Cell Surface Glycoproteins of Corneal Epithelium

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Purpose. To identify those plasma membrane glycoproteins of corneal epithelial cells that are synthesized in a higher amount or are downregulated during cell migration.

Methods. Primary cell cultures of rabbit corneal epithelium were used. Sialic acid and terminal galactose/N-acetylgalactosamine residues of plasma membrane glycoproteins of migrating and nonmigrating corneal epithelial cells were labeled using two well-characterized cell surface carbohydrate labeling techniques. The labeled glycoproteins were extracted in phosphate-buffered saline containing nonionic detergents and various protease inhibitors, and then they were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and fluorography.

Results. At least 12 to 13 radiolabeled components (molecular weight, 240 kd to 21 kd) were detected in the fluorographs of both sialic acid and galactose-labeled cells. Regardless of the labeling technique used, one sialoglycoprotein (CP1, 240 kd) was found in a higher amount in the extracts of nonmigrating cells than in migrating cells, and another glycoprotein (GP12, 28 kd) was present in a higher amount in migrating than in nonmigrating cells. Furthermore, one sialoglycoprotein (GP13, 21 kd) was detected only in migrating cells, and two glycoproteins (GP10, 42 kd; GP11, 32 kd) with terminal galactose/N-acetylgalactosamine residues were present in a higher amount in migrating than in nonmigrating cells.

Conclusions. This study has demonstrated that during corneal epithelial cell migration, the level of one membrane glycoprotein is markedly reduced, and the levels of four membrane glycoproteins are elevated. Further characterization of these glycoproteins should contribute to a better understanding of the mechanisms that modulate corneal epithelial sheet migration and wound healing. Invest Ophthalmol Vis Sci. 1995;36:355-363.

After accidental injury to the cornea and various surgical procedures, initial healing of the wound takes place by the migration of adjacent cells to the injured area.1,2 The failure of epithelium to migrate to the wound surface or the failure of migrated epithelium to remain adherent to the substratum may result in severe clinical problems, leading to the development of persistent epithelial defects and corneal ulceration.3,4 Molecular events that mediate migration of epithelium to the wound and adhesion of epithelium to the underlying substrata have not been fully elucidated, but a number of studies have suggested that plasma membrane glycoconjugates of corneal epithelium play a central role in these events.5-13

Studies aimed at identifying individual membrane glycoproteins of corneal epithelium have shown that integrins, which are widely expressed transmembrane glycoproteins and are known for their role as receptors that mediate attachment of cells to extracellular matrix and cell-cell adhesion, are localized in the cornea along the basal cell membrane of corneal epithelium,14-17 Other known plasma membrane glycoproteins of corneal epithelium include bullous pemphigoid antigens (BPAG 1+2), found along the basal membrane of corneal epithelium,18,19 and urothelin,20 also known as E-cadherin, which is a member of a family of cell adhesion molecules that regulate cell-cell contact.14-17 Other known plasma membrane glycoproteins of corneal epithelium include bullous pemphigoid antigens (BPAG 1+2), found along the basal membrane of corneal epithelium,18,19 and urothelin,20 also known as E-cadherin, which is a member of a family of cell adhesion molecules that regulate cell-cell contact. It is well established that plasma membrane glycoconjugates of different cell types are diverse and cell-type specific. Integrins, BPAGs, and cadherins most probably constitute only a portion of cell surface glycoproteins of corneal epithelium, and it is most likely that other glycoproteins unrelated to any known com-
ponents are also present and play an important role in the regulation of cell function in health and disease.

