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Mucin-like glycoprotein secreted by cultured hamster tracheal epithelial cells

Biochemical and immunological characterization

Reen WU,*§ Charles G. PLOPPER* and Pi-Wan CHENG†‡
*California Primate Research Center, University of California at Davis, Davis, CA 95616, U.S.A., and
†Department of Pediatrics and ‡Department of Biochemistry, University of North Carolina,
Chapel Hill, NC 27599-7220, U.S.A.

We isolated mucin-like glycoproteins from the conditioned medium of primary hamster tracheal epithelial (HTE) cell culture and characterized them biochemically and immunologically. These glycoproteins were purified on Sepharose CL-4B after *Streptomyces* hyaluronidase treatment and then by CsCl-density-gradient centrifugation in the presence of 4 M-guanidinium chloride. The purified glycoproteins were resistant to digestion by chondroitin AC lyase, heparinase, heparitinase and endo-*N*-acetylglucosaminidases A, D and H, but susceptible to endo-β-galactosidase and keratanase. SDS/PAGE demonstrated no contamination by low-molecular-mass proteins. The purified glycoproteins showed a peak buoyant density of 1.56 g/ml in CsCl-density-gradient centrifugation, and contained 10% peptide and 90% carbohydrate by weight. Carbohydrates in these glycoproteins contained *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose, fucose, sialic acid and a trace amount of mannose, but no uronic acid. Serine and threonine together accounted for 27% of the total amino acid residues. In addition, the mucin-like glycoproteins exhibited blood-group A and B activities, and very strong inhibitory activity for influenza A virus haemagglutination. With the use of the purified glycoprotein as an antigen, six monoclonal antibodies that stained mucus granules in hamster tracheal epithelium were obtained. We characterized the antibody produced by one of the clones, HM D46. We conclude that HTE cells cultured in the serum-free medium secrete a glycoprotein with physicochemical properties similar to those known in various airways mucins.

INTRODUCTION

Mucin is a high-molecular-mass glycoprotein containing oligosaccharide chains linked to serine/threonine via *N*-acetylgalactosamine [1–3]. Tracheobronchial mucin, rich in carbohydrates (70–90%) and low in peptide (10–20%), exhibits a peak buoyant density of 1.45–1.60 g/ml in CsCl-density-gradient centrifugation [4–6]. The typical sugars present in these mucins are D-*N*-acetylgalactosamine, D-*N*-acetylglucosamine, D-galactose, L-fucose and sialic acid. Mucin peptide typically contains 30–50 serine + threonine residues/100 amino acid residues, high amounts of glycine, alanine, glutamic acid and proline and low amounts of cysteine and aromatic amino acids [2,7–9].

Most of the information about tracheobronchial mucins comes from studies of mucins isolated from sputa of patients with cystic fibrosis or chronic bronchitis [2,7,8], secretions in tracheal pouch [10–14] or conditioned media of tracheal explant cultures [6,15–18]. There are limitations and potential problems associated with these types of studies. Mucins are secreted by goblet cells in the surface epithelium and by mucus-secreting cells in submucosal glands, and therefore the physicochemical data for tracheobronchial mucins reported in the literature represent composite properties of mucins from these two sources. In addition, mucins isolated from the sputa of patients with airways infection or inflammation are subjected to various modifications such as proteinase degradation [19–21] and possibly glycosidase digestion [22]. To study the products secreted from each of these two secretory elements, cultures of isolated surface epithelial cells [23,24] and submucosal gland cells [25] have been developed. However, it has never been fully demonstrated that these cultured cells secrete mucins.

We have previously developed a serum-free hormone-supplemented culture system for growing the surface epithelial cells of hamster trachea (HTE cells) [26]. The defined medium developed was based on the growth requirements of the cells [27]. These requirements include insulin, transferrin, epidermal growth factor, cortisol, cholera toxin and a crude extract of bovine hypothalamus. Cultured HTE cells were able to form new cilia and mucus granules in confluent culture [26]. We [26] have demonstrated the presence of mucin-type *O*-glycosidic linkages in a high-molecular-mass glycoprotein secreted by HTE cells cultured in this serum-free medium. Using a serum-supplemented medium that we had established earlier [28], Kim *et al.* [29] also reported a similar observation. In the present investigation we have performed the detailed chemical analyses of the composition of this glycoprotein and shown that this glycoprotein has physicochemical properties similar to those of tracheobronchial mucins. In addition, we have generated several monoclonal antibodies with the use of this glycoprotein as an immunogen, and shown them to stain secretory granules in the secretory cells of hamster tracheal epithelium. One of these monoclonal antibodies recognizes a poly-*N*-acetyl-lactosamine structure.

