Glycoconjugates on the Surface of the Pulmonary Epithelial Cells in Surfactant Free Rat Lungs

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ABSTRACT. Glycoconjugates on the cell surface of type I and type II pulmonary epithelial cells were examined after airway irrigation by means of colloidal iron (CI), high iron diamine (HID) and ferritin-conjugated lectin stainings, in combination with digestion with carbohydrate-degrading enzymes, methylation or extraction with acid ethanol. The acid saccharide on the surface of type I cells was sialic acid, but the surface of type II cells contained a mixture of a large amount of sialic acid and a small amount of sulfated saccharide. As to the sensitivity on acid ethanol extraction, the sialo-oligosaccharide on type I cells differed from that on type II cells, although the terminal trisaccharides of sialo-oligosaccharides on the both type I and II cells were sialic acid → galactose → N-acetylgalactosamine. Sulfated saccharide, being mainly composed of glucuronic acid and N-acetylgalactosamine, distributed on the surface of the microvilli of type II cells. As to the constituents of glycoconjugates, large amounts of N-acetylglucosamine, galactose, mannose and glucose were found on type I cells, but type II cells had only small amounts of N-acetylglucosamine, mannose and glucose, and a minute amount of galactose.

Key words: glycoconjugates — lung — pulmonary epithelial cell — cell membrane — histochemistry

The pulmonary epithelium mainly consists of two different cell groups, designated type I and type II cells. Numerous histochemical studies have demonstrated that sialic acid, sulfated saccharide and lectin binding sugar residues exist on the alveolar surface of both types of epithelial cells (for Review see Meban[1]). Atwal and Brown[2] reported that sulfated saccharide existed on the surface of both type I and II cells, whereas several other studies demonstrated sulfated saccharide only on the surface of type II cells.[3,4] Meban[5] reported that no sulfated saccharides existed on the surface of pulmonary epithelial cells. Thus, agreement has not been quite unanimous regarding the distribution of these saccharides on the pulmonary epithelial cells.

The alveolar surface of the pulmonary epithelial cells is covered with a thick layer of surfactant lining.[6] This layer contains abundant carbohydrates[7,8] and interferes with the penetration of various histochemical agents.[9] Airway irrigation can completely eliminate the surfactant lining layer from the alveolar surface.[9] In the present study, the airways of the lungs were washed well with Ringer's solution before fixation with glutaraldehyde.

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The purpose of the present study was to clarify the chemical features of sialo-oligosaccharides and sulfated oligosaccharides on the surface of type I and II pulmonary epithelial cells, and to examine sugar residues in the oligosaccharides using surfactant free rat lungs.

MATERIALS AND METHODS

For histochemical staining procedures, a total of twenty Wistar rats about eight weeks of age were used. Under sodium pentobarbital anesthesia, the trachea was exposed and then the lungs were cannulated through the trachea. After the thorax cavity was exposed, the airways of the lungs were washed three times with Ringer's solution through the cannula to remove the surfactant lining layer on the pulmonary epithelium. The lungs were gently inflated and fixed with 2.5% glutaraldehyde in 70 mM cacodylate buffer, pH 7.4 for 20 min at room temperature. The hardened lungs were removed and cut into thin strips, approximately 0.1-0.2 mm thick. The tissue strips were immersed in the same fixative for 1 hr at room temperature. After fixation, the tissues were successively washed in chilled 50 mM cacodylate buffer, pH 7.4, containing 150 mM NaCl, rinsed with chilled 150 mM NaCl, and processed for the following histochemical staining.

Histochemical staining

1) *Colloidal iron (CI) staining*

To identify the specific character of acid saccharide, CI staining procedures were performed in combination with predigestion with carbohydrate-degrading enzymes. The lung strips were incubated in one of the following six enzyme solutions containing 50 mM NaCl for 18 hr at 37℃ :

1. Neuraminidase (EC 3.2.1.18, from *C. perfringens*, Sigma Type 5) at a concentration of 1 U/ml in 100 mM acetate buffer, pH 5.0.
2. Testicular hyaluronidase (EC 3.2.1.35, from bovine testes, Sigma Type 4) at a concentration of 3,000 NFU/ml in 100 mM acetate buffer, pH 5.5.
3. Streptomyces hyaluronidase (EC 4.2.2.1, from *Streptomyces hyalurolyticus*, Seikagaku) at a concentration of 100 TRU/ml in 100 mM acetate buffer, pH 5.0.
4. β-Glucuronidase (EC 3.2.1.31, from *E. coli*, Sigma Type 1) at a concentration of 50 U/ml in 100 mM cacodylate buffer, pH 7.0.
5. β-Galactosidase (EC 3.2.1.23, from bovine liver, Sigma) at a concentration of 0.15 U/ml in 100 mM Tris-HCl buffer, pH 7.2.
6. Chondroitinase ABC (EC 4.2.2.4, from *Proteus vulgaris*, Seikagaku) at a concentration of 5 U/ml in 100 mM Tris-HCl buffer, pH 8.0.

As controls for these enzyme digestion experiments, lung tissues were examined after incubation in buffer solutions containing heat-inactivated enzymes or in buffer solutions without enzymes.

