

Allergen-containing immune complexes used for immunotherapy of allergic asthma

II. IgE and IgG immune response during and after hyposensitization of sensitized guinea pigs

L. K. POULSEN^{1,2}, L. LUNDBERG³, I. SØNDERGAARD⁴ & B. WEEKE¹

¹Laboratory of Medical Allergology, and ²Biotechnological Research Centre for Immunology, State University Hospital, Copenhagen, ³Institute of Medical Microbiology, University of Copenhagen, and ⁴Chemistry Department, Royal Veterinary and Agricultural University, Copenhagen, Denmark

In a previous study guinea pigs inbred for their ability to develop respiratory anaphylaxis to experimental antigens have been used for comparison of different forms of immunotherapy (IT). Passive, active and combined (immune complexes prepared from antigen and specific IgG) IT was compared with placebo. In the present study methods were evaluated for determination of the allergen-specific IgE and IgG. IgE was determined by the passive cutaneous anaphylactic test (PCA) and the variability of this test on different strains of the recipient guinea pig was investigated. The same strain as used for the IT study was found to produce the most potent response. Radioimmunoassays (RIA) were developed and validated for determination of specific IgG₁ and IgG₂. The IgE and IgG immune response in animals from the IT study were then evaluated by means of PCA and RIA. Animals from all four treatment groups were sensitized during the first part of the IT study, and responded with a marked IgE synthesis which later stabilized on a more moderate level. In spite of notably reduced symptoms in groups treated with active and combined IT, no difference in the IgE level was found between the four groups. In contrast to IgE, mean group titers of IgG₁ and IgG₂ in the groups receiving active or combined IT rose drastically during the first part of therapy and closely paralleled the clinical response during the rest of the study period. However, in the individual animals, no correlations were found between immune response and clinical symptoms. Thus, the strong IgG response during immunotherapy may not be causally related to the outcome of treatment.

Key words: allergic asthma; guinea pigs; IgE; IgG; immunotherapy; immune complexes; PCA.

Accepted for publication 16 October 1990

CLINICAL ASPECTS

An animal model for immunotherapy of allergic asthma is described. Guinea pigs respond with asthma during bronchial provocation test, and the IgG and IgE immune response resembles that described in man. With reference to recent findings in man, possible mechanisms behind the beneficial role of immunotherapy are discussed.

Allergen-specific immunotherapy (IT) is a well-established form of treatment given to patients with a clear diagnosis of asthma or rhinitis to major inhalant allergens (13). Almost 80 years have passed since its introduction (16), but we still do not know the mechanisms through which

its effects are exerted (13, 22). In our laboratory we have focused on the IgG immune response produced during the first months of specific immunotherapy, and have investigated the total specific reactivity against an allergen extract (4, 15), and the IgG specifically directed against the

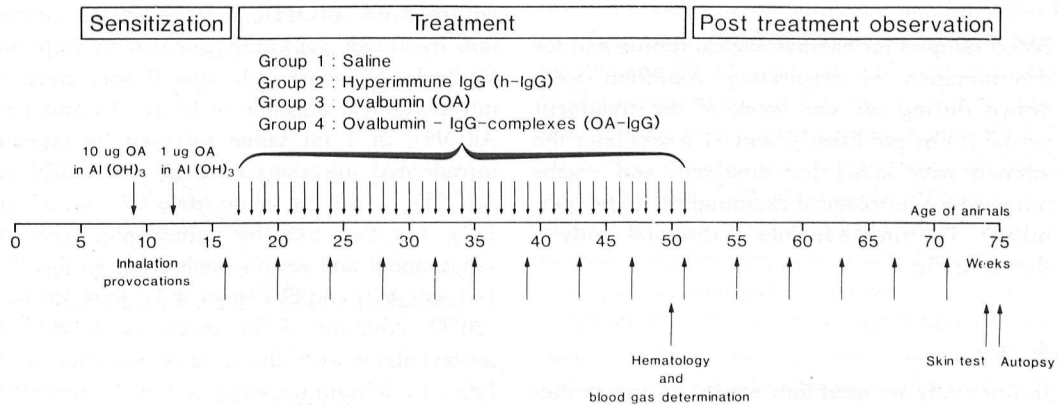


Fig. 1. Study protocol.

individual allergen components (7, 25). Based on these studies we proposed that immune complexes formed *ex vivo* between IgG and allergen might represent a therapeutic alternative (20, 24, reviewed in 22). To further test the hypothesis we carried out experiments using an animal model which could be specifically sensitized with an experimental antigen, and then treated with specific immunotherapy (21). We have previously reported the clinical outcome of these experiments which showed a strong and comparable effect of normal specific immunotherapy and allergen-containing IgG immune complexes. These treatments were also compared with placebo and passive administration of IgG, neither of which had any effects on the allergic condition of the animals evaluated by regular bronchial provocation tests. The aim of the present work was to develop and validate methods for measurement of the antigen-specific IgE and IgG_{1/2} immune responses, and to determine these immune responses during the above-mentioned IT study.

