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Review

Terminal glycosylation in cystic fibrosis

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Abstract

Cystic fibrosis (CF) is a common genetic disease for which the gene was identified within the last decade. Pulmonary disease predominates in this ultimately fatal disease and current therapy only slows the progression. CF transmembrane regulator (CFTR), the gene product, is an integral membrane glycoprotein that normally functions as a chloride channel in epithelial cells. The most common mutation, ΔF508, results in mislocalization and altered glycosylation of CFTR. Altered fucosylation and sialylation are hallmarks of both membrane and secreted glycoproteins in CF and the focus here is on these investigations. Oligosaccharides from CF membrane glycoproteins have the Lewis x, selectin ligand in terminal positions. In addition, two major bacterial pathogens in CF, *Pseudomonas aeruginosa* and *Haemophilus influenzae*, have binding proteins, which recognize fucose in α1,3 linkage and asialoglycoconjugates. We speculate that the altered terminal glycosylation of airway epithelial glycoproteins in CF contributes to the chronic infection and robust inflammatory response in the CF lung. Understanding the effects of mutant CFTR on glycosylation may provide further insight into the regulation of glycoconjugate processing as well as therapy for CF.

Keywords: Cystic fibrosis; Terminal glycosylation; Cystic fibrosis transmembrane regulator; Glycosylation in cystic fibrosis

Contents

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1. Introduction

Interest in glycosylation has been rekindled in the field of cystic fibrosis (CF) research since the identification of the CF gene, named the CF transmembrane regulator (CFTR) in 1989 [52,82,85]. The renewed interest has been fueled by several recent developments resulting from attempts to reconcile the proposed function of CFTR with phenomena known to be involved in the pathogenesis of the disease. Prior to the identification of the CFTR gene, many laboratories had described alterations in the glycosylation of CF glycoproteins, but no connection between the altered glycosylation and the pathogenesis of CF was established. The definition of the structure of oligosaccharides in CF glycoproteins which are similar to those involved in the adherence of bacterial pathogens and the recruitment of inflammatory cells is among the most promising of the new developments. In this article we review the background of altered glycosylation in CF and discuss glycosylation in relation to knowledge about CF which has accumulated since the identification of the CFTR gene.

2. Clinical presentation

CF is the most common, lethal, genetic disease of Caucasians. CF demonstrates an autosomal recessive pattern of inheritance. In the USA, the incidence of the disease in Caucasians is approx. 1 in 3300 live births and in African Americans, 1 in 15 300. The frequency of unaffected heterozygote carriers of a CF mutation is 1 in 29 in persons of Northern European ancestry. Chronic, progressive pulmonary disease dominates the clinical picture. However, all exocrine glands are affected to some degree in CF patients and abnormalities of the pancreas, liver, gall bladder, sweat glands and reproductive organs are among the protean manifestations of CF [83,115]. Advances in treatment have extended the life expectancy to approx. 30 years. Current treatment is only supportive and slows, but does not stop the progression of the disease.

The survival of patients with CF is determined in large measure by the progression of their lung disease. Mucous gland hyperplasia is an early pathologic manifestation [103] and presumed hypersecretion of the abnormally viscous mucous [12,86,87] plays a role in the airways obstruction. Colonization of the airways with pathogenic bacteria, predominately *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Staphylococcus aureus*, is a hallmark of CF. The course of the disease is usually marked by recurrent exacerbations of the pulmonary infection, each of which causes further destruction of airway tissue. The marked predominance of neutrophils in the airway inflammatory response has been known for some time [105]. However, only recently has the possible primary nature of the inflammatory response been appreciated. This latter concept was prompted by the finding of neutrophils in bronchoalveolar lavage fluid from asymptomatic infants who were diagnosed with CF in a newborn screening program [53].

3. CFTR

CF is caused by mutations in a single gene named the *CF transmembrane regulator* (CFTR). The CFTR gene spans approx. 230 kb of DNA and contains 27 exons. The most common CF mutation, and the first to be described, is a three-base pair deletion in exon 10 that causes a deletion of phenylalanine from position 508 (ΔF508) of the CFTR glycoprotein. This mutation accounts for 66% of CF mutations. More than 700 CF mutations have now been reported, and the list continues to grow.

