

Allergen-containing immune complexes used for immunotherapy of allergic asthma

Preparation of complexes and evaluation of their clinical performance in guinea pigs

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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 between antigen and specific IgG) IT were compared with placebo. The bronchial reactivity of the animals to the antigen was monitored regularly before, during (35 weeks) and after IT (20 weeks). Animals treated with passive IT did not improve clinically. Active and combined IT abolished most symptoms within 7 weeks of treatment. During the post-treatment period, animals from both groups surprisingly recovered their original sensitivity to inhalation of the antigen.

Key words: active-passive immunization, allergic disease, guinea pigs, hyposensitization, immunotherapy, immune complexes.

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CLINICAL ASPECTS

Two aspects of this study, might have clinical interest concerning future strategies for development of immunotherapy (IT) for IgE-mediated allergic diseases: 1) complexes between antigen and specific IgG antibody show a promising ability to produce hyposensitization comparable to conventional, and 2) the success of IT given for an extended period of an animal lifetime declines after stop of treatment. Further studies are necessary for evaluation of similar effects in allergic patients in order to determine the long-term cost/benefit of the treatment.

At the beginning of this century immunotherapy of allergic asthma and hay fever with allergen extracts was introduced by Noon and Freeman (5, 18). Cooke et al. (3, 4) found that during therapy a factor blocking the Prausnitz-Küstner reaction, i.e. the allergic reaction mediated by homocytotropic antibodies, was formed in serum. Cooke proposed this factor to be an antibody different from homocytotropic antibody, and this was later confirmed by Lichtenstein (14) who showed that IgG specific to allergen was produced during immunotherapy. However, the exact mechanism by which the allergic symptoms are reduced, still remains obscure.

Attempts have been made to reduce allergenicity while retaining antigenicity (i.e. capability of eliciting and IgG antibody response) of allergen extracts for immunotherapy (6). Several investigations suggest that polymerization of allergens by means of glutaraldehyde offers a possibility of achieving this goal, as utilization of glutaraldehyde-treated allergens for immunotherapy has shown good efficacy and a low rate of side effects (7, 8).

Recently, a method has been proposed for purification of allergen extracts by means of affinity chromatography, employing the patients' own antibodies (22). This method will

produce immune complexes containing the patient's antibodies and the single allergens to which the patient reacts. It is anticipated that such immune complexes have characteristics comparable with the polymerized allergen extracts, i.e. high molecular weight, low diffusibility from the injection site and reduced ability to crossbind IgE on mastcells. Further, IgG-containing immune complexes might be more easily opsonized by macrophages, leading to a better antigen presentation. Thus, it is possible that immunotherapy with immune complexes prepared *in vitro* would represent a more specific and improved form of treatment.

To study the efficacy and possible side effects of such immune complexes, we used a strain of inbred guinea pigs which could be inflicted with allergic asthma to an experimental antigen (15). We produced complexes between antigen and IgG *in vitro* and tested them in comparison with conventional active and passive immunotherapy. In this report we described preparation of immune complexes and the clinical outcome of immunotherapy. Studies are in progress to describe the immunological events and possible side effects accompanying the treatment.

MATERIAL AND METHODS

Animals

Guinea pigs of the strain IMM/S-209 were used for production of hyperimmune serum and for immunotherapy experiments. This strain, selectively bred for high respiratory anaphylactic response to antigen-inhalation, has been brother $\times$ sister mated for about 30 generations. The animals show a uniform ability to produce allergic asthma when sensitized with an appropriate antigen by inhalation or by injections of the antigen alone or adsorbed on $\text{Al}(\text{OH})_3$ (15). The asthma is reversible and easily elicited when the animal is challenged with an aerosol containing the relevant antigen (see later).

The homogeneity of the strain has been demonstrated in skin transplantation experiments, in screening for erythrocyte enzyme polymor-

phism (23) and in examinations of the immune response to different synthetic polypeptides (16).

The animals were used for two purposes: In the first part of the study, animals were *immunized* in order to produce hyperimmune serum. In the second part, other animals were *sensitized* and allocated into 4 study groups for evaluation of the efficacy of the different forms of immunotherapy.

Preparation of hyperimmune IgG and immune complexes

For this preparation we used serum from IMM/S-209 guinea pigs, rich in antibodies against ovalbumin (OA). The animals were immunized by intradermal injections at two abdominal sites with 0.1 ml of OA (grade V, Sigma) solution emulsified in equal parts of Freund's Complete Adjuvant (FCA), containing 0.5 mg/ml of *Mycobacterium tuberculosis*, (kindly supplied by the Tuberculin Department, Statens Serum-institut, Copenhagen). A total amount of 10 μg and 1 μg of OA was injected on days 0 and 21, respectively. On day 28 the animals were de-sanguinated, and the serum was stored at -20°C .

