

Antigen-Specific Down-Regulation of Bronchial Reactivity in Rats Sensitized Daily without Adjuvant

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Daily sensitization with ovalbumin (OA) and dog serum albumin (DSA) without adjuvant was performed in rats for 2-week periods. When the antigen was administered subcutaneously (s.c.), antibody responses were induced, as assessed in serum and bronchial lavage, and strong increases in transpulmonary pressure (TPP) after intravenous (i.v.) challenge with antigen. Sensitization without adjuvant with antigen as aerosol for similar periods also evoked pronounced antibody formation, although only weak increases of TPP were seen after challenge. Animals sensitized s.c. with OA and simultaneously exposed to OA as aerosol exhibited suppression of the TPP increase after challenge, whereas the antibody responses were not affected to any great extent. In contrast, the increase of TPP after challenge in animals similarly sensitized s.c. with DSA were not suppressed by OA given simultaneously as aerosol or vice versa.

Key words: bronchoconstriction; immunization; rats.

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CLINICAL ASPECTS

Antigen-specific desensitization occurred in rats sensitized s.c. and simultaneously exposed to aerosolized antigen despite a good antibody response. This may represent the normal mechanism to airway exposure to allergen, in contrast to the case in atopic individuals who develop hyperreactivity. The described animal model using sensitization and desensitization without the use of any adjuvants may provide a tool for studies of the mechanisms responsible for the regulation of bronchial reactivity.

Exposure of the airways of both humans and animals to various kinds of antigens evokes responses of immunity and inflammation, resulting in pathophysiological bronchial reactions (1, 3, 9, 16, 18, 20, 23). The reactions are accompanied by formation of antibodies of IgE, IgG, IgA and IgM isotypes as well as by accumulation and activation of lymphocytes, mast cells and goblet cells (2-4, 9, 10, 23). In some individuals, this inflammatory response persists, whereas in most individuals it is transient despite continuous exposure to the antigen (7, 19, 20). The antibodies formed may

bind the antigen (6, 15, 22, 24, 25) and the mucin produced may absorb the antigen-antibody complexes preventing antigen penetration. The declined tissue involvement may also be due to altered interactions between inflammatory cell mediators as well as neuropeptides and their receptors. Thus, to improve treatment of allergic patients, the exact events responsible for this phenomenon must be evaluated.

In the present study we have analysed the immune-specificity of this "desensitization" of the airways in rats immunized without adjuvants by daily aerosol inhalation and/or sub-

cutaneous injection for 2-week periods. The increase in transpulmonary pressure (TPP) after i.v. challenge in subcutaneously (s.c.) immunized rats was abolished by exposure to 1% of aerosolized homologous antigen, whereas lower doses did not cause this effect.

MATERIAL AND METHODS

Animals

Specific pathogen-free hybrid rats of BNxWi/Fu strain of both sexes, 6–10 weeks old, weighing 140–250 g were used (A-LAB AB, Sollentuna, Sweden).

Antigens

Ovalbumin (OA) grade V and Dog Serum Albumin (DSA) fraction V (SIGMA Chemical Co., St. Louis, Mo) were used.

Immunizations

As previously described (3, 4, 23), groups of rats were exposed to antigen Monday through Friday for either a 2-week period or for a 2-week period plus a 1- or 2-week booster period after a 4-week interval. The antigens were administered either by subcutaneous injections in the neck or by aerosol inhalation, or both.

For the s.c. sensitizations, antigen in 0.2 ml sterile saline was injected. Ovalbumin was given at daily doses of 100–300 ng and DSA of 10–100 ng. For the aerosol sensitizations, antigen was dissolved in sterile distilled H₂O to a concentration of 0.0001–1%, and 5 ml was delivered into a nebulization chamber (56.5 × 56.5 × 32 cm) using a Bird micronebulizer. The chamber was first saturated for 5 min with a nebulizer air pressure of 10 psi/in². The immunization then proceeded for 30 min at a nebulizer air pressure of 6 psi/in² and an air flow of 2 l/min through the chamber caused by a pump device, resulting in a chamber air pressure of 2 cm H₂O below atmospheric pressure. Air was then blown through the chamber for 15 min. Bleeding was performed

from the left external angular vein (*vide infra*), and the animals were then killed using ether. The bronchi were washed 3 times by injection of 2 ml Hank's balanced salt solution (HBSS) (Flow Labs. Svenska AB, Solna, Sweden) containing 0.1% EDTA into the surgically exposed trachea (Venflon® i.v. cannula, Viggo AB, Helsingborg, Sweden). Serum and bronchial lavage fluid were stored at –20°C until analysed.

