

Histamine-induced changes in rat tracheal goblet cell mucin store and mucosal edema

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Abstract The pathology of chronic asthma in human and mouse is characterized by inflammation and remodeling of airway tissues. As a result of repeated inflammatory insults to the lower airways, smooth muscle thickening, mucin secretion and airway hyperreactivity may develop. In ovalbumin (OVA)-sensitized mice with repeated challenges with OVA to the lower airways, the trachea and bronchi are characterized by goblet cell hyperplasia and mucus hypersecretion from goblet cells. Previous study reports that intravenous (i.v.) application of a high dose of capsaicin releases tachykinin from capsaicin-sensitive nerves, producing acute plasma leakage and mucosal edema formation and causing depletion of mucin granules in goblet cells that results in a reduction in the number and size of Alcian blue (AB)-positive goblet cells in the rat trachea within a few minute after capsaicin application. Histamine is an important non-neural mediator of asthma from mast

cells. The present study investigated whether i.v. application of a high dose of histamine (18 $\mu\text{mol/ml/kg}$) could result in these acute changes and the similar time-course changes in rat trachea. The tracheal whole mounts stained with chloroacetate esterase reagent and AB and tracheal methacrylate sections stained with AB and periodic acid-Schiff reagent were used for evaluation of histological and cellular changes. At 5 min after histamine application, mucosal leaky venules were numerous and subepithelial edema ratio (% of length of edema along the mucosal epithelial circumference of tracheal cross section) was found to be 48.2 ± 4.9 , which was greater ($P < 0.01$) than saline-treated rats. But, the number of AB-positive goblet cells, $2,030 \pm 170/\text{mm}^2$ of mucosal surface epithelium, was similar to saline-treated group ($P > 0.05$). One day later, edema ratio remained large and the number of AB-positive goblet cells was $1,140 \pm 150/\text{mm}^2$ epithelium, reduced to half the number of the group at 5 min after histamine ($P < 0.01$). It is suggested that mucus hypersecretion occurred at this time point. At 3 or 5 days after histamine, edema ratio gradually decreased. The number of AB-positive goblet cells continued to remain small on day 3. On day 5 after histamine, the number of AB-positive goblet cells restored to the level of rat group at 5 min after histamine application. At 7 days after histamine, edema ratio returned to the level of saline-treated group. It is concluded that degranulation and thinning of tracheal goblet cells and mucus hypersecretion lagged behind histamine-induced acute plasma leakage and edema, and restoration of mucin store in goblet cells was associated with remission of mucosal edema.

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Introduction

The pathology of chronic asthma in human is characterized by inflammation and remodeling of airway tissues. As a result of repeated inflammatory insults to the lower airways, smooth muscle thickening, hypersecretion of mucin and airway hyperreactivity may develop (Bousquet et al. 2000). In ovalbumin (OVA)-sensitized mice with repeated OVA challenges to the lower airways, the trachea and bronchi are characterized by goblet cell hyperplasia and mucus hypersecretion from goblet cells (Yamauchi et al. 2008; Cowden et al. 2010).

The epithelium of the tracheal mucosal surface of mammals is pseudostratified, consisting predominantly of ciliated cells, goblet cells and basal cells. In addition, the tracheal epithelium also contains other epithelial cells, such as neuroendocrine and brush cells. In human and guinea pig, goblet cells are mucin-secreting cells. There are many globule leukocytes encountered in the epithelium (Tam et al. 1988). These are invaded immune cells.

In the specific pathogen-free rats, the tracheal epithelium is pseudostratified cuboidal or low columnar type. The predominate secretory cells are not goblet cells, but are serous cells which contain a large number of calcitonin gene-related peptide (CGRP)-containing secretory granules instead of mucin granules (Baluk et al. 1993). When pathogen free rats are exposed to *Mycoplasma pulmonis* or combined mycoplasmal and viral infections, the cuboidal or low columnar epithelium develops into pseudostratified high columnar type with numerous ciliated cells and goblet cells containing a large number of mucin granules (McDonald 1988; Huang et al. 1989; McDonald et al. 1991). Cuboidal-shaped serous cells with CGRP-containing granules are rare. Serous cells may develop into goblet cells and ciliated cells in the tracheal epithelium of infected rats (Huang et al. 1989). The situation in mouse is similar to the rat in that goblet cells are not present in the tracheal epithelium under physiological conditions and appear as such only in pathological conditions.

Goblet cells are characterized by the presence of numerous large mucin-containing secretory granules that fill the bulged supranuclear cytoplasm of the cell, and the nucleus is located in the cell base (Adler et al. 1982; Basbaum et al. 1988; Furuya et al. 1998).

Goblet cell mucin secretion in mammals is under neural and non-neural control. Vagus nerve stimulation causes mucin discharge and is blocked by either atropine or by capsaicin pre-treatment, indicating that both cholinergic nerves (Tokuyama et al. 1990) and capsaicin-sensitive sensory nerves (McDonald 1988; Huang et al. 1989; Tokuyama et al. 1990; Kuo et al. 1990; Huang 1993 1995) contribute to control of goblet cell secretion. Capsaicin is an irritant with a strong inflammation-producing ability in the airways.

Capsaicin depletes substance P from the sensory nerve endings that act on the venular endothelial cells to form endothelial gaps (McDonald 1994) where plasma leaks to the perivascular tissue. Associated with the acute inflammation and edema formation in the airway mucosa induced by either capsaicin or substance P (McDonald 1988), airway goblet cells release a large amount of mucin granules from the supranuclear cytoplasm (Guo et al. 2003). It is suggested that substance P acts directly on the goblet cells to cause release of content from mucin granules in the apical cytoplasm.

The magnitude of goblet cell secretion can be assessed in histologic sections of the trachea stained with AB and periodic acid-Schiff (PAS) by the two semiquantitative morphometric analysis methods, called mucus score and secretory ratio. An increase in mucin discharge is inversely related to a decrease of mucus score (Kuo et al. 1990; Tokuyama et al. 1990; Takeyama et al. 1996). In contrast, the degree of goblet cell mucin secretion is proportional to the secretory ratio (Kuo et al. 1990). When goblet cell mucin secretion is partially depleted by a low dose of capsaicin (Kuo et al. 1990), sensory neuropeptides (Kuo et al. 1990) or histamine (Takeyama et al. 1996), mucus score is useful. The evaluation using mucus score is not applicable to a high dose of capsaicin which produces acute extensive plasma leakage associated with prominent edema in the tracheal mucosa and causes many tracheal goblet cells to completely deplete their secretory granules associated with mucus hypersecretion (Guo et al. 2003); because the number and size of AB-positive mucin granule-containing goblet cells are considerably reduced as shown by the tracheal whole mounts and tissue sections (Guo et al. 2003). Secretory ratio is useful for evaluation of goblet cell secretion in tracheal tissue sections (Guo et al. 2003).