The CFTR gene codes for a transmembrane glycoprotein with 12 membrane spanning domains and

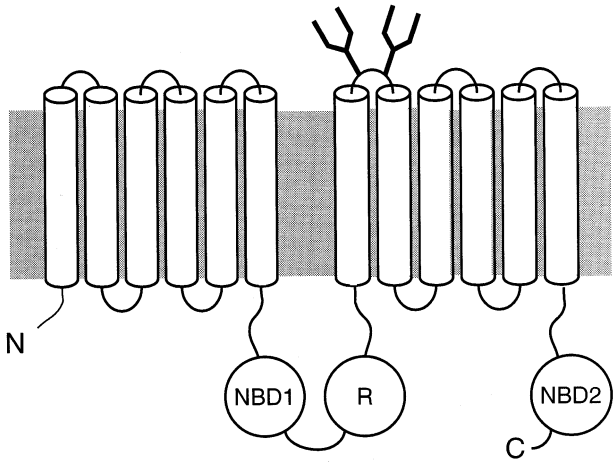

Fig. 1. Domain model of the cystic fibrosis transmembrane conductance regulator (CFTR). Based on hydrophobicity plots, CFTR has 12 transmembrane spanning domains, two nucleotide (N) binding domains (NBD 1 and NBD 2), and a regulatory R domain. The two potential *N*-linked glycosylation sites are at positions 894 and 900 in the fourth extracellular loop. Used with permission from [83].

two Asn-linked *N*-glycosylation sites (Fig. 1). The glycoprotein has been reported to occur in three forms: an immature form (M_r 145–160 000), a mature form (M_r 180–200 000), and an intermediate sized form. The immature form is not fully processed and is most prominent with the ΔF508 mutation. Pulse and chase experiments with wtCFTR were used initially to follow the maturation to the 180–200 000 form [19].

The CFTR gene was first proposed to encode an integral membrane glycoprotein, which functioned as a regulated chloride channel in epithelial cells [82]. While it has been clearly shown that the CFTR glycoprotein does function as a chloride channel at the apical surface of epithelial cells which are involved in the pathology of CF, other compelling evidence supports additional functions for CFTR in endosomes, clathrin coated vesicles, endoplasmic reticulum and surface membranes [5,6,14,30,44,47,59,68,73,75,80,89,93,112]. The high degree of cross-species conservation of CFTR suggests a crucial role in the functions of a cell [30]. However, how the altered function of CFTR is directly related to the pathogenesis remains an unanswered question. The solution of this problem is of paramount importance to the design of more effective therapeutic strategies.

4. Gene therapy for CF with glycosylated vectors

Gene therapy for CF was proposed as a simple solution for the treatment of the disease [23,24]. The initial efforts were focused on using recombinant adenoviral vectors to treat the lung disease since this organ is directly accessible through aerosol delivery and adenoviruses were known to be trophic for human respiratory epithelia. However, despite the employment of a variety of strategies, the use of viral vectors has not yet been successful [108]. The main obstacle to date has been the immune response, which was generated to the viral proteins [88].

Another approach took advantage of endogenous lectins on the airway cell surface and targeted carbohydrate-substituted polylysines to the CF cells. Lactosylated polylysine was demonstrated to be the most efficient vector for airway epithelial cells in culture [54]. Lactosylated polylysine was also shown to achieve the same efficiency as viral vectors in transferring reporter genes [55] or the CFTR gene [56] into either immortalized airway cells or airway epithelial cells in primary culture. It was proposed that the airway cells contained galactose/lactose binding proteins on their cell surface. Support for this proposal was provided by the inhibition of lactosylated polylysine-mediated gene transfer by lactose but not by mannose [54]. The uptake of polylysine and BSA into liver and macrophages following glycosylation with galactose and fucose, respectively [36,72,109], provides proof of principle that such methods are potentially feasible for targeted gene or drug delivery to a variety of diseased organs. While this topic may be unrelated to the role of altered glycosylation in the pathogenesis of CF, it serves to illustrate that an understanding of the functional roles of carbohydrates in cell biology may provide another approach to the therapy of CF.

