

Cross-Species Immunoreactivity of Airway Mucin as Revealed by Monoclonal Antibodies Directed Against Mucins from Human, Hamster, and Rat

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ABSTRACT

Airway mucin plays crucial role in host-defense and has been implicated in pathophysiology of various airway diseases including asthma and cystic fibrosis. The analysis of airway mucin has been hampered mostly by the lack of specific and efficient methods for the detection of mucin. Recent production of antibodies against airway mucin from several species and also the development of immunoassay procedures make it more efficient to study the airway mucin. However, the cross-species immunoreactivity of antibodies against airway mucin has not been clearly demonstrated and this prompted us to investigate the cross-species immunoreactivity of monoclonal antibodies against human (HM02), hamster (HTA), and rat airway mucin (RT03), which is three most widely used species in the study of mucin. All the monoclonal antibodies (MAbs) used in this study is IgM isotype and recognizes *N*-acetyl-galactosamine-linked carbohydrate core or backbone portion of airway mucin. In enzyme-linked immunoadsorbent assay (ELISA), Western blot, immunoprecipitation, and immunohistochemical staining experiments, it was demonstrated that human and hamster airway mucin showed strong cross-species immunoreactivity. However, rat airway mucin did not show any cross-species immunoreactivity against human and hamster airway mucin. Endotoxin-induced secretory cell metaplasia and hence the increase in mucin release from hamster airway mucin could be detected with antibodies against hamster and human airway mucin *in vivo* and *in vitro*. However, the same increase from rat airway could only be detected with antibody against rat airway mucin but not with antibodies against human and hamster airway mucin. In addition, the increase in mucin release from asthmatic patients could be detected with antibodies against human and hamster airway mucin but not with the antibody against rat airway mucin. The data from the present study implicates that the carbohydrate chain of human and hamster airway mucin, but not that of rat airway mucin, share common antigenic structure. In case of the interspecies use of the antibodies against airway mucin, it would be more desirable to clearly identify the cross-species immunoreactivity otherwise might lead to erroneous results.

INTRODUCTION

AIRWAY MUCINS, the major components of airway mucus, play an important role in airway defense mechanism by trapping and removing the airborne particles.^(1,2) Detection of mucin has been performed by using pharmacological and biochemical methods using isotopes and lectins.⁽³⁻⁵⁾ While these

methods have suffered from varying degrees of nonspecificity, immunological methods using monoclonal antibodies (MAbs) can provide more specific methods, which make the research more effective and accurate. Several antimucin antibodies have been reported that recognize airway mucins obtained from various animal species.⁽⁶⁻¹⁵⁾ These antibodies have been successfully applied to studying mucins *in vitro*^(16,17) or *in vivo*.^(18,19)

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In some cases, antibodies directed against airway mucin from one species have been used also for the study of mucin from other species. However, the cross-species reactivity of these antibodies was not demonstrated rigorously and in some cases the analysis of mucin was less efficient most probably due to the lack of cross-species immunoreactivity.⁽¹⁸⁾

In human subjects, the alterations of the structure and release of airway mucin have been assumed to be one of the underlying factors in various airway disease statuses. Therefore, it has been a focus of intense investigation.^(20–23) For the study of mucin release *in vitro*, primary hamster tracheal surface epithelial cell (HTSE) culture system has been most widely used.^(24–26) For the study of mucin release *in vivo*, SO₂ or endotoxin-induced rat airway metaplasia models were commonly used.^(27,28) In these cases, the development and successful use of specific antibodies against the airway mucin should be essential because the immunological procedure is practically the only way to analyze the mucin *in vivo*. Recently, we reported the production of MAbs against human, hamster, and rat airway mucin. These antibodies were proven to be useful for the study of mucin release from human, hamster, and rat, respectively.^(13–15)

In this study, we investigated cross-species immunoreactivity of the human, hamster, and rat airway mucin using MAbs directed against mucins from the three species. Most notably, human and hamster airway mucin and their cognate antibody showed strong cross-species reactivity with each other while rat airway mucin and the cognate antibody showed little, if any, cross-species reactivity against human and hamster mucin. These data suggest that the structure of human and hamster airway mucin is similar while that of rat is somewhat different from human and hamster airway mucin. We strongly suggest that it would be better to use antibodies directed against the cognate species' airway mucin in studying airway mucin.

