Modification of Cell Surface Glycoprotein: Addition of Fucosyl Residues during Epidermal Differentiation

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ABSTRACT   When cutaneous sections from the newborn rat were treated with α-fucosidase, *Ulex europeus* agglutinin I (UEA) binding to the cell surface of the differentiated cells in the epidermis was diminished and there was an appearance in these cell layers of binding by *Bandeiraea simplicifolia* I-B$_4$ lectin (BS I-B$_4$), which normally is specific for the basal cells. A similar treatment with α-galactosidase resulted in a loss of BS I-B$_4$ binding, but had no effect on UEA binding. Glycoproteins isolated from the membranes of epidermal cells showed a threefold increase in the ratio of binding to UEA versus BS I-B$_4$ affinity columns as the proteins were derived from the more differentiated cell populations. These data suggest that α-fucosyl residues are added to the glycoproteins on the cell surfaces of differentiated cells, thus blocking α-galactosyl residues and changing the lectin binding specificity as epidermal cells move out of the basal cell layer.

Alterations of cell surface glycoconjugates have been observed in many differentiating and developing systems by specific binding with various lectins. These systems include the slime mold (6, 14, 40), the intestine (10) and colon (9, 12,). prenatal cerebral tissue (30), developing pancreas (21), leukemic cells (32–34), and the epidermis (4, 16, 19, 26, 28, 38). These modifications in cell surface glycoconjugates may be physiologically significant, because the carbohydrate moiety has been implicated in cell differentiation (17, 36), proteolytic stability (8, 29), secretion (18), membrane insertion (20), and enzymatic activity (11).

The epidermis of the newborn rat is composed of four relatively distinct layers of keratinocytes. The lowermost (basal cell) layer consists of a single layer of cells resting on a basement membrane. DNA replication and cell division occur only in the basal layer. Cells leave the basal layer and migrate into the spinous layer, then into the granular layer, and finally are exposed to the environment when they reach the outer cornified layer or stratum corneum. When sections of the newborn rat are exposed to the isolectin BS I-B$_4$ (α-D-galactopyranoside-binding specificity), a preferential binding to surfaces of the epidermal basal cells is seen (4). This isolectin binds progressively less to the differentiated cells that have migrated from the basal layer towards the stratum corneum. A second lectin, UEA (α-L-fucose-binding specificity) binds strongly to the surfaces of the spinous and lower granular cells

showing little specific binding of the basal or upper granular and cornified layers (4). Using lectins as markers, other studies have indicated the presence of different cell surface glycoconjugates in the various epidermal layers of human and mouse epidermis (16, 19, 27, 28, 29).

This differential lectin-binding specificity may reflect changes in glycoconjugates or alterations in their distribution within the plasma membranes as the keratinocytes differentiate. The intention of this investigation was to define the molecular alteration in the cell surafce that accounts for the change from BS I-B$_4$ binding to UEA binding as the cell leaves the basal layer and migrates toward the cutaneous surface. Several possible explanations were considered. First, α-D-galactopyranoside (D-Gal) residues could be responsible for masking UEA sites in the basal cells and could be removed as the basal cells differentiate, allowing the expression of the UEA sites. Second, α-L-fucose (6-deoxy-L-galactopyranoside) (L-Fuc) residues could be added in the differentiated cells masking the α-D-Gal sites. This mechanism by which one sugar masks another has been reported in lymphocytes (31, 32) and in mouse embryo (15). Third, the two events might not be directly related. Instead, the change in lectin-binding properties could be the result of the appearance of glycoconjugates containing α-L-Fuc residues and the simultaneous disappearance of α-D-Gal-terminating glycoconjugates as differentiation occurs.

The data from this investigation suggest that α-D-Gal resi-.

dues are masked by α-L-Fuc in the plasma membranes of the differentiated cells in the cutaneous epidermis of the newborn rat.

