Role of cell surface glycosylation in mediating repair of human airway epithelial cell monolayers

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Dorscheid, Delbert R., Kimberly R. Wojcik, Kelly Yule, and Steven R. White. Role of cell surface glycosylation in mediating repair of human airway epithelial cell monolayers. Am J Physiol Lung Cell Mol Physiol 281: L982–L992, 2001.—Our laboratory recently demonstrated the pattern of cell surface glycosylation of nonsecretory central airway epithelium (Dorscheid DR, Conforti AE, Hamann KJ, Rabe KF, and White SR. Histochem J 31: 145–151, 1999), but the role of glycosylation in airway epithelial cell migration and repair is unknown. We examined the functional role of cell surface carbohydrates in wound repair after mechanical injury of 1HAEo- human airway epithelial and primary bronchial epithelial monolayers. Wound repair stimulated by epidermal growth factor was substantially attenuated by 10-7 M tunicamycin (TM), an N-glycosylation inhibitor, but not by the inhibitors deoxymannojirimycin or castanospermine. Wound repair of 1HAEo- and primary airway epithelial cells was blocked completely by removal of cell surface terminal fucose residues by a-fucosidase. Cell adhesion to collagen matrix was prevented by TM but was only reduced -20% from control values with prior a-fucosidase treatment. Cell migration in Blind Well chambers stimulated by epidermal growth factor was blocked by pretreatment with TM but a-fucosidase pretreatment produced no difference from control values. These data suggest that cell surface N-glycosylation has a functional role in airway epithelial cell adhesion and migration and that N-glycosylation with terminal fucosylation plays a role in the complex process of repair by coordination of certain cell-cell functions.

Airway epithelial cells are known to have specific surface glycosylation patterns. Secretory cells express a number of O- and N-linked glycoproteins as demonstrated by lectin histochemistry (1, 2, 27, 31). These can differ substantially based on location in the large and small airways (1, 31). Our laboratory (7) has recently characterized cell surface glycosylation in nonsecretory cells of central human airway epithelium and airway epithelial cell lines that utilize lectin-binding patterns. In that study (7), galactose- or galactosamine-specific lectins labeled basal epithelial cells and cell lines derived from basal cells. Lectins specific for several different carbohydrate structures bound columnar epithelial cells, and certain fucose-specific lectins labeled subsets of the airway epithelial cells. These differences may be relevant in various cellular functions of these cells.

The airway epithelium is a target of inflammatory and physical stimuli in obstructive airway diseases such as asthma and bronchopulmonary dysplasia. The epithelium provides a physical barrier to the external environment and regulates several key metabolic functions of airways including fluid and ion transport to the airway lumen, mucociliary clearance, and regulation of airway diameter. Repair of a damaged epithelium may have been associated with specific combinations of ABO or Lewis antigens (18). More recently, it has been noted that human cells express a class of proteins known as selectins that recognize specific carbohydrate structures (11). The general carbohydrate composition of selectin ligands includes fucose, galactose, and sialic acid residues. Functions associated with this receptor family include lymphocyte homing, inflammatory cell migration, metastasis, hemopoietic cell maturation (13), and coordination of repair of endothelial cell layers (38). P-selectin, which recognizes specific carbohydrate structures, is similarly required in endothelial cell migration during repair (9). Many cellular functions that utilize the selectin family do not yet have an identified ligand core structure.

Complex carbohydrate structures attached to cell surface proteins and lipids have functional roles in cell adhesion via integrin proteins (24, 29), proliferation (3, 26a), and growth potential (26, 30) in several cell types. Glycoconjugate expression is an essential element for migration of corneal epithelial cells in surface repair (28). The expression of terminal sugars in the epithelium of central airways has been associated with blood group antigen expression of complex carbohydrates (27), and several diseases, including asthma, have
be a necessary part of restoring airway function to its normal state and may thus play a role in chronic airway remodeling as seen in asthma. Repair generally involves several steps including spreading of epithelial cells at the margin followed by migration of distant cells into the damaged region and, finally, proliferation of new epithelial cells (10, 19).

The purpose of this study was to determine if cell surface glycosylation mediated the repair of airway epithelium after mechanical injury. Human airway epithelial cell lines and primary bronchial epithelial cells grown in monolayer culture were studied with the use of time-lapse videomicroscopy after mechanical injury. Stimulated repair was studied in the presence of specific inhibitors of glycosylation or in the presence of endo- and exoglycosidases. Cell migration in Blind Well chambers and adhesion to collagen-coated plates were studied in the presence of these inhibitors and enzymes. Our data demonstrate that N-glycosylation of cell surface glycoprotein molecules, along with terminal fucose residues, regulate both monolayer repair and migration of epithelial cells in culture.