MATERIALS AND METHODS

Cell isolation and culture conditions

Syrian hamsters aged from 2 to 5 months, obtained from Charles River Co. (Wilmington, MA, U.S.A.), were used in this study. Differences in age and sex had no apparent effects on yields of cell isolation and the performance of the cells in culture. Cells were isolated from trachea by the proteinase method as described in previous publications [23,26,28].

Abbreviation used: HTE cells, hamster tracheal epithelial cells.
§ To whom correspondence should be addressed.

Primary cultures were normally initiated by plating 5×10^4 cells/60 mm-diam. culture dish coated with 1 ml of collagen gel as previously described. The final concentration of collagen in the substratum was 0.24%. The serum-free culture system developed in our laboratory was suitable for HTE cell growth and differentiation [23]. The medium consisted of Ham's F12 nutrients (GIBCO, Grand Island, NY, U.S.A.) supplemented with insulin (5 μg/ml) (Sigma Chemical Co., St. Louis, MO, U.S.A.), transferrin (5 μg/ml) (Sigma Chemical Co.), epidermal growth factor (10 ng/ml) (Upstate Biotechnology, Lake Placid, NY, U.S.A.), cortisol (1 μM, or 0.1 μM-dexamethasone) (Sigma Chemical Co.), cholera toxin (20 ng/ml) (List Biological, Campbell, CA, U.S.A.) and bovine hypothalamus extract (15 μg/ml). Vitamin A (retinol) (Sigma Chemical Co.) was added at 1 μM. Bovine hypothalamus was obtained from Pel-Freeze Biologicals (Rogers, AR, U.S.A.) and the extract was prepared in the laboratory according to the method of Maciag *et al.* [30]. Hormonal stocks were prepared at dilutions of 1/500 or 1/1000 as described in the literature [31–33]. Retinol was handled under yellow lighting to minimize photodegradation, and it was dissolved in dimethyl sulphoxide and stored in liquid N_2 until needed. Medium was changed 2 days after plating and every other day thereafter. Conditioned media were collected from day 7 to day 14 and stored at −20 °C after centrifugation to remove cell debris. The radiolabelled precursor [^{3}H]glucosamine (20 μCi/ml; specific radioactivity 25–40 Ci/mmol) (ICN, Irvine, CA, U.S.A.) was added to the medium of a few dishes for monitoring glycoprotein synthesis and secretion in culture.

Biochemical analyses of glycoproteins secreted in culture

About 5 litres of conditioned media were collected from both vitamin A-treated and untreated cultures. The conditioned media were extensively dialysed against water at 40 °C and freeze-dried. The freeze-dried materials were reconstituted in 50 ml of a SDS sample buffer containing 3% (w/v) SDS and 5% (v/v) 2-mercaptoethanol [34–36] and treated at 100 °C for 3–5 min. The solution was spun at 10000 *g* for 20 min to remove the precipitate before being subjected to Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) chromatography as described previously [26]. The void-volume (V_0) materials were further dialysed against water at room temperature. After being freeze-dried, the sample was then treated with *Streptomyces* hyaluronidase (10 units/ml) (Calbiochem, San Diego, CA, U.S.A.) at pH 6.0 at 37 °C for 24 h in the presence of the proteinase inhibitor phenylmethanesulphonyl fluoride (2 mM) (Sigma Chemical Co.). The sample was rechromatographed on Sepharose CL-4B. The V_0 materials from the second Sepharose CL-4B column chromatography were subjected to CsCl-density-gradient centrifugation at a starting density of 1.50 g/ml in the presence of 4 M-guanidinium chloride (Pierce Chemical Co., Rockford, IL, U.S.A.) [4–6]. The fractions at densities between 1.50 and 1.62 g/ml were combined, dialysed against water and used for compositional analyses and as an immunogen for monoclonal antibody production.