After incubation in enzyme solutions, the lung tissues were washed with 150 mM NaCl, rinsed in 12% acetic acid, and immersed in a dialyzed CI solution prepared according to the method of Mowry[10] for 3 hr at 25℃. Excessive CI was removed by three successive washings in 12% acetic acid and the tissues were then rinsed with 150 mM NaCl.

To examine the chemical features of sialo-oligosaccharide, mild extraction

procedure was performed prior to CI staining. Fixed lung tissues were incubated in 2% acetic acid in 50% ethanol for 4 hr at room temperature. After being washed with 150 mM NaCl, the tissues were examined with the CI staining as described above.

Active and mild methylation procedures were performed in combination with the CI staining procedure to differentiate sulfated saccharides from non-sulfated acid saccharides. For active methylation, the lung tissues were incubated in absolute methanol acidified with 0.1N HCl for 8 hr at 60℃, and, for mild methylation, they were incubated in the same acidified methanol solution for 4 hr at 37℃.[11] Some lung tissues were also methylated after neuraminidase digestion. After methylation, the tissues were washed with 150 mM NaCl and stained with CI.

To examine the chemical features of neuraminidase-resistant acid saccharides, combined digestions with neuraminidase and successive carbohydrate-degrading enzymes were carried out, prior to CI staining. Lung strips were incubated in the neuraminidase solution for 4 hr at 37℃. After they were well washed in 150 mM NaCl, the lung strips were incubated in one of the following seven solutions containing carbohydrate-degrading enzymes and 50 mM NaCl for 18 hr at 37℃ :

1. β-Glucuronidase (EC 3.2.1.31, from beef liver, P-L Biochem.) at a concentration of 2 mg/ml in 100 mM citrate buffer, pH 6.5.
2. N-Acetylglucosaminidase (EC 3.2.1.30, from bovine kidney, Boehringer-Mannheim) at a concentration of 5 U/ml in 100 mM acetate buffer, pH 4.2.
3. N-Acetylgalactosaminidase (EC 3.2.1.49, from *Charonia lampas*, Seikagaku) at a concentration of 0.25 U/ml in 100 mM acetate buffer, pH 4.1.
4. N-Acetylhexosaminidase (EC 3.2.1.51, from jack beans, Seikagaku) at a concentration of 2.5 U/ml in 100 mM acetate buffer, pH 5.0.
5. β-Galactosidase solution.
6. Testicular hyaluronidase solution.
7. Chondroitinase ABC solution.

Control tissues were incubated in heat-inactivated enzyme solutions or in buffer solutions without enzymes. After incubation in the enzyme solutions, the lung tissues were well washed with 150 mM NaCl and examined by CI staining.

2) *High iron diamine (HID) staining*

HID procedures were carried out according to the method of Spicer[12] to visualize sulfated saccharides. The lung tissues were exposed to the HID solution for 16 hr at 25℃ and rinsed in 150 mM NaCl. Some tissues were also stained with HID after neuraminidase digestion.

3) *Lectin staining*

For the detection of specific saccharide residues of glycoconjugates, glutaraldehyde-fixed lung tissues were labeled with the following six ferritin-conjugated lectins (E-Y Laboratories) : Concanavalin agglutinin (Con A), *Ricinus communis* agglutinin I (RCA I), *Dolichos biflorus* agglutinin (DBA), *Ulex europaeus* agglutinin I (UEA I), peanut agglutinin (PNA), and wheat germ agglutinin (WGA). The tissues were incubated for 16 hr at 25℃ in 20

mM cacodylate buffer solution, pH 7.4, containing each ferritin-conjugated lectin at a concentration of 0.5-0.3 mg/ml and 150 mM NaCl. One mM $CaCl_2$ and 1 mM $MnCl_2$ were added to the test solution of Con A. Excessive lectins were removed by successive washings in 150 mM NaCl at room temperature. Control tissue strips were incubated in lectin solutions containing the following inhibitory sugars (200-400 mM) ; methyl α-D-mannose for Con A, lactose for RCA I, N-acetylgalactosamine for DBA, α-L-fucose for UEA I, β-methyl-galactose for PNA, N-acetylglucosamine for WGA.

To examine the character of sialo-oligosaccharide, the lung tissues were incubated in either neuraminidase solution or acid ethanol, and then they were treated with each ferritin-conjugated lectin.

After the histochemical staining procedures, the tissue samples were immersed in 1% osmium tetroxide in 150 mM cacodylate buffer, pH 7.4, for 60 min at 4℃. Following washing twice in 150 mM NaCl, the tissues were stained in block for 20 min at 4℃ with 0.5% uranyl acetate with 150 mM NaCl added.[9] Then they were rinsed in 150 mM NaCl, dehydrated and embedded in Epon 812. Ultrathin sections were cut and examined in a Hitachi H-500 electron microscope.

The following designations were used in tables to denote the subjective binding intensity of CI and ferritin-conjugated lectin particles on the alveolar surface of pulmonary epithelial cells: +++, heavy bindings showing two or more layers of particles; ++, bindings with a single uninterrupted layer of particles; +, scanty and discontinuous bindings; −, absence.