IgG and complexes of ovalbumin and IgG were prepared using protein A affinity chromatography. 3 g of protein A-sepharose (Pharmacia, Uppsala, Sweden) were swollen in PBS and packed in a column according to the instructions of the manufacturer. The column was placed in a chromatographic system comprising a pump, Pharmacia P-1; a UV-recorder (280 nm), Pharmacia UV-1; and a fraction collector, Pharmacia Frac-100. A valve between the pump and the UV-recorder permitted recycling of the buffer through the column. Chromatography was performed at room temperature at a flow rate of 48 ml/h and fractions of 4.8 ml were collected.

For the preparation of hyperimmune IgG (h-IgG) the following procedure was employed: PBS buffer (sodium phosphate 0.01 M, sodium chloride 0.5 M, pH 7.4) was flushed through the column for 1 h. 3 ml of serum were applied on the column and recycled for 1 h. Finally, the column was eluted for 1 h with PBS, and for 90 min with glycine buffer (0.1 M, pH 2.8). To neutralize fractions eluted at low pH, 1.2 ml of

phosphate buffer 1 M, pH 8.0, was added to the tubes before starting.

IgG subclasses were separated, employing a pH-gradient to the protein A-column (17). After application of serum and wash with PBS (pH 7.4) a stepwise pH elution was performed by means of PBS (pH 6.0), acetate buffer (0.1 M, pH 4.2) and glycine buffer (pH 2.8).

For preparation of immune complexes containing OA and hyperimmune IgG (OA-IgG) the above procedures were followed with some modifications: After application and recycling of serum, the column was washed for 1 h with PBS. Then OA 1.5 ml of 2 mg/ml, was applied and allowed to recycle for 1 h. OA not bound to the immobilized IgG was washed out at neutral pH, and, finally, the immune complexes and free IgG could be eluted at low pH with glycine buffer.

h-IgG- and OA-IgG-fractions were concentrated to 1 ml using a Millipore Immersible® (cut-off: 10 kd). h-IgG and OA-IgG were prepared every second week, respectively: h-IgG one week and OA-IgG the next. Thus, the preparations were used for treatment 1 and 8 days after production. Meanwhile they were stored at 4°C with addition of 0.5 % phenol to ensure sterility.

For optimization of the method and characterization of the immune complexes, OA was radio-labelled using a modification of the chloramine-T method (9): To 100 µg of OA in 100 µl of PBS were added 700 µCi of 125-I (sodium iodide, Amersham, England, Code No. IMS 30) and 50 µl (100 µg) of chloramine-T. The reaction was stopped after 60 s by the addition of 50 µl (100 µg) of sodium metabisulphite. Desalination was done on a Pharmacia PD-10 column in PBS containing 10 mg of bovine serum albumin/ml. The labelled OA was further purified on a Sephadex-75 Fine (Pharmacia) column (28 × 2.6 cm, flow-rate 12 ml/h). Trichloroacetic acid precipitability was > 90 %. Due to the low number of tyrosine residues available for iodination in OA (21) this method produces a rather low specific activity (0.5 mCi/mg). However, it was sufficient for the present purpose.

Sensitization

25 guinea pigs, 2 months of age, were sensitized to OA by intraperitoneal injections at days 0 and

21 with 10 µg and 1 µg of OA, respectively, adsorbed on 20 µl of 2 % Al(OH)₃ gel (Alhydrogel from Superfos, Vedbæk, Denmark). Four weeks after the latest injection the animals were challenged with OA in the inhalation provocation test (see below) in order to establish the degree of sensitization.

Inhalation provocation test

The respiratory anaphylaxis of the animals was evaluated clinically as described (15). In brief, the animals are placed in an inhalation chamber divided into individual sections by a rough wire mesh. A glass cover enables observation of the animals during the challenge. An aerosol of OA is introduced in one side of the chamber by nebulizing 0.1 % OA in saline (0.1 ml/min). Several animals from each of the four research groups were tested simultaneously in a manner blinded to the observer. In this way it was ensured that evaluations of the respiratory anaphylactic reactions were comparable between the groups. The observation period was initially 12 min (unless the animals had to be removed, see below) but as the sensitivity of the animals in the two groups treated with OA and OA-IgG decreased during the study, this period was increased to 20 min for these groups. The responses of the animals are evaluated and described using three parameters:

- 1) the severity of the response:

0	No signs of anaphylactic reaction – same as controls.
+	Incidental or slightly forced respiration.
++	Clearly forced respiration.
+++	Strongly forced respiration, fits of coughing.
++++	As 3+, but in addition the animal is shocked, and its general condition is poor.
+++++	As 4+, but in addition an interruption of inhalation for treatment with aerosolized epinephrine is necessary;

- 2) the time until appearance of the first positive response (*reaction time*);