Histamine is one of the important non-neural mediators of mast cells in the pathogenesis of asthma (Yamauchi et al. 2008; Hirota et al. 2012). It is an inflammatory agent when administered to the animals at a high dose. Histamine acts directly on the venular endothelial cells in the tracheal mucosa and produces acute plasma leakage in the venules (Brokaw et al. 1990). It was hypothesized that a high dose of histamine application could also produce acute extensive plasma leakage associated with prominent edema in the tracheal mucosa and cause many tracheal goblet cells to completely deplete their secretory granules associated with mucus hypersecretion. To test this hypothesis, the present study was designed to investigate the time-course of changes in the number and size of AB-positive goblet cells in the rat tracheal whole mounts, to analyze the mucus score and secretory ratio of goblet cell secretion and to evaluate the degree of mucosal edema in the plastic sections of the trachea at 5 min, and at 1, 3, 5, and 7 days after

acute inflammation induced by i.v. application of a high dose of histamine (18 $\mu\text{mol/ml/kg}$).

Materials and methods

Neonatal rats of both sexes and their mother of Sprague–Dawley strain were raised in clean animal rooms, but not kept pathogen free, in the animal center of the Department of Biological Sciences, National Sun-Yat Sen University. When rats grew to 3–4 months of age, experiments that were approved by the Committee on Animal Care and Use in the university were performed.

Animal experiments

Six groups of adult rats were used in the following experiments. For evaluation of the permeability-producing ability of histamine, India ink was used as a tracer dye to label the leaky blood vessels in the mucosal tissue (Lo and Huang 1997; Hwang et al. 1999). After anesthesia with an intraperitoneal injection of sodium pentobarbital (Koch-Light Laboratories, Colnbrook, Buckinghamshire, UK; 50 mg/kg), the first group of six rats received an intravenous injection of India ink (Chroma-Gesellschaft, Kongen, Germany; 1 ml/kg, over 5 s) via the femoral vein and followed by an injection of histamine (Sigma-Aldrich, Saint Louis, MO, USA; 18 $\mu\text{mol/kg}$, over 20 s) via the vein on the opposite side. The second group of six rats received injection of India ink followed by saline (1 ml/kg), the vehicle of histamine. Five minutes after dye injection, the rat chest was opened and rat was perfused through the aorta with 0.05 M phosphate buffer, pH 7.4 for 2 min, and then followed by 0.05 M phosphate-buffered 1 % paraformaldehyde and 0.5 % glutaraldehyde, pH 7.4, for additional 2 min at a pressure of 120–140 mmHg. Afterwards, fixative was retrogradely perfused through a cut in the mainstem bronchus into the tracheal lumen to wash off the covering mucus layer on epithelial surface and further fix the tracheal tissue.

For the remaining four groups of rats, six rats each group, following an i.v. injection of histamine alone, the wound skin at the groin was closed with metal pins. Rats were allowed to survive for 1, 3, 5, or 7 more days before perfusion fixation.

The rostralmost portion of trachea with the 1st–6th cartilage rings was removed for preparation of glycol methacrylate sections for the measurement of edema ratio in the mucosa and the measurement of mucus score and secretory ratio of goblet cell secretion. The remaining portion of trachea was used for the preparation of whole mount for the measurement of AB-positive goblet cell size and number.

Tissue processing

Tracheal whole mounts

Globule leukocytes and goblet cells in tracheal epithelium of whole mounts were differentiated. Whole mounts with the 8th–25th cartilage rings were pinned on the Sylgard base (Dow Corning, Midland, MN, USA) and incubated with chloroacetate esterase medium containing naphthol AS-D chloroacetate as the substrate and hexazotized pararosanilin (Sigma-Aldrich) as the chromogen for 14–18 h at 4 °C, followed by a wash in distilled water for 2 h to show the globule leukocytes with their cytoplasmic globule granules stained orange to red in the tracheal surface epithelium (Tam et al. 1988). Whole mounts were further stained with 0.5 % AB (Sigma-Aldrich) in 3 % acetic acid, pH 2.5, for 30 min. At present, mast cells and globule leukocytes appeared dark red, because they were also stained with AB (Guo et al. 2003; Figs. 1, 2, 3, 4 in the present paper). AB-positive goblet cells with their cytoplasm accumulated with mucin granules appeared brilliantly blue (Guo et al. 2003). To avoid that tracheal surface epithelium might be lost during tissue processing, a cover slip was attached onto the mucosal surface of tracheal whole mount before it was flattened between two glass slides, dehydrated with ethanol, cleared with toluene, and mounted with Permount (Fisher Scientific, NJ, USA).

Tracheal methacrylate sections

Rostral part of the trachea was dehydrated with a graded series of acetone and embedded with glycol methacrylate. Cross sections of tracheal ring 3 μm in thickness were stained with 0.5 % AB in 3 % acetic acid, pH 2.5, for 30 min, treated with 1 % periodic acid for 5 min, and further stained with the Schiff reagent for 5 min (Huang et al. 1989). Some tissue sections were only stained with toluidine blue.

Measurement of histamine-induced plasma leakage

The magnitude of inflammation was expressed by the area density of India ink-labeled leaky blood vessels in the mucosal tissue of tracheal whole mounts, which was determined by a point counting method (Lo and Huang 1997; Hwang et al. 1999).

