Glycoconjugate Journal 18, 685–697, 2001

Biosynthesis of mucin type *O*-glycans: Lack of correlation between glycosyltransferase and sulfotransferase activities and CFTR expression

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Structural differences have been reported in the glycosylation patterns of cystic fibrosis glycoproteins. Although the gene mutated in cystic fibrosis (CFTR) has been cloned and characterized as a chloride channel, its relationship to the highly viscous mucus and structural glycoprotein and mucin abnormalities in cystic fibrosis still remains to be defined. We have evaluated *O*-glycan biosynthesis in CHO and BHK cells that express CFTR and ΔF508 CFTR as *in vitro* models, and utilized the cftr knockout mouse as an *in vivo* model of CFTR dysfunction. Activities of glycosyltransferases and sulfotransferases synthesizing mucin type *O*-glycan chains were determined in these models. Differences in transferase activity levels were found between tissues and cell types and during mouse development. No specific patterns of activities were associated with the lack of CFTR or with ΔF508CFTR expression. This suggests that it is not the presence or absence of normal CFTR, or the presence of mutant CFTR alone, but rather cell specific additional factors or pathophysiological consequences that determine the changes in mucin glycosylation in cystic fibrosis.

Keywords: **glycosyltransferases, mucins, *O*-glycans, CFTR, ΔF508 CFTR, CHO cells, BHK cells, CFTR knock out mice**

Abbreviations: **BHK cells, baby hamster kidney cells; Bz = 4-benzoyl-phenylalanine; CF, cystic fibrosis; CF mice, cftr(−/−) mice; CFL mice, CF mice fed a liquid diet; CFTR, cystic fibrosis transmembrane conductance regulator; CHO cells, Chinese hamster ovary cells; N mice, normal control mice; NL mice, normal control mice fed a liquid diet; MAA, Maackia amurensis; PHA-L, Phytohemagglutinin-L; PNA, Peanut agglutinin; SNA, Sambucus nigra.; UNC, University of North Carolina; WGA, Wheat germ agglutinin.**

Introduction

Cystic fibrosis (CF) is a common autosomal recessive genetic disease resulting from mutations in the gene encoding CFTR, the cystic fibrosis transmembrane conductance regulator (CFTR). Associated with the disease is a salt and water imbalance, viscous mucous secretions, structural and functional abnormalities of the mucus barrier, chronic inflammation, and obstruction of epithelial ducts and exocrine glands. Although many organs are affected in CF, intestinal pathology is prevalent, as well as respiratory infections and chronic inflammation. Since the discovery of the CF gene, many studies have described the structure and function of CFTR [1,2], an ATP binding cAMP regulated chloride channel glycoprotein, expressed on the apical membrane of epithelial cells. Several hundreds of mutations can cause a variety of structural abnormalities and functional dysfunction [3]. The most common mutation that is found in about 70% of CF patients is the lack of Phe at position 508 in the protein (ΔF508). This mutation causes the protein to be misprocessed and dysfunctional resulting in defective trafficking and extensive degradation [4–6].

A number of different abnormalities have been described in CF mucins, as well as in glycoproteins from fibroblasts and other cell types from CF patients [7–9]. CF mucins are abnormal in their carbohydrate content, chain length and sulfate content [10–12]. CF mucins appear to be more acidic and hypersulfated, but have less sialic acid [13]. However, other studies have demonstrated an increased sialic acid content as well as more sulfate and Fuc of CF salivary mucins [8]. An increase in Fuc content, and in the Fuc/sialic acid ratio of glycoproteins and mucins from several cell types from CF patients is a common finding [14]. These changes can alter mucin receptors for

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bacterial binding, can change the adhesive properties of cells, and generally are expected to play a role in the overall structures and functions of glycoproteins [8,15–17].

The mechanisms for the altered glycosylation differences in CF could reflect alterations in the Golgi environment of the transferases, the rate of transport of substrates for glycosylation and sulfation reactions, mRNA levels for the glycosyltransferases and/or sulfotransferases, or in the metabolic regulation of enzyme activities. For example, hypersulfation in CF could be explained by hyperactive sulfotransferases. Since sulfated glycans are often no longer substrates for sialylation, hypersulfation is expected to suppress sialylation, or conversely, a decrease in sialylation could lead to hypersulfation. Changes of the backbone structure of *O*-glycans may also have a significant impact on the overall structures of mucins.

Several experimental models have been produced to study CFTR function. Cell lines have been transfected with mutant and wild type CFTR, and include fibroblasts [18], oocytes [19], CHO cells [20] and BHK cells [21]. CFTR function can also be studied in a number of CFTR expressing cell lines, such as intestinal cancer cells Caco-2 [22] and HT29 [23].

Cultured CF airway cells [24] and skin fibroblasts [25] were previously used to study the relationship between mutant CFTR and glycosylation. Terminal sialylation was reported to be affected in airway cells lacking CFTR [26]. Similarly, mouse mammary epithelial cells expressing ΔF508 CFTR exhibit decreased amounts of sialic acid in glycoconjugates, compared with those expressing normal CFTR [27]. Peanut lectin staining of immortalized CF airway epithelial cells and in a CFTR transfected clone suggested a higher degree of sialylation in CFTR expressing cells. However, this difference appears to be independent of CFTR, since CFTR rescued cells did not show glycosylation differences [28]. In order to evaluate mucin glycosylation, colon cancer cells that differ in CFTR expression have been transfected with epitope-tagged MUC1 cDNA. Glycosylation and sulfation, determined by the reactivity of antibodies towards MUC1 peptide epitopes, as well as by metabolic labeling, was not affected by CFTR expression. This indicated that neither glycosylation nor sulfation of MUC1 mucin-derived peptides were dependent on CFTR expression [29]. Another model to study mucin sulfation is the xenograft model in which CF tissue had been transplanted into nude mice. A higher degree of incorporation of radioactive sulfate was found in mucins isolated from the CF tissue xenograft, compared with normal tissue xenograft [30]. Thus, some of the models used to date have shown an effect of CFTR on glycosylation patterns although others have failed to demonstrate a direct effect of CFTR on glycosylation.

In order to create an animal model for CF, cftr(−/−) mice have been produced by disruption of the cftr gene [31]. These UNC (University of North Carolina) mice suffer mainly from intestinal obstructions commonly found at the ileocecal sphincter or in the colon [32]. These mice show abnormal intestinal histology, are underweight, and die shortly after weaning. Lung disease is not present in these mice, and liver, pancreas and reproductive tract show no signs of lesions. However, Cftr(−/−) mice express highly sulfated glycoproteins in the duodenum and pancreas [33]. The intestinal pathology resembles that found in the human disease. When kept on a liquid diet, cftr(−/−) mice have a prolonged but not normal life span, and still have considerable intestinal disease. The intestinal pathology of UNC cftr(−/−) mice and the mucus accumulation is thought to involve altered mRNA expression of secreted intestinal mouse mucin peptide backbone [34,35]. UNC cftr(−/−) mice seem to be in a state of hypersecretion with lumenal accumulation of mucus, as demonstrated by abundant periodic acid/Schiff (PAS) stained material in the crypt lumen. It is possible that some of the observed changes of PAS positive material are due to altered glycosylation and possibly hypersulfation of mucins. Using lectin staining techniques, lungs from cftr(−/−) knock out mice were found to contain increased amounts of sialic acid as well as Fuc residues although without signs of pathology [36].