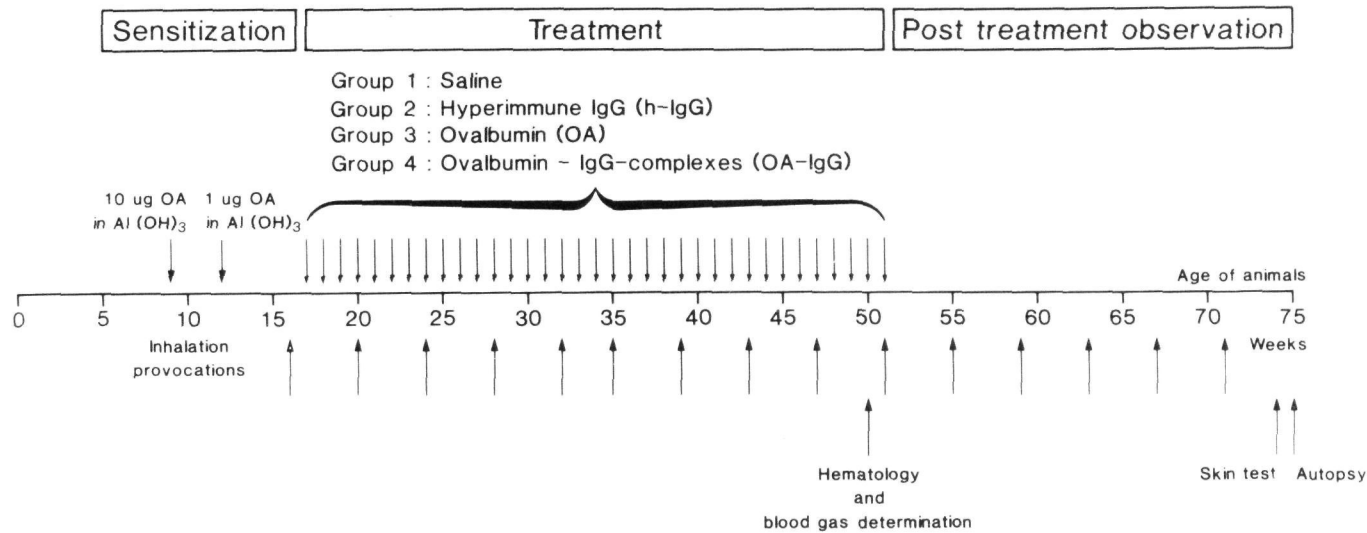


Fig. 1. Study protocol.

3) the total time until the animal was removed from the inhalation chamber due to severe signs of anaphylaxis or the termination of the 12 or 20 min (*total provocation time*).

Skin testing

The intensity of the immediate and cellular hypersensitivity to OA was evaluated 1 h and 24 h after intradermal injections of 1 µg and 10 µg of the antigen dissolved in saline. The reactions are given as the average of two ortogonal diameters of the erythematous area.

Protocol

It has previously been established (Lundberg, unpublished results) that immunotherapy with a dose of 20 µg OA in 100 µl of isotonic buffer would cause a significant reduction of the respiratory anaphylaxis when sensitized animals undergo BPT. Accordingly doses equivalent to this were utilized in the investigations. The sensitized animals (about 4 months old) were allocated to 4 groups according to the first inhalation provocation test:

1. Receiving weekly intradermal injections of saline for 35 weeks (5 animals).
2. Receiving weekly intradermal injections of h-IgG (containing the same amount of IgG as in the OA-IgG-immune complexes given to Group 4, see results) for 35 weeks (6 animals).

3. Receiving weekly intradermal injections of OA (20 µg) in saline for 35 weeks (6 animals).
4. Receiving weekly intradermal injections of OA-IgG (containing 20 µg of OA, see results) for 35 weeks (6 animals).

After the termination of therapy the animals were observed for an additional 24 weeks. The clinical status was evaluated regularly, and every 4 weeks an inhalation provocation test was performed during the entire study (during the treatment period, the provocations were always made immediately prior to the weekly injection). During the last weeks of the treatment period, blood samples were drawn for haematological examinations and for determination of respiratory status (see below). Blood samples for immunological studies (to be published) were drawn every 4 weeks on the day prior to inhalation provocation. After the observation period the animals were dissected and organs removed for histological examinations (to be published).

The time schedule of the study is shown in Fig. 1.

Statistics

Differences between groups were tested using the Wilcoxon two-sample test (two-tailed). The maximum time during which the animals were