Measurement of the number and size of AB-positive goblet cells in the tracheal whole mounts

The tracheal whole mounts stained with chloroacetate esterase reagent and AB were used for measuring the number and transverse diameter of AB-positive goblet cells

Fig. 1 Photomicrographs focusing at India ink-labeled leaky venules in the lamina propria of tracheal whole mounts stained with chloroacetate esterase medium (CAE) and AB. Bar 120 μ m. **a** 5 min after saline application. A few leaky venules (*arrows*) and numerous mast cells (*red dots*) were observed. **b** 5 min after histamine. Many leaky vessels (*arrows*) and mast cells were observed

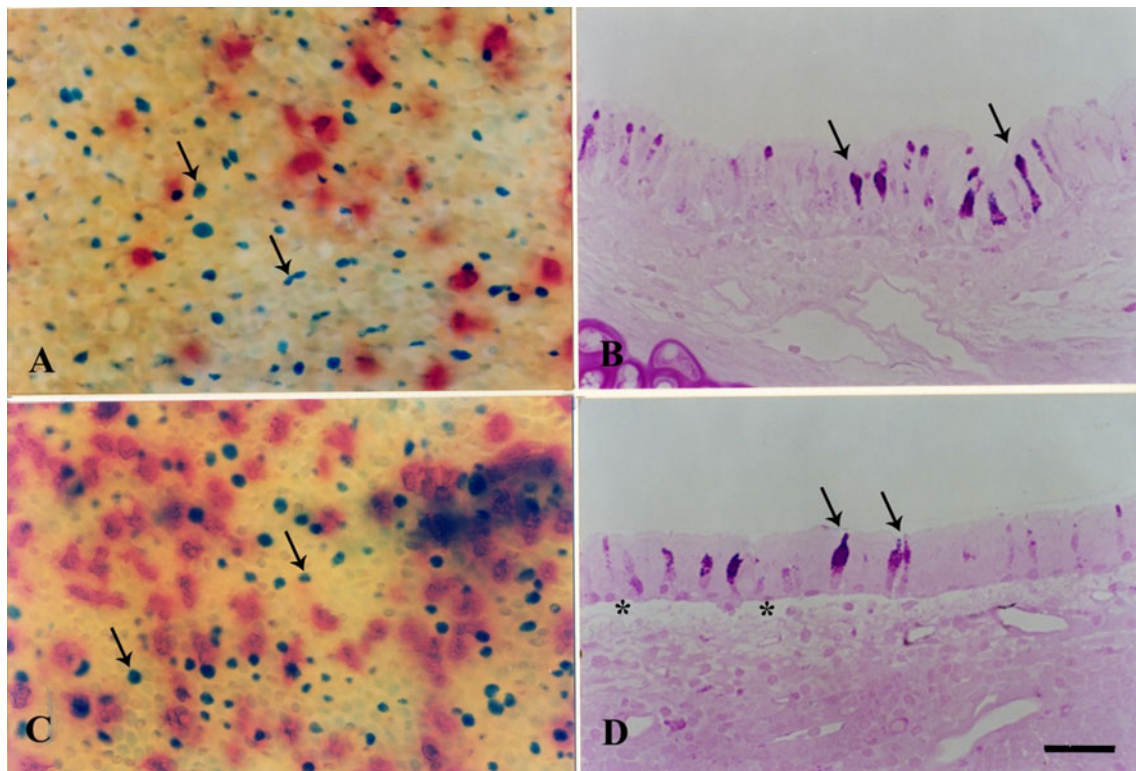
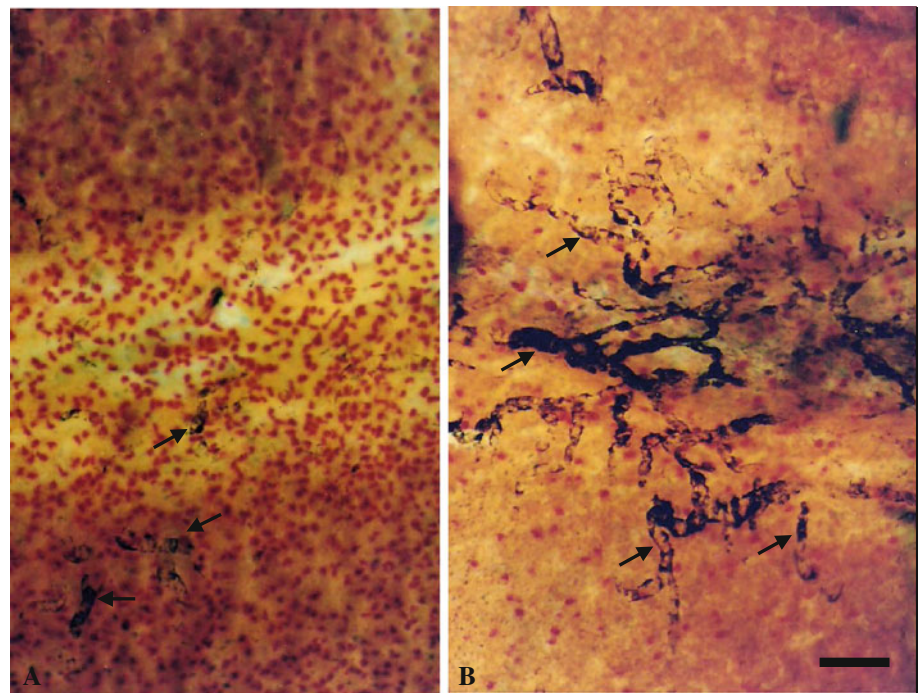


Fig. 2 Photomicrographs focusing at the surface epithelium of tracheal whole mounts stained with CAE and AB, and of tracheal cross sections stained with AB-PAS. Bar 30 μ m. **a** Whole mount of trachea of rat 5 min after saline application showing that, in the surface epithelium, CAE-positive reddish cells were globule leukocytes and round to oval blue masses were cytoplasm of AB-positive goblet cells (*arrows*). **b** Section of trachea 5 min after saline showing

that goblet cells were bulged and filled with AB-PAS-positive purple mucin granules (*arrows*). **c** Whole mount 5 min after histamine. The number and size of AB-positive goblet cells (*arrows*) were similar to Fig. 2a. **d** Section 5 min after histamine. *Prominent edema underneath the epithelium was observed. As the saline-treated rat, goblet cells were bulged and filled with mucin granules (*arrow*)

