

Hyperresponsiveness of bronchial but not tracheal smooth muscle in a murine model of allergic bronchial asthma

Y. Chiba, A. Ueno, H. Sakai and M. Misawa

Department of Pharmacology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan, Fax: ++81 3 5498 5787, e-mail: chiba@hoshi.ac.jp

Received 22 April 2004; returned for revision 10 June 2004; accepted by M. Katori 9 July 2004

Abstract. *Objective:* To determine a change in airway smooth muscle contractility in a murine model of allergic asthma, the responsiveness of airway smooth muscles isolated from ovalbumin (OA)-sensitized and -challenged mice was compared with that from control animals.

Methods: Actively sensitized mice were repeatedly challenged by ovalbumin (OA) antigen inhalation. Twenty-four h after the last antigen challenge, tracheal and bronchial smooth muscle responsiveness to acetylcholine (ACh) and endothelin-1 (ET-1) were measured. Airway microvascular leakage and histochemistry were also determined as indices of airway inflammation.

Results: Both the ACh and ET-1 responsiveness of bronchial, but not tracheal, smooth muscles were significantly augmented in OA-challenged mice, whereas no significant change in the expression levels of M_2 , M_3 and ET_B receptors was observed. The Evans blue dye extravasation in the main bronchial, but not tracheal, tissue of OA-challenged mice was significantly increased as compared with that of sensitized control animals. A marked inflammatory cells infiltration was also observed in bronchial but not tracheal tissues of OA-challenged mice.

Conclusion: Repeated antigen challenge to sensitized mice caused a hyperresponsiveness of bronchial, but not tracheal, smooth muscle accompanied with bronchial tissue inflammation.

Keywords: Airway hyperresponsiveness – Bronchial smooth muscle – Airway inflammation – Asthma

Introduction

Airway hyperresponsiveness (AHR) is a critical component in allergic bronchial asthma [1, 2], although the mechanism(s) underlying the development of AHR is not fully understood. The importance of AHR in the pathogenesis of bronchial asthma has been suggested by its correlation with

the severity of this disease [1, 3]. It is thus very important to clear the underlying mechanisms of AHR for asthma therapy. One possible mechanism of AHR might be an increased contractility of airway smooth muscle because an inhalation of β -stimulant can reverse bronchospasm quickly in most asthmatics. Indeed, an increased responsiveness of airway smooth muscle has been reported in asthmatic patients with in vivo AHR [4, 5].

To understand the mechanisms of AHR, various animal models of allergic asthma have been developed including dogs [6], guinea pigs [7], rats [8, 9] and mice [10, 11]. Among these animal models, mouse might be one of the most useful species because of the easy access to genetic information. Indeed, the mice that have airway inflammation induced by an antigen are widely used as animal models of allergic asthma including AHR. For instance, Kato et al. [10] reported that repeated ovalbumin (OA) inhalation to OA-sensitized mice caused AHR to intravenously administered acetylcholine (ACh) with significant increase in eosinophils and inflammatory cytokines in bronchoalveolar lavage fluids and plasma IgE level, all of which are usually observed in allergic asthmatic patients. Schramm et al. [11] also reported a murine model of AHR induced by OA antigen. However, little information is available on change in the function of airway smooth muscle itself in these murine models of AHR.

In the present study, the contractility of airway smooth muscles isolated from OA-sensitized and -challenged mice was compared with that from control animals to determine whether the mice that have in vivo AHR also have hyperresponsiveness of airway smooth muscles. We used two regions of airway smooth muscles, i.e., upper tracheal and main bronchial smooth muscles, because our previous study suggested a regional difference in the development of airway smooth muscle hyperresponsiveness in a rat model of AHR [12]. The microvascular permeability of tracheal and bronchial tissues was also measured to determine the correlation between smooth muscle contractility and tissue inflammation.

Materials and methods

Sensitization and antigen challenge

Male BALB/c mice (6-week old, specific pathogen-free) were used. All experiments were approved by the Animal Care Committee at the Hoshi University (Tokyo, Japan). The induction of *in vivo* airway hyperresponsiveness (AHR) was performed as described by Kato et al. [10]. In brief, mice were actively sensitized by intraperitoneal injections of 8 µg ovalbumin (OA; Seikagaku Co., Tokyo, Japan) with 2 mg Imject Alum (Pierce Biotechnology, Inc., Rockford, IL, USA) on day 0 and day 5. The sensitized mice were challenged with aerosolized OA-saline solution (5 mg/mL) for 30 min on days 12, 16 and 20. A control group of mice received the same immunization procedure but inhaled saline aerosol instead of OA challenge. The aerosol was generated with an ultrasonic nebulizer (Nihon Kohden, Tokyo, Japan) and introduced to a Plexiglas chamber box (130 × 200 mm, 100 mm height) in which the mice were placed.

