Involvement of immunologic mechanisms in a guinea pig model of Western red cedar asthma

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Western red cedar asthma is the most common form of occupational asthma in the Pacific Northwest. Plicatic acid (PA) is the chemical component of Western red cedar that causes asthma. The role of immunologic processes involved in the PA-induced asthmatic reaction has not been established. To characterize the mechanisms of PA-induced asthmatic reaction, guinea pigs were sensitized to PA through biweekly injection of PA-ovalbumin conjugate with aluminum hydroxide as an adjuvant for a period of 6 months. Specific IgG_1 antibodies to PA were detected in the blood 3 months after sensitization of animals. The level of specific IgG_1 antibodies to ovalbumin after 6 months was about two times the level of specific IgG_1 to PA. At 6 months, tracheal tissue from PA-ovalbumin–sensitized guinea pigs contracted after exposure to either PA or ovalbumin in vitro. The degree of contraction induced by PA was two to three times less than the contraction induced by ovalbumin. PA caused histamine, prostaglandin D_2, and leukotriene D_4 release from both lung mast cells and blood basophils. The amount of histamine and eicosanoids released by PA was also two to three times less than the amount of mediators released by ovalbumin. When the trachea of normal guinea pigs was passively sensitized with serum from PA-ovalbumin–sensitized guinea pigs, it contracted in response to PA or ovalbumin in an organ bath. When the serum of PA-ovalbumin–sensitized guinea pigs was depleted of immunoglobulins and then used for passive sensitization of normal trachea, no contraction was observed when challenged with PA, suggesting that IgG_1 antibodies mediate the tracheal reaction to PA. The results demonstrated that PA-induced reaction in guinea pigs is an immunologic process that involves IgG_1 antibodies. (J Allergy Clin Immunol 1994;93:877-84.)

Key words: *Occupational asthma, guinea pig, plicatic acid, histamine, leukotrienes, prostaglandin, IgG_1*

Occupational asthma caused by Western red cedar has been shown to be caused by plicatic acid (PA) present in the wood.[1] Because PA has a molecular weight of only 440 d, red cedar asthma is a prototype of asthma caused by low molecular weight compounds.

Inhalation challenge tests with PA induced isolated late asthmatic reaction in about 45% and dual asthmatic reaction (immediate with a late component 4 to 6 hours later) in 48% of patients with red cedar asthma. Isolated immediate reaction is uncommon.[2] This is different from inhalation reactions induced by high molecular weight compounds, when isolated immediate reaction and dual asthmatic reaction are common but isolated late reaction seldom occurs.[3] Mediators of anaphylaxis such as leukotrienes and histamine have been found in the bronchoalveolar lavage of patients with red cedar asthma during immediate reaction induced by PA,[4] similar to those released

Abbreviations used
HSA: Human serum albumin
LTD_4: Leukotriene D_4
NADs: New antigenic determinants
PA: Plicatic acid
PGD_2: Prostaglandin D_2

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during immediate asthmatic reaction induced by high molecular weight allergens. However, specific IgE antibodies to PA conjugated to human serum albumin (HSA) are detected in only 20% of patients.[5]

The inability to detect specific IgE antibodies can be due to the fact that HSA is not the correct protein carrier. New antigenic determinants (NADs) may be formed once PA reacts with tissue proteins, thus making the detection of specific IgE antibodies difficult, as in occupational asthma caused by other low molecular weight compounds.[6]

In a previous study we were able to sensitize rabbits to PA-ovalbumin by parenteral route and to demonstrate the production of specific IgE antibodies.[7] However, we were unable to detect airway response when sensitized animals were challenged with PA by inhalation.[7] In this study we explore the significance of humoral antibodies to PA-sensitized guinea pigs in order to understand further the pathogenic mechanism of red cedar asthma.

METHODS

Materials

Fetal bovine serum and tissue culture media (F12 and Dulbecco's modified eagle medium) were purchased from Gibco Laboratories (Grand Island, N.Y.). Histamine carbamylcholine, leukotriene D_4 (LTD_4), collagenase, elastase, HSA, and other chemicals (unless otherwise indicated) were purchased from Sigma Chemical Co. (St. Louis, Mo). Tritiated PA was custom prepared by Du Pont Diagnostic Imaging Division Wilmington, Del.). Radioimmunoassay kits for LTD_4 and prostaglandin D_2 (PGD_2) were purchased from Du Pont Diagnostic Imaging Division.