MATERIALS AND METHODS

Primary hamster and rat tracheal surface epithelial (HTSE and RTSE) cell culture

Tracheas were obtained from male Syrian golden hamsters or Sprague–Dawley rats of 8 to 10 weeks of age. Preparation and plating of HTSE and RTSE cells were carried out as described.^(13,29) About 5×10^5 dissociated cells per dish were plated on a thick collagen gel prepared in 100-mm tissue culture dishes (Falcon, Lincoln Park, NJ) as described.⁽³⁰⁾ Cells were plated in a complete medium containing 5% fetal bovine serum (Gibco, Gaithersburg, MD). The serum-depleted condition was achieved by gradually reducing the concentration of serum in the medium: 5% on Day 0; 2.5% on Day 1 and Day 2; 1% on Day 3 and Day 4; and 0% from Day 5 to Day 8. Each dish was washed twice with Ca²⁺, Mg²⁺–free phosphate-buffered saline (PBS) before adding new media. The serum-free spent media were collected for the purification of hamster and rat tracheal mucins and stored at -70°C until required.

Preparation of human sputum, bronchial washing

To obtain human sputum, 20 mL of saline solution was inhaled by nose and was expelled immediately.⁽¹⁴⁾ To the spu-

tum, 5 volumes of PBS was added and dithiothreitol was added to a final concentration of 10 mM. After vigorous vortexing, the soluble fraction of the sputum was obtained by centrifugation and used for further experiment. In times of bronchoscopy, 10 mL saline was applied into the human airway and expelled immediately to obtain bronchial washings (BW). This procedure was repeated once more and the fluid was combined and centrifuged to remove cell debris. Sputum and BW were adjusted to contain 0.1% SDS and 50 mM sodium acetate, pH 7.2, and were boiled for 3 min.

Purification of human, hamster, and rat airway mucins

Hamster and rat tracheal mucins or human airway mucins were purified by the established procedure.^(14,25) Briefly, the concentrated HTSE or RTSE cell culture spent media or human airway secretions were applied to Sepharose CL-4B columns (2.5×100 cm), and eluted with PBS at a flow rate of 50 mL/h by using a peristaltic pump at 4°C . The void volume fractions were pooled and concentrated to 1 mL. The concentrates were treated with chondroitinase ABC (0.25 U/mL) at 37°C for 16 h and also with hyaluronidase (from bovine testis, 500 U/mL) at 37°C for 16 h. The digested sample was concentrated to 200 μL with Centricon (Mw cut off: 30,000 Da, Amicon, Beverly,

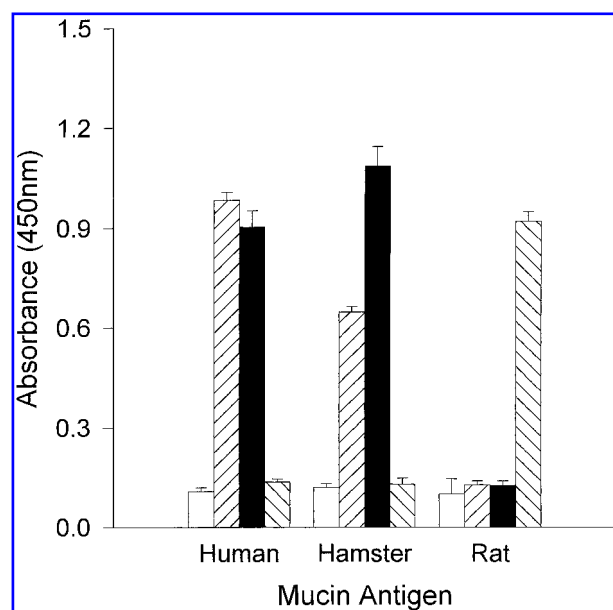


FIG. 1. ELISA of airway mucins from human, hamster and rat. The Purified airway mucins from human, hamster, and rat were diluted in PBS (10 ng/mL) and were adsorbed on 96-well ELISA plate (100 μL /well). After blocking, monoclonal antibodies against human airway mucin (HM02), hamster airway mucin (HTA), or rat airway mucin (RT03) was added and incubated for 1 h. Each well was probed with horseradish peroxidase labeled goat anti-mouse immunoglobulins (dilution, 1:3000 in Blotto). After addition of tetramethyl-benzidine developing solution (100 μg /mL), the absorbance was read at 450 nm. As a negative control, the spent media of sp2/0-Ag14 myeloma cell was used at the same condition. Each point and bar indicates a mean $\pm$ S.D. of triplicate determinations. □: Negative control, ▨: HM02, ■: HTA and ■: RT03.

