ALLERGY INDUCED ASTHMA WITH ASCARIS SUUM ADMINISTRATION TO DOGS

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Abstract—Bronchial asthma such as is seen in humans was successfully produced in male mongrel dogs sensitive to Ascaris suum worms. Seventy to eighty percent of all dogs showed a positive skin reaction to Ascaris antigen. In dogs with high skin reactivity, an inhalation of Ascaris antigen produced increases in airway resistance and respiratory rate. Ascaris extract given intravenously caused systemic anaphylactic shock in the form of prolonged hypotension and dyspnea. Toxocara canis showed the same responses as seen with Ascaris, and a cross reaction between these antigens was suggested. Hypersecretion of respiratory tract fluids was also observed with inhalation of Ascaris. On the other hand, Ascaris extract had no effect in guinea pigs and rats, either in vivo or in vitro. The pulmonary and systemic responses produced by Ascaris extract are, therefore, considered to be the result of antigen-antibody reactions, and not pharmacological effects of Ascaris extract. The increase in airway resistance produced by the antigen in dogs was significantly inhibited by disodium cromoglycate, atropine or hexamethonium, and reduced by chlorpheniramine, suggesting that mast cells and cholinergic pathways are both significantly involved in the responses. The Ascaris-induced bronchial asthma resembled the asthma which occurs in humans, both symptomatically and pathophysiologically, and we propose that the model may be feasible for studies of asthma related to allergies.

Recent increases in bronchial asthma require that we extend our knowledge on the pathophysiology of this bronchial disorder. The mechanism of asthmatic attacks is, however, poorly understood as there is no suitable laboratory model for producing asthma. The guinea pig has hitherto been used for the study of allergic asthma, however, experimental asthma in guinea pigs is considered to be different from that in humans, both immunologically (1-3) and pathophysiologically (4-8). In addition, there is the limitation for measuring parameters responsible for asthma as these animals are small. Patterson (9) reported the naturally occurring pollen sensitivity in dogs, and Booth, et al. (10) who found that dogs inhaling Ascaris suum extract developed dyspnea suggested that the spontaneously sensitized dog to Ascaris would be a promising model for bronchial asthma.

We induced asthma in dogs with Ascaris suum, and attempted to determine whether the Ascaris-induced asthma was an allergy related reaction, was mediated by mast cells, accompanied by an increase in airway secretion and conclusively resembled asthma such as is seen in humans.
MATERIALS AND METHODS

Experimental animals

Male mongrel dogs weighing 7–16 kg, male guinea pigs and Wistar rats weighing 250–400 g were used. Dogs were anesthetized with sodium pentobarbital 30 mg/kg i.v. and the other animals with 30–45 mg/kg i.p. of the same drug.

Antigens

Ascaris suum worms were obtained from freshly killed swine in a slaughter house and Toxocara canis worms were obtained from puppies. Ascaris antigen (Asc-Ag) and Toxocara antigen (To-Ag) were prepared with veronal buffered saline (VBS) according to routine procedures as shown in Fig. 1. The undiluted solution of prepared Asc- or Tox-Ag contained 6 mg protein/ml.

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\begin{array}{c}
\text{Ascaris suum worm} \\
\text{Desiccation in frozen state} \\
\text{Defatting with ether} \\
\text{Centrifugation (10000 rpm 30 min 4°C)} \\
\text{Supernate} & \text{Precipitate} \\
\text{Extraction with VBS (24-48 hr 4°C)} \\
\text{Centrifugation (10000 rpm 30 min 4°C)} \\
\text{Supernate (Asc-Ag)} & \text{Precipitate} \\
\text{VBS : Veronal buffered saline}
\end{array}
\]

Fig. 1. Preparation of the Ascaris suum antigen.

Skin reactivity test

Antigen extract solutions diluted with VBS (3×10^{-3} and 10^{-3} to 10^{-7} g protein/ml) were used for skin tests. Anesthetized dogs and guinea pigs were given 0.5% Evans Blue dye i.v. in volumes of 0.2 ml/kg and 0.5 ml/body respectively 15 min before skin testing. Skin tests were done by giving an intracutaneous injection of 0.1 ml of each dilution of antigen solution. Thirty min after injections of the antigens, blueing diameters at the injected sites were measured, and skin reactivity of the animals was estimated.