RESULTS

In the lung washed before fixation, the surfactant lining was completely removed so that the surface of pulmonary epithelium was exposed to the histochemical agents (Fig. la). After airway washing, the CI and lectin binding sites could be observed on the entire surface of the pulmonary epithelial cells, and the distribution profiles of these binding sites were highly reproducible. Macrophages were rarely observed on the alveolar surface after airway washing. As shown in Fig. la, type I cells were flattened with a smooth alveolar surface. Type II cells had many short microvilli on their cell surface.

Sialic Acid and Sulfated Saccharide

The distribution of acid saccharide on the alveolar surface of the pulmonary epithelial cells was studied by examination of the lung tissues stained with CI. As shown in Fig. 1b, electron-dense CI particles were bound to the alveolar surface of the two types of pulmonary epithelial cells. The stained layer was 15-20 nm thick on type I cells and 20-25 nm on type II cells. Type II cells had a markedly thicker stained layer than type I cells.

Pre-digestion in neuraminidase solution markedly decreased the number of CI particles on the surface of both types of epithelial cells (Fig. 2). A small amount of CI could be observed on the surface of the microvilli of type II cells, but the CI particles were absent on the intermicrovillar regions of type II cells. CI binding on the surface of type I cells was completely inhibited by neuraminidase digestion. Digestion with testicular hyaluronidase, Streptomyces

hyaluronidase, β-glucuronidase, β-galactosidase or chondroitinase ABC, however, had no influence on the CI binding on the surface of either type I or II epithelial cells. No detectable alterations in CI bindings were shown by incubation in an inactivated enzyme solution or in a buffer solution without the enzyme. Therefore, the CI-positive acid saccharide on the surface of type I cells was demonstrated to be sialic acid. The acid saccharide on type II cells was a mixture of a large proportion of sialic acid and a small amount of neuraminidase-resistant acid saccharide.

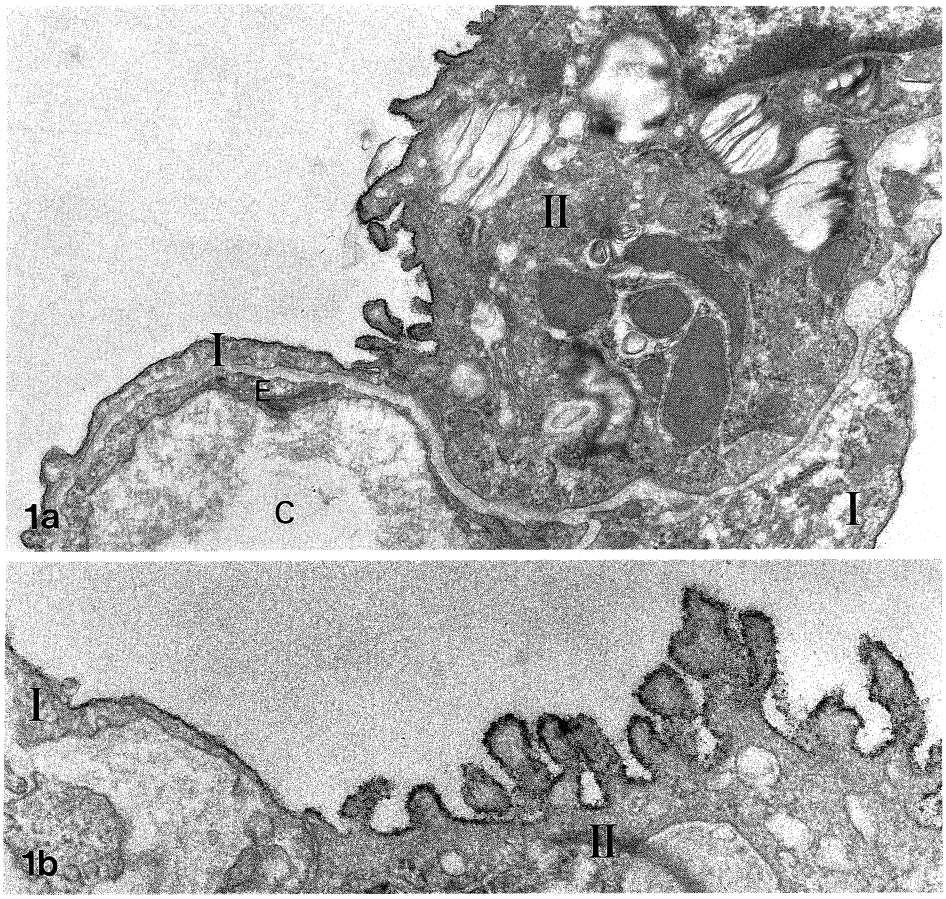

Fig. 1. CI bindings on the surface of pulmonary epithelial cells. (a) CI particles are bound to the entire alveolar surface of both type I cells (I) and type II cells (II), C ; capillary, E ; endothelial cells. × 15,000, (b) The type II cells had a higher density of CI binding sites than that of type I cells. × 36,000

To identify the specific character of neuraminidase-resistant acid saccharide on the surface of the microvilli of type II cells, mild and active methylation procedures were performed after neuraminidase digestion prior to staining with CI. As shown in Fig. 3a, mild methylation did not produce any evident changes in the CI binding of the acid saccharide, and weak CI bindings remained on the microvilli. Active methylation, however, completely inhibited

the CI binding on the microvilli (Fig. 3b). Thus, the neuraminidase-resistant acid saccharide on the surface of the microvilli of type II cells was considered to be sulfated saccharide.

