

## Receptor-specific Mediation by Immunoglobulin E of Antigen-induced Contraction of Tracheal and Lung Parenchymal Strips Isolated from the Guinea Pig

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**A**bstract. The guinea pig is much like humans in the cells and mediators involved in immediate hypersensitivity reactions. However, the major anaphylactic antibody in this species is IgG<sub>1</sub>, not IgE. Recently, we have been successful in producing IgE antibody in guinea pigs. The current study examined whether guinea pig IgE antibody could mediate pulmonary smooth muscle contraction. IgE antibody to picryl and oxazolone determinants was induced by immunizing Hartley strain guinea pigs pretreated with cyclophosphamide. Hyperimmune serum from these animals was passed through a heavy chain-specific anti-IgG<sub>1</sub> affinity column. The presence of IgE anti-hapten antibody in the filtrate fraction was verified by passive cutaneous anaphylaxis (PCA) testing with a 7-d period of local passive sensitization and by heat lability (56°C × 4 h) of PCA activity. This IgE-rich fraction, and purified IgG<sub>1</sub> anti-hapten antibody were transferred to normal guinea pigs. Both fractions sensitized trachea and pulmonary parenchyma for antigen-induced smooth muscle contraction. The IgG<sub>1</sub>-mediated antigen-induced contractile response was not affected by heat (56°C × 4 h) and was inhibited in a dose-dependent fashion by IgG<sub>1</sub> blocking antibody (anti-OA). The IgE-mediated antigen-induced contractile response was significantly decreased by heat and was not affected by the anti-OA blocking antibody even at a concentration of 100 mg/kg.

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Thus, two antigen-specific factors in guinea pig serum can mediate antigen-induced pulmonary smooth muscle contraction: IgG<sub>1</sub> and IgE antibodies. Our data also suggests that these antibodies mediate the contractile response through separate receptors. The finding that guinea pig IgE can mediate pulmonary smooth muscle contraction suggests this species can be a model for IgE-mediated events in the lung.

### Introduction

In humans, IgE is the principal antibody involved in antigen-provoked mediator release from the lung and appears important in the pathogenesis of asthma (1-2). The guinea pig has long been used as a model of human asthma since the airways from the two species respond in a similar way to a variety of contractile and relaxant substances (3). Furthermore, antigen-induced contraction of airway smooth muscle from the two species has been suggested to involve similar primary mediators—histamine and slow reacting substance of anaphylaxis (4-8). Unlike humans, however, the major homocytotropic antibody of the guinea pig is IgG<sub>1</sub> (9-11).

The guinea pig, suitably sensitized, is known to be capable of producing IgE antibody (12-15). Guinea pig IgE is similar to human IgE in the length of time it persists in skin after passive sensitization and in loss of activity after reduction and alkylation or heat treatment (16). However, guinea pig IgE has been difficult to study because of the lack of a myeloma protein in this species.

Several recent studies have used the guinea pig, actively sensitized for the production of IgE antibody, as a model of human IgE-mediated pulmonary responses (17-21). A drawback to these studies is the fact that both IgE and IgG<sub>1</sub> antibodies are produced to the antigen with the immunization protocol (17, 18). Therefore, in an actively sensitized animal, it is difficult to determine the contribution of each antibody to specific antigen responses.

We have produced IgE antibody in guinea pigs by pretreating the animals with cyclophosphamide and immunizing with low doses of antigen in alum (22). Furthermore, the IgE and IgG<sub>1</sub>

antibodies have been successfully separated by affinity chromatography (22), and the IgE antibody was demonstrated to mediate cutaneous basophil hypersensitivity (23) and histamine release from circulating basophils (24) of the guinea pig. In this communication, we report the ability of guinea-pig IgE to sensitize, via its own receptors, guinea-pig tracheal and lung parenchymal strips for antigen-induced contraction.

## Methods

**Animals.** Outbred Hartley albino guinea pigs were obtained from Bio-Lab Corp., St. Paul, MN. Females weighing 350–450 g were used throughout the study. Outbred New Zealand white rabbits used for some immunization procedures were obtained from Klubertanz Farms, Edgerton, WI. Animals were maintained by the Research Animal Resource Center of the University of Wisconsin.

**Preparation of hapten-protein conjugates.** An ascaris protein extract was prepared from a homogenized dessicate of whole worms (George S. Tulloch and Associates, San Antonio, TX) (22). To conjugate picryl or oxazolone groups to ascaris proteins, 10 ml of the extract at 10 mg/ml was adjusted to pH 9.0 by addition of 5% Na<sub>2</sub>CO<sub>3</sub>. Then, 0.5 ml of 10% picryl chloride or oxazolone (Gallard Schlesinger, Carle Place, NY) in dioxane (Fisher Scientific Company, Pittsburgh, PA) was added dropwise while the pH was maintained at 9.0 by addition of 5% Na<sub>2</sub>CO<sub>3</sub>. After stirring for 2 h, the mixture was passed through a 2.4 × 20-cm column of Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with phosphate-buffered saline, pH 7.4 (PBS). The conjugated material in the void volume was concentrated by negative pressure dialysis, and the protein content determined by the Micro-Kjeldahl method. The resulting picryl-Ascaris (Pic-Asc)<sup>1</sup> and oxazolone-Ascaris (Ox-Asc) conjugates contained  $1.1 \times 10^{-7}$  and  $5 \times 10^{-7}$  mol of hapten/mg of protein, respectively. Picryl (Pic) and oxazolone (Ox) haptens were conjugated to human serum albumin (Pic-HSA and Ox-HSA) in a manner similar to that described above.

