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# Effects of Epithelium on the Mechanism of Mediator Release from Guinea pig Tracheal Tissues Sensitized by $IgG_1$ *versus* IgE Antibody*

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*In the present work, we have examined the effect of PAF, removal of epithelium, the mechanism of desensitization, and the substances that increases the level of intracellular c-AMP on the differences of mediator release from superfused tracheal strips after passive sensitization with $IgG_1$ versus IgE Ab. In the passive sensitized tracheal tissues, the effect of PAF and the mechanism of desensitization have been examined by PAF antagonist, CV 3988 and DFP, respectively. The epithelium was stripped from one-half of each trachea by mechanical means. Both superfused tracheal tissues were challenged with Ox-Ag. Inhibitors of mediator release were added into a superfused buffer. Hist released was determined by spectrophotofluorometer, and LT by radioimmunoassay. PAF known to mediate the allergic reaction was not released by Ag after both Ab sensitization. Epithelium removal resulted in similar contraction, Hist and LT release after $IgG_1$ Ab activation, but in the IgE Ab activation, epithelium removal resulted in smaller contraction and Hist release. In the L-cysteine and indomethacin pretreatment after two Ab sensitization, epithelium removal decreased the release of Hist and LT. The compound 48/80 pre-challenge and epithelium removal resulted in the increase of Hist release, but in the decrease of LT release after $IgG_1$ or IgE sensitization. The Amount of LT released by Ag after compound 48/80 pre-challenge increased in the absence or presence of epithelium after both Ab sensitization. Mediator release from tissues sensitized with both Abs was not changed by DFP. The responses of inhibitors to prevent the mediator release were more effective on the IgE Ab than on the $IgG_1$ Ab sensitization.*

*These studies suggest that the tracheal epithelium can act to inhibit immune- and non-immune-induced airway responses. Non-immunological responses may in part reflect the role of epithelium as a diffusion barrier and modulator of mediator release. These data also suggest that immunological responses are related to the localization and functional heterogeneity of tissue mast cells.*

**Key Words:** $IgG_1$ (Immunoglobulin G), IgE (Immunoglobulin E), Epithelium, histamine, leukotrienes.

Abbreviations were used in this paper: Ox-HSA, oxazolone-human serum albumin; Ox-Asc, oxazolone-ascaris; Hist, histamine; LT, leukotrienes; Ag, antigen; Ab, antibody; NDGA, nordihydroguaiaretic acid; DFP, Diisopropylfluorophosphonate; PAF, platelet-activating factor; EpDRF, epithelium-derived relaxing factor[1].

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In the guinea pig pulmonary tissues, the existance of separate Fc receptors for $IgG_1$ and IgE Ab have previously been reported (Graziano *et al.* 1981; Graziano *et al.* 1984). We have previously reported that Hist and LT were released during Ag-induced smooth muscle contraction of guinea pig pulmonary tissues after passive sensitization with $IgG_1$ or IgE Ab (Graziano *et al.* 1984; Ro *et al.* 1991).

Heterogeneity of mast cells has been well characterized for rats in some sites (Shanahan *et al.* 1984; Shanahan *et al.* 1985; Befus *et al.* 1985; Schwartz *et al.* 1987).

Platelet activating factor (PAF) is a phospholipid that was first derived from rabbit basophils upon IgE-mediated immunological challenge (Benveniste *et al.* 1972). It has been reported that PAF causes a long lasting, nonspecific increase in bronchial responsiveness in experimental animals (Mazzoni *et al.* 1985; Barnes *et al.* 1987; Robertson *et al.* 1988). Recently, it was shown that PAF increased the in vivo responsiveness of dog airway smooth muscle to parasympathetic stimuli (Bethel *et al.* 1989). It has also been demonstrated that airway hyperresponsiveness elicited by PAF results from regional stimulation and the release of mediators that augment contractility of airway smooth muscle (Tashiro *et al.* 1992).

It has also been established that the contractile activity of tracheobronchial smooth muscle is influenced by the presence of the epithelial layer (Hogg and Eggleston 1984). The epithelium has the potential to modulate pharmacological responses of the underlying smooth muscle through a number of different mechanisms. These include 1) a physical barrier that restricts movement of pharmacological agents across the wall (Yang *et al.* 1991), 2) metabolizing peptides and modulating reactivity to vasoactive intestinal polypeptide, tachykinins, and endothelin (Tschirhart *et al.* 1989; Farmer and Togo, 1990), 3) release of pharmacological mediators, such as prostaglandin $E_2$ (Braunstein *et al.* 1988; Widdicombe *et al.* 1989; Folkerts *et al.* 1989; Raeburn, 1990), and EpDRF (Flavahan *et al.* 1985; Hay *et al.* 1986a; Vanhoutte, 1988; Goldie *et al.* 1990) which interact with the smooth muscle and diminish its reactivity, and 4) a substance that acts selectively to inhibit mediator release from mast cells.

The respiratory tract epithelium has been considered to be involved in the pathophysiology of asthma since the damage or dysfunction of this layer may contribute to the bronchial hypersensitivity in asthmatic patients (Laitinen *et al.* 1985; Jeffery *et al.* 1989; Fabbri *et al.* 1990). The airway epithelial cell lining of the guinea pig trachea has been reported to protect the underlying smooth muscle from contraction induced by immunologic challenge in vitro (Hay *et al.* 1986b, 1987; Undem *et al.* 1988). The protection from immune-induced mast cell activation are postulated to be the predominent influence exerted by the epithelium (Grundstrom *et al.* 1990). It has also been reported that the removal of epithelium and the presence of inflammatory mediators increase additively the cholinergic sensitivity of peripheral human airways (Jongejan *et al.* 1991).

Compound 48/80, a potent Hist-liberating agent, is employed as a classic mast cell secretogogue which releases Hist and 5-hydroxytryptamine (5-HT) (Rohlich *et al*, 1971). It has been previously indicated that compoud 48/80 is bound to and can cause noncovalent cross-linkage to mast cell membrane proteins (Ortner and Chignell, 1981). It has also been reported that phospholipase C activity become affected by compound 48/80 through guanine nucleotide-binding regulatory protein (Bronner *et al.* 1987; Wu *et al.* 1993).