MATERIAL AND METHODS

Design

The design of the IT study has previously been described (21) and will only be mentioned briefly: animals from an inbred strain (IMM/S-209) (11) of guinea pigs were sensitized by subcutaneous

injections (10 and 1 µg) of ovalbumin (OA, grade V, Sigma) in Al(OH)₃. After sensitization, the animals (about 4 months old) were subjected to a pretreatment inhalation provocation test and allocated to four groups aiming at a uniform distribution of bronchial reactivity:

1. Weekly intradermal injections of saline.
2. Weekly intradermal injections of anti-OA hyperimmune-IgG (containing the same amount of IgG as in the OA-IgG-immune complexes given to Group 4).
3. Weekly intradermal injections of OA (20 µg) in saline.
4. Weekly intradermal injections of OA-anti-OA hyperimmune IgG complexes (containing 20 µg of OA).

The treatment was carried out for 35 weeks and, subsequently, the animals were observed for additionally 24 weeks. The clinical response to the treatments was evaluated every 4 weeks by an inhalation provocation test (during the treatment period, the provocations were always made immediately prior to the weekly injection). Blood samples for immunological studies were drawn every 4 weeks on the day prior to inhalation provocation. At the end of the 24-week post-treatment observation period, the cutaneous reactivity of the animals was evaluated 1 h and 24 h after intradermal injections of 1 µg and 10 µg of the antigen dissolved in saline.

Blood samples for haematological testing and for determination of respiratory condition were drawn during the last week of the treatment period (to be published), and 24 weeks later the animals were killed and dissected, and organs removed for histological examination (to be published). The time schedule of the total study is shown in Fig. 1.

Animals

In this study we used four strains of guinea pigs genetically different as to their respiratory anaphylactic response. These strains were selectively bred (brother X sister) for about 30 generations for either high (IMM/S 209 and 740) or low (IMM/R 201/16 and 203) responsiveness to inhalations of aerosolized aqueous ovalbumin as measured by the respiratory anaphylaxis induced (11). The genetic homogeneity obtained within each strain has already been demonstrated by skin grafting and immunological studies (12, 26). All four strains were compared during the optimization of the passive cutaneous anaphylactic test, whereas only the IMM/S 209 strain was used in the IT study.

Preparation of hyperimmune IgG and immune complexes for IT

Serum from IMM/S 209 guinea pigs, rich in antibodies against OA, was used for preparation of hyperimmune IgG and immune complexes. The animals were immunized intradermally with OA (10 and 1 µg, days 0 and 21) emulsified in Freund's complete adjuvant. On day 28 the animals were desanguinated, and the sera pooled (= pool # 1) and stored at -20°C. IgG and complexes of IgG and ovalbumin were prepared using protein A affinity chromatography as previously described (20, 21).

Preparation of reference sera for IgE and IgG determinations

Sera used as reference serum in the IgE PCA test were prepared by repeated injections of OA

adsorbed on $\text{Al}(\text{OH})_3$, a standardized aluminium hydroxide gel kindly donated by Superfos, Vedbæk, Denmark. On day 0 was given an intraperitoneal injection of 10 µg OA and 1 mg $\text{Al}(\text{OH})_3$ in 1 ml saline followed by repeated intradermal injections of 1 µg OA and 1 mg $\text{Al}(\text{OH})_3$ in 0.1 ml saline (days 23, 51, 87 and 124). On day 135 the guinea pigs were desanguinated and serum pools from strains 201/16 (pool #2) and 209 (pool #3), were stored at -20°C. Aliquots of the serum pool (#1) described above were also used as references in the IgG_{1,2} radioimmunoassays and in the IgG₁ PCA test (see below).

Quantitative cutaneous anaphylactic test (PCA)

This assay was used to quantitate the production of homocytotropic antibodies of the IgE- and IgG₁-class as described by Ovary et al. (17, 18) and Parish (19).

Sera from the individual animals in a group drawn on the same day were pooled and each serum pool was diluted in saline just before injection. 0.1 ml aliquots of each dilution were injected intradermally in the back of the recipient guinea pig.

After the required time (14 days for measuring antibodies of the IgE class (unheated serum) and 2 days for antibodies of the IgG₁ class (serum heated to 56°C for 4 h)) 1 ml of antigen in dye solution (1 mg OA and 10 mg Evans Blue/ml saline) was injected intracardially in the ether-anaesthetized recipient guinea pig. 30 min after the challenge, the blue skin lesions were measured and the results expressed in mm of the mean diameter of the blue spots.

The two-fold titration of each pool was repeated in at least two but most often four guinea pigs. The IMM/S 209 serum pool was titrated in the same way on each recipient guinea pig as reference, and the result was expressed relatively to this serum pool.

Radioimmunoassay of OA-specific IgG₁ and IgG₂

To the test tubes ("Maxisorp", polystyrene tubes, 70 * 11 mm, NUNC, DK-4000 Ros-

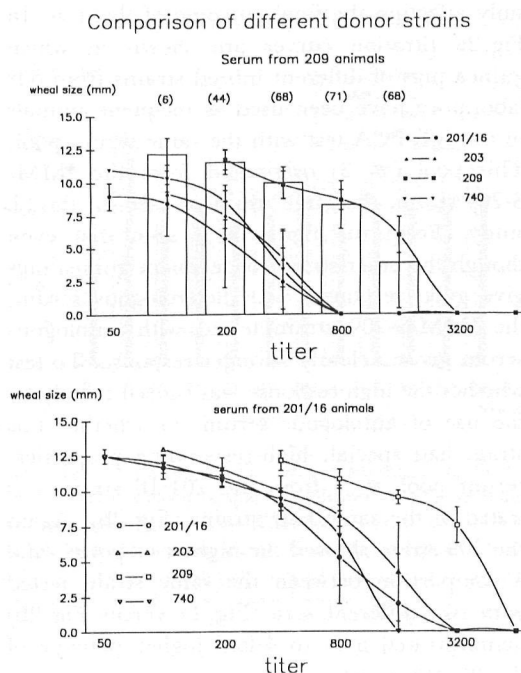


Fig. 2. Dependency of the PCA response on the recipient strain and the origin of the sensitizing serum.

