

Antigen-Induced Bronchial Anaphylaxis in Actively Sensitized Guinea-Pigs

Pattern of Response in Relation to Immunization Regimen

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This work studies the temporal development of the acute anaphylactic bronchoconstriction in guinea-pigs sensitized to ovalbumin by different regimens, including IgE-antibody promoting ones. The results show that guinea-pigs sensitized with low amounts (1-10 μ g) of ovalbumin together with alum produce the most pronounced bronchospasm when challenged with an intravenous injection of a low dose of antigen. Examination of the antibody classes by PCA technique shows that guinea-pigs sensitized with small amounts of antigen together with alum produce IgE and IgG₁ antibodies. However, in sera from animals immunized with large amounts of antigen, only IgG₁ antibodies could be detected.

Key words: bronchial anaphylaxis; IgE; IgG; ovalbumin; sensitized guinea-pig.

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Antibodies with reaginic properties have been shown to belong to the minor IgE class of human immunoglobulins (3, 4, 15, 16, 19, 34). In the pathogenesis of human asthma, IgE antibody mediated reactions seem to play a major role (15). Thus, IgE antibodies are able to sensitize respiratory tissue and to mediate the release of chemical mediators that contract bronchial smooth muscle or are chemotactic for eosinophils (20, 26). Anaphylactic bronchoconstriction in the guinea-pig shows a common feature with bronchial asthma in that kinins, SRS-A, and histamine participate in the acute anaphylactic bronchoconstriction (10). The main homocytotropic antibody in the guinea-pig has been characterized as IgG₁. Furthermore, in recent years, it has been shown that suitably sensitized guinea-pigs are able to produce IgE-antibodies (2, 5-7,

11, 21, 24, 25, 27-31). Guinea-pigs and human reaginic antibodies behave identically concerning optimum latent period at passive sensitization and persistence in the skin and concerning loss of activity after reduction and alkylation or heat treatment. The present study examines the acute anaphylactic bronchoconstriction in guinea-pigs sensitized to ovalbumin (OA) by different regimens, including those that promote IgE-antibody, and characterizes the serum anti-OA antibodies produced by the animal. Special reference has been made to the dose of antigen needed for sensitizing the animals after a single intraperitoneal injection together with alum and the temporal development of the anaphylactic bronchoconstriction produced by various provocation doses. The work determined the best conditions for testing the effect of

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antiallergic drugs on the anaphylactic bronchoconstriction in the guinea-pig.

MATERIAL AND METHODS

Chemicals

Ovalbumin, OA (SIGMA, grade III), is the antigen used in the present investigation. In some experiments, $\text{Al}(\text{OH})_3$ obtained as dry powder (from a local dealer) is used as an adjuvant. Evans blue (Merck, Switzerland), is used to indicate the reaction in the passive cutaneous anaphylaxis in guinea-pigs. The animals were anaesthetized with pentobarbital (Mebumal®, ACO, Sweden) 30 mg/kg i.p.

Animals

Outbred guinea-pigs (Duncin-Hartley) of either sex (250–300 g) were used, bred by Sahlins, Malmö, Sweden.

Sensitizing procedures

Two major sensitization procedures were used:

A) The animals were injected i.p. with OA, 5,000 μg on day 0 and 10,000 μg on day 2. The OA was dissolved in 0.9% NaCl; the injection volume was 0.1 ml. This immunization procedure is extensively used in our laboratory.

B) The animals were sensitized by one i.p. injection of 0.5 ml saline containing 100 mg $\text{Al}(\text{OH})_3$ and OA. The OA doses were 0.1, 1, 10, or 100 μg . The antigen was added to the adjuvant suspension 1 h before injection.

Respiratory measurements

The animals were tracheotomized and ventilated by a Braun constant volume respirator (frequency 60/min, volume 7 ml/kg). Pulmonary mechanics, i.e. lung resistance (R_L) and dynamic lung compliance (C_{DYN}), were estimated by the method of Amdur & Mead (1) modified for anaesthetized guinea-pigs. Airflow ($\dot{V}$) was measured by a mech screen pneumotachograph (Fleisch No. 000),

connected to a Statham differential pressure transducer (PM 15). Tidal volume (V_T) was obtained as the electrical integral of airflow. The transpulmonary pressure (P_{TP}) was obtained by connecting one inlet of a differential pressure transducer (Statham PM5) to a needle inserted through the 5th or 6th intercostal space to measure intrapleural pressure, and the other inlet to a small piece of rubber tubing placed between the endotracheal tube and the pneumotachograph to measure the intratracheal pressure. Output signals representing P_{TP} , $\dot{V}$, and V_T were registered simultaneously on a Grass polygraph model 7. R_L and D_{DYN} were obtained by manual calculations according to the method of Amdur & Mead (1). The animals were challenged with various doses of OA (1–320 $\mu\text{g}/\text{kg}$) injected intravenously through the left jugular vein.