MATERIALS AND METHODS

Materials

BS I-B$_4$ was prepared as described by Murphy and Goldstein (25). One hundred grams of *Bandeiraea simplicifolia* (BS) seeds (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) were used. Purity was determined by comparing the product with purified isolectin (obtained from Dr. I. J. Goldstein, Department of Biological Chemistry, The University of Michigan Medical School) on slab gels using the method of Brewer and Ashworth (7), at pH 9.5. BS I-B$_4$-fluorescein isothiocyanate (FITC) was a gift from Dr. Goldstein. UEA and UEA-FITC were purchased from Vector Laboratories, Inc. (Burlingame, CA).

Photography

Slides were examined for fluorescence using a Zeiss photomicroscope II equipped with a 490-nm excitation filter and 530-nm barrier filter. Micrographs were taken by using the camera photometer, which determined the exposure time based upon the amount of fluorescence emitted by the sample. Kodak Tri-X film was exposed, boosted to 1,600 or 6,300 ASA, and developed in Diafine for 4 min each in solutions A and B at 20°C.

Localization of FITC-Labeled Lectins

Sections of skin were prepared as described by Brabec et al. (4). Cross sections were cut from these samples at 4–6 μm thickness with a cryostat and placed on uncoated cover slips. Cryostat sections were allowed to dry in air for 15–20 min. FITC-lectins were applied at various concentrations in a volume of ~20 μl. The sections were incubated in a moist, darkened chamber for 20 min at 22°C. Unbound lectin was then removed with three washes of phosphate buffered saline (10 mM sodium phosphate, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM CaCl$_2$, pH 7.3) (PBS) for 5 min each. The coverslips were then mounted on slides with a polyvinyl alcohol medium (4).

Isolated cells were labeled by suspending ~10^5 cells in 0.1 ml of PBS containing either BS I-B$_4$-FITC or UEA-FITC at 50 μg/ml. The cells were incubated for 30 min at 22°C. Unbound lectin was removed by adding 3 ml of PBS and centrifuging cells at 1000 g for 4 min. The supernatant solution was discarded and the wash was repeated three times. The cells were resuspended in PBS, placed on a coverslip, and mounted while still wet.

Lectin Affinity Chromatography

Affinity columns were prepared by coupling the UEA and BS I-B$_4$ lectins to CNBr-activated Sepharose 4B (3). For both columns, 10 mg of lectin were coupled to 1 g of CNBr-activated Sepharose 4B. ~90% of the lectin was bound in both cases. Columns were prepared identically except that 0.1 M L-Fuc was present in the UEA reaction mixture whereas 0.1 mM methyl-α-D-galactopyranoside (Me-α-Gal) was present in the BS I-B$_4$ mixture.

BS I-B$_4$-Sepharose 4B

A column of BS I-B$_4$-Sepharose 4 B (0.5 × 6 cm) was washed extensively with 0.5% (vol/vol) Nonident P-40 (NP-40)-PBS before use. The sample was applied in the same buffer with a flow rate of 2–4 ml/h. The column was rinsed with at least 5 column volumes of buffer to remove material binding nonspecifically. Specifically bound material was eluted with 5 mg/ml (21 mM) of Me-α-Gal. L-Fuc (10 mg/ml) had no effect on elution. Rechromatography of the unadsorbed material indicated that >99% of glycoconjugates that could be specifically bound by BS I-B$_4$ were removed by the first application. The recovery of sample material was 90–100%. The BS I-B$_4$ column was less effective below pH 7.0, therefore all samples were adjusted to pH 7.0 or higher before application.

UEA-Sepharose 4B

A column of UEA-Sepharose 4B (0.5 × 6 cm) was washed extensively with 0.5% NP-40-PBS before use. The sample was applied and the column rinsed in the same manner as above. Material specifically bound to the UEA column was eluted with 10 mg/ml (60 mM) of L-Fuc. Me-α-Gal had no effect on elution. The UEA-affinity column also bound >99% of the material that could be bound by UEA upon the first chromatographic pass. ~90% of the sample was recovered routinely. The remaining 10% could be recovered with extensive washing with higher concentrations of L-Fuc.