- a. PCA-response in different strains using sensitizing serum from strain 209. A strongly IgE-positive serum from strain 209 (pool #3) was tested on animals from different strains: 201/16, 203, 209 and 740. As 209 demonstrated the highest sensitivity and lowest variation, this strain was chosen for test of the serum pools from the IT study. When the IT-serum pools were tested, these were quantified relatively to a two fold standard curve (from pool #3) applied on each animal. Bars show the means and SD of these standard curves (number of animals in parenthesis).
- b. PCA response in different strains using sensitizing serum from strain 201/16. A strongly positive serum of strain 201/16 (pool #2) was tested on animals from different strains: 201/16, 203, 209 and 740.

kilde, Denmark) 500 μ l OA (10 μ g/ml) were added, and the tubes were incubated overnight at room temperature. The following day, the tubes were washed 4 times (PBS + 0.1% Tween), and 500 μ l of the test sera were added in dilution buffer (sodium phosphate 0.1 mol/l, sodium chloride 0.15 mol/l, sodium azide 10 mg/l, EDTA 7.4 g/l and fetal calf serum 5% (v/v), pH 7.0) in dilutions of 10^5 (IgG₁) or 10^4 (IgG₂). Following incubation (2 h at 37°C) and washing, 500 μ l of the relevant anti-IgG sub-

class antibody (rabbit antibody against guinea pig IgG₁ and IgG₂, respectively, (Code Nos. 64-320 and 64-321, lot Nos. 16 and 15) from ICN Immunobiologicals, Lisle, IL, USA, dilution 1:1000) were added and incubated for 2 h at 37°C. Finally, the tubes were incubated overnight with 500 μ l of iodine-labelled goat anti-rabbit IgG (see below) overnight, and on the following day they were washed and counted in a gamma-scintillation counter.

All serum samples from each individual animal were tested in duplicate. The titre of an unknown sample was expressed relatively to the reference serum (defined as 100 arbitrary units/ml of IgG₁ or IgG₂, respectively). When tested in a 10- or 100-fold stronger concentration than that of the unknown samples, normal guinea pig serum repeatedly produced results below 5 AU/ml. Accordingly, the cut-off value was defined as 10 AU/ml.

Radiolabelling of secondary antibody

The secondary antibody (goat anti-rabbit IgG, R-3128 lot 107F-8804, Sigma Chemicals, St. Louis, MO, USA) was iodinated by means of a modification of the chloramin T method (5): to 100 μ g of the antibody in 100 μ l of PBS, 700 μ Ci of ^{125} I (sodium iodide, Amersham, England, Code No. IMS 30) and 50 μ l (100 μ g) of chloramin T were added. The reaction was stopped after 60 s by addition of 50 μ l (100 μ g) of sodium metabisulphite. Desalting was made on a Pharmacia PD-10 column (Pharmacia) in PBS containing 10 mg of HSA/ml. In the assay, the ^{125} I-goat anti-rabbit IgG was diluted to 200,000 cpm. More than 97% of radiolabelled preparation was precipitable by trichloroacetic acid.

Statistics

Serum titres were expressed relatively to reference sera as described above. For statistical analysis all serum titres were transformed by $\log_{10}$. IgG values below detection limit (10 AU) were set to 5 in order to permit statistical

analysis. Differences between groups were tested using the Mann-Whitney two-sample test (two-tailed). Differences within groups were tested using the Wilcoxon matched pair test. Correlations between different parameters were described by means of Spearman's Rho.

RESULTS

Variability of PCA test results related to strain of recipient guinea pig

To optimize the PCA test, the strain of the recipient animal was tested as a parameter pos-

sibly affecting the final outcome of this test. In Fig. 2a titration curves are shown in which guinea pigs of different inbred strains from our laboratory have been used as recipient animals in the IgE-PCA test with the same serum pool. This pool (# 3) originated from the IMM/S-209 strain, the same strain as used for the IT study. From the figure it is seen that even though the other strains of recipient guinea pigs give good responses with heterologous serum, the IMM/S-209 strain tested with autologous serum gives a clearly stronger response. To test whether the high response was caused merely by the use of autologous serum, or whether this strain had special, high-responding properties, serum pool # 2 from the 201/16 strain was tested in the same four strains (Fig. 2b). Again the 209 strain showed the highest response, and a comparison between the same strain tested with two different sera (Fig. 2a versus Fig. 2b) demonstrated a 2- to 4-fold higher potency of the 201/16 serum.

Accordingly, the autologous 209 system was used for testing all serum pools from the IT study. The bars in Fig. 2a depict the mean diameter of wheals (number of animals given in parentheses) when standard curves were made in the autologous 209 system. From this it is seen that highly reproducible standard curves were obtained during the actual PCA testing of the sera from the IT study.