Respiratory measurements

The animals were anaesthetized with Inactin® (Promota, Hamburg, FRG), 125 mg/kg body weight, given as a single intraperitoneal injection and placed on a heating pad connected to a thermostat to keep the body temperature at 37°C. They were tracheostomized with a polyethylene tubing (o.d. 2.4 mm) and polyethylene catheters were inserted into the right common carotid artery and the left external jugular vein for blood pressure recordings and intravenous (i.v.) injections, respectively. The animals were given 0.5 mg/kg i.v. of pancuronium bromide (Pavulon®) for muscle relaxation and were ventilated with a Braun constant volume animal respirator as previously described (1, 23).

The respiratory frequency was set to 76–80 strokes/min with a tidal volume determined by the formula ($v = m \times 6.26 \times 10^{-3} + 0.727$) (v in ml, m in g). This resulted in an arterial pCO₂ of 4.7–5.3 kPa. Ventilation was performed with a positive end-expiratory pressure of 2 cm H₂O to avoid spontaneous atelectasis.

Transpulmonary pressure (TPP) was measured by connecting one outlet of a differential pressure transducer (Statham) PM 131 TC) to a side arm of the tracheal tube (tracheal pressure), and the other to an indwelling teflon catheter (Braunüle®, o.d. 2.0 mm) which was inserted through the 5th or 6th intercostal space (intrapleural pressure). Mean arterial blood pressure (MAP) was measured with an IPM-transducer and both TPP and MAP were continuously recorded on a UV oscillograph recorder (Ultralette, ABEM, Stockholm). Arterial blood samples were collected before and after the airway provocation and after the

i.v. provocation for analysis of blood gases using an automatic blood gas analyser (model IL 1302, Instrumentation Lab., Metric AB, Solna, Sweden). Arterial blood was also drawn before the provocations for later antibody analyses.

The animals were challenged with an antigen aerosol (OA or DSA) of 10 mg/ml in sterile distilled H₂O for 10 min using an Ultrasonic nebulizer model 108 (Gambro-Engström Medical AB, Stockholm) connected to the respirator. This resulted in a total delivery of 4 ml antigen solution to the nebulization chamber. After the TPP and MAP had stabilized after 10–15 min the animals were challenged i.v. with 10 mg/kg antigen in sterile saline, and TPP and MAP were recorded for 5 min before blood gas analysis. From control experiments with animals given one or the other challenge it was evident that the challenges performed in the described way did not influence each other. Thus, increases in TPP showed very similar patterns after aerosol and i.v. challenge. Since i.v. challenge resulted in stronger responses, the results shown are from this type of provocation.

Antibody analyses

The IgE antibody levels were determined with the radioallergosorbent test (RAST) modified for maximum sensitivity according to Hemady et al. (11) as described previously (4). Serum was diluted 1:9 with a phosphate-tween-dextran (PDT) buffer and bronchial lavage fluid 1:1 with HBSS containing 10% fetal calf serum. Duplicate aliquots of 100 µl were incubated with OA- or DSA-conjugated paper discs (8) for 24 h at +18°C with slow shaking. The discs were washed using a DA-300 dispenser-aspirator (Pharmacia AB). Rabbit anti-rat IgE antibodies (MIAB, Uppsala, Sweden) labelled with ¹²⁵I were diluted in PDT buffer to a concentration of 5.65–22.6 ng/ml giving a radioactivity of 35,000–50,000 cpm/200 µl. Aliquots of 200 µl were incubated with the discs as described above. The discs were washed again and the bound radioactivity was determined.