Treatment of Skin Sections with Glycosidases

A solution of α-galactosidase from green coffee beans (Sigma Chemical Co., St. Louis, MO) was used to remove α-galactosyl residues. Cryostat sections of newborn rat skin were treated with 50 μl of α-galactosidase (1 U/ml) in McIlvaine's phosphate-citrate buffer, pH 6.0 (22) for 30 min at 22°C. The sections were washed with PBS three times, 5 min per wash. The sections were then exposed to FITC-labeled lectins as described above. The preparation of α-galactosidase activity had no β-galactosidase activity.

α-Fucosidase from beef kidney (Boehringer Mannheim Biochemicals, Indianapolis, IN) was used to remove α-fucosyl residues. Cryostat sections of newborn rat skin were treated with 10 μl of α-fucosidase (1.4 U/ml) in 0.1 M sodium acetate, pH 5.0, for 1 h at 37°C. As a control, some sections were incubated with α-fucosidase containing 60 mM L-Fuc to inhibit the enzyme. The sections were washed with PBS three times for 5 min per wash. They were then labeled with lectins conjugated to FITC. This preparation of α-fucosidase had insignificant glycosidase activity on galactose, glucose, mannose, glucosamine, or galactosamine as the nitrophenyl glycoside.

Cell Separation, Cell Surface Labeling, and Solubilization

Epidermal cells were isolated using the method described by Vaughan and Bernstein (39) with slight modifications. The cell surfaces were radiolabeled with galactose oxidase and NaB^{3}H$_4$ (13) and then solubilized with NP-40. Briefly, dorsal skin was removed from newborn rats (1–2 d old) and the fascia was scraped off with a scalpel. The skin was stretched one and one-half to two times its original size by this scraping procedure. Next the skin was placed in a petri dish with the stratum corneum side down and allowed to cool at 4°C for 30 min. 15 ml of 1% crude trypsin (Difco Laboratories, Detroit, MI; 1:250) in Earle's Balanced Salt Solution (EBSS) were added to the dish and the contents were incubated for 45–60 min at 4°C. The trypsin was removed and the skins were washed twice with EBSS. The epidermis was then separated from the dermis and the epidermis was cut into small pieces (<0.5 cm square). The pieces were stirred gently in Minimum Essential Medium (MEM) containing 2.5% (wt/vol) bovine serum albumin (BSA) and 1 mM PMSF (1 ml/skin). Buffer and liberated cells were decanted after 15 min, new buffer was added, and the procedure was repeated for three successive periods of 15 min. The cells were sedimented at 1,500 g. This method gives populations of cells that contain a higher percentage of morphologically more differentiated cells at each later interval.

~10^6 isolated cells were resuspended in 0.45 ml of PBS. To these cells 0.05 ml of 10 mM NaBH$_4$ in 10 mM NaOH was added. The cells were incubated 30 min at 22°C and then washed three times with PBS. They were resuspended in 0.5 ml of PBS containing 10 U of galactose oxidase, incubated at 37°C for 30 min with shaking, and washed three times with PBS. The cell surfaces were then radiolabeled by suspending cells in 0.05 ml of PBS and 0.05 M of NaB^{3}H$_4$ (0.5 mCi) in 10 mM NaOH. The mixture was incubated for 30 min at 22°C and washed three times with PBS. Controls in which the galactose oxidase step was omitted resulted in 5% as much radioactivity being incorporated into BS I-B$_4$- or UEA-binding material as when the enzyme was used.

Cell surface glycoproteins were solubilized with a nonionic detergent, NP-40. Radiolabeled cells were suspended in 1 ml of PBS containing 0.5% NP-40 and incubated for 1 h. The mixture was centrifuged at 12,800 g for 10 min and the pellet was discarded. Before further use, the supernatant solution was dialyzed against 0.5% NP-40-PBS.