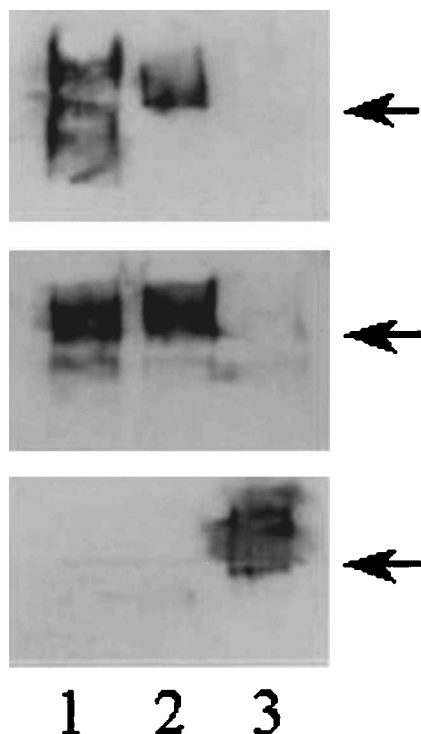


FIG. 2. Western blot analysis of mucins from human, hamster, and rat airway mucin. The purified human airway mucins (Lane 1) from hamster airway mucin (Lane 2), or rat airway mucin (Lane 3) were electrophoresed (8% SDS-PAGE) and proteins were transferred onto nitrocellulose membrane. After blocking, the strip was incubated with MAbs against human airway mucin (HM02, upper panel), hamster airway mucin (HTA, middle panel), or rat airway mucin (RT03, lower panel) for 1 h at room temperature. Each strip was probed with horseradish peroxidase labeled goat anti-mouse IgM. The band was visualized by ECL. The MAbs showed diffuse immunoreactivity on the stacking gel (4%) region as predicted from the high molecular weight of airway mucin. The boundary between stacking and running gel was indicated by an arrow.

MA), and was adjusted to 0.1% SDS and 50 mM sodium acetate (pH 7.2). The samples were heated at 100°C for 5 min and centrifuged at 12,000 g. The supernatants were applied to a Sepharose CL-4B column (1 × 40 cm), and eluted with 50 mM sodium acetate, 0.1% SDS (pH 7.2). The void volume fractions were pooled and stored at -20°C and used as purified mucins.

Nebulization of ATP and determination of mucin release from hamster and rat trachea

ATP (1 mM in PBS) was administered by nose only inhalation for 10 min, as described previously.⁽³¹⁾ The animals were confined in a conical restraint that allowed the nose to protrude into the path of an airstream containing nebulized adenosine 5'-triphosphate (ATP). The nebulized ATP aerosol was generated with a Wright nebulizer, supplied with compressed air by a Pari Mater portable compressor (Pari, Starnberg, Germany). During this procedure, 8 mL of PBS or ATP solution was delivered in

the form of aerosol with a droplet size less than 2 μ m. After 1 h, animals were sacrificed by cervical dislocation and the tracheas were removed. The tracheal lumen was flushed with 1 mL of PBS and the washout solutions were adjusted to 0.1% SDS and 50 mM sodium acetate. The tracheal washout solutions were boiled for 5 min and centrifuged at 12,000 g for 15 min. The supernatants were diluted 10-fold with PBS and analyzed for their mucin contents by enzyme-linked immunosorbent assay (ELISA).

MAbs

MAbs that are directed against airway mucins from human, hamster, and rat were used in this study. The names of the antibodies were HM02, HTA and RT03, respectively. The production and characterization of the MAbs used in this study were reported previously.⁽¹³⁻¹⁵⁾ All the MAbs were produced by fusion of immunized spleen cell and sp2/0-Ag14 myeloma

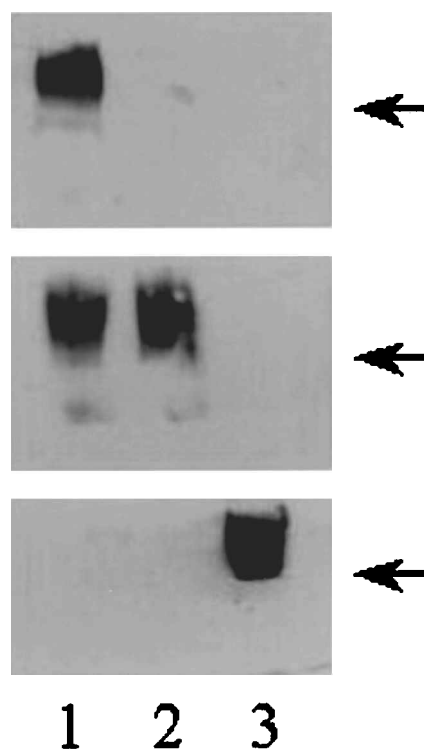


FIG. 3. Indirect immunoprecipitation of human, hamster, and rat airway mucin. The purified airway mucins (1 μ g/mL in PBS) from human (upper panel), hamster (middle panel), or rat (lower panel) were mixed with MAbs against human airway mucin (Lane 1), hamster airway mucin (Lane 2), or rat airway mucin (Lane 3) and were incubated overnight at 4°C. To this mixture, 40 μ L of 1:1 slurry of anti-mouse IgM agarose was added and incubated for 1 h at room temperature. After centrifugation, the precipitates were washed three times with PBS-Tween. The precipitates were added 2 × SDS-sample buffer and analyzed by Western blot, as described in the Materials and Methods section using MAbs HM02 (upper panel), HTA (middle panel), and RT03 (lower panel). The boundary between stacking and running gel was indicated by an arrow.

cells according to the method of Köhler and Milstein, which was slightly modified.^(13–15,32) The isotypes of all these antibodies were IgM. These antibodies recognize carbohydrate chain of mucin, especially *N*-acetyl galactosamine containing portion. These antibodies showed specific and strong immunoreactivities against crude or purified mucin from the cognate species *in vivo* and *in vitro* as revealed by ELISA, Western blot, indirect immunoprecipitation, and immunohistochemistry.