The IgG and IgA antibody levels were determined with the enzyme-linked immu-

nosorbent assay (ELISA) (4), using specific rabbit anti-rat-Ig γ-globulin fractions (Nordic Labs., Tilbury, The Netherlands), conjugated with alkaline phosphatase (Sigma). The enzyme activity reflected by OD₄₀₅ after 100 min enzyme reaction was determined using a Titertek multiscan spectrophotometer (Flow Labs). The serum dilution reciprocal giving an OD₄₀₅ of 0.25 units above that of a non-immune control serum was considered as the antibody titer. The background OD₄₀₅ of negative serum was below 0.10 units throughout the experiments.

RESULTS

Sensitization with the same antigen given subcutaneously and as aerosol

Subcutaneous sensitization with 100 to 300 ng of OA per injection induced a strong clinical reactivity in the airways determined after i.v. challenge (Fig. 1). Similarly, considerable lung reactivity to i.v. challenge with DSA was noted when sensitizing s.c. with 10 to 100 ng of DSA. In all experiments, i.v. challenge resulted in a stronger and more persistent reaction than did the aerosol challenge (results not shown).

Aerosol sensitization with 1% OA induced a small clinical reactivity to challenge by the i.v. route (Fig. 1). At time of challenge, considerable antibody responses of IgG and IgA isotypes and only little of IgM and IgE isotypes were noted in the serum of the animals exposed to antigen for two periods (Fig. 1 showing the IgG and IgE titers). Less antibodies were found after one such sensitization period (not shown). Antibody analysis of the bronchial lavage demonstrated large amounts of IgG and less amounts of IgA and IgE antibodies (Table 1). Sensitization with lower doses than 1% of aerosolized OA resulted in weaker clinical reactivity and lower antibody responses (not shown).

To determine whether the poor clinical airway reactivity after aerosol exposure was due to "desensitization", experiments were performed using combinations of both aerosol and s.c. sensitization with OA. The exposure to 1% OA

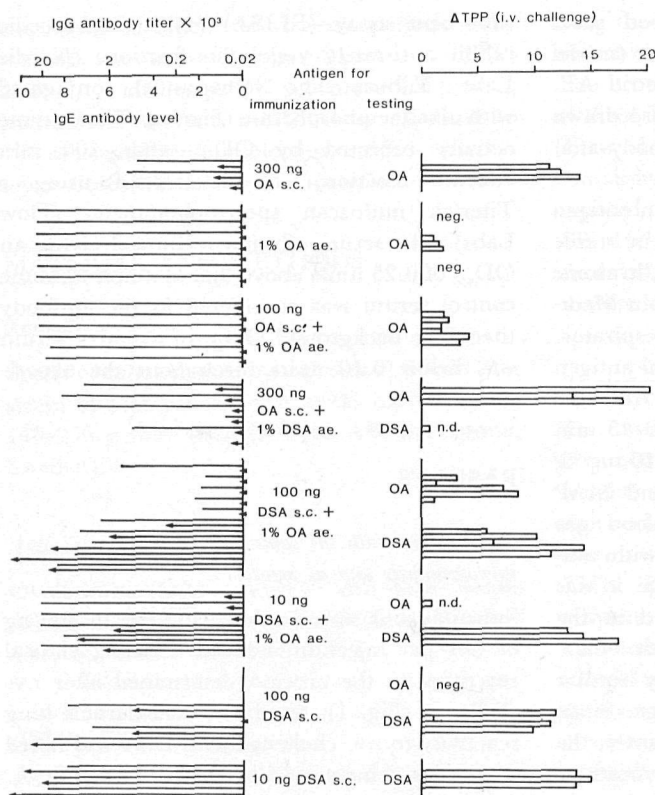


Fig. 1. Responses after s.c. sensitization, by aerosol or in combinations determined as IgG (—) antibodies with ELISA, IgE (←) antibodies with RAST and increase of TPP after i.v. challenge (□). Results of individual rats are given.

aerosol simultaneously with the s.c. injections profoundly decreased the clinical reactivity (Fig. 1). The serum (Fig. 1) and bronchial (Table 1) antibody titers picked up with the two antigens in the ELISA were not suppressed but rather enhanced by such combined antigen treatment.