The mechanism underlying glycosylation differences in CF may involve abnormal biosynthesis of mucin *O*-glycans [15,37,38]. In this paper we examined whether glycosyltransferase and sulfotransferase activities were affected by the expression of CFTR in three model systems. We investigated the direct effect of the presence of CFTR and ΔF508CFTR on glycosyltransferase and sulfotransferase activities *in vitro* in clones of cultured CHO and BHK cells. These cells synthesize simple mucin type *O*-glycans with core 1 structures [39–41] (Figure 1). Using an *in vivo* model of CFTR dysfunction, we investigated glycosyltransferases and sulfotransferases in different tissues of UNC cftr(−/−) mice throughout their development, and compared results to normal mice. Our results suggest that mucin biosynthesis in mouse tissues is more complex than in the *in vitro* systems, and that the levels of glycosyltransferase and sulfotransferase activities in the three models are cell type and tissue specific but not significantly dependent on CFTR expression. The *in vivo* studies in mice indicate that activities vary during development, and are abnormal in several tissues of severely diseased animals. The differences in glycosylation of CF mucins are therefore likely due to a secondary effect of the pathophysiology as a consequence of CFTR dysfunction.

Experimental

Materials

Mucin peptide derivatives Ac-PTPTGTQTPTTTPITTTTT-VTPTNH_2, TPTPTGTQTG and AcBzPTPPPNH_2 were synthesized by Dr. Hans Paulsen, University Hamburg, Germany (Bz = 4-benzoyl-phenylalanine). VTSAPDTRPAPGST was a kind gift of Dr. Joyce Taylor-Papadimitriou, Imperial Cancer Research Fund, London, UK. GlcNAc, GalNAcα-Bn, Galβ3-GalNAcα-Bn, GlcNAcβ3GalNAcα-pnp, Galβ4GlcNAc, GlcNAcβ-Bn and PHA-L lectin were purchased from Sigma.

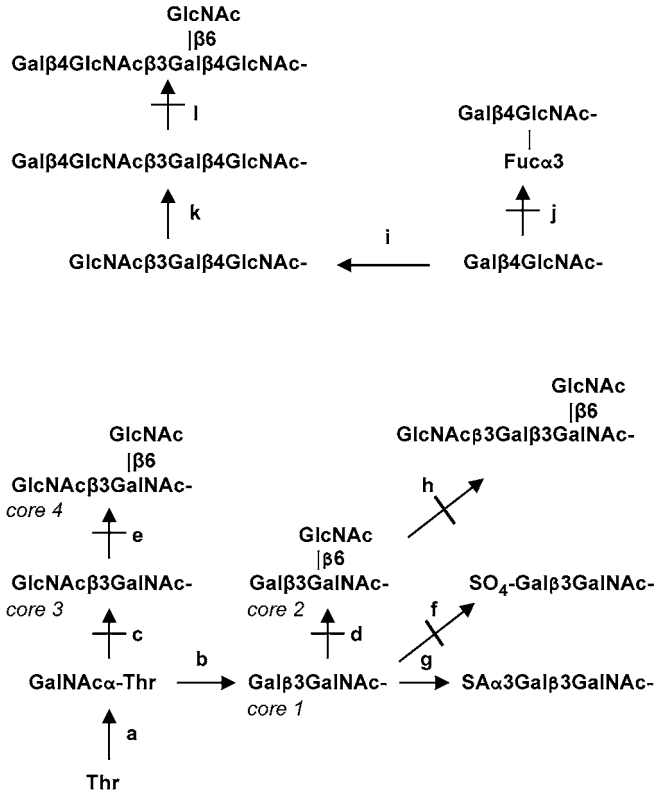

Figure 1. Biosynthesis of *O*-glycans in BHK cells. The biosynthetic pathways for the synthesis of mucin type *O*-glycans in BHK cells are shown, based on the activities of glycosyltransferases and sulfotransferases measured in this study. a, Polypeptide GalNAc-transferase; b, core 1 β3-Gal-transferase; c, core 3 β3-GlcNAc-transferase; d, core 2 β6-GlcNAc-transferase; e, core 4 β6-GlcNAc-transferase; f, core 1 sulfotransferase; g, core 1 α3-sialyltransferase; h, elongation β3-GlcNAc-transferase; i, i β3-GlcNAc-transferase; j, α3-Fuc-transferase; k, β4-Gal-transferase; l, centrally acting I β6-GlcNAc-transferase. BHK cells can only synthesize core 1 but not core 2,3, or 4 structures. The activities in CHO cells are likely to be similar to those in BHK cells, in that they are only able to synthesize significant amounts of core 1 but not other core structures. BHK cells transfected with CFTR have a less active sialyltransferase (path g) while cells transfected with ΔF508CFTR have a less active core 1 β3-Gal-transferase (path b). A similar situation is seen in CHO cells. However, CHO cells transfected with ΔF508CFTR maintain a high level of sialyltransferase (path g). Paths a, b, c, d, f and h have been demonstrated in mouse colonic tissue. All of the pathways shown are highly active in rat colon. Blocked arrows denote inactive pathways.

Other lectins were from Vector Laboratories. Galβ3-GalNAcα-pnp was from Toronto Research Chemicals, and Galβ4GlcNAcβ3Galβ4Glc was from Calbiochem. GalNAcβ4GlcNAcβ-Bn, GlcNAcβ6[Galβ3]GalNAcα-Bn and Galβ3GalNAcα-allyl were kindly provided by Dr. Khushi Matta, Roswell Park Cancer Institute, Buffalo, NY. Galβ4-GlcNAcβ-Bn was synthesized using milk β4-Gal-transferase (Sigma) in a large scale β4-Gal-transferase assay (as described below). Product was purified by AG1x8, followed by HPLC using a C18 column and acetonitrile/water 10/90 as the mobile phase. HPLC analysis showed that the product was >90% pure Galβ1-4GlcNAcβ-Bn. All other materials were obtained as described in Brockhausen et al. [42,43].

Mice

Heterozygous mice obtained from the University of North Carolina were bred by Dr. G. Kent in the animal facility of the Hospital of Sick Children, Toronto, to obtain homozygous cftr(−/−) mice. The cftr(−/−) mice that had solid food died of natural causes within 10 days after weaning and were collected postmortem. Three cftr(−/−) mice were small and fragile, and died at the age of 30 days of intestinal obstruction (pooled samples CF). Two cftr(−/−) mice were kept on a liquid diet (CFL) and died at ages 30 and 52 days (pooled samples CFL). Nine normal mice were also either kept on normal solid food after weaning (pooled samples N) or on liquid diet (pooled samples NL), and were sacrificed in the CO_2 chamber.

In addition, both normal and cftr(−/−) mice on liquid diet were sacrificed at different stages and pooled, i.e. 21 days (pooled samples stage 1, just before weaning), at 6 days post weaning (pooled samples stage 2), at 20 days post weaning (pooled samples stage 3), and 50 days post weaning (pooled samples stage 4). Tissues were excised from dead mice, carefully cleaned from blood, fat and mucus, and then cut in small pieces and homogenized with a 5-fold excess volume of 0.25 M sucrose. Protein was determined by the method of Bradford (Bio-Rad) using bovine serum albumin as the standard. Enzymes were stored at −50°C.

