

ANTI IgE-INDUCED ASTHMA IN GUINEA PIGS; A NEW MODEL FOR ASTHMA
Physiological and Pathological Studies

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We established an experimental animal model for IgE-mediated asthma by the use of intravenous injection of rabbit anti guinea pig IgE in normal guinea pigs. The degree of asthmatic airway obstruction was quantitatively estimated by Mead's apparatus which enabled us to use unanesthetized guinea pigs. With the injection of anti IgE, only an early asthmatic response was observed. Inhalation of anti IgE did not trigger asthma.

Histo-pathological changes in the lung obtained immediately after the injection of anti IgE resembled those reported in human asthma. This system may be used for pathological and pharmacological studies of the IgE-dependent early asthmatic response.

Key words: anti guinea pig IgE — experimental asthma — pathology of asthma

INTRODUCTION

Guinea pigs have been widely used in experimental asthma, because their bronchial smooth muscles contract easily on nonspecific or specific stimulation. For instance, the animals have lower threshold for inhaled histamine than other animals, and sensitized guinea pigs show asthmatic symptoms to antigen challenge¹⁾.

In humans, IgE antibodies are thought to play a leading role in immediate allergic reactions²⁾, although short-term sensitizing antibodies belonging to another class of immunoglobulin may contribute³⁾. Guinea pigs have two types of tissue sensitizing antibodies: IgE- and IgG1 antibodies⁴⁾⁻⁸⁾. The former is long-term sensitizing and heat labile, and the latter is short-term sensitizing and heat stable. For studies of allergic reactions due to only IgE antibodies, guinea pigs

passively sensitized with an isologous serum containing IgE antibodies are usually challenged 8 days later to avoid confusion with reactions due to IgG1 antibodies⁸⁾.

As previously reported⁹⁾, intravenous injection of an appropriate amount of rabbit anti guinea pig IgE (a-GPIgE) caused immediate asthmatic response, whereas intravenous injection of anti-IgG1 or anti-IgG2 did not cause such a response⁹⁾. The narrowing of the airway was not judged quantitatively but by symptoms of airway obstruction. Highly diluted anti IgE still caused immediate skin reactions in guinea pigs⁹⁾. Recently, a very potent a-GPIgE became available for further investigation¹⁰⁾.

The purpose of this study is to examine quantitatively the degree of asthmatic response in unanesthetized guinea pigs and to examine the pathological changes in the lungs of animals injected with this a-GPIgE.

Received: January 31, 1990

Accepted: May 14, 1990

Abbreviations: a-GP IgG1 rabbit anti guinea pig IgG1; a-GP IgG2 rabbit anti guinea pig IgG2; a-GP IgE rabbit anti guinea pig IgE; DSCG disodium cromoglycate; Rfp respiratory flow pattern; Rrs respiratory resistance

MATERIALS AND METHOD

Test animals (Guinea pigs)

Hartley-strain guinea pigs were used throughout this study.

Preparation of rabbit a-GPIgE

The detailed procedure for preparing a-GPIgE has been described previously¹⁰. Briefly, 5 guinea pigs (600 g) were orally infected with 1,000 larvae of *Trichinella spiralis*. One month after infection, the animals were sacrificed. Their sera were pooled and fractionated to obtain an IgE-rich fraction. This fraction containing 5 mg protein was mixed with complete Freund's adjuvant and was injected twice with a two week interval between the injections into each of two rabbits. The rabbits were bled two weeks after the last injection and the sera were pooled. The rabbit serum was absorbed with an equal volume of normal guinea pig plasma. The absorbed serum was monospecific to guinea pig IgE when examined by immunodiffusion and radioimmunoassay using guinea pig serum containing IgE antibodies to ovalbumin¹⁰. The antiserum contained an excess of IgG1, IgG2 and IgM, as shown when tested with antisera specific to these immunoglobulins in double diffusion. IgE antibodies determined by ELISA using this antiserum correlated well with the titers of passive cutaneous anaphylaxis (PCA)¹¹. A gamma-globulin fraction was obtained from the absorbed antiserum by 33% ammonium saturation, dialyzed against pH 7.4 phosphate buffered saline and passed through a milipore filter (0.22 μ m, Milipore Corporation, Bedford, MA). The protein concentration of the fraction was adjusted to 10 mg/ml. This gamma-globulin was used as a-GPIgE in our animal experiments.

