

Influence of indomethacin and L-cysteine on histamine and peptidoleukotriene release from superfused tracheas taken from guinea pigs passively sensitized with IgG1 and IgE antibodies

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We have previously reported differences in mediator release during equivalent levels of antigen (Ag)-induced smooth muscle contraction of guinea pig pulmonary tissues after passive sensitization with IgG1 versus IgE antibodies (Abs). In the present study, we have examined the influence of indomethacin (5×10^{-6} mol/L) and L-cysteine (3 or 10 mmol/L) on mediator release from superfused trachea taken from guinea pigs passively sensitized with IgG1 or IgE Ab 1 day before in vitro studies. Tissues were challenged with Ag (oxazolone-human serum albumin conjugate), and contractions and superfusate histamine and peptidoleukotrienes were monitored at discrete time intervals thereafter. Superfusate mediator contents were determined by spectrophotofluorimetry (histamine) and RAST (peptidoleukotrienes). The profiles of peptidoleukotrienes were examined with high-pressure liquid chromatography. At equivalent levels of contraction, significantly less histamine and peptidoleukotrienes were found in superfusate samples after sensitization with IgE Abs. None of the drug pretreatments significantly altered Ag-induced histamine release after IgG1 or IgE sensitization. Indomethacin resulted in an increase in total measurable peptidoleukotrienes found only after IgG1 receptor activation, but it did prolong tracheal contractions with both Abs. L-cysteine, 10 mmol/L, resulted in an increase in total measurable superfusate peptidoleukotriene content under all experimental conditions. The percentage increase in peptidoleukotriene content from that found without drug pretreatment was larger in the case of IgE compared to IgG1 sensitization. During early time periods, after Ag challenge, measurable peptidoleukotriene levels in superfusate samples were similar for both Abs in the presence of L-cysteine, 10 mmol/L. These data suggest that there is a differential pattern of peptidoleukotriene metabolism after activation of IgG1 versus IgE receptors in guinea pig trachea. (J ALLERGY CLIN IMMUNOL 1991;87:1150-60.)

IgE is the principal Ab involved in Ag-provoked mediator release from the human lung, and this mediator-release process appears important in the

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Abbreviations used

Ag:	Antigen
Ab:	Antibody
LTE ₄ , LTC ₄ , LTD ₄ :	Leukotrienes E ₄ , C ₄ , and D ₄
Ox:	Oxazolone
Asc:	Ascaris
i.p.:	Intraperitoneal
GPA:	Guinea pig albumin
PBS:	Phosphate-buffered saline
PCA:	Passive cutaneous anaphylaxis
HSA:	Human serum albumin
HPLC:	High-pressure liquid chromatography
RIA:	Radioimmunoassay
AA:	Arachidonic acid

pathogenesis of allergic asthma.¹⁻⁵ The guinea pig has long been used as a model for human asthma because the airways from the two species respond in a similar way to a variety of contractile and relaxant substances.³ Furthermore, Ag-induced contraction of airway smooth muscle from the two species has been suggested to involve similar primary mediators, namely, histamine and peptidoleukotrienes (slow-reacting substance of anaphylaxis⁴⁻⁸). A major difference is that IgG1 is the predominant homocytotropic Ab in the guinea pig.⁹⁻¹¹ We have previously reported, however, that the guinea pig is capable of producing an IgE Ab and that these animals can be passively sensitized for Ag-induced pulmonary smooth muscle contraction with either homologous IgG1 or IgE Ab.^{12, 13} These studies also provided evidence for the existence of separate Fc receptors for IgG1 and IgE in guinea pig pulmonary tissues. An additional study demonstrated that smaller amounts of histamine and peptidoleukotrienes (slow-reacting substance of anaphylaxis bioactivity) were released by Ag after IgE than after IgG1 sensitization, even though activation of the two Ab receptors resulted in equal magnitudes of pulmonary smooth muscle contraction.¹⁴

Our present study was designed to study further the differences observed in mediator release on activation of the two Ab receptors. We examined the hypotheses that the differences in mediator release were due to (1) greater AA metabolism via the cyclooxygenase pathway and/or (2) a greater degree of metabolism in the peptidoleukotriene cascade leading to LTE_4 (less bioactive and immunoreactive than LTC_4 and LTD_4) as a result of activation of the IgE Ab receptor. For these experiments, indomethacin was used as an inhibitor of the cyclooxygenase pathway of AA metabolism and L-cysteine as an inhibitor of the aminopeptidase involved in the degradation of LTD_4 to LTE_4 .

MATERIAL AND METHODS

Preparation of hapten-protein conjugates

Preparation of hapten-protein conjugates was performed as detailed previously.^{12, 13} Briefly, in the preparation of the hapten-protein conjugate Ox-Asc, 10 ml of an Asc protein extract (10 mg/ml) was adjusted to pH 9.0 at 25° C by addition of 5% Na_2CO_3 . Then, 0.5 ml of 10% Ox (Gallard Schlesinger Chemical Manufacturing Co., Carle Place, N.Y.) in dioxane (Fischer Scientific Co., Pittsburgh, Pa.) was added dropwise, whereas the pH was maintained at 9.0 by addition of 5% Na_2CO_3 . After 2 hours of stirring, the mixture was dialyzed extensively against PBS, pH 7.4. The dialyzed material was concentrated by negative pressure filtration, and the protein was determined by the micro-Kjeldahl's method. The Ox-Asc conjugate contained 5×10^{-7} mol of hapten per milligram of protein. Ox was conjugated to HSA and GPA in a similar manner to that described above. Ox-Asc and Ox-GPA were used for the

production of IgE and IgG1 anti-Ox antibody, respectively (see below). The hapten-protein conjugate Ox-HSA was used as antigen to detect anti-Ox Ab responses.