To understand better the cell surface architecture of the corneal epithelium with respect to glycoprotein constituents, two well-characterized cell surface carbohydrate labeling techniques were used in this study to identify the plasma membrane glycoproteins of primary cell cultures of rabbit corneal epithelium. To identify the specific molecules that may be involved in cell–cell and cell–matrix interactions, electrophoretic patterns of plasma membrane glycoproteins of confluent–nonmigrating cell cultures were compared with those of migrating cell cultures, and it was shown that during cell migration, levels of four 3-[3-cho

Preparation of Rabbit Corneal Epithelial Cell Cultures

Rabbit corneal epithelial cells were grown in tissue culture using eyes from Pel-Freeze Biologicals (Rogers, AR), as described by Jumblatt and Neufeld. Epithelial fragments from corneas were cultured in 100-mm dishes using supplemental hormonal epithelial medium. Within 3 days of starting the culture, approximately 30% to 50% of the dish was populated with migrating cells migrating away from the explants. These cells were designated as migrating (M) corneal epithelial cells. Within 10 to 12 days, 90% to 95% of the dish was populated with tightly packed polygonal cells, designated as nonmigrating (N) corneal epithelial cells. Migrating cells were also obtained by treating monolayer, nonmigrating cultures with Dispase II (2 mg/ml) (Boehringer Mannheim, Indianapolis, IN) for 20 minutes at 37°C. Cells from one dish were replated onto three dishes and were allowed to spread for 16 to 18 hours, at which time approximately 50% to 70% of the dish was populated with migrating cells.

Radiolabeling of Plasma Membrane Sialoglycoproteins of Corneal Epithelial Cells

Sialic acid residues of epithelial membrane glycoproteins were radiolabeled by sodium periodate (NaIO4)–tritiated sodium borohydride (NaBH4) treatment using the Gahmberg and Andersson method, with an additional prerredution step to minimize nonspecific labeling. This procedure is known to label externally exposed cell surface sialic acid residues specifically and involves cleavage of carbons 8 and 9 of the sialic acid molecule by NaIO4, leaving an aldehyde group at carbon 7 followed by reduction of CHO to HC\(\text{CHO}^+\) groups with NaBH4. Various reaction times and reagent concentrations were obtained from parameters determined by Panjwani and Baum. Cells were labeled directly on the monolayer in 100-mm dishes by sequential treatment with 45 \(\mu\)mol of NaBH4 for 15 minutes at room temperature, NaIO4 at a final concentration of 1 mM at 4°C for 10 minutes, and 2 or 3 mCi of NaB\(\text{H}^4\) (5 to 15 Ci/mmol) for 5 minutes at room temperature. The cell membranes were solubilized by incubating the labeled cells with 0.5% CHAPS or 0.5% Triton X-100 in phosphate-buffered saline containing various protease inhibitors for 1 hour at 4°C with gentle shaking. The solubilized membrane extracts were then centrifuged at 12,000g for 15 minutes in a Sorvall centrifuge to remove any insoluble material, the supernatants were transferred to Centricon-10 tubes (Amicon, Beverly, MA) for further centrifugation at 6000g to remove detergent and salts, and the labeled glycoproteins were analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Control cells underwent the same labeling procedure except that the periodate step was omitted. Because similar results were obtained regardless of whether the membranes were solubilized by CHAPS or Triton X-100, with the exception of a few initial experiments, the membranes of the labeled cells were solubilized with buffers containing CHAPS in all studies, mainly because it can be removed more efficiently than Triton X-100 by dialysis or centrifugation in Centricon tubes.

Neuraminidase Treatment of Cell Surface Sialoglycoproteins of Corneal Epithelial Cells

To ensure that the label was introduced specifically to cell surface sialic acid residues, membrane sialoglycoproteins were radiolabeled with NaIO4/NaB\(\text{H}^4\) treatment as described above and were then treated with neuraminidase (1 U, Type VI from Clostridium perfringens, Sigma, St. Louis, MO) or with enzyme buffer alone for 1 hour at room temperature. After enzyme treatment, cell membranes were solubilized, as described above, using the CHAPS buffer and were electrophoresed.

