Differentiation-associated decrease in the proportion of fucosylated polylactosaminoglycans of CaCo-2 human colonic adenocarcinoma cells

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CaCo-2 cells are human colonic adenocarcinoma cells which can differentiate spontaneously into enterocytes when maintained confluent for extended periods of time. Cells kept in culture for 4 days (rapidly growing), 7–9 days (early confluence) and 19–22 days (late confluence) were incubated for 24 h with L-[5,6-³H]fucose or D-[6-³H]glucosamine in order to examine the changes in glycoprotein carbohydrate structure that occur during this differentiation. Labelled glycopeptides obtained by exhaustive Pronase digestion of the cell-surface and cell-pellet fractions were fractionated on Bio-Gel P-6. A high-*M_r* glycopeptide fraction which was excluded from Bio-Gel P-6 was present in all cases. These glycopeptides were then fractionated by affinity chromatography on *Datura stramonium* agglutinin–agarose. The glycopeptides which were specifically bound to the lectin column were largely degraded by endo- β -galactosidase, thereby indicating that they consisted of fucosylated polylactosaminoglycans. The proportion of labelled polylactosaminoglycans decreased with increasing time in culture, whereas sucrase activity, which is characteristic of differentiated enterocytes, increased. These results demonstrate that a relatively large decrease in the proportion of fucosylated polylactosaminoglycans occurs with differentiation of CaCo-2 cells.

INTRODUCTION

Differentiation of intestinal epithelial cells is characterized by morphological and functional changes in which the cells become polarized and develop a characteristic brush border, containing specific enzymes such as sucrase–isomaltase and aminopeptidase [1]. Although there are studies *in vivo* of the changes in carbohydrate of glycoproteins which take place during intestinal differentiation in other species [2], the structural changes that occur in membrane glycoproteins of human intestinal cells during this process have not been characterized. Human colonic tumour cell lines which can differentiate *in vitro*, and which can serve as useful models to study enterocytic differentiation, have now become available [3,4]. One of these lines, known as CaCo-2, was shown to undergo spontaneous differentiation to form polarized cells expressing brush-border-specific enzymes, when maintained confluent for extended periods [3]. Differentiated CaCo-2 cells have been used to study the biosynthesis and processing of intestinal brush-border enzymes [5]. It has been shown that the expression of endogenous retroviral LTR sequences depends on the state of differentiation of CaCo-2 cells, with the highest activity found in undifferentiated cells [6]. Monoclonal antibodies produced against differentiated CaCo-2 cell membrane components have been used to identify proteins characteristic of crypt and villus cells [7]. In the present work, we report the structural changes in fucosylated glycoproteins which occur during differentiation of CaCo-2 cells. We showed previously that high-*M_r* fucosylated glycopeptides were obtained after exhaus-

tive pronase digestion of undifferentiated CaCo-2 cells [8]. Similar glycopeptides were also obtained from two other human colonic adenocarcinoma cell lines, HCT-8R and HCT-15, but not in a cell line (CCL 239) derived from normal adult colonic mucosa [8]. In the present study, we demonstrate that the high-*M_r* glycopeptide fraction of CaCo-2 cells contains fucosylated polylactosaminoglycans and that the proportion of these carbohydrate groups is greatly decreased when CaCo-2 cells differentiate.

MATERIALS AND METHODS

Materials

The following materials were obtained from the companies indicated: α -minimal essential medium, fetal-calf serum, non-essential amino acids, trypsin and EDTA, Grand Island Biological Laboratories, Burlington, Ontario, Canada; penicillin and streptomycin, Flow Laboratories, McLean, VA, U.S.A.; L-[5,6-³H]fucose (sp. radioactivity 55 Ci/mmol) and D-[6-³H]glucosamine (sp. radioactivity 40 Ci/mmol), ICN Radiochemicals, Irvine, CA, U.S.A.; Pronase, Calbiochem, La Jolla, CA, U.S.A.; chitin, Pfanstiehl Laboratories, Waukegan, IL, U.S.A.; Bio-Gel P-6 (200–400 mesh), Bio-Rad Laboratories, Richmond, CA, U.S.A.; *Datura stramonium* agglutinin (DSA)–agarose, E-Y Laboratories, San Mateo, CA, U.S.A.; phenylmethanesulphonyl fluoride, aprotonin and trypsin inhibitor, Sigma Chemical Co., St. Louis, MO, U.S.A.; Falcon 75 cm² tissue-culture flasks, Becton–Dickinson, Oxnard, CA, U.S.A.