Rabbit anti guinea pig IgG1 (a-GPIgG1) and anti guinea pig IgG2 (a-GPIgG2)

a-GPIgG1 and a-GPIgG2 were purchased from Miles Laboratories Inc. Naperville, Ill., U.S.A. A gamma-globulin fraction containing 10 mg/ml protein was obtained from each antiserum by a procedure similar to that described above. The fraction was used as a-GPIgG1 or a-GPIgG2 in the experiments.

Measurement of airway responsiveness to intravenous injection or inhalation of anti-immunoglobulins

Normal guinea pigs weighing approximately 400 g were used in the experiments. Pulmonary function of the guinea pigs was estimated according to

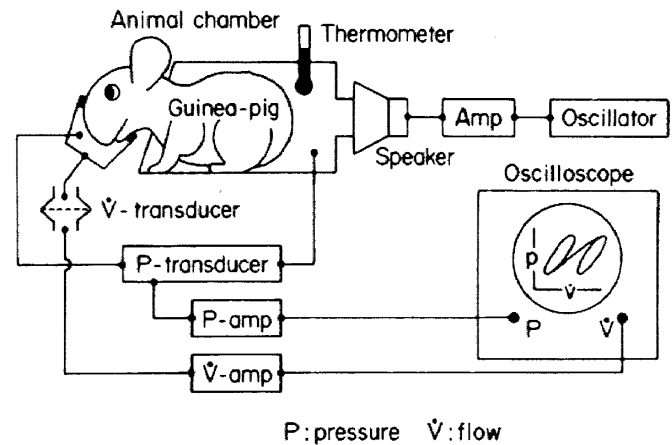


Fig. 1. Apparatus used to measure respiratory resistance. The respiratory flow pattern was also recorded with this apparatus. P: pressure. $\dot{V}$: flow. Amp: amplifier.

Mead's method¹² with some modifications¹³. A diagram of the apparatus is shown in Fig. 1. The detailed description of the apparatus was given in our previous paper¹³. The guinea pig was placed in a small box, and the head protruded out of box through a hole in the center of the lid to allow breathing. A soft rubber board with a hole, a little smaller than the animal's neck, was attached to the front of the lid to form an airtight seal around the neck. The chamber was supplied with an oscillating pressure of over 10 cmH₂O with a 6–20 Hz sine wave through a loud speaker attached behind the animal. The air flow of the guinea pig was measured via a plastic cone mask fitted to the animal's face. At the top of the mask cone, there was a small opening of 8 mm diameter containing a 400 mesh wire screen which provided a small but constant resistance to air flow.

The flow through the screen due to breathing of the guinea pig was measured with a pressure transducer as the pressure difference between the interior of the mask and the room. The pressure signal from the chamber and the flow from the mask were recorded by a differential pressure transducer (Nihon Kagaku Kogyo, Ltd., Tokyo, Japan, Model TP 602 T). After amplification by a carrier amplifier, the signal was transferred to an oscilloscope. The respiratory resistance (Rrs) was obtained from oscilloscope vectors of flow and pressure. The respiratory flow pattern (Rfp) was recorded with a polygraph.

The procedure for measuring the airway respon-

siveness to injection or inhalation of anti-immunoglobulin was as follows. The animal was placed in the apparatus, Rrs was measured, and the Rfp was recorded. Then, the animal was given anti-immunoglobulins by intravenous injection or by inhalation. Thereafter, the time course of Rrs and Rfp was examined. Inhalation of anti-immunoglobulin was achieved with a jet nebulizer and a single phase induction motor (Hitachi Co. Ltd., Tokyo, Japan) at a flow rate of 100 ml/sec.

Our criteria of asthma in guinea pigs were as follows. 1) Rrs reached twice the level before injection or inhalation, or 2) the expiratory phase in Rfp was prolonged; duration time of the expiratory phase (E)/duration time of the inspiratory phase (I) was more than 1.5.