METHODS

Materials. Bovine serum albumin (BSA), trypsin, epidermal growth factor (EGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), human placental collagen IV, and EDTA were obtained from Sigma (St. Louis, MO). Fetal calf serum (FCS) was obtained from HyClone (Logan, UT) and was heat denatured before use. Tunicamycin (TM), doexymannojirimycin (DMJ), α-fucosidase, endoglycosidase H, O-glycosidase, sialidase, β-galactosidase, amylglucosidase, N-acetyl-β-D-glucosaminidase, and castanospermine (CSP) were obtained from Boehringer Mannheim (Indianapolis, IN). The biotinylated lectins Aleuria aurantia agglutinin (AAA), Ulex europaeus agglutinin (UEA)-1, Lens culinaris agglutinin (LCA), and Pisum sativum agglutinin (PSA) were obtained from EY Laboratories (San Mateo, CA). Streptavidin- horseradish peroxidase was obtained from DAKO (Carpinteria, CA).

Cell culture. 1HAEo− cells are SV40-transformed normal human airway epithelial cells that have been characterized previously (5, 14) and express multiple surface carbohydrate markers of primary basal airway epithelial cells (7). Cells were grown on collagen IV-coated flasks or plates in Eagle’s modified essential medium (MEM) containing 10% FCS, 2 mM L-glutamine, 100 μg/ml of streptomycin, and 100 U/ml of penicillin G (medium A) and incubated at 37°C in 5% CO2. Cells were passaged into matrix-coated six-well plates for wound repair experiments. Primary normal human bronchial epithelial (NHBE) cells were purchased from Clonetics (Walkersville, MD). These cells were derived from a single donor and were supplied as first-passage cells. Cells were placed in defined medium (Clonetics) containing 5 μl/ml of insulin, 0.5 μg/ml of human EGF, 10 mg/ml of transferrin, 6.5 μg/ml of triiodothyronine, 0.5 mg/ml of epinephrine, and 2 ml/l of bovine pituitary extract. Cells were subcultured and used between passages 3 and 7. Experiments were done in the same manner as those conducted with the 1HAEo− cell line except that cells were kept in defined medium and not in 10% FCS, and wound repair was performed at ~70% confluence.

Monolayer wound repair assay. Our laboratory has described this method previously (20–22). Cells were grown until confluent in medium A and then placed in 2 ml of medium B (medium A without FCS). A small wound (1 mm²) was made in the confluent monolayer with a rubber stylus, and epithelial cells were removed without disturbing the underlying matrix. Wound closure was documented serially for 24 h starting immediately after wound creation. Microscope images were photographed with a Sony Iris charge-coupled device camera (Sony, Rolling Meadows, IL) on a Nikon Diaphot inverted stage microscope. Video images were digitized with a Macintosh computer and Apple Video Player software (Apple Computer, Cupertino, CA). Analysis of perimeter length and area of the remaining wound in each image was performed with NIH Image software (Wayne Rasband, National Institutes of Health, Bethesda, MD).

In each experiment, one well was used as a negative control (no EGF, inhibitor, or glycosidase), and one well was treated with 15 ng/ml of EGF, which has previously been demonstrated to be a potent accelerant for epithelial monolayer wound closure (20). In six experiments, cells were treated concurrently with both EGF and 10–10 to 10–5 M TM or 10–2 M DJM, and monogalactosyl N-acetylα-fucosidase (12, 38). In five experiments, cells were treated concurrently with EGF and 10–6 to 10–5 M CSP, an α-glucosidase inhibitor that prevents processing of some N-linked glycans (15, 28, 30). In four experiments, 1HAEo− cells were treated concurrently with both EGF and 10–250 mU/ml of α-fucosidase. In an additional four experiments, NHBE cells were similarly treated. This exoglycosidase removes all available α-1,6-fucose, α-1,3-fucose, and α-1,4-fucose linkages (17), where activity decreases as the complexity of the oligosaccharide increases but is stable for terminal residues. In four experiments, cells were treated concurrently with both EGF and 10–2000 U/ml of endoglycosidase H (32). In an additional four experiments, initial treatment with sialidase, β-galactosidase, amylglucosidase, and N-acetyl-β-D-glucosaminidase was followed by concurrent treatment with EGF and O-glycosidase during wound closure. This pretreatment was to allow for maximal digestion by O-glycosidase per recommendation of the manufacturer.