Throughout the entire experiment, a catheter inserted in the right a. carotis registered the blood pressure.

Passive cutaneous anaphylaxis in guinea-pigs

Passive cutaneous anaphylactic reactions were estimated according to the principles given by Watanabe & Ovary (38). Sera from guinea-pigs (250–300 g) sensitized according to procedure A or B (1 or 10 μg OA) were collected and pooled 14 or 50 days after immunization. Each group consisted of seven animals. Each serum pool was examined by passive cutaneous anaphylaxis both before and after heat treatment (56°C; 1 h). 0.1 ml of the serum for examination, appropriately diluted in saline, was injected into the shaved abdominal skin of normal animals. After a latent period of 3 h, 4 days, or 14 days the animals were challenged by an i.v. injection of 2 mg OA together with 10 mg/kg Evans blue. These latent periods are considered to reflect mainly the activity of IgG_{1a} , IgG_{1b} and IgE -like antibodies respectively (27–30). The antibody titre was estimated by the end point dilution technique. Titres given are the highest dilution that showed blueing in four out of six animals.

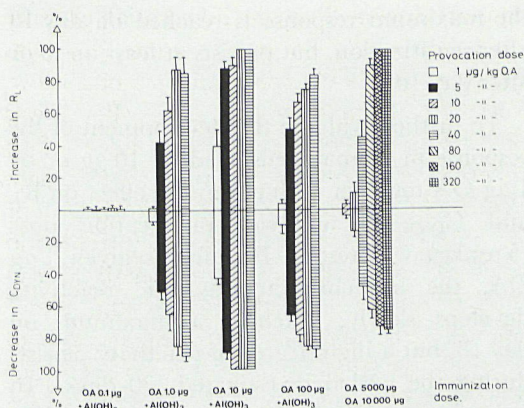


Fig. 1. Effect of ovalbumin challenge on pulmonary resistance and dynamic pulmonary compliance in actively sensitized, anaesthetized guinea-pigs.

Guinea-pigs were sensitized with 0.1, 1.0, 10.0 or 100 µg OA together with 100 mg $\text{Al}(\text{OH})_3$ according to procedure B or with 5,000 µg OA + 10,000 µg OA according to procedure A. The experiments were performed on day 14 after sensitization. Each animal was challenged only once with the indicated provocation dose of OA. The bars indicate the mean $\pm$ S.E.M. ($n = 6-8$).

The sera were stored at -80°C and thawed just before test.

RESULTS

Respiratory measurements in anaesthetized guinea-pigs

Anaesthetized guinea-pigs sensitized to OA showed a markedly fast-developing, dose dependent increase in R_L and decrease in C_{DYN} when challenged with an intravenous injection of OA. In groups where a sufficient number of tests were performed, the individual determinations were found to be normally distributed.

Effect of provocation dose

Animals sensitized by increasing doses of OA (0.1–100 µg) together with $\text{Al}(\text{OH})_3$ 0.1 g were compared 14 days after immunization. The groups sensitized with 0.1 µg did not respond to the provocation doses (1–40 µg/kg OA). The optimum effect was seen in the

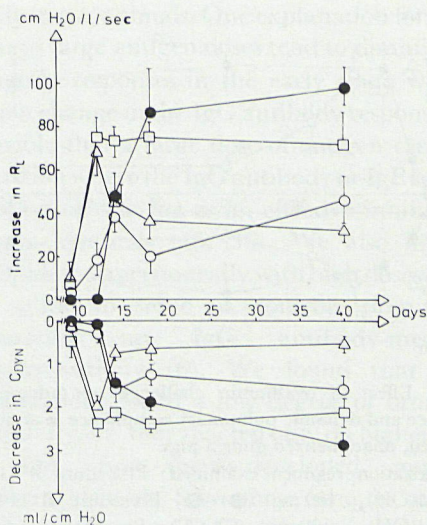


Fig. 2. Effect of ovalbumin challenge on pulmonary resistance and dynamic pulmonary compliance in actively sensitized, anaesthetized guinea-pigs.

Sensitization regimen examined:

- Procedure B (1 µg OA + $\text{Al}(\text{OH})_3$, 100 mg) ●—●.
- Procedure B (10 µg OA + $\text{Al}(\text{OH})_3$, 100 mg) □—□.
- Procedure B (100 µg OA + $\text{Al}(\text{OH})_3$, 100 mg) △—△.
- Procedure A (5,000 µg OA (day 0) + 1,000 µg OA (day 2)) ○—○.