Enzyme-linked immunosorbent assay (ELISA)

Purified or crude mucins were diluted with PBS, pH 7.4 and 100- μ L aliquots were coated onto 96-well assay plates at 32°C for 2 h. The plates were blocked with Blotto (5% non-fat dried milk in PBS-0.2% Tween 20) and washed with PBS-Tween. MAbs were added to the wells and incubated at 32°C for 1 h. The plates were washed again and incubated with horseradish peroxidase (HRP) labeled goat anti-mouse IgM at 32°C for 1 h. Finally, the plates were washed four times and

incubated with freshly prepared tetramethylbenzidine (TMB) substrate solution (Sigma, St. Louis, MO) for 10 min at room temperature. The Absorbance was read at the optical density (O.D.) of 450 nm on a Titertrek ELISA reader (Flow Laboratory, U.K.).

Western blot

Mucin samples were treated with 2 $\times$ SDS-sample buffer at 100°C for 3 min prior to electrophoresis on 8% SDS polyacrylamide gel. The resolved band was electro-transferred onto nitrocellulose (NC) membrane.⁽³³⁾ The NC membrane was blocked with Blotto and then incubated with the MAbs at room temperature for 2 h. After three 10-min washes with PBS-Tween, the NC membranes were incubated with peroxidase-labeled goat anti-mouse IgM at room temperature for 2 h. After extensive washing with PBS-Tween, the membranes were developed with diaminobenzidine (DAB) substrate solution or by enhanced chemiluminescence (ECL).