Abbreviations used: DSA, *Datura stramonium* agglutinin; LTR, long terminal repeat.

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Cell culture

CaCo-2 cells [9], obtained from Dr. J. Fogh, Memorial Sloan-Kettering Cancer Centre, Rye, NY, U.S.A., were used between passages 24 and 30. The cells were grown in α -minimal essential medium containing 15% (v/v) fetal-calf serum, streptomycin (50 μ g/ml), penicillin (50 units/ml) and 0.1 mM non-essential amino acids. The cells were grown in 75 cm² flasks and maintained in an atmosphere of 5% CO₂ in air at 37 °C. Cells were subcultured when they were 75–80% confluent by using 0.5% trypsin/0.02% EDTA, and seeded at a density of 7.5×10^5 /75 cm² flask.

Labelling of cells and isolation of glycopeptides

Cells were maintained in culture for 4 days (rapidly growing), 7–9 days (early confluence) or 19–22 days (late confluence). At each time interval, two flasks were incubated for 24 h with L-[5,6-³H]fucose (5 μ Ci/ml) or D-[6-³H]glucosamine (5 μ Ci/ml) in 20 ml of α -minimal essential medium supplemented as described above. After incubation, the medium was removed and the cells were washed three times with phosphate-buffered saline (8 mM-Na₂HPO₄/1.5 mM-KH₂PO₄/13 mM-KCl/137 mM-NaCl, pH 7.4), and incubated at room temperature for 10 min with 4 ml of the saline, containing 0.1% trypsin/0.05% EDTA. The trypsin-released material, which was enriched in cell-surface components, was collected and centrifuged at 700 g for 5 min to remove any detached cells. The cells remaining in the flasks were scraped and suspended in phosphate-buffered saline. The suspension was centrifuged as described above, and the cell pellet was extracted with 3 $\times$ 3 ml of chloroform/methanol/water (10:10:3, by vol.) to remove any labelled lipid-linked oligosaccharides. The delipidated material was the source of cell-pellet glycopeptides. The cell-surface and cell-pellet fractions were exhaustively digested with Pronase (4 mg/ml; 1 ml for the cell surface, and 2 ml for the cell pellet) at 60 °C for 24 h [10]. The Pronase was preincubated at 37 °C for 90 min before use, to eliminate any contaminating glycosidase activity.

Fractionation of the labelled glycopeptides

The cell-surface and cell-pellet glycopeptides obtained after Pronase digestion were fractionated by gel-filtration chromatography on a column of Bio-Gel P-6 (1 cm $\times$ 110 cm) with bovine serum albumin (to determine the excluded volume). The glycopeptides were eluted from the column with 0.1 M-acetic acid, pH 5.0, containing 0.02% NaN₃; 1 ml fractions were collected and samples were counted for radioactivity in a LKB model 1212 scintillation counter. Recovery of radioactivity was always greater than 90%.

DSA-agarose affinity chromatography

Labelled glycopeptide fractions obtained after Bio-Gel P-6 chromatography were pooled, dried and further fractionated on a 1 ml column of DSA-agarose as described by Cummings & Kornfeld [11]. The samples were applied in 0.5 ml of phosphate-buffered saline (6.7 mM-KH₂PO₄/150 mM-NaCl, pH 7.4, containing 0.02% NaN₃); the column was eluted with the same buffer (10 ml) and then with the buffer containing 8 mg of chitin hydrolysate/ml (5 ml). Fractions (1 ml) were collected and portions (0.4 ml) were counted for radioactivity. The unbound fractions were pooled, dried and

desalted on Bio-Gel P-6, and were re-applied to DSA-agarose to ensure complete removal of the lectin-binding species. Recovery of radioactivity was always over 85%. The chitin hydrolysate was prepared by strong-acid hydrolysis of chitin [12].