Affinity Chromatography of Membrane Glycoproteins of Corneal Epithelial Cells on Agarose-Bound Wheat Germ Agglutinin

To ensure further that the NaIO4/NaB\(\text{H}^4\) treatment specifically introduced the label to sialic acid residues, the radiolabeled sialoglycoproteins were chromatographed on a wheat germ agglutinin (WGA)-agarose column expected to bind specifically to glycoproteins containing sialic acid and N-acetylgalcosamine residues. For this, CHAPS-solubilized material from NaIO4/NaB\(\text{H}^4\)-treated corneal epithelial cells was applied to the WGA-agarose column (0.7 X 2.0 cm) and
was allowed to incubate for 20 minutes. The columns were washed with 15 ml of equilibration buffer (50 mM Tris-HCl, pH 7.8, 0.15 M NaCl, 0.2% CHAPS), and subsequently the bound glycoproteins were eluted with 0.3 M N-acetylglucosamine made in the equilibration buffer. Fractions of 1 ml were collected and analyzed for radioactivity using 10-μl aliquots. Fractions comprising peaks were pooled, dialyzed, and electrophoresed as described below. The entire affinity chromatography procedure was carried out at 4°C.

**Radiolabeling of Terminal Galactose Residues of Cell Surface Glycoproteins of Corneal Epithelial Cells**

To label terminal galactose–N-acetylgalactosamine residues of the plasma membrane glycoproteins of migrating and nonmigrating rabbit corneal epithelial cells in culture, the procedure described above to label the cell surface sialoglycoproteins was used. However, instead of treatment with NaIO₄, the cells were incubated with galactose oxidase (5 U, Dactylium dendroides; Sigma) at 37°C for 1 hour.⁴⁵

**Protein Determination**

The total protein concentration for each preparation was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL) using bovine serum albumin as a standard.

**Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate**

Electrophoresis was carried out according to the procedure of Laemmli in reducing conditions using 10% separating gels (70-mm × 80-mm × 1.5-mm) and 4% stacking gels. The gels were stained with Coomassie blue to visualize protein components and then were processed for fluorography. The following proteins were added as molecular weight standards: myosin (200 kd), phosphorylase b (97 kd), bovine serum albumin (69 kd), ovalbumin (46 kd), carbonic anhydrase (30 kd), soybean trypsin inhibitor (22 kd), and lysozyme (14 kd).

**RESULTS**

**Cell Cultures**

Under phase-contrast microscope, confluent/nonmigrating cultures appeared to contain polygonal cells with marked contact inhibition, whereas sparse/migrating cell cultures contained mostly nonpolarized cells (Fig. 1).

**Radiolabeling of Membrane Glycoproteins of Migrating and Nonmigrating Corneal Epithelium**

Cell viability, by trypan blue exclusion test, was at least 99% at the end of labeling. Treatment with 0.5%

### TABLE 1. Incorporation of Tritium From NaB³H₄ Into Periodate- or Galactose Oxidase-Treated Migrating and Nonmigrating Rabbit Corneal Epithelial Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Treatment</th>
<th>DPM X 10⁶/μg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmigrating</td>
<td>NaIO₄/NaB³H₄*</td>
<td>7.0 ± 1.3 (N = 6)</td>
</tr>
<tr>
<td>Migrating</td>
<td>NaIO₄/NaB³H₄*</td>
<td>14.0 ± 1.8 (N = 4)</td>
</tr>
<tr>
<td>Nonmigrating</td>
<td>Galactose oxidase/ NaB³H₄†</td>
<td>4.9 ± 0.8 (N = 3)</td>
</tr>
<tr>
<td>Migrating</td>
<td>Galactose oxidase/ NaB³H₄†</td>
<td>10.6 ± 3.9 (N = 5)</td>
</tr>
<tr>
<td>Nonmigrating</td>
<td>NaB³H₄*</td>
<td>1.0 ± 0.5 (N = 3)</td>
</tr>
<tr>
<td>Migrating</td>
<td>NaB³H₄†</td>
<td>3.9 (N = 2)</td>
</tr>
</tbody>
</table>

Values are given as means ± SEM. N = number of preparations.

* 2 μCi of NaB³H₄ was used for reduction.