Determination of airway smooth muscle responsiveness

Airway smooth muscle responsiveness was determined as follows. Briefly, 24 h after the last antigen challenge, the mice were sacrificed by exsanguination from abdominal aorta under urethane (1.6 g/kg, *i.p.*) anesthesia. Then the airway tissues below the larynx to lungs were immediately removed. About 3 mm length of the trachea and left main bronchus were isolated and epithelium was removed by gently rubbing with keen-edged tweezers [8]. The resultant each tissue ring preparation was then suspended in an organ bath at a resting tension of 0.5 g. The organ bath contained modified Krebs-Henseleit solution with the following composition (mM); NaCl 118.0, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25.0, KH₂PO₄ 1.2 and glucose 10.0 (pH 7.4). The isometrical contraction of the circular smooth muscle was measured as described previously [13]. After an equilibration period, smooth muscle responsiveness to cumulatively administered acetylcholine (10⁻⁷–10⁻³ M) or endothelin-1 (10⁻⁹–10⁻⁶ M) was determined.

Evaluation of airway microvascular leakage

Airway microvascular leakage was also evaluated as an index of airway inflammation by the method described previously [12]. The tissues were dissected 24 h after the last antigen challenge. In brief, mice were anesthetized with urethane (1.6 g/kg, *i.p.*) placed in the supine position. Evans blue dye (30 mg/kg) and heparin (600 U/kg) were administered via a cannula introduced into the right femoral vein. Twenty min after the administration of Evans blue dye, the animals were exsanguinated from the abdominal aorta and the thorax was opened. Then a blunt-ended 23-gauge needle was passed through the right ventricle into the pulmonary artery. The pulmonary veins were incised to allow the outflow

of perfusate, and the airways and lungs were perfused with 2–3 mL of 1% paraformaldehyde in citrate-buffered saline (pH 3.5) to remove intravascular dye and to fix the tissues. After then, the trachea, main bronchus and lungs were removed and their wet weights were measured. Evans blue dye was extracted by incubating tissues in 1 mL of 100% formamide at 60°C for 24 h, and its concentration was determined by light absorbance at 620 nm (U-2000 spectrophotometer; Hitach, Tokyo, Japan) with standard Evans blue dye solution. Evans blue dye content was expressed as nanograms per milligram wet weight of tissue.

Histological examination

Airways were removed from animals 24 h after the last antigen challenge and the upper trachea, main bronchus and lungs were cut into 2–3 mm chips. Then the tissue chips were fixed in 10% formaldehyde and embedded in Paraplast X-TRA paraffin (Fisher Healthcare, Houston, TX, USA). Four-µm sections were obtained from blocks and mounted on silane-coated glass slides, deparaffinized with xylene and graded ethanol, and processed for histological examination. The sections were stained with hematoxylin and eosin by a standard technique.

Competitive reverse transcription-polymerase chain reaction (RT-PCR)

Twenty-four h after the last antigen challenge, total RNAs were extracted from upper trachea and main bronchus, individually, by using TRI Reagent (Sigma, Saint Louis, MO, USA) according to the manufacturer's instructions. The mRNA levels of m₂ and m₃ muscarinic and ET_B endothelin receptors, which are known as the receptors contributing to ACh- and ET-1-induced contractile response respectively [14–16], were examined by competitive RT-PCR. Briefly, cDNAs were prepared from the total RNA (1.0 µg) by using a RevertAid First Strand cDNA Synthesis Kit (#K1622, Fermentas Inc., Hanover, MD, USA) in a total volume of 100 µL reaction buffer containing 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM MgCl₂, 1 mM dNTP mixture, 1 U/µL RNase inhibitor, 10 ng/µL random 6 mers, and 10 U/µL M-MuLV reverse transcriptase. The reaction mixture was incubated for 10 min at 25°C followed by 60 min at 42°C to initiate the synthesis of the cDNAs. Reverse transcriptase was inactivated at 70°C for 5 min. Then the RT reaction mixture (2 µL) was subjected to PCR (0.2 µM forward and reverse primers, 0.025 U/L *Taq* DNA polymerase, 2 mM MgCl₂, 0.2 mM dNTPs) in the absence or presence of corresponding competitor (1–10,000 copies/µL) in a final volume of 10 µL. The PCR primer sets used were summarized in Table 1. The thermal cycle profile used was 1) denaturing for 15 s at 95°C, 2) annealing primers for 15 s at 55°C, 3) extending the primers for 60 s at 72°C. The PCR amplifications were performed 30 cycles. A portion (5 µL) of the PCR mixture was subjected to electrophoresis on 1.2% agarose gel and visualized by ethidium bromide staining. The band intensity was quantified by a densitometer (Atto Densitograph; Atto Co., Tokyo, Japan). The ratios of the

Table 1. PCR primers for target genes used in the present study.