**Immunization procedures.** Serum rich in IgE antibody to the hapten-protein conjugates Pic-Asc or Ox-Asc was obtained by a modification of techniques described previously (22). Briefly, 6–8 guinea pigs per group received an intraperitoneal injection of 250 mg/kg of cyclophosphamide (Mead Johnson, Evansville, IN) 2 d before primary intraperitoneal immunization with 1 μg of conjugate adsorbed to 1 mg of Al(OH)<sub>3</sub> (alum) in 1 ml of normal saline. Every month thereafter for 5 mo, a similar dose of antigen in alum was given intraperitoneally. 7 d after antigen injection in months 3, 4, and 5, the animals were bled daily for 3 d by cardiac puncture. At the end of 5 mo, the animals were exsanguinated. The sera were stored in aliquots at –90°C until time of use.

Serum rich in IgG<sub>1</sub> antibody to ovalbumin (OA), Pic-guinea pig albumin, or Ox-guinea pig albumin was obtained with the immune deviation procedure as described previously (25). In this procedure, separate groups of guinea pigs were given a primary intraperitoneal immunization with 10 μg of the respective antigen in Alum (1 mg). 2 wk later, 50 μg of antigen emulsified with complete Freund's adjuvant

(CFA) was injected into the four foot pads. Every 2 wk for 2 mo thereafter, 50 μg of antigen in CFA was injected subcutaneously in multiple sites on the animals. 7 d after the last injection, the animals were bled daily for 5 d by cardiac puncture. On the sixth day, the animals were exsanguinated. Serum obtained from the bleedings was stored at –90°C until time of use.

Rabbit anti-guinea pig IgG<sub>1</sub> antibody was prepared by immunizing rabbits monthly with subcutaneous injections of 500 μg of column-purified IgG<sub>1</sub> (anti-OA) protein (see below) emulsified in CFA. Blood was obtained from ear veins, and serum was stored in aliquots at –20°C.

**IgG<sub>1</sub> purification.** IgG<sub>1</sub> antibody was purified from immune serum by a combination of DE-52 cellulose (Whatman Laboratory Products, Inc., Clifton, NJ) and G-200 Sephadex (Pharmacia Fine Chemical) column chromatography by using modifications of methods described previously (22). Briefly, DE-52 cellulose columns (2.4 × 100 cm) were equilibrated with 5 mM phosphate buffer, pH 8.0, at 4°C. Immune serum equilibrated by dialysis with the same buffer was applied to the column and eluted in a stepwise fashion with the equilibrating buffer followed by 10 mM, pH 6.45; 40 mM, pH 6.2; 60 mM, pH 6.1; 0.1 M, pH 5.9; and 0.3 M, pH 5.2 phosphate buffers. Those fractions rich in IgG<sub>1</sub> antibody (fractions eluted by 10 and 40 mM phosphate), as measured by Ouchterlony immunodiffusion using rabbit anti-IgG<sub>1</sub>, were pooled and rechromatographed on Sephadex G-200 equilibrated in 10 mM Tris, 0.2 M NaCl at 4°C. A portion of the IgG<sub>1</sub> fraction was iodinated by using lactoperoxidase and tested for purity by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Use of murine myeloma immunoglobulin standards showed that 5–10% nonantibody protein contaminated the preparation.

**Affinity column chromatography.** The methods of Axen et al. (27) were used to prepare affinity columns. A guinea pig IgG<sub>2</sub> affinity column was prepared by linking IgG<sub>2</sub> (anti-OA) antibody, purified as described previously (26), to 50 g of Sepharose 4B (Pharmacia Fine Chemical) via cyanogen bromide. Upon completion of the coupling procedure, the Sepharose was treated with 1 M ethanolamine for 2 h at 25°C and then washed with low (0.2 M acetate, pH 4.5) and high (0.2 M bicarbonate, pH 9) pH buffer solutions. The coupling efficiency of IgG<sub>2</sub> antibody to Sepharose was 90%.

Heavy chain-specific anti-guinea pig IgG<sub>1</sub> was prepared by passing an 18% Na<sub>2</sub>SO<sub>4</sub> fraction of the rabbit antiserum through the IgG<sub>2</sub> affinity column. The gamma globulin eluate was concentrated by negative pressure dialysis to 20 mg/ml and tested for heavy chain specificity by immunodiffusion against guinea pig IgG<sub>1</sub> and IgG<sub>2</sub> (both at 3 mg/ml). Only one precipitin band to IgG<sub>1</sub> was noted.