Each point represents the mean $\pm$ S.E.M. ($n = 12-40$) of measurements performed in individual animals at provocation by 40 µg OA/kg.

groups sensitized with 10 µg. The groups sensitized with two high doses of OA (regimen A) and challenged 14 days after sensitization showed less response with respect to R_L and C_{DYN} than the groups sensitized according to procedure B; higher doses of OA (10–320 µg/kg) were needed at the challenge to produce an effect (Fig. 1).

Temporal development of the anaphylactic response

The temporal development of the sensitized state was then examined in four series of experiments at different times (9–40 days) after sensitization. The challenge dose in these series of experiments was 40 µg/kg of OA.

The investigated immunization regimens were procedure A and procedure B, with 1, 10, or 100 µg OA. Fig. 2 shows that the most pronounced response 14 days after immuniza-

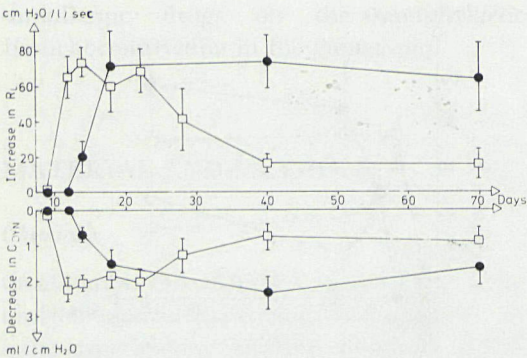


Fig. 3. Effect of ovalbumin challenge on pulmonary resistance and dynamic pulmonary compliance in actively sensitized, anaesthetized guinea-pigs.

Sensitization regimen examined: Procedure B (1 µg OA + Al(OH)₃, 100 mg) ●—●, Procedure B (10 µg OA + Al(OH)₃, 100 mg) □—□. Provocation dose: 5 µg/kg OA i.v. Each point represents the mean ± S.E.M. (n = 8–10) of measurements performed in individual animals.

tion is achieved when the animals are sensitized by 10 or 100 µg OA together with alum. Animals sensitized by 10 µg OA show a persistent effect on R_L and C_{DYN} up to 40 days, whereas the effect on these two parameters decreases up to 40 days in the group sensitized by 100 µg of OA. The development of the sensitized state is delayed in the group of animals sensitized by 1 µg together with alum;

Table 1

Titre of homocytotropic anti-OA-antibodies in sera from animals sensitized according to procedure B (1 or 10 µg OA), or with 5,000 + 10,000 µg OA according to procedure A

Sensitizing regimen	Day	Latent time					
		3 hours		4 days		14 days	
		Before heating	After heating	Before heating	After heating	Before heating	After heating
5,000 µg OA + 10,000 µg OA	14	128	N.D.	8	8	N.R.	N.R.
	50	512	N.D.	256	256	N.R.	N.R.
10 µg OA + Al(OH) ₃ , 100 mg	14	512	N.D.	64	64	16	2
	50	32	N.D.	256	256	4	N.R.
1 µg OA + Al(OH) ₃ , 100 mg	14	128	N.D.	32	32	32	4
	50	32	N.D.	64	64	2	N.R.

The titre given is the highest dilutation of pooled sera taken from seven animals in each group after 14 days or 50 days sensitization which gives a positive PCA-reaction in four out of six animals. The reaction was examined at a latent time of 3 h, 4 days or 14 days. Heating sera was performed at 56°C for 1 h. N.D.; experiments not done; N.R.; no reaction.

the maximum response is reached on day 18 after sensitization, but persists at least up to or after day 40.

To further evaluate the development of the response in animals sensitized by 10 µg or by 1 µg OA together with alum, the effect on R_L and C_{DYN} of a lower provocation dose (5 µg/kg) was tested. In animals given 1 µg OA, the bronchial anaphylactic reactivity develops slowly, reaching a maximum on day 18, but a high degree of reactivity persists during the period investigated (70 days). In animals given 10 µg OA, the response to 5 µg OA/kg reaches a peak at day 12–22 after sensitization; thereafter a decrease in reactivity is seen (Fig. 3).

Passive cutaneous anaphylaxis

Serum samples from animals sensitized to OA according to procedure A or B (1 µg or 10 µg OA) were examined for homocytotropic antibodies by passive cutaneous anaphylactic reactions in the guinea-pigs. The sera were sampled at 14 or 50 days after immunization and pooled as described in the experimental section (for results, see Table 1).