It has also been suggested that the reason for the inability of Ag to provoke 100% release of Hist from basophils is the presence of ongoing desensitization (MacGlashan *et al.* 1983). This suggestion can deduce a hypothesis that the smaller mediator release observed with IgE sensitization of tracheal tissues results from a more rapid or more complete desensitization of mediator release in this tissue than the mediator release in the tissues provoked with $IgG_1$ antibody. This hypothesis could be examined using diisopropylfluorophosphonate which decreases the desensitization (MacGlashan *et al.* 1983).

Moreover, several substances that are known to increase intracellular levels of c-

AMP also cause the inhibition of immunological mediator release. The most predominant substances are β-adrenergic agonists (e.g., isoproterenol) (Sorenby, 1975; Undem and Buckner, 1984; Zaagsma *et al.* 1984), phosphodiesterase inhibitors (e.g., theophylline), and forskolin (Seamon and Daly, 1981; Seamon *et al.* 1981). A differential action of receptor agonists against the two Abs at equivalent levels of functional antagonism would imply the existance of different populations of activated receptors.

In the present study, we examined if PAF or epithelium denudation has influence on the Ag-induced contraction as well as on the release of mediators from tracheal tissues sensitized with $IgG_1$ or IgE antibody sensitization, if the mechanism of desensitization has influence on the differences of mediator release after both Ab sensitizations, and also, if several substances which are known to increase intracellular levels of c-AMP have influence on the differences of mediator release after both receptor activations.

## MATERIALS AND METHODS

### Animals

Hartley albino female guinea pigs (Sam Yook Experimental Animal, Osan), weighing, approximately 250~350 g were used. Animals were maintained in the Research Animal Care of Yonsei University.

### Immunologic technique

Preparation of hapten-protein conjugates, immunization procedures for development of guinea pig $IgG_1$ and IgE Ab, Ab separation techniques with affinity-column chromatography, and quantitation of serum Ab titers by passive cutaneous anaphylaxis (PCA) have been comprehensively described in previous articles (Graziano *et al.* 1981; Ro *et al.* 1991). The titers of $IgG_1$ and IgE Abs were 6,400~12,800 and 640, respectively.

### Sensitization and pulmonary tissue mediator release and contraction

Guinea pig tracheal tissues were used in this study according to the previously described techniques (Ro *et al.* 1991). Guinea pigs were passively sensitized for specific Ag challenge. Passively sensitized animals received intravenous injections of Ab serum 1 day before being killed for study. These "standard" doses of Abs were selected on the basis of preliminary experiments in which they were found to sensitize the guinea pig trachea for similar magnitude of contractions (0.5ml/kg of serum in $IgG_1$, 2.5 ml/kg of serum in IgE). The trachea was cut in spiral fashion and bisected. The two tracheal halves from each animal were studied as paired tissues. Tracheal spirals were suspended in air-filled, water-jacked tissue chambers with kerbs biocarbonate solution, and superfused at a rate of 1.5 ml/min. Changes in tension were recorded via force transducers(FT-03) on a Grass model 5D(Grass Instruments, Quincy, Mass). When required, the epithelium was removed from the trachea by rubbing the luminal surface with gauze. The effectiveness of this procedure to remove the epithelium was verified in initial experiments by histology. The intact epithelium of the unrubbed half was also checked by histology.

Immunologic challenge of the trachea with Ox-HSA or Ox-Asc (0.1 mg/ml) was conducted using tissues taken from passively sensitized guinea pigs. Pretreated drugs were added into a superfused buffer 5 min before Ag challenge and during the entire experimental period. 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 $LTE_4$.

### Histamine assay

Hist was determined by an automated, continuous flow extraction, and a fluorometric analyzer (with dialyzer) as described by Siraganian (1974). The sensitivity of the assay is 5 ng/ml of Hist.

### Radioimmunoassay (RIA) of Leukotrienes

The LT content of each superfusate sample was determined by RIA as described previous-

ly (Aharony *et al.* 1983). The LT Ab (#332) was diluted in buffered saline (5 mM MES, HEPES adjusted to pH 7.4 with 1N NaOH) containing 0.1% gelatin. Each assay tube contained 100 $\mu$l of supernatant, Ab (50 $\mu$l of a 1: 1000 dilution), and 50 $\mu$l of $^3$H-LTD$_4$ (2500 to 3000 cpm) in buffered saline. Incubations were for 2 h at 4℃ and the reaction was terminated by an addition of 0.5 ml dextran coated charcoal (200 mg charcoal and 20 mg dextran mixed with 100 ml buffered saline). After 5 mins of incubation, the mixture was centrifuged at 3000 rpm at 4℃ and 0.4 ml of the supernatant was added to Aquasol (NEN Research Products) and counted by a liquid scintillation spectrometry (Packard, Model 3225). Standard curves were constructed in the presence of Ag using LTD$_4$. The detection limit of the assay was 0.045 pmol LTD$_4$. LT release was expressed as pmole/g tissue.

### Statistics

Means and S.E.M.S were calculated for values in each experimental series. Differences between means were determined using analysis of variance and Students unpaired t test for the unpaired samples and paired t test for the paired samples. The n values refer to the number of guinea pigs.

### Drugs and solutions

The following substances were used: histamine free base, MES, HEPES, indomethacin, oxazolone, human serum albumin, complete Freund's Adjuvant, incomplete Freund's Adjuvant, platelet activating factor (PAF)-acether, NDGA (Sigma Chemical Co., ST. Louis, MO); carbamylcholine chloride (carbachol), (Aldrich Chemical, Inc., Milwaukee, WI); gelatin (Difco Labs, Detroit, MI); CV3988 (Takeda chemical Industries, Ltd ); LT Ab for RIA (Stuart Pharmaceuticals, Division of ICI Americas, Inc., Wilmington, Del); $^3$H-LTD$_4$ (specific activity, 39 Ci/mmol, New England nuclear, Boston, Mass); $\alpha$-Ox-HSA, $\alpha$-Ox-Asc (Prepared by our laboratory).