Administration and evaluation of antigens

In anesthetized dogs, antigens were inhaled for 10 min via a branch of inserted tracheal cannula using an ultrasonic nebulizer (TUR-3000, Nihon Kohden, Co., Ltd.) or injected i.v. into the cephalic vein. Airway resistance (R_{aw}) was measured every 5 min for 70 min after administration of the aerosol of antigen with a respiratory resistance meter (MRP-6, Nihon Kohden, Co., Ltd.) connected with an endotracheal tube. Respiratory movement was recorded with a respiratory pick-up (MCR-2TA, Nihon Kohden, Co., Ltd.) applied
around the thorax of animals. Femoral blood pressure and heart rate were also recorded using a pressure transducer and a tachometer. Sixty min after the end of Ag inhalation, the volume of airway secretions accumulated in the trachea between the cut end for inserting the endotracheal cannulae and the bifurcatio tracheae was measured with a syringe of 0.25 or 0.5 cc.

In anesthetized guinea pigs and rats, antigen was injected i.v. into the femoral vein. Blood pressure, heart rate and respiratory movement were recorded.

The guinea pig isolated trachea, ileum and atria were also used. Guinea pigs were sacrificed by a blow on the head and exsanguinated. The trachea, ileum and heart were excised. A tracheal ring of 4 mm in width was cut at the cartilage so that two pieces of cartilage remained attached to both ends of the muscle strip. The muscle was suspended in an organ bath containing Tyrode solution at 37°C gassed with a mixture of 95% O₂-5% CO₂. The ileum, approx. 2 to 3 cm in length, was suspended in an organ bath of 32°C containing Tyrode solution and bubbled with air. The atria were dissected from the heart and suspended in an organ bath containing Krebs-Henseleit solution at 37°C oxygenated with a mixture of 95% O₂-5% CO₂. Mechanical responses were isometrically recorded on a polygraph.

**Drugs**

Acetylcholine chloride (Daiichi Seiyaku), disodium cromoglycate (Fujisawa), chlorpheniramine maleate (Sankyo), atropine sulfate (Tokyo Kasei) and hexamethonium bromide (Yamanouchi) were used. All doses were expressed as salts.

**RESULTS**

**Effects of Ascaris- and Toxocara-antigens in dogs**

Asc-Ag was inhaled for 10 min in a total dose of 3 mg protein, and injected for 20 sec in a dose of 1 mg protein/kg i.v. Typical recordings of the effect of Asc-Ag are shown in Fig. 2. An Asc-Ag aerosol caused remarkable increases in Rₐᵥ and respiratory rate (R.R.) as well as a slight fall in blood pressure and bradycardia. An intravenous injection evoked

![Fig. 2. Effects of aerosol and i.v. administrations of Ascaris suum antigen (Asc-Ag) in anesthetized dogs. B.P.: femoral arterial blood pressure, H.R.: heart rate, Rₐᵥ: airway resistance, and Resp.: respiratory movement. Administration of Asc-Ag, 3 mg protein aerosol for 10 min, or, 1 mg protein/kg i.v., was carried out.](image-url)
responses similar to systemic anaphylactic shock, such as a remarkable and persistent fall in blood pressure, bradycardia, arrhythmia and tachypnea after 0.5–5 min lag following administration. The durations of responses were at least 1 hr with either route of administration. Similar responses were observed with Tox-Ag aerosol (3 mg protein) and an i.v. injection (1 mg protein/kg) (Fig. 3). When the blood pressure had returned to the initial level following Tox-Ag injection (1 mg protein/kg i.v.), the effects of subsequent Asc-Ag challenge (1 mg protein/kg i.v.) were no longer produced (Fig. 3). In addition, there were no effects of subsequent Tox-Ag challenge following initial Asc-Ag injection. The second challenge of Asc-Ag following the first Asc-Ag also produced no response. Administrations of VBS by either route had no effect on any parameter.