† 5 μCi of NaB³H₄ was used for reduction.
CHAPS solubilized approximately 45% of total protein. The radioactivity incorporated in the NaIO₄/NaB³H₄ and galactose oxidase/NaB³H₄-treated cells was always greater than in the control cells treated with NaB³H₄ alone (Table 1). In spite of prior reduction of the cells with cold NaBH₄, some nonspecific incorporation of radioactivity was always observed in the control cells, which, however, were found to contain no radiolabeled components upon electrophoresis (see below).

Polyacrylamide Gel Electrophoresis of Plasma Membrane Glycoproteins of Migrating and Nonmigrating Corneal Epithelium

We analyzed electrophoretic patterns of CHAPS solubilized material of NaIO₄/NaB³H₄-treated migrating (M) and nonmigrating (N) cells to identify the major membrane sialoglycoproteins of rabbit corneal epithelium and to determine if the alteration in membrane sialoglycoproteins occurs during cell migration. Fluorography of the SDS-PAGE gels of material derived from M cells revealed at least 13 components (GP1-GP13, molecular weight 21 kd to 240 kd, Fig. 2). Comparison of the fluorography patterns of M and N cells revealed significant differences: component GP1 (240 kd) was present in a higher amount in N cells than in M cells; component GP12 (28 kd) was present in a higher amount in M cells compared to N cells; and component GP13 (21 kd) was detected only in the fluorograph of M cells (Fig. 2). Differences between M and N cells with respect to GP12 and GP13 were consistent in all four preparations of M and N cells analyzed in this study. GP1, which appeared as an intense band in fluorographs of N cells in all four preparations analyzed in this study, was either undetected (three preparations) or was detected only as a weak band (one preparation) in M cells. Several other differences between the fluorography patterns of M and N cells (for example, the presence of GP4 in higher amounts in N cells than in M cells; Fig. 2) are not discussed here because they were not found consistently in fluorographs of all preparations analyzed. Similar fluorography patterns of the labeled cells were obtained regardless of whether the cell membranes were solubilized in buffers containing Triton X-100, or CHAPS, and whether the migrating cells used were from explant cultures or were prepared by subculture of primary confluent cells with a split ratio of 1:3 (passage 1). Fluorography of the control (C) cells that underwent only NaB³H₄ treatment did not reveal any bands, whereas Coomassie blue staining pattern of NaB³H₄ as well as NaIO₄/NaB³H₄-treated cells was identical (Fig. 2). These observations suggest that the components detected in the NaIO₄/NaB³H₄-treated cells were specifically labeled, and the C cell

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933410/)

The molecular weight scale is based on the electrophoretic mobility of reduced standard proteins. The glycoproteins GP1 through GP13 are designated in order of increasing mobility on the gel. Note that: (1) no radiolabeled components were detected in fluorography patterns of nonmigrating (C) or migrating (not shown) control cells treated with NaB³H₄ alone, (2) GP1 is present only in N cells, (3) GP12 is present in a higher amount in M cells than in N cells, and (4) GP13 is present only in M cells. To visualize GP13 more clearly, some gels were overexposed (bottom). Note that GP13 is detected only in M cells even when gels are overexposed. BPB = bromophenol blue tracking dye; CHAPS = 3-[((3-Cholamidopropyl) dimethylammonio]-l-propanesulfonate.
FIGURE 3. Neuraminidase treatment of cell surface sialoglycoproteins of rabbit corneal epithelium in culture. Membrane sialoglycoproteins of migrating epithelial cell cultures were radiolabeled with NaIO₄/NaB³H₄ treatment as described in the Methods section and were then treated with neuraminidase or with enzyme buffer alone for 1 hour. After enzyme treatment, cell membranes were solubilized and were electrophoresed in 10% gels using 37 µg of protein in each lane. Note that, with the exception of a portion of GP12 (arrow), no other components were resistant to enzyme treatment.