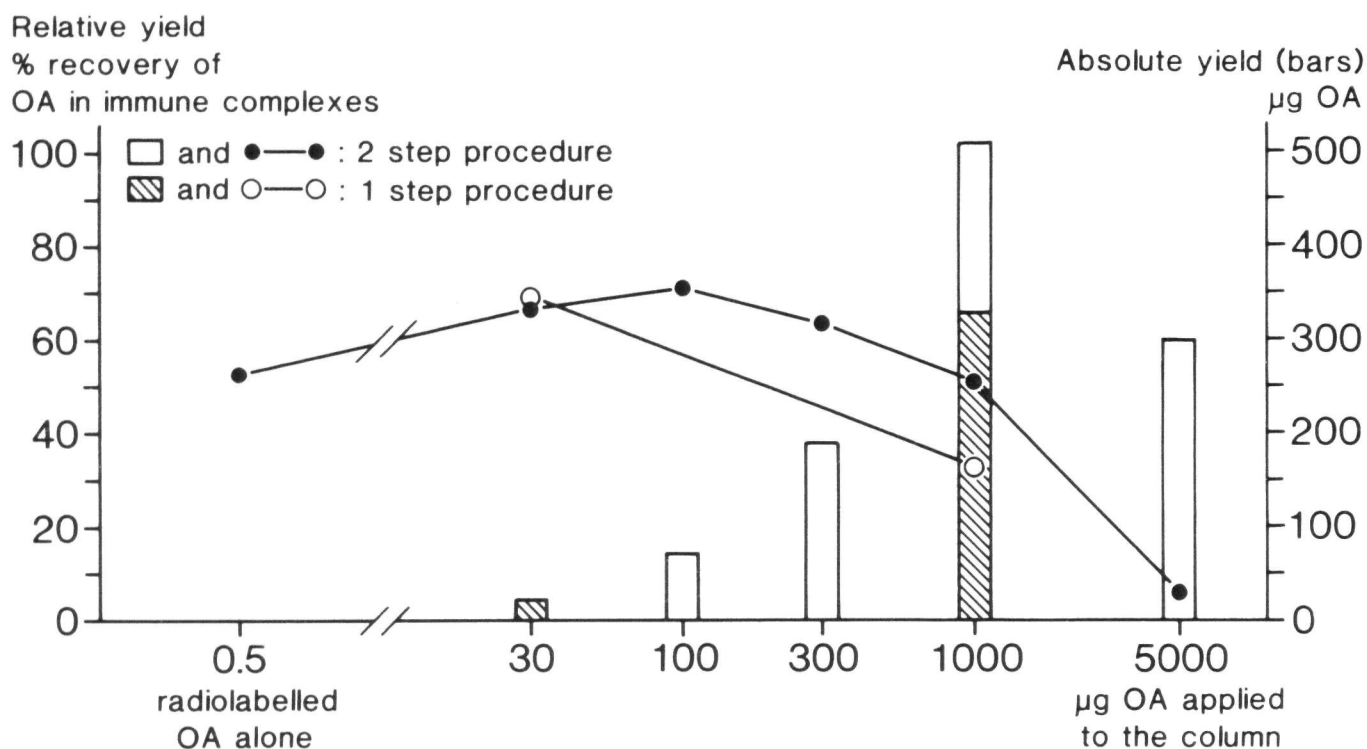


Fig. 2. Preparation of OA-IgG complexes by protein A affinity chromatography (See Material and Methods). In a series of experiments 1 ml of serum was applied to the column, and varying amounts of OA including radiolabelled OA were applied at the same time (one-step method) or sequentially (two step method). The absolute (bars) and relative (lines) recoveries are indicated in the figure.

challenged with the inhalation provocation test was longer for Groups 3 and 4 (Group 1: 12 min, Groups 3 and 4: 20 min from week 28). To make the results comparable in the statistical analysis, longer reaction times and total reaction times from these 2 groups were treated as 12 min. This procedure gives a weaker test, the "true" differences tending to be larger as can be seen in Figs. 3 and 4.

RESULTS

Characterization of the hyperimmune IgG

When the preparation of h-IgG was tested in crossed immunoelectrophoresis against anti-guinea pig whole serum only one anodic precipitate appeared (result not shown), indicating a fairly high purity of IgG.

An attempt was made to separate the homocytotropic subclasses of IgG (IgG_{1 a+b}) from IgG₂. Whole serum was applied to the protein A-column followed by wash and stepwise elu-

tion with pH 6.0, 4.2 and 2.8. The first step did not lead to any elution of protein, whereas the latter two steps produced two peaks ("IgG_{1 a+b}-rich" and "IgG₂-rich") of comparable size. The two peaks were collected, the eluate neutralized, and reappplied to the column. Radio-labelled ovalbumin was then applied, and after washing with PBS pH 7.4, a stepwise pH-gradient was flushed through the column as described above. These experiments showed that only the latter peak, presumably IgG₂, had the ability to bind labelled OA. The radioactivity was only eluted at pH 2.8, indicating that the antibodies had bound the antigen with a high avidity. These results indicated that the antibody raised in immunized animals with FCA as adjuvant was dominated by an IgG₂ response.

Preparation and characterization of immune complexes

From earlier experiments (Lundberg, unpublished observations) it is known that immunotherapy with repeated injections of 20 µg OA/100 µl buffer produces significant relief of