Endo- β -galactosidase treatment of labelled glycopeptides

Endo- β -galactosidase from *Escherichia freundii* was generously provided by Dr. M. Fukuda, La Jolla Cancer Research Foundation, CA, U.S.A. The labelled glycopeptides fractionated by DSA-agarose were desalted on Bio-Gel P-6 and were then incubated for 24 h at 37 °C with 20 munits of endo- β -galactosidase in 0.1 ml of 0.1 M-sodium acetate buffer, pH 5.8, containing 0.02% NaN₃. Untreated glycopeptides were incubated under the same conditions in the absence of enzyme. The samples were boiled for 2 min and were chromatographed on Bio-Gel P-6 as described above.

Isolation of brush-border membranes and assay of sucrase activity

Cells in culture for 5–26 days were processed by the method of Schmitz *et al.* [13] for the preparation of brush-border membranes. Cells from five to ten 75 cm² flasks were scraped, and resuspended in ice-cold Tris/mannitol buffer (2 mM-Tris/HCl, 50 mM-mannitol), pH 7.1, containing 1 mM-phenylmethanesulphonyl fluoride, 0.1 mg of aprotinin/ml and 0.1 mg of trypsin inhibitor/ml. The cell suspension was transferred to a Dounce homogenizer and was disrupted with approx. 70 strokes. CaCl₂ was added to give a final concentration of 10 mM. The homogenate was left on ice for 15 min, and was then centrifuged at 2800 g for 10 min at 4 °C. The supernatant was further centrifuged at 33 500 g for 30 min at 4 °C. The resulting pellet, containing a crude membrane fraction, was resuspended in distilled water. This fraction was assayed for sucrase activity [14] and for protein [15].

RESULTS

Fractionation of fucose-labelled glycopeptides

The changes in structure of fucosylated glycoproteins that occur with differentiation of CaCo-2 cells were studied. Cells which had been in culture for various periods of time were incubated with L-[5,6-³H]fucose for 24 h, and the cell-surface components were released by mild trypsin treatment of the cells. The trypsin-released material and the cell pellets were exhaustively digested with Pronase, and the resulting glycopeptides were fractionated on a column of Bio-Gel P-6 into two major fractions (Fig. 1): Fraction I, which contained high-*M_r* glycopeptides excluded from Bio-Gel P-6, and Fraction II, which was included in the column. The most striking difference in the elution profiles of the fucose-labelled glycopeptides is the decrease in the proportion of Fraction-I glycopeptides with increasing time in culture. This fraction, which accounted for approx. 25–30% of the fucose-labelled cell surface glycopeptides in the rapidly growing cells (Fig. 1a), decreased to about 15% in the early-confluent cells (Fig. 1b) and to about 5% in the late-confluent cells (Fig. 1c). A similar trend was seen in the cell pellet fraction, where the proportion of radioactivity in Fraction I was about 45, 36 and 25% in the rapidly growing, early- and late-confluent cells respectively (Figs. 1d–1f). Although there was some

Fig. 1. Gel filtration of cell-surface and cell-pellet glycopeptides after incubation with L-[5,6- ^3H]fucose

CaCo-2 cells, maintained in culture for various periods of time, were incubated with L-[5,6- ^3H]fucose for 24 h. Cell-surface and cell-pellet glycopeptides were obtained and fractionated on a column of Bio-Gel P-6 as described in the Materials and methods section. Fractions I and II were pooled separately and dried. (a)–(c), Cell-surface glycopeptides; (d)–(f), cell-pellet glycopeptides. (a) and (d),

variation in the percentage of radioactivity found in Fraction I in different experiments, the decrease with time in culture was always about the same.

The fucose-labelled glycopeptides in Fraction I were resistant to the mild alkaline-borohydride treatment which released *O*-linked oligosaccharides from the high-*M_r* glycopeptides obtained from the HCT-8R cells [8], suggesting that, in CaCo-2 cells, these fucosylated oligosaccharides are primarily *N*-linked to protein (results not shown).

