F(ab')_2$

Tubes containing test sera were incubated with pepsin digested rabbit IgG 1:2 for 24 hours at 5°C. The sera so treated were control tested for negative reactions to anti- $F(ab')_2$, and then tested for anti-IgG (Fc) reactivity as described above.

Precipitation with polyethylene glycol (PEG)

Precipitation of the aggregates was carried out with PEG 3000. The samples were diluted with phosphate buffered saline to a final concentration of 1:37 as described by Riha et al. (17). By this concentration of PEG 3000 the normal serum globulins are left diluted, whereas immune complexes and aggregates precipitate (17).

Assay for antinuclear antibodies

The sera were investigated for the occurrence and titres of IgG, IgM, IgA, IgE and complement C3 fixing granulocyte specific and organononspecific GS- and ON-ANA with IIF previously described (22, 23). All conjugates used in both IIF techniques were tested for monospecificity by crossed immunoelectrophoresis and direct immunofluorescence technique of monoclonal bone marrow specimens from patients with myeloma (IgG, IgA, IgE and IgD) and macroglobulinaemia (IgM) (23).

Other laboratory studies

IgM anti-IgG antibodies were searched for with the F II Latex fixation test (Behringwerke, West Germany). Titres ≥ 20 were considered abnormal. The total concentration of IgE in serum was determined by paper radioimmunosorbent technique (PRIST) using Phadebas IgE kits (Pharmacia, Sweden). The concentrations were expressed in units according to WHO standard serum (1 u = 2.4 ng IgE). Serum con-

centrations of IgG, IgA, IgM, IgD and α_2 -macroglobulin were determined by quantitative immunoelectrophoresis using monospecific antisera (Dako, Denmark, and Behringwerke, West Germany).

Normal ranges in the present study: IgG 7.2–15.1 g/l, IgM 0.23–1.33 g/l, IgA 0.74–3.06 g/l and α_2 -macroglobulin 1.3–4.6 g/l.

Statistical method

The Mann-Whitney test and the fourfold table test were used (5).

RESULTS

IgG anti-IgG occurred in 77% of patients with type I allergy, in 40% of the intrinsic asthmatic patients and in 7% of the healthy controls (Table 2). Both prevalence and titres were significantly higher in type I allergic patients and significantly higher in patients ≤ 40 years old ($P < 0.001$).

IgA anti-IgG occurred only in one type I allergic patient and in none of the controls. IgM anti-IgG tested with Latex fixation test was found in 3% of type I allergic patients, but in none of the intrinsic asthmatic patients and in none of the controls.

The IgG anti-IgG antibodies were present in the patients' sera mainly in an aggregated state or as complexes, as will be seen from the following experiments.

When the patients' sera were not pretreated with dithiothreitol (DTT) before being assayed, positive reactions were obtained both with the smear of sheep red cells coated with rabbit IgG and with the control smear of non-sensitized sheep red cells, with almost the same titres, whereas in the few sera of control persons which were positive, these positive reactions were obtained with IgG coated red cells only; the nude red cells gave negative reactions, as they should do. This suggested either that the allergic patients' sera contained heterophil-like antibodies to sheep red cells in addition to anti-IgG antibodies, or that some of their IgG,

Table 2
Occurrence of IgG anti-IgG in Type I allergic patients, intrinsic asthmatics and healthy controls

	No. of Type I patients	No. of intrinsic asthmatics	No. of normal controls
Positive	74	8	8
Negative	22	12	108
Total	96	20	116

$P < 0.002$ $P < 0.001$

probably aggregates or complexes, might adhere nonspecifically to the surface of both the IgG coated cells of the test system and the nude control red cells.

In some experiments the formalin fixed sheep erythrocytes were replaced with smears of formalin fixed swine erythrocytes, which gave positive reactions in the same titres as the sheep cells, suggesting a non-immunological binding, and arguing against the possibility of heterophil-like anti-sheep antibodies.

In further experiments 10 sera were treated with polyethyleneglycol, which precipitates aggregated and complexed IgG. The super-

natants did not react with the control smears of nude sheep cells but gave positive reactions with rabbit IgG coated cells, although in lower titres than the corresponding sera, implying that the reactions obtained with sera were due partly to specific binding of anti-IgG antibodies and partly to a possibly non-specific binding of aggregated or complexed IgG. In a series of experiments patients' sera were treated with DTT for 30 min. After this the sera did not react with smear of nude sheep cells, but solely with smear of IgG coated cells, implying that the non-specific binding has been abolished because of an as yet unclarified split of aggregates or complexes due to DTT treatment. The results for IgG anti-IgG in the present study therefore originate in DTT treated sera only.