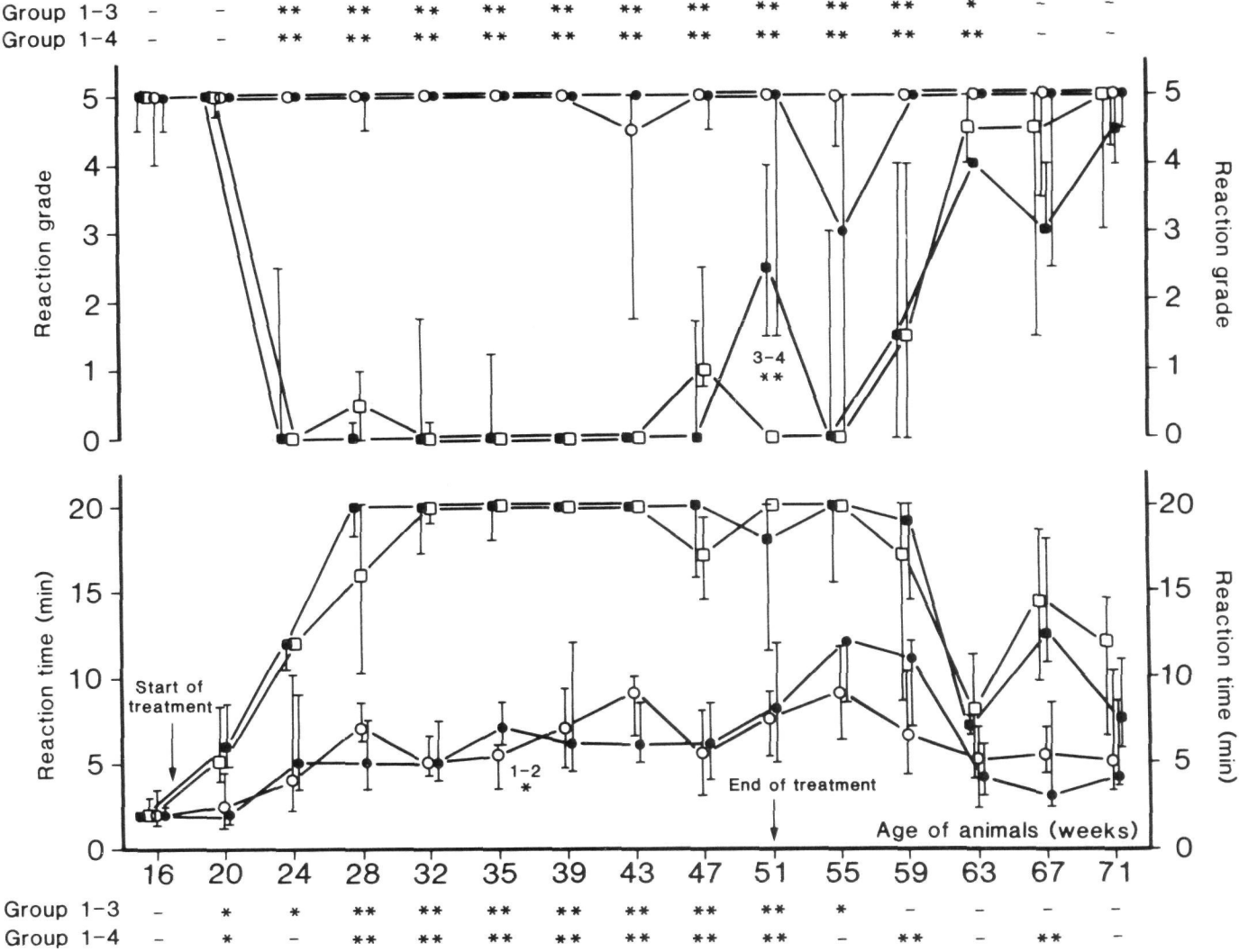


Fig. 3. Respiratory reactivity of the four groups (median and interquartiles) after challenge with aerosolized OA in the inhalation provocation test (see Material and Methods). Statistically significant differences between Groups 1-3 and 1-4 indicated as: - $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; ○—○ Group 1: saline; ●—● Group 2: h-IgG; □—□ Group 3: OA; ■—■ Group 4: OA-IgG. Upper part: Reaction grade. Differences between Groups 1 and 2 were never significant, between Groups 3 and 4 at week 51 ($P < 0.01$). Lower part: Reaction time (i.e. time elapsed until first reaction). Differences between Groups 1 and 2 were significant at week 35 ($P < 0.05$).

symptoms in sensitized animals. Thus we aimed at preparing immune complexes containing this amount of OA. Affinity chromatography experiments were conducted to establish the binding capacity of the hyperimmune serum prepared. In these experiments 1 ml of serum and a constant amount of radio-labelled OA were used together with varying amounts of “cold” OA. The procedure was sequential application of antiserum and antigen as described in Material and Methods. The results appear from Fig. 2, in which, as was to be expected, it is seen that with increasing amounts of OA applied to the column, in-

creasing yields are obtained. Surprisingly, the relative yield increases up to 100 μg , and only when higher doses are applied does the relative yield decrease as anticipated from the mass-equilibrium-law. With the highest amount of 5 mg, however, a form of saturation occurs resulting in a decreased yield.