5. Alteration in terminal glycosylation

CFTR when mutated, alters the terminal glycosylation of other glycoproteins through a mechanism which is yet to be defined. Tables 1 and 2 present a selected rather than an exhaustive summary of the literature. The tables provide substantial evidence concerning the alteration of terminal glycosylation

Table 1
Abnormal terminal glycosylation in CF determined by chemical/structural analysis[a]

Alterations in CF[b]	Source	Fraction examined	Year	Ref.
↑ Ratio Fuc:NeuAc	Duodenum	Mucins	1959	[31]
↑ Ratio Fuc:NeuAc	Rectum	Mucins	1967	[84]
↑ SO_4	Lung	Mucins	1967	[60]
↑ Fuc; ↓ NeuAc	Serum	Heparin bound glycoproteins	1979	[77]
↓ NeuAc	Plasma	α_2-Macroglobulin	1979	[9]
↑ Fuc	Meconium	Mucus glycoproteins	1979	[21]
↑ Fuc	Fibroblasts	Membrane glycopeptides	1980	[97]
Novel Fuc oligosaccharide sequences	Lung	Mucins	1982	[107]
↑Fuc	Fibroblasts	Med; Heparin bound glycoproteins	1982	[98]
↑ Ratio Fuc:NeuAc	Intestine	Mucins	1983	[116]
↓ NeuAc and Gal	Serum	Immunoglobulin G	1983	[70]
NeuAc and Fuc novel oligosaccharides	Lung	Mucins	1984	[61]
↑ Fuc	Fibroblasts	Peripheral glycopeptides	1985	[99]
↑ Fucα-1,6 and α1,3-linkage; ↓ NeuAc	Fibroblasts	Peripheral glycopeptides	1990	[110]
↑ Ratio Fuc:Gal	Fibroblasts	*N*-Linked glycopeptides	1991	[10]
↑ Fuc; ↑ NeuAc; ↑ SO_4	Salivary gland	Mucins	1993	[16]
↓ NeuAc	Immortalized CF AEC[c]	Peripheral glycoconjugates	1998	[100]
Tri Fuc heptasaccharide	Sputum	Mucins	1998	[106]

[a]Selected summary.
[b]↑ increased; ↓ decreased.
[c]Airway epithelial cells.

in CF, not only in the mucins, but also in *N*-linked glycoconjugates which are cell-associated or secreted. An earlier review provides additional background [3].

On reviewing Tables 1 and 2, it is apparent that the pursuit of the altered glycosylation in CF received less attention immediately after the CFTR gene was cloned in 1989 [82] and after the subsequent definition of the gene product as an apical Cl^- channel in 1990 [33]. The process of unraveling the details of the function and processing of CFTR has been the subject of many investigations [114]. It is only in the past 5 years that alterations in glycosylation are once again being actively pursued. This results from two reasons: (1) the inability to explain the pathology of the disease based solely on a defective apical epithelial Cl^- channel; and (2) a cure via gene therapy has proven elusive.

It is clear from the earlier studies that the mucin carbohydrate compositions are different (Tables 1 and 2). However it was not until differences from normal in CF fibroblast membrane glycoproteins and CF serum glycoproteins were reported that a generalized defect of glycosylation in CF was considered. Most importantly, improvements in carbohydrate analytical methods, e.g., lectin chromatography, purified enzymes which are specific for sugar and linkage, ^{1}H-NMR and FAB spectroscopy, as well as general advances in molecular biology and cloning, have increased the capabilities of glycobiologists to probe cell function and disease pathogenesis.