Target gene	Accession No.	Sequence	Product size (bp)
Chrm2	AF264049	Sense: 5'-TTCAGTTCTTTTCCAACGCTGC-3' Antisense: 5'-CCGTGTTTTTCATCCTGGGTATC-3'	390
Chrm3	NM_033269	Sense: 5'-GGCTGACTACTTAATCTTGG-3' Antisense: 5'-GGATGTTGCCGATGATGGTC-3'	325
Ednrb	NM_007904	Sense: 5'-GCGCAATGGTCCCAATATCTTG-3' Antisense: 5'-CCTATGGCTTCGGGGACAGC-3'	321
Gapdh	NM_001001303	Sense: 5'-ACCACAGTCCATGCCATCAC-3' Antisense: 5'-TCCACCACCCTGTTGCTTGT-3'	452

Chrm2; m₂ muscarinic cholinergic receptor, Chrm3; m₃ muscarinic cholinergic receptor, Ednrb; ET_B endothelin receptor, and Gapdh; glyceraldehyde-3-phosphate dehydrogenase.

target gene/competitor to corresponding Gapdh/Gapdh competitor in the presence of 100 copies/ μ L of respective competitor were calculated.

The DNA competitors used in the present study were prepared by PCR using primer sets (Table 2) with pGBKT7 plasmid (Clontech Laboratories, Inc., Palo Alto, CA, USA) as a template. After purification of the PCR product by GeneElute PCR Clean-Up kit (Sigma), concentration of DNA competitor (copies/ μ L) was calculated by the following formula: $[\text{OD}_{260} \times 50 \text{ (ng}/\mu\text{L)} \times 10^{-9} \times 6 \times 10^{23}] / [\text{length of competitor (bp)} \times 660]$.

Statistical analyses

All data were expressed as mean with S.E. Statistical significance of difference was determined by unpaired Student's *t*-test or two-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered significant.

Results

Figure 1 shows the acetylcholine (ACh) and endothelin-1 (ET-1) responsiveness of airway smooth muscles isolated from sensitized control and repeatedly ovalbumin (OA)-challenged mice. Both ACh and ET-1 elicited contractile responses of the mouse tracheal and bronchial smooth muscles in a concentration-dependent manner, respectively. The concentration-response curve to ACh of bronchial smooth muscles from OA-challenged mice was significantly shifted upward as compared with that from control animals ($p < 0.01$ by ANOVA; Fig. 1B), whereas no significant difference in the ACh responsiveness of tracheal smooth muscles was observed between groups (Fig. 1A). Similar results were also obtained in case of ET-1 responsiveness (Fig. 1C and 1D). The pD_2 (-log for 50% effective concentration (M) of ACh or ET-1) and Emax (maximal contractile response) values obtained from individual concentration-response curve are summarized in Tables 3. On the other hand, no significant difference in the isotonic high K^+ (10–90 mM)-induced tracheal and bronchial smooth muscle contraction was observed between groups (Fig. 2).

To examine the changes in the expression levels of m_2 and m_3 muscarinic and ET_B endothelin receptors in the hyperresponsive bronchial smooth muscle, mRNA levels of m_2 , m_3 and ET_B receptors were measured by competitive RT-PCR. Figure 3 shows typical PCR products for target genes (in the absence or presence of respective competitors) obtained from

bronchial RNA samples of sensitized control and OA-challenged mice. In the absence of competitors, single bands for target genes were obtained with expected sizes, respectively (Fig. 3, each lane 1), indicating the existence of these receptors in bronchial tissue. In the presence of respective competitors (1–10,000 copies/ μ L), synthesis of PCR products for target genes was inhibited in a competitor concentration-dependent manner. The same results were also observed in tracheal tissues (not shown). The ratios of the target gene/competitor to corresponding Gapdh/Gapdh competitor in the presence of 100 copies/ μ L of respective competitor were calculated and summarized in Table 4. No significant difference in the expression of m_2 , m_3 and ET_B receptors was observed between the sensitized control and OA-challenged groups (Table 4).

Changes in microvascular leakage of the tracheal and main bronchial tissues were measured 24 h after the last antigen challenge. The levels of Evans blue dye exudation in sen-