Since sucrase is a brush-border enzyme found in mature enterocytes [1], it is a useful index of functional differentiation of CaCo-2 cells [3]. The appearance of sucrase activity was therefore monitored at the same time as the changes in fucose-labelled glycopeptides, between 5 and 26 days in culture (Fig. 2). In this experiment, about 35% of the cell-surface fucose radioactivity of the rapidly growing cells (5 days in culture) was found in Fraction I. As the cells reached confluence (10 days in culture), this proportion decreased to about 20%. When the cells were maintained confluent for an additional 2 weeks, only 5–10% of the radioactivity was found in Fraction I. A longer period was required before some decrease was seen in the proportion of radioactivity in Fraction I of the cell pellet. During the first 10 days in culture about 50% of the cell-pellet radioactivity was associated with Fraction I. After 12 days in culture, the proportion of radioactivity found in Fraction I decreased continuously to about 30% by 22 days. Sucrase activity was measured only in cells which had been in culture for at least 10 days, because it was difficult to obtain enough cells to prepare membranes at earlier times. At 10 days

Rapidly growing cells (4 days in culture); (b) and (e), early-confluent cells (9 days in culture); (c) and (f), late-confluent cells (22 days in culture). In this and subsequent Figures, the arrow indicates the elution of bovine serum albumin (V_0).

Fig. 2. Effects of time in culture on [^3H]fucose-labelled Fraction-I glycopeptides and sucrase activity

Cells were maintained in culture for various periods. The labelled glycopeptides were analysed as described in Fig. 1, and the radioactivity is expressed as the percentage of total radioactivity in cell-pellet glycopeptides (▨) and in cell-surface glycopeptides (■). Sucrase activity (munits/mg) (▤) was assayed as described in the Materials and methods section (1 munit hydrolyses 1 nmol of sucrose/min at 37 °C). In this experiment, cells were confluent by day 10.

Fig. 3. Gel filtration of cell-surface and cell-pellet glycopeptides after incubation with D-[6-³H]glucosamine

Cells maintained in culture for various periods of time were incubated with D-[6-³H]glucosamine for 24 h. The glycopeptides were obtained and fractionated on a column of Bio-Gel P-6 as described in the Materials and methods section. Fractions I and II were pooled separately and dried. (a)–(c), Cell-surface glycopeptides; (d)–(f), cell-pellet glycopeptides. (a) and (d), Rapidly growing cells (4 days in culture); (b) and (e), early-confluent cells (9 days in culture); (c) and (f), late-confluent cells (22 days in culture).

there was no measurable sucrase activity, but by 12 days sucrase became detectable. At subsequent times, sucrase activity increased about 7-fold.

Fractionation of glucosamine-labelled glycopeptides

We then analysed the labelled glycopeptides from cells incubated with D-[6-³H]glucosamine to determine if differentiation of the CaCo-2 cells was accompanied by changes in the types of oligosaccharides. There was only a small difference between the Bio-Gel P-6 elution profiles of the cell-surface glycopeptides obtained at various times in culture (Figs. 3a–3c). The labelled glycopeptides in Fraction I accounted for 45, 44 and 36% of the total cell-surface radioactivity of the rapidly growing, early- and late-confluent cells respectively. However, the cell-pellet glycopeptides showed a much greater decrease in the proportion of radioactivity associated with Fraction I with increasing time in culture (Figs. 3d–3f). The labelled glycopeptides excluded from the column accounted for 45, 44 and 25% of the total cell-pellet radioactivity of the rapidly growing, early- and late-confluent cells respectively. Ion-exchange chromatography

Fig. 4. DSA-agarose affinity chromatography of [³H]fucose-labelled glycopeptides

The [³H]fucose-labelled glycopeptides in Fraction I (Fig. 1) were fractionated on a DSA-agarose column as described in the Materials and methods section. The arrow indicates the point at which the chitin hydrolysate was added to elute bound glycopeptides. The glycopeptides in fractions 1–6 (unbound) and 11–16 (bound) were pooled separately. (a)–(c), Cell-surface glycopeptides; (d)–(f), cell-pellet glycopeptides. (a) and (d), Rapidly growing cells; (b) and (e), early-confluent cells; (c) and (f), late-confluent cells.