PA-ovalbumin and PA-HSA conjugates

PA was conjugated to HSA as described previously.[5] Briefly, HSA (1 mmol) was dissolved in 0.5 ml of bicarbonate buffer saline solution (0.5 mol/L, pH 10.0). PA (100 ml) was added in small quantities to the HSA saline solution, and the reaction mixture was stirred at room temperature for 18 hours. The solution was passed through a 1.5 × 30 cm column containing Sephadex G 25 (Pharmacia, Uppsala, Sweden) to separate the conjugated PA-HSA from the free PA. The conjugated PA-HSA was passed through another 2.6 × 90 cm column containing Ultrogel ACA 34 to obtain a purified fraction of PA-HSA. The number of PAs conjugated to HSA was found to be 30.[5] The purified PA-HSA was dialyzed against distilled water, lyophilized, and stored at 4° C until use. The PA-ovalbumin conjugate was prepared in the same manner.

Guinea pig sensitization

Cam-Hartley male guinea pigs were sensitized to PA-ovalbumin conjugate by injection of 10 μg PA-ovalbumin adsorbed to 1 mg of $Al(OH)_3$ intraperitoneally. Animals were injected intraperitoneally with 1 mg of PA-ovalbumin–$Al(OH)_3$ each month for a total period of 6 months.

IgG_1 and IgG_2 measurement

Homologous passive cutaneous anaphylaxis assay was used to detect IgG_1 antibodies. Three recipient guinea pigs were used to test for IgE antibodies to PA, ovalbumin, or HSA. Duplicates of 0.1 ml of serum from sensitized guinea pigs were used in dilutions of 1:80, 1:160, and 1:320 for PA-HSA challenge; 1:160, 1:320, and 1:640 for ovalbumin challenge, and 1:10 for HSA challenge. As a control, a 1:10 dilution of normal serum was used. Forty-eight hours later, one recipient guinea pig was challenged intravenously with 5 mg of PA-HSA in 1 ml of Evans blue dye for the detection of anti-PA activity. The second recipient was challenged with 5 mg of HSA in 1 ml of Evans blue dye. The third recipient was challenged with 5 mg of HSA in 1 ml of Evans blue dye. Bluing of individual skin sites of passive sensitization was measured 30 minutes after the challenge. A blue spot of 5 mm or greater in diameter was considered a positive response.

The ELISA method was used for the measurement of IgG_1 and IgG_2 antibodies. Plates (96 wells) were coated with either PA-HSA or ovalbumin in bicarbonate buffer (pH 8.6) overnight at 4° C. The next day, wells were washed three times with phosphate-buffered saline–Tween 20. The following reagents were added sequentially to the wells and incubated for 1 hour at room temperature, followed by washing with phosphate-buffered saline–Tween 20, 0.1% bovine serum albumin, 1:100 dilutions of serum from immunized guinea pigs, rabbit anti-guinea pig IgG_1 or IgG_2, alkaline phosphatase–labeled goat anti-rabbit IgG, and finally p-nitrophenyl phosphate. The absorbance was measured at 405 nm. The amounts of IgG_1 or IgG_2 in the samples were obtained from a standard curve.

Tracheal contraction in vitro

Spirals of guinea pig tracheal tissue approximately 0.5 cm long, were placed in oxygenated Krebs-Henseleit solution in a 5 ml jacketed organ bath under 1*g* resting tension. Isometric force was measured with a Grass FT .03 transducer (Grass Instrument Co., Quincy, Mass.) and recorded on a polygraph (Grass model 7D). Before the addition of antigens, the tissues were allowed to equilibrate for 30 minutes to obtain a stable baseline. Agonists were added in 50 μl aliquots. Cumulative dose-response curves were obtained with the maximal contractile activity of carbachol (1 mmol/L), assessed at the end of each experiment.

Histamine release assay

Lung fragments of approximately 100 mg (wet weight) or blood leukocytes (10^6 cells) were incubated with various concentrations of antigens (ovalbumin or PA) for 30 minutes at 37° C. At the conclusion of this period, lung tissues or leukocytes were centrifuged at 300*g*, and supernatants were removed and assayed for the presence of histamine with a radioimmunoassay technique as reported earlier.[8] Lung tissues or leukocytes were frozen and thawed twice in Tyrode's buffer, and the supernatant was removed for measurement of remaining histamine. The samples were frozen at −80° C. Histamine released was expressed as a percentage of total histamine content.