Thirty patients were hyposensitized. Before treatment 87% were positive for IgG anti-IgG (titre 9-72) (Table 1). After at least 13 months of hyposensitization 100% were positive. The increase in titres was significant ($P < 0.001$) (Fig. 1). No correlation was found to duration of treatment or to the type of allergens administered. IgM anti-IgG did not vary significantly during hyposensitization. IgE anti-IgG occurred rarely in allergic patients and only in low titres

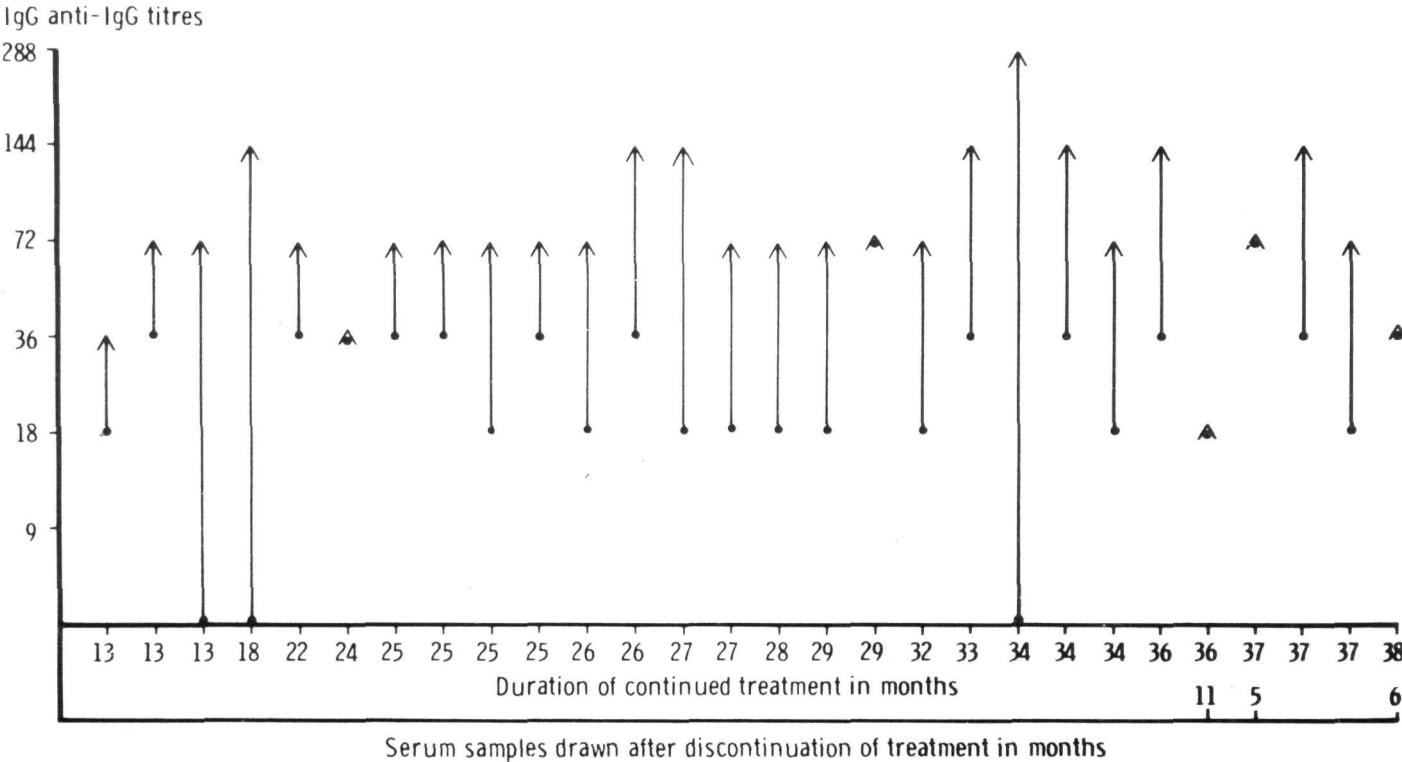


Fig. 1. The variation of IgG anti-IgG titres during hyposensitization. • before treatment. △ during treatment.

(≤ 16). Eight patients (26%) had IgE anti-IgG previous to, and nine (30%) after, hyposensitization. Six of these patients had IgE anti-IgG on both occasions. None of the controls had IgE anti-IgG. Eight patients were reexamined at a follow-up study after at least 12 months during which period the treatment had been discontinued. IgG anti-IgG titres had then reverted to the level obtained before hyposensitization (Table 1).