To ensure that OA did not bind unspecifically to the column, 30 μg and 1000 μg were applied as described above, but with the exclusion of serum. Elution from the column showed that 2.6% (0.8 μg) and $< 0.1\%$ ($< 1 \mu\text{g}$), respectively, of the radioactivity could be recovered in the fractions in which the immune

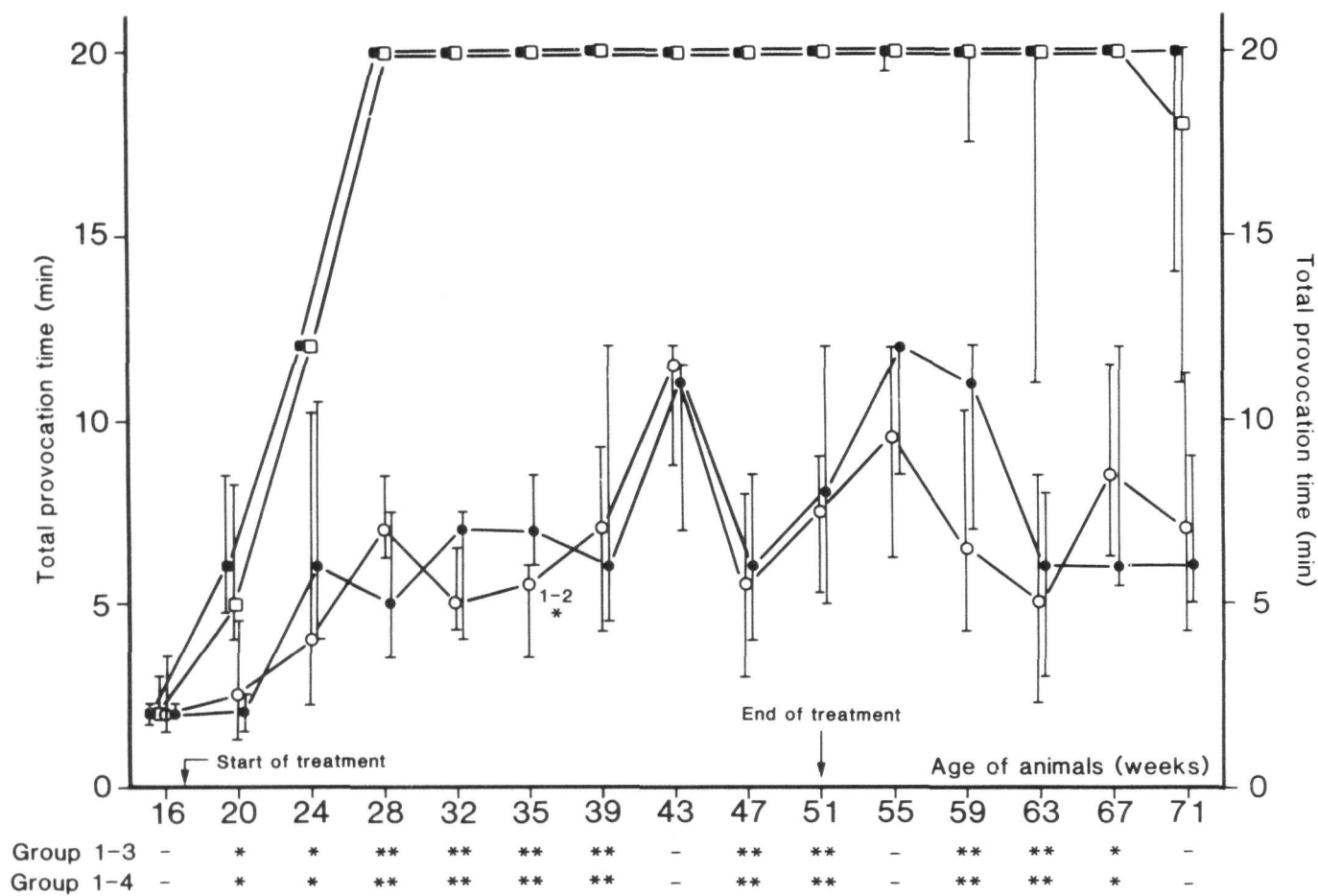


Fig. 4. Total provocation time (time elapsed from start to end of provocation or until removal of the animals was necessary) for the four groups (medians and interquartiles). For explanation of symbols and statistically significant differences, see legend to Fig. 3. Groups 3 and 4 showed no significant differences, Groups 1 and 2 varied at week 35 ($P < 0.05$).

complexes were normally found. The theory was advanced that mixing of antiserum and antigen before application to the column might increase the yield, as more immune complexes might be formed by preincubation. A marginal increment was indeed seen when 30 μg of OA was preincubated with serum, but for 1 mg of OA the yield was substantially lower than with the two-step method. As a favourable yield of immune complexes was obtained with a ratio of 1 mg of OA to 1 ml of hyperimmune serum this ratio was used for later experiments. With the use of radio-labelled OA it was additionally shown that 50% of the immune complexes appeared in the two central fractions of the peak eluted with low pH. These fractions could be concentrated to 1 ml with a loss of 50%. Thus, from 3 ml of serum and 3 mg of OA a final product with a concentration of 400 μg of OA/ml could be produced. To approach iso-

tonicity 50 μl of this product was mixed with the same volume of PBS before injection into the animals.

When the method described above was employed, a faint precipitate appeared when the immune complexes were eluted from the column and pH neutralized. Tracer studies showed that a majority of OA was associated with this precipitate, indicating that it was kept in suspension in the final product. The precipitation reaction was not due to denaturation of IgG, as it did not occur during the preparation of h-IgG.