Additional control experiments determined monolayer wound closure rates in the presence of HGF (0.1–10 ng/ml) and PDGF (0.3–30 ng/ml) with and without the inclusion of 10–10 to 10–6 M TM or 10–250 mU/ml of α-fucosidase. Heat-denatured (56°C for 15 min) α-fucosidase (250 mU/ml) or BSA (comparable mass to 500 mU of α-fucosidase) was added to the 1HAEo− and NHBE monolayers at the initiation of wound repair. These conditions served as controls for the exogenous protein and chemicals added as part of the commercial preparation of α-fucosidase.

Lectin histochemistry. Cell monolayers grown on two-chamber slides were treated for 12 or 24 h with α-fucosidase (100 mU/ml), CSP (10–5 M), or DJM (10–5 M); fixed with 4% paraformaldehyde at room temperature (RT) for 4 h; and subsequently dehydrated in sequential alcohol baths. Endogenous peroxidases were quenched by incubation in 0.3% H2O2 for 30 min. Slides were blocked in HEPES buffer (10 mM HEPES, 150 mM NaCl, 0.1 mM MgCl2, and 0.1 mM CaCl2 at pH 7.2) containing 0.1% BSA before incubation for 60 min at RT with biotinylated lectins (5 μg/ml) in HEPES buffer without BSA. Slides were rinsed and incubated in 1:300 streptavidin-horseradish peroxidase for 20 min at RT. Diaminobenzidine augmented with nickel plus a 1:1,000 dilution of 30% H2O2 was applied for 7 min followed by a rinse in distilled, deionized water. Slides were counterstained with hematoxylin and mounted for viewing. Control slides were processed, but the lectin was omitted. Specificity of the reactions for fucose was verified with the use of HEPES buffer.
containing 50 mM fucose during incubation for the AAA and UEA-1 lectins, 100 mM glucose for LCA lectin, and 50 mM mannose for the PSA lectin.

Cell death determination. Cells were grown until confluent in medium A and then incubated for 24 h in 2 ml of medium B, 2 ml of medium B plus 15 ng/ml of EGF, or concurrently with EGF plus the addition of either 5 × 10⁻⁷ M TM or 250 mM/ml of α-fucosidase. Cells were lysed in the culture wells by the addition of 500 µl of hypotonic propidium iodide (PI) solution (50 µg/ml of PI and 0.1% Triton X-100 in 0.1% sodium citrate), followed by flow cytometric analysis of the intact nuclei with a Becton Dickinson FACScan flow cytometer. At least 5,000 nuclei were examined for each sample to determine the proportion of nonviable nuclei or cell death. Cell death is expressed as the percentage of total counted nuclei (cells) that demonstrated <2 N chromosomal content on flow cytometry by PI staining.

Cell adhesion assay. Cells were grown until confluent in medium A and then incubated overnight with medium B alone or with either 5 × 10⁻⁷ M TM or 250 mM/ml of α-fucosidase. The next day, after treatment with 0.02% EDTA for 20 min at 37°C, the cells were collected with a rubber policeman and replated on collagen IV-coated plates. Cells previously incubated in medium B alone were continued in medium B, and cells previously incubated with either inhibitor were continued in either medium B alone or medium B plus the same concentration of inhibitor. Plates were washed gently after 2, 6, or 24 h to remove nonadherent cells, and adherent cells were collected and counted with a Coulter counter.

Cell chemotaxis assay. Our laboratory previously described this assay (21, 22). Briefly, cells were grown until confluent in medium A and then incubated overnight with medium B with and without the inhibitors TM (5 × 10⁻⁷ M) or α-fucosidase (250 mM/ml). Cells were collected in medium B and adjusted to 10⁶ cells/ml. Chemotaxis assays were performed with 48-well Blind Well chambers (NeuroProbe, Cabin John, MD). In order, 26 µl of medium B containing 0–100 ng/ml of EGF were placed in the bottom wells of the chamber, a gelatin-coated 8-µm pore polycarbonate filter (Poretics, Livermore, CA) was placed over the bottom wells, and the upper plate was placed securely on top with 50 µl of the cell suspension placed in each of the top wells. The chamber was then incubated at 37°C in 5% CO₂ for 6 h, an incubation period that elicits maximal migration (21, 22). The filter was then removed, with the adherent cells on the top of the filter removed by gentle scraping in 3% acetic acid. The cells on the bottom of the filter were then fixed in methanol with Mala-chite Green (0.2 mg/l) overnight. The next day, filters were stained with traditional methylene blue stain (0.47 g of methylene blue, 0.44 g of azure A, 4.0 g of dibasic sodium phosphate, and 5.0 g of monobasic potassium phosphate per 1.0 liter of distilled, deionized water). Migration through the filter was measured as the total number of cells counted in 10 high-power fields (HPF) with a Nikon light microscope at ×400 magnification.