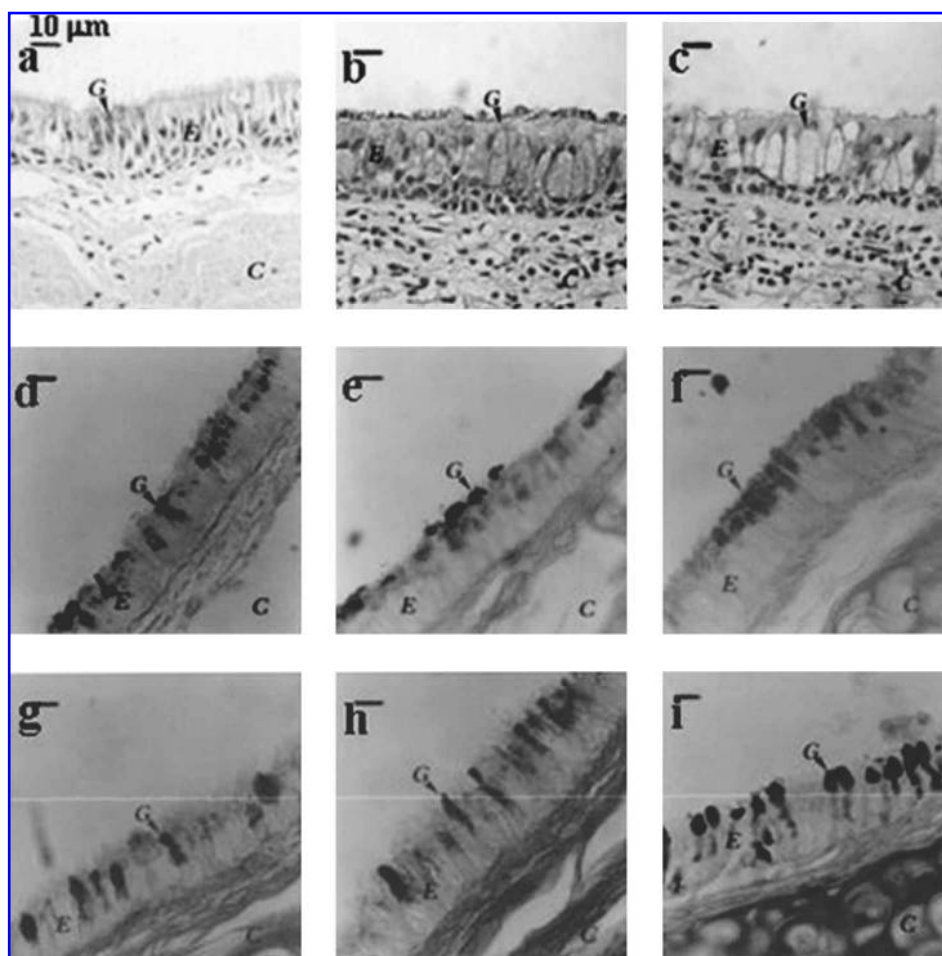


FIG. 4. Immunohistochemical localization of airway mucins of human, hamster and rat. Human bronchiole (upper panel; a,b,c), hamster trachea (middle panel; d,e,f), or rat trachea (lower panel; g,h,i) was removed and paraformaldehyde-fixed thin tissue sections (5 μ m) were prepared. After blocking with 3% normal goat serum, the sections were probed with MAbs against human (HM02, right; a,d,g), hamster (HTA, middle; b,e,h), and rat (RT03, left; c,f,i). After reaction with HRP-conjugated secondary antibody, the immunoreactivity was visualized with diaminobenzidine followed by counter-staining with periodic acid Schiff's reagent. The abbreviations used were as follows: G = goblet cell; E = epithelium; and C = connective tissue. Scaling bars represents 10 μ m. (Original magnification: $\times 200$.)

Indirect immunoprecipitation

The purified human, hamster, and rat airway mucins (0.5 $\mu\text{g/mL}$) were mixed with the MABs. The mixture was incubated at room temperature for 2 h and precipitation was accomplished by the addition of 50 μL of 1:1 slurry of anti-mouse IgM agarose (Sigma, St. Louis, MO). The precipitates were washed three times with the PBS-Tween and analyzed by Western blot.

Immunohistochemistry

Paraformaldehyde-fixed paraffin tissue sections (5 μm in thickness) of human bronchiole, hamster and rat trachea were blocked with 3% normal goat serum for 1 h at room temperature. After washing with PBS, the sections were incubated with the MABs at room temperature for 1 h. Finally, the sections were incubated with peroxidase-labeled goat anti-mouse IgM for 1 h at room temperature. After extensive washing with PBS-Tween, the sections were developed with the diaminobenzidine substrate solution. To induce airway secretory cell metaplasia in hamster or rat, endotoxin was treated to normal animals. Endotoxin was dissolved in PBS and nebulized to 8-week-old male hamsters or rats.^(13,34) After 5 days, the animals were sacrificed by CO_2 asphyxiation and the trachea was removed for immunohistochemical examination.

RESULTS

In ELISA with purified mucin, MAB HM02, a MAB directed against human airway mucin, showed strong immunoreactivity against purified human airway mucin. The immunoreactivity of HM02 was slightly lower against purified hamster airway mucin and was negligible against purified rat airway mucin at the same concentration of antigen (Fig. 1). The MAB HTA, which was directed against hamster airway mucin, showed strong immunoreactivity against purified hamster and human airway mucin. However, HTA showed little, if any, immunoreactivity against purified rat airway mucin (Fig. 1). The MAB RT03, which was directed against rat airway mucin, showed immunoreactivity only against purified rat airway mucin (Fig. 1). In ELISA with crude mucin, which was obtained from human sputum and HTSE or RTSE culture spent media, all the MABs showed similar results (data not shown).

In Western blot analysis, MABs HM02 and HTA revealed diffuse immunoreactive band on stacking gel region against both purified and crude airway mucin from human and hamster, but not against airway mucin from rat (Fig. 2). In addition, MAB RT03 showed immunoreactivity only against rat airway mucin, but not against human or hamster airway mucin (Fig. 2).

All the MABs specifically immunoprecipitated purified airway mucin obtained from cognate species, which was used for the preparation of mucin antigen for the production of the antibody (Fig. 3). The MAB HTA immunoprecipitated hamster and human airway mucin, but not rat airway mucin. In case of HM02, it immunoprecipitated only human airway mucin. Likewise, MAB RT03 did not immunoprecipitate either human or hamster airway mucin (Fig. 3).

When airway cross section was analyzed immunohisto-

chemically, all the antibodies showed immunoreactivities on the surface of airway epithelium and goblet cell granule of cognate species (Fig. 4). As in the case of ELISA and Western blot, HM02 and HTA showed cross-reactivity against hamster and human airway mucin, respectively, but not against rat airway mucin. Likewise, RT03 showed no cross-reactivity against human and hamster airway mucin (Fig. 4). In case of HM02, it also showed immunoreactivity on submucosal gland of human bronchiole. None of the other antibody, HTA and RT03, showed immunoreactivities against human airway mucin on submucosal gland (data not shown). The secretory cell metaplasia induced by nebulization of endotoxin into hamster and rat airway increased mucin content in airway epithelium. The increase in mucin contents could be also detected with HM02 in hamster airway but not in rat airway. The increase in mucin contents in rat airway could only be detected with RT03 but not with either HM02 or HTA (Fig. 5).