Histological examination

Animals which died from the immediate asthmatic response after the injection of a-GPIgE were immediately dissected, and the lungs were frozen with liquid nitrogen. Animals which showed asthmatic symptoms but survived were sacrificed immediately or 6 hours after the injection, and the lungs were frozen as above mentioned. Animals injected with a-GPIgG1 or a-GPIgG2 were sacrificed immediately or 6 hours after the

injection.

Animal care and management

We followed "Standards Relating to the Care and Management of Experimental Animals" (Notification No. 6, March 27, 1980 of the Prime Minister's Office, Tokyo, Japan) for the care and use of the animals.

RESULTS

Intravenous injection of anti-immunoglobulins

1) a-GPIgE

0.02, 0.05, 0.1, or 0.2 ml a-GPIgE was injected in each group of 5 normal guinea pigs. a-GPIgE was mixed with saline and the final volume injected was 0.4 ml. All animals which got 0.2 ml died within a few minutes. Three of 5 animals injected with 0.1 ml died, 2 showed retardation of the expiratory phase in Rfp and also more than a doubling of Rrs. At the same time, wheezing was audible like in asthmatic patients. The attack disappeared within 30 minutes. All animals injected with 0.05 ml showed similar Rfp and elevation of Rrs and improved similarly. Three of 5 animals injected with 0.02 ml showed an as-

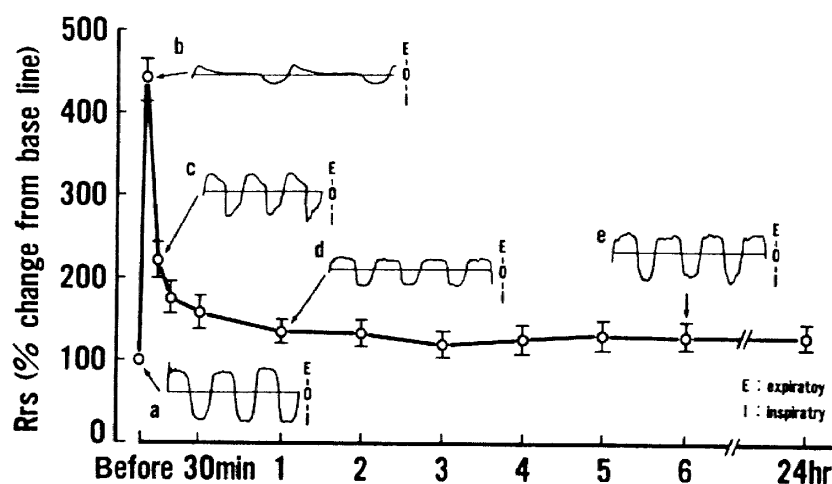


Fig. 2. Changes in respiratory resistance (mean $\pm$ SE) in 5 guinea pigs after anti-IgE injection. A typical respiratory flow pattern was shown in a-e.

(a) respiratory pattern before the injection of anti IgE

(b) 3 minutes after the injection

(c) 10 minutes after the injection

(d) 1 hour after the injection

(e) 6 hours after the injection

E: expiratory phase. I: inspiratory phase. O: base line.

Table 1 Relation between volume of a-GPIgE intravenously injected a-GPIgE and appearance of asthma

Volume ml of a-GPIgE injected iv.	0.02*	0.05*	0.1*	0.2*
Number of GPs injected	5	5	5	5
Number of GPs getting asthma	3	5	5	5
Number of GPs dying due to asthma	0	0	3	5

*Saline added to a volume of 0.4 ml.

The optimal volume for non-lethal asthma was around 0.05 ml.

thmatic response. Control guinea pigs injected with 0.5 ml of normal rabbit serum showed no asthmatic response. These results are summarized in Table 1. Fig. 2 shows the time course of Rrs and Rfp seen in 5 animals injected with 0.05 ml of a-GPIgE. The typical Rfp of asthma is the appearance of a sharp and small peak in the expiratory phase and retardation of the expiratory phase. The 5 animals which survived injection of 0.05 ml a-GPIgE were followed, and their Rrs and Rfp were recorded 1, 2, 3, 4, 5, 6 and 24 hours after the injection. No changes were observed in Rfp or