RESULTS

Effects of Glycosidases on Lectin-binding Properties of Epidermal Cell Surfaces in Tissue Sections of Skin

Sections of skin were treated with α-galactosidase and α-fucosidase, respectively, to test whether the change in lectin-binding properties could be explained by the subtraction of α-D-Gal or the addition of α-L-Fuc residues. If the first model presented in the introduction is correct, exposure of sections to α-galactosidase from green coffee beans should remove BS I-B$_4$-binding sites and allow the expression of UEA sites in the basal cells. The results shown in Fig. 1 are not in agreement with this mechanism. Treatment with α-galactosidase removed the BS I-B$_4$-binding sites (Fig. 1 *a* and *b*) but did not result in UEA binding in the basal cell layer (Fig. 1 *c* and *d*). If the
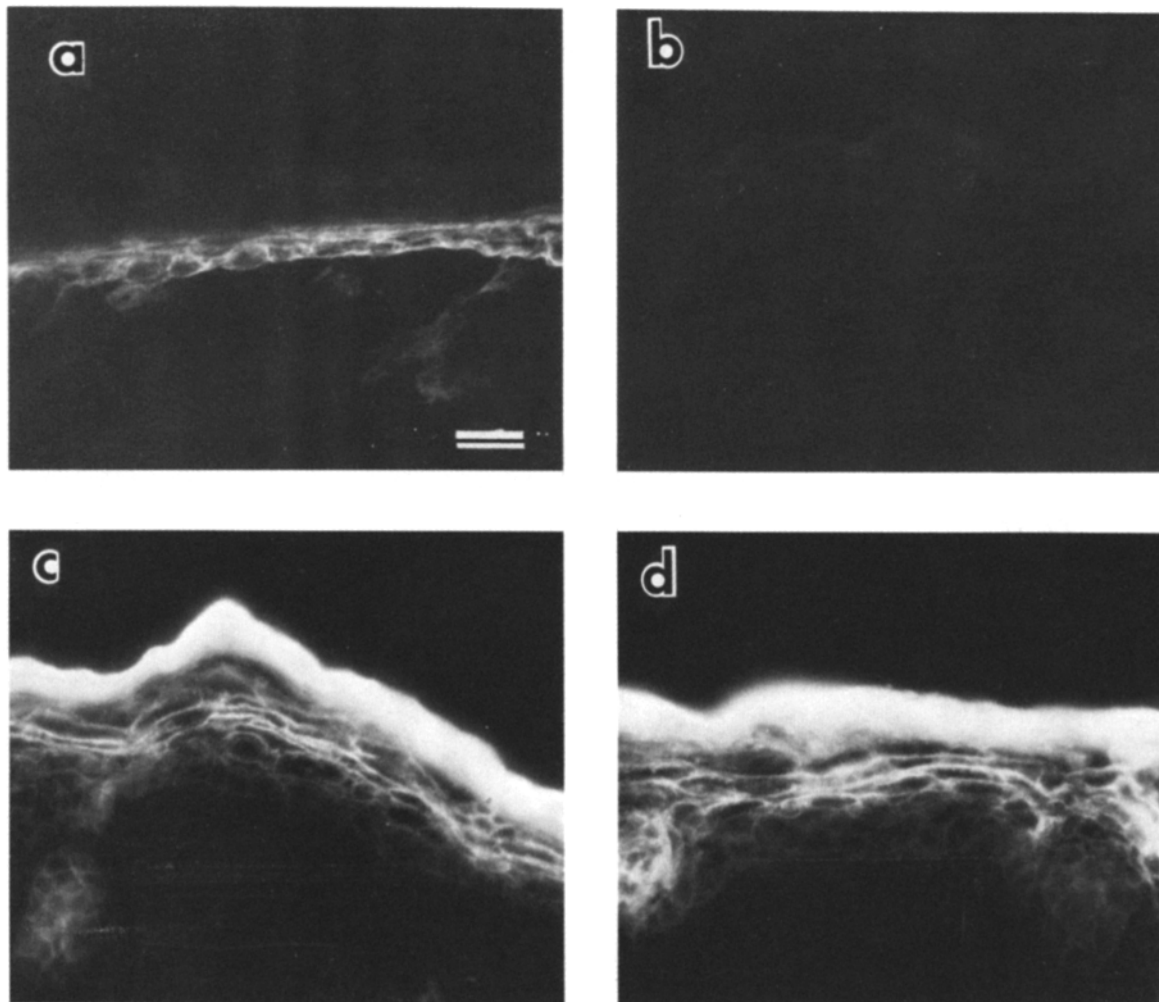
FIGURE 1   Photomicrographs of skin sections after exposure to α-galactosidase. (a) BS I-B₄-fluorescein isothiocyanate (FITC) (150 μg/ml) applied to section after incubation in 50 μl McIlvaine's (22) phosphate-citrate buffer, pH 6.0, for 30 min at 22°C. Bar, 25 μm. × 360. (b) BS I-B₄-FITC (150 μg/ml) applied to section after incubation with 50 μl of α-galactosidase (1 U/ml) in phosphate-citrate buffer, pH 6.0, for 30 min at 22°C. × 360. (c) UEA-FITC (100 μg/ml) applied to section after incubation in 50 μl of buffer, pH 6.0, for 30 min at 22°C. × 360. (d) UEA-FITC (100 μg/ml) applied to section after incubation with α-galactosidase as described in b. × 360.

