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Human airway mucin glycosylation: A combinatory of carbohydrate determinants which vary in cystic fibrosis

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Human airway mucins represent a very broad family of polydisperse high molecular mass glycoproteins, which are part of the airway innate immunity. Apomucins, which correspond to their peptide part, are encoded by at least 6 different mucin genes (*MUC1, MUC2, MUC4, MUC5B*, *MUC5AC* and *MUC7*). The expression of some of these genes (at least *MUC2* and *MUC5AC*) is induced by bacterial products, tobacco smoke and different cytokines.

Human airway mucins are highly glycosylated (70–80% per weight). They contain from one single to several hundred carbohydrate chains. The carbohydrate chains that cover the apomucins are extremely diverse, adding to the complexity of these molecules. Structural information is available for more than 150 different *O*-glycan chains corresponding to the shortest chains (less than 12 sugars).

The biosynthesis of these carbohydrate chains is a stepwise process involving many glycosyl- or sulfo-transferases. The only structural element shared by all mucin *O*-glycan chains is a GalNAc residue linked to a serine or threonine residue of the apomucin. There is growing evidence that the apomucin sequences influence the first glycosylation reactions. The elongation of the chains leads to various linear or branched extensions. Their non-reducing end, which corresponds to the termination of the chains, may bear different carbohydrate structures, such as histo-blood groups A or B determinants, H and sulfated H determinants, Lewis a, Lewis b, Lewis x or Lewis y epitopes, as well as sialyl- or sulfo- (sometimes sialyl- and sulfo-) Lewis a or Lewis x determinants. The synthesis of these different terminal determinants involves three different pathways with a whole set of glycosyl- and sulfo-transferases.

Due to their wide structural diversity forming a combinatory of carbohydrate determinants as well as their location at the surface of the airways, mucins are involved in multiple interactions with microorganisms and are very important in the protection of the underlying airway mucosa.

Airway mucins are oversulfated in cystic fibrosis and this feature has been considered as being linked to a primary defect of the disease. However, a similar pattern is observed in mucins from patients suffering from chronic bronchitis when they are severely infected. Airway mucins from severely infected patients suffering either from cystic fibrosis or from chronic bronchitis are also highly sialylated, and highly express sialylated and sulfated Lewis x determinants, a feature which may reflect severe mucosal inflammation or infection.

These determinants are potential sites of attachment for *Pseudomonas aeruginosa*, the pathogen responsible for most of the morbidity and mortality in cystic fibrosis, and the expression of the sulfo- and glycosyl-transferases involved in their biosynthesis is increased by TNFα.

In summary, airway inflammation may simultaneously induce the expression of mucin genes (*MUC2* and *MUC5AC*) and the expression of several glycosyl- and sulfo-transferases, therefore modifying the combinatory glycosylation of these molecules.

Keywords: **airway mucin, *O*-glycosylation, sulfation, inflammation, cystic fibrosis**

Introduction

The lower human airway mucosa is normally sterile due to the mucociliary system, phagocytosis, and both innate and immune defenses. The mucociliary system comprises a mucus layer, which covers the airway epithelium and is separated from the

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epithelium surface by a liquid phase where beat the cilia of the ciliated cells. The ciliary beating draws the mucus layer upward along the trachea up to the pharynx and this mucociliary escalator traps bacteria, viruses, particles and molecules inhaled during respiration and carries them back to the pharynx where they are normally swallowed.

Mucus production is an evolutionary ancient defense mechanism, which protects host mucosal surfaces against pathogens and irritants. Like other mucus, the human respiratory mucus has rheological properties (elasticity and viscosity) and is an interface between the environment and the "milieu intérieur". Airway mucus is a mixture of molecules mostly secreted by goblet cells of the surface and by mucous and serous cells of the submucosal bronchial glands. It contains peptides and proteins, which have protective properties: secretory antibodies, antiproteases, antimicrobial peptides such as lysozyme (which lyses bacterial cell wall) or defensins (which introduce pores in bacterial cell wall), lactoferrin, an iron-chelating protein, which sequesters iron needed for bacterial metabolism, trefoil peptides, and mucins which are its main constituents.