Immunization procedures

Serum rich in IgE Ab to the hapten-protein conjugate, Ox-Asc, was obtained with techniques described previously.¹² Briefly, six to eight outbred female Hartley albino guinea pigs (Bio-Lab, St. Paul, Minn.), weighing approximately 300 gm, received i.p. injections of 250 mg/kg of cyclophosphamide (Mead Johnson Co., Evansville, Ind.) 2 days before primary i.p. immunization with 1 μg of conjugate (Ox-Asc) adsorbed to 1 mg of $\text{Al}(\text{OH})_3$ (alum) in 1 ml of normal saline. Every month thereafter for 5 months, a similar dose of Ag in alum was administered i.p. Seven days after Ag injection in months 3, 4, and 5, the animals were bled daily for 3 days by cardiac puncture. At the end of 5 months, the animals were exsanguinated. The sera obtained from the bleedings were stored in aliquots at -70°C until time of use.

Serum rich in IgG1 antibody to Ox-GPA was obtained with the immune-deviation procedure as described previously.¹⁵ Briefly, separate groups of guinea pigs were administered a primary i.p. immunization with 10 μg of the Ag in alum (1 mg). Two weeks later, 50 μg of Ag emulsified within complete Freund's adjuvant was injected into the four footpads with care not to cause excessive pain or reaction. Every 2 weeks for 2 months thereafter, 50 μg of Ag in incomplete Freund's adjuvant was injected subcutaneously in multiple sites on the animals. Seven days after the last injection, the animals were bled daily for 5 days by cardiac puncture. On the sixth day, the animals were exsanguinated. Serum obtained from the bleedings were stored at -90°C until time of use. Our protocol was approved by the Animal Care Committee at the University of Wisconsin that follows the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences.

Affinity column chromatography

In the current study, all IgG Ab was removed from guinea pig serum by passage through a column of protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N.J.) with a modification according to the method of Martin.¹⁶ Protein A-Sepharose was equilibrated in pH 7.3 citrate (0.1 mol/L)-phosphate buffer (0.2 mmol/L), and 10 to 20 ml of the material was packed into a column. Serum to be adsorbed of IgG Ab was applied (underloaded according to predetermined conditions) to this column and allowed to incubate in the column bed for 35 to 45 minutes at 4°C . At the end of this time period, the column was washed with the equilibrating buffer. The eluted serum was concentrated with negative pressure filtration and dialyzed against PBS. IgG Ab was desorbed from the protein A affinity column with 0.1 mol/L of citric acid, pH 2.1. This material was concentrated and dialyzed against PBS.

Quantitation of guinea pig IgG1 and IgE Ab

A quantitative estimation of IgG1 and IgE anti-Ox Ab in guinea pig serum was obtained with PCA. In this assay,

IgG1 Ab PCA activity is heat stable and can be detected in guinea pig skin for up to 5 days.¹⁷ IgE Ab PCA activity is heat sensitive and can be detected in guinea pig skin for up to 21 days.^{12, 18}

PCA Ab titrations were performed as described by Ovary.¹⁹ In this procedure, 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 gm guinea pigs (done in triplicate). A sensitization period of 4 hours or 10 days was used, and PCA reactions were developed by injecting, intravenously, 1 ml of 0.5% Evans blue containing 1 mg of Ag (Ox-HSA). The PCA titer was taken as the highest dilution of antiserum eliciting a 6 × 6 mm blue reaction on the reflected skin surface in at least two of three recipients. Heat sensitivity of the PCA Ab activity was determined by incubating antisera at 56° C for 4 hours before making dilutions for the PCA titration.

Estimation of IgG1 Ab remaining in IgE-rich serum, passed through a protein-A Sepharose affinity column, was obtained with immunodiffusion and immunoelectrophoresis in agar (1% purified Difco certified, Difco Laboratories, Detroit, Mich.) with heavy chain-specific rabbit antiguinea pig IgG1 obtained as described previously.¹³ We have previously demonstrated (by ELISA) that <20 ng/ml of IgG1 Ab remains in affinity column-adsorbed IgE-rich serum.¹³

Sensitization of pulmonary tissue mediator release and contraction

Female Hartley albino guinea pigs, weighing 250 to 350 gm, were passively sensitized for specific Ag challenge. Passively sensitized animals received intravenous injections of Ab-rich serum 1 day before being killed for study. Passive sensitization was achieved by administration of (1) 0.5 ml/kg of serum rich in IgG1 Ab directed against Ox-GPA (anti-Ox IgG1; PCA titer, 2560) or (2) 2.5 ml/kg of protein A-adsorbed serum devoid of IgG1 Ab and containing IgE Ab directed against Ox-Asc (anti-Ox IgE; PCA titer, 160 to 640). These "standard" doses of the anti-hapten IgG1 and IgE serums were selected on the basis of preliminary experiments in which they were found to sensitize the guinea pig trachea for similar magnitudes of contractions induced by a maximally effective concentration (0.1 mg/ml of Ox-HSA). In all cases, the doses of sera administered were optimal for contraction and mediator release. Doses of serum higher than these levels did not elicit further contraction or mediator release.^{13, 14}

Tracheas were trimmed of excess tissue and cut in a spiral fashion.²⁰ Tracheal spirals were suspended in air-filled, water-jacketed tissue chambers and superfused²¹ with a Krebs bicarbonate solution of the following composition (millimolar): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 0.5; NaH₂PO₄, 1; NaHCO₃, 25; and glucose, 11. The solution was gassed with a mixture of oxygen (95%) and carbon dioxide (5%) and pumped from a heated (37.5° C) reservoir through tygon tubing to a water-jacketed, coiled, glass tube (heat exchanger) with a Gilson Minipuls II peristaltic pump (Gilson Medical Electronics, Middleton, Wis.). From the heat exchanger, the physiologic salt solution su-

perfused the tissues at a rate of 1.5 ml/min. The tracheal spirals were maintained at initial tensions of 5 g for a 90-minute equilibration period before Ag challenge.¹⁴ Changes in tension were recorded via force transducers (FT-03) on Grass model 5D or 79B polygraphs (Grass Instruments, Quincy, Mass.). Histologic examination of selected trachea demonstrated that the epithelium was 85% to 95% intact with these procedures.