CHO cells

CHO cells (untransfected, transfected with CFTR, and transfected with ΔF508CFTR) were obtained from Dr. David Williams, University of Toronto [44]. The transfection procedure using the pNUT vector has been described in Lukacs et al. [4] and Tabcharani et al. [20]. CHO cells were grown in alpha-MEM medium supplemented with antibiotics, 8% FCS and 200 μM methotrexate. CHO cells were also grown without methotrexate. Cells were harvested at confluency, washed 3 times in PBS, and homogenized with 0.25 M sucrose (1 ml sucrose/10^8 cells) for enzyme assays. Enzyme preparations were stored at −50°C.

BHK cells

BHK cells (untransfected, transfected with CFTR, and transfected with ΔF508CFTR) were obtained from Dr. Gergely Lukacs, Hospital for Sick Children, Toronto [21]. BHK cells were grown in 1:1 mixtures of DMEM (high glucose) and F12 (Gibco), supplemented with 5% FCS. 500 μM methotrexate was added to the medium for transfected cells. Cells were harvested at confluency and cell homogenates were prepared as described above and stored at −80°C.

Lectin binding assays

Cells were grown to superconfluency on 96 well plates. Cells were washed in PBS containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$, and fixed for 15 minutes with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. Cells were washed again with PBS containing 0.2% gelatin (PBS-G), and incubated with biotin-labeled lectin (1 μg/ml): Phytohemagglutinin-L (PHA-L), peanut agglutinin (PNA), wheat germ agglutinin (WGA), Sambucus nigra (SNA) or Maackia Amurensis (MAA lectin I). This was followed by washing with PBS-G. Cells were then subjected to avidin-alkaline phosphatase conjugate for 1 hour, and 2 hours with p-nitrophenyl-phosphate substrate in 0.5 $MgCl_2$, pH 9.5, at room temperature. Plates were read at 405 nm using a microplate spectrophotometer (Bio-Tek Instruments). Each sample was analyzed 8 times.

Glycosyltransferase and sulfotransferase assays

Glycosyltransferase assays were carried out as described by Yang et al. [45] and Brockhausen et al. [42,43] by Dowex (AG1x8) assays, HPLC assays (AG1x8, followed by separation on C18 and amine columns) or Sep-Pak (C18) assays. Sialyltransferase and sulfotransferase activities were measured by high voltage electrophoresis [45]. HPLC separations were carried out using acetonitrile/water mixtures as the mobile phase. Protein concentrations were 50 to 200 μg/assay of CHO or BHK cell homogenate, 100 to 1200 μg/assay of mouse tissue homogenates. Rat colon mucosal homogenate (100 μg protein/assay) was used as the positive control for all glycosyl- and sulfotransferases. All enzyme assays were carried out at least in duplicate determinations under conditions that were linear with time, and proportional to enzyme concentration.

Galβ1-3GalNAc α3-sialyltransferase assays

The assay mixtures for Galβ1-3GalNAc α3-sialyltransferase contained in 40 μl total volume: 4 mM acceptor substrate Galβ1-3GalNAcα-Bn, 10 mM AMP, 0.125 M Tris-HCl, pH 7, 0.5% Triton-X100, 1.5 mM CMP-[^{3}H]-sialic acid, 2000 cpm/nmol, (or CMP-[^{14}C]-sialic acid, 1900 cpm/nmol) and 10 to 20 μl enzyme homogenate. Assay mixtures were incubated for 1 hour at 37°C; 10 μl 20 mM EDTA/1% Na-tetraborate were added to stop the reaction, and mixtures were spotted on Whatman 3M paper and separated by high voltage electrophoresis in 1% Na-tetraborate at 2 kV and 150 mA for 1 to 1.5 hours. Product moved about 5 cm from the origin. Paper strips were cut out and counted in scintillation fluid.

Sulfotransferase assays

Sulfotransferase was assayed in a total volume of 40 μl with 2 mM acceptor substrate (Galβ1-3GalNAcα-Bn, Galβ1-4GlcNAc, Galβ4GlcNAcβ-Bn, GlcNAcβ-Bn or GlcNAcβ1-3Galβ-Me), 2.5 mM ATP, 40 mM Tris-HCl, pH 6.3, 10 mM NaF, 10 mM 2,3-dimercapto-1-propanol (BAL), 2.5 mM Mg-acetate, 0.25 to 1% Triton-X100, 4.5–8 μM PAP^{35}S (1100–4700 dpm/pmol), 10 to 20 μl enzyme homogenate. Assay mixtures were incubated for 1 hour at 37°C; 10 μl 20 mM EDTA/1% Na-tetraborate were added to stop the reaction, and mixtures were separated on paper by high voltage electrophoresis as described above. Sulfotransferase products from rat colon enzymes migrated about 9 cm on paper during high voltage electrophoresis to distinct peaks, separated from the remaining radioactivity.

GlcNAc-transferase assays

Core 2 β6-GlcNAc-transferase activity was measured in a total volume of 40 μl with 2 mM Galβ1-3GalNAcα-pnp acceptor substrate, 0.125 M GlcNAc, 5 to 10 mM AMP, 5 mM γ-galactono-lactone, 0.125 M MES pH 7, 0.125% Triton-X100 and 0.25–1 mM UDP-[^{3}H]-GlcNAc (2,000–11,500 dpm/nmol). Mixtures were incubated for 1 hour at 37°C, passed through AG-1x8 (0.4 ml), and following washing, counted in scintillation fluid (Dowex assays). For HPLC assays, eluate from AG1x8 columns were lyophilized and subjected to HPLC analysis [45]. Some assays were done by passing incubation mixtures through C18 Sep-Pak cartridges, followed by washing with water and elution of product with methanol. Core 3 β3-GlcNAc-transferase activity was measured similarly by HPLC assays using a C18 column, with 12.5 mM $MnCl_2$ in the assay mixture and 4 mM GalNAcα-Bn acceptor substrate. i β3-GlcNAc-transferase was assayed by HPLC with 2 mM Galβ1-4GlcNAcβ-Bn acceptor substrate in the presence of 12.5 mM $MnCl_2$. Elongation β3-GlcNAc-transferase was measured similarly in the presence of 12.5 mM $MnCl_2$ and 2 mM GlcNAcβ6(Galβ3)GalNAcα-Bn substrate, followed by HPLC. Centrally acting I β6-GlcNAc-transferase activity was assayed using 2 mM Galβ4GlcNAcβ3Galβ4Glc substrate, followed by separation of product on an amine column using acetonitrile/water 80/20 as the mobile phase.

Gal-transferase assays

Core 1 β3-Gal-transferase activity was measured in a total volume of 40 μl with 2 or 4 mM acceptor substrate GalNAcα-Bn, 5 to 10 mM AMP, 5 mM γ-galactono-lactone, 0.125 M MES pH 7, 0.125% Triton-X100, 12.5 mM $MnCl_2$, 0.9 to 1.6 mM UDP-[^{3}H]-Gal (5,200 dpm/nmol), and 10 to 20 μl enzyme homogenate. Mixtures were incubated for 1 hour at 37°C, and product was identified by HPLC. β4-Gal-transferase assays were done similarly either with GlcNAc or GlcNAcβ-Bn as the substrate.

Fuc-transferase assays

α3-Fuc-transferase activity was measured in a total volume of 40 μl with 2 mM acceptor substrate GalNAcβ4GlcNAcβ-Bn,

2.5 mM AMP, 0.125 M MES pH 7, 0.125% Triton-X100, 12.5 mM $MnCl_2$, 1 mM GDP-[^{3}H]-Fuc (5000 dpm /nmol), and 20 μl enzyme homogenate. Mixtures were incubated for 1 hour at 37°C, and product was measured by the Dowex assay.