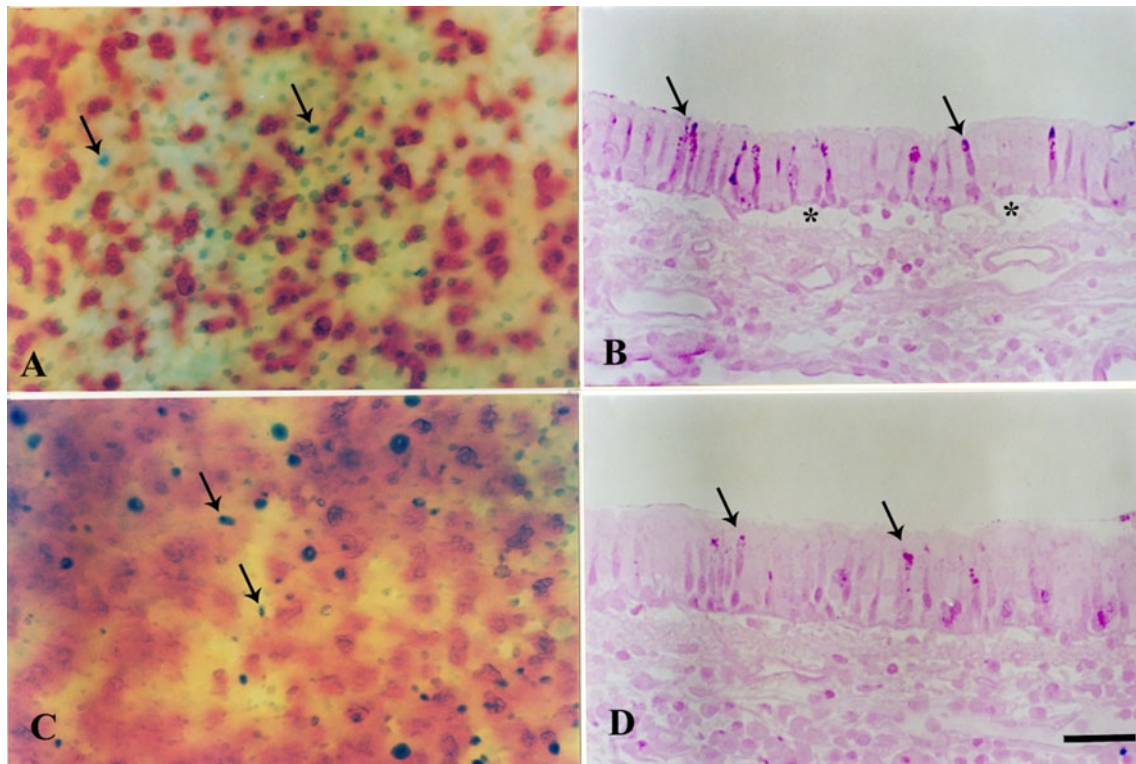


Fig. 3 Photomicrographs of whole mounts and sections of trachea. Bar 30 μm . **a** Whole mount 1 day after histamine showing that the number and size of AB-positive goblet cells (arrows) was strikingly reduced. **b** Tissue Section 1 day after histamine. Goblet cells (arrows) became thin and long. *Subepithelial edema was as prominent as the trachea at 5 min after histamine. **c** Whole mount

of trachea 3 days after histamine showing that AB-positive goblet cells (arrows) were still small in number. **d** Tracheal Section 3 days after histamine showing that goblet cells were still thin and long and contained a little mucin granules in the apical cytoplasm (arrows). Subepithelial edema could be observed

(Guo et al. 2003). AB-positive goblet cells were sampled from the surface epithelium in the areas between cartilages where they were near the posterior membrane of trachea. Twelve visual fields, 6 from the right side and 6 from the left side, were magnified to $400\times$ under a conventional light microscope. An ocular micrometer with a grid of 100 squares was seen on the visual field of tissue. The area of each square measured $773 \mu\text{m}^2$. The number and transverse diameter of goblet cells were recorded. In the tracheal epithelium of each rat, about 500–1,000 cells were studied.

Measurement of goblet cell mucin secretion

Two values of goblet cell secretion for each rat were obtained from two tracheal cross sections that were not consecutive. Mucus score (in arbitrary units) was calculated as $(1 \times n_1 + 2 \times n_2)$ where 1 and 2 were the gradings, and n_1 and n_2 the total numbers of cells in each grade, respectively (Kuo et al. 1990; Tokuyama et al. 1990). Secretory ratio was calculated as the ratio of the number of grade 1 cells to the number of grade 2 cells in tissue sections of each animal (Kuo et al. 1990).

Measurement of mucosal edema

To measure the tracheal inner circumference and the length of subepithelial edematous loci, the montage of a tracheal cross section of each rat was prepared. It was formed from 6 to 8 low-power photomicrographs taken at the original magnification of $40\times$ along the circular tracheal wall using the software of Adobe Photoshop 7.0. The neighboring photomicrographs were partially overlapped with each other (Fig. 5a). The mucosal surface of a tracheal montage was drawn along the tracheal mucosal epithelium using the software Simple PCI (Compix Inc., USA). Subepithelial edematous loci were marked with short line segments underlying the tracheal epithelium (Fig. 5b). Edema ratio was proposed as the degree of edema that was expressed as the percentage of the total length of subepithelial edematous regions to the epithelial circumference.

Statistical analysis

Data were expressed as mean $\pm$ SEM. Sample size was 6 for each data of rat group. Data were analyzed with one-way ANOVA followed by the Newman–Keuls method. P values <0.05 were considered significantly different.