3H represents volatile radioactivity. It is known that during the reduction with borohydride, volatile radioactivity representing tritiated hydrogen and water is generated, and the amount of such radioactivity present after the reaction varies depending on the lot of NaB³H₄ crystal used in the reaction. ²³⁻²⁵

Effect of Neuraminidase Treatment on Radiolabeled Sialoglycoproteins of Corneal Epithelium

To ensure that the NaIO₄/NaB³H₄ treatment specifically labeled the cell surface sialic acid residues, the radiolabeled cells were treated with neuraminidase or the enzyme buffer alone before extraction with the CHAPS buffer and electrophoresis. Treatment with neuraminidase removed 50% of the radioactivity. All components, with the exception of a portion of GP12, were susceptible to the enzyme treatment (Fig. 3), suggesting that NaIO₄/NaB³H₄ treatment specifically labeled the cell surface sialic acid residues of epithelial cells. The neuraminidase-resistant fraction of GP12 also migrated faster on electrophoresis gels than the fraction that was susceptible to the enzyme. The Coomassie blue staining pattern of samples treated with the enzyme and the control samples incubated with the enzyme buffer alone were indistinguishable (not shown).

Affinity Chromatography of Sialoglycoproteins of Corneal Epithelium on WGA-Agarose Lectin

To confirm that NaIO₄/NaB³H₄ treatment specifically labeled the cell surface sialic acid residues, the CHAPS-solubilized material obtained from NaIO₄/NaB³H₄-treated cells was chromatographed on an agarose-bound lectin column, which is expected to bind specifically to glycoconjugates containing sialic acid and N-acetylglucosamine residues. ²⁴ Electrophoresis and fluorography of the bound and unbound material eluted from the WGA-agarose column revealed that, with the exception of a portion of GP12, all components bound to the WGA-agarose column (Fig. 4), providing additional evidence that NaIO₄/NaB³H₄ treatment specifically labeled the sialoglycoproteins. In the unbound fraction, only a small portion of GP12 was detected. Again, GP12 eluted in the unbound fraction migrated faster than that eluted in the bound fraction.

Polyacrylamide Gel Electrophoresis of Galactose Oxidase/NaB³H₄-Labeled Migrating and Nonmigrating Corneal Epithelial Cells

To identify the major membrane glycoproteins of rabbit corneal epithelium that contain oligosaccharides with terminal galactose/N-acetylgalactosamine residues and to determine whether alterations in these glycoproteins occur during cell migration, we analyzed electrophoretic patterns of CHAPS-solubilized material of galactose oxidase/NaB³H₄-treated M- and N cells. The most dramatic difference between the electrophoretic patterns of M and N cells was that GP1, which appeared as an intense band in the N cells, was barely detectable in the M cells (Fig. 5) in all three preparations. Furthermore, three components (GP12, 28 kd; GP11, 32 kd; and GP10, 42 kd) were present in a higher amount in M cells than in N cells. Although these three components were present in a higher amount in M cells than in N cells in all the preparations analyzed, their relative levels varied
FIGURE 4. Fluorography patterns of corneal epithelial cell surface sialoglycoproteins eluted from the WGA-agarose column. Membrane sialoglycoproteins of migrating epithelial cell cultures were radiolabeled with NaIO₄/NaBH₄ and were then chromatographed on a WGA-agarose column. Unbound fraction was eluted with 0.2% CHAPS in 50 mM Tris/HCl, pH 7.8, 0.15 M NaCl, and the bound fraction was eluted with the same buffer containing 0.3M N-acetylglucosamine. Samples applied to each lane originated from 330 μg of the original cell protein. Note that with the exception of a portion of GP12 (arrow), no components were seen in the WGA unbound fraction. WGA = wheat germ agglutinin; CHAPS = 3-((3-Cholamidopropyl)dimethylammonio)-1-propanesulfonate.