Polypeptide GalNAc-transferase assays

Polypeptide GalNAc-transferase activity was assayed in a total volume of 40 μl with 0.5 to 1 mM acceptor substrate, 2.5 mM AMP, 0.125 M MES, 0.125% Triton-X100, 0.7 mM UDP-[^{14}C] or [^{3}H]-GalNAc (1600 to 9100 dpm/nmol) and 10 to 20 μl enzyme homogenate by Dowex and HPLC assays.

Results

The CHO cell model: Glycosyltransferase and sulfotransferase activities in CHO cells before and after transfection with CFTR or ΔF508 CFTR

CHO cells transfected with wild type CFTR (CHO-CFTR) and with ΔF508CFTR (CHO ΔF508CFTR), and untransfected cells (without methotrexate) grew well in culture, with CHO ΔF508CFTR showing the slowest growth. The conditions to assay glycosyltransferase and sulfotransferase activities have been previously established [42,43,45,46]. CHO cells contained enzymes for the synthesis of sialylated *O*-glycans with core 1 structures, but there was no detectable activity of core 2 β6-GlcNAc-transferase (Figure 1, path d) in untransfected CHO cells. The activities of core 1 β3-Gal-transferase (Figure 1 path b) were similar in untransfected and CHO-CFTR cells. CHO-ΔF508CFTR cells, however, only had one third of the activity (Table 1). By comparison, the activity of α3-sialyltransferase acting on core 1 (Figure 1 path g) was the same in untransfected and ΔF508CFTR-transfected cells but this enzyme had only 20% of the activity in CFTR-transfected cells (Table 1). We were unable to detect sulfotransferase activities towards Galβ1-3GalNAcα-Bn and Galβ4GlcNAc in the three cell lines.

The BHK cell model: Glycosyltransferase and sulfotransferase activities in BHK cells before and after transfection with CFTR or ΔF508 CFTR

In order to determine whether the pattern of transferase activity differences seen in CHO cells was associated with CFTR expression in other models as well, we examined BHK cells as a second model. In contrast to CHO cells, BHK cells transfected with Δ508CFTR (BHK-ΔF508CFTR) grew fastest compared to untransfected cells, and CFTR transfected cells (BHK-CFTR) grew slowest. BHK cells were found to synthesize mucin type *O*-glycans with core 1 structure (Figure 1), and exhibited good activity of polypeptide GalNAc-transferase (Figure 1, path a) with 1 mM AcBzPTPPPNH$_2$ as a substrate. Core 1 β3-Gal-transferase, α3-sialyltransferase acting on core 1 and β4-Gal-transferase (Figure 1, path k) were also active (Table 2) while i β3-GlcNAc-transferase activity was barely detected (<0.1 nmol/h/mg) (Figure 1, path i). We were unable to detect activities of core 3 β3-GlcNAc-transferase (Figure 1, path c), elongation β3-GlcNAc-transferase (Figure 1, path h) and core 4 β6-GlcNAc-transferase (Figure 1, path e), centrally acting I β6-GlcNAc-transferase (Figure 1, path l) or α3-Fuc-transferase (Figure 1, path j). However, it could not be excluded that very low activities of core 2 β6-GlcNAc-transferase were present. These results indicate that only non-elongated core 1 structures can be synthesized in BHK cells. Although sulfotransferase activities were at the limits of detection, very low levels of sulfotransferase activities towards Galβ3GalNAcα-Bn, Galβ4GlcNAcβ-Bn and GlcNAcβ-Bn substrates could not be excluded. By comparison, rat colon homogenate, which was used as the control tissue, had high activities of all the glycosyltransferases and sulfotransferases. The activities of sulfotransferases in rat colon homogenate were 313 pmol/h/mg towards Galβ3GalNAcα-Bn substrate, 76 pmol/h/mg towards Galβ4GlcNAcβ-Bn and 17 pmol/h/mg towards GlcNAcβ-Bn.

Compared with untransfected cells, CFTR-transfected BHK cells had similar activities of core 1 β3-Gal-transferase and β4-Gal-transferase but 2-fold lower polypeptide GalNAc-transferase and α3-sialyltransferase activities. In

Table 1. Glycosyltransferase and sulfotransferase activities in normal and transfected CHO cells

		Activity (nmol/h/mg)		
Enzyme	*Substrate*	*CHO*	*CHO-CFTR*	*CHO-ΔF508CFTR*
core 1 β3-Gal-T	2mM GalNAcα-Bn	4.1	3.4	1.4
core 2 β6-GlcNAc-T	2mM Galβ3GalNAcα-pnp	<0.1		
α3−sialyl-T	2mM Galβ3GalNAcα-pnp	21.6	4.2	21.0
Sulfo-T	2mM Galβ3GalNAcα-Bn	0	0	0
	2mM Galβ4GlcNAc	0	0	0

Enzymes were assayed as described in the Methods section in at least duplicate determinations by HPLC assays for core 1 β3-Gal-transferase and core 2 β6-GlcNAc-transferase, and by high voltage electrophoresis for α3-sialyltransferase acting on core 1 and sulfotransferases. Positive control tissue was rat colon homogenate. -T, transferase. CHO, untransfected CHO cells grown without methotrexate. CHO-CFTR, CHO cells transfected with CFTR; CHO-ΔF508CFTR, CHO cells transfected with ΔF508CFTR.

Table 2. Glycosyltransferase activities in normal and transfected BHK cells

		Activity (nmol/h/mg)		
Enzyme	*Substrate*	*BHK*	*BHK-CFTR*	*BHK-ΔF508CFTR*
Polypeptide GalNAc-T	1 mM AcBzPTPPPNH$_2$	1.4	0.7	2.4
Core 1 β3-Gal-T	2 mM GalNAcα-Bn	2.4	2.7	1.5
β4-Gal-T	2 mM GlcNAcβ-Bn	11.3	9.3	6.6
α3-Sialyl-T	2 mM Galβ3GalNAcα-pnp	2.9	1.2	1.0
Sulfo-T	2 mM Galβ3GalNAcα-Bn	$<$0.0001	0	0
	2 mM Galβ4GlcNAcβ-Bn	$<$0.0004		
	2 mM GlcNAcβ-Bn	$<$0.0003		

Enzymes were assayed as described in the Methods section in at least duplicate determinations. Gal-transferases were measured by HPLC assays, polypeptide GalNAc-transferase by the Dowex assay; α3-sialyltransferase acting on core 1 and sulfotransferases were assayed by high voltage electrophoresis. Positive control tissue with high activities for all enzymes was rat colon homogenate. Bz, 4-benzoylphenylalanine; -T, transferase. BHK, untransfected BHK cells grown without methotrexate. BHK-CFTR, BHK cells transfected with CFTR; BHK- ΔF508CFTR, BHK cells transfected with ΔF508CFTR.

ΔF508CFTR-transfected BHK cells, polypeptide GalNAc-transferase activity was high but the other three transferases had lower activities. i β3-GlcNAc-transferase activities and core 1 sulfotransferase activities could not be detected in transfected cells.