Finally, we investigated the applicability of these antibodies for the determination of mucin release from human airway disease patients. It was also investigated whether the use of these MABs is adequate for the analysis of *in vivo* mucin release from hamster and rat by nebulizing ATP, a known strong secretagogue of airway mucin, into the animals. When the sputum obtained from asthmatic patients was analyzed by ELISA, HM02 revealed the 120% increase of mucin release in asthmatic patients as compared with normal subjects. With HTA, the results were similar as compared with that obtained with HM02 though the actual absorbance value was somewhat lower in case of HTA (Fig. 6a). With RT03, it was practically impossible to detect any differences in mucin release between normal and asthmatic patients (Fig. 6a). Nebulization of PBS itself, which was used for the preparation of ATP solution, caused increase in mucin release but nebulization of 1 mM ATP caused further increase in mucin release from hamster and rat airway (Figs. 6b and 6c). The increase in mucin release from hamster airway lavage fluid could be detected with HM02 and HTA (Fig. 6b), but those from rat airway could be detected only with RT03 (Fig. 6c).

DISCUSSION

We and several other researchers reported the production and characterization of MABs against airway mucin from several species.^(6–15,35) However, there is little demonstration of cross-species immunoreactivity of these antibodies against airway mucins. To clarify this issue, we examined the cross-species immunoreactivity of human, hamster, and rat airway mucin, which are the three most widely used species for the biochemical and pharmacological study of mucin in physiological and pathological situations.

In the present study, cross-species immunoreactivity of airway mucins were investigated using MABs against airway mucins from human (HM02), hamster (HTA), and rat (RT03). The major findings of this study were that human and hamster airway mucins showed strong cross-species immunoreactivity with each other, whereas rat airway mucin did not show any cross-species immunoreactivity against human and hamster airway mucin.