Prostaglandin and leukotriene measurement

Lung fragments (100 mg, wet weight) and leukocytes (10^6) were incubated in the presence of either PA, ovalbumin, or PA-HSA for 30 minutes at 37° C. The incubation was terminated with the addition of 1 volume of methanol (total volume = 1 ml). Cells and denatured proteins were centrifuged at 160*g* for 10 minutes. The supernatants were extracted on a Sep-Pak column (Pharmacia) as reported earlier.[9] The extracted materials were divided, and LTD_4 was separated by high-performance liquid chromatography.[10] The fractions corresponding to LTD_4 were collected, and the solvent was removed. The residues were assayed for LTD_4 with radioimmunoassay kits.[11] One portion of the Sep-Pak–extracted materials was analyzed for prostaglandins as reported.[12] The eluates corresponding to PGD_2 were collected, the solvent was evaporated, and the residues were assayed for PGD_2 by radioimmunoassay.

PA binding assays

Heparinized blood was obtained from normal and PA-sensitized guinea pigs. Leukocytes were prepared by dextran sedimentation and low-speed centrifugation (to remove platelets). Red blood cells were lysed with Tris-Al(OH_3) buffer. Leukocytes were suspended in Tyrode's buffer with calcium and incubated at 37° C with 0.1 mg/ml tritiated PA (Du Pont Diagnostic Imaging Division, 147 Ci/mmol) in the presence or absence of 50 mg/ml of unlabeled PA. After 15 minutes, the reaction mixture was filtered through presoaked Whatman GF/C glass fiber filters (Whatman, Inc., Clifton, N.J.) under vacuum. Filters were washed with 5 ml Tyrode's buffer to remove unbound tritiated PA. The filters were then dried and counted in a scintillation counter.

Passive sensitization

Trachea rings from normal guinea pigs were incubated with sera from sensitized guinea pigs (1:4, serum to buffer, respectively). They were incubated at 37° C for 2 hours under intermittent oxygenation, then washed and placed in an organ bath. Antigen (PA or ovalbumin) was added, and the contractile response of the tracheal rings was recorded on a polygraph.

Immunoglobulin removal procedure

The serum was passed through a column of protein A-Sepharose-C1-4B to remove immunoglobulins. The column was washed with phosphate-buffered saline. The serum was reconstituted to its original volume and tested for the absence of IgG_1 by immunodiffusion. The serum was then used for passive sensitization experiments.

Statistical analysis

Data were expressed as means ± SD. Tracheal contractile responses to cumulative drug dosages were compared with a repeated measures analysis of variance. Data were considered significant when the p value was less than 0.05 according to Student's t test.

RESULTS

Passive cutaneous anaphylaxis assays with sera from all sensitized guinea pigs were strongly positive with titers greater than 1:320 when challenged with PA-HSA and 1:640 when challenged with ovalbumin but were negative when challenged with HSA, indicating that the sera from the sensitized animals contained IgG_1 antibodies against both the hapten and carrier proteins.

Guinea pigs generated specific IgG_1 antibodies to PA and ovalbumin after 3 months. The levels of antibodies increased gradually, reaching a maximum after 5 to 6 months. At 6 months, the mean level of circulating IgG_1 against ovalbumin was about 1.5 times higher than those against PA when the ELISA technique was used (41 μg/ml vs 27 μg/ml, respectively). The levels of IgG_2 antibodies against PA and ovalbumin were 19 μg/ml and 16 μg/ml, respectively.