second model were the mechanism involved, α-fucosidase from beef kidney should remove the α-L-Fuc residues and expose α-D-Gal residues resulting in the loss of UEA binding and the appearance or increase in BS I-B₄ binding to the spinous and granular cells. The α-fucosidase treatment greatly reduced UEA binding (Fig. 2a and b) and resulted in BS I-B₄-binding through all cell layers except the stratum corneum (Fig. 2c–e). The specificity of this reaction was indicated by the blockage of this appearance of new BS I-B₄-binding sites when 60 mM L-fucose was added to the fucosidase solution (Fig. 2f). This experiment strongly suggests that α-L-Fuc residues mask potential BS I-B₄-binding sites in the differentiated cells.

Lectin-binding Properties of Isolated Epidermal Cells

Cell surface glycoproteins were obtained from populations of epidermal keratinocytes having a high proportion of basal and differentiated cells, respectively, in order to show that the amount of UEA binding glycoproteins relative to BS I-B₄ binding-glycoproteins increases with differentiation. The differential cell separation technique of Vaughan and Bernstein

(39) allows the isolation of epidermal cell populations containing varying proportions of basal and differentiated cells depending on the length of time the tissue is stirred in MEM after exposure to trypsin (cf. Materials and Methods). The first 15 min gives ~80% basal cells, whereas the proportion of differentiated cells in the isolated cell population increases to 80% at 60 min. The results in Fig. 3 indicated that the separation procedure (including trypsinization) did not qualitatively alter the lectin binding properties of either cell type with respect to the lectins of interest. BS I-B₄ still bound the basal cells preferentially (Fig. 3a and b), and UEA bound the differentiated cells preferentially (Fig. 3c–e).