Airway mucin is a complex molecule composed of 80–90%

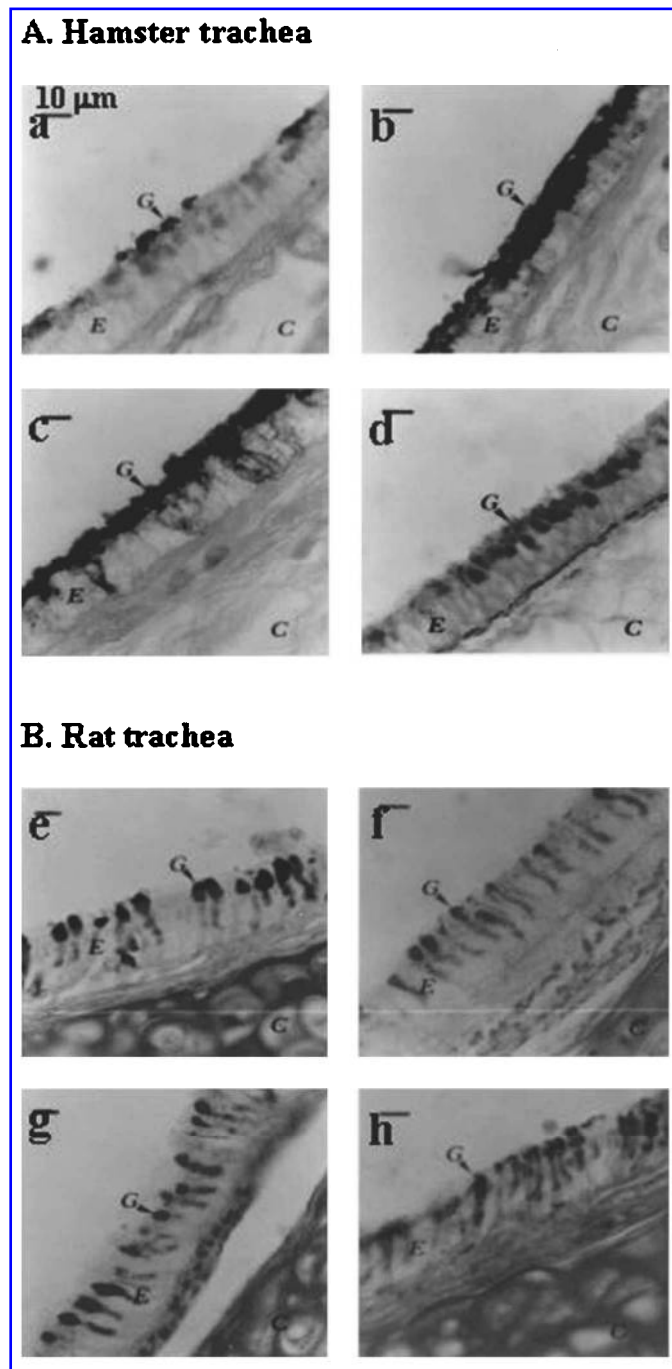


FIG. 5. Immunohistochemical localization of mucins on the endotoxin-treated hamster and rat trachea. Endotoxin (2 mg/mL) was treated, as described in the Materials and Methods section. Five days later, control (a,e) or endotoxin-treated (b,c,d,f,g,h) hamster (A) or rat (B) trachea was removed and paraformaldehyde-fixed, paraffin-embedded cross sections (5 μ m in thickness) were prepared. After blocking with 3% normal goat serum, each section was probed with MAbs against human (HM02; b,f), hamster (HTA; c,g), or rat airway mucin (RT03; d,h). Sections from control animals were stained with MAbs against hamster (HTA; a) or rat airway mucin (RT03; e), respectively. After reaction with horseradish peroxidase-conjugated anti-mouse IgM, the immunoreactivity was visualized with diaminobenzidine and counter-stained with periodic acid Schiff's reagent. The immunoreactivity was markedly increased with endotoxin treatment. The abbreviations used were as follows: G = goblet cell; E = Epithelium; and C = connective tissue. Scaling bars represents 10 μ m. (Original magnification: $\times 200$.)

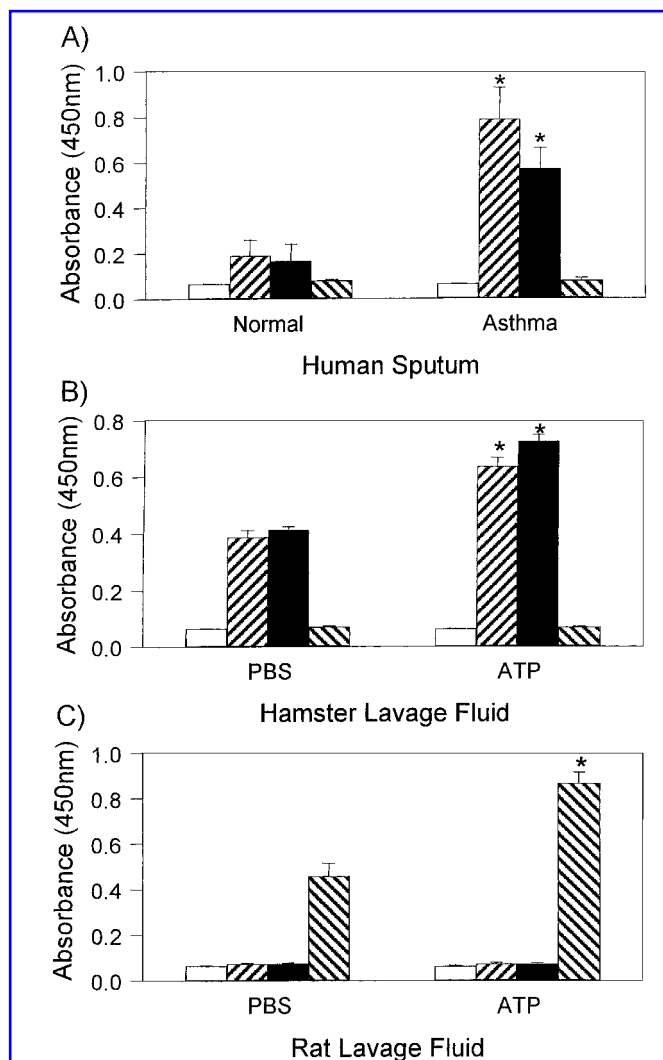


FIG. 6. Determination of airway mucin release from asthmatic patients and ATP-nebulized hamster and rat. (A) Sputum of normal and asthmatic patient ($n = 27$) was analyzed in their mucin contents by ELISA with MAbs against human, hamster, or rat airway mucin. ATP (1 mM in PBS) or PBS-nebulized hamster (B) or rat (C) trachea was removed and tracheal lumen was washed with PBS, as described in the Materials and Methods section. The mucin contents in the tracheal lavage fluids were determined by ELISA ($n = 16$). □: Negative control, ▨: HM02, ■: HTA, ▩: RT03. *Represents a significant difference from normal subjects (a) or PBS treated animals (b,c).

carbohydrate chain, which is attached to 10–20% of peptide backbone moiety by weight.⁽³⁶⁾ These structural features make the carbohydrate chain more important for the rheologic properties of airway mucin. Several researchers focused on the alterations of structural or physicochemical alterations of the carbohydrate chain in the pathophysiology of several airway diseases including cystic fibrosis and asthma.^(37–40)

The exact structure of carbohydrate chain in respiratory mucin is extremely difficult to examine. It has been reported that the linkage of sugar moieties obtained from human respiratory mucin could be classified to several different types.⁽⁴¹⁾

All the three MAbs used in this study recognized carbohydrate portion including *N*-acetyl galactosamine as an epitope and did not recognize terminal sugar of mucin carbohydrate chain including fucose, galactose, and neuraminic acid.^(13–15) *N*-acetyl galactosamine is a core sugar moiety linking carbohydrate chains to serine or threonine residue of a mucin peptide backbone.⁽⁴¹⁾ These implies that MAbs used in this study recognizes core and backbone structure of mucin carbohydrate chain.