The response of sensitized trachea to PA or ovalbumin was investigated. Tracheal tissue obtained from PA-ovalbumin–sensitized guinea pigs contracted after exposure to PA or ovalbumin. PA initiated contraction of tracheal tissue at concentrations greater than 1 μmol/L, whereas ovalbumin initiated contraction at concentrations greater than 1 nmol/L. PA-induced contraction of tracheal tissue reached a plateau rapidly at about 100 mmol/L (Fig. 1). At the maximal concentration PA (1 mmol/L) was only able to contract tracheal tissue by about 25% of maximal contraction induced by carbachol. At concentrations greater than 1 mmol/L, PA did not induce further contraction, suggesting that the effect of PA was

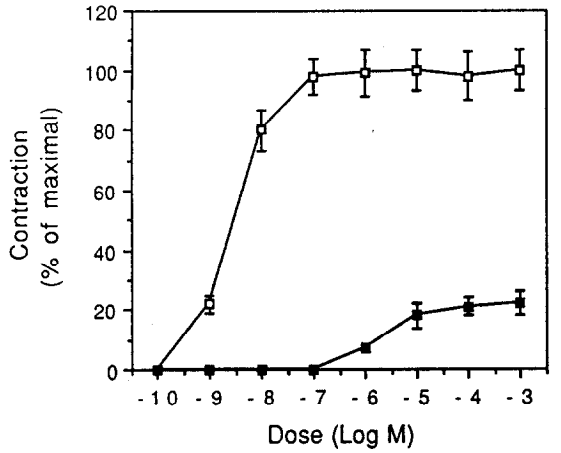

FIG. 1. Isometric response of isolated tracheal tissue from PA-ovalbumin–sensitized guinea pigs challenged with ovalbumin (□) or PA (■). Data are means ± SD of nine experiments.

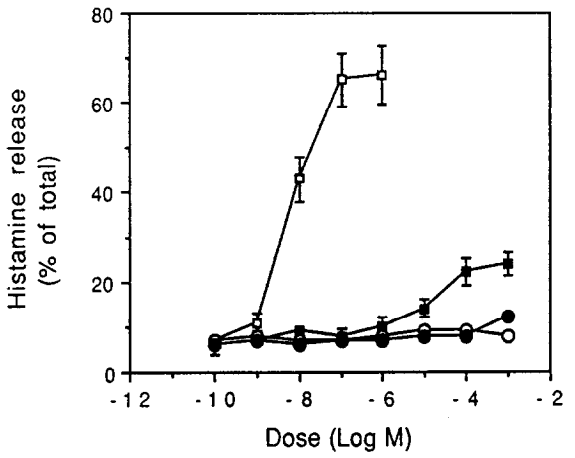

FIG. 2. Release of histamine from lung fragments of PA-ovalbumin–sensitized guinea pigs in response to ovalbumin (□) or PA (■). The release of histamine from lung fragments of normal guinea pigs in response to ovalbumin (○) and PA (●). Histamine was measured after 5 minutes of tissue challenge with the antigens. Results are means ± SD of eight experiments in triplicate.

saturated at that concentration. However, ovalbumin at maximal concentration induced tracheal contraction by more than 90% of that maximally induced by carbachol. Fig. 1 shows that the maximal contraction caused by PA was about three times less than that caused by ovalbumin.

In an attempt to elucidate the mechanism of low contractile response to PA, experiments were performed to evaluate the effects of PA on mediator release from mast cells in lung fragments and from basophils in blood leukocytes. Lung fragments from PA-ovalbumin–sensitized guinea

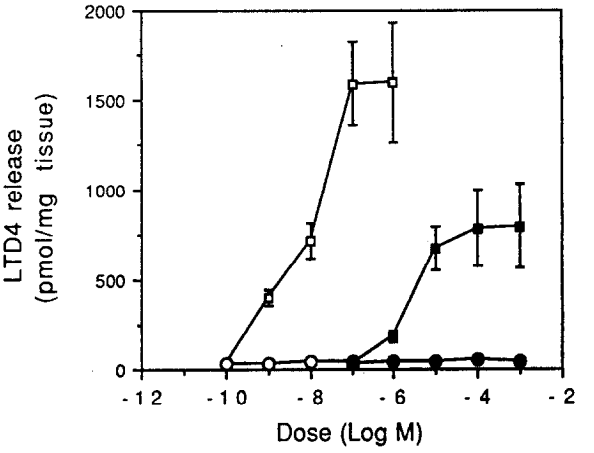

FIG. 3. Generation of LTD_4 from lung fragments of PA-ovalbumin–sensitized guinea pigs in response to ovalbumin (□) and PA (■). The release of LTD_4 from lung fragments of normal guinea pigs in response to challenge with ovalbumin (○) and PA (●). LTD_4 was measured 5 minutes after challenge. Data are means ± SD of eight experiments in triplicate.