DISCUSSION

The respiratory effects of antigen provocation were examined in animals sensitized to favour IgG and IgE reagenic antibody development. The recording of the bronchial responses, i.e. pulmonary resistance and dynamic pulmonary compliance, was performed according to the principles developed by Amdur & Mead (1). Previous investigators (12, 23, 33, 35) reported changes in R_L and C_{DYN} caused by antigen challenge of sensitized guinea-pigs. We observed an almost instantaneous change in lung mechanics after intravenous challenges of appropriately sensitized animals with a sufficient dose of allergen. These mechanical changes presumably result from pharmacological action of chemical mediators released by cells participating in the immediate type hypersensitivity reaction (9, 10, 13).

Experimentally induced reagenic antibody formation in animals greatly depends on the dose of antigen and the adjuvant (17, 36). The temporal development of the sensitized state and the effect of antigen dose on the antibody production have been extensively investigated in rat (for references, see (17)). The present work used ovalbumin as antigen and injected it i.p. in low doses together with alum. This mode of immunization raises preferentially IgE-like homocytotropic antibodies (36). However, models of reagin-mediated bronchoconstriction in guinea-pigs are not yet amply documented. Hicks & Okpaka (14) examined in some detail the influence of the size of the sensitization dose of antigen in the development and the duration of anaphylactic hypersensitivity. The immunization regimen that these authors used—single intraperitoneal injections of ovalbumin in saline—cannot be considered to promote IgE antibody production. The present investigation achieved the most pronounced and sustained bronchial response to OA challenge with low doses of antigen (1.0 μ g OA) together with alum as an adjuvant. Higher doses of antigen in alum (10 and 100 μ g OA) produced a comparably shorter persistence of the high bronchial reactivity seen

early in our animals. One explanation for this is that as large antigen doses tend to diminish the reagenic responses in the early stage without much change in the IgG antibody response, it is possible that a large dose of antigen creates a situation where the IgG antibody or IgE specific suppressor cells act as an effective inhibitor of reagin synthesis (15, 36). We also injected animals intraperitoneally with high doses of OA in saline to serve as controls presumably possessing an IgG antibody-mediated hypersensitivity (6). We found that these animals developed a low degree of bronchial reactivity, i.e. they reacted only on higher doses of OA, but then developed responses indistinguishable from those seen in animals given low doses of OA in alum.

The changes in bronchial response to antigenic challenge are reflected in the antibody titre as examined by PCA, as far as the IgG_{1a} titre is concerned. The IgG_{1b} titre seems to rise later in the temporal development of the sensitized state. To examine the relative importance of IgG_{1a} and IgG_{1b} in the fast anaphylactic response specific measurement of IgG-antibodies will be used in the future. It is worth noting that in guinea-pigs sensitized with 10 μ g OA together with alum, the bronchial hyperreactivity is decreasing from 14 to 50 days while the IgG_{1b} titre is increasing.

There is as yet no way of assessing IgE-antibodies in guinea-pigs other than with PCA reactions. In the present study, we found moderate titres of heat-sensitive antibodies mediating PCA reactions with 14-day latency periods in pools of sera from guinea-pigs immunized with 1 μ g or 10 μ g OA in alum. A rough qualitative assessment of the effect of heat treatment of the serum shows that treatment at 56°C for 1 h drastically reduces the capacity to mediate reactivity. Thus, according to the criteria of Ovary et al. (28) and Watanabe & Ovary (38), these groups of animals synthesize IgE-antibodies. Sustained production of IgE in mice in response to low doses of antigen has been reported by Levine & Vaz (22), Vaz et al. (37) and Platteau &

Bazin (32). In the present communication, animals sensitized with 1 μ g OA together with alum show a high bronchial sensitivity even 40 to 70 days after sensitization at low provocation dose (5 μ g/kg OA), but the titres of homocytotropic antibodies in sera from these animals have decreased at day 50 compared with those observed 14 days after immunization. In the rat system the correlation is not clear between serum IgE-antibody titre, on one hand, and the bronchial anaphylactic sensitivity, the skin reactivity, or the capacity to release mediators from lung tissue, on the other (8, 18, 39). Jarrett & Stewart (18) suggested that detectable levels of IgE antibodies possibly do not appear in serum until IgE fixing sites in pertinent tissues have been saturated.

In conclusion, this report shows that guinea-pigs sensitized with low amounts (1 or 10 μ g) of ovalbumin together with alum produce the most pronounced bronchospasm when challenged with an intravenous injection of a low dose of antigen. An examination of the antibody classes by PCA technique shows that guinea-pigs sensitized with small amounts of antigen together with alum produce both IgE and IgG₁ antibodies, whereas in sera from animals immunized with larger amounts of antigen, only antibodies of the IgG₁ could be detected. We therefore consider bronchial anaphylaxis in guinea-pigs sensitized with low doses of ovalbumin, 6–7 weeks before the experiment, a suitable model for the evaluation of antiallergic properties of drugs. In this model, it is considered that IgE antibodies play, at least partly, a role in the anaphylactic reaction.

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