Table 1 summarizes some of the most influential reports describing glycosylation alterations in CF. Roussel et al. [90] provided an early analysis of sulfate in CF mucins. Boat and colleagues described an increased sulfate incorporation in CF mucus glycoproteins [11]. Additional reports have confirmed this increased sulfation of CF mucins (Tables 1 and 2). One report correlated the increase in sulfation in CF with increasing severity of illness [17]. Gerkin and Gupta [38] provided a comprehensive review of the alterations in the size, structure, sulfation and glycosylation of CF mucins. In their review, areas of conflicting results and interpretations are identified and discussed. In the present discussion of terminal glycosylation in CF, the emphasis is placed primarily on integral cell membrane glycoconjugates.

Dische's laboratory [31] was the first to describe an altered ratio of fucose to sialic acid in CF duodenal mucins. Two decades later, it was shown that CF

Table 2
Abnormal terminal glycosylation in CF detected by a variety of methods[a]

Alterations in CF[b]	Method	Source	Fraction examined	Year	Ref.
↓ NeuAc	Asialo-GM_1 binding	CF patients	*Pseudomonas*	1988	[57]
↑ SO_4	Isotope ratio	Primary nasal polyp	Glycoconjugates	1989	[18]
Defective processing	Western blot	COS cells[c]	Mutated CFTR	1990	[19]
↑ Fuc	LTG binding	Small intestines	Mucins	1990	[104]
↓ NeuAc	Isotope ratio	Immortalized AEC[d]	Glycoconjugates	1991	[5]
↓ NeuAc	*Pseudomonas* binding	Pili	Asialo-GM_1	1993	[92]
↑ Con A; WGA	Binding	Serum and meconium	α1-Antitrypsin	1993	[35]
↓ NeuAc	Transferase activity	CF-PAC cells[e]	α2,6-NeuAc transferase	1993	[6]
↑ NeuAc	*Pseudomonas* binding	Salivary gland	Mucins	1993	[16]
↑ Fuc	Lentil lectin binding	AEC	Membrane glycopeptides	1994	[62]
↓ NeuAc	FITC lectins	Transfected C127 cells[f]	Cell histochemistry	1994	[32]
↓ NeuAc	*Pseudomonas* binding	Lung	Mucins	1994	[29]
↓ NeuAc; ↑ Fuc	Lectin binding	ΔF508 transfected	Cell surface	1995	[117]
↑ SO_4	Xenograft mouse	Human bronchial cells	Respiratory mucous	1995	[121]
↓ NeuAc	Cholera toxin binding	ΔF508 AEC	Cell surface	1995	[58]
↓ NeuAc	*Pseudomonas* binding	Immortalized AEC	Asialo-GM_1	1995	[47]
↑ NeuAc; ↓ Fuc	α1,3/4-Fucosidase	Transfected CF/T1 cells[g]	Membrane glycoconjugates	1997	[76]
↑ Fuc	PA II fucose binding lectin	Primary cells	Cilia	1997	[1]
↑ SO_4	HPLC	*cftr* knockout mouse	Liver and ileum	1997	[46]
↓ NeuAc	Immunohistochemistry	Immortalized AEC	Asialo-GM_1	1998	[15]
↑ NeuAc; ↑ Fuc	Lectin binding	*cftr* knockout mouse	Lung sections	1998	[22]

[a]Selected summary.
[b]↑ increased; ↓ decreased.
[c]COS, monkey kidney cells.
[d]Airway epithelial cells.
[e]CF pancreatic tumor.
[f]Mammary tumor.
[g]CF cell line transfected with wtCFTR.

serum glycoproteins and CF fibroblast surface membrane glycoproteins had an altered ratio of fucose to sialic acid [77,97]. The latter report [97] was confirmed by GLC and lectin chromatography [99] and subsequently by high resolution, ^{1}H-NMR spectroscopy [110]. The data obtained permitted the definition of a structure that accounted for the increased fucosylation and decreased sialylation of oligosaccharides from CF fibroblast surface membranes (Fig. 2). The specific alterations observed were a de-