Isolation and Characterization of UEA and BS I-B₄ Binding Glycoproteins

Using these cell populations it was possible to prepare and compare cell surface glycoconjugates from basal and differentiated cells. Groups of cells obtained by 15, 30, 45, and 60 min of stirring were exposed to radiolabeling with galactose oxidase/NaB³H₄ and the glycoconjugates of the plasma membranes were solubilized with the nonionic detergent, NP-40.
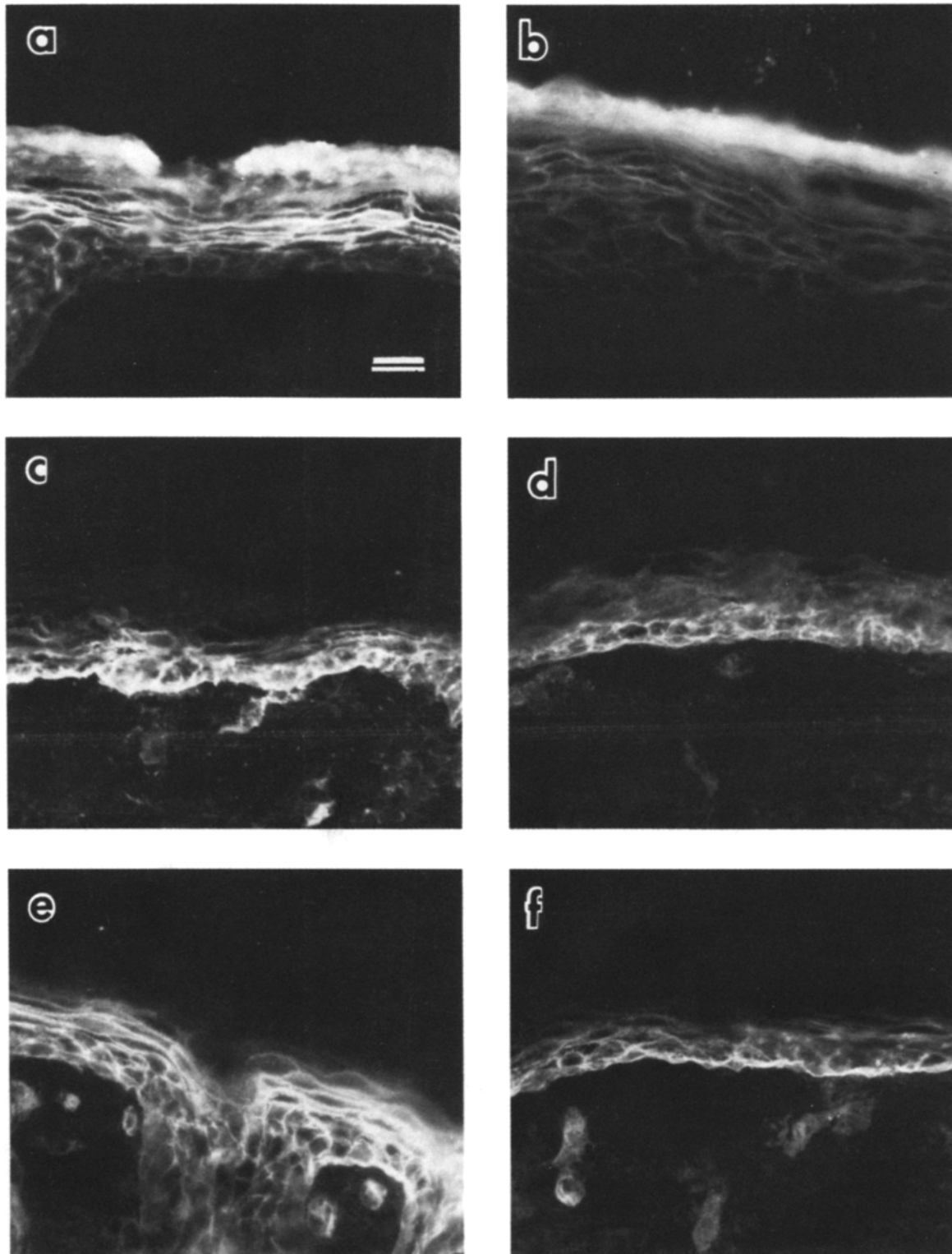
FIGURE 2 Photomicrographs of skin sections after exposure to α-fucosidase. (a) UEA-FITC (100 μg/ml) applied to section after incubation with 0.1 M sodium acetate, pH 5.0, for 1 h at 37°C. Bar, 25 μm. × 350. (b) UEA-FITC (100 μg/ml) applied to section after pretreatment with 10 μl of α-fucosidase (1.4 U/ml) in 0.1 M sodium acetate, pH 5.0, for 1 h at 37°C. × 360. (c) BS I-B₄-FITC (45 μg/ml) applied to section as described by Brabec et al. (4). × 360. (d) BS I-B₄-FITC (45 μg/ml) applied to section after pretreatment with 0.1 M sodium acetate, pH 5.0, for 1 h at 37°C. × 360. (e) BS I-B₄ (45 μg/ml) applied to section after pretreatment with α-fucosidase as in B. × 360. (f) BS I-B₄ (45 μg/ml) applied to section after pretreatment with α-fucosidase solution containing 60 mM α-L-fucose. × 360.