The CI binding patterns on both type I and II cells after mild methylation without neuraminidase digestion were similar in appearance to those after mild methylation in combination with neuraminidase pre-digestion. Weak CI binding could not be seen on type I cells, but it was observed on

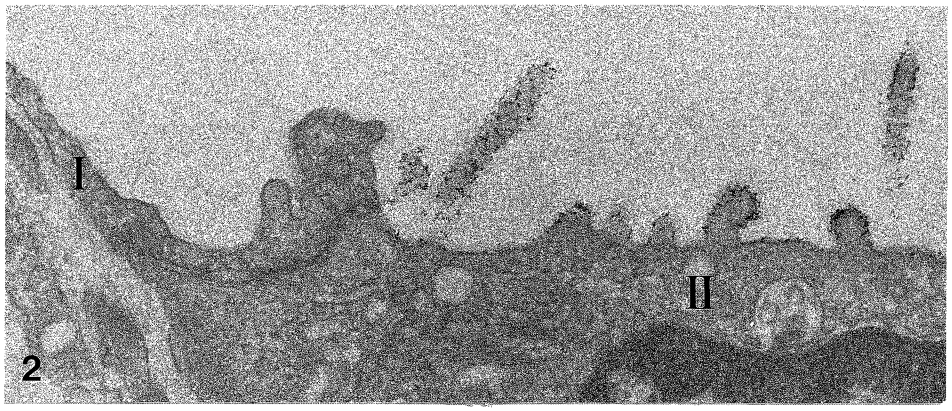

Fig. 2. Effects of neuraminidase digestion on CI bindings. A small amount of CI can be observed on the surface of the microvilli of type II cells. × 36,000

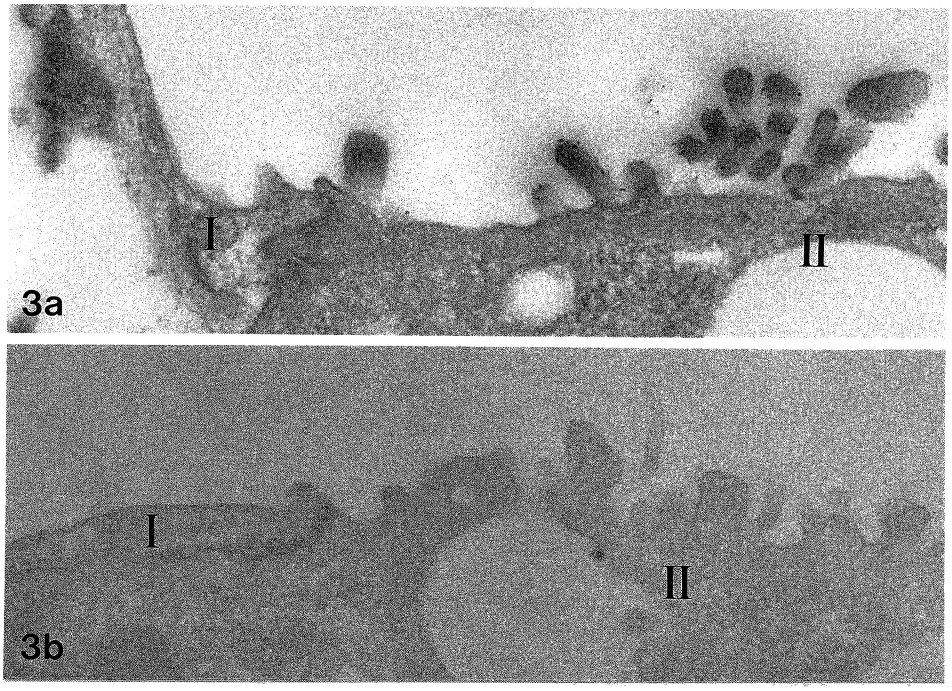

Fig. 3. Effects of methylation on CI binding after neuraminidase digestion. (a) A weak CI binding is present on the microvilli of type II cells after mild methylation. (b) The CI binding disappears after active methylation. × 36,000

the surface of the microvilli of type II cells. Active methylation without pre-digestion completely inhibited the CI bindings on both type I and II cells. Thus, it was confirmed that neuraminidase pre-digestion could not remove sulfated saccharide from the epithelial surface.

HID staining was employed to confirm the presence of sulfated saccharide on the surface of the microvilli of type II cells (Fig. 4a). The reaction products consisted of two components : small electron-dense particles, 3-5 nm in diameter, and an amorphous substance with a moderate electron density (Fig. 4b). A large amount of the amorphous substance was distributed over the entire surface of type II cells, forming a continuous layer, 10-25 nm in thickness. Small electron-dense particles, however, displayed a patchy distribution along the surface of the microvilli of type II cells. No reaction products could be observed on type I cells. In tissues digested with neuraminidase prior to the HID staining, amorphous products could not be seen on type II cells, but small numbers of electron-dense particles were patchily distributed over the surface of the microvilli of type II cells (Fig. 4c).