pigs were incubated with various concentrations of PA for 5 minutes. Histamine release from lung fragments after PA challenge was evaluated. As can be seen in Fig. 2, PA caused histamine release from concentrations similar to those that caused tracheal contraction. Addition of ovalbumin to PA-ovalbumin–sensitized lung fragments caused histamine release. Once again, ovalbumin induced several times more histamine release than PA. Ovalbumin was able to stimulate histamine release at a concentration of 10^{-9} mol/L, whereas PA initiated histamine release at a concentration of 10^{-6} mol/L. Histamine release induced by ovalbumin reached a plateau after 10^{-6} mol/L (Fig. 2).

In addition to the release of histamine by lung fragments, it has been shown previously that immunologic stimulation of mast cells also releases LTD_4. Fig. 3 demonstrates that lung fragments from PA-ovalbumin–sensitized guinea pigs responded to stimulation with PA by generating an appreciable amount of LTD_4. Similarly, lung fragments from these guinea pigs also responded to stimulation with ovalbumin by releasing LTD_4. Once again, ovalbumin released several times more LTD_4 than PA. Neither PA nor ovalbumin was able to cause LTD_4 release from normal lung fragments, indicating that the action of PA or ovalbumin on mast cells is immunologic.

The release of histamine and eicosanoids from basophils of sensitized animals when challenged with PA or ovalbumin was also investigated (Fig.

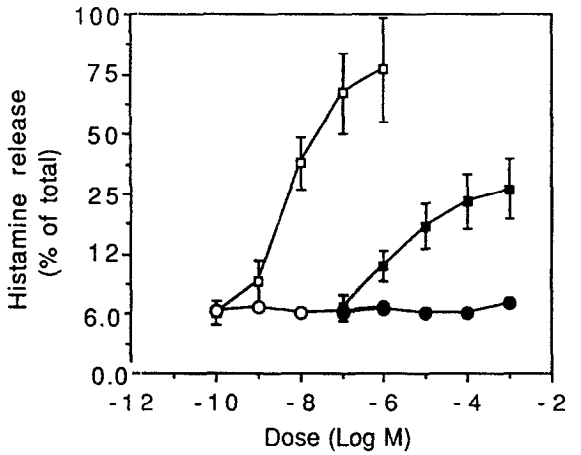

FIG. 4. Release of histamine from 10^6 leukocytes of PA-ovalbumin–sensitized guinea pigs after exposure to ovalbumin (□) and PA (■). Release of histamine from 10^6 leukocytes of normal guinea pigs after exposure to ovalbumin (○) or PA (●). Results are means ± SD of six experiments in triplicate.

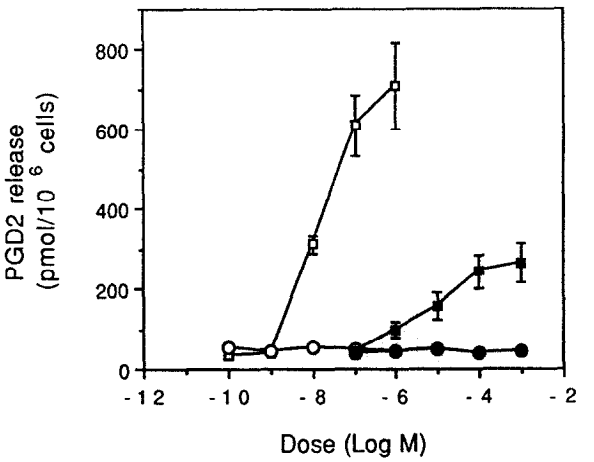

FIG. 5. Generation of PGD_2 from 10^6 leukocytes of PA-ovalbumin–sensitized guinea pigs after exposure to ovalbumin (□) or PA (■). Generation of PGD_2 from leukocytes of normal guinea pigs after exposure to ovalbumin (○) or PA (●). Data are means ± SD (n = 6).

4). PA and ovalbumin caused the release of histamine when added to leukocytes of PA-ovalbumin–sensitized guinea pigs but not from leukocytes of normal guinea pigs. PA caused significantly less histamine release than ovalbumin. Ovalbumin was effective in releasing mediators in concentrations as low as 10^{-9} mol/L, and the maximal effect was seen at 10^{-7} mol/L. PA, on the other hand, was effective at a concentration of 10^{-6} mol/L, and the maximal effect was seen at 10^{-3} mol/L.