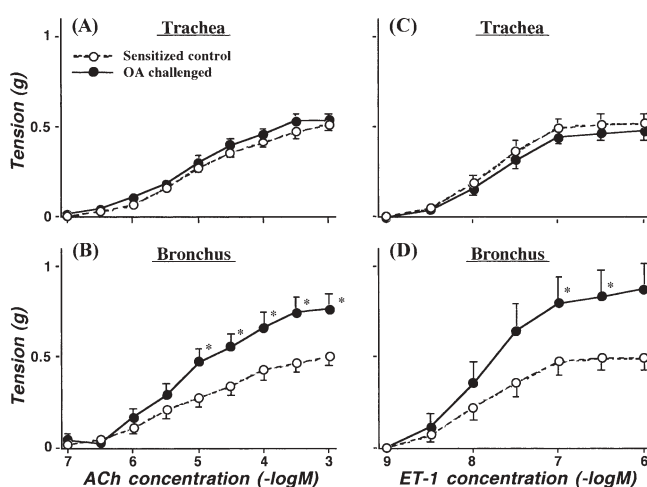


Fig. 1. Acetylcholine (ACh; A and B) and endothelin-1 (ET-1; C and D) concentration-response curves of isolated tracheal (A and C) and bronchial (B and D) rings from the sensitized control (open circles) and repeatedly ovalbumin (OA)-challenged mice. Each point represents the mean $\pm$ S.E. from 11 (Sensitized control) and 10 (OA challenged) experiments, respectively. Both the bronchial smooth muscle responsiveness to ACh (B) and ET-1 (D) were significantly augmented in OA challenged mice ($p < 0.01$ by ANOVA, respectively) whereas no significant difference between groups was observed in case of tracheal smooth muscle responsiveness (A and C).

Table 2. PCR primers for competitor synthesis.

Competitor for	Sequence	Product size
Chrm2	Sense: 5'-TTCAGTTCCTTTTCCAACGCTGCTAATACGACTCACTATAGGGC-3' Antisense: 5'-CCGTGTTTTCATCCTGGGTATCTAAGAGTCACTTTAAAATTGTAT-3'	327
Chrm3	Sense: 5'-GGCTGACTACTTAATCTTGGTCATCGGAAGAGAGTAG-3' Antisense: 5'-GGATGTTGCCGATGATGGTCTAAGAGTCACTTTAAAATTGTAT-3'	380
Ednrb	Sense: 5'-GCGCAATGGTCCCAATATCTTGTCTATCGGAAGAGAGTAG-3' Antisense: 5'-CCTATGGCTTCGGGGACAGCTAAGAGTCACTTTAAAATTGTAT-3'	382
Gapdh	Sense: 5'-ACCACAGTCCATGCCATCACTATCGGAAGAGAGTAG-3' Antisense: 5'-TCCACCACCCTGTTGCTGTTAAGAGTCACTTTAAAATTGTAT-3'	379

The DNA competitors used were prepared by PCR using the above primer sets with pGBKT7 plasmid as a template. Chrm2; m_2 muscarinic cholinergic receptor, Chrm3; m_3 muscarinic cholinergic receptor, Ednrb; ET_B endothelin receptor, and Gapdh; glyceraldehyde-3-phosphate dehydrogenase.

Table 3. Summary of the acetylcholine (ACh)- and endothelin-1 (ET-1)-induced contractile responses of tracheal and bronchial smooth muscles in mice.

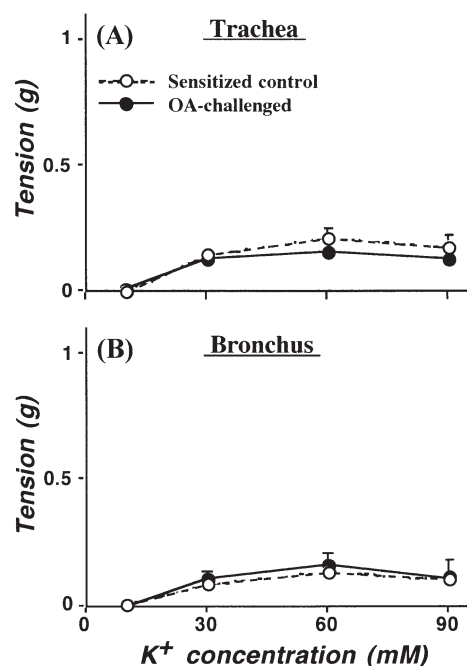
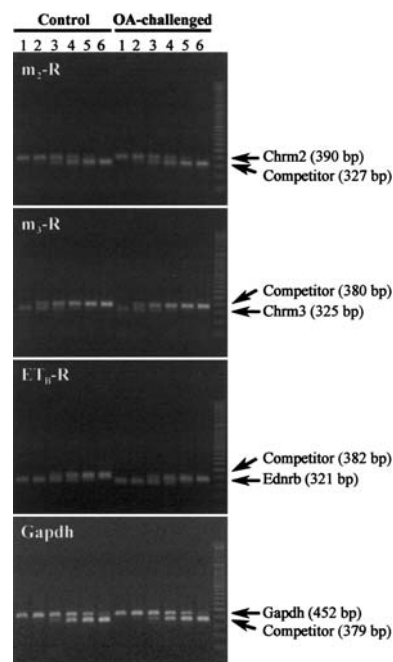
Group	<i>n</i>	Agonist	Trachea		Bronchus	
			pD ₂	E _{max} (g)	pD ₂	E _{max} (g)
Control	11	ACh	4.84 ± 0.11	0.52 ± 0.04	5.06 ± 0.18	0.51 ± 0.04
		ET-1	7.52 ± 0.12	0.49 ± 0.04	7.82 ± 0.14	0.50 ± 0.06
OA-challenged	10	ACh	4.99 ± 0.12	0.54 ± 0.04	5.11 ± 0.06	0.77 ± 0.06*
		ET-1	7.79 ± 0.12	0.51 ± 0.05	7.88 ± 0.14	0.87 ± 0.12*