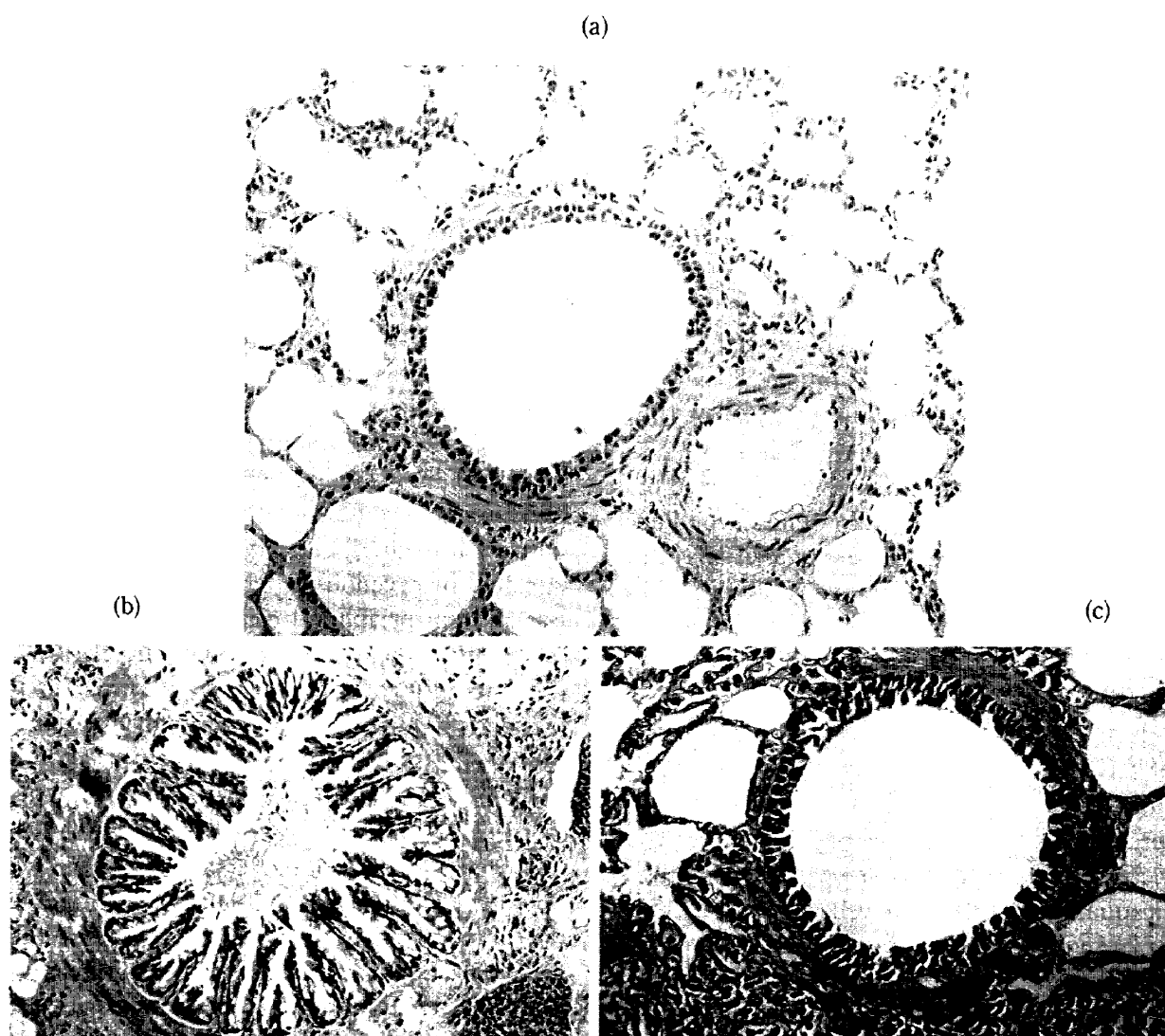


Fig. 3. Histologic observation of guinea pig bronchi.

(a) Normal guinea pig bronchi.

(b) Immediately after the injection of a-GPIgE: Narrowing of the bronchial lumen, increased plication of the epithelial lining cells into the bronchial lumen due to contraction of the bronchial smooth muscle, desquamated bronchial epithelial cells and edema in the peribronchial space are observed. The bronchial lumen is filled with mucous plugs.