After equilibration, all tissues were challenged with Ox-HSA dissolved in the superfusion solution. Superfusate samples were collected at the base of the tissue chamber, and each collection tube contained an appropriate amount of gelatin to make 0.1% at the end of the collection period. Superfusate samples were collected for a 2-minute period before (spontaneous release) and continuously from the beginning of challenge with Ox-HSA. Histamine and peptidoleukotrienes were not found in spontaneous-release samples. During Ag challenge, consecutive time intervals of superfusate collection were for 5, 10, and 5 minutes, with additional 5-minute collection intervals as needed. The maximum duration for Ag challenge and superfusate collection was 45 minutes. After collection, the superfusate samples were placed in an ice bath and stored at 4° C until samples were analyzed for histamine and peptidoleukotrienes (see below).

Tracheal tension was monitored throughout the experiment and quantified at 1-minute intervals during Ag challenge. Peak changes in tension were determined independent of time interval. At the end of Ag challenge, trachea were superfused with carbachol, 10⁻³ mol/L, to elicit the maximum contractile response from each tissue. Responses to Ox-HSA were expressed as a percentage of the maximum contractile response in each tissue. Ox-HSA did not cause contraction of tissues taken from unsensitized animals.

At the end of each experiment, the trachea was weighed and placed in 3 ml of 0.4 N perchloric acid, and the tubes were placed in a boiling water bath for 15 minutes before homogenization. After centrifugation, the supernatant was analyzed for histamine content. There were no statistical differences in total histamine content between tissues in any of the experimental groups and, for 60 tissues, averaged 13.5 ± 0.4 µg/gm of wet weight.

Histamine assay

Histamine content of each sample was determined by an automated continuous-flow extraction (with dialyzer) and fluorometric analyzer as described by Siraganian.²² The sensitivity of the assay is approximately 5 ng/ml of histamine. The amount of histamine in each superfusate sample was expressed as a percentage of the total tissue histamine content remaining after the preceding collection period. In preliminary studies, it was found that the Ag and drugs did not elicit nonspecific fluorescence or quenching of histamine standard solutions.

Peptidoleukotriene RIA

The peptidoleukotriene content of each superfusate sample was determined by RIA as described previously.²³ Ab (No. 332; percent cross-reactivity: LTC₄, 145; LTD₄,

100; and LTE_4 , 41) was diluted in buffered saline (5 mmol/L maintenance electrolyte solution and *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid adjusted to pH 7.4 with 1 N of NaOH) containing 0.1% gelatin. Ab, 50 μl of the appropriate dilution (1:1000), was added to 100 μl of sample and mixed with 50 μl (2500 to 3000 cpm) of ^3H -LTD $_4$ diluted with buffered saline. Incubations were performed at 4° C for 2 hours, and reactions were terminated by addition of 0.5 ml dextran-coated charcoal (200 mg of charcoal and 20 mg of dextran mixed with 100 ml of buffered saline). After 5 minutes, the mixture was centrifuged at 2000 *g* for 5 minutes at 4° C. A volume of 0.4 ml of the supernatant was added to 5 ml of Aquasol (NEN Research Products, Boston, Mass.) and counted by liquid scintillation spectrometry (model 3255, Packard Instrument Co., Downers Grove, Ill.). Standard curves were constructed in the presence of the drugs and Ag with LTD $_4$ (4.5×10^{-10} to 1×10^{-7} mol/L). The detection limit was 0.045 pmol of LTD $_4$.

Peptidoleukotrienes HPLC

A $\mu\text{Bondapak C}_{18}$ reverse-phase column (3.9 mm $\times$ 30 cm, 10 μm particles), solvent-delivery system, model 660 solvent programmer, equipped with model M6000 pump (A pump) and model 45 pump (B pump), model U6K injector, and model 450 ultraviolet detector were obtained from Waters Associates (Milford, Mass.). The chromatographic profiles were recorded on an Omniscrite recorder (Houston Instrument, Austin, Texas).

The superfusate samples for HPLC were processed by Sep-Pak C $_{18}$ cartridge (Waters Associates). The remainder of all superfusate samples from each tissue type used in a single experimental protocol were pooled and passed through Sep-Pak cartridges that were prewashed with 100% methanol and equilibrated in distilled water. After samples were washed with 10 ml of 10% ethanol (ethanol:water, 1:9 vol/vol) to eliminate contaminating substances, the samples were eluted with 2 ml of 100% methanol. The eluates were dried under nitrogen gas. The residues were dissolved in 250 μl of methanol/water (65/35) and centrifuged at 10,000 *g* for 5 minutes in a microcentrifuge. This solution (30 to 150 μl) was injected into the HPLC column.

The peptidoleukotrienes in each sample were analyzed by a modification of a procedure described previously.²⁴ The reconstituted and clarified eluates from Sep-Pak were chromatographed by linear-gradient elution. Two elution buffers were used: (1) buffer A: methanol/water/ acetic acid, 60/40/0.02 (vol/vol/vol), adjusted to pH 5.7 with 10% NH_4OH , and (2) buffer B: 100% methanol. These solvents were filtered by Millipore filter (Millipore Corp., Bedford, Mass.) and degassed before use.

Before sample application, the column was equilibrated with buffer A and 10% buffer B (initial condition, 10%). After sample injection, the separation of peptidoleukotrienes was effected by a linear 20-minute gradient elution to 70% buffer B (final condition, 70%). The flow rate was 1 ml/min. The ultraviolet detector wavelength was set at 280 nm to monitor peptidoleukotriene elution.