FIGURE 5. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of corneal epithelial cell surface glycoproteins containing terminal galactose/N-acetylgalactosamine residues. Migrating (M) and nonmigrating (N) cells in culture were treated with galactose oxidase/NaBH₄ and the radiolabeled glycoproteins were solubilized in 0.5% CHAPS containing protease inhibitors as described in the Methods section. Electrophoresis was performed in 10% gels using 75 μg of protein in each sample. Protein components were visualized by Coomassie blue staining (CB, top left), and the same gel was subsequently processed for fluorography to visualize radioactive components (FG, top right). The molecular weight scale is based on the electrophoretic mobility of reduced standard proteins. The glycoproteins GP1 through GP12 are designated in order of increasing mobility on the gel. Note that GP1 is present in a higher amount in N cells than in M cells, and GP10, GP11, and GP12 are present in a higher amount in M cells than in N cells. To visualize GP10 to GP13 more clearly, some gels were overexposed (bottom). BPB = bromophenol blue; CHAPS = 3-((3-Cholamidopropyl)dimethylammonio)-1-propanesulfonate.
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among different preparations so that in one preparation they were present in a moderately (Fig. 5, top) higher amount and in two preparations they were present in a significantly higher amount in M cells than in N cells (Fig. 5, bottom).

DISCUSSION

To understand the role of corneal epithelial cell surface glycoproteins in cell migration and cell–cell and cell–matrix interactions, the present study focuses on identifying major surface glycoproteins of migrating and nonmigrating corneal epithelium using two well-characterized cell surface carbohydrate labeling techniques. That the mild periodate treatment followed by reduction with NaB₃H₄ specifically labeled the cell surface sialic acid residues was demonstrated by the fact that most components labeled by the technique were susceptible to neuraminidase treatment and reacted with WGA, a lectin known to bind specifically to sialic acid and N-acetylgalactosamine residues. A small portion of GP12 that was resistant to neuraminidase and did not react with WGA may represent a modified form of sialic acid. The most commonly found sialic acid in higher animals is N-acetylneuraminic acid, but various modifications, such as hydroxylation of the N-acetyl group (N-glycolyneuraminic acid) and acetylation of N-acetyl and N-glycolyneuraminic acid, are also found, though to a lesser degree. The modified sialic acids differ with respect to their susceptibility to neuraminidases from different sources. Heterogeneity within GP12 was also evident by the observation that its neuraminidase-resistant fraction migrated faster on electrophoresis gels than the fraction that was susceptible to the enzyme.

The most significant finding of the present study is the identification of a 240-kd membrane glycoprotein (GP1) that appeared as an intense band in fluorographs of nonmigrating epithelium but was barely detectable in the extracts of migrating epithelium. This glycoprotein was labeled by NaIO₄/NaB₃H₄, as well as galactose oxidase/NaB₃H₄ labeling techniques, indicating that it contains oligosaccharides with terminal sialic acid and galactose/N-acetylgalactosamine residues. Because GP1 is not detected in significant amounts until the cells reach confluence and it disappears again when confluent cells are induced to migrate by subculture of primary cells, it appears likely that the synthesis of this glycoprotein is induced upon establishment of cell–cell contact and that there is a downregulation of this component during cell migration. Downregulation of GP1 may be of significance in events that mediate breakage of cell–cell contact in response to injury, an event prerequisite for the initiation of reepithelialization. It remains to be determined whether GP1 is a novel glycoprotein identified for the first time or whether it represents a known component. Furthermore, although we have assumed that the 240-kd glycoprotein labeled by both NaIO₄/NaB₃H₄ and galactose oxidase/NaB₃H₄ treatment is the same component, the possibility remains that both techniques have labeled different components of similar molecular weight. Although positive identification of GP1 must be made, it is unlikely to be BPAG1, a known 250-kd glycoprotein found along the basal membrane of corneal epithelium, because it could not be precipitated by human autoimmune bullous pemphigoid antiserum that cross-reacts with the rabbit antigen (Mohan et al, unpublished observations). Furthermore, recent studies suggest that BPAG1 may be a transmembrane glycoprotein, the intracellular domain of many transmembrane glycoproteins forms a complex with molecules of cytoskeleton, and such complexes are not usually extractable, in buffers containing nonionic detergents (see below). Nor is GP1 likely to be a subunit of fibronectin because it did not bind to a gelatin-Sepharose (Pharmacia, Piscataway, NJ) column (Ahmed and Panjwani, unpublished observations).