Data analysis. Wound closure is expressed either as area (µm²) or as a percentage of area at time 0. In previous videomicroscopy experiments with cell monolayers (22), intraobserver variability was <2%, and interobserver variability was <4% for all measurements. Cell adhesion is expressed as the percentage of cells adhered for a particular condition compared with the total number of cells initially plated. Cell chemotaxis is expressed as the number of cells migrating per 10 HPF. Comparisons between multiple groups were made by analysis of variance; when significant differences were found, further comparisons were made by Fisher’s least significant difference test. Comparisons between two groups were made by paired Student’s t-test. Bonferroni’s correction was made as appropriate for multiple comparisons. Differences were considered significant when P < 0.05.

RESULTS

Effect of glycosylation inhibitors on epithelial cell monolayer wound repair. Mean initial wound area for the 136 HAEo⁻ cell monolayers used in this study was 949,380 ± 37,330 µm². Initial wound area and perimeter for monolayers within each experimental series were equivalent and consistent. Control monolayers closed modestly over 24 h, whereas wounds in monolayers treated with 15 ng/ml of EGF closed substantially in the same time period. In control and EGF-only experiments pooled across experimental series, the remaining wound area after 24 h was 64 ± 5% in control cultures (n = 35) and 24 ± 4% in EGF-treated wounds (P < 0.0001; n = 31).

Treatment of monolayers with TM inhibited wound repair in a concentration-dependent manner where treatment with 10⁻⁶ M TM blocked wound repair completely (Fig. 1). The remaining wound area 24 h after treatment with both 10⁻⁶ M TM and EGF was 76 ± 12% of the original area (n = 8 monolayers) compared with 41 ± 8% for monolayers treated with EGF alone (P = 0.0005; n = 9). Concentrations of TM > 10⁻⁶ M caused lifting of cells and ablated wound margins (Fig. 2).

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Fig. 1. Closure of HAEo⁻ cell monolayer wounds in the presence of tunicamycin (TM). Confluent monolayers were wounded as described in METHODS. Epidermal growth factor (EGF; 15 ng/ml) and TM (10⁻⁶ to 10⁻¹⁰ M; TM-10 to TM-6, respectively) were added to the wounds in culture. Remaining wound area was measured by time-lapse videomicroscopy at selected intervals. Ctl, control. Values are means ± SE. SE for each data set was similar. For clarity, some SE bars are not shown. TM attenuated wound closure in monolayers treated with EGF starting at 12 h. P < 0.0005 for 10⁻⁶ M TM + EGF vs. EGF alone at 24 h.
Because TM prevents glycosylation of nascent peptides, preexisting surface glycoproteins would still have representative N-glycosylations. These preexisting glycosylations could still mediate wound closure. To test this, additional monolayers were pretreated with \(5 \times 10^{-7} \text{ M} \) TM for 12 h before wound creation. Monolayers were then treated with 15 ng/ml of EGF and \(5 \times 10^{-7} \text{ M} \) TM for an additional 24 h to permit wound closure. The remaining wound area in these monolayers was 104 ± 5% of the original area (\(n = 4\)) compared with 41 ± 8% for monolayers treated with EGF without either pre- or concomitant treatment with TM (\(P < 0.0001; n = 9\)) and 86 ± 5% for control monolayers treated with neither EGF nor TM (\(P = \text{not significant (NS)} \) vs. EGF + TM; \(n = 9\)).