Standard solutions were prepared from methanolic stock

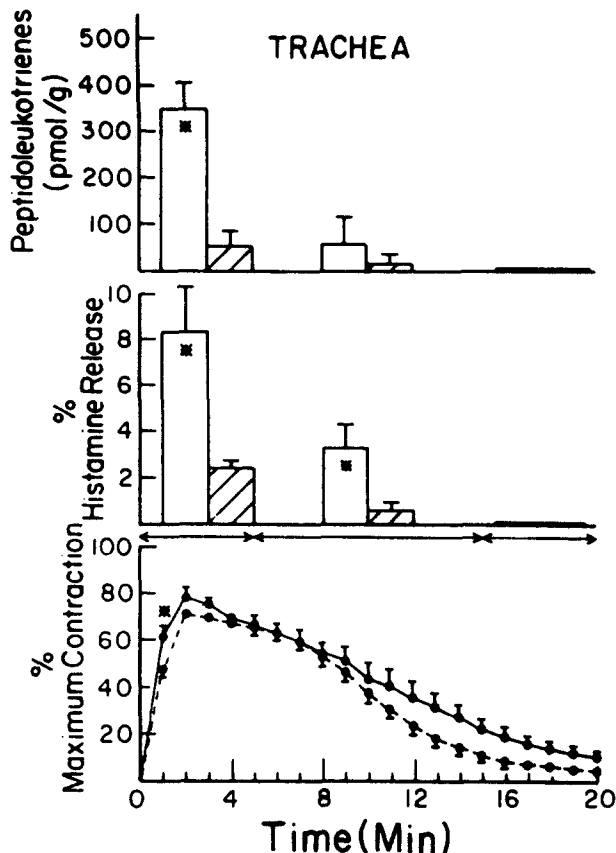


FIG. 1. Time course for tracheal contractions and superfusate histamine and peptidoleukotriene contents after challenge with Ox-HSA, 0.1 mg/ml, in the absence of drug pretreatment. Animals were passively sensitized with IgG1 (solid lines, open bars) or IgE (dashed line, hatched bars) Abs 1 day before each experiment. Horizontal arrows beneath the histamine release portion indicate superfusate collection periods. Vertical lines indicate SEM and are not illustrated where they lie within the limits of the symbol or overlap another. An asterisk denotes a value obtained in the IgG1-sensitized state that is statistically different ($p < 0.05$) from value obtained in the IgE-sensitized state during the same collection interval or time point after Ag challenge. These data are summarized in Table I. Peak percentage contractile responses produced by Ox-HSA were $78\% \pm 3\%$ and $71\% \pm 2\%$ with IgG1 and IgE sensitization, respectively. The respective maximum contractile responses to carbachol, 10^{-3} mol/L, were 47 ± 8 and 42 ± 5 gm/gm of wet-weight tissue.

solutions of the peptidoleukotrienes stored at -70°C under argon. Quantitation was based on comparative peak area measurements between standards and samples. The peak area was obtained by the product of peak height and the width at the midpoint of the peak. Retention times for LTC $_4$ (0.51 nmol), LTD $_4$ (0.39 nmol), and LTE $_4$ (0.72 nmol) were 9.5, 13, and 15 minutes, respectively.

Analyses

Means and standard errors of the means were calculated for values obtained in each experimental protocol. Differ-

TABLE 1. Ag-induced responses of superfused trachea after passive sensitization of guinea pigs with IgG1 or IgE antibody*

Drug pretreatment† Ab	% Total histamine release‡ (±SEM)	Total peptidoleukotriene release (pmol/g)§ (±SEM)	% Total peptidoleukotriene			
			LTC ₄	LTD ₄	LTE ₄	N¶
None						
IgG1	11.6 ± 2.9¶	407 ± 57¶	14	10	76	6
IgE	3.0 ± 0.6	69 ± 33	12	2	87	6
Indomethacin, 5 × 10 ⁻⁶ mol/L						
IgG1	21.6 ± 5.4¶	684 ± 45¶#	11	9	80	6
IgE	6.6 ± 2.1	136**	17	1	82	6
L-cysteine, 3 mmol/L						
IgG1	13.3 ± 2.3¶	753 ± 111¶#	6	27	68	6
IgE	4.3 ± 0.9	213 ± 97	39	1	60	6
L-cysteine, 10 mmol/L						
IgG1	20.7 ± 2.2¶	1970 ± 228¶#	34	60	7	6
IgE	3.3 ± 0.9	1030 ± 122	33	31	36	6

N, Number of animals.

*Animals were passively sensitized by intravenous injection of anti-Ox IgG1 or IgE Ab 1 day before removing tissues for study.

†Drugs, at each indicated concentration, were added to the superfusion solution and in contact with the tissues from the time of extirpation.

‡Values represent total histamine found in all superfusate samples after antigen (Ox-HSA) challenge expressed as a percentage of the total tissue histamine content.

§Values represent total peptidoleukotrienes found by RIA in all superfusate samples after Ag challenge expressed as picomoles per gram of wet-weight tissue.

||Values represent the percentage of each peptidoleukotriene in the total peptidoleukotrienes determined by HPLC in pooled residual superfusate samples for each experimental protocol.

¶A value that is statistically different ($p < 0.05$) from the value obtained after IgE sensitization in the same experimental protocol.

#A value that is statistically different ($p < 0.05$) from the value obtained with the same Ab in the absence of drug pretreatment.

**Total peptidoleukotrienes found in superfusate samples from one trachea after Ag challenge. Peptidoleukotrienes were not detected in any of the superfusate samples from the other tissues studied in this group.

ences between means were determined with analysis of variance and Student's *t* test for unpaired samples. A *p* value of <0.05 was considered statistically significant.