graphy on QAE (quaternary aminoethyl)-Sephadex of the glucosamine-labelled glycopeptides from Fraction I showed that about 95% of the radioactivity was eluted with 250 mM-NaCl (results not shown), a concentration lower than that required for elution of glycosaminoglycans [16]. These results suggest that the glucosamine label in Fraction I is almost entirely associated with glycoproteins. Mild alkaline-borohydride treatment of the glucosamine-labelled glycopeptides in Fraction I released 30–40% and about 25% of the radioactivity from the cell-surface and cell-pellet fractions respectively. These results suggest that a significant proportion of the glucosamine-labelled glycopeptides, particularly in the cell-surface fraction, contain O-linked oligosaccharides.

DSA-agarose affinity chromatography

Since the fucose-labelled glycopeptides in Fraction I (Fig. 1) were resistant to alkaline-borohydride reduction, suggesting that the large size of these glycopeptides was not due to the presence of many O-linked oligosaccharides such as those found on mucins, we examined the possibility that these glycopeptides contain polylactosaminoglycans. Affinity chromatography on DSA-agarose was used, as DSA has been shown to bind oligosaccharides and glycopeptides containing repeating N-acetyl-lactosamine units [11]. For cell-surface components of the rapidly growing cells, a large proportion of fucose-labelled Fraction-I glycopeptides bound specifically to DSA-agarose. This proportion decreased with

Fig. 5. DSA-agarose affinity chromatography of the [^3H]-glucosamine-labelled glycopeptides

The [^3H]glucosamine-labelled glycopeptides in Fraction I (Fig. 3) were fractionated on DSA-agarose as described in the Materials and methods section. The arrow indicates the point at which the chitin hydrolysate was added to elute bound glycopeptides. The glycopeptides in fractions 1–6 (unbound) and 11–16 (bound) were pooled separately. (a)–(c), Cell-surface glycopeptides; (d)–(f), cell-pellet glycopeptides. (a) and (d), Rapidly growing cells; (b) and (e), early-confluent cells; (c) and (f), late-confluent cells.

time of culture, from 43 % for the rapidly growing cells to 27 and 29 % for the early- and late-confluent cells respectively (Figs. 4a–4c). Similarly, in the cell pellet, 50, 45 and 36 % of the labelled glycopeptides were bound to the lectin column in the rapidly growing, early- and late-confluent cells respectively (Figs. 4d–4f). A similar decrease in the proportion of glucosamine-labelled glycopeptides of Fraction I bound to DSA-agarose was also observed (Fig. 5). In the cell-surface fraction 30, 15 and 5 % of the labelled glycopeptides were bound in the rapidly growing, early- and late-confluent cells respectively (Figs. 5a–5c). The corresponding values for the cell pellet fraction were 50, 30 and 30 % respectively (Figs. 5d–5f). The fucose- and glucosamine-labelled glycopeptides from Fraction II (Figs. 1 and 3) did not bind to DSA (results not shown).

Table 1 summarizes the results of the lectin-binding experiment in which the amount of labelled glycopeptides bound to DSA-agarose is expressed as a percentage of the total glycopeptide radioactivity in the cell-surface and cell-pellet fractions. The results clearly demonstrate that, with increasing time in culture, the proportion of labelled glycopeptides bound to DSA-agarose decreased 5–6-fold in the cell-surface fraction and about 3-fold in the cell-pellet fraction.

Endo- β -galactosidase treatment of labelled glycopeptides

Since DSA can also bind structures other than polylectosaminoglycans [11,17], the glycopeptides were treated with endo- β -galactosidase from *Escherichia*

Table 1. Proportion of fucose- and glucosamine-labelled glycopeptides bound to DSA-agarose

The fucose- and glucosamine-labelled glycopeptides obtained from cells in culture for various periods of time were fractionated on Bio-Gel P-6 and then on DSA-agarose as described in the Materials and methods section. The results are expressed as the proportion of the total radioactivity in Fractions I and II bound to DSA-agarose.