Tracheal and main bronchial smooth muscles were isolated from the sensitized control (Control) and the repeatedly ovalbumin (OA)-challenged mice (OA-challenged). The isometrical force developed was measured. Data represent the mean ± S.E. The pD₂ (-log for 50% effective concentration (M) of acetylcholine) and E_{max} (maximal contractile response; g) values were obtained from individual concentration-response curves. *n*; number of experiments, **p* < 0.05 vs. respective control group.

Table 4. Expression levels of mRNAs for m₂ (Chrm2) and m₃ muscarinic (Chrm3) and ET_B endothelin (Ednrb) receptors in tracheal and bronchial tissues of sensitized control (Control) and ovalbumin (OA)-challenged mice determined by competitive RT-PCR.

	<i>n</i>	Trachea			Bronchus		
		Chrm2	Chrm3	Ednrb	Chrm2	Chrm3	Ednrb
Control	5	0.37 ± 0.10	0.18 ± 0.09	0.13 ± 0.02	0.60 ± 0.11	0.29 ± 0.11	0.26 ± 0.08
OA-challenged	5	0.41 ± 0.05	0.34 ± 0.15	0.21 ± 0.03	0.52 ± 0.17	0.10 ± 0.06	0.26 ± 0.09

The mRNA levels were determined by competitive RT-PCR as described in the Methods section. Data are expressed as the ratios of the target-gene/target-gene-competitor to corresponding Gapdh/Gapdh-competitor in the presence of respective competitors (100 copies/μL) and represent the mean ± s.e.mean. *n*; number of experiments.

**Fig. 2.** High K⁺ depolarization-induced contractile responses of isolated tracheal (A) and bronchial (B) rings from the sensitized control (open circles) and repeatedly ovalbumin (OA)-challenged mice. Each point represents the mean ± S.E. from 11 (Sensitized control) and 10 (OA challenged) experiments, respectively. No significant difference in the tracheal (A) or bronchial (B) smooth muscle responsiveness was observed between groups.**Fig. 3.** Typical photographs of PCR products for m₂ (Chrm2), m₃ (Chrm3) and ET_B (Ednrb) receptors and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) obtained from bronchial RNA samples of sensitized control (Control) and OA-challenged mice. Each target gene was determined by competitive RT-PCR as described in the Methods section. The PCR amplification was performed as follows: lane 1; in the absence of competitor, lanes 2–6; in the presence of 1, 10, 100, 1,000 and 10,000 copies/μL of competitor, respectively. Arrows indicate the bands for target genes and respective competitors with molecular weight in parentheses, respectively.

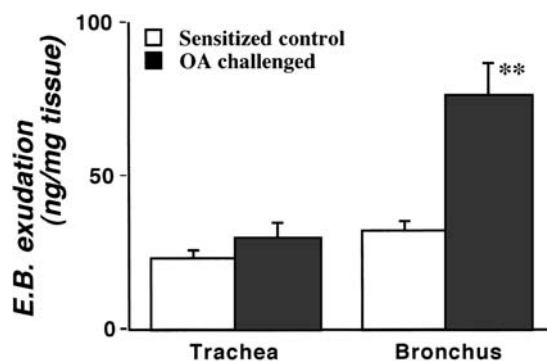


Fig. 4. Changes in amount of extravasated Evans blue dye (E.B.; ng E.B./mg wet weight tissue) in the trachea (*left*) and main bronchi (*right*) of sensitized control (*open columns*) and ovalbumin (OA)-challenged mice (*closed columns*). Each column represents the mean $\pm$ S.E. from 6 animals, respectively. ** $p < 0.01$ vs. sensitized control by unpaired Student's *t*-test.

sitized control mice (trachea, 23.3 ± 2.7 ng/mg tissue; bronchus, 32.2 ± 3.1 ng/mg tissue; $n = 6$; Fig. 4) were almost the same as naive animals (trachea, 23.3 ± 3.2 ng/mg tissue; bronchus, 35.5 ± 3.8 ng/mg tissue; $n = 6$), indicating that the sensitization procedure alone had no effect on the dye exudation. As shown in Fig. 4, the Evans blue dye extravasation in the main bronchial tissue of OA-challenged mice was significantly increased as compared with that of sensitized control animals, whereas no significant difference in the dye extravasation of tracheal tissues was observed between groups. The dye exudation in lungs of the OA-challenged mice (30.0 ± 2.9 ng/mg tissue, $n = 6$) was also significantly increased as compared with that of sensitized control group (22.7 ± 2.2 ng/mg tissue, $n = 6$; $p < 0.05$).