Validation of the IgG_{1,2} assay

The specificity of the isotype-specific IgG-radioimmunoassay was checked in several ways. Initially, it was demonstrated that preincubation of serum with OA could inhibit the response in the IgG_{1/2}-RIA. To check the possible cross-reactivity between the antibodies directed against the two IgG-subclasses, columns were prepared by coupling 1) OA, 2) anti-IgG₁, 3) anti-IgG₂, and 4) rabbit antibody to an unrelated antigen (timothy grass pollen) to cyanogen-bromide activated Sepharose. A hyperimmune anti-OA guinea pig serum and a normal guinea pig serum were then passed through the columns and the effluents tested in the IgG_{1/2}-

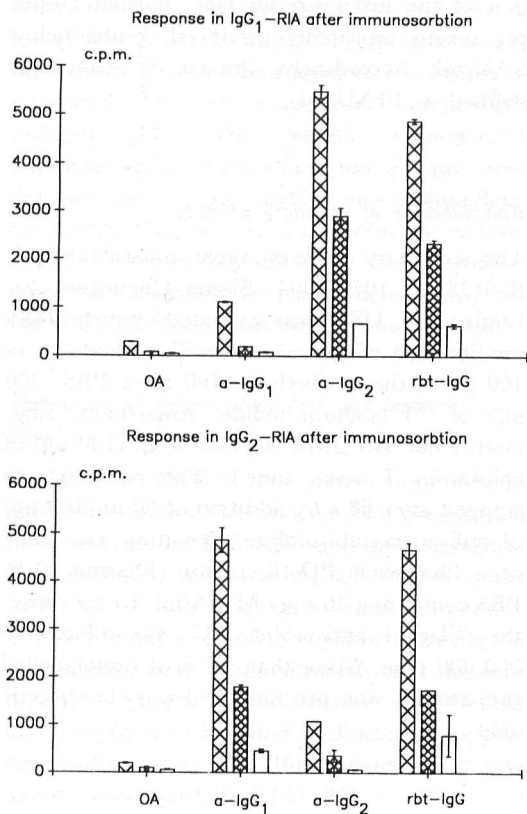


Fig. 3. Check of specificity of IgG_{1/2} radioimmunoassay. OA, a-IgG₁, a-IgG₂ and rbt-IgG were coupled to cyanogenbromide activated Sepharose, and a strongly positive serum was passed through the column. The effluents were tested in the IgG₁- (a) or IgG₂- (b) radioimmunoassays (see Material and methods) in dilutions of 1:10, 1:100 and 1:1000.

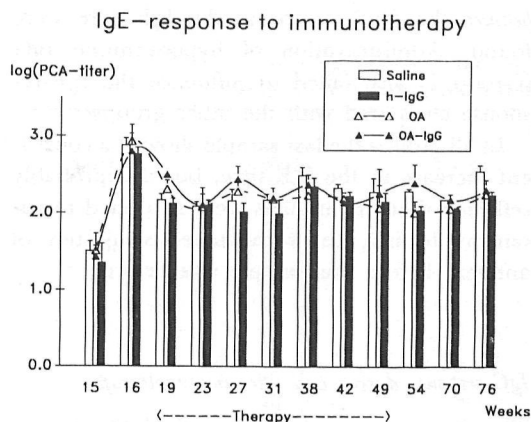


Fig. 4. IgE titre during the IT study as determined by PCA. On 12 occasions blood samples were drawn from the animals and sera pooled group-wise. These serum pools were tested in IgE-PCA (14 days, unheated; Controls: heated for 4 h at 56°C for the two most concentrated solutions) and results expressed relatively to a standard serum (pool # 3).

RIA. In no case did the normal serum produce any positive response in the radioimmunoassay. The hyperimmune serum lost almost all its activity after absorption with the OA-sepharose (Fig. 3). The anti-IgG₁ does not bind any IgG₂ activity as compared with the non-relevant rabbit antibody, and the same holds true for anti-IgG₂/IgG₁-combination. In the control experiments it is shown that the antibodies are able to absorb more than 95% of their own isotype.

Correlation between radioimmunometric determination of IgG₁ and biological determination by means of the PCA assay was tested for nine serum samples. Although a good correlation was observed between the two tests ($r = 0.90$, $P < 0.001$, $n = 9$), the absolute values were different, i.e. PCA-titres ranging from 3 to 66 gave RIA responses between 5 and 2450. To eliminate a possible uncertainty of the absolute values of the RIA, only non-parametric statistics were used.