Time in culture (days)	Radioactivity bound to DSA-agarose (%)			
	Cell surface		Cell pellet	
	Fucose	Glucosamine	Fucose	Glucosamine
4	10	13	23	22
9	4	7	16	13
22	2	2	7	8

freundii, an enzyme which cleaves R-Gal-GlcNAc sequences found in polylectosaminoglycans between the Gal and GlcNAc residues [18]. The results of a 24 h enzyme treatment of the fucose-labelled glycopeptides obtained from the rapidly growing cells are shown in Fig. 6. Treatment of glycopeptides which did not bind to DSA-agarose released only about 20 % of the radioactivity (Figs. 6a and 6c). On the other hand, treatment of the DSA-agarose-bound glycopeptides released 80–85 % of the radioactivity (Figs. 6b and 6d). Similarly, treatment of the glucosamine-labelled glycopeptides which did not bind to DSA-agarose released less than 15 % of the radioactivity (Figs. 7a and 7c), whereas 75–85 % of the radioactivity of the bound glycopeptides was released (Figs. 7b and 7d). In both cases, the DSA-bound glycopeptides were degraded to a heterogeneous mixture of low- M_r products. Increasing the time of endo- β -galactosidase treatment to 48 h did not change these results. Similar results were obtained with the Fraction-I glycopeptides from the early- and late-confluent cells (results not shown). Endo- β -galactosidase treatment of either the fucose- or glucosamine-labelled glycopeptides from Fraction II did not release any radioactivity (results not shown).

DISCUSSION

In the present work, we demonstrate that in CaCo-2 cells the high- M_r glycopeptide fraction excluded from Bio-Gel P-6 [8] contains fucosylated polylectosaminoglycans, since they are bound to DSA-agarose and are largely degraded by endo- β -galactosidase. We also show that there is a large decrease in the proportion of these fucosylated polylectosaminoglycans during differentiation of CaCo-2 cells. Rapidly growing cells, which are morphologically and functionally undifferentiated [3], have relatively high contents of fucosylated polylectosaminoglycans, whereas in late-confluent cells, which are polarized and contain active brush-border enzymes [3], the relative amount of fucosylated polylectosaminoglycan is greatly decreased.

A significant proportion of the CaCo-2 polylectosaminoglycans appears to be branched, as deduced from the heterogeneous pattern of degradation products

Fig. 6. Effects of *E. freundii* endo- β -galactosidase on [^3H]fucose-labelled glycopeptides

[^3H]Fucose-labelled glycopeptides from rapidly growing cells obtained after DSA-agarose chromatography (Figs. 4a and 4d) were treated with endo- β -galactosidase and fractionated on Bio-Gel P-6 as described in the Materials and methods section. (a) and (b), cell-surface glycopeptides; (c) and (d), cell-pellet glycopeptides. (a) and (c), DSA-unbound glycopeptides; (b) and (d), DSA-bound glycopeptides. —●—, Untreated glycopeptides; —, endo- β -galactosidase-treated glycopeptides. The arrows indicate the elution positions of bovine serum albumin and the disaccharide GlcNAc-Gal respectively. Similar results were obtained with the glycopeptides from the early- and late-confluent cells (results not shown).

Fig. 7. Effect of *E. freundii* endo- β -galactosidase on [^3H]glucosamine-labelled glycopeptides

[^3H]Glucosamine-labelled glycopeptides from rapidly growing cells obtained after DSA-agarose chromatography (Figs. 5a and 5d) were treated with endo- β -galactosidase and fractionated on Bio-Gel P-6 as described in the Materials and methods section. (a) and (b), cell-surface glycopeptides; (c) and (d), cell-pellet glycopeptides. (a) and (c), DSA-unbound glycopeptides; (b) and (d), DSA-bound glycopeptides. —●—, Untreated glycopeptides; —, endo- β -galactosidase-treated glycopeptides. The arrows indicate the elution position of bovine serum albumin and the disaccharide GlcNAc-Gal respectively. Similar results were obtained with the glycopeptides from the early- and late-confluent cells (results not shown).