All drug solutions were prepared on the day of each experiment. Indomethacin was dissolved in 95% ethanol and diluted 10,000 fold in the physiological salt solution used for tissue superfusion.

### Acknowledgements

CV3988 was kindly provided by Dr. Yoshikazu Oka of Takeda Chemical Industries, Ltd.

## RESULTS

### The role of platelet activating factor (PAF) in IgG$_1$ and IgE antibody mediated tracheal contraction

In order to determine the optimum time for pre-exposure and apparent $K_B$ (dissociation constant) for CV3988 (PAF antagonist) in guinea pig tracheal tissues using PAF-acether as agonist, normal guinea pig tracheal tissues were first superfused by PAF from $10^{-9}$M to $10^{-5}$M. The results showed that any concentration of PAF did not cause the contraction in the tracheal tissues. Therefore, the optimum time for pre-exposure and apparent $K_B$ for CV3988 in guinea pig tracheal tissues using PAF could not be determined(data not shown).

However, in order to confirm our examination of the relationship and differences between mediator release and the contractile response when IgG$_1$ and IgE Ab-bound receptors are stimulated by specific Ag, guinea pig tracheal tissues sensitized by IgG$_1$ or IgE antibody were superfused by 0.1 mg/ml Ox-HSA or Ox-Asc after CV3988 (from $10^{-8}$M to $10^{-4}$M) pretreatment for 10 min. The results showed that tissue contractile responses of IgG$_1$ or IgE sensitized trachea evoked by adding each concentration of CV3988 did not decrease, and did not change the difference of mediator release (especially LT) when tracheal tissues were sensitized by IgG$_1$ or IgE antibody (Table 1).

### The role of epithelium on the mediator release in tracheal tissues sensitized with IgG$_1$ or IgE antibody.

**1) Influence of the epithelium;** The time-course for contraction, Hist release, and LT

**Table 1. Antigen-induced responses of superfused tracheal tissues after passive sensitization of guinea pigs with $IgG_1$ or IgE antibody***

| Ab | N | contraction(%) | | Histamine(%) | | LT(pmol/g tissue) | |
|---|---|---|---|---|---|---|---|
| | | (−)CV3988 | (+)CV3988+ | (−)CV3988 | (+)CV3988 | (−)CV3988 | (+)CV3988 |
| $IgG_1$ | 8 | 79±3.2 | 81±4.7 | 12.1±2.3* | 14.5±2.7* | 431±65* | 453±70* |
| IgE | 8 | 70±5.9 | 73±6.2 | 3.9±0.8 | 4.5±1.8 | 87±44 | 156±87 |

N, Number of animals

a. Animals were passively sensitized by iv injection of anti-Ox $IgG_1$ or IgE 1 day before removing tissues for study. Isolated tracheal tissues were challenged with Ox-antigen.

+. PAF($10^{-5}$M) was added to the superfusion solution with antigen(0.1 mg/ml) concomitantly.

*. Avalue that is statistically different($P<0.05$) from the value obtained after IgE sensitization in the same experimental protocol.

**Table 2. Responses of epithelium removal on Ox-antigen-induced contraction and mediator release in the passively sensitized guinea pig superfusion studied in the absence and presence of drug pretreatment[a]**

| Ab pretreatment[b] | Maximum Contraction(%) | | Total HT(%) | | LT(pmol/g tissue) | | N |
|---|---|---|---|---|---|---|---|
| | Epi(+) | Epi(−) | Epi(+) | Epi(−) | Epi(+) | Epi(−) | |
| $IgG_1$ | 75.9±1.9 | 73.5±7.6 | 14.3±2.1 | 15.2±4.6 | 653±78 | 621±164 | 11 |
| Indomethacin & L-cysteine | 97.6±2.7 | 89.4±6.0 | 29.3±4.6 | 16.0±3.4* | 2727±216 | 2800±176 | 12 |
| Indomethacin & NDGA | 88.8±5.2 | 85.4±3.4 | 25.1±4.2 | 13.1±3.6* | 185±84 | 94±48 | 10 |
| IgE | 71.8±3.7 | 33.3±4.4*+ | 4.2±0.8+ | 0.2±0.1*+ | 63±24+ | 0+ | 11 |
| Indomethacin & L-cysteine | 73.4±5.7+ | 51.1±11.0 | 7.6±0.7+ | 0.8±0.4*+ | 1091±165+ | 0*+ | 10 |
| Indomethacin & NDGA | 77.8±7.1 | 52.3±5.0*+ | 8.2±1.1+ | 0.7±0.1*+ | 0+ | 0+ | 10 |

a. Animals were passively sensitized by iv injection of anti-Ox-HSA($IgG_1$) or anti-Ox-Asc(IgE) Ab 1day before removing tissues for study. The precedure detailed indicated at "Material & Method".

b. The concentration of drugs used: Indomethacin, $5\times10^{-6}$M; L-cysteine, 10mM; NDGA, $10^{-5}$M

*A value that is statistically different($P<0.05$) from the value obtained with the same Ab in the absence of epithelium

+A value that is statistically different($P<0.05$) from the value obtained with the defferent Ab in the presence or absence of epithelium.

release induced by Ox-HSA or Ox-Asc, 0.1 mg/ml, in trachea with and without epithelium are illustrated in Figure 1. The data from these and other similar experiments are summerized in Table 2. Epithelium removal resulted in similar contraction as in Hist and LT release when the tracheal tissues passively sensitized with $IgG_1$ Ab were challenged by Ox-HSA. However, in the tracheal tissues sensitized with IgE Ab, epithelium removal resulted in smaller contraction and Hist release (Table 2). Therefore, in the epithelium removal, less amounts of mediators released by the contracted tracheal tissues sensitized with IgE Ab were still observed than those released by the contracted tracheal tissues sensitized with $IgG_1$ Ab (Fig 1 and Table 2). This results showed that the differences for