The generation of eicosanoids by leukocytes in response to activation by PA or ovalbumin was investigated (Fig. 5). Cell supernatants were analyzed for the release of PGD_2. Again, ovalbumin caused the release of a significantly greater amount of PGD_2 than PA. The effect of PA was dose-dependent and maximal at approximately 10^{-4} mol/L. Neither PA nor ovalbumin released PGD_2 from normal cells, confirming further the involvement of an immunologic process in PA-induced mediator release.

To determine whether the conjugate PA-HSA increases tissue and leukocyte responses to PA, experiments were performed with various concentrations of PA and PA-HSA (the ratio of PA-HSA was 30:1, mol/mol). PA-HSA conjugate did not induce greater histamine release than PA alone up to 10^{-4} mol/L (Fig. 6, *A*). Similarly, the release of LTD_4 was not affected by the coupling of PA to HSA (Fig. 6, *B*). The dose-response curves showed that PA-HSA only slightly, but not significantly, shifted the histamine release curve to the left of the PA curve. However, the maximal release with either antigen remained the same. PA-induced tracheal contraction was not significantly different from that induced by PA-HSA (Fig. 6, *C*).

Studies were then designed to investigate the binding of PA to leukocytes of normal and PA-ovalbumin–sensitized guinea pigs (Fig. 7). The binding of tritiated PA to leukocytes of normal animals was not reduced in the presence of an excess amount of nonlabeled PA, indicating that there was no specific binding sites for PA in normal animals. However, in sensitized animals the binding of tritiated PA to leukocytes was reduced in the presence of nonlabeled PA in sensitized animals, indicating the presence of specific binding sites.

To establish the role of immunoglobulins in PA-induced anaphylactic reaction, two sets of experiments were performed. In one set plasma from PA-ovalbumin–sensitized guinea pigs was added to the tracheal tissue of normal guinea pigs for the purpose of passive sensitization. After 2 hours of passive sensitization, the tracheal tissue was washed and evaluated for contraction in response to PA or ovalbumin in a tissue bath. The contraction induced by PA or ovalbumin in the passively sensitized tracheal tissue was compared with the contraction induced in PA-ovalbumin–sensitized guinea pigs. As can be seen in Fig. 8, the passively sensitized tracheal tissue responded