The word mucin is one of the oldest words in the field of glycobiology. For more than a century the concept of mucin, or mucus glycoprotein, was associated with material synthesized and secreted by mucosae or mucous exocrine glands. Mucins were probably the first type of compounds to be clearly recognized as glycoproteins. The definition of mucins is based on their chemical composition (from 50 to 80% carbohydrate) and molecular mass (from several hundred to several thousands kDa) [1]. Mucins are glycoproteins containing from one single to several hundred carbohydrate chains attached to the peptide by *O*-glycosidic linkages between *N*-acetylgalactosamine and a hydroxylated amino acid (serine or threonine). Frequently the carbohydrate chains are clustered in highly glycosylated domains and the usual representation of mucins is that of a "bottle-brush". Apomucins, the peptide part of the mucins, have a high proportion of hydroxylated amino acid (serine + threonine) especially present in tandem repeats. They are encoded by different mucin genes (*MUC* genes). Typical human mucins contain fucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine and sialic acid. They may also contain sulfate and small quantities of mannose. The physical polydispersity and several other lines of evidence clearly indicate that there is a very large family of mucin molecules differing from each other, at the peptide as well as at the carbohydrate levels: the different mucin genes encode different apomucins which are sometimes polymorphic, generating different variants, and there are probably several glycoforms for each apomucin or variant. Mucins are synthesized by epithelial cells: some mucins forming visco-elastic gels are secreted by goblet cells or by exocrine glands, whereas others are bound to the apical membrane of the epithelial cells, but may be shed [1]. Unfortunately, the word mucin (or "mucin-like") is also sometimes utilized to designate membrane-bound glycoproteins from endothelial origin [2].

In electron microscopy, mucins purified from human bronchial secretion appear as long, polydisperse, linear and apparently flexible threads [3–6]. Their size varies from a few hundred nm up to more than 5 μm, and decreases after reduction of disulfide bridges.

The carbohydrate content of human airway mucins is in the 70 to 80% (by weight) range and, on average, there are about 30 carbohydrate chains per 100 amino acid residues [7].

In the human respiratory mucosa, several types of cells are involved in the synthesis and secretion of mucins, especially goblet cells of the surface and mucous cells of the bronchial submucosal glands, which are strongly stained by Schiff-periodate [8].

The present review is focused on the glycosylation and sulfation of mucins secreted or shed by the human airway mucosa. Recent reviews on apomucins [9–12] or *O*-glycosylation in cancer cells [13] have been published elsewhere.

Apomucins of human airways

A whole range of secreted or membrane-bound mucin genes have been cloned. MUC1 and MUC4 are membrane-bound mucins, which extend out of the surface of epithelial cells. The high molecular mass secreted mucins MUC2, MUC5AC, MUC5B and MUC6 are encoded by a cluster of genes on chromosome 11p15.5 [14,15]. They exist as multimers linked end to end via disulfide-bridges. The mechanism of multimerization of these high molecular mass mucins is probably analogous to the process described for the biosynthesis of the pig submaxillary mucin [11]. MUC7 is a rather small secreted mucin which does not contain disulfide bridges.

At least seven mucin genes are expressed in the human airways, *MUC1, MUC2, MUC4, MUC5B, MUC5AC, MUC7* and *MUC8*, and their expression may vary during development [16–20]. The surface epithelium expresses *MUC1, MUC2, MUC4* and *MUC5AC*. Mucous glands express *MUC5B*, and *MUC1* and serous cells express the low-molecular mass *MUC7* [17,19].

Mucin production is part of the airway innate immunity. The number and size of PAS positive epithelial cells increase in response to noxious elements [21]. Mucins may be excessively produced in response to pathogens, inhaled particles and irritants. *Pseudomonas aeruginosa*, the major pathogen found in the lung of patients suffering from cystic fibrosis is able to activate NF-κB via a Src-Ras-MEK-MAPK-pp90RSK pathway and to induce *MUC2* expression via the binding of NF-κB to a κB site upstream of *MUC2* [22,23]. It simultaneously up-regulates *MUC5AC* and this action can be mimicked by lipopolysaccharide probably acting on a Toll-like receptor [22,24,25]. Cytoplasmic proteins from non-typeable *Haemophilus Influenzae* also up-regulate *MUC5AC* transcription [26]. Gram-positive bacteria, such as *Staphylococcus aureus*, also increase the expression of *MUC2*; bacterial lipoteichoic acid activates the platelet-activating factor receptor

(PAFR), which is G-protein coupled. PAFR transduces the signal to epidermal growth factor acting on Ras to activate mucin production [25]. Tobacco smoke also induces mucin transcription through c-Src and MAP kinase, but does not seem either to involve NF-κB activation [23]. All-trans retinoic acid enhances the promoter activity of the 5′cis flanking region of *MUC5B* [27]. Finally, different cytokines influence mucin genes expression. TNFα induces *MUC2* expression in primary cultures of human airway epithelial cells [28] and *MUC5AC* message levels in NCI-H292 cells [29]. IL-9 present in allergic airway fluid is capable of stimulating *MUC5AC* and *MUC2* transcription [30,31] and IL-4 induces *MUC2* expression and goblet cell metaplasia *in vitro* and *in vivo* [32]. Conversely dexamethasone suppresses mucus production and *MUC2* and *MUC5AC* gene expression by NCI-H292 cells [33].