Lectin binding assays for BHK cells

In order to correlate glycosyltransferase activities with cell surface glycan structures, untransfected BHK cells, BHK-CFTR and BHK-ΔF508CFTR cells were subjected to lectin staining in enzyme-linked lectin assays (ELLA). WGA, SNA, MAA, PNA and PHA-L lectins, tested in 8 different wells, all showed significant staining in the three cell lines (Figure 2). CFTR transfected BHK cells, compared to untransfected cells, demonstrated significantly higher binding of WGA (that binds to GlcNAc/sialic acid), SNA (that stains sialylα2-6Gal(NAc) termini), PNA (that binds to unmodified core 1), MAA lectin I (that recognizes Galβ1-3GlcNAc termini), and PHA-L (that recognizes branched *N*-glycans). These lectin binding patterns suggest that more Galβ1-3GlcNAc and sialylα2-6 linkages are present on cell surfaces of CFTR transfected cells. Increased amounts of unmodified core 1, and branched *N*-glycans could also be detected. ΔF508CFTR transfected BHK cells were stained significantly more with WGA, SNA and PNA, indicating an increase of core 1 structures as well as sialylα2-6 linkages on their cell surfaces but this increase was less than that seen in CFTR-transfected cells.

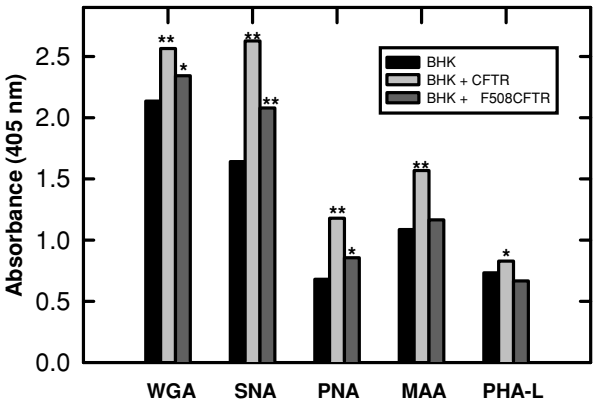

Figure 2. Enzyme-linked lectin binding to BHK cells. WGA, SNA, PNA, MAA lectin I, PHA-L were used to stain untransfected, CFTR-transfected and ΔF508CFTR transfected BHK cells in enzyme-linked lectin assays as described in the Methods section. Lectin binding was carried out 8 times per sample, and binding was quantified by absorbance at 405 nm. Mean values of intensities are shown, with a star for significant variation ($p < 0.05$) and 2 stars for $p < 0.001$. Specificities of the lectins are sialic acid/GlcNAc (WGA), sialyl α2-6Gal(NAc) (SNA), Galβ1-3GalNAc (PNA), Galβ1-3GlcNAc (MAA), and branched *N*-glycans (PHA-L).

The mouse model: Distribution of glycosyltransferase activities in tissues from normal and cftr(−/−) mice fed solid and liquid diet

Tissues were obtained from normal (N) and homozygous cftr(−/−) knock out mice (CF), normal mice fed a liquid diet after weaning (NL), cftr(−/−) mice that died of natural causes shortly after weaning due to intestinal obstruction (CF), and cftr(−/−) mice that died after being kept on a liquid diet post weaning (CFL). CF mice were small and frail, and had obstructed intestines. It had been observed that CF mice on a liquid diet had about 90% normal life span [32], although they were smaller than normal mice. Therefore, to reduce suffering from intestinal obstruction and to extend their lifespan, CF and normal control mice were kept on liquid diets (CFL and NL mice), and were sacrificed at different stages of their development (21 days of age, 6 days post weaning, 20 and 50 days post weaning). These mice did not show any signs of obstruction but the intestines had thin and delicate appearance. Mice were pooled in order to obtain sufficient material for enzyme assays, and to obtain average activity values. Specific enzyme

Table 3. Glycosyltransferase activities in mouse tissues

Tissue	*Substrate*	*Assay*	*Activity (nmol/h/mg)*			
			N	*NL*	*CF*	*CFL*
Polypeptide GalNAc-transferase						
colon	0.25 mg/ml Ac-PTPTGTQTPTTTPITTTTTVTPTNH$_2$	Dowex	22.6	9.9	10.9	1.5
ileum	1 mM VTSAPDTRPAPGST	Dowex	10.9	2.3	10.6	11.5
lung	1 mM VTSAPDTRPAPGST	Dowex	1.9	2.7	0.5	1.0
liver	1 mM VTSAPDTRPAPGST	Dowex	1.5	0.5	0.6	1.0
Core 1 β3-Gal-transferase						
colon	4 mM GalNAcα-Bn	HPLC	16.6	12.1	8.0	15.7
ileum	4 mM GalNAcα-Bn	Dowex	23.5	30.3	12.8	28.5
lung	4 mM GalNAcα-Bn	Dowex	0.8	2.1	0.3	<0.1
liver	4 mM GalNAcα-Bn	Dowex	5.9	4.1	4.7	3.0
kidney	4 mM GalNAcα-Bn	Dowex	0.5	6.0	1.4	11.3
duodenum	4 mM GalNAcα-Bn	HPLC	8.8	19.8	6.2	5.2
stomach	4 mM GalNAcα-Bn	Dowex	14.5	9.8	10.0	0.2
β4-Gal-transferase						
colon	4 mM GlcNAc	Dowex	13.1	9.7	6.1	0.3
Core 2 β6-GlcNAc-transferase						
colon	2 mM Galβ1-3GalNAcα-Bn	HPLC	21.8	18.0	14.3	1.2
Core 3 β 3-GlcNAc-transferase						
colon	4 mM GalNAcα-Bn	HPLC	1.4	<0.1	<0.1	0
ileum	4 mM GalNAcα-Bn	HPLC	0			
lung	4 mM GalNAcα-Bn	HPLC	<0.1			
liver	4 mM GalNAcα-Bn	HPLC	0			
kidney	4 mM GalNAcα-Bn	HPLC	0			
duodenum	4 mM GalNAcα-Bn	HPLC	<0.1			
stomach	4 mM GalNAcα-Bn	HPLC	0			
Core 1 sulfotransferase[a]						
colon	2 mM Galβ1-3GalNAcα-allyl	HVE	14.6	33.3	<0.1	8.6
ileum	2 mM Galβ1-3GalNAcα-allyl	HVE	1.3	0.7	0.2	4.7
lung	2 mM Galβ1-3GalNAcα-allyl	HVE	1.7	0.7	0.6	0.2
liver	2 mM Galβ1-3GalNAcα-allyl	HVE	0.4			
kidney	2 mM Galβ1-3GalNAcα-allyl	HVE	15.6			
duodenum	2 mM Galβ1-3GalNAcα-allyl	HVE	3.2			
stomach	2 mM Galβ1-3GalNAcα-allyl	HVE	5.1			

Glycosyltransferase and sulfotransferase activities were assayed as described in the Methods section in at least duplicate determinations with the substrates indicated. Rat colon homogenate was used as a positive control for all enzymes. Pooled tissues from the four groups (N, NL, CF, CFL) were assayed. N, normal mice; NL, normal mice fed liquid diet; CF, CF mice that died a natural death shortly after weaning; CFL, CF mice that were fed a liquid diet. HVE, high voltage electrophoresis.
[a]Activity expressed in pmol/h/mg.

assays of N, NL, CF and CFL mice for each tissue were always carried out simultaneously. The activities determined within the same experiment did not vary more than 5 to 10%. However, the activities in different pooled samples varied up to 50% in the cftr(−/−) population, indicating that there was considerable individual variation, possibly due to the pathology present.