These isolated materials were judged to be glycoproteins because they became dialyzable after extensive treatment with crystalline trypsin. These solubilized glycoproteins were isolated upon affinity columns made from BS I-B₄ or UEA (cf. Materials and Methods). There was a steady increase in the ratio of UEA binding material to BS I-B₄ binding material as
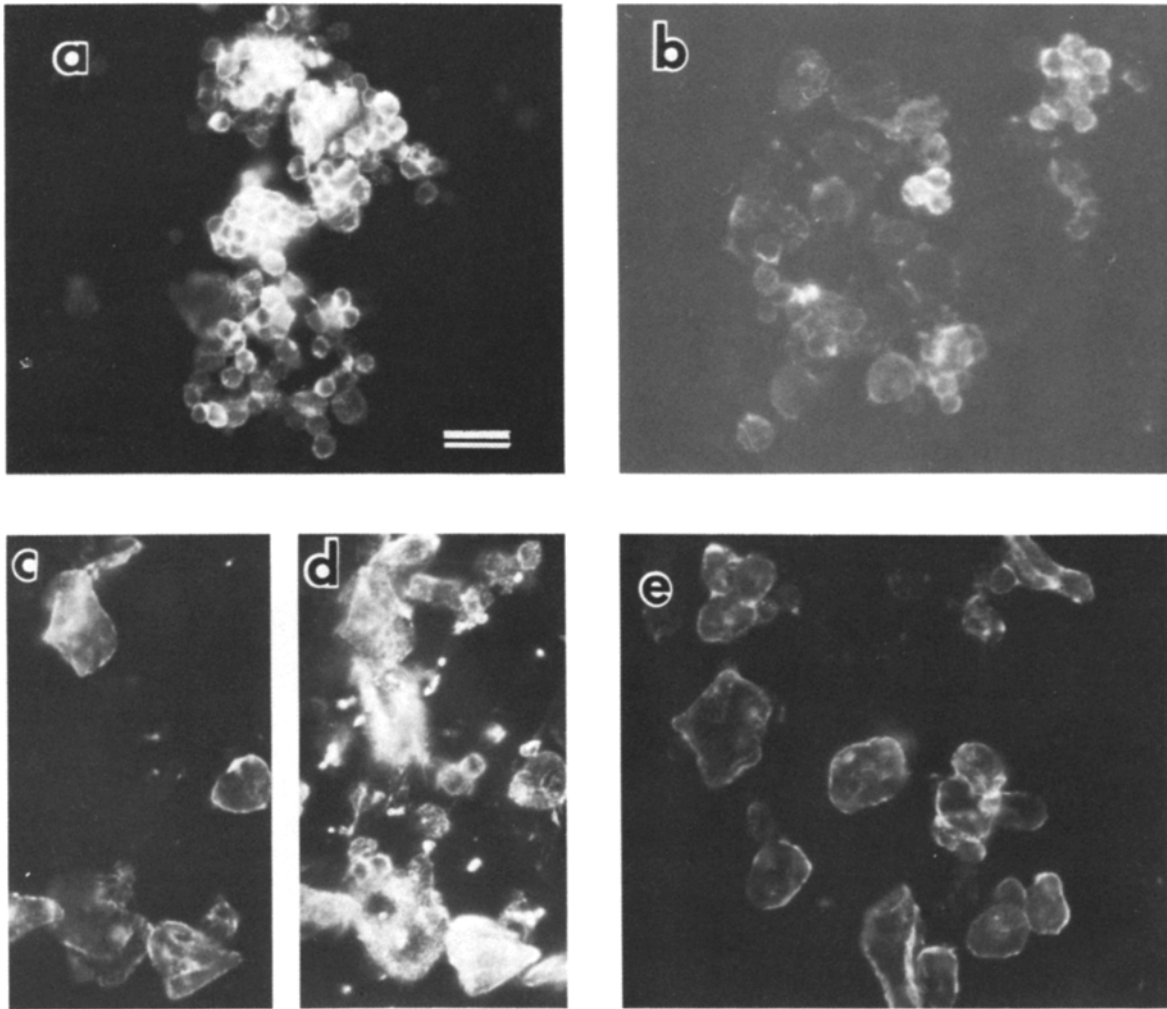
FIGURE 3   Photomicrographs of lectin binding to isolated epidermal cells after separation with 1% crude trypsin. (a) BS I-B₄-FITC (50 µg/ml) applied to cells in 15 min preparation. Bar, 25 µm. × 350. (b) BS I-B₄-FITC (50 µg/ml) applied to cells in 45 min preparation. Note minimal response of differentiated (large) cells. × 360. (c) UEA-FITC (50 µg/ml) applied to epidermal cells in early time preparation. × 360. (d) Same field as in c with no fluorescence filters. Note basal (small) cells seen in d that are not visible in C. × 360. (e) UEA-FITC (50 µg/ml) applied to cells in 60 min preparation. × 360.