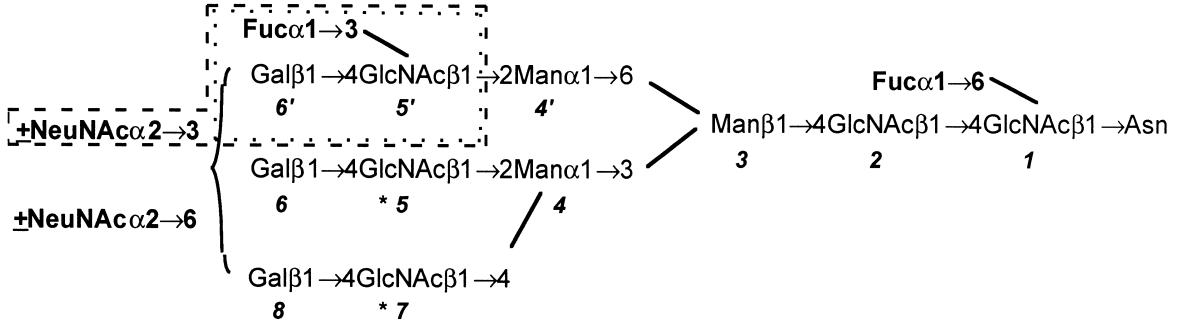

Fig. 2. The proposed structure for a surface membrane glycopeptide isolated from CF fibroblasts. The salient terminal glycosylations are presented in bold. * represents additional sites for α1,3 fucosyl residues; dotted lines enclosing the glycosyl residues are potential bacterial or selectin binding sites which may be sialylated as represented by the dashed lines. Adapted from Wang et al. [110].

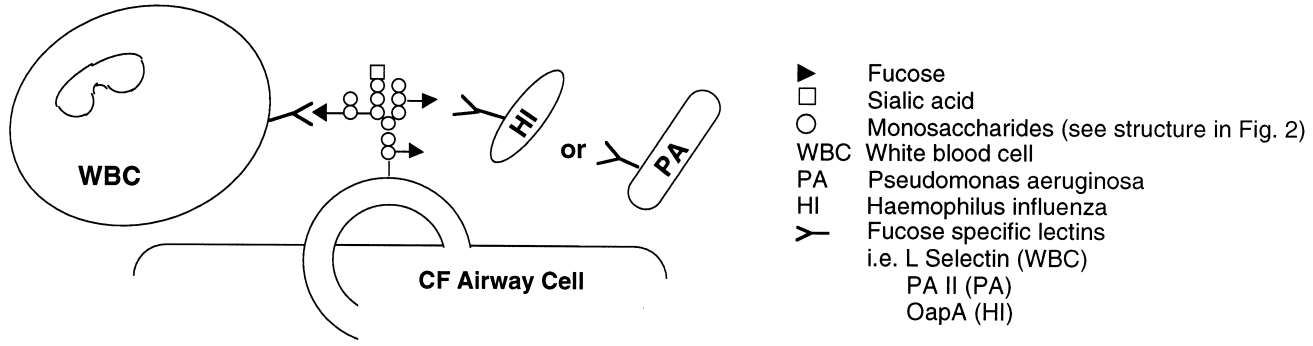

Fig. 3. Schematic depicting potential binding sites of bacterial pathogens and neutrophils on CF airway epithelial cells. The airway cell is depicted to have the glycosylation sites (see Fig. 2) necessary for fucose binding lectins from bacteria or the selectins from WBC. The CF cell glycoconjugates are low in sialic acid and *Pseudomonas* (PA) binds to the terminal Gal/GalNAc residues.

crease in terminal sialic acid residues and an increase in fucosyl residues linked α1,3 and α1,6 to antennary or core GlcNAc residues, respectively. In this comparison, it was reported that the CF glycopeptides examined had 25% less sialic acid and two times more Fuc α1,6 linked to core GlcNAc residues. That is, 50% and 25% of the GlcNAc-1 residues of CF and control glycopeptides, respectively, contained α1,6 linked fucose. There was no Fuc α1,3 GlcNAc in the non-CF glycopeptides. Recently, these carbohydrate alterations were shown in airway epithelial cells by lectin chromatography for α1,6 fucosyl residues [62] and by sialic acid analysis [100] of membrane glycoproteins. At the same time, the presence of fucosyl residues linked α1,3/4 to GlcNAc was shown with the use of an α-L-fucosidase, specific for these linkages [76]. The difference in sialic acid content [100] of CF airway cells corrected with wtCFTR was significant (130 nmoles vs. 9 nmoles per mg protein for the cells corrected with wtCFTR vs. CF cells, respectively).