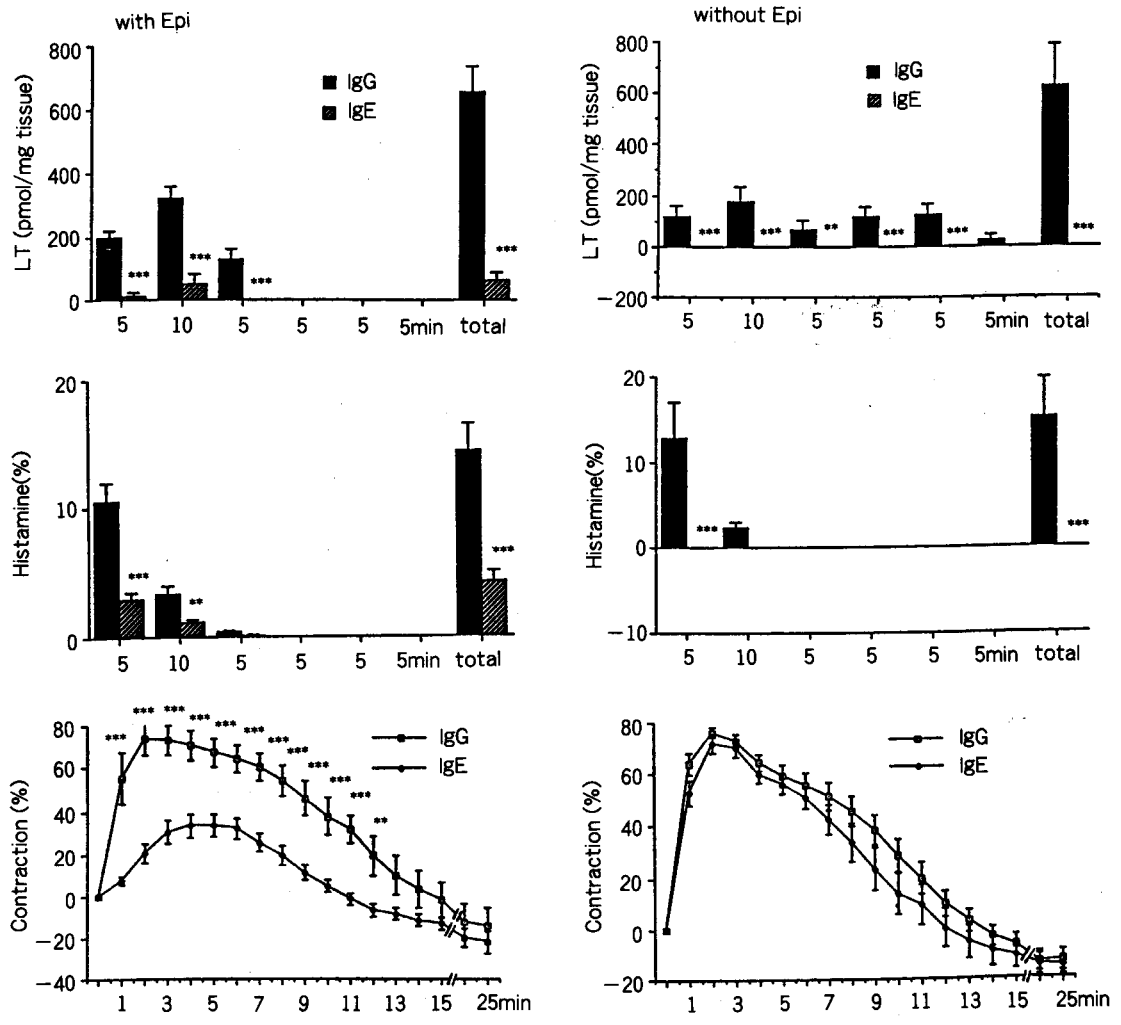


***Fig. 1.*** *Time-dependent contraction (lower panels), histamine (middle panels), and leukotriene (upper panels) release induced by Ox-HSA or Ox-Asc, 0.1 mg/ml, in superfused tracheal strips obtained from passive sensitization with $IgG_1$ (opened squares and closed bars) or IgE (closed circles and hatched bars) Ab 1day before each experiment and studied in the absence or presence of the epithelium. The number beneath histamine and leukotriene release portion indicate superfusate collection periods(min). Vertical lines represent SEM. Statistically significant (*, $P<0.05$; **, $P<0.01$) difference between values obtained at each collection period with $IgG_1$ or IgE Ab in the absence or presence epithelium. The data are summerized in table 2.*

the amounts of mediators released by trachea sensitized with $IgG_1$ vs IgE Ab may be not caused due to the epithelium.

**2) Influence of L-cysteine and indomethacin;** L-cysteine(10mM) and indomethacin($5\times10^{-6}$ M) pretreatment resulted in larger increases of Hist release in responses to Ox-HSA in the presence than in the absence of epithelium of tracheal tissues sensitized with $IgG_1$ Ab. However, in the tracheal tissues sensitized with IgE Ab, epithelium removal resulted in a smaller Hist release. LT release was not af-