Fig. 4 shows the changes in $R_{aw}$ and R.R. after Asc-Ag inhalation in nine dogs. $R_{aw}$ values increased significantly viz., 3.5, 1.8 and 1.4 times as high in 10, 40 and 70 min after inhalation as before. R.R. values changed similarly to $R_{aw}$.

Ratio of duration of expiration to inspiration (E/I ratio) following an Asc-Ag inhalation was about 1.5 fold as high as the pre-inhalation level.

![Figure 3](image1.png)

**FIG. 3.** Effects of aerosol and i.v. administrations of Toxocara canis antigen (Tox-Ag) in anesthetized dogs. B.P.: femoral arterial blood pressure, H.R.: heart rate, $R_{aw}$: airway resistance, and Resp.: respiratory movement. Administration of Tox-Ag, 3 mg protein aerosol for 10 min, or 1 mg protein/kg i.v. was carried out. Asc-Ag challenge following Tox-Ag produced no effect.

![Figure 4](image2.png)

**FIG. 4.** Effects of Ascaris suum antigen (Asc-Ag) on airway resistance ($R_{aw}$) and respiratory rate (R.R.) in dogs. Asc-Ag was inhaled for 10 min in a total dose of 3 mg protein. Each point represents the mean of nine dogs for Asc-Ag, and of five dogs for veronal buffered saline (VBS) control. Vertical bars indicate the S.E. of means.
Relationship between airway and skin reactivities

In the skin reactivity test performed on 14 dogs, the animals were classified into two groups, namely, one group (10 of these 14 animals) showed significant blueings at a low concentration of $10^{-2}$ g protein/ml (the positive skin reaction group and the other group (4 of these animals) showed blueings at high concentrations over $10^{-4}$ g protein/ml (Fig. 5). When the total effect on airway resistance ($J_{Raw}$) by an Asc-Ag aerosol was expressed as the area delineated by the graph and over the pre-injection control value, only the former group increased $J_{Raw}$ (29±6) while the latter did not (3±1). This result demonstrated that animals showing a positive skin reaction to antigen solution below concentrations of $10^{-5}$ g protein/ml were naturally sensitive to Asc-Ag. Natural sensitization occurred in 70–80% of all dogs used herein. The blueing responses to over $10^{-4}$ g protein/ml were attributed to a nonspecific irritant effect of Ascaris suum extracts.

Effect of Ascaris antigen on airway secretion

Aerosols of Asc-Ag caused a remarkable increase in highly viscous airway secretion (Fig. 6). At 60 min after the end of antigen inhalation, the mean accumulated secretion volume was 0.42±0.09 ml, while that after VBS buffer inhalation was 0.15±0.04 ml. The increase by the antigen was significant at $P<0.05$.

Effect of Ascaris-antigen in guinea pigs and rats

Guinea pigs showed negative skin reaction to Asc-Ag. Antigen solutions over $10^{-4}$ g
protein/ml, however, evoked blueings such as were seen in dogs and ascribed to a nonspecific irritant effect of the extracts. In anesthetized guinea pigs or rats, an i.v. administration of 3 mg protein/kg of Asc-Ag had no influence on blood pressure, heart rate (H.R.) and respiratory movement (Resp.) (Fig. 7). In the guinea pig isolated trachea, ileum or atria, concentrations up to $6 \times 10^{-1} \text{protein/ml}$ had no significant effect as shown in Fig. 7.

**Effects of some drugs on responses to Ascaris antigen**

In Fig. 8 the effect of disodium cromoglycate on the $R_{aw}$ response to Asc-Ag in dogs is shown. Disodium cromoglycate was inhaled for 20 min from 10 min before starting antigen inhalation to the end of the inhalation. The increase in $R_{aw}$ produced by Asc-Ag was inhibited by 50 (5 dogs) or 100 mg (5 dogs) of the drug. The depression of the total effect on airway resistance by 100 mg was significant at $P<0.05$ (Fig. 9). Pretreatment of...
atropine 2 mg/kg i.v. or hexamethonium 5 mg/kg i.v. 5 min before Asc-Ag aerosol also significantly inhibited the increase in $J_{R_{aw}}$ produced by the antigen. Chlorpheniramine 2 mg/kg i.v. reduced the $R_{aw}$ response (Fig. 9).