IgE response during and after immunotherapy

Specific IgE against ovalbumin was determined by means of the PCA test. The PCA titre was interpreted as exclusively IgE-related as IgG₁

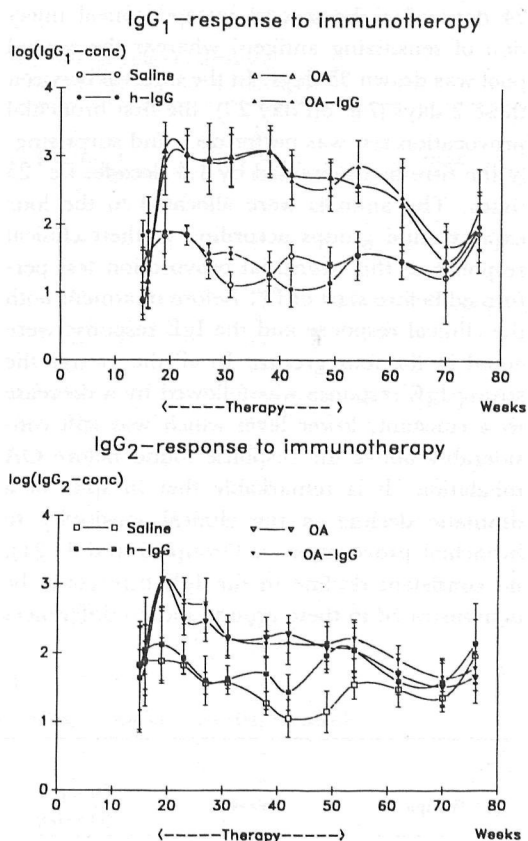


Fig. 5. IgG titre during the IT study as determined by RIA. IgG₁ (a) and IgG₂ (b) immune responses were determined by radioimmunometric assays in individual blood samples obtained during and after immunotherapy. Results are given as mean ($\pm$ SD). Results of statistical analysis of differences between sample #1 and the rest are given in Table 1. Statistical differences in comparison between groups are described in Table 2.

was shown to be unable to sensitize the skin for 2 weeks (a heat-treated serum expressed a titre of 3200 when animals were passively sensitized for 2 days, but less than 25, when sensitizing for 14 days). Further, 8-16 times more concentrated aliquots of heat-treated (56°C, 4 h) serum pools were regularly applied as controls. In all cases but one these controls showed no response. Thus, less than 1/10 of the response could theoretically be ascribed to IgG₁.

Fig. 4 shows the IgE response in the four groups during the total experimental period. The first serum pool was made from sera drawn

24 days after the second intraperitoneal injection of sensitizing antigen, whereas the second pool was drawn 35 days. In the interval between these 2 days (i.e. on day 29), the first bronchial provocation test was performed, and surprisingly the titre had increased by 1.4 decade, i.e. 25 times. The animals were allocated to the four experimental groups according to their clinical response to this bronchial provocation test performed before start of IT. Before treatment both the clinical response and the IgE response were equal in the four groups. In all the groups the strong IgE response was followed by a decrease to a constant, lower level which was still considerably above the response found before OA inhalation. It is remarkable that in spite of a dramatic decline in the clinical sensitivity to bronchial provocation in Groups 3 and 4 (21), no consistent decline in the IgE titre could be demonstrated in these groups and no differences

between the four groups in the IgE titre were found. Administration of hyperimmune IgG (Group 2) also failed to influence the IgE-response compared with the other groups.

In all groups the last sample showed a consistent increase in the IgE titre, but this probably reflected that the animals were subjected to cutaneous testing, i.e. intracutaneous injection of antigen, before this sample was drawn.

IgG-response during and after immunotherapy

The development of OA-specific IgG_{1,2} in the four experimental groups is shown in Figs. 5a, b. As the pool used for preparation of hyperimmune IgG and immune complexes was used as reference, the immune response is expressed relatively to this serum pool (defined as 100 units = 2 log steps), which was obtained by

Table 1.
Statistical differences between the IgG titre before and during/after immunotherapy

IgG ₁					
Sample	Week	Group 1 (placebo)	Group 2 (h-IgG)	Group 3 (OA)	Group 4 (OA-IgG)
2	16	-	-	-	-
3	19	-	*	*	*
4	23	-	-	*	*
5	27	-	-	*	*
6	31	-	-	*	*
7	38	-	-	*	*
8	42	-	-	*	*
9	49	-	-	*	*
10	54	-	*	*	*
11	62	-	-	*	*
12	70	-	-	-	*
13	76	-	*	*	-

IgG ₂					
2	16	-	*	-	-
3	19	-	*	*	*
4	23	-	-	*	*
5	27	-	-	-	*
6	31	-	-	-	-
7	38	-	-	-	-
8	42	-	-	-	-
9	49	-	-	-	-
10	54	-	-	-	-
11	62	-	-	-	-
12	70	-	-	-	-
13	76	-	-	-	-

| Treatment period; - *P* > 0.05; * *P* ≤ 0.05.

Table 2.

Statistical differences between the IgG titre in different groups.
Group No. 1 (placebo); No. 2 (h-IgG); No. 3 (OA) and No. 4 (OA-IgG)

Sample	Week	IgG ₁			
		1 versus 2	1 versus 3	1 versus 4	3 versus 4
1	15	*	*	*	-
2	16	-	-	-	-
3	19	-	**	**	-
4	23	-	**	**	-
5	27	-	**	**	-
6	31	-	*	*	-
7	38	-	**	**	-
8	42	-	**	**	-
9	49	*	**	**	-
10	54	-	**	**	-
11	62	-	-	**	-
12	70	-	-	-	-
13	76	-	-	-	-

Sample	Week	IgG ₂			
		1 versus 2	1 versus 3	1 versus 4	3 versus 4
1	15	-	-	-	-
2	16	-	-	-	-
3	19	-	**	**	-
4	23	-	**	**	-
5	27	-	**	**	-
6	31	-	*	*	-
7	38	-	**	**	-
8	42	-	**	**	-
9	49	*	**	**	-
10	54	*	-	**	-
11	62	-	-	*	-
12	70	-	-	-	-
13	76	-	-	-	-

† Treatment period; - $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$.

immunization with OA in Freund's complete adjuvant.