The purified glycoproteins were further treated with various glycosidases (Seikagaku Kogyo Co., Tokyo, Japan) according to the procedures suggested by manufacturer. Both endoglycosidase A and endoglycosidase H treatments were carried out at pH 6.0 at 0.02 unit/ml, and endoglycosidase D treatment was at pH 6.5 at 0.02 unit/ml. Heparinase and heparitinase were both used at 5 units/ml at pH 7.0. Chondroitin AC lyase was used at 1 unit/ml at pH 6.0. Neuraminidase and α-fucosidase (Boehringer Mannheim Biochemical, Indianapolis, IN, U.S.A.) were used at pH 7.0, at 0.2 unit/ml and 100 μg/ml respectively. Two types of endo-β-galactosidase, from *Escherichia freundii* (Seikagaku Kogyo Co.) and *Bacteroides fragilis* (Boehringer Mannheim Biochemical), were used at 0.02 unit/ml at pH 6.0. Keratanase from *Pseudomonas* sp. IFO-13309 (Seikagaku Kogyo Co.) was used at 1 unit/ml at pH 7.0. All of these treatments were carried out in the presence of 2 mM-phenylmethanesulphonyl fluoride and at 37 °C for 20 h. Periodic acid treatment was carried out at pH 4.5 in the presence of 0.01 M-periodate for 20 h at 4 °C.

Amino acid compositions of isolated mucin-like glycoproteins were determined in a Beckman 6300 high-performance amino acid analyser (Beckman Instruments, Palo Alto, CA, U.S.A.) after acid hydrolysis (6 M-HCl at 100 °C for 24 h under vacuum). Duplicate samples were analysed. Hexosamines were determined by an h.p.l.c. method [37] after acid hydrolysis (4 M-HCl at 100 °C for 5 h), neutral sugars were determined by a g.l.c. method after methanolysis and trimethylsilylation [7] and sialic acid was determined by a modified thiobarbituric acid method [38]. For monitoring the carbohydrate (neutral sugar) in the column fractions, the phenol/H_2SO_4 method [39] was employed.

Immunology methods

Mice were immunized with purified mucin-like glycoproteins at 10 μg of protein per mouse per injection. After four injections over a period of 2 months, mice spleen cells were fused with myeloma SP2/0 [40]. The hybrids were selected in the HAT (hypoxanthine/aminopterin/thymidine) medium. A radioimmunoassay was developed to screen these hybrids. Micro-titre plates were coated with 5 ng of purified mucin-like glycoconjugate/well (based on the protein determination). After being washed three times with phosphate-buffered saline (0.15 M-NaCl/20 mM-sodium phosphate buffer, pH 7.0) containing 0.05% (v/v) Tween 20 and then treated with BSA (2 mg/ml) in phosphate-buffered saline containing 0.05% Tween 20, each well was exposed to the conditioned medium of hybridoma cells. The control well was exposed to the original culture medium, RPMI medium containing 10% (v/v) foetal bovine serum. ^{125}I-labelled goat anti-(mouse IgG) antibody (from NEN, Boston, MA, U.S.A.) was used to identify the positive clone. The radioactivity bound to each well was determined with a γ-radiation scintillation counter. Positive clones were further cloned by a serial-dilution method until every well of diluted culture produced antibody that reacted with purified mucin antigen. The immunohistochemical staining of HTE cells was carried out in frozen sections of hamster trachea with the Vectastain ABC kit (peroxidase-based) (Vector Laboratories, Burlingame, CA, U.S.A.) to identify the cells that react with the monoclonal antibodies. For classifying the type of immunoglobulin secreted by hybridoma clones, a screening subtype kit was used. This kit detects mouse forms IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgM and κ and λ light chains.