Sensitization and evaluation of respiratory anaphylactic response

After the animals had been sensitized to OA an inhalation provocation test was conducted. 22 of 25 animals reacted with + + + + + (5), where-

as the remaining 3 had a + + + (3) reaction. The time until appearance of response varied between 1 and 4 min (median = 2.0 min).

Two animals died from anaphylactic shock and respiratory failure during the first inhalation provocation in spite of immediate removal from the challenge chamber and treatment with aerosolized epinephrine – all within 2 min of the start of provocation. The remaining 23 animals were allocated to the four study groups as described in Material and Methods.

Response to therapy

Four groups of guinea pigs sensitized to OA were subsequently treated with saline, h-IgG, OA and OA-IgG, respectively. The bronchial reactivity to antigen inhalation in the 4 groups throughout the study is shown in Figs. 3 and 4.

Starting with the control group receiving saline, it appears that the bronchial reactivity measured as degree of affection, is more or less unchanged throughout the study. In fact, the animals were so responsive that one animal from this group died from shock in week 20. Reaction time and total provocation time appear to be more sensitive parameters, as increases are observed throughout the period, indicating that respiratory reactivity has slightly declined.

Treatment with h-IgG produced no significant decrease in respiratory reactivity compared to saline-treatment. One animal in this group died from shock in week 20, as in the saline-treated group.

Contrary to h-IgG, it appears that both OA and OA-IgG have a striking effect on respiratory reactivity. Already 3 weeks after the start of treatment an elevation of the reaction time is seen, and after 7 weeks of treatment the animals are irresponsive to inhalation provocation with OA in a concentration of 1 mg/ml. In week 28 the provocation time was increased to 20 min for these two groups, but this did not lead to any significant reactions. It was not possible to differentiate between the effect of treatment with OA and OA-IgG, either on the reaction grade or reaction time.

After cessation of therapy the animals were regularly monitored for a further 20 weeks. In spite of the long period during which the asthma symptoms were almost completely suppressed, both groups gradually developed increased responses to inhalation provocations. In fact, the responsiveness increased to a level that was not significantly different from that of the saline-treated group. In the OA-group one animal died from shock in week 67. Fig. 3 shows that the time until onset of anaphylactic reaction in the OA and OA-IgG groups compared to the other groups is still higher, although not significantly.

To reveal possible differences between groups with similar clinical responses, the total provocation time tolerated by the animals was compared (Fig. 4). The differences between the OA/OA-IgG and saline/h-IgG are expanded using this parameter, and it appears that even at the last provocation in week 71, the two former groups show considerably higher resistance to the provocation. Even though this

Table 1

Intracutaneous test performed in week 74 just before termination of the post-treatment observation period. Diameter of wheal (median and interquartile) (in mm) read 1 and 24 h after injection of 1 or 10 µg of ovalbumin (OA)

	Group 1 Saline (n = 4)	Group 2 h-IgG (n = 5)	Group 3 OA (n = 5)	Group 4 OA-IgG (n = 6)
1 µg of OA (after 1 h)	5.0 (0-12.3)	10.0 (4.5-11)	12.0 (0-14)	7.0 (0-15)
(after 24 h)	5.5 (0-14.8)	12.0 (5.5-13)	15.0 (0-16.5)	10.0 (0-16.3)
10 µg of OA (after 1 h)	3.5 (0-8.5)	5.0 (0-9)	5.5 (1.3-6.8)	3.5 (0-8)
(after 24 h)	10.5 (2.5-11.8)	9.0 (0-10.5)	7.0 (2.5-17)	6.6 (4.5-10.3)

parameter seems a better discriminator, no significant differences are revealed between either OA/OA-IgG or saline/h-IgG groups.

Cutaneous reactivity

Attempted transfer of the skin prick test procedure used in humans for evaluation of allergic status was not successful, as the thickness of guinea pig skin made it difficult to produce even a wheal and flare reaction with histamine 10 mg/ml (results not shown). As intracutaneous tests are known to produce a profound stimulation of the immune system, it was decided not to use this test until immediately before cessation of the study, i.e. 1 week before dissection (Table 1). From the table it appears that at that time, nearly 6 months after termination of the treatment period, no significant differences were found between the four groups.

DISCUSSION

In this investigation different forms of immunotherapy were administered. To institute meaningful comparisons of efficacy it was essential to ensure that the doses of the different treatments were similar. The tracer studies established that the amount of OA in the immune complexes was equal to that given in normal active immunotherapy with OA only. Using the same amount of serum for the preparation of hyperimmune IgG it was moreover ensured that animals treated with h-IgG received the same amount of IgG as the OA-IgG-treated animals.