(c) 6 hours after the injection of a-GPIgE: There was no marked change compared with the normal guinea pig lung.

in Rrs after that acute attack had subsided.

After the intravenous injection of anti IgE, almost all guinea pigs showed scratching of noses or/and sneezing before the starting of asthma or anaphylatic shock.

2) *a-GPIgG1*

a-GPIgG1 was injected similarly to GPIgE. However, no change was observed in Rfp or Rrs in any of the animals. Therefore, 0.5 or 1.0 ml of a-GPIgG1 was injected in another group of animals, but again no asthma was observed.

3) *a-GPIgG2*

a-GPIgG2 was injected similarly to a-GPIgG1. No change in respiratory mechanics was observed in any of the animals injected.

Inhalation of anti-immunoglobulins

0.05, 0.2 or 1.0 ml a-GPIgE was given by inhalation to one of 3 groups. Each group contained 3 animals. The a-GPIgE was mixed with added saline to a final inhaled volume of 1.0 ml. Three guinea pigs which inhaled 1.0 ml of normal rabbit serum were used as controls. An inhalation period took approximately 4 minutes for 1.0 ml of the antiserum. All animals were followed as above. No change in respiratory mechanics was observed in any of the animals within 24 hours. Similar experiments were carried out using a-GPIgG1 or a-GPIgG2, but again no asthma was observed.

Pulmonary histopathology

1) *a-GPIgE injection*

The lung obtained immediately after the injection of a-GPIgE showed narrowing of the bronchial lumen and increased plication of the epithelium protruding into the bronchial lumen due to contraction of the bronchial smooth muscle (Fig. 3b). Desquamated bronchial cells and edema in the peribronchial space were also observed. The bronchial lumen was filled with mucous plugs. For comparison, the bronchial tissue from a normal guinea pig is shown (Fig. 3a).

Six hours after the injection of a-GPIgE, there was no marked change compared to the normal lung (Fig. 3c). Cellular infiltration was not also observed in the tissue of injected animals.

2) *a-GPIgG1 injection*

Immediately after the injection of a-GPIgG1, there was no narrowing of the bronchial lumen

and no plication in most bronchi, but in some bronchi of relatively large diameter, slight digitation of the epithelium was observed. Six hours after the injection, some bronchi showed weak plication (data are not shown).

3) *a-GPIgG2 injection*

Immediately after the injection, some bronchi showed epithelial shedding and slight digitation. Six hours after the injection, there was no narrowing or plication (data are not shown).

DISCUSSION

Guinea pigs actively or passively sensitized show symptoms similar to human asthma when challenged with appropriate antigens. As shown by our results, asthmatic responses induced by the injection of a-GPIgE resemble the immediate asthmatic responses in humans, although the duration time was shorter than antigen induced asthma in humans. The respiratory mechanics of the asthmatic responses confirms our previous observation⁹.

The pathological changes such as narrowing of bronchial lumen and shedding of cells into the lumens also resembles the pathological changes in human bronchial asthma¹⁴. As anti IgE was injected intravenously, chemical mediators released from peripheral cells might contribute to the bronchoconstrictions. However, these reactions in guinea pigs may be mainly due to a reaction of anti-IgE with IgE molecules in the bronchial tissues, because a prior inhalation of Disodium cromoglycate (DSCG) completely blocked the asthmatic response caused by an intravenous injection of anti IgE¹⁵.

Guinea pigs can produce homocytotropic antibodies which belong to two separate classes; IgG1 and IgE⁸. IgG1 antibodies are heat stable, while IgE antibodies are destroyed by heating at 56°C for 2-4 hours. IgE antibodies are able to persist in the skin much longer than IgG1 antibodies. Davis et al. report that, when guinea pigs were sensitized for 24 hours with heated antiserum, anaphylaxis was still observed although the severity decreased by about 20%¹¹. This would suggest that IgG1 antibodies are also responsible for anaphylaxis which is supported by our previous finding

that a-GPIgG1 contracted the guinea pig trachea in vitro⁹). However, as shown in the present study, an injection of a-GPIgG1 did not provoke anaphylaxis or asthmatic symptoms, while a small amount of a-GPIgE did. Although the precise reason for these observations is not clear, one reason may be that the quantity of IgG1 present in the serum was sufficient to neutralize the a-GPIgG1 injected intravenously, which partly or totally inhibited the reaction with IgG1 in the bronchial tissues, even if IgG1 could fix to the bronchial tissues. On the other hand, IgE is present in normal guinea pig serum only at low concentrations. Therefore, the IgE in the serum may not be enough to neutralize the a-GPIgE injected intravenously. We examined up to one ml of a-GPIgG1 containing 10 mg of γ -globulin, and it did not cause any reaction. However, higher amount of a-GPIgG1 might cause anaphylactic shock.