The structure of carbohydrate chain is complex and heterogeneous with numerous backbone or peripheral sugars such as *N*-acetyl-galactosamine, *N*-acetyl-glucosamine, galactose, mannose, sialic acid and fucose.^(42,43) Also, the varying degree of sugar modification including sulfation make it more complex to understand the structure of carbohydrate chain of airway mucin.⁽²⁰⁾ For the detection of carbohydrate chain of airway mucin, many researchers used general histochemical techniques including periodic acid Schiff's (PAS) staining and alcian blue (AB) staining.⁽⁴⁴⁾ However, St. George et al.^(7,35) suggested that although the carbohydrate portions of respiratory mucins are histochemically homogeneous as revealed by PAS-AB staining, the structure of glycoconjugates of the airway mucin are antigenically more complex. The data from the present study implicate that the microstructure in the vicinity of *N*-acetyl galactosamine containing core- and backbone-region of carbohydrate chain of airway mucin is different between human (hamster) and rat.

Very limited reports regarding the cross-species immunoreactivity of antimucin antibodies are available at present. Lin et al.⁽⁴⁵⁾ reported that an antimucin antibody produced against primate airway secretion is strongly cross-reactive with human airway mucin and they successfully used this antibody for the quantitation of tracheal mucin from human and nonhuman primates. Koshino et al.⁽⁴⁶⁾ reported that a MAb produced against epithelial glycoprotein from human hypersecretory mucus is only weakly cross-reactive to antigens from canine airway. Li et al.⁽¹¹⁾ reported the production and characterization of antibodies against guinea pig tracheal mucin. They reported that those antibodies showed varying degree of cross-species immunoreactivity with ferret airway mucin in ELISA and with bovine airway mucin in immunofluorescence staining. Kai et al.⁽¹²⁾ reported that a MAb against hamster tracheal mucin (4H6) was cross-reactive to rat, dog, and guinea pig airway mucin in ELISA. However, this antibody was not specific against airway mucin in that it also showed cross-reactivity against hamster or pig digestive tract mucin. The relatively non-specific antigen recognition of 4H6 made it difficult to directly compare the data from the results of the present study.

At present, increasing number of researchers are using immunoassay procedures for the determination of mucin from *in vivo* and *in vitro* systems, largely due to the its simplicity and specificity.^(45,47) In some cases, they used antibody against one species for the analysis of another species' mucin without rigorous characterization. For example, Steiger et al.⁽¹⁹⁾ used MAbs directed against human airway mucin for the study of mucin storage and release from endotoxin treated rat airway. In agreement with the results from the present study, these antibodies showed very weak cross-reactivity in Western blot. In ELISA, they incorporated additional experimental steps such as dialysis and concentration steps of airway secretions instead of directly using crude airway secretion, which may be possible

by using more specific antibodies with stronger immunoreactivity.⁽³¹⁾ In addition, we have shown that it is relatively safe to use specific antibodies in the analysis of airway mucin because using weakly or noncross-reactive antibodies may lead to different output (Fig. 6).

In the present study, MABs against human and hamster mucin showed strong cross-species immunoreactivity toward airway mucins from the other species. Although exact structure of mucin carbohydrate chain is currently unclear, it is noteworthy that the overall carbohydrate composition is similar between human and hamster mucin. Steiger et al.⁽¹⁸⁾ reported the composition of mucin-like antigen purified from human airway secretion. They found that the carbohydrate chain of human airway mucin is composed of 17.5% fucose, 11.3% galactosamine, 48.5% glucosamine, 19.2% galactose, 2.8% glucose, and 0.03% mannose. Wu et al.⁽²⁶⁾ reported the composition of mucin purified from hamster tracheal epithelial cell culture. They found that the carbohydrate chain of hamster airway mucin is composed of 2.3% fucose, 10.1% galactosamine, 36.3% glucosamine, 27.6% galactose, 12.2% sialic acid, and 1.8% mannose. Currently, whether rat airway mucin has similar or different carbohydrate composition as compared with human or hamster airway mucin awaits further investigation.

Although human and hamster airway mucin showed strong cross-species immunoreactivity in ELISA (Fig. 1), Western blot (Fig. 2) and immunohistochemistry (Fig. 4), indirect immunoprecipitation experiment revealed that MAB against human airway mucin did not immunoprecipitate hamster airway mucin (Fig. 3). These data suggest that there may be subtle differences in the microstructure of the human and hamster epitope.

In summary, MABs against human and hamster airway mucin, but not rat airway mucin, showed strong cross-species immunoreactivity, which indicates shared antigenic structure of human and hamster airway mucin. It is strongly recommended to use species-specific antibody in the analysis of airway mucin.

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