Blood-group A, B and H (O) titres for the purified glycoprotein were measured by haemagglutination inhibition with a 1% suspension of human erythrocytes according to the procedure described previously [41]. Anti-A and anti-B sera were obtained from Hyland Co. (Costa Mesa, CA, U.S.A.) and anti-H lectin (*Ulex europeus*) was from Sigma Chemical Co. Influenza-virus haemagglutination inhibition activity was measured similarly [41] with heat-inactivated influenza A virus and a 1% suspension of chicken erythrocytes. Immunoblot analysis was carried out as described by Towbin *et al.* [42].

RESULTS

Isolation and characterization of mucin-like glycoproteins

Fig. 1(*a*) shows the Sepharose CL-4B elution profiles of the concentrated conditioned media from retinol-treated and untreated HTE cell cultures and of the original culture medium. The two peaks detected by the 280 nm absorbance were only observed in the conditioned media from retinol-treated and

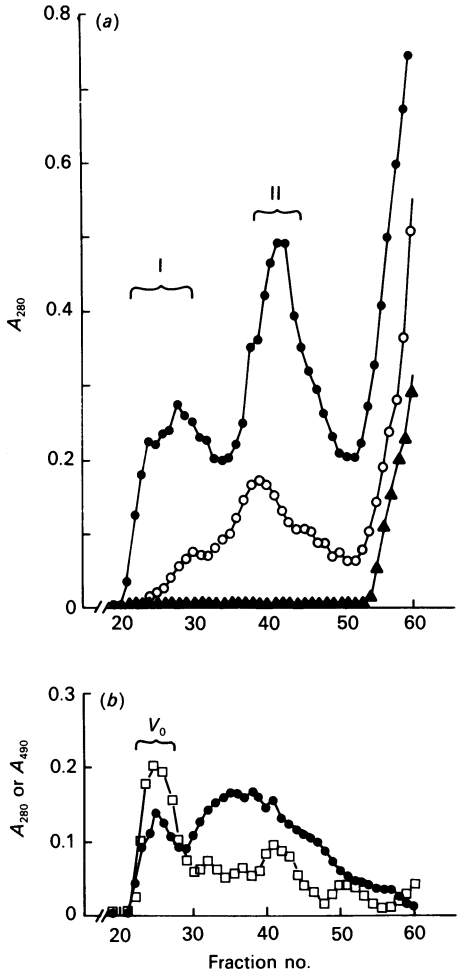

Fig. 1. Sepharose CL-4B (5 cm × 120 cm) chromatography profile of concentrated conditioned media from retinol-treated (●) and untreated (○) HTE cultures and the original culture medium (▲)

Concentrated conditioned media were prepared from HTE cultures as described in the text. These concentrated conditioned media (50 ml) were adjusted to contain SDS and 2-mercaptoethanol at final concentrations of 3% (w/v) and 5% (v/v) respectively. The entire fluids were then loaded on to a Sepharose CL-4B column (5 cm × 120 cm). The column was eluted with phosphate-buffered saline containing 0.1% SDS and 0.5% 2-mercaptoethanol. Protein was monitored by absorbance at 280 nm. The recovery for the column was 85%, based on the protein determination. (*a*) Peak I and II fractions were separately pooled and dialysed. The peak I fractions, after being freeze-dried, were further treated with hyaluronidase and chromatographed on the same column (*b*). Protein (●) and carbohydrate (□) measurements were carried out for each fraction by the absorbance at 280 nm and 490 nm (phenol/H_2SO_4 method) respectively. Void-volume fractions (V_0) were combined.

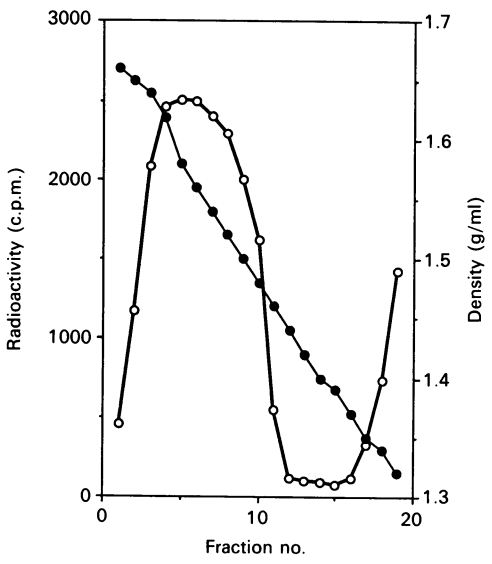

Fig. 2. CsCl/guanidinium chloride-density-gradient centrifugation of the V_0 materials from the second Sepharose CL-4B run of culture medium obtained from the retinol-treated cultures