Although methods of structural analyses of carbohydrates have been refined with higher resolution ^{1}H-NMR spectroscopy and with FAB mass spectroscopy, investigators have turned to cell and molecular biology to examine the CF cells. The ready availability of airway epithelial cell lines since the early 1990s has also contributed to more recent progress [42,49,74,78,81,120]. Recent approaches include histochemistry with lectins or antibodies and binding of bacteria or toxins to immortalized or primary airway cells. In addition, lectin affinity chromatography has been used to characterize the glycoproteins. The change in analytical emphasis is seen by comparing the reports in the literature obtained by chemical/structural determinations (Table 1) with those obtained by other methods (Table 2). Table 2 summarizes selected investigations of CF cells, which lend support for altered terminal glycosylation. For example, Weyer et al. [117] reported that transfection of epithelial cells with mutant CFTR led to the expression of the CF glycosylation phenotype, decreased sialyl and increased fucosyl residues at the cell surface. Kube et al. [58] demonstrated that transfection of normal airway epithelial cells with ΔF508 CFTR cDNA resulted in decreased binding to elderberry bark lectin which is specific for sialic acid linked α2,6. They also showed decreased binding of cholera toxin, which binds GM_1 [57], thus, suggesting decreased sialylation of both glycoproteins and glycolipids. In most, but not in all cases, [50] cells transfected with the CFTR gene have provided the most striking evidence that glycosylation of the CF cells is altered. These studies prompted Barasch et al. [5] to propose that altered Cl^- conductance within the CF cell affects glycoprotein processing.

6. α-L-Fucosidase

Abnormalities in CF α-L-fucosidase have been reported in respect to distribution [95,96], carbohydrate composition [4], growth cycle of the cell [69], and isoenzyme patterns [2,97]. It was reasoned that any of these abnormalities could be explained by processing defects. However, in contrast to other acid hydrolases, which had been previously examined [63], α-L-fucosidase from normal fibroblasts was not

proteolytically processed. Recently, the increased activity of α-L-fucosidase was also observed in CF airway epithelial cells [62] when compared to that of non-CF cells. At the same time, the activities of other lysosomal hydrolases, which were examined, were not increased. Not all investigators observed an increase in α-L-fucosidase [43].

The fact that α-L-fucosidase activity as well as the enzyme protein in CF airway epithelial cells were elevated led to the suggestion that CFTR may play a role in the regulation of glycoprotein processing and/or secretion. It is possible that the altered distribution of α-L-fucosidase is secondary to altered glycosylation of this enzyme, which is processed without proteolytic cleavage [51,63].

7. CFTR glycoprotein trafficking

CFTR mutations have been grouped into four or five classes, depending on the effect of the mutation on the expression, processing, and function of the protein [25]. The most common mutation, ΔF508, is a processing mutation in which very little of the mutant protein reaches the apical surface. Some mutations (e.g., G542X) produce a truncated transcript and no protein, while other mutations (e.g., R117H) produce a protein that has impaired conduction properties.

A number of experiments [19,28,34,41,118] have supported strongly the mislocation of CFTR as a cause of defective function. It has been proposed that ΔF508 CFTR does not leave the endoplasmic reticulum (ER) in a timely manner [19] and that this may be due to the binding to calnexin, a molecular chaperone of the ER [79]. However, mutated CFTR has been shown to have Cl^- channel activity in the ER [75]. Moreover, ΔF508 CFTR is functional in the surface membrane under certain conditions such as reduced temperature [27] and overexpression in amphibian [34] or insect [64] cells or when overexpressed in butyrate-treated CF cells [20,91]. These results suggest that ΔF508 CFTR can, in some circumstances, bypass the quality control of the ER [45] and assume its role as an apical Cl^- channel. Complicating the issue is the fact that ΔF508 CFTR protein has Cl^- channel activity when isolated and reconstituted into an artificial lipid bilayer [64].