In order to evaluate the antigen specificity of IgG anti-IgG antibodies, 10 sera from allergic hyposensitized patients were assayed with sheep red cells coated with F(ab')₂ fragments of rabbit IgG instead of whole IgG. It was found that these sera in addition to the previously demonstrated anti-IgG reactivity, contained reactivity to the F(ab')₂ fragment in titres ranging from 10 to 20. This reactivity was inhibited completely by the addition of F(ab')₂ fragments to the sera as described in "Methods", whereas the reactivity to whole rabbit IgG was reduced with never more than one titre step. This implies that the IgG anti-IgG antibodies demonstrated are different from anti-F(ab')₂ antibodies, although the latter are present also in sera from allergic patients.

Concerning ANA 9% of the type I allergic patients had IgM ANA, 6% had IgG ANA, 4% had IgA ANA and none had IgE ANA or C3-fixing ANA. In intrinsic asthmatic patients 25% were positive for IgM ANA and 10% had IgG ANA. Of the controls, 6% were positive for IgM ANA, 10% for IgG ANA and 8% for IgA ANA. IgE ANA and C3-fixing ANA were present neither in intrinsic asthmatic patients nor in the controls. In patients with type I allergy the prevalence of ANA of the various immunoglobulin classes was the same as in the controls. In patients with intrinsic asthma, the prevalence of IgM and IgG ON-ANA was higher than, but not statistically different from, those of controls. ANA occurred mostly in hospitalized patients with active disease. The titres were low and only exceeded 32 in three type I allergic patients and in one patient with intrinsic asthma. There was no correlation between the presence of ANA and the titres of anti-IgG antibodies, and the variations during hyposensitization were negli-

gible. IgA, IgM, IgG and α_2 -macroglobulin levels were within normal range in all patients.

DISCUSSION

The study demonstrated that allergic patients produce IgG anti-IgG antibodies detectable when sera had been treated with DTT. Whether the aggregated state of the antibodies was due to immune complexes or to the known ability of IgG anti-IgG to self-associate is not clarified. Neither is the procedure by which DTT was able to break up bindings between the linked serum globulins clear. It has previously been noted by Carson et al. (3) that reduction with DTT was able to influence IgG binding in whole serum.

The anti-IgG antibodies may have properties in common with the anti-idiotypic antibodies described by Nasu et al. (12). Similar antibodies have earlier been demonstrated by Mellbye & Natvig (11) as a result of immunizing rabbits with precipitates containing autologous IgG. It is likely that immunization of allergic patients may be compared with Mellbye & Natvig's experiments. In the present material, those patients having not received immunotherapy also had IgG anti-IgG but with lower frequency and in the same rather low titres as found in patients with intrinsic bronchial asthma. This suggests that the stronger reaction which can be observed during hyposensitization may be regarded as caused by magnification of the exposure to allergen of allergic patients in their daily life, whether they are of endogenous or exogenous character. Suggestions have previously been made that asthma and other allergic disorders might imply some involvement of autoimmunity (15) and of complex mediated type III hypersensitivity (7). If formation of antibodies could be caused not only by exogen allergen stimulation but also by autoimmunological disturbances this might explain why anti-IgG antibodies were present in patients with intrinsic asthma, where no exogen allergens can be demonstrated.

Circulating IC-containing IgG have previously been demonstrated in atopic patients by Cano et al. (2) and these authors observed a decrease in the contents of IC when hyposensitization was discontinued. We, too, noticed a decrease in IgG anti-IgG titres when hyposensitization was discontinued, an observation which indicates that development of IgG anti-IgG may be related to the presence of IC probably formed between specific "blocking" IgG and allergens.

In children with cystic fibrosis IC have frequently been found (18), and such patients have with the indirect immunofluorescence test shown high prevalence of IgG anti-IgG (19), which might be similarly explained on the basis of IC stimulation. The titres of IgG anti-IgG were positively correlated to the number of pseudomonas precipitins by which the CF patients were chronically infected and it was suggested in the paper that anti-IgG antibodies may be regarded as a secondary event following repeated immunization with foreign antigen. This suggestion was further actualized when Carson et al. (4) showed that patients with subacute bacterial endocarditis had IgG anti-IgG in high titres falling to normal values after treatment of the underlying bacterial disease.

Generally the patients in the present study had received a combination of many allergens administered at the same time. The study has been followed by an examination of 40 patients during hyposensitization with highly purified timothy extracts (26). We found that the levels of IgG anti-IgG were significantly lower than in the present patients and furthermore the titres did not vary significantly during hyposensitization. The patients were examined once before and 4 times during treatment.

The results indicate that the composition and the purity of allergens administered during hyposensitization therapy may be of significance for development of anti-IgG antibodies.

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