Immunoelectrophoretic investigation of h-IgG showed only one precipitate, and this conforms with our experience that binding of other immunoglobulin-classes to protein A can be eliminated by utilization of a high ionic strength (22). The immunological potential of both antibody and antigen seemed to be unspoiled by the chromatographic preparation, as evidenced by several indications: when the antibody was rechromatographed on protein A, it showed an unreduced ability to bind the antigen. This is in accordance with our own results (19) and those of others (11). Concerning the antigen, tracer studies showed that

a major part of OA was presented in a precipitate observed after chromatography. This suggests that OA was still bound to the OA-specific IgG. Attempts to increase the yield by means of preincubation of serum and antigen were not successful, as a lower yield was obtained when a large amount of antigen was used. This can readily be explained as the protein A-sepharose exposes the IgG molecules in such a way that each of the two antigen-binding sites is able to bind one ovalbumin molecule, as opposed to the formation of larger immune complexes in solution, where several immunoglobulin molecules can bind to each antigen molecule.

Sensitization of the animals with two intraperitoneal injections of OA-Al(OH)₃ made the animals extremely vulnerable to inhalation provocation with aerosolized OA, and previous investigations by us (Lundberg, unpublished observations) and others (2) have shown that treatment with antigen-Al(OH)₃ is accompanied by formation of antigen-specific IgE. This vulnerability decreased only to a minor degree during a 1-year period, indicating that hyposensitization caused by antigen challenge of 1 mg OA/ml in the provocation tests every 4 weeks was minimal. However, compared to the first provocation, significant increases in reaction time and total provocation time were observed during the study period. As the animals were challenged several times during the experiment, the increments in reaction time and total reaction time could be explained by the elevated age during the experiment. In a previous study (10) animals also inbred for their susceptibility to respiratory anaphylaxis, but from a different strain, were passively sensitized by one injection of serum containing homocytotropic antibody. Inhalation provocation tests were done every 2 weeks, and within 24 weeks the animals showed a spontaneous loss of respiratory reactivity. The turnover of the passively administered homocytotropic antibody is likely to have caused the change in sensitization. The sensitization produced by administration of antigen with Al(OH)₃ as adjuvant seems to produce a long-lasting sensitization which does not decline in spite of inhalation provocation tests every 4 weeks. Thus it seems verified that

the sensitization and provocation protocol used in the present study constitutes an animal model that allows for long-term experiments of respiratory anaphylaxis. Preliminary studies indicate that the strain used in the present study has an elevated involvement of the eosinophilic cell evidenced by eosinophilia compared to the strain used in the earlier experiments (unpublished results).

In the present study continuous passive immunization (i.e. treatment with h-IgG) did not cause any hyposensitization. In spite of 35 treatments with h-IgG, no difference from the control group was detected. From the protocol it can be calculated that the amount of IgG given in one dose of h-IgG (or OA-IgG), corresponds to the amount of IgG in 150 μ l of whole blood, or about 0.3 % of the total blood volume. The negative result is in accordance with experience obtained in humans (1, 13, 20), where passive immunization did not cause any change in allergic symptoms compared to treatment with allergens.

Animals treated with OA and OA-IgG show a remarkable hyposensitization, which is fully established within 11 weeks after start of treatment. Surprisingly, the bronchial sensitivity of the OA- and OA-IgG-treated animals gradually increased after the cessation of 35 weeks of treatment. This indicates that the specific hyposensitization of the animals is reversible and overcome by an underlying allergic reaction, which gradually becomes manifest when treatment is withdrawn. The implications of this finding in humans remain obscure. To our knowledge no controlled studies exist in which the clinical course after the cessation of immunotherapy has been compared with placebo.

Compared to conventional immunotherapy (1) treatment of allergic patients with a combination of antigen and autologous antibody has been shown to improve the clinical outcome. Furthermore, in mice a hapten coupled to IgG has been shown to be efficient as a tolerogen in suppressing formation of reaginic antibody (12). In light of these results we formed the theory that antigen and antibody combined in immune complexes would have strong hyposensitizing properties. The present

investigation shows that immune complexes effectively reduce respiratory anaphylaxis. However, we are unable to distinguish this response from that obtained when treating the animals with OA alone. If the immune complexes are compared with unbound OA, one would anticipate two opposite changes in stimulation of the immune system: 1) due to antibody molecules themselves and steric hindrance, fewer free antigenic determinants of OA would be expected in the complexes, and 2) immune complexes will be detected and processed by macrophages and interact with other IgG-receptor-bearing cells better than free OA. From the present data we are not able to reveal whether any of these (or other) explanations are correct, because both the OA- and OA-IgG-treated groups showed a maximal response in terms of diminishing respiratory reactivity.

To detect a discrepancy between the two treatments a less favourable protocol might have been applied. Thus, a suboptimal dose or an increased interval between injections might have disclosed a potential difference. A weekly dose equivalent to 20 μ g OA was chosen as we wanted to establish the OA-treated group as a positive control. Alternatively, more symptoms might be produced by raising the total provocation time or the concentration of allergen in the aerosol. However, we preferred to avoid these measures so as not to hyposensitize the animals by inhalation of high doses. Thus, it was still possible to make comparisons with the two other groups – untreated and h-IgG. The antibody response in the animals during the treatments could reveal differences which might be obscured by the rather crude clinical measurements. We are at present analysing sera for OA-specific IgG and IgE developed during the study period.

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