Group 1, i.e. the placebo group, showed some variation during the treatment and observation periods, but the changes from the pretreatment value never reached significance either for IgG₁ or for IgG₂ (Table 1). The last sample (drawn after intracutaneous test) showed a consistently higher response in all of the four groups.

Group 2, i.e. the group treated with hyperimmune IgG, showed a significant increase (Table 1) in both IgG subclasses early in the experiment, but only the titre for IgG₁ varied significantly from that of the placebo group in week 15 (Table 2). During late IT, the IgG_{1,2} titres increased significantly compared with that of the placebo group.

Groups 3 and 4, treated with either OA or OA-IgG, showed parallel responses to treatment (Fig. 5, Table 2). Both IgG subclasses increased after the initiation of therapy, although throughout the study only IgG₁ remained statistically different from the pretreatment value (Table 1). As opposed to the IgE response, the IgG titre declines after cessation of therapy.

Correlation to clinical response

To investigate whether the IgG response played any role in the clinical response of the individual animals within a group, a total clinical score was defined as the sum of symptoms during each bronchial provocation test carried out from the start of therapy until the end of the study.

Symptoms were defined as 1) the clinical score, 2) the time until onset of symptoms, and 3) the total provocation time, as each of these symptoms proved to be the most sensitive parameter in the experiment. As no statistical differences in either symptoms or immune response had been detected between Groups 1/2 (placebo, h-IgG) and 3/4 (OA, IgG-OA) respectively, these groups were paired in order to increase the strength of the statistical test.

The statistical analysis revealed no definite correlation between IgG response and symptoms. For Groups 1 and 2, however, the IgG-responses in both subclasses showed a

slightly positive correlation with the total clinical response to bronchial provocation tests. This correlation reached significance for samples drawn in the late study period. In Table 3, this is shown as negative correlation to clinical score (S) and positive correlations to reaction time (R) and total reaction time (T). In Groups 3 and 4 the same tendency, although weaker, was seen. Contrary to the former two groups, a negative correlation between IgG₂ and clinical response was seen before the start of therapy. When the single (i.e. every provocation test for each individual guinea pig) clinical responses during the study were compared with the IgG of a

Table 3.

Correlation between the IgG immune response and total clinical response. Total clinical response was defined as sum of S, R or T scores in all bronchial provocations performed during and after IT

No active treatment: placebo and h-IgG (Groups 1 and 2)								
Sample	Week	IgG ₁			IgG ₂			
		S	R	T	S	R	T	
1	15	-	-	-	-	-	-	
2	16	-	-	-	-	-	-	
3	19	-	-	-	-	-	-	
4	23	-	-	-	-	-	-	
5	27	-	-	-	-	-	-	
6	31	-	-	-	-	-	-	
7	38	-	-	-	-	-	-	
8	42	-	-	-	-	-	-	
9	49	* -0.75	-	-	** -0.78	-	-	* 0.59
10	54	** -0.80	* 0.70	* 0.72	*** -0.93	* 0.62	* 0.65	
11	62	-	-	-	-	-	-	
12	70	-	** 0.85	** 0.81	* -0.74	* 0.60	* 0.67	

Active treatment: OA and OA-IgG (Groups 3 and 4)								
Sample	Week	IgG ₁			IgG ₂			
		S	R	T	S	R	T	
1	15	-	-	-	-	** -0.72	** -0.58	
2	16	-	-	-	-	-	*	-0.54
3	19	-	-	-	-	-	-	
4	23	-	-	-	-	-	-	
5	27	-	-	-	-	-	-	
6	31	-	-	-	-	-	-	
7	38	-	-	-	-	-	-	
8	42	-	-	-	-	-	-	
9	49	-	-	-	-	* 0.66	-*	0.60
10	54	-	-	-	-	-	-	
11	82	-	-	-	-	-	-	
12	70	-	-	-	-	-	-	

S = symptom score, R = time until onset of reaction, T = total provocation time tolerated by the animal, | = treatment period; - $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

blood sample drawn consecutively with the provocation test, no significant correlations were found (results not shown).

DISCUSSION

Previously, during this study, we have demonstrated clinical efficacy of treatment with either OA alone or as immune complexes in combination with hyperimmune IgG (21). Because it was of great interest to elucidate whether this was related to levels of specific IgE or IgG, tests for these isotypes were evaluated.

A significant variance in PCA response between inbred strains has been reported for the murine system (3). Thus, different strains of guinea pigs were compared. The 209 strain which was used in the IT study, showed a markedly higher skin responsiveness compared with three other inbred strains. The differences could not be explained solely by the use of autologous serum (Fig. 2a), as a heterologous serum (Fig. 2b) also gave higher responsiveness in these animals.