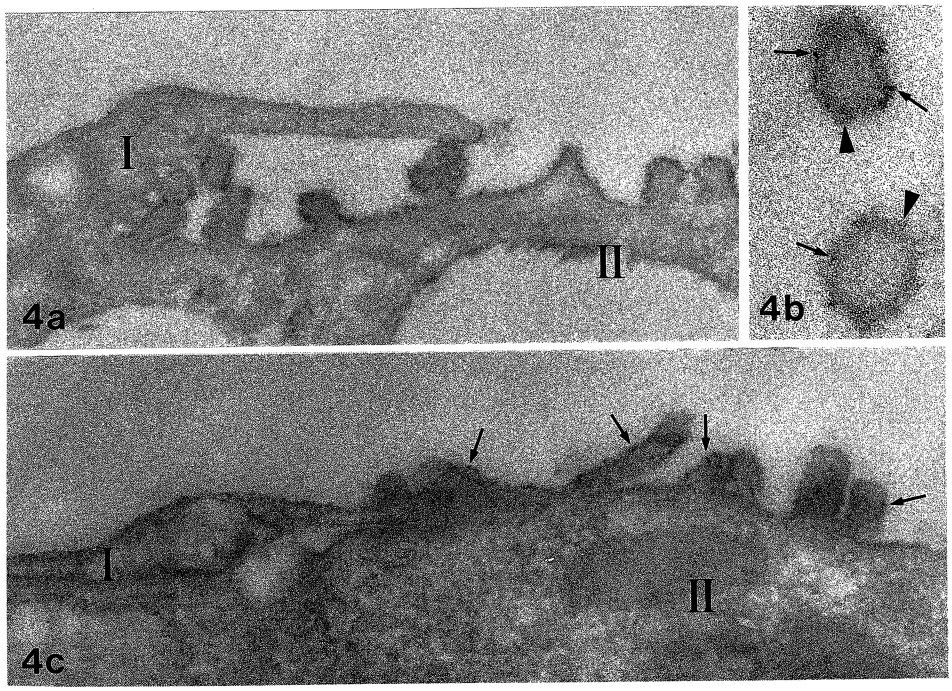

Fig. 4. Effects of neuraminidase digestion on HID staining. (a) The reaction products distribute over the entire surface of type II cells before the enzyme digestion. × 36,000, (b) A high-power view of cross sections of the microvilli of type II cells. The reaction products consist of two components: small electron-dense particles (arrow) and an amorphous substance having moderate electron density (arrowhead). × 99,000, (c) Small electron-dense particles (arrow) remain on microvilli after the enzyme digestion. × 36,000

TABLE 1. Lectin bindings to the alveolar surface of the pulmonary epithelial cells before and after neuraminidase digestion

Lectins	Specificity	Type I cells	Type I cells (N.ase)	Type II cells	Type II cells (N.ase)
Con A	Man, Glc	++	++	+	+
RCA I	Gal	++	++	+	+++
WGA	GlcNAc, SA	++	++	+++	+
UEA I	Fuc	−	−	−	−
DBA	GalNAc	−	−	−	−
PNA	Gal → GalNAc	−	+	−	++

The binding intensity is based on a subjectively estimated scale from − (unreactive) to +++ (most reactive). (N.ase); neuraminidase pre-digestion, Fuc; fucose, Gal; galactose, GalNAc; N-acetylgalactosamine, Glc; glucose, GlcNAc; N-acetylglucosamine, Man; mannose, SA; sialic acid

Sugar Residues of Oligosaccharides

The sugar residues of oligosaccharides on the surface of pulmonary epithelial cells were determined by staining with ferritin-conjugated lectins (Table 1). Con A and RCA I similarly bound to the alveolar surfaces of both types of epithelial cells, but the lectin binding patterns of type I and type II cells were different. The density of the binding sites for these lectins was high on the surface of type I cells, but sparse and patchy on that of type II cells (Fig. 5a, 5b). Although WGA binding sites could be observed on the surface of both types of epithelial cells, the density of the binding was higher on type II cells than on type I cells. UEA I, DBA and PNA did not appear to bind to the plasma membrane of either of these pulmonary epithelial cells (Fig. 5c). Incubation in a mixture of ferritin-conjugated lectins and inhibitor sugars interfered with lectin bindings on the surface of both epithelial cells.

Nature of Sialo-oligosaccharides

To determine the penultimate sugar residues of sialo-oligosaccharide after the removal of terminal sialic acid residues, neuraminidase digestion was performed, prior to lectin stainings (Table 1). Neuraminidase digestion caused an increase in number of PNA binding sites on both types of epithelial cells (Fig. 6a). Compared with type I cells, there was a noticeable increase in the number of PNA binding sites on type II cells. After digestion by neuraminidase, a marked increase was noted in number of RCA I binding sites on the surface of type II cells, but the enzyme digestion did not produce any detectable alterations in RCA I bindings on type I cells. The digestion with neuraminidase significantly decreased the number of WGA binding sites, particularly on the surface of type II cells (Fig. 6b). The digestion, however, had no influence on the binding intensity of Con A, UEA I and DBA to both types of epithelial cells.