The wide diversity of carbohydrate chains and glycosyltransferase expression in the human lung

Goblet cells and mucous cells of the bronchial submucosal glands are the main cells involved in the synthesis and secretion of mucins. When using electron microscopy with specially adapted techniques equivalent to Schiff-periodate, differences in the density of mucin granules may be observed from one cell to the other, sometimes from one granule to the other within the same cell [34], already suggesting a diversity of the carbohydrate moieties of mucins synthesized even by an individual cell.

The general scheme of a mucin molecule is that of a bottlebrush, with hundreds of carbohydrate chains attached to the apomucin (Figure 1). As already mentioned, most mucin carbohydrate chains are joined to the apomucin through *N*-acetylgalactosamine in α-*O*-glycosidic linkages to the hydroxyl

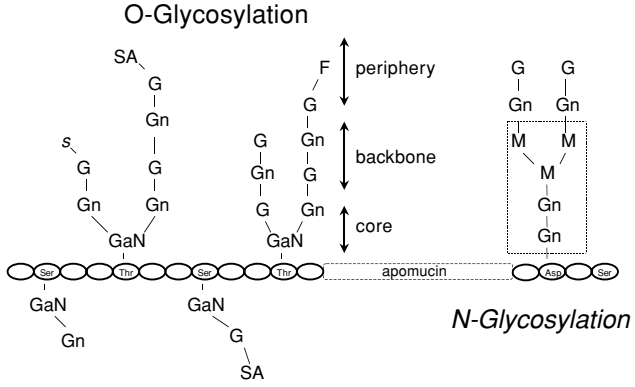

Figure 1. Schematic representation of mucin *O*-glycans, i.e. carbohydrate chains *O*-glycosidically linked to apomucin by linkages involving *N*-acetylgalactosamine (GaN) and a hydroxyamino acid (serine or threonine). Each *O*-glycan can be described with a core, a backbone and a periphery [66]. There may be a few *N*-glycans having the typical central pentasaccharide. F = fucose; G = galactose; Gn = *N*-acetylglucosamine; M = mannose; SA = sialic acid; s = sulfate.

oxygen of serine or threonine: these linkages are alkali-labile [35]. Besides *N*-acetylgalactosamine, mucins contain fucose, galactose, *N*-acetylglucosamine and sialic acid. Using the protocol developed by Klein et al. [36], human airway mucins were found to contain predominantly, if not exclusively, *N*-acetylneuraminic acid as sialic acid (unpublished data). In addition, human airway mucins may contain sulfate groups and a small amount of mannose [1]. In this respect, the amino-acid sequence of several mucin genes contain the characteristic Asn-X-Ser [or Thr] motifs (sequons) enabling *N*-glycosylation of apomucins (Figure 1), a cotranslational process which occurs in the rough endoplasmic reticulum and creates a few *N*-glycans.

Most carbohydrate chains of human airway mucins can be released by β-elimination and these chains can be purified by the combination of various techniques of HPLC, allowing their subsequent structural elucidation using MS spectrometry and ^{1}H-NMR spectroscopy [37–59]. Many carbohydrate epitopes can be also identified in mucins or mucin-secreting cells using various lectins and monoclonal antibodies [60–65]. From the five types of monosaccharide residues commonly found in the *O*-glycan chains of human mucins, the biosynthetic process leads to a wide spectrum of oligosaccharide structures, varying in composition, length, branching and acidity [37–59].

Structural information is only available for the shortest (less than 12 sugars) carbohydrate chains. Due to the difficulty in collecting the normal human bronchial secretion, all the structural studies can only be performed on the mucins hypersecreted by patients. So far, the primary structure of more than 150 different carbohydrate chains has been established, mostly using a combination of ^{1}H-NMR spectroscopy and MS spectrometry, but important structural work still needs to be done. Examples of the most frequent carbohydrate sequences identified in human airway mucins are presented in Figures 2–10. The carbohydrate chains of the airway mucins of any individual are very diverse. For example, 88 different chains have been isolated from the respiratory mucins of a single individual with blood group O [44–48,54] and it can be assumed that airway mucins from a single individual contain several hundreds of different carbohydrate chains.