resulting from the endo- β -galactosidase treatment (Figs. 6 and 7). Previous studies on the linear poly-lactosaminoglycans of undifferentiated K562 cells by using comparable endo- β -galactosidase treatment released a trisaccharide (Gal-GlcNAc-Gal) and a disaccharide (GlcNAc-Gal) as the major products [19]. Since branching in poly-lactosaminoglycans generates linkages resistant to endo- β -galactosidase [20], the products released in branched poly-lactosaminoglycans are larger and more heterogeneous than those obtained from linear structures. Since there was no apparent difference in the pattern of degradation products observed for the labelled poly-lactosaminoglycans obtained from the CaCo-2 cells in culture for different periods of time, there seems to be no major change in branching with differentiation of these cells.

The fucosylated poly-lactosaminoglycans on CaCo-2 cells are probably *N*-linked to protein, since fucose-labelled Fraction-I glycopeptides are resistant to mild alkaline-borohydride treatment. In previous studies fucosylated poly-lactosaminoglycans were detected on colonic-tumour cells, but these were not shown to be associated with glycoproteins, since they were identified immunologically either in tissue sections [21,22] or on purified glycolipids [23].

Some of the glucosamine-labelled glycopeptides in Fraction I were sensitive to mild alkaline-borohydride treatment, indicating the presence of *O*-linked structures, but these glycopeptides did not bind to DSA-agarose and were not degraded by endo- β -galactosidase (A. Youakim & A. Herscovics, unpublished work).

Poly-lactosaminoglycans, which were first described on glycoproteins [24] and glycolipids of erythrocytes [25], have also been found on haemopoietic cells [26,27], teratocarcinoma cells [28], CHO cells [29] and thyroid cells [30]. It has been shown that the synthesis and structure of poly-lactosaminoglycans change with differentiation of specific types of cells. For example, poly-lactosaminoglycans from fetal and adult erythrocytes differ in their degree of branching; the fetal form is made up of linear chains [31], whereas the adult form is highly branched [32]. A similar change in branching occurs during differentiation of K562 human erythroleukaemia cells [33]. In contrast, differentiation of HL60 human promyelocytic cells [34], F9 mouse teratocarcinoma cells [28] and PC12 mouse pheochromocytoma cells [35] is accompanied by a relative decrease in poly-lactosaminoglycan synthesis. The results of our study are the first demonstration *in vitro* of a differentiation-associated decrease in the proportion of protein-bound poly-lactosaminoglycans in a human epithelial cell.

Poly-lactosaminoglycans have also been detected in several tumour cells [21,22,26,27,36,37], and in one model system the presence of poly-lactosaminoglycan was correlated with the metastatic properties of the cells [36]. In colonic cells, poly-fucosylated poly-lactosaminoglycans appear to be tumour-associated, since monoclonal antibodies directed against these types of structures were more reactive with tumour tissue than with normal tissue by immunohistochemical analysis [21,38].

Differentiated CaCo-2 cells resemble cells of adult small intestine both morphologically and functionally [3], although they are of colonic origin [9]. Since colonic cells express brush-border enzymes only in the fetus at mid-gestation, it has been suggested that CaCo-2 cells may represent a model for fetal colon differentiation [39].

Immunological studies using a monoclonal antibody, FH4, which recognizes a poly-fucosylated antigenic determinant associated with poly-lactosaminoglycans on glycolipids [23], showed reactivity with colon from early embryos (6–7 weeks) but not from later embryos [22]. These observations are consistent with the notion that undifferentiated CaCo-2 cells, which express relatively high amounts of fucosylated poly-lactosaminoglycans but no detectable brush-border enzymes, represent early-embryonic cells, whereas differentiated CaCo-2 cells, which express relatively low amounts of fucosylated poly-lactosaminoglycans and high activities of brush-border enzymes, represent fetal cells at mid-gestation.

The present work shows that CaCo-2 cells provide a useful model *in vitro* to investigate the changes and control of glycosylation during differentiation of intestinal epithelial cells.

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