The nature of sialo-oligosaccharide was examined by treatment with acid ethanol before CI staining. The acid ethanol treatment significantly decreased the number of CI particles on the alveolar surface of type II cells, and a weak CI reactivity could be seen on the surface of microvilli (Fig. 7). As shown in

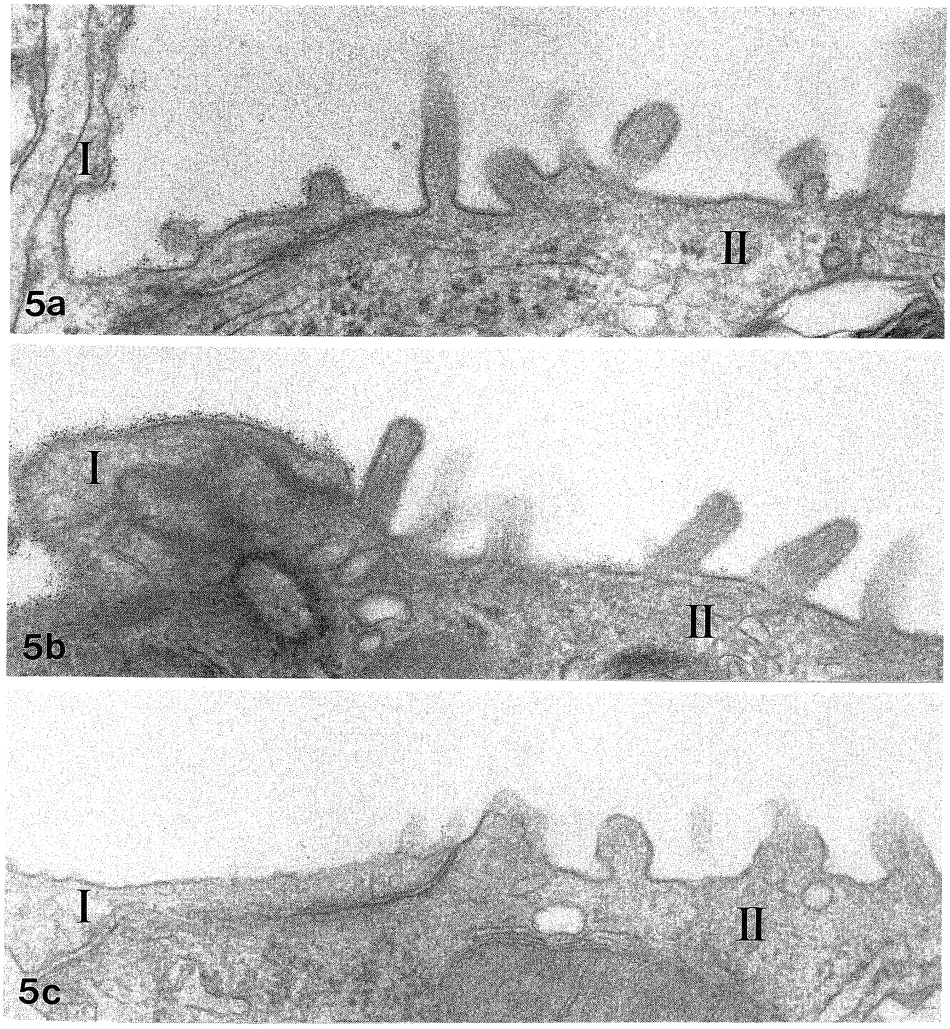

Fig. 5. Lectin binding sites on the surface of both type I and type II cells. (a) Con A, (b) RCA I, (c) PNA. The densities of Con A and RCA I binding sites are higher on type I cells than on type II cells. PNA binds to neither type I nor type II cells. × 45,000

Fig. 2, the pattern of CI bindings on type II cells was similar to that after digestion with neuraminidase. There was little change in the CI labeling on type I cells after the treatment. Acid ethanol treatment had no influence on the binding intensity of PNA on the surface of either type of pulmonary epithelial cell.

Sulfated Oligosaccharides on the Surface of Microvilli of Type II Cells

Table 2 illustrates the effects of digestion with carbohydrate-degrading enzymes after neuraminidase digestion prior to staining with CI. Digestion with β-glucuronidase eliminated the CI bindings on the microvilli of type II