Anti-IgG<sub>1</sub> affinity columns were prepared by linking 6 ml (17 mg/ml) of the heavy chain-specific anti-IgG<sub>1</sub> to 100 g of Sepharose 4B with cyanogen bromide (88% coupling efficiency). Upon completion of the coupling procedure, the Sepharose was treated with ethanolamine and washed with low and high pH buffer solutions as described above.

IgG<sub>1</sub> was removed from guinea pig serum containing IgE antibody by slowly (1.5–2 h) passing the serum through an anti-IgG<sub>1</sub> affinity column at 4°C. The material passing through the column was concentrated to the original serum volume, and tested for residual IgG<sub>1</sub> antibody by immunodiffusion against the rabbit anti-IgG<sub>1</sub> and by enzyme-linked immunosorbent assay (ELISA). IgE antibody activity in this fraction was measured by using passive cutaneous anaphylaxis (PCA). The column was then washed thoroughly with 10 mM PBS, pH 7.4. IgG<sub>1</sub> bound to the affinity column was eluted with 10 ml of 0.2 M sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), pH 11.3, which was subsequently removed from the preparation by dialysis against PBS.

**PCA.** PCA antibody titrations were performed as described by Ovary

1. **Abbreviations used in this paper:** alum, Al(OH)<sub>3</sub>; CFA, complete Freund's adjuvant; ED<sub>50</sub>, median effective dose; ELISA, enzyme-linked immunosorbent assay; OA, ovalbumin; Ox, oxazolone; Ox-Asc, oxazolone-Ascaris; PCA, passive cutaneous anaphylaxis; Picryl; Pic-Asc, picryl-Ascaris.

(28). Tenfold dilutions of the serum (in PBS) to be tested were made in test tubes and 0.1 ml of each dilution was injected intradermally into the flank skin of 300-g guinea pigs. A sensitization period of 4 h or 7 d was employed, and PCA reactions were developed by injecting intravenously 1 ml of 0.5% Evans Blue containing 1 mg of antigen. The PCA titer was taken as the highest dilution of antiserum giving a 6 × 6-mm blue reaction on the reflected skin surface in at least 3 of 4 recipients (24, 28). Heat sensitivity of the PCA antibody activity was determined by incubating antisera at 56°C for 4 h before performing dilutions for the PCA titration.

*Quantitation and immunochemical identification of guinea pig IgG<sub>1</sub>.* A modification of a previously described ELISA method (29) was used to quantitate IgG<sub>1</sub>. Each well of plastic microtiter plates (Flow Laboratories, Inc., McLean, VA) was coated with 100 μl of purified IgG<sub>1</sub> (anti-OA, 25 μg/ml) in carbonate buffer, pH 9.0. Sites on the well surface not bound by IgG<sub>1</sub> protein were saturated by the addition of 10% bovine serum albumin (BSA) in carbonate buffer. Coated plates were washed with PBS containing 0.05% Tween 20, pH 7.5, and aspirated dry. A competitive binding standard curve for IgG<sub>1</sub> was obtained by adding purified IgG<sub>1</sub> at various concentrations (10 ng–1 mg/ml) to test tubes containing a 1:50 dilution of heavy chain-specific rabbit anti-guinea pig IgG<sub>1</sub>. After incubation at 37°C for 1 h and subsequent centrifugation, 100 μl of the adsorbed anti-IgG<sub>1</sub> was added to the IgG<sub>1</sub>-coated plates. The plates were incubated at 37°C for 1 h and subsequently, washed three times. Horseradish peroxidase (Sigma Chemical Co., St. Louis, MO) conjugated sheep anti-rabbit Fc (100 μl, diluted 1/1000 in PBS with 5% BSA) was added to the wells and the plates were incubated at 37°C for 30 min. After three washes, 100 μl of 2,2-Azino-di(3-ethylbenzthiazinesulfonic acid) in a 0.05 M citrate buffer, pH 4.0, was added to each well and the reaction allowed to develop for 30 min at room temperature. The reaction was stopped by the addition of 100 μl of 0.001 M editic acid in 0.1 M hydrofluoric acid. Optical density was measured at 414 nm in a micro-sampling spectrophotometer (Guilford, Oberlin, OH). This assay could detect 10–20 ng of IgG<sub>1</sub> per milliliter.

Immunodiffusion and immunoelectrophoresis in agar (1% purified-Difco Certified, Difco Laboratories, Inc., Detroit, MI) were performed on 2.5-cm slides as described previously (26). IgG<sub>1</sub> protein concentration determinations were performed according to the method of Lowry et al. (30).

*Measurement of tracheal and parenchymal smooth muscle contraction.* Normal guinea pigs were given intravenous injections of 3 ml/kg of either unadsorbed IgE-rich serum, heated (56°C × 4 h) or nonheated IgE-rich serum after adsorption with anti-IgG<sub>1</sub> linked to Sepharose, or with varying doses of affinity column or DE-52, G-200 Sephadex-purified IgG<sub>1</sub>. The animals were sacrificed 1, 4, or 7 d later and the proximal half of the trachea and lower right lung lobe removed. Tracheal segments were trimmed of excess tissue and cut in a spiral fashion. One parenchymal strip, containing the pleural edge and measuring ~3 × 3 × 20 mm, was cut from the lung lobe. The techniques have been previously described (31, 32).