Hounsell and Feizi [66] have proposed to identify three regions in an *O*-glycan chain: the core, the backbone and the periphery (Figure 1). In contrast to *N*-glycosylation, there is no lipid intermediate involved in the biosynthesis of these chains. The *O*-glycosylation process is a stepwise process, which starts in the *cis*-Golgi [67] or in an intermediate compartment between the endoplasmic reticulum and the Golgi apparatus [68]. It implies different transferases, which act successively in the different compartments of the Golgi apparatus and the *trans*-Golgi-network, and nucleotide sugars synthesized in the cytosol, which are transferred to these compartments by nucleotide sugar antiports. The initiation process corresponds to the biosynthesis of the core. Then, elongation of the chains generates the backbone of the chain, which is ultimately modified by termination reactions (Figure 1).

Table 1. UDP-GalNAc-polypeptide-GalNAc-transferases and core enzymes possibly involved in the biosynthesis of human airway mucins

Transferases	*Chromosome localization*	*mRNA expression in the lung*	*Ref. of cloning*	*Ref. of expression*
UDP-GalNAc-polypeptide-GalNAc transferases				
GalNAc-T1	18q12.1	+	[69]	[70]
GalNAc-T2	1q42	+	[69]	[70]
GalNAc-T3	2q24–31	−/+[a]	[70]	[70]
GalNAc-T4	12q21.3–q22	−/+[a]	[71]	[71]
GalNAc-T6	12q13	−/+[a]	[72]	[72]
GalNAc-T8	12p13.3	+	[73]	[73]
UDP-GalNAc-glycopeptide GalNAc-transferases				
GalNAc-T4	12q21.3–q22	−/+[a]	[71]	[71]
GalNAc-T7	4q31.1	+	[74]	[74]
GalNAc-T9	7q11.22	−	[75]	[75]
UDP-Gal: GalNAc-peptide-β1,3-Gal transferase (core 1 enzyme)	7p14–p13	+	[76]	[76]
UDP-GlcNAc: GalNAc-peptide-β1,3-GlcNAc transferase (core 3 enzyme or β3Gn-T6)		+	[77]	[77]
UDP-GlcNAc: GalNAc-peptide-β1,6-GlcNAc transferases (core 2/4 enzymes)				
C2GnT1 or C2GnTL (core 2 enzyme)	9q13	+	[78]	[79]
C2GnT2 or C2GnTM (core 2/4 enzyme + IGnT)	15q22.1	−	[79,80]	[79,80]
C2GnT3 (core 2 enzyme)	5q12 (Yeh)	−	[81]	[81]
UDP-Gal: GalNAc-peptide-α1,3-Gal transferase (core 8 enzyme)			Not cloned	
CMP-NeuAc:R-GalNAcα1-Ser(Thr)-α2,6-NeuAc transferases				
ST6GalNAc-I (acceptor : GalNAc-R or Galβ1-3GalNAc-R)	17	+	[82]	[82]
ST6GalNAc-II (acceptor : Galβ1-3GalNAc-R or NeuAcα2-3Galβ1-3GalNAc-R)	17	++	[83,84]	[83,84]
ST6GalNAc-IV (acceptor : NeuAcα2-3Galβ1-3GalNAc-R)	9q34.1	+	[84,85]	[84,85]

[a]Tetaert D, (personal communication).

Many glycosyl- and sulfo-transferases have already been cloned and found to be expressed in the human lung (Tables 1–3) [69–120]. Therefore, the combination of structural information obtained from secreted airway mucins and transferases expression allows the description of tentative pathways for the biosynthesis of many carbohydrate epitopes of human airway mucins.

Synthesis of carbohydrate-peptide linkages and *O*-glycan cores

The only structural element shared by all mucin *O*-glycans is the GalNAc residue linked to the apomucin. Mucin oligosaccharide synthesis is initiated by the action of very specific enzymes, UDP-GalNAc-polypeptide-α-*N*-acetylgalactosaminyltransferases (GalNAc-T), which attach GalNAc from UDP-GalNAc onto apomucins. Nine enzymes have been described so far [69–75]. Mammalian GalNAc-T1 to T5 are multisubstrate enzymes with extensive active sites containing at least nine amino acid residues. They catalyze the glycosylation of both serine and threonine with a higher efficiency for threonine and appear to have distinct but overlapping *in vitro* acceptor substrate specificities [121]. They recognize different peptide sequences around the hydroxy-amino acid involved in the O-glycosidic linkage. At least seven different polypeptide-GalNAc transferases, GalNAc-T1, -T2, -T3, -T4, -T7 and –T8, are expressed in the human lung (Table 1). GalNAc-T7 is a UDP-GalNAc-glycopeptide-GalNAc transferase: it has no activity with a large group of non-glycosylated peptides, but is selectively activated by partial GalNAc glycosylation of peptides derived from the tandem repeats in MUC2 [74], suggesting that the complete glycosylation of apomucins requires different GalNAc-transferases acting in a coordinate manner. In other tissues, this is also true for GalNAc-T9 [122], an enzyme which is not expressed in the lung.