The present study demonstrated that a-GPIgG2 did not cause asthma in guinea pig. Probably the injected a-GPIgG2 was neutralized by serum IgG2. In our previous study, a-GPIgG2 did not cause contraction of the guinea pig trachea in vitro, while a-GPIgG1 did⁹). This may support the finding of Ovary⁵) that IgG2 antibodies do not fix to homologous tissues.

There is possibility that immune complex consisting of immunoglobulin and anti immunoglobulin may contribute to the reactions through activation of complement or Fc receptors, although only a-GPIgE caused allergic reactions in guinea pigs, whereas a-GPIgG1 and a-GPIgG2 did not, or if any, caused only slight reaction. Therefore, comparison between the reaction to whole molecule of a-GPIgE and that to $F(ab)_2$ of a-GPIgE should be done.

Remarkably enough inhalation of a-GPIgE did not induce any asthmatic response in the guinea pigs. This may indicate that the molecules of a-GPIgE do not reach to bronchial mast cells through inhalation. It is not clear that the inhaled a-GPIgE did not reach to bronchial airways or the inhaled a-GPIgE could not react with bronchial mast cells. Our recent study shows that a nasal drop of a-GPIgE causes bronchoconstriction in normal guinea pigs. Therefore, there may be some mechanical reasons for that the nebulized a-GPIgE did not reach to the bronchial airways. The

details will be reported in another paper.

In the present study, the change in lung mechanics due to the asthmatic response by a-GPIgE could be quantitatively estimated by Mead's method. The presented method for experimental asthma has advantages in comparison with the conventional systems, namely 1) Normal guinea pigs can be used without any active or passive sensitization. 2) Only the reaction due to IgE, not due to IgG1 or IgG2, can be observed. 3) For observations of allergic reactions due to IgE, this system is time-saving because an intravenous injection of a-GPIgE can cause immediate asthmatic responses, while the conventional challenge tests take at least 8 days to show reactions due to only IgE. The presented system may be advantageous for pathological and pharmacological studies of IgE-dependent asthma.

Intravenous injection of a-GPIgE in our system did not produce late asthmatic responses (LAR). This may indicate that LAR needs factors other than IgE reactions. However, Shampain showed that rabbits passively sensitized with IgE antibodies to *Alternaria* showed dual asthmatic responses upon challenge with the antigen¹⁶). We are now performing further studies on LAR by the use of the a-GPIgE system, comparing it with conventional systems.

We have reported that inhalation of DSCG dose-dependently inhibited asthmatic responses caused by injection of a-GPIgE¹⁵). We are now examining several antiallergic agents using this system.

Thus, the system may be useful for studying the effects of antiasthmatic agents and the pathological changes related to asthma.

We are thankful to Mr. Shinya Murakami for his technical assistance. We are also thankful to Miss Kaoruko Onozawa for her secretary assistance.

This work was supported by grant No. 62570343 from Ministry of Education, Science and Culture, Japanese Government and by Manabe Medical Foundation.

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抗 IgE 惹起モルモット喘息（新しい喘息モデル）の

生理学的、病理学的研究

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渡辺直熙

我々は、IgE 依存性の喘息モデルとして正常モルモットにウサギ抗モルモット IgE を静注する事によって惹起される実験喘息を確立した。その喘息の程度は、非麻酔下のモルモットを使用できる Mead の装置を用いて定量的に測定した。

抗 IgE の静注によって、即時型反応のみが認められた。抗 IgE の吸入によっては喘息反応は認められなかった。抗 IgE の注射後ただちに得られた肺の病理学的変化はヒトの喘息に類似していた。

この系は、IgE 依存性即時型喘息反応の病理学的、薬理学的研究の良好な手段となり得ると思われる。