A tissue survey showed that colon tissue had all the activities of the enzymes tested, including glycosyltransferases synthesizing core 1, 2 and 3 structures, β4-Gal-transferase and sulfotransferase acting on core 1 (Table 3). These model tissues therefore had the enzymes synthesizing more complex *O*-glycan structures. Colon, compared to other tissues, had high activity levels of polypeptide GalNAc-transferase using MUC2 mucin-derived substrate. Ileum also had high activities of polypeptide GalNAc-transferase using MUC1 mucin-derived substrate, and of core 1 β3-Gal-transferase and core 2 β6-GlcNAc-transferase, but had a lower activity of core 1 sulfotransferase and no detectable activity of core 3 β3-GlcNAc-transferase. Stomach mucosa and duodenal tissue were also active in core 1 synthesis and core 1 sulfotransferase but lacked core 3 β3-GlcNAc-transferase. High β4-Gal-transferase activities were found in the duodenum. However, it was difficult to establish exact activity values for transferases in duodenum, since this tissue had extremely high activities of enzymes that degrade

nucleotide sugars. Kidney homogenates showed low activity of core 1 β3-Gal-transferase but a relatively high core 1 sulfotransferase activity, comparable to the activity in the colon. Lung tissue exhibited relatively low activities of polypeptide GalNAc-transferase and core 1 β3-Gal-transferase, but had significant activity of core 1 sulfotransferase. Liver tissues, in contrast, had a similar polypeptide GalNAc-transferase activity but a high core 1 β3-Gal-transferase and a low core 1 sulfotransferase activity. In addition, significant sulfotransferase activity was also detected in ileum tissue from normal and CFL mice with 1 mM Galβ1-4GlcNAc as the substrate.

The patterns of relative tissue distribution of activities were similar in normal mice and normal mice fed a liquid diet. Some differences were observed; e.g. the activity of polypeptide GalNAc-transferase was relatively low in the ileum of NL mice, and of core 1 β3-Gal-transferase was relatively high in the duodenum. Core 3 β3-GlcNAc-transferase activity was not detected in the colon of NL mice.

Transferase differences between normal and cftr(−/−) mice

Generally, glycosyltransferases and sulfotransferases involved in *O*-glycan biosynthesis were active in the tissues isolated from CFTR knockout mice. Normal levels of polypeptide GalNAc-transferase were found in the ileum, lung and liver of CFL mice, while the colon had low activity. Core 1 β3-Gal-transferase appeared normal in all CFL tissues tested (Figure 1, Table 3) except for the lung where the activity was below detectable levels, and in the stomach which showed very low activity. β4-Gal-transferase and core 2 β6-GlcNAc-transferase were also very low in the colon of CFL mice. Core 3 β3-GlcNAc-transferase was not detected in the colon of CF, CFL and NL mice. Sulfotransferase acting on core 1 (Figure 1, path f) showed low levels in the lung but high levels of activity in the colon and especially in the ileum of CFL mice. Significant sulfotransferase activity was also detected in ileum tissue from normal and cftr(−/−) mice with 1 mM Galβ1-4GlcNAc as the substrate. Core 1 sulfotransferase was not detected in the colon of CF mice that were very ill and died of obstructive disease, and the activity in the ileum was very low while the lung showed normal levels. Thus intestinal obstruction particularly affected sulfotransferases in the colon and ileum, while other tissues, and other glycosyltransferases were much less affected in CF mice.

Alterations of activities during development of mice

In order to follow the activities during the development and during the course of the disease of cftr(−/−) mice, tissues were obtained from normal and CFTR(−/−) mice fed a liquid diet that were sacrificed at four stages pre- and post weaning. A general decrease in glycosyltransferase activities was observed in the colon of aging animals, in both normal and cftr(−/−) mice (Figures 3 to 7). Compared to normal mice, the activities of core 1 β3-Gal-transferase (Figure 4) and β4Gal-transferases

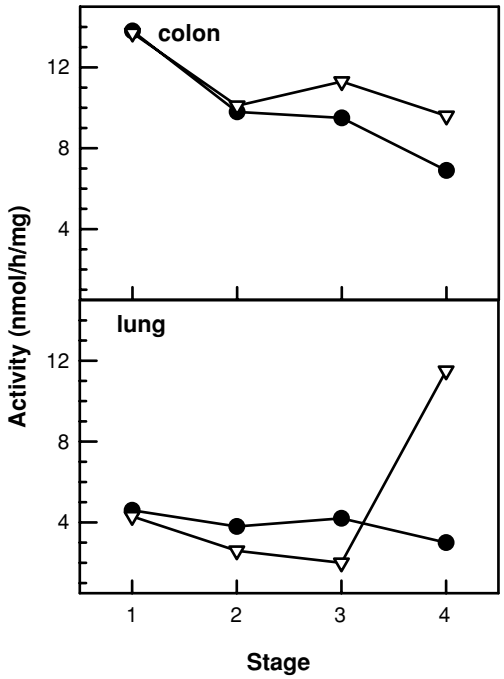

Figure 3. Polypeptide GalNAc-transferase activities in mouse tissues. Polypeptide GalNAc-transferase activities were assayed with 1 mM AcBzPTPPPNH$_2$ as the substrate by HPLC assays, in at least duplicate determinations, as described in the Methods section. Tissue homogenates from colon and lung at different stages of mouse development were obtained from mice at 21 days of age, just before weaning (stage 1), and 6 days (stage 2), 20 days (stage 3) and 50 days (stage 4) post weaning. Significant pathology was present in CF mice. NL, normal mice fed a liquid diet (closed circles); CFL, CF mice fed a liquid diet (open triangles).

(Figure 6) were lower in cftr(−/−) mice at all stages but that of core 2 β6-GlcNAc-transferase was similar (Figure 5), and polypeptide GalNAc-transferase activity (Figure 3) in colon and lung was higher in aging animals.

The ileum which was particularly affected by obstruction and inflammation did not show the same trends. Core 2 β6-GlcNAc-transferase activity in the ileum was similar in normal and cftr(−/−) mice, and did not fluctuate greatly throughout the four stages. Core 1 β3-Gal-transferase and β4-Gal-transferase activities were lower in the ileum of cftr(−/−) mice and declined in the later stages. Core 1 sulfotransferase activity showed an increase in stage 3, particularly in cftr(−/−) mice, but then declined in stage four.

Most activities in lung tissue were similar between normal and cftr(−/−) mice. Interestingly, at the late stage, most enzymes, e.g. polypeptide GalNAc-transferase, β4-Gal-transferase and core 1 β3-Gal-transferase, showed a noticeable increase in cftr(−/−) mice. Glycosyltransferase and sulfotransferase activities in liver, kidney and stomach were comparable in normal and cftr(−/−) mice. However, at the early stage of development activities were higher in normal mice compared to cftr(−/−) mice.

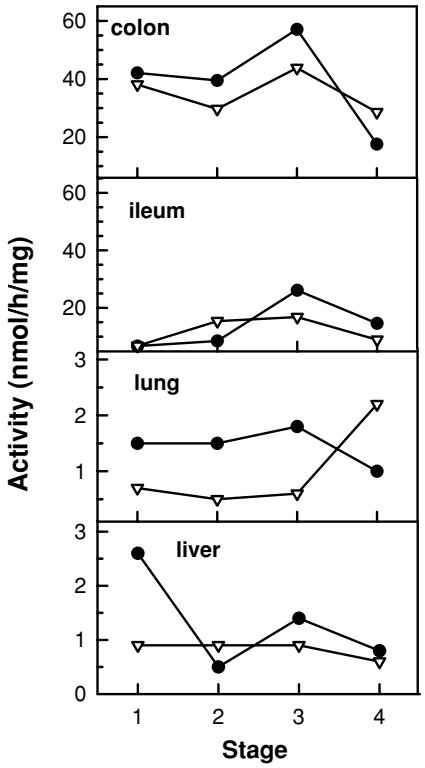

Figure 4. Core 1 β3-Gal-transferase activities in mouse tissues. Core 1 β3-Gal-transferase activities were assayed with 2 mM GalNAc-Bn as the substrate by HPLC in at least duplicate determinations, as described in the Methods section. Tissue homogenates from colon, ileum, lung and liver were obtained from mice at different stages of development, as indicated in Figure 3. NL, normal mice fed a liquid diet (closed circles); CFL, CF mice fed a liquid diet (open triangles).