The peptide-linked GalNAc and the sugar(s) directly attached to it constitute the "core" region of the mucin oligosaccharides [66] (Figure 1). The GalNAc can be substituted on the hydroxyl of C3 either by a Galβ1-3 or by a GlcNAcβ1-3 [38] to give respectively core 1 and core 3 [1,66] (Figure 2). The enzymes responsible for these syntheses have been recently cloned [76,77]. Moreover, there are indications

Table 2. GlcNAc- and Gal-transferases expressed possibly involved in the biosynthesis of human airway mucins

Transferases	*Chromosome localization*	*mRNA expression in the lung*	*Ref. of cloning*	*Ref. of expression*
UDP-GlcNAc:Gal -β1,6-GlcNAc transferase				
IGnT	6p24	+ (weak) fetal lung	[86]	[87]
UDP-GlcNAc:Gal -β1,3-GlcNAc transferases				
iGnT	11q13.1	+	[87]	[87]
β3GnT2	2p12–13	+	[88]	[88]
β3GnT3	19p	−	[88]	[88]
β3GnT4	12	−	[88]	[88]
β3GnT6 (= core 3 enzyme)	11q14	+	[77]	[77]
UDP-Gal:GlcNAc -β1,4-Gal transferases				
β4GalT1	9p13	+	[89]	[90]
β4GalT2	1p32–p33	−	[91]	[91]
β4GalT3	1q23	+	[91]	[90]
		−		[91]
β4GalT4	3q13.3	+	[91]	[91]
β4GalT5	20q13.1–13.2	+	[92]	[90]
β4GalT6	18q11	−	[90]	[90]
UDP-Gal:GlcNAc -β1,3-Gal transferases				
β3GalT1	?	−	[93]	[94]
β3GalT2	1q31	−	[94]	[94]
β3GalT3	3q25	+	[94]	[94]
β3GalT4 (active on gangliosides)	6p21.3	+	[94]	[94]
β3GalT5	21q22.3	−	[95]	[95]

that the activity of these enzymes is controlled by the amino acid sequence and glycosylation of the glycopeptide substrates [123,124].

Addition of GlcNAc in β1-6 linkage to core 1 and core 3 produces two other cores, cores 2 and 4 (Figure 2). So far, three UDP-GlcNAc: GalNAc-peptide-β1,6-GlcNAc-transferases, which are possibly involved in the biosynthesis of core 2, have been cloned (Table 1) [78–81]. C2GnT1, and C2GnT3 to a low level, are expressed in the lung. Additional cores have been described in other mucins (cores 5, 6, 7) [1], but they have never been found in human respiratory mucins.

In rare occasions, the core GalNAc can be substituted by a Gal(α1-3) residue (Figure 2) to generate core 8 [57], but the enzyme responsible for this synthesis is still unknown.

The GalNAc residue of cores 1, 3 and 8 can also be substituted by an *N*-acetylneuraminic residue in α2-6 linkage (Figure 2) and three CMP-NeuAc:R-GalNAcα1-Ser(Thr)-α2,6-NeuAc-transferases are expressed in the lung [82–85] (Table 1). This diversity of cores already exists in the mucins secreted by a single individual which may contain up to five different cores [44,46–48].

The galactose residue of core 1 can also be directly sialylated by a α2,3-sialyltransferase (possibly ST3Gal-I or -II; see below) to generate NeuAcα2-3Galβ1-3GalNAc- [46] or fucosylated by a α1,2-fucosyltransferase to generate a trisaccharide chain Fucα1-2Galβ1-3GalNAc- [38]. In bovine submaxillary mucin, the apomucin polypeptide may influernce the fucosylation [125].

O-glycan chain elongation

The synthesis of the "backbones" of the different carbohydrate chains results from the successive action of other glycosyltransferases allowing the transfer of galactose and *N*-acetylglucosamine from UDP-Gal and UDP-GlcNAc to determined positions [77,86–96] (Table 2).