Subcutaneous and aerosol exposure to different antigens

To determine whether the "desensitization" was antigen-specific, experiments were performed giving different antigens s.c. and as aerosol (Fig. 1). Administration of DSA as 1% aerosol simultaneously with OA s.c. did not alter the clinical reactivity to OA, but the OA IgG antibody responses were enhanced. The administration of OA as aerosol combined with DSA as s.c. injections resulted in slightly higher lung reactivity and lower IgG antibody titers to OA compared with OA aerosol alone. The responses to DSA were similar with and without

simultaneous exposure to OA aerosol, both regarding antibody formation and clinical reactivity (Fig. 1).

Dose-dependency of the desensitization

To determine whether the "desensitization" of the aerosol exposure was dose-dependent, experiments were performed using combinations of various doses of OA aerosols together with OA given s.c. The exposure to OA given as 0.0001 and 0.01% aerosol simultaneously with the s.c. injections did not decrease this reactivity, whereas the 1% aerosol dose did (Fig. 2). The serum (Fig. 2) and bronchial (Table 1) antibody titers were not suppressed but rather enhanced by such combined antigen treatment.

Time-dependency of desensitizing aerosol exposure

Experiments analysing the time-dependency for

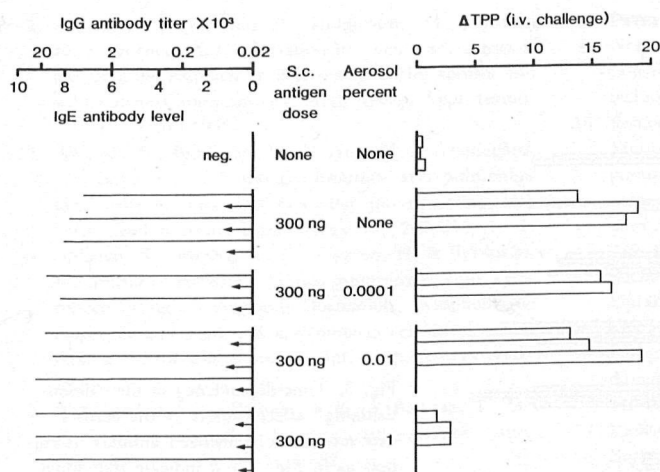


Fig. 2. Dose-response of the "desensitizing" effect caused by the aerosol on the increase of TPP after i.v. challenge and effects on antibody responses in s.c. sensitized rats. (Symbols as in Fig. 1 and indicate individual rats).

Table 1

Antibody titers of IgG and IgA isotypes determined with ELISA and levels of IgE antibodies determined with RAST in bronchial lavage

Immunization Rat No.	IgE	IgG	IgA
300 ng OA s.c.			
1	0.62	< 2	n.d.
2	0.59	< 2	n.d.
3	0.45	< 2	< 2
4	0.62	< 2	< 2
300 ng OA s.c. + 0.01 % OA aerosol			
1	0.64	34	< 2
2	0.58	11	< 2
3	0.69	57	< 2
4	0.76	45	4
5	0.71	44	4
300 ng OA s.c. + 1 % OA aerosol			
1	1.14*	302	7
2	0.69	383	10
3	0.78	410	10
4	0.69	338	8
5	0.76	416	8
1 % OA aerosol			
1	0.77	745	5
2	0.60	221	3
3	0.78	555	2
4	0.78	407	4
Control	0.54	< 2	< 2

* Significantly elevated level

the desensitization by aerosol exposure were performed. Exposing the animals to one period of aerosol between the periods for s.c. sensitization did not desensitize the animals. Only the exposure for two periods to aerosolized OA antigen simultaneously (Figs. 1, 2) and 1 week before the s.c. sensitization (Fig. 3) had this desensitizing effect.