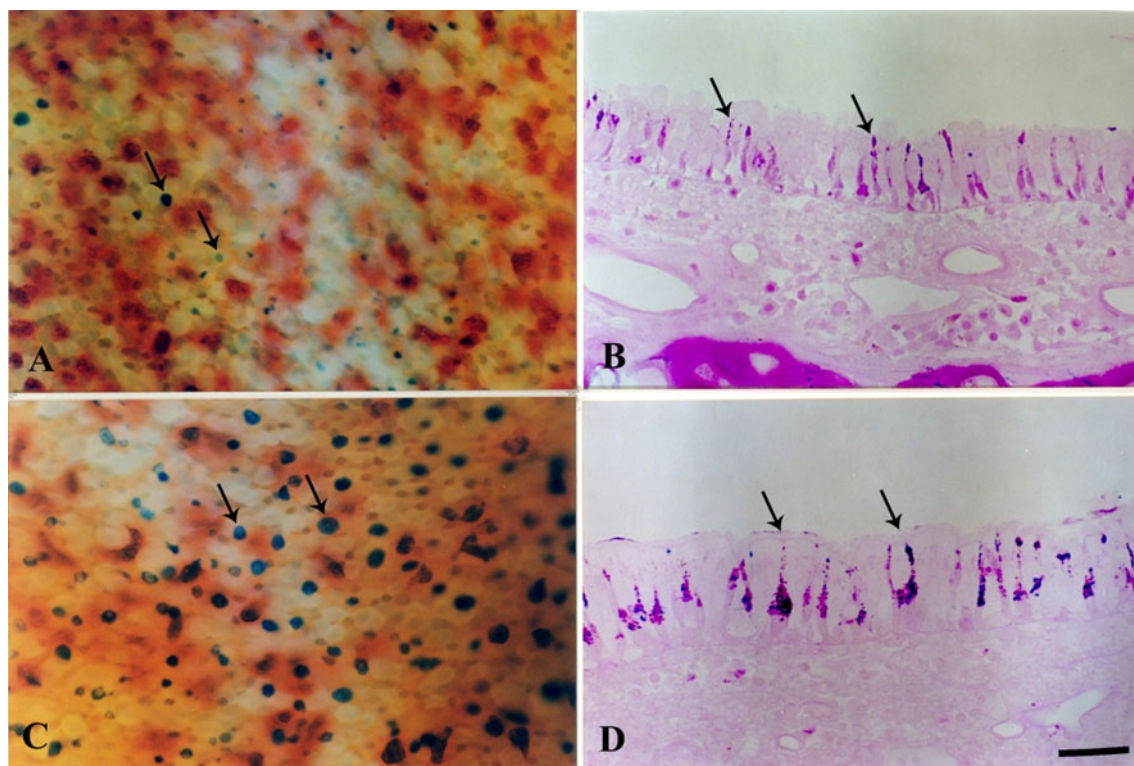


Fig. 4 Photomicrographs of tracheal whole mounts and tissue sections. *Bar* 30 μ m. **a** Whole mount of trachea 5 days after histamine. The cell size of AB-positive goblet cells was still small. **b** Tracheal Section 5 days after histamine. Edema underneath the epithelium was still present. Goblet cells were also thin and long, but their cytoplasm were filled with mucin granules (*arrows*). **c** Whole

mount 7 days after histamine showing that the number and size of AB-positive goblet cells (*arrows*) were similar to the rat 5 min after histamine application. **d** Tracheal Section 7 days after histamine. The basal portion of many goblet cells were bulged and filled with mucin granules (*arrow*). The apical portion of goblet cells was still thin

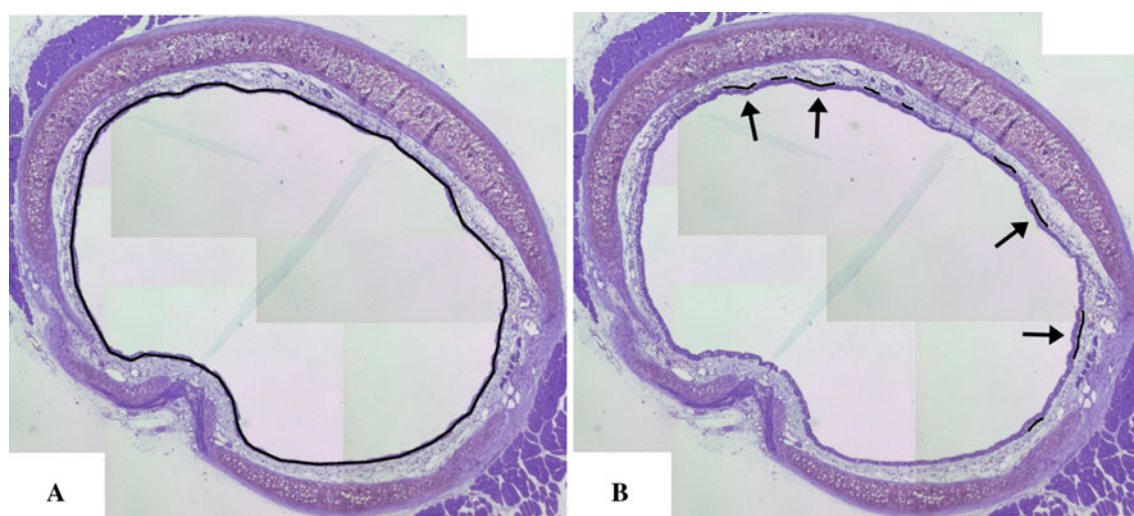


Fig. 5 Montage of 6 low-power photomicrographs taken from a single tracheal cross section that were overlapped successively to form a tracheal ring. **a** The *black line circle* was drawn along the tracheal mucosal epithelium at the inner circumference. **b** Subepithelial edema

was marked with *short black line segments (arrows)*. Edema ratio was proposed as the edema index, expressed by the percentage of the summation of length of intermittent edema formations for the whole length of inner circumference

Results

Histamine-induced plasma leakage

At 5 min after application of saline, a little extravasation of India ink was produced in the tracheal mucosa (Fig. 1a); the area density of India ink-labeled leaky venules was $0.31 \pm 0.11\%$ ($n = 6$) which was statistically much less ($P < 0.01$) than histamine-treated group. Histamine at the dose of $18 \mu\text{mol/kg}$ produced extensive India ink leakage as evidenced by many leaky venules (Fig. 1b) in the tracheal mucosa at 5 min after histamine application. The area density of leaky venules was $15.06 \pm 1.15\%$ ($n = 6$).

Density and size of Alcian blue-positive goblet cells

Goblet cells were clearly differentiated from globule leukocytes in the surface epithelium of the tracheal whole mounts stained with chloroacetate esterase reagent and AB (Figs. 2, 3, 4). Saline-treated rat trachea exhibited numerous AB-positive goblet cells, the number of which was $1,980 \pm 110/\text{mm}^2$ tracheal epithelium (Fig. 6). The average transverse diameter of AB-positive goblet cells was $4.28 \pm 0.22 \mu\text{m}$ (Table 1). At 5 min after histamine application, the number of AB-positive goblet cells was $2,030 \pm 170/\text{mm}^2$ tracheal epithelium that was similar to saline-treated group ($P > 0.05$). At 1 day after histamine application, the number of AB-positive goblet cells was $1,140 \pm 150/\text{mm}^2$ epithelium, reducing to half the number of the group at 5 min after histamine or saline ($P < 0.01$). AB-positive goblet cells were even smaller in size (Table 1).

Goblet cell number remained small on day 3 (Fig. 6). On day 5, the cell number restored to the level (Fig. 6) as the saline-treated group and the group at 5 min after histamine application.

Mucus score

Mucus score between groups was not significantly different when analyzed with one-way ANOVA ($P > 0.05$). This might be due to a large variation in the number of AB-PAS-positive goblet cells in different rat groups (Fig. 7).

Secretory ratio

Secretory ratio of goblet cell secretion in saline-treated group was not significantly different ($P > 0.05$) from that in the group at 5 min after histamine application (Fig. 8). Secretory ratio of goblet cell secretion was significantly different ($P < 0.01$) between the groups at 5 min and at 1 day after histamine. At 3 or 5 days after histamine, secretory ratio was still significantly different ($P < 0.01$) from that of the group at 5 min after histamine. At 7 days after histamine, it was not significantly different from rat group at 5 min after histamine.