Mucin carbohydrate backbones are made up of disaccharides formed by alternating galactose and *N*-acetylglucosamine residues, always β-linked, with two types of linkages: Galβ1-3GlcNAc (type 1 disaccharide) or Galβ1-4GlcNAc (type 2 disaccharide or *N*-acetyl lactosamine) [1] (Figures 3 and 4). In the oligosaccharides from human airway mucins, the type 1 disaccharide has always been found at the periphery of the chains (Figures 3 and 4). The backbone of the chains can be linear or branched, generating i or I antigen respectively (Figure 3). There are evidences from the studies of mucins secreted by airway cells in culture [126] and of mucins purified from sputum (unpublished data) that few carbohydrate chains may correspond to poly-*N*-acetyllactosamine.

The biosynthesis of the backbones can be deduced from the isolation of incomplete chains corresponding to the different intermediate steps of the process. It starts by the addition of either

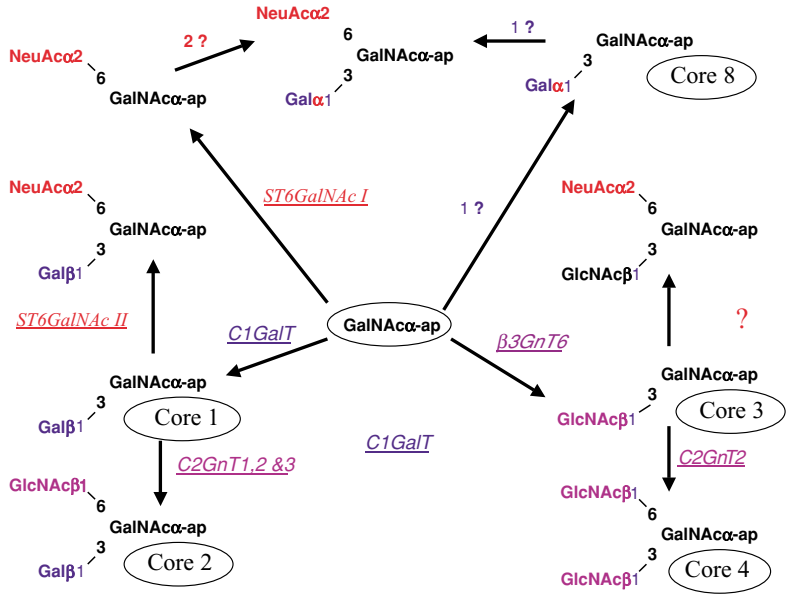

Figure 2. *O*-glycan core structures of human airway mucin and their biosynthesis. First, the GalNAc residue is linked to an apomucin (*ap*) and then the biosynthesis of the different cores implies several enzymes: core 1 enzyme (C1GalT), core 2, 3 and 4 enzymes (C2GnT, C3GnT and C4GnT). The structural elucidation of these different cores is described in [38,39,43,46]. Core 8 has not yet been isolated as such but in a sialylated form [57]. Therefore, there are two hypothetical pathways for the biosynthesis of the sialylated core 8. The GalNAc residue may also be directly sialylated by a sialyltransferase.