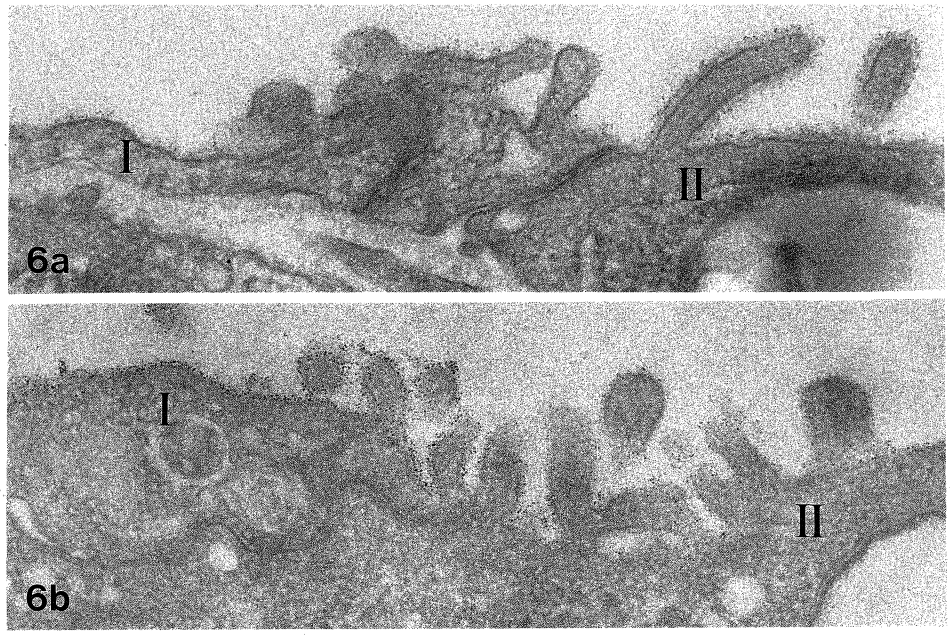

Fig. 6. Effects of neuraminidase digestion on lectin binding sites. (a) PNA heavily binds both type I and type II cells after the digestion, (b) A decrease in density of WGA binding sites can be observed on type II cells after the enzyme digestion. × 45,000

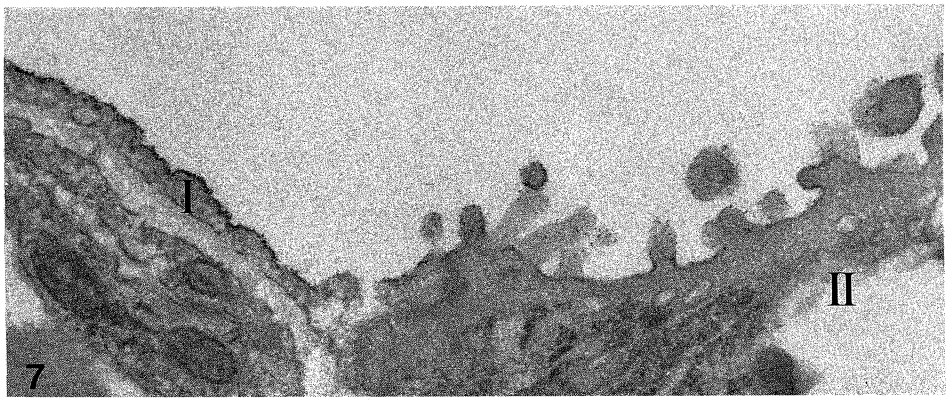

Fig. 7. Effects of acid ethanol treatment on CI binding. The CI binding intensity is decreased on type II cells. A weak binding can be observed only on microvilli. The CI binding sites on type I cells are not changed by the treatment. × 36,000

cells. Digestion with either N-acetylgalactosaminidase or N-acetylhexosaminidase also abolished the CI binding on the microvilli of type II cells. However, digestion with β-galactosidase, N-acetylglucosaminidase, testicular hyaluronidase or chondroitinase ABC did not produce any evident changes in the CI binding on the microvilli of type II cells.

TABLE 2. Effects of carbohydrate-degrading enzymes on the CI bindings on the alveolar surface of pulmonary epithelial cells after neuraminidase digestion

Enzymes	Type I cells	Type II cells
Neuraminidase	−	+
+ β-Glucuronidase	−	−
+ N-Acetylgalactosaminidase	−	−
+ N-Acetylhexosaminidase	−	−
+ β-Galactosidase	−	+
+ N-Acetylglucosaminidase	−	+
+ Testicular hyaluronidase	−	+
+ Chondroitinase ABC	−	+
Control	++	+++

The binding intensity is based on a subjectively estimated scale from − (unreactive) to +++ (most reactive).

DISCUSSION

Numerous investigators have observed acid saccharide on the alveolar surface of pulmonary epithelial cells using dialyzed iron,[3,13] CI,[9,14] and ruthenium red.[9,15] As shown in the present results, the acid saccharide on type I cells is sialic acid and on type II cells, a mixture of sialic acid and neuraminidase-resistant acid saccharide. These findings are in agreement with previous studies by Kuhn[13] and by Katsuyama and Spicer[4] using neuraminidase digestion prior to dialyzed iron staining. According to Spicer,[11] mild methylation selectively blocks the CI binding of non-sulfated acid saccharide, and active methylation blocks the CI binding of non-sulfated and sulfated acid saccharide. As to the chemical features of the acid saccharide, the present results indicated that not mild but active methylation abolishes the CI binding of neuraminidase-resistant acid saccharide on the surface of the microvilli of type II cells. Thus, the neuraminidase-resistant acid saccharide on the surface of the microvilli of type II cells can be considered to be a sulfated saccharide. Further, the present results have demonstrated the existence of a HID-positive layer, approximately 15 nm thick, on the entire surface of type II cells. The reaction products observed in the layer consist of two components; small electron-dense particles, 3-5 nm in diameter, and an amorphous substance with moderate electron density. The distribution pattern of the electron-dense particles is similar to that of the CI binding sites after mild methylation, and electron-dense particles could be observed on the microvilli of type II cells in the tissues stained with HID after removal of sialic acid residues by neuraminidase digestion. Therefore, sulfated saccharide may morphologically correspond to the electron-dense particles.