**DISCUSSION**

Guinea pigs have been used for the study of allergy induced asthma. There are, however, differences between asthmatic pathological states in guinea pigs and those in man. The antibody responsible for an allergy related asthma in guinea pigs actively or passively sensitized with agents such as bovine serum albumin and ovalbumin is found to be IgG (1). In contrast, that in the human is IgE (2, 3). The asthma in guinea pigs is effectively protected by antihistamines such as diphenhydramine and chlorpheniramine (11, 12), but in humans antihistamines have almost no effect (4). Disodium cromoglycate (7) and adrenocortical steroids (8) are ineffective against bronchoconstriction in the guinea pig. Furthermore, it is relatively difficult to explore the mechanisms of asthma with guinea pigs, since there is a limitation for measuring parameters related to asthma. Dogs have been spontaneously sensitized by ragweed (9), but the relative unavailability of dogs with this sensitivity prevents widespread investigations. Booth, et al. (10) found that Ascaris suum extracts produce a respiratory anaphylactic reaction in dogs, and suggested that the reaction is a reaginic immediate-type hypersensitivity as determined by the homologous passive cutaneous anaphylaxis test.

We also observed herein that 70-80% of all our mongrel dogs had positive skin reactions to Ascaris antigen. From cross reaction experiments with Ascaris suum and Toxocara canis extracts, this reactivity to Ascaris was attributed to a cross reaction with Toxocara canis by which most of dogs were infested parasitically in their childhood. Namely both antigens probably have a common antigenic determinant. Such evidence indicates that
Ascaris suum which is quite procurable can be used to induce asthmatic attacks in dogs instead of the Toxocara canis.

In dogs with negative skin tests or in guinea pigs and rats which appear not to have antibodies to Ascaris, Ascaris extract caused no effect. Moreover, in the guinea pig isolated preparations, relatively high concentrations of Ascaris extract solution produced almost no effect. Therefore, the pulmonary and systemic responses produced by Ascaris may be concluded to result from antigen-antibody reactions, and not from any other pharmacological effects of Ascaris extract.

The secretory activity of the respiratory tract has not yet been documented in experimental asthma in laboratory animals. In the present study, we determined the airway secretory activity by measuring secretory volume accumulated in the trachea in dogs. The increased airway secretion which had a high viscosity was observed by the challenge with Ascaris antigen, indicating that in this experimental asthma model, hypersecretion of tracheobronchial fluids as well as bronchoconstriction shown by an increase in airway resistance occurred, such as is the case in asthma in humans, therefore, this model may be useful for investigating mechanisms of airway secretion in asthma and screening newly developed expectorants.

Gold, et al. (13) found that the increase in airflow resistance by Ascaris extract in dogs was blocked by atropine or bilateral cervical vagotomy. We also confirmed the critical importance of the parasympathetic nervous system in antigen-induced bronchoconstriction in sensitized dogs, by the fact that pretreatment of atropine or hexamethonium markedly inhibited the increase in airway resistance produced by Asc-Ag.

Furthermore, the critical participation of mast cells in the asthmatic attack of the dog is suggested from the finding that disodium cromoglycate, a mast cell stabilizer, effectively inhibited the responses to Ase-Ag. Histamine is considered to be one of the main chemical mediators released from the mast cells. Antigen-induced bronchoconstriction was reduced by chlorpheniramine, suggesting that H1-receptor, a histamine receptor, is, at least, in a part associated with the responses to Asc-Ag.

Recently, Reed (15) proposed a working hypothesis on the mechanisms involved in the human asthmatic attacks. In his theory, the participation of mast cells and the cholinergic pathway are given attention. Both mechanisms were also found to be involved in the canine Ascaris system in the present work.

The Ascaris-induced asthma in dogs resembles asthma in humans both pathologically and symptomatically. Moreover, Ascaris antigen is readily available and since most dogs appear to be sensitive to Ascaris, the Ascaris-induced canine asthma can be feasibly carried out. This canine-Ascaris system is considered to be a promising model not only to clarify the pathophysiology involved in allergy-induced asthma but also to develop new therapeutic drugs for asthma.

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