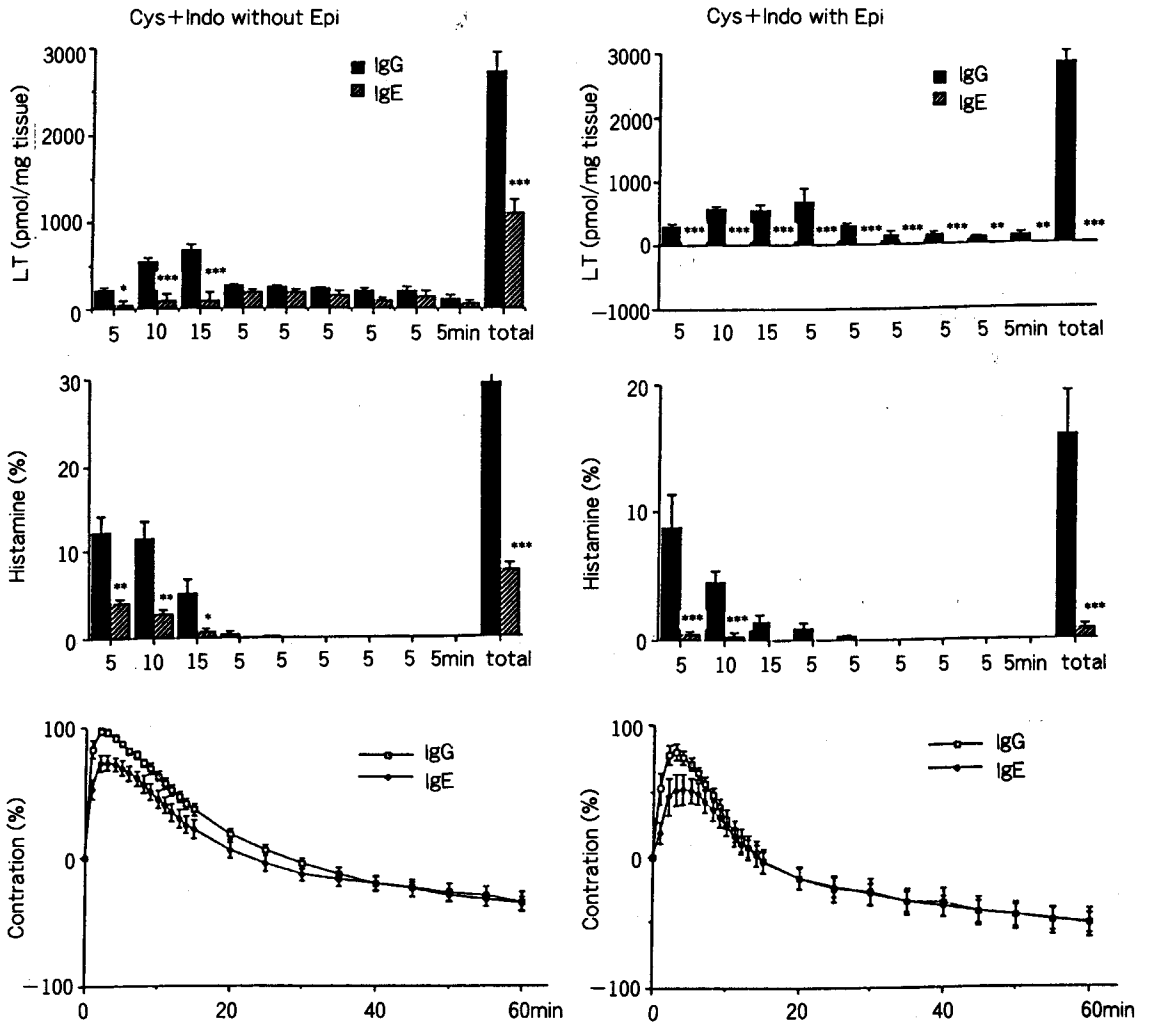


***Fig. 2.*** *Time-dependent contraction (lower panels), histamine (middle panels), and leukotriene (upper panels) release induced by Ox-HSA or Ox-Asc, 0.1 mg/ml, in the presence of indomethacin, $5x10^{-6}$ M and L-cysteine, 10 mM. Animals were passively sensitized with $IgG_1$ (opened squares and chosed bars) or IgE (closed cicles and hatched bars) Ab 1 day before each experiment and studied in the absence or presence of the epithelium. The number beneath the histamine and leukotriene release portion indicate superfusate collection periods (min). Vertical lines represent SEM. Statistically significant (*, $p<0.05$; **, $p<0.01$: ***, $p<0.005$) difference between values obtained at each collection period with $IgG_1$ and IgE Ab in the absence or presence epithelium. The data are summerized in table 2.*

fected by removing the epithelium of tracheal tissues sensitized with $IgG_1$ but affected by IgE Ab (Table 2). Therefore, in the epithelium removal, less amounts of Hist and LT released by the contracted tracheal tissues sensitized with IgE Ab were observed than those released by the contracted tracheal tissues sensitized with $IgG_1$ Ab (Fig 2 and Table 2).

We also examined effects of indomethacin($5 \times 10^{-6}$M) and NDGA($10^{-5}$M), which inhibit the