To evaluate the IgG antibody response, radioimmunometric assays for the two subclasses 1 and 2 were developed. Only a marginal degree of cross-reactivity was found between the two subclasses (Fig. 3). The IgG₁ RIA, however, did not correlate very well with the biological PCA test. In both tests the samples were expressed relatively to the same standard serum, but the difference may be due to different slopes of the titration curves. For example, in the PCA test, the sera had to be used in concentrations which differed from the standard in order to produce skin reactions of a size comparable to the standards. Thus, the total protein and polyspecific IgG₁ concentration were much higher in the samples than in the standard. A possible affinity of the anti-IgG₁ antibody for IgE, seems to be of less importance as the RIA shows a large difference in IgG₁ titre between Groups 1/2 and 3/4 in spite of the fairly constant IgE level in all four groups (Fig. 4).

When sera from the IT study were tested, it was found that the IgE titres of all four groups rose dramatically from samples 1 to samples 2

which were drawn 24 and 35 days after the second intraperitoneal injection. Compared with a rat model (8), this peak came later than expected for a secondary immune response. Several reasons could account for this: it is possible that the bronchial provocation boosted the IgE response. Thus samples 2 were drawn 6 days after the inhalation provocation test, whereas later in the study blood samples were always drawn the day before a provocation test (Fig. 1), i.e. at least 27 days after the previous inhalation. Another reason for the late-occurring peak value could be that the major part of the newly synthesized IgE during the first couple of weeks after the second intraperitoneal injection becomes fixed to different cell types as mast cells, eosinophils, basophils and monocytes, and therefore might not be detected as circulating antibody.

After the peak value in week 16, constantly lower levels of titre 100–200 in all four groups were maintained throughout the study. The half-life of serum IgE has been determined to 12 h in the rat (1) and to 2.5 days in humans (9). If guinea pig-IgE has comparable decay, this implies that the immunization protocol results in a significant ongoing IgE synthesis. When comparing the four groups, no clear differences could be found at any time. Again, it cannot be excluded that bronchial provocation helped maintain the IgE level but it is remarkable that the four groups have identical IgE levels in spite of varying doses due to different exposure times. Neither does immunotherapy appear to influence the IgE level. In this respect, the animal model resembles the human immunotherapy situation, in which the IgE level does not decrease substantially during or after therapy (7, 22, 25).

Although in this study (Fig. 5) and other studies IT is evidently associated with an increase in IgG, it has not been possible to establish a firm, causal relationship (7, 21). If allergen-specific IgG had any regulatory effect, we speculated that administration of hyper-immune IgG should reduce symptoms and/or IgE serum-levels. Indeed, passive immunization has been used in conjunction with immunotherapy (2, 10, 23, reviewed in 22), and administration

of low levels of hyperimmune IgG has reduced the IgE level in mice (6). We could not confirm any of these findings. It is possible, however, that in our study the IgG dose was insufficient. In preparing the hyperimmune IgG (21) 3 ml of serum were used with an estimated final yield of 50% after concentration. The content of both IgG₁ and IgG₂ in this serum was defined to 100 AU/ml, i.e. each week during the treatment period 7.5 AU were administered to each animal. During the entire experiment the serum level of IgG in the hyperimmune IgG-treated group was substantially above 20 AU/ml, which indicates that the IgG administered to the animals comprised only a small fraction of the total specific IgG in the body.

Another indication of causal relationship between IgG and clinical outcome of therapy would be clear correlations between clinical response and IgG titre in the individual animals. In the groups receiving active IT (Groups 3 and 4), negative correlations between IgG₂ before IT and clinical response were observed. In the groups receiving non-active IT (Groups 1 and 2), a correlation was found between overall symptoms and IgG response in the late part of study. This could mean a small effect of IgG formed as a consequence of provocation tests, but still the animals of Groups 1 and 2 had substantially higher reactivity than those of Groups 3 and 4.

Several mechanisms have been proposed to account for the clinical efficacy of IT. In a recent placebo-controlled study of human IT, serum samples drawn before and during IT were used for passive sensitization of IgE-depleted basophils from a non-allergic donor (14). Results suggested a possible, lower sensitizing capacity of IT sera compared with placebo sera drawn at the same time of year. In the present study, the PCA response did not decrease during IT compared with placebo. This suggests that the overall sensitizing capacity of the sera, including the interference from other isotypes, did not change. In the guinea pig model it is still possible, however, that (cell-bound?) specific IgG₁ or IgG₂ may bind some of the offending antigen, thus inhibiting the allergic reaction. This cannot be determined in the PCA, as the IgG

isotypes will be removed at the time of the antigen challenge (14 days). The lack of difference in clinical response between Groups 1 and 2, indicates that the (homocytotropic) IgG₁ in the h-IgG-preparation, does not cause a high degree of *in vivo* sensitization.

From the data mentioned above we conclude that the animal model employed in the present study has several features in common with human allergy and immunotherapy. Presently, we are investigating possible differences in the pathology of the animals. The aim of these studies is to elucidate whether the treatment with immune complexes had any adverse effects, for example in the form of immune complex diseases.

ACKNOWLEDGEMENTS

The excellent and skilful technical assistance of Lisbeth Abrahamsen and the secretarial assistance of Anne Larsen are highly appreciated. This study was supported by grants from the P. Carl Petersens Fond, the Dagmar Marshalls Fond, the Fabrikant Einar Willumsens Mindelegat, Fonden til Lægevidenskabens Fremme and the Dansk Asthma og Allergiforbund.