Each strip was suspended vertically in a 10-ml water-jacketed (37–38°C) tissue bath containing a physiological salt solution of the following composition: NaCl, 118 mM; KCl, 4.7 mM; CaCl<sub>2</sub> · 2H<sub>2</sub>O, 2.5 mM; MgCl<sub>2</sub> · 6H<sub>2</sub>O, 0.5 mM; NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O, 1 mM; NaHCO<sub>3</sub>, 25 mM; glucose, 11 mM. The solution bathing the tissue and the stock salt solution were gassed with a mixture of oxygen (95%) and carbon dioxide (5%). Initial tensions on the tracheal and parenchymal strips were adjusted to 5 and 2 g, respectively, and maintained at these levels throughout a 90–120-min equilibration period. Changes in tension were recorded via force transducers (FT-03) on a Grass model 5D or 7C polygraph.

Cumulative dose-response effects of antigen were obtained by increasing the concentration by a factor of 10 after the effect of the previous concentration reached a plateau (33). Only one cumulative dose-response curve was obtained on each strip. Responses to each concentration of antigen were calculated as a percentage of the maximum contraction produced in the trachea by carbachol, 10<sup>-3</sup> M, and in the parenchymal strip by histamine, 10<sup>-3</sup> M, both added at the end of each experiment.

Median effective dose (ED<sub>50</sub>) values were obtained visually from a plot of log concentration (milligrams per milliliter) vs. percent of the maximum response produced by the antigen. All ED<sub>50</sub> values were converted to negative log values (-log ED<sub>50</sub>) and standard errors of the mean calculated for values obtained in each series of experiments. Differences between two means were determined using Student's *t* test for unpaired samples.

Inhibition studies were conducted by injecting intravenous various doses of competing IgG<sub>1</sub> (anti-OA) into normal guinea pigs ~30 min before injecting intravenous 3 ml/kg of either unadsorbed IgE-rich serum, nonheated IgE-rich serum after adsorption with anti-IgG<sub>1</sub> linked to Sepharose, or with 1 mg/kg of purified IgG<sub>1</sub> (anti-Ox). The animals were sacrificed the following day (20–24 h), and measurement of tracheal and parenchymal smooth muscle contraction was performed as described above.

## Results

*Affinity column adsorption of IgG<sub>1</sub> from IgE-rich serum.* Guinea pigs pretreated with cyclophosphamide and immunized with monthly intraperitoneal injections of either Pic-Asc or Ox-Asc in alum had peak, heat-labile, 7-d sensitization PCA titers, representing IgE responses, at months 4 and 5 of the immunization protocol. IgG<sub>1</sub> antibody is also produced in response to this protocol (22). Therefore, to exclude the participation of IgG<sub>1</sub>, serum rich in IgE was passed through an affinity column of heavy chain-specific anti-IgG<sub>1</sub>. The capacity of this column to deplete guinea pig serum of IgG<sub>1</sub> antibody was measured by serial passage of 0.2 ml serum aliquots through a column containing 10 ml of antibody-linked Sepharose. Immunodiffusion and ELISA analysis showed that virtually all the IgG<sub>1</sub> in 1 ml of serum was adsorbed by this column.

When adsorbing IgG<sub>1</sub> from IgE-rich serum, 50 ml of anti-IgG<sub>1</sub> affinity column was underloaded with 4 ml of immune serum to obtain complete adsorption of IgG<sub>1</sub> antibody. Material passing through such a column was concentrated to the original starting volume (4 ml) and tested for the presence of IgE antibody by evaluating PCA activity in heated (56°C × 4 h) and nonheated samples. In Fig. 1, it can be observed that unadsorbed antiserum (heated or nonheated) produced significant PCA titers when a 4-h sensitization period was used, and had some heat-stable PCA activity when a 7-d sensitization period was used. This suggests the presence of IgG<sub>1</sub> antibody that is heat insensitive and also IgE antibody that is heat sensitive. Column-adsorbed serum retained PCA activity at 4-h and 7-d skin sensitization periods and this PCA activity was completely heat-labile (Fig. 1). Accordingly, our adsorbed serum contains PCA activity that is due to IgE and not IgG<sub>1</sub> antibody, and this is as we have described previously (22). The PCA activity of serum samples

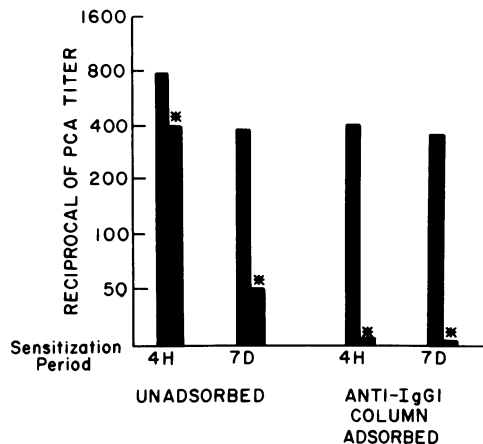


Figure 1. 4-h and 7-d sensitization period PCA titers of unadsorbed and anti-IgG<sub>1</sub> affinity column-adsorbed serum obtained 5 mo after immunization for IgE antibody response by cyclophosphamide pretreatment and monthly injection of guinea pigs with 1  $\mu$ g Pic-Asc plus Alum. \*, heated IgE-rich serum.

was evaluated prior to being used in studies measuring pulmonary smooth muscle contraction.