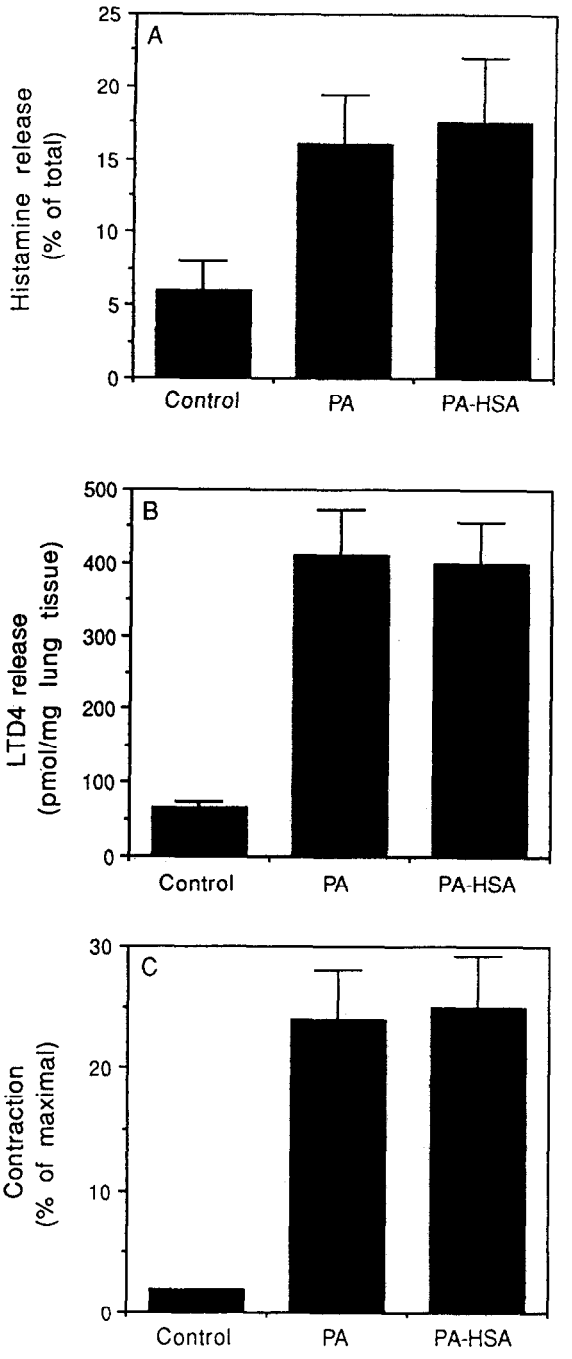

FIG. 6. Effect of addition of PA or PA-HSA conjugate to tracheal tissue from PA-ovalbumin–sensitized guinea pigs. **A,** Histamine release. **B,** LTD_4 release. **C,** Contraction. Data are mean ± SD of seven experiments. The PA-HSA dose is based on the moles of PA in the solution.

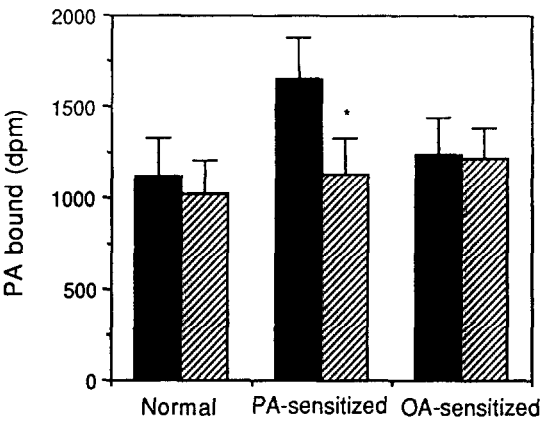

FIG. 7. Binding of tritiated PA to leukocytes of normal PA-ovalbumin–sensitized and ovalbumin-sensitized guinea pigs in the presence (▨) and absence (■) of 100-fold excess amount of nonlabeled PA. Data are means ± SD ($n = 5$). *$p < 0.05$.

to both PA and ovalbumin. The response to PA stimulation of the passively sensitized tracheal tissue was approximately three times less than the response to ovalbumin. In the second set of experiments, humoral antibodies were removed by column chromatography. Affinity columns made from the protein A-Sepharose-C1-4B, which binds specific immunoglobulins, were used. Plasma from PA-ovalbumin–sensitized animals was passed through the affinity column, and the eluates were collected as described in the Methods section. The eluates (proteins) were concentrated to approximately the original volume with the use of Amicon filters (Amicon Inc., Beverly, Mass.). The concentrated proteins were used to passively sensitize the trachea. After 2 hours of incubation with concentrated proteins, the trachea was washed and then challenged with the antigens (PA or ovalbumin). Tracheal tissue passively sensitized with serum depleted of immunoglobulins did not contract in response to either PA or ovalbumin (Fig. 8). The data demonstrated the involvement of immunoglobulins in PA- or ovalbumin-induced tracheal muscle contraction.

DISCUSSION

Agents that cause occupational asthma can be divided into two broad groups: high molecular weight organic compounds, such as organic proteins, and low molecular weight compounds, such as toluene diisocyanates, acid anhydrides, and PA.[3] In human beings high and low molecular weight agents appear to cause occupational asthma through different mechanisms. High molecular weight allergens readily induce specific IgE antibodies and behave similarly to common aeroallergens that cause atopic asthma.[13] In contrast, low molecular weight allergens are more heterogeneous. Some agents such as acid anhydrides[6] and platinum salts[14] induce specific IgE

antibodies. With other low molecular weight compounds such as PA and toluene diisocyanate, specific IgE antibodies are not found or are found in only a small percentage of patients.[5, 15] In human beings it has been shown that basophils from patients with atopic asthma degranulate when exposed to appropriate allergens, and the mechanism responsible for this response is generally accepted to be IgE-dependent.[16] This mechanism is also responsible for the asthmatic reaction to high molecular weight allergens. However, the mechanism for asthma caused by low molecular weight compounds is not clear.