Drugs and solutions

The following substances were used: histamine free base, maintenance electrolyte solution, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, indomethacin, L-cysteine (Sigma Chemical Co., St. Louis, Mo.), carbamylcholine chloride (carbachol, Aldrich Chemical, Inc., Milwaukee, Wis.), gelatin (Difco Laboratories), methanol (HPLC grade, Aldrich Chemical Co. Inc.), LTC₄, LTD₄, LTE₄, and leukotriene Ab for RIA (Stuart Pharmaceuticals, Division of ICI Americas, Inc., Wilmington, Del.), and ³H-LTD₄ (specific activity, 39 Ci/mmol, New England Nuclear, Boston, Mass.). All drug solutions were prepared on the day of each experiment. Indomethacin was dissolved in 95% ethanol and diluted 10,000-fold in the physiologic salt solution used for tissue superfusion. All other drugs were prepared in 0.9% sodium chloride and diluted or dissolved directly in the superfusion solution. When this is indicated, tissues were exposed to indomethacin or L-cysteine from the time of extirpation. The con-

centrations of indomethacin and L-cysteine were selected on the basis of demonstrated effects in inhibiting release of prostaglandins²⁵ and formation of LTE₄ from LTD₄,²⁶ respectively, in guinea pig airways. In the current experiments, indomethacin (5 × 10⁻⁶ mol/L) resulted in virtually complete inhibition of tracheal prostaglandin D₂ release after activation of both IgG1 and IgE Abs (RASTs conducted by F. Atkinson, Jr., and B. J. Undem, The Johns Hopkins University; data not presented). In addition, nordihydroguaiaretic acid 10⁻⁴ mol/L, a lipoxygenase pathway inhibitor, abolished immunoreactive leukotriene release after IgG1 (*n* = 2) and IgE (*n* = 2) activation. These data would suggest that immunoreactive leukotrienes were being measured (by RIA) in nonnordihydroguaiaretic acid-treated superfusate samples.

RESULTS

IgG1- and IgE-mediated pulmonary responses

Consistent with our previous findings,¹⁴ exposure to Ag (Ox-HSA), 0.1 mg/ml, resulted in tracheal contraction and mediator (histamine and peptidoleu-

kotriene) release after guinea pigs were sensitized with either IgG1 or IgE Abs (Fig. 1; Table I).

Contractile responses during most collection periods and peak contractions (independent of time after challenge) were similar for the two Abs. However, the magnitude of histamine and peptidoleukotrienes released was consistently smaller after sensitization with IgE. Peptidoleukotrienes were detected in superfusate samples from only three of the six tracheas examined after IgE sensitization.

Effects of indomethacin

In the presence of indomethacin, 5×10^{-6} mol/L (Fig. 2; Table I), peak tracheal contractions were not altered. Rather, indomethacin pretreatment resulted in a prolongation of the Ag-induced contraction for both Abs.

Total measurable peptidoleukotriene release was increased by indomethacin pretreatment after IgG1, but not after IgE sensitization. With this experimental condition, peptidoleukotrienes were detected by RIA in superfusate samples from only one of the six tracheas examined after IgE sensitization. Total histamine release was not significantly altered by indomethacin after sensitization with either Ab.

Effects of L-cysteine

Neither concentration of L-cysteine (3 or 10 mmol/L) produced a dramatic alteration in the pattern of tracheal contraction produced by Ox-HSA after sensitization with IgG1 or IgE. Generally, except for later time periods after tracheal challenge, contractile amplitudes were only slightly smaller after IgE versus IgG1 sensitization (Figs. 3 and 4). Neither concentration of L-cysteine produced a significant change in total histamine release or the pattern of histamine release from trachea with IgG1 or IgE sensitization (Table I).

With the smaller concentration of L-cysteine (3 mmol/L), a significant increase in measurable superfusate peptidoleukotrienes was observed only for the tracheas sensitized with IgG1. However, there were significantly larger amounts of measurable peptidoleukotrienes found in all cases in the presence of L-cysteine, 10 mmol/L. In the case of IgG1 sensitization, this was primarily related to the presence of measurable peptidoleukotrienes in superfusate samples collected during later time intervals after challenge (Fig. 4). With L-cysteine, 10 mmol/L, much larger amounts of measurable peptidoleukotrienes were found in all early (first 25 minutes) superfusate samples collected after IgE sensitization. During the first three collection periods (20 minutes total), su-

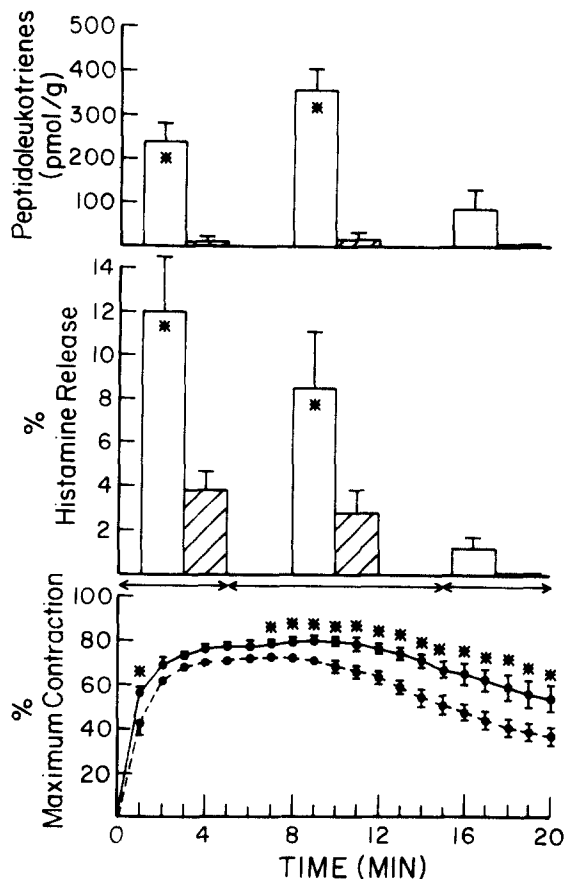


FIG. 2. Time course for tracheal contraction and superfusate histamine and peptidoleukotriene contents after challenge with Ox-HSA, 0.1 mg/ml, in the presence of indomethacin, 5×10^{-6} mol/L. Animals were passively sensitized with IgG1 (solid lines, open bars) or IgE (dashed lines, hatched bars) Abs 1 day before each experiment. Horizontal arrows beneath the histamine release portion indicate superfusate collection periods. Vertical lines indicate SEM and are not illustrated where they lie within the limits of the symbol or overlap another. An asterisk denotes a value obtained in the IgG1-sensitized state that is statistically different ($p < 0.05$) from value obtained in the IgE-sensitized state during the same collection interval or time point after Ag challenge. These data are summarized in Table I. Peak percentage contractile responses produced by Ox-HSA were $80\% \pm 2\%$ and $72\% \pm 2\%$ with IgG1 and IgE sensitization, respectively. The respective maximum contractile responses to carbachol, 10^{-3} mol/L, were 65 ± 7 and 53 ± 4 gm/gm of wet-weight tissue.