Unadsorbed sera obtained from three animals immunized for IgG<sub>1</sub> antibody by using immune deviation were found to have 4-h PCA titers ranging from 1:1600 to 1:3200. These titers were unaffected by heating the serum at 56°C  $\times$  4 h. PCA titers of these sera with a 7-d sensitization period were 1:20 to 1:50. Adsorption of the sera with an anti-IgG<sub>1</sub> affinity column eliminated all PCA activity. These data strongly suggest that IgE antibody is not present in the IgG<sub>1</sub> antisera.

**Immunochemical identification and quantitation of IgG<sub>1</sub>.** Ouchterlony immunodiffusion analysis using heavy chain-specific rabbit anti-guinea pig IgG<sub>1</sub> in the center well consistently resulted in a strong precipitin line of identity against unadsorbed serum and guinea pig IgG<sub>1</sub>. No lines were observed against purified IgG<sub>2</sub> or concentrated IgE-rich serum, that was depleted of IgG<sub>1</sub> by adsorption with the anti-IgG<sub>1</sub> column. It has been shown previously that as little as 35  $\mu$ g of purified IgG<sub>1</sub> can transfer cutaneous basophil hypersensitivity (34), and in the current study, 0.01 mg/kg of purified IgG<sub>1</sub> could transfer to normal animals a detectable pulmonary smooth muscle contraction. This amount of antibody is below the detection limits of the Ouchterlony gel diffusion system; thus, a competitive binding ELISA assay was established to measure lower levels of IgG<sub>1</sub> that might be present in adsorbed serum.

The lower limit of detection of IgG<sub>1</sub> in the ELISA was 10–20 ng/ml. The concentration of IgG<sub>1</sub> in unadsorbed IgE-rich serum was found to be 500–1,100  $\mu$ g/ml. When this serum was depleted of IgG<sub>1</sub> by passage through the anti-IgG<sub>1</sub> affinity column, IgG<sub>1</sub> levels were consistently <20 ng/ml. All column-adsorbed sera were assayed similarly before being used in any study.

Guinea pig IgG<sub>1</sub> bound to the anti-IgG<sub>1</sub> affinity column was eluted with 0.2 M Na<sub>2</sub>CO<sub>3</sub>, pH 11.3. The eluates from multiple columns were concentrated back to the original volume and pooled. Immunoelectrophoretic analysis of this material vs. either rabbit anti-guinea pig whole serum, heavy chain-specific anti-IgG<sub>2</sub>, or anti-IgG<sub>1</sub>, revealed only IgG<sub>1</sub> to be present in this fraction. The protein concentration of the preparation by Lowry assay was 820  $\mu$ g/ml, and the IgG<sub>1</sub> content by ELISA was 710  $\mu$ g/ml.

**Tracheal and parenchymal strip contractions.** Preliminary experiments ( $n = 18$ ) were conducted to establish the optimum dose of IgG<sub>1</sub> antibody that could sensitize pulmonary smooth muscle for antigen-induced contraction. Purified IgG<sub>1</sub> anti-picryl or anti-oxazolone antibodies were administered in doses of 0.001–6 mg/kg at 1 d prior to isolation of tracheal and parenchymal strips. From this series of experiments, we found that detectable contractions of tracheal and parenchymal strips (7% of maximum) to hapten antigen could be obtained with 0.01 mg/kg of antibody. No responses were observed with 0.001 mg/kg of the IgG<sub>1</sub> antibody. The maximum contractile responses to antigen increased with antibody dose. A dose of 1 mg/kg of IgG<sub>1</sub> ( $n = 4$ ) consistently resulted in maximum responses to antigen of 60–70% (Fig. 2 and Table I). While doses of 2 and 6 mg/kg ( $n = 6$ ) of IgG<sub>1</sub> occasionally resulted in slightly larger responses (82 $\pm$ 6%), the remainder of the study was conducted using 1 mg/kg of IgG<sub>1</sub> to passively sensitize the animals.