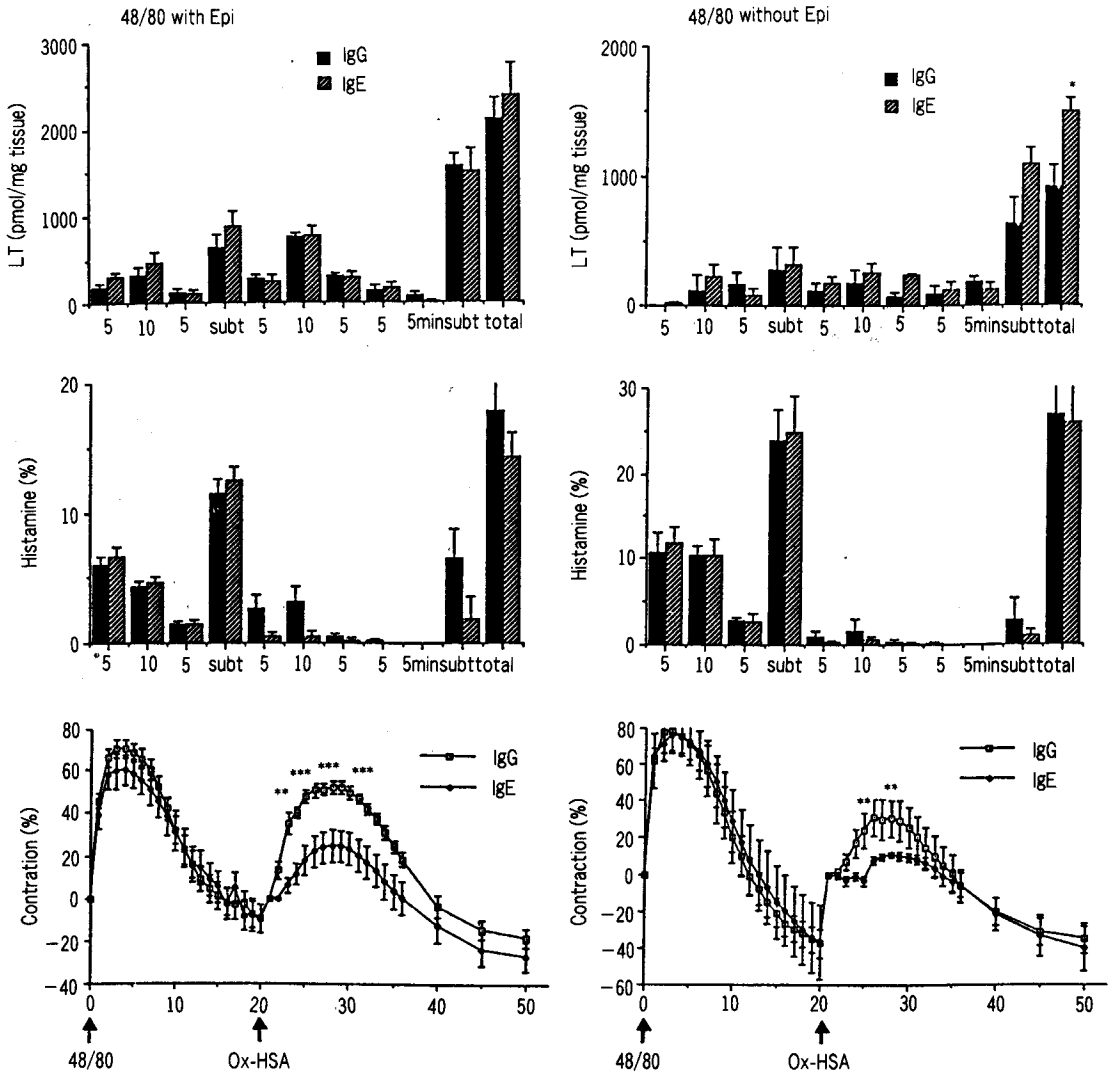


***Fig. 3.*** *Time-dependent contraction (lower panels), histamine (middle panels), and leukotriene (upper panels) release induced succesively by compound 48/80 and Ox-HSA or Ox-Asc, 3 mg/ml and 0.1 mg/ml, respectively, in the superfused tracheal strips obtained from passively sensitized guinea pigs and studied in the absence or presence of epithelium. The number, subt, and total beneath histamine and leukotriene release portion indicate superfusate collection periods (min), subtotal, and total contents, respectively. 48/80 is abbreviation of compound 48/80. Vertical lines represent SEM. Statistically significant (*, $p<0.05$; **, $p<0.01$) difference between values obtained at each collection period with $IgG_1$ and IgE Ab in the absence or presence epithelium. The data are summerized in table 3.*

lipoxygenase enzyme required for the LT biosynthesis, on the mediator release induced by specific Ag after $IgG_1$ or IgE Ab sensitization. Indomethacin and NDGA pretreatment resulted in a larger increase of Hist release in responses to Ox-HSA in the presence than in the absence of epithelium of tracheal tissues sensitized with $IgG_1$ Ab. However, in the tra-

**Table 3. Responses of epithelium removal on compound 48/80- and antigen-induced contraction and mediator release in the passively sensitized guinea superfused trachea studied in the absense and presence of drug pretreatment***

| Ab | Pretreatment[b] | Challenge | Maximum Contraction(%) | | Total HT(%) | | LT(pmol/g tissue) | | N |
|---|---|---|---|---|---|---|---|---|---|
| | | | Epithelium (+) | Epithelium (−) | Epithelium (+) | Epithelium (−) | Epithelium (+) | Epithelium (−) | |
| $IgG_1$ | − | 48/80 | 70.4±4.4 | 78.6±11.0 | 11.5±1.0** | 23.9±9.0 | 639±149 | 279±182 | 12 |
| | | Ox-HSA | 51.9±3.1* | 31.0±9.7 | 6.4±2.3 | 2.9±2.5 | 1562±141** | 635±194 | |
| | Indomethacin | 48/80 | 71.2±3.5 | 78.1±5.1 | 12.6±1.9** | 26.4±6.2 | 740±167 | 357±155 | 10 |
| | | Ox-HSA | 58.3±3.8* | 42.8±5.9 | 8.7±2.1 | 3.1±1.8 | 1848±175** | 756±182 | |
| | Indomethacin +NDGA | 48/80 | 69.6±3.7 | 72.8±4.7 | 10.9±1.3** | 21.5±3.7 | 149±52 | 0 | 11 |
| | | Ox-HSA | 55.9±5.6 | 40.3±3.9 | 6.1±1.8 | 3.5±0.9 | 82±38 | 0 | |
| IgE | − | 48/80 | 60.0±7.0 | 76.0±9.9 | 12.5±1.1* | 24.8±4.3 | 879±172* | 320±134 | 10 |
| | | Ox-Asc | 24.8±7.4*+ | 10.8±1.6+ | 1.9±1.6+ | 1.1±0.7 | 1499±270* | 1091±222 | |
| | Indomethacin | 48/80 | 63.8±4.1 | 70.5±3.8 | 12.9±1.0* | 26.1±4.7 | 890±164* | 439±172 | 13 |
| | | Ox-Asc | 30.3±3.7*+ | 15.4±2.0+ | 2.5±1.3+ | 1.8±0.3 | 1745±251 | 1287±234 | |
| | Indomethacin +NDGA | 48/80 | 62.4±3.3 | 67.6±2.9 | 13.2±1.8* | 23.2±3.8 | 0 | 0 | 10 |
| | | Ox-Asc | 33.2±2.1*+ | 15.9±1.8+ | 2.7±1.1+ | 1.5±0.6 | 120±57 | 182±87 | |

a. Animals were passively sensitized by iv injection of anti-Ox-HSA($IgG_1$) or anti-ox-Asc(IgE) Ab 1day before removing tissues for study. Isolated tracheal tissues were challenged with compound 48/80 (0.3 mg/ml) for 20 min and washed with superfused buffer until returning of tissue contraction to base line (approximately 10 min). After that, tissues were challenged with Ox-antigen(0.1 mg/ml).

b. The concentration of drugs used: Indomethacin, $5\times10^{-6}$M; L-cystein,e 10mM; NDGA, $10^{-5}$M.