In contrast to the inhibition of wound repair elicited by the complete inhibition of nascent peptide N-glycosylation, altered processing of the high-mannose structures did not affect monolayer repair after injury. In six experiments, the remaining wound area 24 h after treatment with both EGF and \(10^{-5} \text{ M} \) DJM, which inhibits mannosidase I, was 23 ± 6% of time 0 vs. 16 ± 8% for EGF alone (\(P = \text{NS}; n = 6\); Fig. 3A). Similarly, altered processing of these high-mannose structures with CSP, which inhibits \(\alpha\)-glucosidase, did not affect monolayer repair. In five experiments, the remaining wound area 24 h after treatment with both EGF and \(10^{-5} \text{ M} \) CSP was 39 ± 10% of time 0 compared with 41 ± 5% for EGF alone (\(P = \text{NS}; n = 5\); Fig. 3B). To determine if CSP and DJM treatments were altering the composition of cell surface complex carbohydrates, treated monolayers were stained with LCA and PSA lectins. These lectins identify terminal glucose and mannose, respectively, and do not bind to control 1HAEo cells, as previously determined (7). If CSP and DJM were inhibiting the processing of the high-mannose structures, the treated monolayers, relative to the control monolayers, would demonstrate the accumulation of terminal glucose and mannose, respectively. As demonstrated by the lectin-binding patterns shown in Fig. 3C, only the treated monolayers were positive (brown) for LCA and PSA staining. Regardless of these changes in the processing of N-glycosyl residues, terminal fucose was still identified on the treated monolayers as demonstrated by the staining with lectin AAA of both treated and control cells (Fig. 3C).

Endoglycosidases facilitate the sequential breakdown of mature N-glycosylations. Treatment with endoglycosidase H did not slow wound closure compared with EGF treatment alone. The remaining wound area 24 h after treatment with both 15 ng/ml of EGF and 300 mU/ml of endoglycosidase H was 12 ± 4% compared with 3 ± 1% for cells treated with EGF alone (\(P = \text{NS}\)). Similarly, after initial treatment with the
enzymes sialidase, β-galactosidase, amyloglucosidase, and N-acetyl-β-D-glucosaminidase, followed by concurrent treatment with EGF and O-glycosidase digestion, there was no impaired wound closure. The remaining wound area at 24 h was 1 ± 0 vs. 0 ± 0% for EGF alone (P = NS).

Certain terminal carbohydrate residues such as fucose may be available as a modification of N-glycosyla-
tions to participate in cell migration and spreading. To test this hypothesis, we treated monolayers with the exoglycosidase α-fucosidase immediately after wound creation and followed wound closure over 24 h. Treatment with α-fucosidase inhibited wound repair completely. The remaining wound area 24 h after treatment of 1HAEo− cells with both EGF and 250 mU/ml of α-fucosidase was 82 ± 8% of that at time 0 compared with 3 ± 1% for monolayers treated with EGF alone (P < 0.001) and 27 ± 6% for monolayers without either (P = 0.005 vs. EGF alone; P < 0.0001 vs. EGF + α-fucosidase; n = 6 monolayers/group; Fig. 4). Additionally, the remaining wound area 24 h after treatment of NHBE cells with both EGF and 250 mU/ml of α-fucosidase was 91 ± 9% of that at time 0 compared with 36 ± 5% for monolayers treated with EGF alone (P < 0.01; n = 6/group; Fig. 4). Lower concentrations of the enzyme did not significantly impair wound closure as measured at 24 h for either cell type (Fig. 4). The addition of 250 mU/ml of heat-inactivated α-fucosidase or an equivalent mass of BSA did not impair wound closure rates (data not shown).

The EGF receptor is glycosylated and contains terminal fucose linkages that regulate affinity for EGF (6). This structure-function relationship may be altered by modifying its glycosylation. To assess if the effect of inhibited wound repair was due to alterations in the glycosylation of the EGF receptor, we tested the effects of TM and α-fucosidase on wound repair rates accelerated by the addition of either HGF or PDGF. Both growth factors elicited concentration- and time-dependent acceleration of wound closure in 1HAEo− cell monolayers similar to that generated by EGF (Fig. 5A). Additionally, wound closure in the presence of either HGF (10 ng/ml) or PDGF (30 ng/ml), concomitant with the presence of either 5 × 10−7 M TM or 250 mU/ml of α-fucosidase, demonstrated an inhibition of closure similar to that seen with either inhibitor during EGF treatment (Fig. 5, B and C, and Table 1).