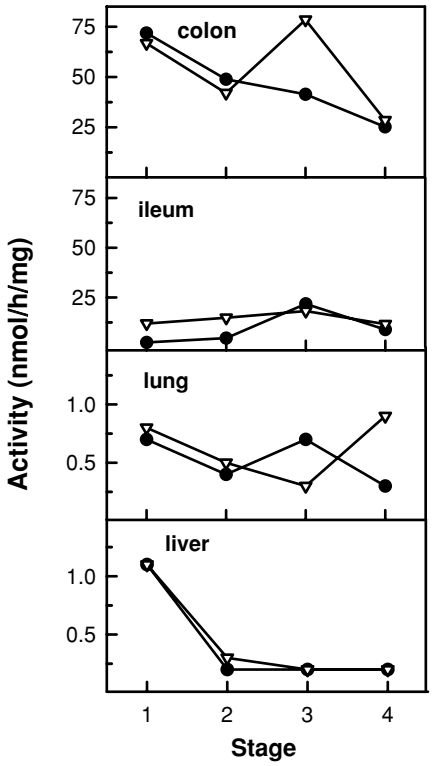

Figure 5. Core 2 β6-GlcNAc-transferase activities in mouse tissues. Core 2 β6-GlcNAc-transferase activities were assayed with 2 mM Galβ1-3GalNAcα-Bn as the substrate by Sep-Pak assays, in at least duplicate determinations, as described in the Methods section. Results were confirmed with Dowex and HPLC assays. Tissue homogenates from colon, ileum, lung and liver at different stages of mouse development were obtained as described for Figure 3. NL, normal mice fed a liquid diet (closed circles); CFL, CF mice fed a liquid diet (open triangles).

Discussion

It has been clearly shown that glycoproteins have different glycosylation patterns in cells with dysfunctional CFTR, such as cells or tissues derived from CF patients. The question that remains to be answered is how the lack of CFTR function or mislocalization of CFTR affects glycosylation, or alternatively, how CFTR directly affects glycosylation. One possibility is that the expression or function of CFTR alters the activities of enzymes involved in the biosynthesis of glycoproteins. This could be mediated by affecting the amounts of enzyme proteins produced, their posttranslational modifications, or by altering their activities in the ER and Golgi, for example by the presence of enzyme modifiers, changes in the membrane structure or changes in the lumenal pH, rate of transport of substrates, posttranslational processing of transferases, or other factors. In this study we examined how the maximal activities of Golgi glycosyl- and sulfotransferases involved in the biosynthesis of mucin type *O*-glycans and other glycans were affected by CFTR expression *in vitro* and *in vivo*. We did not discover general trends that could be correlated with CFTR expression. However, transfection with wild type CFTR or ΔF508CFTR caused a selective change of glycosyltransferase activity *in vitro*. *In vivo*, differences in glycosyl- and sulfotransferases were observed between normal and cftr($-/-$) mice. The advantage of using the CHO and BHK cell model was that these cells were less subject to inter-individual variations than the mouse model. The inflammation and obstructive disease in mice has introduced another parameter of complexity in the mouse model. The advantage of using the mouse model was that mouse tissues were similar to human tissues in their ability to synthesize complex mucin type *O*-glycans [38,45,Brockhausen unpublished] while CHO and BHK cells were restricted to the synthesis of *O*-glycans with core 1 structures.

Structural studies of glycoproteins produced in CHO and BHK cells showed the presence of sialylated *O*-glycans with core 1 structures [40,41]. CHO cells have been shown to express polypeptide GalNAc-transferase [47] and α3-sialyltransferase [48], as well as at least six β4-Gal-transferases [49] involved in the synthesis of type 2 *N*-acetyllactosamine backbone structures. Apparently, *N*-glycans can only be α2-3-sialylated but the α2-6Gal linkage cannot be synthesized [50,51]. BHK cells

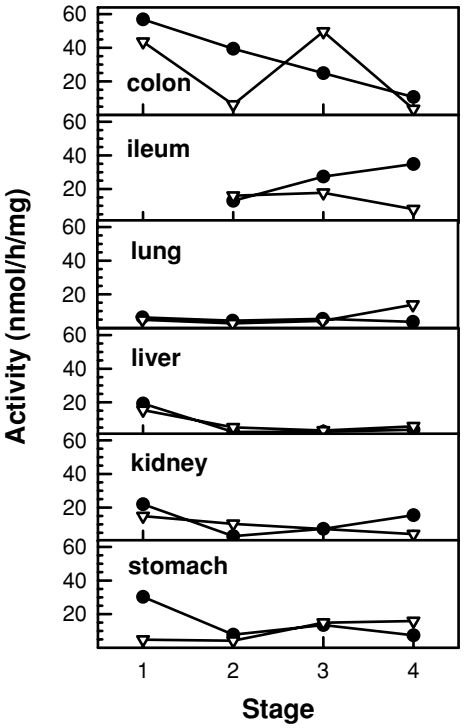

Figure 6. β4-Gal-transferase activities in mouse tissues. β4-Gal-transferase activities were assayed with 4 mM GlcNAc as the substrate by Dowex assays, in at least duplicate determinations, as described in the Methods section. Tissue homogenates from colon, ileum, lung, liver, kidney and stomach mucosa at different stages of mouse development were obtained as described for Figure 3. NL, normal mice fed a liquid diet (closed circles); CFL, CF mice fed a liquid diet (open triangles).

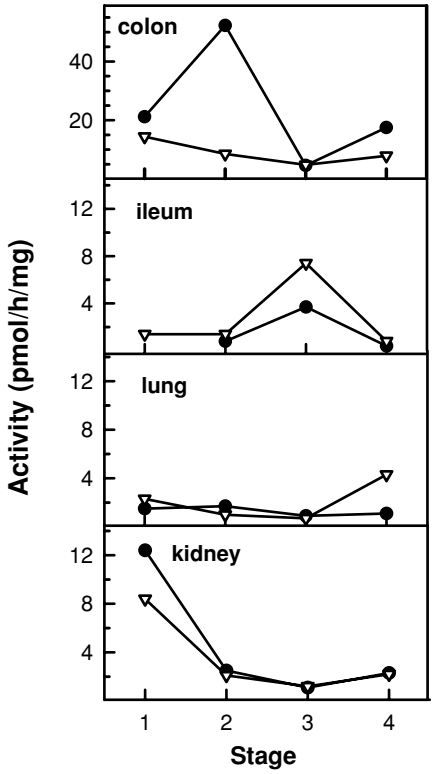

Figure 7. Sulfotransferase activities in mouse tissues. Sulfotransferase activities were measured in at least duplicate determinations by high voltage electrophoresis with 1 mM Galβ1-3GalNAcα-Bn as the substrate, as described in the Methods section. Tissue homogenates from colon, ileum, lung and kidney at different stages of mouse development were obtained as described for Figure 3. NL, normal mice fed a liquid diet (closed circles); CFL, CF mice fed a liquid diet (open triangles).

have previously been reported to contain the core 1 β3-Gal-transferase synthesizing core 1 from GalNAc-mucin and the β4-Gal-transferase synthesizing the Galβ1-4GlcNAc-R linkage [39]. BHK cells also have Fuc- and sialytransferases [52]. Structural analysis of glycoproteins expressed in BHK cells indicate the presence of the enzymes synthesizing α2-3-sialylated, core-α6-fucosylated, and *N*-acetyllactosamine-containing bi-, tri- and tetra-antennary *N*-glycans [40,53]. Although most of the backbone structures are type 2 chains, type 1 chains (Galβ1-3GlcNAc-) can also be found. The lack of α3-Fuc-transferase found in our study is consistent with the absence of Fucα1-3GlcNAc linkages in glycoproteins expressed in BHK cells [53].