CFTR is proposed to be incompletely processed in the ER in some mutations. It has been reported [19] that a size decrease in the oligosaccharides of CFTR is responsible for the immature CFTR observed in CF cells. It is generally accepted that the most common mutation ΔF508 falls into this category [114]. It is believed that due to the lack of processing, mutated CFTR is degraded in the ER and never traffics to the cell surface. As mentioned in a number of reports [20,27,34,64,117], mutated CFTR has been shown to be present in the cell surface membrane but not always fully active. Therefore, although the results are compatible with faulty processing, some ΔF508 CFTR is trafficked to the surface membrane. Of interest also is the fact that trafficking in polarized MDCK cells from the basolateral surface to the apical membrane is influenced by factors [39] which also restore the Cl^- channel activity of CFTR, such as temperature and sodium butyrate.

Degradation of CFTR may be mediated by a pathway involving ubiquitination and proteasomes [48,94,111]. It has been reported that the ER membrane trimeric complex sec61β may transport CFTR into the cytoplasm where after deglycosylation, CFTR is targeted for degradation. Both wild type and mutant CFTR appear to undergo degradation via the same pathways [7]. Wei et al. [112] reported that the degradation of CFTR occurred at a faster rate than new synthesis in the presence of castanospermine, a glycosylation processing inhibitor. It was concluded that the lack of the proper oligosaccharides on mutant CFTR may contribute to its more rapid degradation. The inhibitor-induced lack of glycosylation may be similar to the deglycosylated intermediary reported [7] prior to degradation. Thus although CFTR has only two potential glycosylation sites (Fig. 1), glycosylation appears to be the key to CFTR survival. In both wild type and mutant CFTR, folding and processing is extremely inefficient [71,112].

In a study examining the lungs of a *cftr*(−/−) knockout mouse, it was found that both sialic acid and fucose were increased at the surface of the lung cells. The lectins, SNA and UEA, were used to detect glycosylation in histological sections [22]. In utero treatment of the knockout mice with *cftr* resulted in a change of location of the lectin binding from the surface to internal vesicles of the lung cells. These

studies support a role for CFTR mediating vesicular trafficking as well as altering glycosylation. In this context brefeldin A, which disrupts the Golgi [65], increased the fucosylation difference between CF and non CF airway cells [62].

8. Glycosylation related to pathogenesis

Although a defect in the acidification of intracellular vesicles by mutant CFTR may produce faulty terminal glycosylation [5], it does not, in and of itself, account for the pathology seen in CF. We hypothesize that the structural alterations in the CF surface glycoconjugates (Fig. 2) could be responsible for the differences in binding characteristics of both bacteria and leukocytes (Fig. 3). Since one major alteration in CF cells is an increase in terminal fucosylation, the predominance of these antennary fucosyl residues may be an important factor contributing to bacterial adherence and neutrophil recruitment in the CF airway. *Pseudomonas* [40] and *Haemophilus* [66,113] are known to have fucose binding proteins and these may play an important role in the predominance of these two pathogens in the pathogenesis of CF lung disease.