Histochemical examination also revealed a marked inflammation, i.e., inflammatory cells infiltration in main bronchus (Fig. 5B) and the hilus of the lung (Fig. 5C), but not in upper trachea (Fig. 5A), in OA-challenged mice. Most of the infiltrated cells were eosinophils as determined by Diff-Quik staining of sections (not shown). Eosinophil infiltration was observed even in the bronchial smooth muscle layer of antigen-challenged animals (Fig. 5B). The eosinophilic inflammation was also observed in the peripheral lung tissues of the OA-challenged mice (not shown).

Discussion

Kato et al. demonstrated a marked *in vivo* airway hyperresponsiveness (AHR) with increased IgE production and pulmonary eosinophilia in actively sensitized and repeatedly ovalbumin (OA)-challenged BALB/c mice [10]. By using the same sensitization and challenge protocol in BALB/c mice, the current study demonstrated that repeated antigen challenge induced a distinct bronchial smooth muscle hyperresponsiveness, i.e., enhanced bronchial smooth muscle responsiveness to acetylcholine (ACh) and endothelin-1 (ET-1) without change in expression of M_2 , M_3 and ET_B receptors. Bronchial inflammation, i.e., increased microvascular leakage and inflammatory cells infiltration in bronchial tissue, was also observed in this murine model of AHR. On the

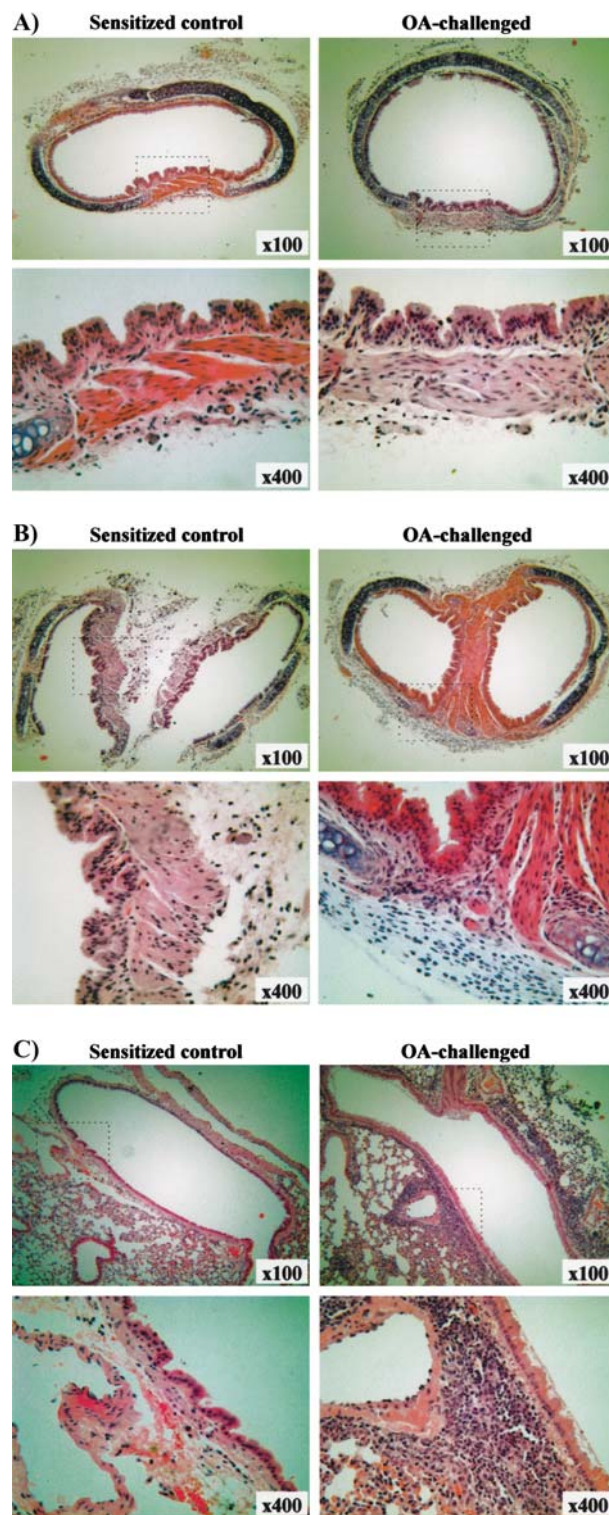


Fig. 5. Histological examinations of airway tissues (A: upper trachea, B: main bronchi, and C: near the hilus of the lungs) 24 h after saline (Sensitized control) or ovalbumin inhalation (OA-challenged) in OA-sensitized mice. Four- μ m sections of formalin-fixed tissues were stained with hematoxylin and eosin before examination by light microscopy. Original magnification: upper panels, $\times 100$; lower panels, $\times 400$ (the area of dotted line indicated in upper panel, respectively). Many eosinophils (polymorphonuclear cells with pink granules) were observed in main bronchus (B, right panels) and the hilus of the lungs (C, right panels) obtained from OA-challenged mice.

other hand, no significant differences in smooth muscle responsiveness and microvascular leakage in the trachea were found between the sensitized control and antigen-challenged groups. These findings indicate a distinct regional difference in the development of airway inflammation and hyperresponsiveness.