The [^{3}H]glucosamine-labelled V_0 materials were dissolved in 4 M-guanidinium chloride and then adjusted with crystalline CsCl to an initial density of 1.50 g/ml. Centrifugation was carried out at 100000 *g* (40000 r.p.m. in a Beckman type 65 rotor) for 72 h at 15 °C. Fractions were collected from the bottom of the centrifuge tube. The radioactivity (○) and the density (●) of each fraction were determined.

untreated cell cultures but not for the culture medium not exposed to HTE cells. About 5-fold more material (on the basis of the absorbance at 280 mm) was secreted by the HTE cells treated with retinol than by the untreated cells. The peak I and peak II materials were separately pooled and dialysed against water. Because peak II materials have a low carbohydrate content (25%) and low serine and threonine contents (12%), suggesting the absence or low content of mucin, they were not studied further. The peak I material was treated with hyaluronidase and subjected to Sepharose CL-4B chromatography (Fig. 1*b*). Approx. 20% of the materials was resistant to hyaluronidase and excluded from the column. The hyaluronidase-resistant V_0 materials were further purified by equilibrium-CsCl-density-gradient centrifugation in the presence of 4 M-guanidinium chloride (Fig. 2). With the use of [^{3}H]glucosamine labelling to monitor the separation, a major but broad radioactive peak at buoyant density between 1.50 and 1.62 g/ml with a peak buoyant density at 1.56 g/ml was obtained. Rechromatography of the materials from different densities on the Sepharose CL-4B column showed that all of them remained in the V_0 peak (results not shown). Therefore the materials with buoyant densities between 1.50 and 1.62 g/ml were pooled, dialysed against water and freeze-dried. A total of 2.7 mg of the purified mucin-like glycoprotein was obtained from 5 litres of culture medium from the retinol-treated HTE cell culture.

The isolated glycoprotein did not enter the 7.5% PAGE on electrophoresis in the presence of SDS and 2-mercaptoethanol, nor was low-molecular-mass protein band detected by silver stain (results not shown). The purified material was treated with various glycosidases specific for *N*-glycoproteins and proteoglycans. Chondroitin AC lyase, heparitinase, heparinase and endoglycosidases A, D and H did not degrade the molecule, as demonstrated by the complete recovery of the glycosidase-treated material in the V_0 of the Sepharose CL-4B column (results not shown). Endo-β-galactosidases from *Escherichia* and *Bacteroides* and keratanase removed 65%, 40% and 30% of the labelling of the mucin-like glycoproteins respectively.

The purified material contains 10% protein and 90% carbohydrate by weight (Table 1). The sugars present in the mucin-like glycoproteins were galactose, *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose and sialic acid (Table 1). Only a trace amount of mannose was detected, and uronic acid was undetectable. Serine and threonine were the major amino acids, which accounted for 27% of the amino acid residues (Table 1). The contents of other amino acids such as glutamic acid, glycine, alanine, aspartic acid and proline were also high, and together with serine + threonine they constituted 80% of the amino acid

Table 1. Amino acid and carbohydrate composition of mucin-like glycoprotein secreted by primary HTE cell culture

Mucin-like glycoprotein was purified as described in the text and the final volume of the purified material was 60 ml. The protein concentration was determined from the amino acid composition. The carbohydrate content was the sum of the individual sugar composition. Abbreviation: N.D., not determined.