Of interest also is the fact that ciliary beating in explants of CF primary tracheal cells was inhibited by PAII lectin [1], a fucose binding protein purified from *P. aeruginosa* [40]. Krivan et al. demonstrated that *Pseudomonas* binds to asialo-GM_1 [57]. Since that time several laboratories have each demonstrated increased binding of *Pseudomonas* to airway cells, which are homozygous for ΔF508 [15,26,47,119]. These investigations confirm that CF airway cells have increased amounts of asialo-GM_1 when compared to non-CF cells, which contain GM_1. These findings also confirm that *Pseudomonas* binds more avidly to the asialo-GM_1 surface receptor. It is possible that *Pseudomonas* binding is facilitated by the interaction of increased terminal fucosyl residues with PAII and that a decrease in sialic acid residues permits additional binding through PAII as well as promoting the binding of *Pseudomonas* to asialo-GM_1 via the action of a Gal/GalNAc binding protein. It is apparent from a comprehensive review by Scharfman et al. [101] that there are multifactorial interactions between *Pseudomonas* and host respiratory glycoconjugates. Bentzmann et al. hypothesized that *Pseudomonas* binds only to damaged airway epithelial cells [8].

Cholera toxin binds to GM_1 [57], CF tissue contains a mixture of glycolipids including asialo-GM_1 and exhibit decreased toxin binding. It is known that CF cells have decreased sialylation (Tables 1 and 2). Therefore, it is not surprising that CF patients may not be susceptible to this toxin. Other toxins may also be included in this effect. It has been hypothesized that CF mutations persisted among caucasians in the middle ages because cholera toxin does not bind readily to CF cells and therefore provided a heterozygote advantage during epidemics [37]. As a result, CF mutations were maintained at a relatively high frequency in the gene pool of European populations. Ironically, the protection provided to heterozygotes against one pathogen in the past may render CF patients more susceptible to the bacteria which ultimately cause the principal morbidity of the disease.

The defined structure (Fig. 2) which is characteristic of the CF glycosylation phenotype contains the basic ligand for selectins [67]. We hypothesize that the glycoconjugates, terminally glycosylated as shown in Fig. 2, are responsible for the leukocytes in the airways since the fucosyl residues are prevalent on the airway epithelial cells from CF patients [76]. The binding of bacteria and leukocytes depicted in Fig. 3 may account for much of the pathology of the CF lung. The use of carbohydrate mimetics may be a valid therapeutic approach.

9. Speculation

In summary, specific mutations in the CFTR gene, especially the most common ΔF508, result in the production of a protein which is mislocalized and which is not properly glycosylated when compared to mature, wild-type CFTR. This triggers a cascade of events, including aberrant glycosylation of membrane glycoconjugates and mucins. We have demonstrated that a major alteration in CF glycosylation is the synthesis of a highly fucosylated oligosaccharide which is only minimally sialylated. The terminal, antennary sugars on this oligosaccharide have been characterized by ^{1}H-NMR and form the Lewis x

antigen with Fuc linked α1,3 to GlcNAc [110]. This structure is characteristic of the selectin ligand which is involved in leukocyte recruitment. The two most important pathogens in CF, *P. aeruginosa* and *H. influenzae*, have binding proteins, which recognize α1,3-linked Fuc and asialoglycoconjugates. We hypothesize that altered glycosylation of CF airway glycoproteins is a key element contributing to the chronic bacterial infections and robust inflammatory response, hallmarks of CF lung disease.

How does CFTR, a Cl^- channel, contribute to the glycosylation in epithelial cells and their secretions? It has been proposed that the defective function of the mutated CFTR protein could affect the pH of intracellular vesicles [6]. As proposed, acidification would inhibit the activity of the terminal sialyltransferases in the Golgi. Decreased activity of the sialyltransferases would lead to decreased sialylation and in turn this could lead to increased fucosylation on CF oligosaccharides. This series of events provides a plausible explanation for the predominance of the increased fucosylation and decreased sialylation of CF glycoproteins listed in Tables 1 and 2. Although the hypothesis of Barasch and Al-Awqati [6] is disputed [102], it remains the basis for the most plausible explanation put forward thus far. An alternative hypothesis is that the turnover rate of mutant CFTR in the surface membrane could effect the recycling time in the Golgi and thus the degree of the terminal glycosylation of the recycled membrane glycoproteins [13,112]. Although there are several proposals of how CFTR effects glycosylation, none has been proven. Full understanding of the function and regulation of CFTR may provide further insight into the regulation of glycoprotein processing as well as information regarding the pathogenesis of CF.

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