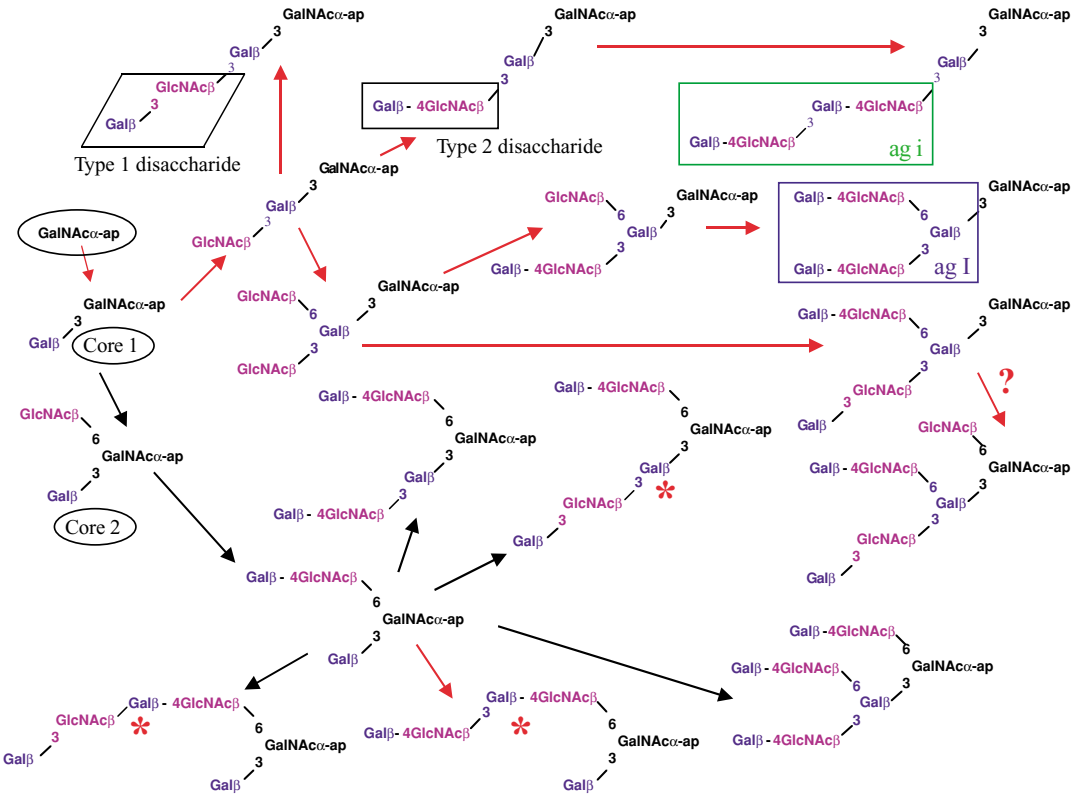

Figure 3. Different types of backbones generated from core 1 and core 2 that have been identified in human airway mucins [see 38–40,43,44,46,48,49,54]. The carbohydrate structures corresponding to type 1 and type 2 disaccharides as well as i and I antigens are indicated. In some chains a galactose residue indicated as Gal (*) can be substituted by a (α 1,2) linked fucose residue generating an internal H2 determinant (see Figure 7).

Table 3. Peripheral glycosyl- and sulfo-transferases possibly involved in the biosynthesis of human airway mucins

Transferases	*Chromosome localization*	*mRNA expression in the lung*	*Ref. of cloning*	*Ref. of expression*
GDP-Fuc:Gal-α1,2-Fuc transferases				
FUT1	19q13.3	−	[97]	[98]
FUT2	19q13.3	+	[99]	[98]
GDP-Fuc:GlcNAc-α1,3/4-Fuc transferases				
FUT3	19p13.3	+	[100]	[98]
FUT4	11q21	+ (weak)	[101]	[98]
FUT5	19p13.3	−	[102]	[98]
FUT6	19p13.3	−	[103]	Personal data
FUT7	9q34.3	−	[104]	Personal data
FUT9	6q16	−	[105]	[106]
CMP-NeuAc:Gal-α2,6-NeuAc transferase				
ST6Gal-I	3q27–q28	+	[107]	[108]
CMP-NeuAc:Galα2,3-NeuAc transferases				
ST3Gal-I	8q24	+	[108]	[108]
ST3Gal-II	16	+	[109]	[109]
ST3Gal-III	1p34–p33	+	[110]	[224]
ST3Gal-IV	11q23–q24	+	[111]	[108,112]
ST3Gal-V (GM3-synthase)	?	+	[113]	[113]
ST3Gal-VI	?	−	[114]	[114]
Gal-3-*O*-sulfo transferases				
Gal3ST-2	2q37.3	+	[115]	[115]
Gal3ST-3	11q13.4	−	[116]	[116]
Gal3ST-4	7q22	+ (weak)	[117]	[117]
GlcNAc-6-*O*-sulfo transferases[a]				
GST-2 or GlcNAc-6-sT or CHST-2	7q31	+	[118]	[118]
GST-3 or HEC-GlcNAc-6-sT	?	−(NB)	[119]	[119]
GST-4 or I-GlcNAc-6-sT	16q23.1	−(NB)	[120]	[120]

[a]Nomenclature of GST = galactose/*N*-acetylglucosamine/*N*-acetylgalactosamine 6-*O*-sulfotransferases according to [120].

an *N*-acetylglucosamine residue by a *N*-acetylglucosaminyl-transferase on the unsubstituted galactose residue of a core 1, or of a galactose residue by a galactosyl transferase on a GlcNAc residue belonging to cores 2, 3 or 4 (Figures 3 and 4).

The elongation of the linear chains requires the alternating action of UDP-GlcNAc:Galβ1,3-GlcNAc transferases and UDP-Gal:GlcNAcβ1,4-Gal transferases (Figure 5). Several galactosyl-transferases are expressed in the lung: β4GalT1, -T3, -T4 and -T5 (Table 2). The only Galβ1,3GlcNAc transferases identified so far in the human lung are β3GalT3 and -T4 (Table 2). The synthesis of i antigens and polylactosamine chains requires the alternative action of UDP-Gal:GlcNAcβ1, 4Gal- and of UDP-GlcNAc:Galβ1,3GlcNAc transferases (Table 2) (Figures 3 and 4). The synthesis of branched chains such as the I antigen requires an additional UDP-GlcNAc: Galβ1, 6GlcNAc transferase (IGnT) (Table 2 and Figures 3 and 4).