REFERENCES

1. Bazin H, Pauwels R. IgG and IgG_{2a} isotypes in the rat. *Prog Allergy* 32, 55-104, 1982.
2. Bernstein IL, Michael JG, Malkiel S, Sweet LC, Brackett RG. Immunoregulatory of specific IgE. II. Clinical evaluation of combined active and passive immunotherapy. *Int Arch Allergy Appl Immunol* 58, 30-37, 1979.
3. De Souza CM, Maia LC, Vaz NM. Susceptibility to cutaneous anaphylaxis in inbred strains of mice. *J Immunol* 112, 1369-1372, 1974.
4. Djurup R. The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 40, 469-486, 1985.
5. Greenwood FC, Hunter WM, Glover JS. The preparation of 131-I labelled human growth hormone of high specific radioactivity. *Biochem J* 89, 114-123, 1963.
6. Hall EP, Gault EA. Suppression of IgE responses by hyperimmune serum in rats. *Immunology* 59, 577-581, 1986.
7. Jarolim E, Poulsen LK, Stadler BM, et al. A long term follow-up study of hyposensibilization using immunoblotting. *J Allergy Clin Immunol* 85, 996-1004, 1990.
8. Jarrett EEE. Stimuli for the production and control of IgE in rats. *Immunol Rev* 41 52-76, 1978.
9. Jeske DJ, Copra JD. Immunoglobulins: Structure and function. In Paul WE (ed): *Fundamental immunology*, pp. 131-165. Raven Press, New York, 1984.

10. Lessof MH, Sobotka AK, Lichtenstein LM. Effects of passive antibody in bee venom anaphylaxis. *Johns Hopkins Med J* 142, 1-7, 1978.
11. Lundberg L. Guinea pigs inbred for studies of respiratory anaphylaxis. *Acta Pathol Microbiol Immunol Scand [C]* 87, 55-66, 1979.
12. Lundberg L, Koch C, Magnusson M, Bertelsen C. Characterization of two strains of selectively bred guinea pigs. 2. Differences in immune response to synthetic polypeptides. *Acta Pathol Microbiol Immunol Scand [C]* 91, 187-195, 1983.
13. Malling H-J. Clinical efficacy of allergen immunotherapy. *ISI atlas of science: Immunology* 1: 215-219, 1988.
14. Malling H-J, Stahl Skov P. Diagnosis and immunotherapy of mould allergy. VIII. Qualitative and quantitative estimation of IgE in *Cladosporium* immunotherapy. *Allergy* 43, 228-238, 1988.
15. Mosbech H, Djurup R, Dreborg S, Poulsen LK, Stahl Skov P, Steringer I. Hyposensitization in asthmatics with mPEG modified and unmodified house dust extract. III. Effect on mite-specific immunological parameters and correlation to changes in mite-sensitivity and symptoms. *Allergy* 45, 130-141, 1990.
16. Noon L. Prophylactic inoculation against hay fever. *Lancet* i, 1572-1573, 1911.
17. Ovary Z, Benacerraf B, Bloch KJ. Properties of guinea pig 7S antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis. *J Exp Med* 117, 951-964, 1963.
18. Ovary Z, Kaplan B, Kojima S. Characteristics of guinea pig IgE. *Int Arch Allergy Appl Immunol* 51, 416-428, 1976.
19. Parish WE. Homologous serum passive cutaneous anaphylaxis in guinea pigs mediated by two gamma₁ or gamma₂-type heat-stable globulins and a non-gamma₁ heat labile reagin. *J Immunol* 105, 1296-1298, 1970.
20. Poulsen LK, Søndergaard I, Weeke B. Preparation of patient-related allergens for hyposensitization. Qualitative aspects. *Allergy* 43, 552-564, 1988.
21. Poulsen LK, Lundberg L, Søndergaard I, Weeke B. Allergen-containing immune complexes used for immunotherapy. Preparation of complexes and evaluation of their clinical performance in guinea pigs. *Allergy* 44, 132-142, 1988.
22. Poulsen LK, Søndergaard I, Weeke B. Improvement of specific immunotherapy by human IgG and modified allergens. *Allergy* 44, 241-255, 1989.
23. Rubinstein E, Fujita Y, Okazaki T, Tripodi D, Reisman RE, Arbesman C. Effect of ragweed hyperimmune human gammaglobulin on in vivo and in vitro parameters of ragweed sensitivity. *J Allergy Clin Immunol* 57, 335-341, 1976.
24. Søndergaard I, Weeke B. Isolation of patient-related antigens from allergen extract by means of protein A-sepharose-patient IgG_{1,2,3,4} bioaffinity chromatography. *Allergy* 39, 551-559, 1984.
25. Søndergaard I, Poulsen LK, Østerballe O, Weeke B. Computational approach to the description of individual immune responses. IgE and IgG-subclass allergen-specific antibodies formed during immunotherapy. *Allergy* 46, 10-19, 1991.
26. Sørensen, M, Jørgensen PN, Lundberg L. Characterization of two strains of selectively bred guinea pigs. 1 skin transplantation experiments and screening for erythrocyte enzyme polymorphism. *Acta Pathol Microbiol Immunol Scand [C]* 91, 181-185, 1983.

Address:

Lars K. Poulsen, Ph.D.

Laboratory of Medical Allergology

Medical Department TTA 7542

State University Hospital

Tagsensvej 20

DK-2200 Copenhagen N

Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.