Another major finding of the present study is the identification of several cell surface glycoproteins found in elevated amounts in migrating epithelium compared to nonmigrating epithelium. Regardless of the labeling technique used, GP-12 (28 kd) was found in higher amounts in migrating epithelium than in nonmigrating epithelium. In addition, a 21-kd sialoglycoprotein was detected only in migrating epithelium, and two glycoproteins (GP10, 42 kd; GP11, 32 kd) containing oligosaccharides with terminal galactose/N-acetylgalactosamine residues were detected in a higher amount in migrating than in nonmigrating epithelium. Because GP12 and GP13 were synthesized in elevated amounts by migrating epithelium even when the cells were cultured in the presence of 5-fluorouracil to inhibit cell mitosis (data not shown), they are likely to be related to cell migration rather than cell mitosis. The glycoproteins, which were detected in a higher amount in migrating compared to nonmigrating epithelium, may play a role in cell migration and cell–cell or cell–matrix interactions. Many studies have shown that in sparse cell cultures, as well as in cells plated in low Ca++ culture media to prevent the formation of cell–cell junction, membrane glycoproteins, especially those that mediate cell–cell and cell–matrix interactions, are evenly distributed over the entire cell surface and, as the cells reach confluence and become tightly packed, there is a redistribution of these glycoproteins such that they are localized at the site of cell–cell or cell–matrix attachment. Many transmembrane glycoproteins
are extractable in nonionic detergents from the sparse cell cultures in the absence of cell–cell contact; upon reaching confluency and the establishment of cell–cell contact, the same glycoproteins form a complex with the molecules of cytoskeleton and become insoluble in nonionic detergents. For example, in the presence of extensive cell–cell contact, approximately 35% of uvomorulin, a glycoprotein found at the cell–cell junction, could be extracted from the MDCK cells, whereas in the absence of extensive cell–cell contact, >90% of this glycoprotein is extracted in buffers containing nonionic detergents. Properties of different plasma membrane glycoproteins vary with respect to their extractability in buffers containing nonionic detergents. Unlike E-cadherin and a number of other plasma membrane glycoproteins that are easily extracted from the membranes of sparse cell cultures in buffers containing nonionic detergents, desmocollins, the membrane glycoproteins found in desmosomes, are insoluble in the nonionic detergents regardless of whether the cell–cell contacts have been induced.

Whether the plasma membrane glycoproteins of rabbit corneal epithelium identified in this study using cells grown in culture are directly relevant to the cells in vivo remains to be established. In this study, only primary cell cultures were used, and therefore there is good probability of preserving many of the in vivo characteristics of the cells. In a recent study, we demonstrated that a glycolipid, paragloboside, is synthesized in a higher amount in migrating epithelium than in nonmigrating epithelium regardless of whether the epithelia are prepared using primary cell cultures, organ cultures, or in vivo techniques. Because of difficulties in obtaining sufficient migrating and nonmigrating epithelium from corneas in vivo and in organ culture, we think that further characterization of plasma membrane glycoproteins identified in the present study using cells grown in culture will provide tools to study the role of plasma membrane glycoproteins of corneal epithelium in an in vivo model of corneal wound healing.

In summary, the present study has identified a number plasma membrane glycoproteins of corneal epithelium and has demonstrated that during cell migration, the level of one cell surface glycoprotein is markedly reduced whereas the levels of four cell surface glycoproteins are elevated. Isolation and characterization of these glycoproteins is under way in our laboratory. Given their strategic location on the cell surface, the glycoproteins identified in this study could serve as mediators of important biologic processes, including cell migration and cell–cell and cell–matrix interactions, both of which are important events in wound healing.

Key Words
corneal epithelium, wound healing, cell migration, plasma membranes, glycoproteins

Acknowledgment
The authors thank Dr. Nikhat Ahmed for performing the cell viability study and for help with the estimation of molecular weight of GPI using cross-linked hemocyanin standards (210 kd, trimer; 280 kd, tetramer). They also thank Drs. Zheng Zhao and Royce Mohan for helpful discussions.

References