The CI binding on the surface of the microvilli of type II cells could be eliminated by additional digestion with either β-glucuronidase or N-acetyl-

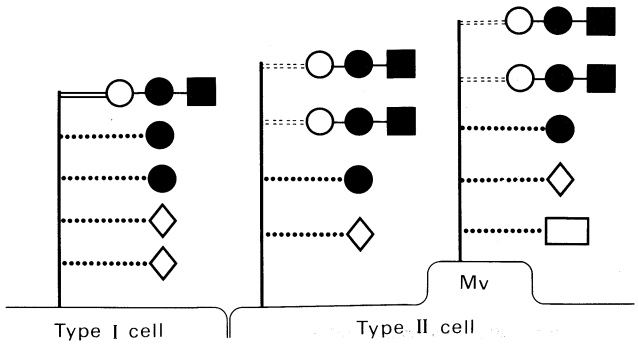

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Fig. 8. Schematic illustrations of oligosaccharides on the cell surface of type I and II cells. Symbols: ■; sialic acid. □; sulfated saccharide. ●; galactose. ○; N-acetylgalactosamine. ◇; mannose, glucose and N-acetylglucosamine. ═══; acid ethanol-resistant oligosaccharide. = = =; acid ethanol-susceptible oligosaccharide. Mv; microvilli.

galactosaminidase after neuraminidase digestion. Thus, the sulfated saccharide on the microvilli may mainly be composed of glucuronic acid and N-acetylgalactosamine. Several investigators have indicated that chondroitin sulfate A, C, D and E are mainly composed of glucuronic acid and N-acetylgalactosamine[16,17] and that these saccharides are hydrolyzed by chondroitinase ABC or testicular hyaluronidase.[17,18] Since the sulfated saccharide on microvilli of type II cells could not be digested by testicular hyaluronidase or chondroitinase ABC, it seems likely that the sulfated saccharide on type II cells could be different in chemical structure from chondroitin sulfate A, C, D and E.

The terminal structure of the sialo-oligosaccharide on the surface of both type I and II cells could be identified by applying ferritin-conjugated lectins after neuraminidase digestion. The PNA binding on the surface of both type I and II cells occurred after terminal sialic acid residues were removed from the sialo-oligosaccharide by digestion. Since PNA binds specifically to the terminal disaccharide, galactose → N-acetylgalactosamine,[19] it was demonstrated that the terminal trisaccharide of sialo-oligosaccharide on both type I and II cells is sialic acid → galactose → N-acetylgalactosamine. Such a terminal structure of sialo-oligosaccharide has been mainly detected in O-glycosidically linked sialo-oligosaccharide, whereas that of N-glycosidic type is mainly sialic acid → galactose → N-acetylglucosamine.[20,21] However, a striking difference in sialo-oligosaccharide on the cell surface was shown to exist between type I and II cells in the present study. Acid ethanol treatment significantly decreased the CI binding on type II cells, but did not reduce that on type I cells. Since the treatment did not increase the PNA binding on the surface of type II cells, it appears that acid ethanol can not liberate terminal sialic acid residues but instead extracts sialic acid in the form of oligosaccharide. The chemical structure of the basal part of sialo-oligosaccharide on type II cells may be different from that on type I cells.

By labeling with ferritin-conjugated lectins, the sugar residues of oligosac-

charides on the cell surface could be determined. Both mannose and glucose residues can be recognized by means of Con A binding[22] and disaccharide, galactose → N-acetylgalactosamine by PNA binding.[19] Galactose can be recognized by RCA I binding,[23] N-acetylgalactosamine by DBA[24] and fucose by UEA I.[25] In addition, N-acetylglucosamine can be identified by WGA binding after neuraminidase digestion.[26] Oligosaccharides on the surface of type I cells contain large amounts of N-acetylglucosamine, galactose, mannose and glucose. In contrast, those on type II cells contain small amounts of N-acetylglucosamine, mannose and glucose, and a minute amount of galactose.

RCA I binding has widely been used as a marker specific for type I cells[27,28] The present results have revealed that a strong binding of RCA I occurs on type I cells, whereas type II cells exhibit a very weak binding. Using PNA staining, Meban[29] reported that galactose residues are present on the surface of both type I and type II cells. Ito *et al.*[30] and Taatjes *et al.*[31] reported that type II cells exhibit weak RCA I binding. In view of this, RCA I binding appears to be a less reliable marker for type I cells.

The features of the identification of oligosaccharides on the surface of both type I and type II cells are summarized in Fig. 8. These differences are considered to be reliable markers for the histochemical identification of type I and II pulmonary epithelial cells.

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