In the present study, the responsiveness to ACh and ET-1 was augmented in bronchial smooth muscle of the antigen-challenged mice. ACh and ET-1 are known to induce airway smooth muscle contraction mainly via activation of muscarinic M_3 [17] and ET_A/ET_B [16, 18, 19] receptors, respectively. In murine airways, it has also been reported that ET-1-induced bronchoconstriction is mediated via ET_B receptors [16]. Because no significant change in the expression levels of these membrane receptors was observed in the bronchial smooth muscle of OA-challenged mice (Table 4), a change in intracellular signaling via these receptors might be involved in the augmented contraction. Both the M_3 and ET_B receptors are linked to a heterotrimeric GTP-binding protein, G_q [20, 21]. The current observation that high K^+ -induced bronchial smooth muscle contraction, which is not mediated by receptor-G protein coupling, was not changed even in the antigen-challenged group suggests that augmentation of intracellular signal transduction mediated by membrane receptors/G proteins might be involved in the hyperresponsiveness of bronchial smooth muscle. Indeed, increased expression of G proteins has been demonstrated in guinea pig [22], rat [23] and murine [24] models of AHR. In addition, augmented agonist-induced Ca^{2+} sensitization of bronchial myofilaments, which is also mediated by heterotrimeric G proteins [21], has been reported in a rat model of AHR [8]. Further studies are needed to clear the underlying mechanism(s) of augmented bronchial smooth muscle contraction in the present murine model of airway hyperresponsiveness.

The current study demonstrated a distinct regional difference in the change in smooth muscle responsiveness after repeated antigen challenge. The results are consistent with our previous study, which demonstrated a bronchial smooth muscle hyperresponsiveness and normal tracheal smooth muscle contractility in a rat model of antigen-induced AHR [12]. Although the exact mechanism of the regional difference between tracheal and bronchial smooth muscle is not clear, a difference in the development of inflammation between tracheal and bronchial tissues (Fig. 5) might be involved. The present study also demonstrated an increased Evans blue extravasation only in bronchial tissues of the OA-challenged mice (Fig. 4). The extravasation of Evans blue dye, which correlates well with extravasation of radio-labeled albumin [25], is frequently used for determining vascular permeability. It has been postulated that airway edema associated with increased vascular permeability may directly contribute to airway hyperresponsiveness by causing airway narrowing and increasing mucosal thickness [26]. The regional difference in the development of smooth muscle hyperresponsiveness may come from the difference in the antigen-induced change in vascular permeability between bronchial and tracheal tissues. Alternatively, differences in the neural control [27] and/or physical adhesion of OA antigen to the airway luminal surface between tracheal and bronchial regions might also reflect the differences in the

development of tissue inflammation and smooth muscle hyperresponsiveness.

In conclusion, repeated antigen challenge to sensitized mice caused a hyperresponsiveness of bronchial, but not tracheal, smooth muscle accompanied by bronchial tissue inflammation. Elucidation of mechanisms of the bronchial smooth muscle hyperresponsiveness may be helpful for development of therapeutic drugs for AHR in asthmatics.

Acknowledgements. We thank Rie Hisatomi, Sachiko Hirose and Motonobu Matsuda for technical assistance.