*A value that is statistically different(*, $p<0.05$; **, $p<0.01$) from the value obtained with the same Ab in the absence of epithelium.

+A value that is statistically different($p<0.05$) from the value obtained with the different Ab in the presence or absence of epithelium.

cheal tissue sensitized with IgE Ab, epithelium removal resulted in a smaller Hist release. The differences of LT release in the absence and presence of epithelium after $IgG_1$ sensitization were not observed but the differences of LT release with IgE Ab were observed by indomethacin pretreatment (Table 2).

**3) Influence of compound 48/80;** We examined the influence of mediator release evoked by successively challenging specific Ag after the prechallenge of non-immunological secretogogue, such as compound 48/80. In the absence or presence of epithelium, the pattern of Hist and LT release induced by successively challenging the compound 48/80 (0.3 mg/ml) and Ox-Ag (0.1 mg/ml) were not different from the $IgG_1$ *versus* IgE Ab sensitization. But, the contractile responses of tracheal tissues induced by specific Ag significantly differed for both Abs (Fig 3 and Table 3).

Hist release evoked by initially compound 48/80 resulted in a larger increases when using epithelium removal than when using intact epithelium of the tracheal tissues sensitized with $IgG_1$ or IgE Ab (Table 3). In intact and denuded epithelium, Hist release induced by sucessively challenging with specific Ag after compound 48/80 challenge in the tracheal tissues sensitized with $IgG_1$ or IgE Ab similarly decreased (Table 3).

**Table 4. Effects of mediator inhibitors on the antigen-induced responses of superfused tracheal tissues after passive sensitization of guinea pig with $IgG_1$ of IgE antibody[a]**

| Ab | Pretreatment | Maximun Contraction (%) | Total Hist (%) | LT (pmol/g tissue) | N |
|---|---|---|---|---|---|
| $IgG_1$ | – | 74.5±1.9 | 15.3±2.1 | 559±78 | 6 |
| | Isoproterenol($10^{-6}$M) | 70.2±3.7 | 8.7±1.6* | 527±95 | 6 |
| | Forskolin($10^{-5}$) | 71.4±2.4 | 8.9±1.2* | 498±103 | 6 |
| | Theophylline($5\times10^{-5}$M) | 73.1±2.5 | 7.8±2.3* | 591±85 | 6 |
| IgE | – | | | | |
| | Isoproterenol($10^{-6}$M) | 71.8±3.7 | 5.0±0.8 | 78±33 | 6 |
| | Forskolin($10^{-5}$M) | 64.5±4.3 | 2.4±0.4** | 0 | 6 |
| | Theophylline($5\times10^{-5}$M) | 66.2±2.9 | 1.2±0.3** | 0 | 6 |
| | | 67.8±3.1 | 1.9±0.2** | 113±46 | 6 |

a. Animal were passively sensitized by iv injection of anti-Ox $IgG_1$ or IgE antibody 1 day before removing tissues for study. Preteated drugs were added into superfused buffer during the all experimental period.

* A value that is statistically different(*, $p<0.05$, **, $p<0.01$) from the value obtained with the same antibody in the absence of each drug.

When the tracheal tissues sensitized with $IgG_1$ or IgE Ab were prechallenged with compoud 48/80, the tissues released LT in intact or denuded epithelium. Amounts of LT release in intact epithelium significantly increased in the tissues rechallenged with Ox-Ag after compound 48/80 prechallenge, but in the epithelium removal, LT release after prechallenge with compound 48/80 decreased, compared to intact epithelium (Table 3).

**Desensitization in tracheal tissues**

In order to confirm whether the smaller mediator release obtained through IgE results from a more rapid or more complete desensitization than that which occurs when release is provoked with $IgG_1$ Ab, we examined mediators released from the superfused trachea passively sensitized with either $IgG_1$ or IgE Ab, and challenged with specific Ag in the absence or presence of diisopropylfluorophosphate (DFP, $10^{-7}$M-$10^{-4}$M) which is known to decrease the desensitization. Desensitization inhibitor (DFP) had no influence on the mediator release evoked by both Ab sensitization. This result still indicated that there are differences in mediator release evoked from tracheal tissues sensitized with $IgG_1$ *versus* IgE Ab (data not shown).

**Influence of mediator release inhibitors in tracheal tissues**

We attempted to examine whether several substances which mediate an inhibition of Ag-induced mediator release, such as isoproterenol ($10^{-6}$M), forskolin ($10^{-5}$M), and theophylline ($5\times10^{-5}$M), have influence on the differences of mediator release after both Ab sensitizations. In all drug pretreatments, Ag-induced Hist release after $IgG_1$ Ab sensitization decreased up to approximately 40% and in the IgE Ab, decreased up to 70%, but had no influence on the LT release. The amount of LT obtained in control samples after IgE Ab was found from three tracheal tissues after Ag challenge and the amount of LT in three tracheal tissues was "0"(Table 4).

## DISCUSSION

We have previously reported that the substantial differences in measurable Hist and LT released during similar levels of Ag-induced contraction in guinea pig pulmonary tissues passively sensitized with $IgG_1$ *versus* IgE Abs may be in part related to a differential

pattern of LT metabolism by the trachea after Ab activation. However, there still is the substantial differences of Hist and LT release after $IgG_1$ *versus* IgE Ab receptor activation. Therefore, we deduced a few hypotheses, 1) that PAF, which is potent mediator in allergic asthmatics, can mediate a different fraction of the airway contractile response to IgE than to $IgG_1$ activation, besides Hist and LT, 2) that epithelium is involved to the contractile response and mediator release in guinea pig tracheal tissues, 3) that the mechanism of desensitization and inhibitors of mediator release associated with tissue mast cell heterogeneity is related to the mediator release after $IgG_1$ *versus* IgE activation.