**Effect of the exoglycosidase α-fucosidase treatment on expression of terminal fucose residues by epithelial cells.** The lectins AAA and UEA-1 bind specific and differing fucose linkages (23, 37) and label basal airway epithelial cells in situ (7). Both lectins had bound to the 1HAEo− cell monolayers before α-fucosidase treatment. Treatment with α-fucosidase for 12 h inhibited subsequent binding of the AAA lectin to 1HAEo− cell monolayers (Fig. 6, right). In contrast, binding of UEA-1 was not affected by exoglycosidase exposure (data not shown).

**Effect of glycosylation inhibitors on epithelial cell viability.** Treatment of 1HAEo− cell monolayers for 24 h with 15 ng/ml of EGF plus either TM or α-fucosidase elicited no difference in cell viability compared with control monolayers as determined by hypotonic PI analysis (Table 2).

**Effect of glycosylation inhibitors on epithelial cell adhesion.** Both TM and α-fucosidase blocked 1HAEo− cell adhesion. Pretreatment with 5 × 10−7 M TM blocked adhesion to collagen IV almost completely (Table 3). The reduction in cell adhesion was similar in control cells and in cells treated with EGF and was not reversed by the removal of TM during the adhesion period (Table 3). Similarly, pretreatment with 250 mU/ml of α-fucosidase blocked adhesion to collagen IV, although the extent of the reduction of adhesion with α-fucosidase treatment was markedly less than that generated by TM (Table 4). As in the experiments with TM, the reduction in cell adhesion was similar in EGF-treated and control cells and was not reversed by removal of α-fucosidase during the adhesion period (Table 4).

**Effect of glycosylation inhibitors on epithelial cell chemotaxis.** EGF elicited migration of 1HAEo− cells over 6 h in chemotaxis chambers. Migration through chemotaxis filters 6 h after treatment with 30 ng/ml of EGF was 18.0 ± 3.9 vs. 5.2 ± 0.9 cells/10 HPF for cells not treated with EGF (n = 5 cultures/group; P = 0.01). Pretreatment with 5 × 10−7 M TM for 12 h blocked subsequent EGF-stimulated migration completely (Fig. 7). Cell migration through chemotaxis filters 6 h after treatment with 30 ng/ml of EGF after pretreatment with 5 × 10−7 M TM was 3.7 ± 0.7 cells/10 HPF (P = 0.03; n = 3 cultures) vs. cells treated with EGF alone. In contrast, pretreatment with α-fucosidase did not abolish the subsequent concentration response to
EGF (Fig. 7). In these experiments, cell migration through chemotaxis filters 6 h posttreatment with 30 ng/ml of EGF after pretreatment with 250 mU/ml of α-fucosidase was 26.1 ± 10.9 cells/10 HPF (P = NS vs. cells treated with EGF alone; n = 4).

**DISCUSSION**

Our laboratory (7) recently characterized cell surface glycosylation in nonsecretory cells of central human airway epithelium and airway epithelial cell lines with the use of lectins that bind specific glycosyl residues. Galactose-specific and galactosamine-specific lectins labeled basal epithelial cells and cell lines derived from basal cells, and fucose-binding lectins specifically labeled subsets of airway epithelial cells. These differences may be relevant in several functions of these cells such as repair after injury. Our data in this study demonstrate that N-glycosylated glycoproteins, particularly those with a terminal fucose residue, participate in the adhesion and migration of airway epithelial cells and facilitate closure of epithelial wounds in monolayer culture.

Treatment of monolayers with TM inhibited wound repair in a concentration-dependent manner such that treatment with 10^-6 M TM blocked wound repair significantly within 12 h (Fig. 1). TM prevents nascent...
peptide N-glycosylation in the Golgi body. As such, pretreatment of the monolayers with TM before wound creation blocked migration and closure completely. This suggests the need for N-glycoproteins to facilitate repair. The inhibition of closure was not the result of cell death as demonstrated by PI staining for cell viability. However, alteration in integrin N-glycosylation elicited by TM treatment decreased the function of these receptors in cell adhesion and migration (3, 25, 33). Integrins are associated with “firm adhesion” of epithelial cells to matrix proteins (8), and TM treatment of airway epithelial cells produces a near total loss of adherence to collagen IV (Table 3). Integrins facilitate a portion of wound closure of damaged airway epithelial cell monolayers (35); the loss of adhesion mediated by changes in N-glycosylation could result in decreased wound closure rates. To the extent that integrins facilitate repair, this remains only a partial explanation of the TM-inhibited closure rates.