In this study we found that guinea pigs developed hypersensitivity to PA after they received intraperitoneal injections of PA-ovalbumin with $Al(OH)_3$ monthly for several months. The optimal duration of sensitization was found to be about 6 months. Both specific IgG_1 and IgG_2 were produced. The levels of IgG_1 antibodies to ovalbumin were about two times higher than that of PA when passive cutaneous anaphylaxis assays were used and 1.5 times higher when the ELISA technique was used.

The sensitization to PA was found to be specific. Although cells from guinea pigs sensitized to PA-ovalbumin released mediators such as histamine, leukotrienes, or prostaglandins when challenged with PA or PA-HSA, normal guinea pigs or guinea pigs sensitized to ovalbumin alone did not release mediators when challenged with PA. When tracheal tissues were incubated with sera of sensitized animals from which specific immunoglobulins were removed, they did not contract in response to antigen challenge, indicating that these immunoglobulins were important in this process. Because specific IgG_2 antibodies are not known to induce mediator release from lung fragments or from basophils, specific IgG_1 antibodies are likely to be involved. The positive passive cutaneous anaphylaxis studies confirmed that it was the specific IgG_1 antibodies that were responsible for the hypersensitivity reaction.

We demonstrated that there were no receptors for PA on the surface of the leukocytes of nonimmunized guinea pigs. Specific receptors were found for PA only on leukocytes of immunized guinea pigs. This finding indicates that the mediators released by lung fragments and leukocytes of sensitized guinea pigs were the results of an immunologic process rather than a direct action of PA.

In sensitized animals we found that the response of the trachea to PA was about three times

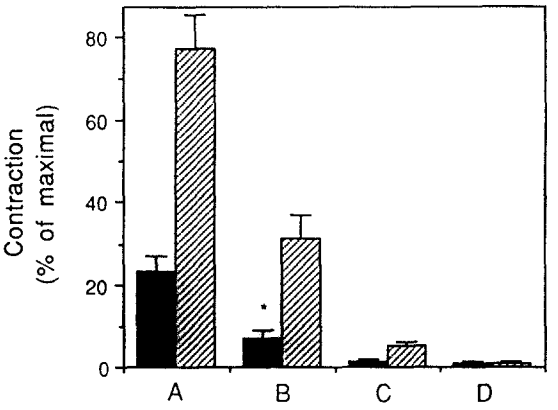

FIG. 8. Effects of PA (■) or ovalbumin (▨) on the contraction of tracheal tissue from PA-ovalbumin–sensitized guinea pigs **(A)**, on passively sensitized normal tracheal tissue **(B,)** on the tracheal tissue exposed to serum depleted of immunoglobulins **(C)**, and on normal tracheal tissue **(D)** are shown. Data are means ± SD ($n = 5$). $*p < 0.05$.

less than the response of the trachea to ovalbumin. Similarly, the release of mediators from lung fragments and leukocytes of sensitized guinea pigs was higher when challenged with ovalbumin than with PA. This could partly be accounted for by the higher levels of specific IgG_1 antibodies against ovalbumin rather than against PA and by the fact that ovalbumin is a multivalent protein allergen, whereas PA is a univalent hapten.

When a hapten is being inhaled, it combines with one of the proteins in the airway to form a complete antigen. This complete antigen stimulates the body to produce antibodies, not only against the hapten-protein conjugate but also to the carrier protein and possibly any NAD that might be formed as a result of the process of conjugation. However, this study does not address whether sensitization has occurred to the NADs and whether antibodies to NADs can elicit mediator release or tracheal contractions in PA-ovalbumin–sensitized animals. Comparison of mediator release after challenge with PA-ovalbumin versus PA alone in PA-ovalbumin–sensitized guinea pigs would be helpful in answering this question. This animal model can be used to delineate the importance of NADs in the pathogenesis of asthma caused by low molecular weight compounds.

In conclusion, the present work demonstrated that the reaction in sensitized guinea pigs to PA is an immunologic reaction similar to those for high molecular weight allergens. We confirmed that PA does not bind directly to basophils to cause

mediator release. Specific binding of labeled PA was only seen on leukocytes from sensitized animals, indicating that the binding was due to the presence of specific immunglobulin receptors for PA. In this model specific immunoglobulins were directed not only to the hapten but also to the carrier protein. The allergic response to the carrier protein was more severe than the allergic response to the hapten. The specific immunoglobulin responsible for the allergic reaction is likely to be IgG_1.

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