TABLE I

Ratio of Specifically-bound ³H from Progressively More Differentiated Cell Populations (BS I-B₄ Column First)

Total time of stir-ring	Cell population % basal/% differen-tiated	Ratio of specifically-bound ³H (UEA/BS I-B₄)
min		
15	80/20	1.19 ± 0.12
30	50/50	1.51 ± 0.17
45	30/70	2.22 ± 0.25
60	20/80	3.22 ± 0.38

In these experiments solubilized material from cell suspensions obtained after galactose oxidase/NaB³H₄ labeling were chromatographed on the BS I-B₄-then UEA-affinity columns. Ratios were determined by dividing the UEA specifically-bound ³H by the BS I-B₄ specifically-bound ³H for each time point. Averages represent six experiments.

the material came from preparations with a progressively higher proportion of differentiated cells (Table I). The results are in agreement with the findings in intact skin in which BS I-B₄ binding decreases and UEA binding increases with epidermal differentiation.

DISCUSSION

This investigation was designed to identify the molecular alterations in the cell surface glycoconjugates that result in the change in lectin-binding from BS I-B₄ to UEA as the epidermal basal cell moves into the spinous cell layer. The data support the hypothesis that the modification from BS I-B₄ to UEA binding is the result of the addition of fucosyl residues that mask the BS I-B₄-binding sites. This model requires the presence of fucosyl transferase activity in the epidermal cells. The presence of such a transferase was indicated by preliminary findings that BS I-B₄-binding glycoproteins incubated with an epidermal homogenate resulted in the conversion to a UEA-binding form that contained [^{14}C]fucose if GDP-[^{14}C]fucose were added to the reaction mixture.

This investigation leads to several intriguing questions concerning the addition of the fucosyl residues. First, where in the oligosaccharide chain is the fucosyl residue being added? The easiest explanation would be that the residue is added directly to the α-D-Gal residue. However, a linkage of this type has never been reported. Second, what is the nature of the control that prevents the addition of α-L-Fuc residues in the basal

cells? Sequential appearance of glycosyl transferases has been reported in several developing systems including human leukemic cells (1), chick pectoral muscle (37), and rat brain (5). The presence of active transferase only in spinous cells could explain the epidermal observations. Third, is the sugar added to existing glycoproteins on the cell surface or is fucose added to new glycoproteins being synthesized inside the cell when the basal cell moves away from the basal lamina?

Classically, epidermal differentiation has been viewed as a terminal process involving only degradative events. The demonstration that a histidine-rich protein was synthesized in the granular cell made that view untenable (35). This report further supports the concept (2) that differentiation of keratinocytes is a highly programmed process that can involve major synthetic events. In this case a change in cell surface properties is accomplished by the addition of α-L-Fuc residues. Because many developing and differentiating systems also reveal changes in cell surface carbohydrates, this process may be applicable to many other systems but, of course, the demonstration of one synthetic system does not rule out the involvement of degradative systems such as glycosidases (23, 24, 27) among differentiative events.

Increased amounts of fucose and fucosyl transferases with increasing differentiation have been reported in several tissues (1, 9, 10, 12, 24, 30). In the mouse embryo (24) the change in antigenic sites accompanying development is apparently the result of the addition of a fucosyl residue to an antigenic site seen in the less differentiated embryo. It is possible that in developing and differentiating systems generally, changes in cell surface properties reflect alterations in the carbohydrate moieties of specific glycoproteins and addition of carbohydrate residues may be a common mechanism during these processes.

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