Edema ratio

Local mild subepithelial edema was found in some saline-treated rats at 5 min after injection of saline. Prominent extensive edema between the epithelium and the lamina propria was observed at 5 min after histamine (Fig. 2d). Edema ratio was 48.2 ± 4.9 ($n = 6$) at 5 min after

Fig. 6 Change in the number of goblet cells/ mm^2 of tracheal epithelium after histamine application. One day or 3 days after histamine, the number of AB-positive goblet cells was significantly decreased ($**P < 0.01$) as compared with the rat group 5 min after histamine application. The group 5 min after histamine was not significantly different ($P > 0.05$) from the group 5 min after saline

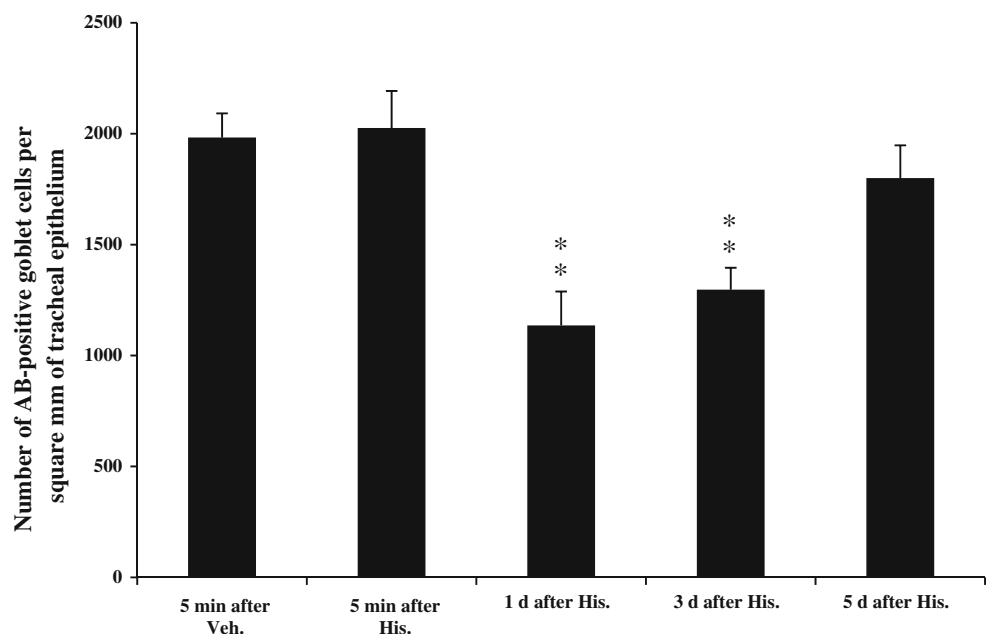


Table 1 Changes in the cell size (transverse diameter in μm) of AB-positive goblet cells measured in tracheal whole mounts after histamine application

Rat group	5 min after saline	5 min after histamine	1 day after histamine	3 days after histamine	5 days after histamine
Cell size	4.28 ± 0.22	4.44 ± 0.16	$3.64 \pm 0.16^{**}$	4.02 ± 0.14	$3.29 \pm 0.09^{**}$
In μm	($n = 6$)	($n = 6$)	($n = 6$)	($n = 6$)	($n = 6$)

The rat number in the parenthesis of each animal group was 6. For each rat over 500 AB-positive goblet cells were measured