Treatment with either endoglycosidases to cleave discrete distal portions of mature polysaccharides or inhibitors to prevent specific modifications to nascent high-mannose N-linked structures did not slow repair. The requirement of N-linked glycoproteins for certain cellular processes has been described (13, 24, 25, 33), but often, the identification of a more specific glycoconjugate has not been possible. Treatment of cell monolayers with CSP and DJM altered the cell surface carbohydrate profiles as determined by lectin-binding patterns (Fig. 3C). These altered patterns were consistent with appropriate enzymatic inhibition, whereas terminal fucosylation remained intact. This suggests that the presence of N-linked glycosidase molecules can serve as a substrate for fucosyltransferases and result in terminal fucosylation. That neither CSP nor DJM inhibited wound closure suggests that the intact terminal fucose linkages facilitated the epithelial wound repair. The removal of fucose residues by the action of α-fucosidase, as identified by the lectin AAA, corresponded to the absolute inhibition of monolayer wound repair (Figs. 4 and 6).

Lectin histochemical surveys of the intact human airway demonstrate the expression of specific fucose linkages on subsets of airway epithelial cells (7). The cell monolayers used in this study express terminal fucose. Although some morphological features of the 1HAEo- cells are not the same as the pseudostratified columnar epithelium seen in vivo (34), the monolayers are similar to those described in other studies of epithelial cell function (26) and have multiple characteristics of primary airway epithelial cells (5, 7, 14). Additionally, confirmation of significant wound inhibition was demonstrated in primary isolated NHBE cells in an attempt to more closely model wound repair by in vivo epithelial cells. To determine if fucose resides on cell surface structural proteins expressed by these cells were involved in cell migration and spreading in vivo,
monolayers were treated with the exoglycosidase α-fucosidase. For both the 1HAEo cell line and NHBE primary cells, the remaining wound area 24 h after treatment with both EGF and 250 μU/ml of α-fucosidase was substantially greater than that in monolayers treated with EGF alone (Fig. 4). Impairment of wound repair after removal of terminal fucose residues did not result from cellular toxicity because viability and cell death were unchanged at all concentrations of α-fucosidase compared with those in control monolayers. Similarly, the use of heat-denatured and inactivated α-fucosidase did not impair wound repair. One past study (18) demonstrated that patients with severe asthma have a deficiency of terminal fucose in their blood group surface antigens. Combined with our data, this suggests that defects in epithelial repair in asthma patients may be due, in part, to such a deficiency.

The EGF receptor can be fucosylated (6). The particular carbohydrate structure determines the affinity of the receptor for the ligand. Loss of the fucose residue reduces receptor affinity for EGF. To demonstrate that the wound repair inhibition is not an EGF receptor-specific phenomenon, we examined the effect of α-fucosidase treatment during wound repair accelerated by two other growth factors, PDGF and HGF, the function of which is not regulated by such structures (6). As for EGF, α-fucosidase treatment inhibited repair stimulated by these growth factors.

Of note is the effect of α-fucosidase on epithelial cell adhesion and migration. Although the effect on wound closure was profound, α-fucosidase treatment elicited only a slight reduction in two processes thought to be significant components of repair. Exposure to α-fucosidase has not been demonstrated to alter integrin function. Intact integrins function to mediate “hard adhesion” of cells to a protein matrix. Repair after injury is a complex event that may require participation of molecules or processes not otherwise utilized during the study, specifically, adhesion or migration. Cell-to-cell

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\text{Fig. 7. Migration of treated 1HAEo cells. Cells were pretreated with either 5 \times 10^{-7} M TM or 250 \mu U/ml of α-fucosidase for 12 h. Cells (50,000/well) were placed in the upper chamber, and EGF (0–100 ng/ml) was placed in the lower chamber. Chambers were incubated for 6 h. Cells migrating through the membrane were counted and are expressed as no. per 10 high-power fields (HPF) and compared with EGF-stimulated chemotaxis of cells not treated with TM or α-fucosidase. TM inhibited EGF-stimulated chemotaxis completely, whereas α-fucosidase did not. *P < 0.09 compared with control cells.}
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### Table 3. Adhesion of 1HAEo cells in the presence of TM

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<th>O/N + experiment</th>
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</tbody>
</table>