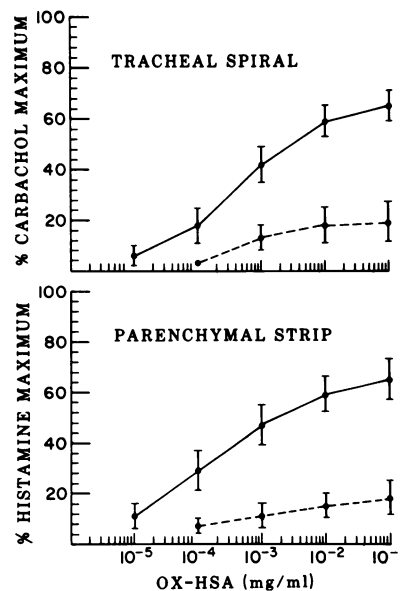


Figure 2. Log dose-response curves for Ox-HSA in producing contraction of guinea pig tracheal spirals and parenchymal strips. Animals were treated with 1 mg/kg purified anti-Ox IgG<sub>1</sub> alone (—) or with 9–15 mg/kg purified anti-OA IgG<sub>1</sub> before the anti-Ox IgG<sub>1</sub> (- - -) at 1 d before the experiment. Vertical lines indicate SEM and the data are summarized in Table I.

Table I. Summary of Ox-HSA-induced Contractile Response in Trachea and Parenchyma

Treatment*		Trachea		Parenchyma		No. <sup>  </sup>
Anti-Ox antibody	Antagonist	-log ED <sub>50</sub> ‡±SEM	% maximum§±SEM	-log ED <sub>50</sub> ‡±SEM	% Maximum§±SEM	
1 mg/kg IgG <sub>1</sub>	—	3.37±0.20	65±6	3.81±0.21	65±8	7
1 mg/kg IgG <sub>1</sub>	9–15 mg/kg IgG <sub>1</sub> anti-OA	3.60±0.25	19±8 <sup>¶</sup>	3.70±0.40	18±7 <sup>¶</sup>	6
3 ml/kg adsorbed IgE filtrate	—	3.45±0.13	57±8	3.60±0.19	64±6	8
3 ml/kg adsorbed IgE filtrate	50 mg/kg IgG <sub>1</sub> anti-OA	3.62±0.19	67±8	3.75±0.23	49±5	7
3 ml/kg unadsorbed IgE serum	—	3.62±0.18	83±2	3.36±0.19	70±2	6
3 ml/kg unadsorbed IgE serum	50 mg/kg IgG <sub>1</sub> anti-OA	3.52±0.12	78±3	3.42±0.27	62±7	8

\* All animals were passively sensitized with antibody intravenously. When IgG<sub>1</sub> anti-OA was administered, it was given 30 min before anti-Ox antibody. ‡ Negative logarithm of the concentration of Ox-HSA (milligrams per milliliter) required to produce a response equal to 50% of its own maximum contraction. § Percent maximum response produced by Ox-HSA relative to the maximum contraction produced by carbachol (trachea) or histamine (parenchyma). || Number of observations. ¶ Designates value that is statistically different ( $P < 0.05$ ) from that immediately preceding in the same column.

Adsorbed IgE-rich serum containing <20 ng/ml of IgG<sub>1</sub> antibody (ELISA) was administered in doses of 1–15 ml/kg at 1, 4, or 7 d prior to isolation of the tracheal and parenchymal strips. A dose of 3 ml/kg ( $n = 6$ ) was found to optimally sensitize the tissues and resulted in maximum responses to antigen (Pic-HSA or Ox-HSA; data shown only for Ox-HSA) of ~40–60% (Fig. 3 and Table I). Contractile responses were similar when examined at all three time periods after serum administration. Usually, tissues isolated from animals sensitized with <3 ml/kg contracted 10–30% at maximum antigen concentrations. Doses >3 ml/kg did not result in an increase in the maximum antigen-provoked contractions.

Contractions to the antigens were not observed when guinea pigs were injected with serum taken from unimmunized animals (3 ml/kg, 1 d), anti-hapten serum obtained from animals immunized for IgG<sub>1</sub> production and depleted of IgG<sub>1</sub> by an anti-IgG<sub>1</sub> affinity column (3 ml/kg, 1 d), or with IgG<sub>2</sub> purified from anti-hapten serum (30 mg/kg, 1 d). Furthermore, neither Pic-HSA nor Ox-HSA caused contractions of strips taken from animals not passively sensitized with antibody. The specificity of the responses was further verified by the demonstration that contractions could not be elicited to an extraneous antigen (OA) in tissues taken from animals sensitized with anti-hapten IgG<sub>1</sub> or IgE.

*Heat sensitivity of IgE-mediated, antigen-induced pulmonary contractions.* Heat treatment (56°C × 4 h) of anti-Pic IgE-rich serum after adsorption with anti-IgG<sub>1</sub> linked to Sepharose resulted in a marked reduction in the contractile responses to antigen in the guinea pig tissues. Peak contractions to Pic-HSA in the trachea and parenchyma were 49±6 and 42±7% of maximum, respectively, when the tissues were taken from animals

receiving 3 ml/kg of unheated adsorbed serum ( $n = 3$ ). The respective values were 19±7 and 18±6% of maximum when animals received an equal dose of heated, adsorbed serum ( $n = 5$ ). Similar heat treatment of purified IgG<sub>1</sub> anti-hapten antibody (1 mg/kg, 1 d) did not alter the contractile response to antigen ( $n = 4$ ).