$^{**} P < 0.01$ as compared with the group 5 min after histamine

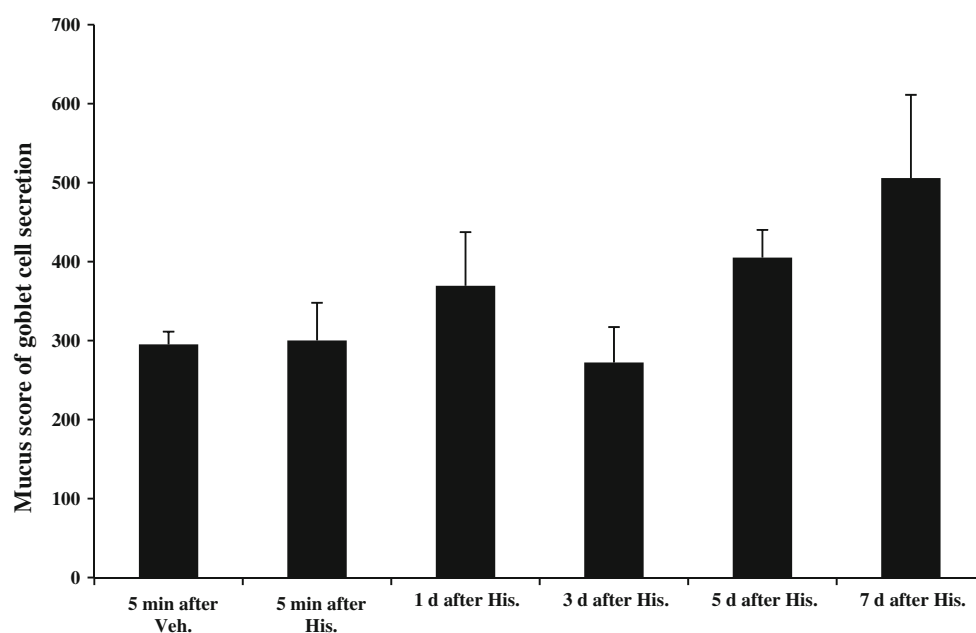
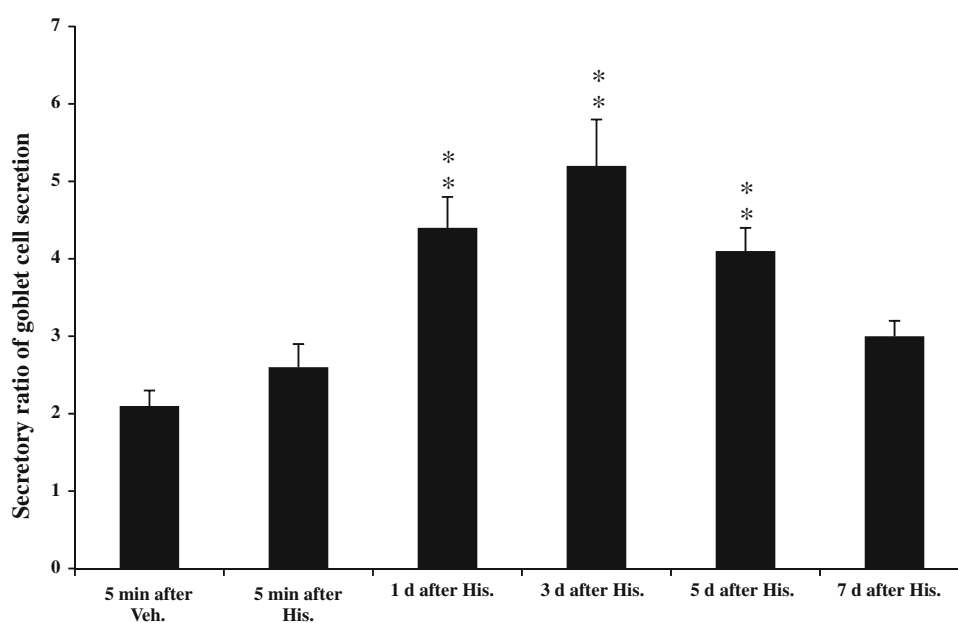
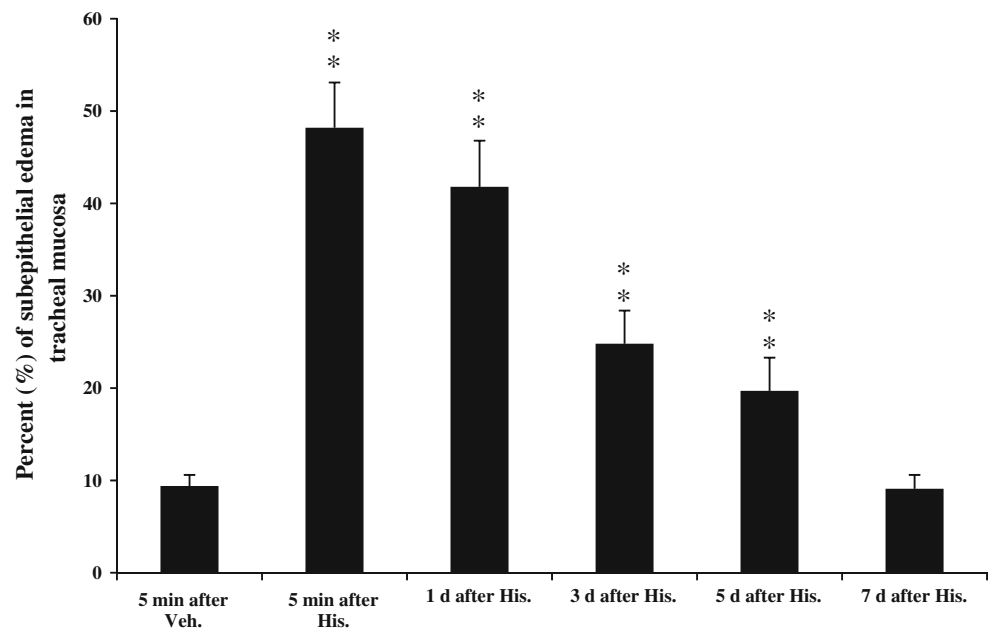
Fig. 7 Time-course of mucus score of goblet cell secretion after histamine. There was no significant difference ($P > 0.05$) between rat groups, probably due to high variation in the number of goblet cells that were depleted of mucin granules**Fig. 8** Time-course of change in secretory ratio of goblet cell secretion after histamine application. One, 3, or 5 days after histamine, secretory ratio of goblet cell secretion was significantly larger ($^{**}P < 0.01$) than rats 5 min after histamine

Fig. 9 Time-course of change in the subepithelial edema ratio (%) after histamine application. Focal edema was observed in the trachea of saline-treated rats. At 5 min, 1 day, 3 days, or 5 days after histamine application, edema ratio was significantly larger (** $P < 0.01$) than saline-treated group



histamine (Fig. 9). Subepithelial edema lasted for several days up to 5 days. It remained large at days 1 and 3 (Fig. 3b, d), then diminished strikingly (Fig. 4b). At day 7, edema ratio restored to the level of saline-treated rat (Figs. 4d, 9).

Discussion

The predominate mucin-secreting cells in the tracheal epithelium of human and guinea pig are goblet cells. In the asthmatic subjects of human and mouse, the allergic or inflammatory lower airways are characterized by goblet cell hyperplasia and mucus hypersecretion from goblet cells (Yamauchi et al. 2008; Cowden et al. 2010; Hirota et al. 2012).

The tracheal epithelium of specific pathogen-free rats is pseudostratified cuboidal or low columnar type which consists of numerous serous cells with CGRP-containing secretory granules and less ciliated cells (Baluk et al. 1993). When pathogen-free rats are subjected to intranasal inoculation of *Mycoplasma pulmonis* or exposed to naturally combine mycoplasmal and viral infections, the cuboidal or low columnar epithelium develops into pseudostratified high columnar type which consists of numerous ciliated cells and goblet cells (McDonald 1988; Huang et al. 1989; McDonald et al. 1991). Extensive angiogenesis and tissue thickening occur in the mucosa within a few weeks after respiratory mycoplasmal infections in rats and mice (McDonald et al. 1991; Ezaki et al. 2001).

In the present study, all rats were not kept pathogen-free, they might be exposed to natural respiratory infections

since neonatal life. Histological examination indicated that the type of tracheal mucosal surface epithelium of all rats was pseudostratified high columnar as reported previously (McDonald 1988; Huang et al. 1989; Guo et al. 2003). In saline-treated rats, goblet cells contained a large accumulation of purple mucin granules in the supranuclear cytoplasm that made the cells appeared goblet-shaped in tissue sections stained with AB-PAS. At 5 min after histamine application, epithelial structure and goblet cell structure were similar to those of saline-treated rats. But, subepithelial edema was prominent and edema ratio was high. Goblet cells seemed to be not affected at 5 min after histamine.

Previous study using capsaicin reports that within a few min after capsaicin application, mucin-discharging secretory activity in rat goblet cells are strikingly enhanced (Guo et al. 2003). It is suggested that i.v. application of a high dose of capsaicin releases tachykinin from capsaicin-sensitive nerves that produce acute plasma leakage and mucosal edema formation and cause depletion of mucin granules in goblet cells, resulting in reduction in the number and size of AB-positive goblet cells and mucus hypersecretion in the rat trachea within a few min after capsaicin application (Guo et al. 2003).

In the present study, depletion of goblet cell mucin granules and mucus hypersecretion lagged behind histamine-induced acute extensive plasma leakage and mucosal edema formation within a few minute after histamine application. The number of tracheal AB-positive goblet cells in rats at 5 min after histamine application was not changed as compared to saline-treated rats. The cell size of AB-positive goblet cells was not decreased. The secretory ratio of goblet cell mucin secretion was also not changed.