	Amino acid or carbohydrate composition		
	(Residues/1000 amino acid residues)	(μg/ml)	(%)
Protein		4.20	9.7
Aspartic acid	88	0.39	
Threonine	135	0.54	
Serine	137	0.48	
Glutamic acid	122	0.60	
Proline	80	0.31	
Glycine	84	0.21	
Alanine	80	0.24	
Valine	66	0.26	
Methionine	4	0.02	
Isoleucine	36	0.16	
Leucine	66	0.29	
Tyrosine	3	0.02	
Phenylalanine	6	0.03	
Histidine	25	0.13	
Lysine	30	0.15	
Arginine	36	0.39	
Cysteine	N.D.	N.D.	
Carbohydrate		39.3	90.3
Fucose	183	1.0	2.3
Galactose	2000	12.0	27.6
N-Acetylgalactosamine	600	4.4	10.1
N-Acetylglucosamine	2157	15.8	36.3
Sialic acid*	515	5.3	12.2
Mannose	133	0.8	1.8
Uronic acid	0	0	0

* Calculated as *N*-acetyl derivative.

Table 2. Blood group and influenza A virus haemagglutination inhibition activities of purified mucin-like glycoprotein

The blood group activity was measured by the minimum concentration of mucin-like glycoprotein that inhibited haemagglutination between anti-A serum (1:64 dilution) and 1% blood-group A erythrocytes, anti-B serum (1:64 dilution) and blood-group B erythrocytes, and *Ulex europeus* lectin and blood-group O erythrocytes as described in ref. [41]. The influenza A virus haemagglutination inhibition was measured as the minimum concentration of the glycoprotein that inhibits chicken erythrocyte agglutination by influenza A virus as also described in ref. [41].

	Minimum concentration (μg/ml)
Blood group A	0.193
Blood group B	0.773
Blood group H (O)	> 12.400
Influenza A virus haemagglutination inhibition	0.003

residues. The aromatic amino acids phenylalanine and tyrosine were present in trace amounts. The purified glycoprotein had blood-group A and B activities but no blood-group H activity, and inhibited influenza A virus haemagglutination (Table 2).

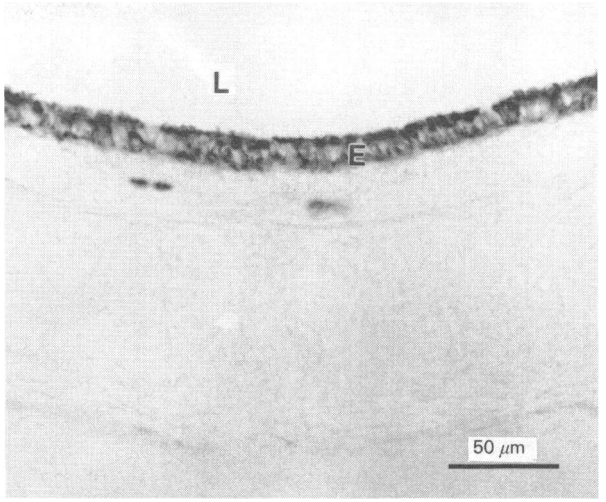

Fig. 3. Immunohistochemical staining of hamster tracheal epithelium with monoclonal antibodies specific to mucin-like glycoprotein

Frozen sections of hamster trachea were fixed and reacted with HM D46 (1:500 dilution). Vectastain ABC kit (peroxidase-based) was used to identify the positive reacting stain. Other monoclonal antibodies, HM D20, HM D5, HM D36, HM F18 and HM G23, had similar results. Key: L, lumen; E, epithelium.

Table 3. Effects of glycosidases and periodate treatment on the radioimmunoassay reactivity of mucin-like glycoprotein to the monoclonal antibody HM D46

Treatments were carried out as indicated in the Materials and methods section. V_0 fractions from Sepharose CL-4B chromatography were used in this study. A 50 μl sample from each fraction was dried in the 96-well micro-titre plate and used for radioimmunoassay as described in the text. The value of each radioimmunoassay count was then divided by the total radioactivity (c.p.m.) in the 50 μl volume and the result expressed as c.p.m./unit. An average from these five fractions in the V_0 peak was used. The background count represents the radioactivity without the primary antibody in the radioimmunoassay.