References

- [1] Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; 7: 235–43.
- [2] Magnussen H, Nowak D. Roles of hyperresponsiveness and airway inflammation in bronchial asthma. *Respiration* 1989; 55: 65–74.
- [3] Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF et al. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981; 68: 347–55.
- [4] Bai TR. Abnormalities in airway smooth muscle in fatal asthma. *Am Rev Respir Dis* 1990; 141: 552–7.
- [5] De Jongste JC, Mons H, Bonta IL, Kerrebijn KF. In vitro responses of airways from an asthmatic patient. *Eur J Respir Dis* 1987; 71: 23–9.
- [6] Jiang H, Rao K, Halayko AJ, Kepron W, Stephens NL. Bronchial smooth muscle mechanics of a canine model of allergic airway hyperresponsiveness. *J Appl Physiol* 1992; 72: 39–45.
- [7] Duan W, Kuo IC, Selvarajan S, Chua KY, Bay BH, Wong WS. Antiinflammatory effects of genistein, a tyrosine kinase inhibitor, on a guinea pig model of asthma. *Am J Respir Crit Care Med* 2003; 167: 185–92.
- [8] Chiba Y, Takada Y, Miyamoto S, Mitsui-Saito M, Karaki H, Misawa M. Augmented acetylcholine-induced, Rho-mediated Ca^{2+} sensitization of bronchial smooth muscle contraction in antigen-induced airway hyperresponsive rats. *Br J Pharmacol* 1999; 127: 597–600.
- [9] Ellis KM, Cannet C, Mazzoni L, Fozard JR. Airway hyperresponsiveness to bradykinin induced by allergen challenge in actively sensitized Brown Norway rats. *Naunyn Schmiedeberg Arch Pharmacol* 2004; 369: 166–78.
- [10] Kato Y, Manabe T, Tanaka Y, Mochizuki H. Effect of an orally active Th1/Th2 balance modulator, M50367, on IgE production, eosinophilia, and airway hyperresponsiveness in mice. *J Immunol* 1999; 162: 7470–9.
- [11] Schramm CM, Puddington L, Wu C, Guernsey L, Gharaee-Kermani M, Phan SH et al. Chronic inhaled ovalbumin exposure induces antigen-dependent but not antigen-specific inhalational tolerance in a murine model of allergic airway disease. *Am J Pathol* 2004; 164: 295–304.
- [12] Misawa M, Chiba Y. Repeated antigenic challenge-induced airway hyperresponsiveness and airway inflammation in actively sensitized rats. *Jpn J Pharmacol* 1993; 61: 41–50.
- [13] Chiba Y, Sakai H, Misawa M. Augmented acetylcholine-induced translocation of RhoA in bronchial smooth muscle from antigen-induced airway hyperresponsive rats. *Br J Pharmacol* 2001; 133: 886–90.
- [14] Chiba Y, Sakai H, Misawa M. Characterization of muscarinic receptors in rat bronchial smooth muscle in vitro. *Res Commun Mol Pathol Pharmacol* 1998; 102: 205–8.
- [15] Chiba Y, Sakai H, Misawa M. Possible involvement of G_{i3} protein in augmented contraction of bronchial smooth muscle from antigen-induced airway hyperresponsive rats. *Biochem Pharmacol* 2001; 61: 921–4.

- [16] Nagase T, Aoki T, Oka T, Fukuchi Y, Ouchi Y. ET-1-induced bronchoconstriction is mediated via ETB receptor in mice. *J Appl Physiol* 1997; 83: 46–51.
- [17] Roffel AF, Elzinga CR, Zaagsma J. Muscarinic M₃ receptors mediate contraction of human central and peripheral airway smooth muscle. *Pulm Pharmacol* 1990; 3: 47–51.
- [18] Goldie RG, D'Aprile AC, Cvetkovski R, Rigby PJ, Henry PJ. Influence of regional differences in ETA and ETB receptor subtype proportions on endothelin-1-induced contractions in porcine isolated trachea and bronchus. *Br J Pharmacol* 1996; 117: 736–42.
- [19] Adner M, Cardell LO, Sjoberg T, Ottosson A, Edvinsson L. Contractile endothelin-B (ETB) receptors in human small bronchi. *Eur Respir J* 1996; 9: 351–5.
- [20] Caulfield MP. Muscarinic receptors – characterization, coupling and function. *Pharmacol Ther* 1993; 58: 319–79.
- [21] Croxton TL, Lande B, Hirshman CA. Role of G proteins in agonist-induced Ca²⁺ sensitization of tracheal smooth muscle. *Am J Physiol* 1998; 275: L748–55.
- [22] Lee JY, Uchida Y, Sakamoto T, Hirata A, Hasegawa S, Hirata F. Alteration of G protein levels in antigen-challenged guinea pigs. *J Pharmacol Exp Ther* 1994; 271: 1713–20.
- [23] Chiba Y, Sakai H, Arimoto T, Takada Y, Yoshikawa T, Misawa M. Gq protein level increases concurrently with antigen-induced airway hyperresponsiveness in rats. *Respir Physiol* 2000; 121: 75–83.
- [24] Gavett SH, Wills-Karp M. Elevated lung G protein levels and muscarinic receptor affinity in a mouse model of airway hyperreactivity. *Am J Physiol* 1993; 265: L493–500.
- [25] Udaka K, Takeuchi Y, Movat HZ. Simple method for quantitation of enhanced vascular permeability. *Proc Soc Exp Biol Med* 1970; 133: 1384–7.
- [26] Evans TW, Chung KF, Rogers DF, Barnes PJ. Effect of platelet-activating factor on airway vascular permeability: possible mechanisms. *J Appl Physiol* 1987; 63: 479–484.
- [27] Stretton D, Belvisi MG, Barnes PJ. The effect of sensory nerve depletion on cholinergic neurotransmission in guinea pig airways. *J Pharmacol Exp Ther* 1992; 260: 1073–80.



To access this journal online:
<http://www.birkhauser.ch>