It is suggested that considerable amount of mucins were not released from goblet cells within 5 min after histamine application.

At 1 day after histamine application, edema ratio remained large and the number of AB-positive goblet cells was $1,140 \pm 150/\text{mm}^2$ epithelium, reduced to half the number of the group at 5 min after histamine. Mucin store was strikingly reduced. Many goblet cells with cytoplasmic mucin granules partially or completely depleted were no longer goblet-shaped. They acquired an elongated, rod shape. The diameter of these cells was obviously reduced. Mucus hypersecretion took place during the interval between 5 min and 1 day after histamine application.

Again, 3 or 5 days after histamine, many goblet cells still looked like an elongated, rod shape with mucin granules partially or completely depleted. The diameter of these cells remained small. At 3 or 5 days after histamine, edema ratio was gradually decreased, but still larger than saline-treated rats. The number of AB-positive goblet cells continued to remain small at day 3. At day 5 after histamine, the number of AB-positive goblet cells restored to the level of rat group at 5 min after histamine or saline application.

At 7 days after histamine, goblet cells refilled with mucin granules with their basal cytoplasm thicker than the apical cytoplasm. At 7 days after histamine, edema ratio returned to the level of saline-treated group.

It is concluded that degranulation and thinning of tracheal goblet cells and mucus hypersecretion lagged behind histamine-induced acute plasma leakage and edema and restoration of mucin store in goblet cells was associated with remission of mucosal edema.

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References

- Adler KB, Hardwick DH, Craighead JE (1982) Porcine tracheal goblet cell ultrastructure: a three-dimensional reconstruction. *Exp Lung Res* 3:69–80
- Baluk P, Nadel JA, McDonald DM (1993) Calcitonin gene-related peptide in secretory granules of serous cells in the rat tracheal epithelium. *Am J Respir Cell Mol Biol* 8:446–453
- Basbaum CB, Carlson D, Davidson E, Verdugo P, Gail DB (1988) Cellular mechanisms of airway secretion. *Am Rev Respir Dis* 137:479–488
- Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM (2000) Asthma. From Bronchoconstriction to airway inflammation and remodeling. *Am J Respir Crit Care Med* 161:1720–1745
- Brokaw JJ, Hillenbrand CM, White GW, McDonald DM (1990) Mechanism of tachyphylaxis associated with neurogenic plasma extravasation in the rat trachea. *Am Rev Respir Dis* 141:1434–1440
- Cowden JM, Riley JP, Ma JY, Thurmond RL, Dunford PJ (2010) Histamine H4 receptor antagonism diminishes existing airway inflammation and dysfunction via modulation of Th2 cytokines. *Respir Res* 11:86–97
- Ezaki T, Baluk P, Thurston G, La Barbara A, Woo C, McDonald DM (2001) Time course of endothelial cell proliferation and microvascular remodeling in chronic inflammation. *Am J Pathol* 158:2043–2055
- Furuya S, Naruse S, Hayakawa T (1998) Intravenous injection of guanylin induces mucus secretion from goblet cells in rat duodenal crypts. *Anat Embryol* 197:359–367
- Guo JJ, Wang DS, Huang HT (2003) Spontaneous remission of edema and regranulation of goblet cells in rat tracheae after capsaicin-induced acute inflammation. *Anat Embryol* 206:301–309
- Hirota N, Risse PA, Novali M, McGovern T, Al-Alwan L, McCuaig S, Proud D, Hayden P, Hamid Q, Martin JG (2012) Histamine may induce airway remodeling through release of epidermal growth factor receptor ligands from bronchial epithelial cells. *FASEB J* 26:1704–1716
- Huang HT (1993) Unilateral cervical vagotomy decreases the magnitude of neurogenic inflammation induced by capsaicin in the ipsilateral bronchial tree of rats. *Anat Embryol* 188:363–370
- Huang HT, Haskell A, McDonald DM (1989) Changes in epithelial secretory cells and potentiation of neurogenic inflammation in the trachea of rats with respiratory infections. *Anat Embryol* 180:325–341
- Huang HT, Huang SH, Luor YG (1995) Postvagotomy changes in neurogenic plasma extravasation in rat bronchi. *J Auton Nerv Syst* 55:9–17
- Hwang T, Huang HT, Tsao CF (1999) Thoracic vagus section distal the recurrent laryngeal nerve reduces substance P-immunoreactive innervation in the rat bronchial tree. *Anat Embryol* 200:153–160
- Kuo HP, Rohde JAL, Tokuyama K, Barnes PJ, Rogers DF (1990) Capsaicin and sensory neuropeptide stimulation of goblet cell secretion in guinea-pig trachea. *J Physiol* 431:629–641
- Lo YK, Huang HT (1997) A novel procedure for mediastinal vagotomy inhibits neurogenic inflammation in rat bronchial tree. *J Auton Nerv Syst* 67:79–88
- McDonald DM (1988) Respiratory tract infections increase susceptibility to neurogenic inflammation in the rat trachea. *Am Rev Respir Dis* 137:1432–1440
- McDonald DM (1994) Endothelial gaps and permeability of venules in rat trachea exposed to inflammatory stimuli. *Am J Physiol* 266:L61–L83
- McDonald DM, Schoeb TR, Lindsey JR (1991) Mycoplasma pulmonis infections cause long-lasting potentiation of neurogenic inflammation in the respiratory tract of the rat. *J Clin Invest* 86:1409–1415
- Takeyama K, Tamaoki J, Nakata J, Konno K (1996) Effect of oxitropium bromide on histamine-induced airway goblet cell secretion. *Am J Respir Crit Care Med* 154:231–236
- Tam EK, Calonic LD, Nadel JA, McDonald DM (1988) Globule leukocytes and mast cells in the rat trachea: their number, distribution, and response to compound 48/80 and dexamethasone. *Anat Embryol* 178:107–118
- Tokuyama F, Kuo HP, Juliette A, Rohde AL, Barnes PJ, Rogers DF (1990) Neural control of goblet cell secretion in guinea pig airways. *Am J Physiol* 259:L108–L115
- Yamauchi K, Piao HM, Nakadate T, Shikanai T, Nakamura Y, Niisato M, Ito H, Mouri T, Kobayashi H, Maesawa C, Sawai T, Ohtsu H, Inoue H (2008) Progress in allergy signal research on mast cells: the role of histamine in goblet cell hyperplasia in allergic airway inflammation—a study using the Hdc knockout mouse. *J Pharmacol Sci* 106:354–360