DISCUSSION

The present study revealed a clear-cut "desensitizing" effect by aerosolized antigen on the bronchial reactivity to aerosol (not shown) and i.v. challenge, which developed with s.c. sensitization. Since the patterns of reactivity were very similar to the two types of challenge, although the response to aerosol challenge was much weaker than to the i.v. challenge, the results can be regarded as representative for an asthmatic reaction. The "desensitization" was antigen-specific. Thus, OA aerosol did not decrease the clinical reactivity to DSA after s.c. immunization with DSA, and vice versa. Some increases of clinical reactivity and IgG antibody levels indicated a cross-reactivity between the antigens when DSA was given s.c. simultaneously with OA as aerosol. Such treatment also somewhat suppressed the IgE antibody levels. This cross-reactivity was unexpected and could not be verified employing immunoprecipitation (unpublished).

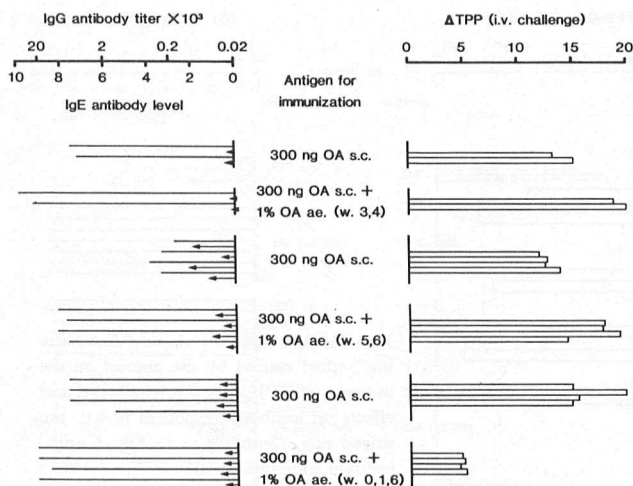


Fig. 3. Time-dependency of the "desensitizing" effect caused by the aerosol exposure of s.c. sensitized animals. (Symbols as in Fig. 1 and indicate individual rats).

Our results, indicating a "desensitization" of the bronchial reactivity without any profound accompanying suppression of the antibody formation, are in agreement with those of Schuyler & Schmitt (21) and Blanchard et al. (5).

In the present study, large amounts of serum IgG antibodies and bronchial lavage IgA and IgG antibodies were formed after aerosol immunization. This is in agreement with our earlier findings (1-4) and with those of Richardson et al. (17). Such antibodies, formed after the simultaneous exposure to 1% aerosol with the homologous antigen, may block the clinical lung reactivity (6, 15, 22, 24, 25). This would be supported by the findings of a number of investigators. Thus, in guinea pigs, Richardson et al. (17) showed a superficial distribution of the antigen on the epithelium and endothelium in the airways of the animals. Accordingly, Wallace et al. (25) showed a minimum penetration of radiolabelled antigen into the blood stream in the presence of high IgG antibody levels, and Braley et al. (6) demonstrated by perfusion experiments that immunization of rabbits decreased the penetration of antigen through the airway epithelium. However, from our antibody titer and clinical reactivity patterns it was evident that alternative mechanisms also have to be searched for.

A specific immune suppression of the cell-mediated immune functions mediating alveolitis

could not be ruled out in the present study. Some other investigators have very clearly demonstrated that lymphoblastic proliferation and generation of migration inhibitory factor is not suppressed in aerosol exposed animals (21), although others have shown suppressed lymphoblastic responses to antigen and mitogen and of plasminogen activator as an acute phase reactant (7).

The suppressive effect by aerosol exposure on the IgE-antibody formation as demonstrated by others (13, 14) could only be verified in the present study when aerosol was given together with s.c. injections. Aerosol alone induced IgE antibodies as was shown in our previous investigations (1-4) and recently by Henderson et al. (12).

In conclusion, the present study reveals that antigen given as 1% aerosol, but not 0.01% or lower, causes a suppression of the clinical reactivity to challenge by aerosol and i.v. administered antigen in s.c. sensitized animals. This suppression is antigen-specific. The humoral immune system does not seem to be a major factor for this desensitization process.

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