perfusate peptidoleukotriene contents were not statistically different for IgG1 and IgE sensitization (Fig. 4). The measurable peptidoleukotriene contents of superfusate samples collected thereafter declined more rapidly in the case of IgE sensitization. When L-cysteine-treated groups were compared with no drug pretreatment groups, L-cysteine, 10 mmol/L, resulted

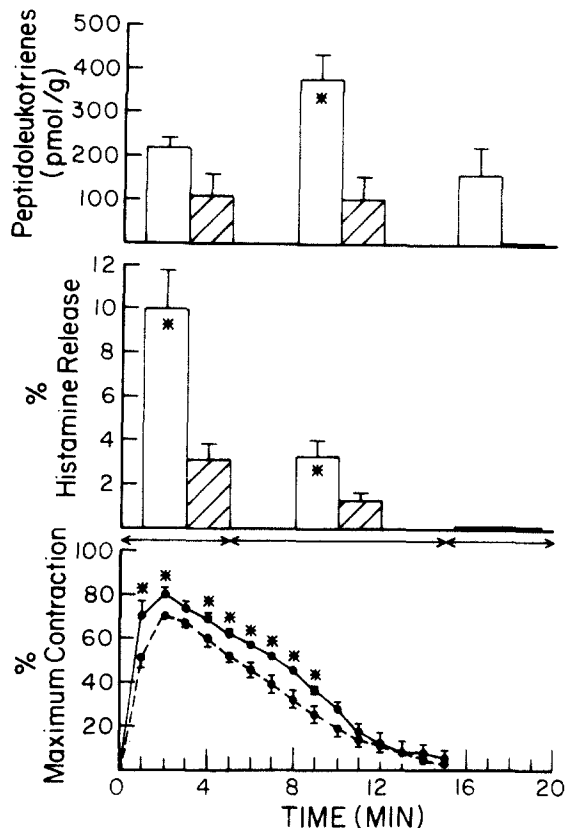


FIG. 3. Time course for tracheal contraction and superfusate histamine and peptidoleukotriene contents after challenge with Ox-HSA, 0.1 mg/ml, in the presence of L-cysteine, 3 mmol/L. Animals were passively sensitized with IgG1 (solid lines, open bars) or IgE (dashed lines, hatched bars) Abs 1 day before each experiment. Horizontal arrows beneath the histamine release portion indicate superfusate collection periods. Vertical lines indicate SEM and are not illustrated where they lie within the limits of the symbol or overlap another. An asterisk denotes a value obtained in the IgG1-sensitized state that is statistically different ($p < 0.05$) from value obtained in the IgE-sensitized state during the same collection interval or time point after Ag challenge. These data are summarized in Table I. Peak percentage contractile responses produced by Ox-HSA were $79\% \pm 3\%$ and $70\% \pm 1\%$ with IgG1 and IgE sensitization, respectively. The respective maximum contractile responses to carbachol, 10^{-3} mol/L, were 32 ± 2 and 32 ± 3 gm/gm of wet-weight tissue.

in a much larger percentage increase in total measurable peptidoleukotriene release with IgE (1393%) than with IgG1 (384%) sensitization. The profiles of superfusate peptidoleukotrienes found by HPLC are presented in Fig. 5 and Table I. The most obvious difference between the two Abs was the smaller relative percentage of LTD₄ in the case of IgE sensitization. From these results, L-cysteine, 10 mmol/L, appeared more effective in decreasing the relative percentage of LTE₄ found after IgG1 than after IgE sensitization.

DISCUSSION

We have previously demonstrated substantial differences in measurable histamine and peptidoleukotrienes released during similar levels of Ag-induced contraction in guinea pig pulmonary tissues after passive sensitization of the animals with IgG1 versus Abs.¹⁴ The results obtained in this study with selected pharmacologic pretreatments of the isolated tissues suggest that these differences may be related to a differential pattern of peptidoleukotriene metabolism by the trachea after Ag activation. This suggestion is supported by observations with L-cysteine, an inhibitor of aminopeptidase, to decrease conversion of LTD₄ to LTE₄. L-cysteine, 10 mmol/L, resulted in larger amounts of measurable peptidoleukotrienes in superfusate samples after sensitization with both IgG1 and IgE Abs. The percentage increase in measurable peptidoleukotrienes was larger after IgE than after IgG1 sensitization, and at early times after challenge with specific antigen, the peptidoleukotriene contents of superfusate samples were similar for both Abs. These results suggest that the apparently smaller superfusate peptidoleukotriene levels after IgE sensitization in the absence of L-cysteine may be related to more rapid conversion of LTD₄ to the less immunoreactive and hence less measurable LTE₄. However, it is possible that L-cysteine increases measurable peptidoleukotriene by mechanisms independent of inhibition of aminopeptidase activity.

The ability of L-cysteine to decrease the formation of LTE₄ was confirmed by HPLC of the residual superfusate samples. Even in the presence of L-cysteine, 10 mmol/L, there was smaller total (all superfusate samples) peptidoleukotrienes measured by RIA after IgE than after IgG1 sensitization (Table I). This finding was a reflection of the more rapid decline in immunoreactive peptidoleukotrienes after the former Ab (Fig. 4). Whether this is related to an apparently weaker ability of L-cysteine to decrease formation of LTE₄ after IgE Ab stimulation can not be ascertained from these studies.