Treatment	Radioimmunoassay (c.p.m./unit)
None	3208
Periodate	570
Chondroitin AC lyase	3315
Endo-β-galactosidase (*Escherichia*)	789
Endo-β-galactosidase (*Bacteroides*)	1045
Keratanase	1278
Heparinase	2989
Heparitinase	3252
Neuraminidase	2872
α-L-Fucosidase	2796
Background	557

Production of monoclonal antibodies that stain mucus granules

The purified mucin-like glycoproteins were used to immunize mice to produce monoclonal antibodies. More than 200 hybridoma clones were screened by the radioimmunoassay method. About 16 active and stable hybridoma clones were obtained. On the basis of the radioimmunoassay results, the six clones with activities 5-fold above the background control were characterized further. Two of the monoclonal antibodies, HM D36 and HM D46, secreted IgG_1, and the rest (HM D5, HM D20,

HM F18 and HM G23) secreted IgM. All of them reacted with mucus granules and the apical surface of epithelium in hamster tracheal epithelium (Fig. 3). Furthermore, the stain was observed only in the epithelial layer and there was no stain in the cartilage and interstitial layer. Treatment of tissue sections with secondary antibody alone did not show any staining (results not shown). Radioimmunoassay analysis of the gel-filtration fractions indicated that these antibodies recognized mainly the V_0 materials (results not shown). These results were confirmed by the Western-blot analysis, which showed that the antibody reacted only with materials that remained at the origin of the 7.5% gel. To characterize the epitope of one of these antibodies, the mucin-like glycoproteins were treated with endo-β-galactosidase, periodic acid and other glycosidases, and then treated with this antibody. As shown in Table 3, the epitope of HM D46 was completely destroyed by the *E. freundii* endo-β-galactosidase and periodic acid treatments. Endo-β-galactosidase from *Bacteroides* and *Pseudomonas* keratanase destroyed 82% and 73% of the epitope respectively. Other glycosidases did not affect the epitope.

DISCUSSION

We have previously demonstrated that HTE cells grown in this serum-free medium produced new cilia as well as new mucus granules [26]. Evidence of mucin-like glycoprotein secretion in culture was based on the incorporation of [^{3}H]glucosamine into glycoproteins excluded by Sepharose CL-4B and the presence of ^{3}H-labelled galactosaminitol in purified glycoprotein after β-elimination and acid hydrolysis [26]. In the present work, we purified enough mucin-like glycoproteins from the culture medium to carry out detailed physicochemical characterization and to address the question of whether or not the differentiated HTE cells in culture secrete mucins. Detailed chemical analyses showed that 10% of the purified mucin-like glycoproteins is protein that has high serine + threonine (27%), glutamic acid, aspartic acid, glycine and proline contents and is low in aromatic acids. The amino acid composition of this glycoprotein is similar to that of mucin but with a minor variance. For example, the serine + threonine content is somewhat lower than that (30–50%) found in other airways mucins. In addition, glutamic acid and aspartic acid contents are somewhat higher than those in other airways mucins [2,6,7,9]. The five sugars *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose, fucose and sialic acid, typically found in tracheobronchial mucins, are present in this purified glycoprotein. The presence of a trace amount of mannose in the purified glycoprotein suggests either a contamination of a small amount of glycoprotein that contains asparagine-linked oligosaccharide, or the oligosaccharides being a part of the mucin-like glycoprotein. In the former possibility, the contamination of *N*-linked glycoprotein in the purified material must be very small, because we observed no change in the elution profile of the purified mucin-like glycoprotein after the treatment with endo-glycosidases A, D and H. The latter possibility may exist because the sequencing data of the cDNA of the intestinal apo-mucin suggest several possible glycosylation sites for asparagine-linked carbohydrates [43]. However, it has never been conclusively demonstrated that mucin contains *N*-linked oligosaccharides. We could not unequivocally distinguish these two possibilities at the present time. Further study is needed to answer this important question.

There is strong evidence, however, that the mucin-like glycoprotein is free of xylose-linked carbohydrates, which is characteristic of glycosaminoglycans excluding keratan sulphate. For instance, we could not detect any uronic acid in the mucin-like glycoprotein by g.l.c. (Table 1). In addition, we have demonstrated a complete resistance of the purified glycoprotein to the enzymes that degrade hyaluronic acid and xylose-linked carbohydrates present in glycosaminoglycans. These enzymes include *Streptomyces* hyaluronidase, chondroitin AC lyase, heparinase and heparitinase.