*Inhibition of positive sensitization.* Having established that two antigen-specific factors in guinea pig serum can sensitize pulmonary smooth muscle for a contractile response on exposure to antigen, we sought to determine if these antibody-mediated responses could be inhibited by IgG<sub>1</sub> of an extraneous specificity. Results of inhibition experiments by using purified guinea pig anti-OA IgG<sub>1</sub> as blocking antibody are illustrated in Figs. 2–4 and summarized in Table I. There was substantial inhibition of the ability of Ox-HSA to contract tissues taken from animals passively sensitized with purified anti-Ox IgG<sub>1</sub> antibody (1 mg/kg, 1 d) after administration of 9–15 mg/kg of anti-OA IgG<sub>1</sub> (Fig. 2 and Table I). Doses of 3 and 6 mg/kg of anti-OA did not alter contractions to Ox-HSA. Doses of 50 and 100 mg/kg completely abolished contraction in four animals sensitized with 1 mg/kg of anti-Ox IgG<sub>1</sub> antibody. At the end of each experiment, the tracheal and parenchymal strips were exposed to OA (10<sup>-1</sup> mg/ml) and substantial contractions were observed.

In contrast, doses of 50 and 100 mg/kg of anti-OA IgG<sub>1</sub> given before either unadsorbed or adsorbed (<20 ng IgG<sub>1</sub>-ELISA) IgE-rich anti-Ox serum (3 ml/kg) did not result in a change in the dose-response curves to the antigen Ox-HSA (Figs. 3 and 4 and Table I).

In an additional experiment, the IgG<sub>1</sub> anti-Ox antibody adsorbed from IgE-rich serum was desorbed from the anti-IgG<sub>1</sub> affinity column and its identity confirmed by immunoelectro-

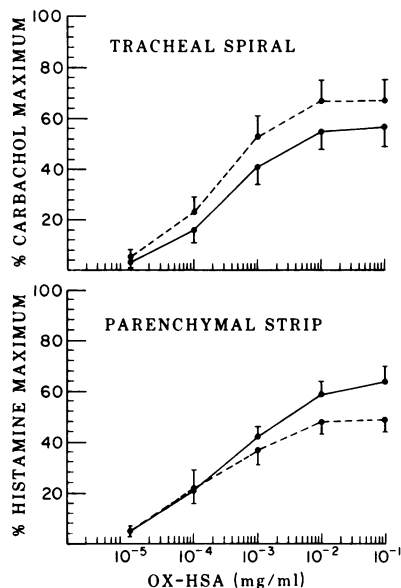


Figure 3. Log dose-response curves for Ox-HSA in producing contraction of guinea pig tracheal spirals and parenchymal strips. Animals were treated with 3 ml/kg anti-IgG<sub>1</sub> affinity column-adsorbed IgE-rich serum (anti-Ox) alone (—) or with 50 mg/kg purified anti-OA IgG<sub>1</sub> before the adsorbed serum (---) at 1 d before the experiment. Vertical lines indicate SEM and the data are summarized in Table I.

phoresis and ELISA. This antibody in a dose of 1 mg/kg was injected into four animals. Two of the animals also received anti-OA IgG<sub>1</sub>, 50 mg/kg, 30 min beforehand. One day later, Ox-HSA-induced contractions in isolated tracheal and parenchymal strips were evaluated. The results are not shown, but are similar to those described above for the inhibition of anti-Ox IgG<sub>1</sub>. Contractions to Ox-HSA were not observed in tissues taken from animals pretreated with anti-OA IgG<sub>1</sub> antibody.

## Discussion

This study has demonstrated that two factors in guinea pig serum can sensitize pulmonary smooth muscle for antigen-induced contraction. Our results provide strong evidence that these factors are IgG<sub>1</sub> and IgE antibodies. Furthermore, we have provided evidence that IgG<sub>1</sub> and IgE sensitize pulmonary tissues by attaching to different receptors.

Since there have been no myeloma proteins described in the guinea pig, IgE antibody can be defined only by characteristics it has in common with IgE from other species: PCA activity after a prolonged sensitization period in skin, loss of PCA activity after heating or reduction and alkylation of the antibody, and passage of PCA activity through an affinity chromatography column that removes IgG<sub>1</sub> antibody (22, 28). Each of these conditions has been satisfied in this study for PCA as well as for antigen-induced tracheal and parenchymal strip contractions.

Since IgG<sub>1</sub> is the major anaphylactic antibody in the guinea pig and is produced in significant concentrations in any IgE-producing immunization protocol (22), it was imperative to deplete the preparation of IgG<sub>1</sub> in order to study the properties of IgE. Effective removal of IgG<sub>1</sub> by affinity chromatography in our study was demonstrated by ELISA. Residual IgG<sub>1</sub> in serum after adsorption was <10–20 ng; a depletion of ~99.995%. The amount of IgG<sub>1</sub> antibody remaining in affinity column adsorbed serum was approximately 1,000 times less than the smallest amount of IgG<sub>1</sub> that could sensitize for detectable tracheal and parenchymal strip contraction. Therefore, it is unlikely that sensitization by IgE-rich adsorbed serum was due to contaminating IgG<sub>1</sub>. IgE anti-hapten activity in the IgG<sub>1</sub>-depleted serum was measured by PCA activity that was destroyed by heat. Injection of adsorbed IgE filtrates into normal animals passively sensitized trachea and parenchyma at 1, 4, and 7 d for antigen-induced contraction. This effect was heat-sensitive. Although antigen-induced contraction was obtained with purified anti-hapten IgG<sub>1</sub> antibody at 1, 4, and 7 d, this was resistant to heat treatment and absent after adsorption with an anti-IgG<sub>1</sub> affinity column.