It does not appear likely that differential cyclooxygenase product formation in response to Ag challenge is responsible for the smaller apparent histamine and peptidoleukotriene release after sensitization with IgE. Indomethacin pretreatment to inhibit the cyclooxygenase pathway of arachidonate metabolism resulted in increased immunoreactive peptidoleukotrienes in superfusate samples after IgG1 and not after IgE sensitization. However, contractile responses to Ag after both Abs were prolonged, but there was no change in the pattern of mediator release after IgE sensitization (Fig. 2). These observations suggest that the effect of indomethacin on tracheal contraction may be primarily related to an inhibition of the formation of

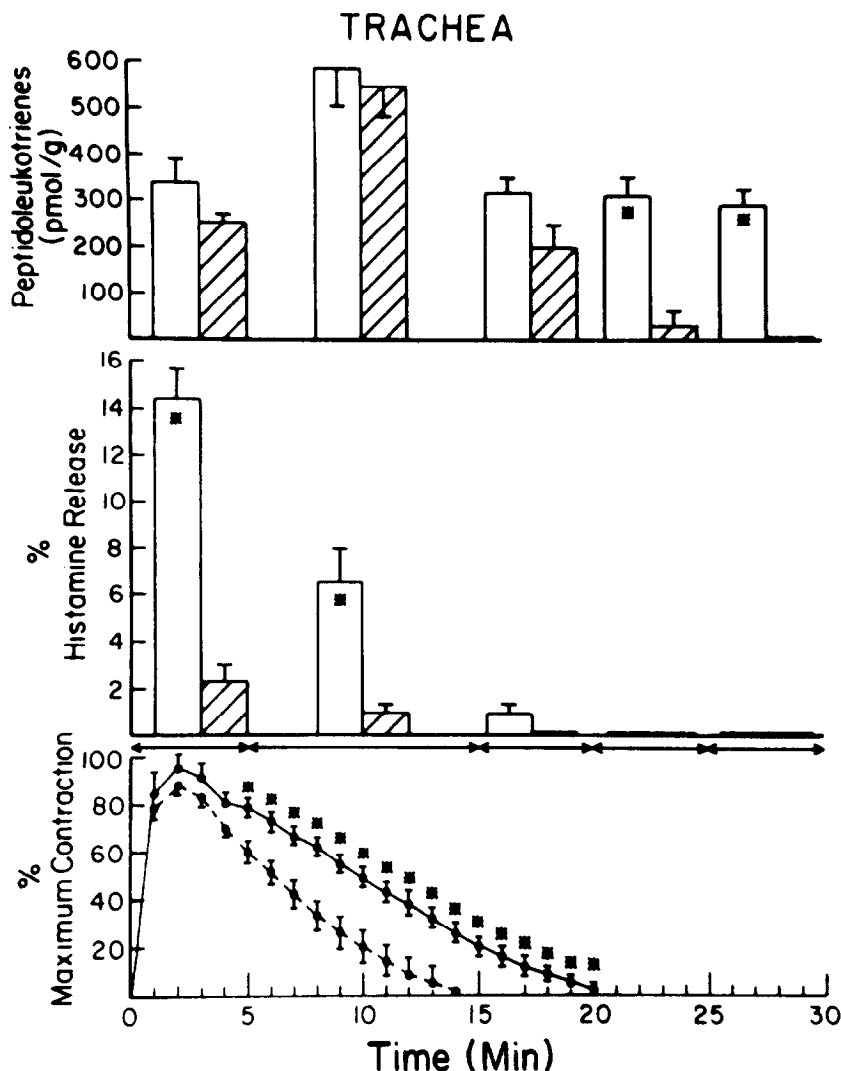


FIG. 4. Time course for tracheal contractions and superfusate histamine and peptidoleukotriene contents after challenge with Ox-HSA, 0.1 mg/ml, in the presence of L-cysteine, 10 mmol/L. Animals were passively sensitized with IgG1 (solid lines, open bars) or IgE (dashed lines, hatched bars) Abs 1 day before each experiment. Horizontal arrows beneath the histamine release portion indicate superfusate collection periods. Vertical lines indicate SEM and are not illustrated where they lie within the limits of the symbol or overlap another. An asterisk denotes a value obtained in the IgG1-sensitized state that is statistically different ($p < 0.05$) from value obtained in the IgE-sensitized state during the same collection interval or time point after Ag challenge. These data are summarized in Table I. Peak percentage contractile responses produced by Ox-HSA were $96 \pm 6\%$ and $88 \pm 4\%$ with IgG1 and IgE sensitization, respectively. The respective maximum contractile responses to carbachol, 10^{-3} mol/L, were 23 ± 3 and 28 ± 2 gm/gm of wet-weight tissue.

bronchodilating (functionally antagonistic) cyclooxygenase products.

The differential influences of indomethacin and, to a lesser extent, L-cysteine on peptidoleukotriene release after IgG1 versus IgE suggest that apparent quantitative differences in mediator release may not be the only feature distinguishing the two Abs. The present observations, coupled with results demonstrating separate Fc receptors for guinea pig IgG1 and IgE Abs

in lung tissue,¹³ suggest the possibility of differential regulation of release by activation of the two Ab receptors. This potential difference has not been extensively explored but is supported by observation that unrelated stimuli may result in differences in AA metabolism in basophils.²⁷

Alternatively, it is possible that these differences may be related to a differential degree of sensitization by IgG1 and IgE of separate mast cell population in