It has been demonstrated that PAF, besides Hist and arachidonic metabolites (e.g. LT), is another mediator potentially involved in tracheobronchial constriction (Darius *et al.* 1986). We examined whether CV3988, a selective PAF receptor antagonist (Terashita *et al.* 1983), antagonizes contractions and LT release evoked by $IgG_1$ or IgE activation. But, CV3988 did not change the contraction and mediator release(especially LT) after $IgG_1$ or IgE Ab activation (Table 1). Therefore, these results indicate that it is unlikely that airway contractile response to IgE than to $IgG_1$ activation is mediated by PAF. These data also suggest that more mediators release from $IgG_1$ than from IgE activation are not released by PAF initially synthetized after Ab activation. These observations agree to such report that lung and skin mast cell or basophils do not degranulate during PAF-induced bronchoconstriction (Louis *et al.* 1993: Thomas and Church, 1990).

In order to obtain the information regarding a potential differential involvement of the epithelial cells in modulating responses to $IgG_1$ *versus* IgE activation, tracheal tissues in intact and denuded epithelium were examined for $IgG_1$ *versus* IgE activation-induced contraction and mediator release. These results are showed in Table 2~3 and Figure 1~3. Epithelium removal resulted in similar contraction and mediator release from trachea sensitized with $IgG_1$ Ab, but in IgE Ab, epithelium removal resulted in smaller contraction and mediator release rather than the intact epithelium (Fig 1 and Table 2). Therfore, it seems that epithelium itself is not involved in modulating responses to $IgG_1$ *versus* IgE activation-induced contraction and mediator release. We can infer a suggestion that IgE Ab mainly activates the mast cell located in epithelium, and that $IgG_1$ Ab mainly activates the mast cell in mucosa surface and around the venule from the fact that the distribution of mast cell in epithelium is small, but large in the mucosa and around the venule.

L-cysteine (an inhibitor of aminopeptidase) and indomethacin (an inhibitor of cyclooxygenase) pretreatment resulted in larger amounts of Hist and LT release in superfusate samples after sensitization with $IgG_1$ or IgE Ab. The percentage increase of LT was larger after IgE than after $IgG_1$ sensitization. These results reconfirmed our results previously reported (Ro *et al.* 1991). Epithelium removal resulted in smaller Hist release from drug pretreated tissues after $IgG_1$ or IgE Ab activation, but in IgE Ab activation, almost no mediators were released. These results can also suggest that IgE Ab mainly activates the mast cell in the epithelium. Also, the fact that the epithelium removal after $IgG_1$ Ab activation resulted in smaller Hist release implies the involvement of epithelial cells in modulating response to $IgG_1$ activation.

The amounts of Hist and LT released by the prechallenge of non-immunological secretogogue, compound 48/80, were similar regardless in the absence or presence of epithelium after $IgG_1$ or IgE activation, but epithelium removal after $IgG_1$ or IgE activation significantly increased Hist release only (Table 3). This result suggestes that epithelium plays a role of a physical barrier that restricts movement of pharmacological agents across the wall. That is, it can be suggested that in the elimination of the barrier, compound 48/80 is bound to the mast cell membrane protein which is located to the mucosa and could cause a non-covalant cross-linkage of membrane protein and ultimately could release the Hist (Ortner and Chignell, 1981). When tracheal tissues after $IgG_1$ or IgE activation and prechallenge with compound 48/80 were suc-

cessively challenged with Ox-antigen in intact or denuded epithelium, Hist release significantly decreased. It can be regarded from this result that preformed Hist storaged in granules is initially degranulated by the compound 48/80 prechallenge. LT as well as Hist after $IgG_1$ or IgE activation are released with compound 48/80 in the intact or denuded epithelium. Amounts of LT release significantly increased in the tissues successively rechallenged with Ox-Ag after prechallenging compound 48/80 in the intact epithelium, but in the epithelium removal, LT release after prechallenge with compound 48/80 decreased (Table 3). The data suggest that the epithelium can release a substance that acts selectively to inhibit or stimulate arachidonic metabolizing pathway (especially, lipoxygenase pathway) from mast cells or suggest that compound 48/80, bound to mast cell membrane protein, can activate an enzyme which can trigger the inhibition of the lipoxygenase pathway as reported by Wu *et al.* (1993). These results also indicate that eicosanoid production (especially arachidonic acid) is associated with the airway epithelium as demonstrated by Lindstrom *et al.* (1992).

The differences for mediator release after $IgG_1$ *versus* IgE Ab activation were not affected by the mechanism for desensitization opposing the suggestion by MacGlashan et al (1983).

Isoproterenol, forskolin, and theophylline have previously been known to cause the inhibition of immunological mediator release due to the increase of intracellular levels of c-AMP after mainly $IgG_1$ Ab activation (Undem and Buckner, 1984; Zaagsma *et al.* 1984; Seamon and Daly, 1984). This study obtained a result that Ag-induced Hist release in the tissues pretreated with all drugs is inhibited more to IgE than to $IgG_1$ Ab activation. However, the inhibition of LT release evoked by these substances have not been conducted after $IgG_1$ Ab activation of guinea pig tracheal tissues (Table 4). Our results suggest that immune-induced airway responses were in part related to the localization and functional heterogeneity of tracheal tissue mast cells.

The data obtained in our experiments suggest that the tracheal epithelium can act to prevent immune- and non-immune-induced airway responses. Non-immune-induced airway responses may in part reflect the role of epithelium as a diffusion barrier and modulator of enzyme activity mediating the mediator release. The data also suggest that immune-induced airway responses are related to the localization and functional heterogeneity of tissue mast cells.

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