Values are means ± SE in percent of cells plated; \( n = 3 \) experiments/time point. 1HAEo cells were pretreated with 5 \times 10^{-7} M TM or sham diluent overnight, collected, and replated in collagen IV-coated wells. TM (5 \times 10^{-7} M) was continued for the duration of the experiment in some wells (O/N + experiment), or wells were treated with TM overnight only (O/N only). Control cells were not treated with TM at any point (none). Adhesion of these pretreated cells to collagen IV was determined in the presence and absence of 15 ng/ml of α-fucosidase only. Control cells were not treated with α-fucosidase overnight only. Significantly different compared with control experiments at the same time point: *\( P < 0.005; † P < 0.01; ‡ P < 0.05; § P < 0.0005; ¶ P < 0.0001; \$ P < 0.00001.\]

### Table 4. Adhesion of 1HAEo cells in the presence of α-fucosidase

<table>
<thead>
<tr>
<th>Time, h</th>
<th>None</th>
<th>O/N only</th>
<th>O/N + experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Without EGF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>69.4 ± 4.2</td>
<td>60.1 ± 5.0</td>
<td>65.0 ± 9.4</td>
</tr>
<tr>
<td>6</td>
<td>108.2 ± 4.0</td>
<td>82.3 ± 2.0‡</td>
<td>77.7 ± 7.6†</td>
</tr>
<tr>
<td>24</td>
<td>97.9 ± 9.6</td>
<td>58.4 ± 6.6§</td>
<td>61.8 ± 1.2‡</td>
</tr>
<tr>
<td><strong>With 15 ng/ml of EGF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>71.9 ± 6.0</td>
<td>57.0 ± 6.0</td>
<td>64.3 ± 4.9</td>
</tr>
<tr>
<td>6</td>
<td>95.3 ± 7.0</td>
<td>79.0 ± 7.1</td>
<td>67.4 ± 4.7*</td>
</tr>
<tr>
<td>24</td>
<td>96.8 ± 3.7</td>
<td>74.2 ± 10.1§</td>
<td>62.9 ± 0.5†</td>
</tr>
</tbody>
</table>

Values are means ± SE in percent of cells plated; \( n = 3 \) experiments/time point. 1HAEo cells were pretreated with 250 μU/ml of α-fucosidase or sham diluent overnight, collected, and replated in collagen IV-coated wells. α-Fucosidase was continued for the duration of the experiment in some wells, or wells were treated with α-fucosidase overnight only. Control cells were not treated with α-fucosidase at any point. Adhesion of these pretreated cells to collagen IV was determined in the presence and absence of 15 ng/ml of EGF for 2, 6, or 24 h. Significantly different compared with control treatments at the same time point: *\( P < 0.05; † P < 0.01; ‡ P < 0.05.\]

\[
\text{Table 3. Adhesion of 1HAEo cells in the presence of TM}
\]

\[
\text{Table 4. Adhesion of 1HAEo cells in the presence of α-fucosidase}
\]
or “soft adhesion” and migration of one cell with respect to another is a possible explanation for the inability of α-fucosidase to inhibit either migration or adhesion as separate processes while inhibiting epithelial wound repair. A human family of lectins, selectins, bind to a fucose-containing ligand and have been demonstrated to be responsible for cell-cell and not cell-matrix mobility (29). The involvement of selectins is possible because the loss of fucose and the selectin ligand are coordinate with wound repair inhibition. Treatment with α-fucosidase during repair removes AAA lectin binding but not that for UEA-1. UEA-1 binds α-1,4-fucose linkages (23), whereas the lectin AAA binds α-1,3- and α-1,2-fucose linkages that include the siaryl-Leβ structures (37), a classic ligand for selectins. Evaluation of selectin function during repair of airway epithelium requires further study.

One potential limitation of our study was the use of a simple matrix, collagen IV, on which our cells were grown. Duplication of the basement membrane with a variety of matrix proteins such as fibronectin, laminins, collagens, proteoglycans, entactin, and nidogen (16) may permit multiple interactions of different glycosylated proteins with the elements of a complex matrix compared with this single matrix protein. Our data demonstrate a role for specific N-glycosylations in the complex functions of epithelial cell migration and adhesion during wound repair over collagen.

In summary, we demonstrated that N-glycosylated proteins participate in the airway epithelial cell processes of adhesion and migration. N-glycosylated proteins and proteins with terminal fucose residues mediate wound closure of airway epithelial cells and repair of epithelial cell monolayers. These data suggest that molecules not previously characterized with reference to cell surface glycosylation have a role in airway epithelial cell migration and repair.

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19. Koyama K and Hughes RC. Identification of fucosylated pro-