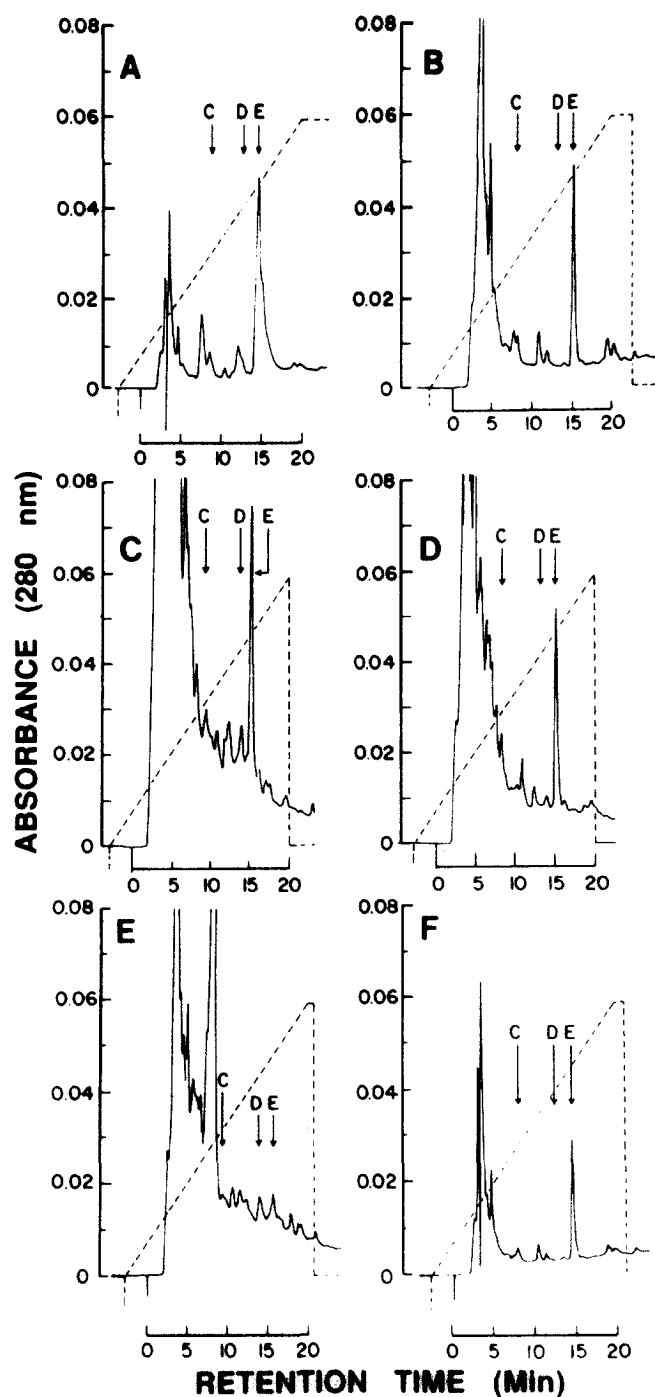


FIG. 5. Peptidoleukotriene (LTC_4 , LTD_4 , LTE_4 , C, D, and E, respectively) HPLC profiles from superfusate samples obtained from Ag (Ox-HSA) stimulation of IgG1 and IgE Ab sensitized tracheal tissue (with and without drug treatment). Each profile represents a pool of superfusate samples from six animals. **A**, Peptidoleukotriene profile from tracheal tissue passively sensitized with IgG1 anti-Ox Ab and stimulated with specific Ag. **B**, Peptidoleukotriene profile from tracheal tissue passively sensitized with IgE anti-Ox Ab and stimulated with specific Ag. **C**, Peptidoleukotriene profile from tracheal tissue sensitized and stimulated as in **A** and in the presence of indomethacin (5×10^{-6} mol/L). **D**, Peptidoleukotriene profile from tracheal tissue sensitized and stimulated as in **B** and in the presence of 5×10^{-6} mol/L of indomethacin. **E**, Peptidoleukotriene profile from tracheal tissue sensitized and stimulated as in **A** and in the presence of 10 mmol/L of L-cysteine. **F**, Peptidoleukotriene profile from tracheal tissue sensitized and stimulated as in **B** and in the presence of 10 mmol/L of L-cysteine.

guinea pig lung tissue. This suggestion is reasonable in light of numerous studies demonstrating biochemical and functional heterogeneity of mast cells from several sources.²⁸⁻³¹ Human lung appears to contain at least two populations of mast cells,³² and mediator release from these mast cells may also be differentially regulated.³³ In the guinea pig, there is a differential effect of histamine release exerted by d-tubocurarine in airways compared to peripheral lung, and it has been suggested that there is a "curare-sensitive" population of mast cells predominantly located at the former sites.³⁴ Whether this is the same population sensitized by IgE Ab remains to be determined. However, it is conceivable that a small, strategically located population of mast cells in the guinea pig airways is selectively sensitized by IgE Ab. On Ag challenge, substantial contraction might then be achieved by release of relatively smaller amounts of mediators. Since it is possible that peptidoleukotrienes are released from other cell types in guinea pig pulmonary tissues,³⁵⁻³⁸ a differential degree of activation of these cells might also account for differences between IgG1 and IgE activation in AA formation, action, and/or degradation.

More specific information about the roles of different potential mediators and modulators and the distribution of the relevant mast cells relative to the contractile elements will eventually be required to correlate mediator release to smooth muscle function.

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Facilitated referral to asthma specialist reduces relapses in asthma emergency room visits

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Facilitated asthma-specialist care delivered by allergists was compared to generalist care on the rate of relapse of asthma emergency room (ER) visits and hospitalizations and on asthma control in a prospective, controlled study of San Diego Kaiser Health Plan members with asthma. Subjects with asthma between the ages of 6 and 59 years presenting for acute ER care for asthma were systematically assigned by alternating, consecutively, the day of their ER visit to receive either (1) facilitated referral to an asthma specialist within the allergy department and concomitant comprehensive ongoing asthma care (intervention group, n = 149) or (2) continued outpatient management from generalist physicians (control group, n = 160). The course of their asthma was evaluated blindly during the subsequent 6 months by review of medical records, initial and follow-up questionnaires, and spirometry. Compared to the control group, the intervention group noted (1) a 75% reduction in the number of, and percent of, subjects with asthma awakenings per night ($p \leq 0.0001$), (2) an almost 50% reduction in asthma ER relapses ($p = 0.017$) resulting from a reduction in the frequency of multiple relapses ($p = 0.005$), and (3) a greater use of inhaled corticosteroids ($p < 0.00001$) and cromolyn ($p = 0.002$). Thus, facilitated referral of subjects with asthma to specialists in asthma therapy after acute ER therapy appears to reduce asthma ER relapses and to improve asthma outcome. (J ALLERGY CLIN IMMUNOL 1991;87:1160-8.)

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