Katayama et al. (35) have shown that a non-IgG<sub>1</sub>, non-IgE serum factor can mediate cutaneous basophil hypersensitivity reactions. These reactions have previously been shown to be mediated only by immune lymphocytes and antibody (36). The serum factor of Katayama et al. has been shown to be antigen specific, to have a molecular weight of 70,000, to be present in

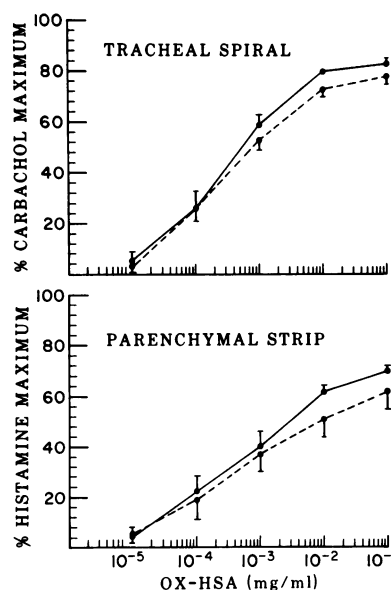


Figure 4. Log dose-response curves for Ox-HSA in producing contraction of guinea pig tracheal spirals and parenchymal strips. Animals were treated with 3 ml/kg unadsorbed IgE-rich serum (anti-Ox) alone (—) or with 50 mg/kg purified anti-OA IgG<sub>1</sub> before the unadsorbed serum (---) at 1 d before the experiment. Vertical lines indicate SEM and the data are summarized in Table I.

immune serum for only a short period of time after sensitization (~1 wk), and to be absent from the globulin fraction of serum. There is no evidence that this factor is heat-sensitive or remains in tissues for a prolonged period of time. In our work, the antigen-induced pulmonary smooth muscle contraction mediated by adsorbed IgE-rich serum met all the criteria for the definition of IgE antibody in the guinea pig. Furthermore, serum from animals immunized for IgG alone (no IgE as shown by PCA) and passed through an anti-IgG<sub>1</sub> affinity column failed to sensitize pulmonary tissues for antigen-induced contraction. Therefore, our data do not support the existence of another factor in guinea pig serum capable of sensitizing pulmonary tissue.

Since a myeloma protein for IgE in the guinea pig is not available, we have not performed inhibition studies with purified IgE preparations. Nevertheless, results from our inhibition studies provide strong evidence for the existence of two distinct receptors for IgG<sub>1</sub> and IgE in the guinea pig pulmonary systems examined. These data also emphasize and strengthen the interpretation that two different factors in guinea pig serum can sensitize for antigen-induced smooth muscle contraction. The inhibition by anti-OA IgG<sub>1</sub> of the ability of anti-Ox IgG<sub>1</sub> to sensitize the pulmonary tissues cannot be attributed to a nonspecific effect since anti-OA IgG<sub>1</sub> did not alter sensitization by adsorbed IgE filtrate or unadsorbed IgE-rich serum. Furthermore, substantial OA-induced contraction was observed in the tissues that did not respond (or weakly contracted) to Ox-HSA. These observations would appear to rule out an alteration by blocking antibody of the desensitization process occurring during cumulative antigen administration.

Since both IgE and IgG<sub>1</sub> anti-hapten antibodies are present in hyperimmune serum and both can mediate a contractile response in pulmonary smooth muscle, it is likely that blockade of sensitization by either antibody alone is not adequate to inhibit antigen-specific contraction. This is supported by the observation that anti-OA IgG<sub>1</sub> did not alter responses to Ox-HSA in animals receiving unadsorbed IgE-rich serum. In these experiments, the contractile response observed in tissues taken from animals receiving the blocking antibody was probably mediated by IgE.

Since we have demonstrated that IgE antibody can sensitize guinea pig pulmonary tissues for antigen-induced contraction, this system can now be further examined as a possible model for IgE-mediated events in the human lung. In our studies, there was little difference in antigen sensitivity or maximum antigen-induced contraction between tracheal and parenchymal strips or between IgG<sub>1</sub>- and IgE-mediated contractions. However, this does not provide information about mediators released or cell types activated by antigen. Furthermore, the magnitude of desensitization occurring during cumulative antigen administration cannot be ascertained from these studies. Preliminary work has suggested differences in mediator release between IgG<sub>1</sub> and IgE-sensitized guinea pig pulmonary tissues (19–21, 37). Additional studies will be needed for a proper comparison of IgE-mediated events in guinea pig and human lung.

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