We found that core 1 β3-Gal-transferase activities were similar in untransfected and CFTR transfected CHO and BHK cells, while ΔF508CFTR transfected cells had lower activities. In BHK cells, the β4-Gal-transferase activities paralleled those of core 1 β3-Gal-transferase. This suggests that less core 1 is being made in ΔF508CFTR-transfected cells. The total amount of unsubstituted core 1 present on BHK cell glycoproteins is expected to be a function of the relative activities of core 1 β3-Gal-transferase and the sialyltransferases modifying core 1. α3-Sialyltransferase was reduced in CFTR transfected CHO cells but in BHK cells was reduced in both transfected cell lines. Thus in CFTR transfected BHK cells, α3-sialyltransferase is 2.3 times lower relative to core 1 β3-Gal-transferase, and this could explain the increase in PNA binding. In ΔF508 BHK cells, α3-sialyltransferase is only 1.5 times lower, and therefore PNA binding is less affected. None of these enzyme activities correlate with CFTR expression, or CFTR function, although an effect of ΔF508CFTR on the expression of Gal-transferases cannot be ruled out. The activity levels did not correspond to the rate of cell growth. The lectin binding reflects the biosynthesis of glycoconjugates in the intact cell, while enzyme assays reflect the maximal activities of transferases under defined conditions. Thus from the lectin binding study it can be concluded that the expression of both CFTR and ΔF508CFTR influences the cell surface glycosylation. It is not likely that the effect is due to the presence of methotrexate in the culture medium of transfected cells (which is not present in untransfected cells) since CFTR and ΔF508CFTR cells differ significantly.

Binding studies with sialic acid binding lectins WGA, SNA and MAA lectin I suggest that BHK cells acquire more Galβ1-3

residues and sialic acid in 2–6 linkages when transfected with cftr, probably due to increased expression or activation of several different transferases. This would be consistent with reports of decreased sialylation in cells with nonfunctional CFTR [27,28]. Since α3-sialyltransferase activity was high and sulfotransferase was not detected, the CHO model does not appear to be satisfactory to explain the hypersulfation and hyposialylation in CF. BHK also are not useful for the study of glycoprotein sulfation, and α3-sialylation does not appear to depend on the presence of functional CFTR. Differences may have been a result of the overexpression or inappropriately targetted CFTR that possibly affected the activities of intracellular membrane bound enzymes. Thus glycosylation differences in these cell models do not appear to be due to CFTR dysfunction and it is possible that clonal variations between cultured cells are entirely responsible for differences in enzyme activities and lectin binding.

The pathways of *O*-glycan biosynthesis in BHK and CHO cells are much simpler than those in rat colon [42], and are restricted to the synthesis of core 1. This finding is consistent with published structures of glycoproteins produced in CHO and BHK cells [40,41]. Neither CHO cells nor BHK cells appear to be able to sulfate glycans with terminal Gal or GlcNAc residues. In contrast, mouse colon, and especially rat colon, have high activities of core 1 sulfotransferase involved in the synthesis of sulfated mucins which may be characteristic of colonic mucosa [46,54] although mouse kidney also seems to be a significant source for this activity. Animal tissues, especially mucin secreting tissues, may have the enzymes synthesizing more complex oligosaccharides than those found in CHO and BHK cells.

We assayed a number of glycosyltransferases in different mouse tissues, and related the activities to liquid diet, CFTR function, and to the development of mice and the course of the disease of cftr(−/−) mice. We could not find consistent alterations associated with CFTR function. Mouse tissues were shown to have the potential to synthesize complex mucin type *O*-glycans and can synthesize core 2 *O*-glycans in colon and ileum, and at lower levels in lung and liver. Activities in the colon and ileum of cftr(−/−) mice, tissues affected by obstruction and inflammation, appeared to be rather specifically regulated. This may be due to a cell type-specific effect of inflammatory cytokines. CF mice generally had lower activities, perhaps because these mice were extremely ill before death. CFL mice which were kept on a liquid diet rather than a solid food diet had a considerably longer life span and were much less affected by the disease. Several glycosyltransferases in a number of tissues in these CFL mice had normal levels, suggesting that CFTR dysfunction does not directly alter the levels of these enzymes but that the alterations seen were a result of a secondary effect of the disease. The present results also suggest that liquid diet had an influence on the activities in normal and cftr(−/−) mouse intestinal tissues.

We observed glycosyltransferase and sulfotransferase changes during mouse development in most tissues. This course of activities was found to be abnormal in cftr(−/−) mice. Transferases were generally fully active throughout the development, and a number of enzymes activities declined in the older animals at the later stage. The decline in activities in both normal and cftr(−/−) mice was especially prevalent in the colon which was affected by the disease. However, this decline was not seen in the ileum which also showed pathology, while the lung, which was apparently free of pathology, showed increases in several glycosyltransferases, and in core 1 sulfotransferase. It therefore appears that the developmental changes in activities are tissue-dependent, and in several tissues differ between cftr(−/−) and normal mice. According to our observations, mice with advanced disease may react to obstruction and mucosal inflammation by increased mucin biosynthesis. Concomitantly, sulfation is decreased in the colon throughout the stages but is increased in some of the animals in the lung and ileum at the later stages of the disease. This may reflect a mechanism by which sulfotransferases are activated similar to the increased sulfation in CF patient's mucins. The diseased tissues may produce significant levels of inflammatory cytokines which may influence the expression and activities of glycosyltransferases [43,55–59]. The *in vitro* models (CHO and BHK cells) studied here did not address this inflammatory effect, while cftr(−/−) mouse tissues such as the ileum and colon, and possibly other organs, especially at the later stages of the disease, are clearly affected by inflammation. Future work will focus on this influence of cytokines on glycosylation as a secondary response to inflammation and infections in CF tissues. It could also be useful to examine human epithelial cells and tissues which may reflect the glycosylation patterns of human mucins.

Acknowledgments

This work was supported by a grant from the Canadian Cystic Fibrosis Foundation and a Research Scientist Award from The Arthritis Society to Inka Brockhausen. The authors are grateful to Dr. Geraldine Kent for supplying the mice; Dr. David Williams for providing CHO cells, and to Dr. Gergely Lukacs for providing BHK cells. We thank Wensheng Qin for growing CHO cells, and Michael Lehotay, Jimmy Yang, Verena Pollmann, Elham Mohammadi, and Lilly Jain for carrying out glycosyl- and sulfotransferase assays. We also thank Dr. John Schutzbach for his help with the preparation of the manuscript.

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Received 10 April 2002; accepted 22 May 2002