Hamster mucin has blood-group A and B activities, and no blood-group H activity, suggesting that all of the blood-group H determinants are capped with either α3-linked *N*-acetylgalactosamine or galactose because the blood-group H determinant is the precursor for blood groups A and B [41]. On the basis of this result we would predict that the activities of α3-*N*-acetylgalactosaminyltransferase and α3-galactosyltransferases would be much higher than the $\alpha 1 \rightarrow 2$-fucosyltransferase activity in the cultured HTE cells. The presence of a strong inhibitory activity of influenza A virus haemagglutination suggests the presence of α2,6-linked sialic acid at the non-reducing termini of sugar chains [44]. Furthermore, the purified glycoproteins exhibit a peak CsCl buoyant density at 1.56 g/ml, which is well within the range expected for tracheobronchial mucins. These results suggest that the differentiated HTE cells in culture secrete a high-molecular-mass glycoprotein that has physicochemical properties similar to those of tracheobronchial mucins with some minor variance in amino acid composition. This is the first time that a detailed chemical composition of mucin-like glycoprotein secreted by cultured tracheal epithelial cells has been reported. In previous studies the tracheobronchial mucins have been isolated *in vivo* from sputum [2,7–9], or pouch [11–14], or *in vitro* from conditioned media of tracheal explant cultures [6,15–18]. These mucins are secreted by both the goblet cells at the surface epithelium and the submucosal mucin-secreting cells. It has not been possible to discriminate between the properties of mucins produced by each of these two mucin-secreting elements. The cells in this primary culture system are derived from the surface epithelium, and the data in this paper may represent the properties of mucins produced by the secretory cells in the surface epithelium of hamster trachea.

Kim *et al.* [29] used the serum-containing culture conditions previously developed in our laboratory [28] to demonstrate the secretion of mucin-like glycoprotein by HTE cells. Our present results show that serum is not required for HTE cell differentiation. The serum-free hormone-supplemented medium provides numerous advantages over the serum-supplemented condition. For instance, serum contains many macromolecules that may hinder the isolation and characterization of mucin-like molecules produced in culture. Furthermore, serum also contains many growth factors and hormones that will interfere with the regulation of mucin synthesis in culture. In the present paper we have shown that it is possible to demonstrate mucin synthesis in a serum-free system. A similar conclusion has been recently demonstrated in human tracheobronchial epithelial cells [45].

The other interesting finding in this study is that the six monoclonal antibodies generated by the mucin-like glycoprotein purified from the conditioned medium react not only with the antigen but also with the mucus granules in airway epithelium. We have obtained six monoclonal antibodies that react with mucin-like glycoconjugates purified from the culture medium. These antibodies recognize not only the mucin-like glycoprotein in cell extract and conditioned medium but also the mucus-secreting granules *in vivo*. These results suggest that mucin isolated from the conditioned medium and the mucin in mucus-secreting granules of hamster tracheal epithelium share similar carbohydrate epitopes. We have observed a total destruction of the epitope of one of the monoclonal antibodies (HM D46) by periodate, which suggests that the epitope is carbohydrate in nature. Extensive damage to the epitope by endo-β-galactosidase treatment further suggests that the epitope contains at least an *N*-acetyl-lactosamine structure.

In this study we also demonstrated that the secretory activity in hamster cells is vitamin A-dependent. Vitamin A enhances the secretion of mucin-like glycoprotein by HTE cells more than 5-fold. This is consistent with a previous study that utilized [^{3}H]glucosamine labelling and histochemical staining methods [26]. The importance of vitamin A in the homoeostasis of mucociliary epithelium is well known. Under vitamin A-depleted conditions squamous-cell metaplasia occurs in hamster airway epithelium. Normally, mucus secretion in the airway lumen is low. However, this phenomenon is reversed with a single treatment with vitamin A or its derivatives (retinoids). At present, the mechanism underlying the vitamin A-controlled airway cell differentiation is not known. The differentiated HTE cell culture system and the mucin-specific antibodies will facilitate further study in understanding the role of vitamin A in the regulation of mucin secretion.

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