O-glycan chain termination

The periphery of the mucin oligosaccharide chains is characterized by the possible presence of four sugars: Fuc, Gal, GalNAc, NeuAc, most often in α anomeric configuration. These sugars are added by a series of glycosyltranferases using the corresponding nucleotide sugars, GDP-Fuc, UDP-Gal, UDP-GalNAc and CMP-NeuAc. Several transferases are polymorphic and confer tissular or histo-blood group antigenic activities to the mucins (ABH, Secretor and Lewis) [127,128]. Sulfate donated by PAPS can also be added by different sulfotransferases at the periphery of mucin chains, either on the C3 of a galactose residue or on the C6 of a GlcNAc residue (Table 3).

The most external disaccharide unit may correspond to Galβ1-3GlcNAc (type 1 disaccharide) or Galβ1-4GlcNAc (type 2 disaccharide) (Figures 3 and 4). Schematically, the biosynthesis of those most external disaccharides (Gal-GlcNAc) units can follow three different pathways (Figures 5–9). First a GlcNAc residue is introduced by a UDP-GlcNAc:Galβ1,3-GlcNAc transferase on the penultimate galactose. Then, three different enzymes may compete for this substrate, UDP-Gal:GlcNAc-β1,4-Gal transferase, UDP-Gal:GlcNAc-β1,3-Gal transferase and GlcNAc-6-*O*-sulfotransferase, which generate three series of different peripheral determinants (Figures 5–9).

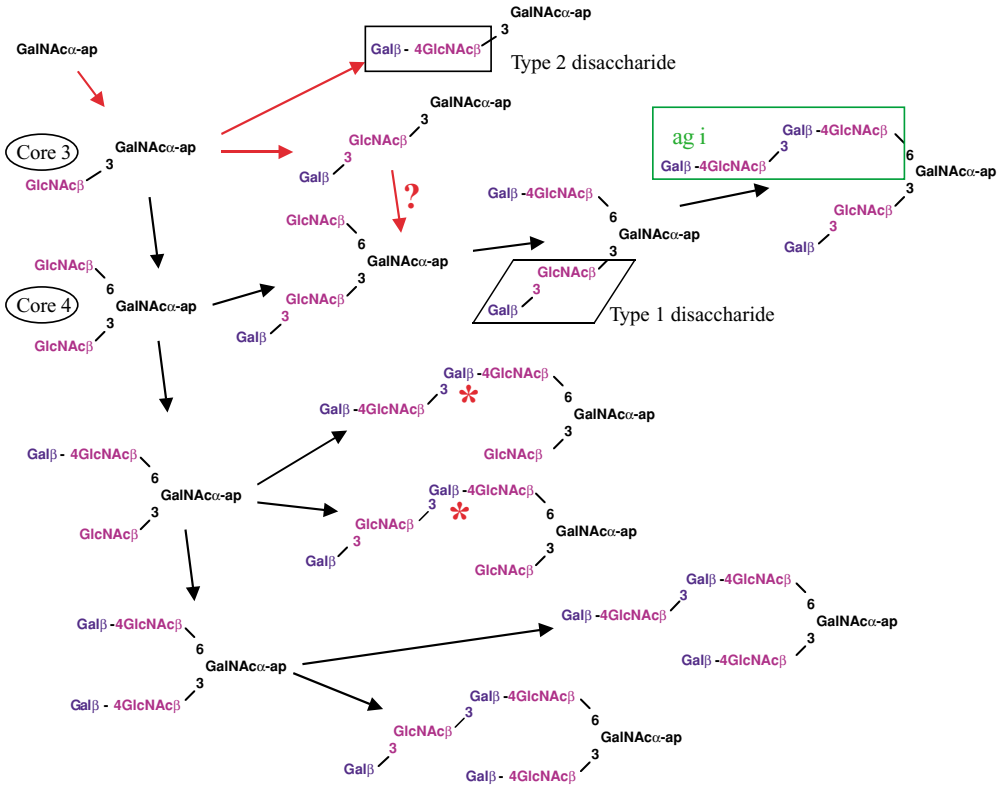

Figure 4. Different types of backbones generated from core 3 and core 4 that have been identified in human airway mucins [see 38–41,43,45–47,49]. The carbohydrate structures corresponding to type 1 and type 2 disaccharides are indicated. In some chains a galactose residue indicated as Gal (*) can be substituted by a (α1,2) linked fucose residue generating an internal H2 determinant (see Figure 7).

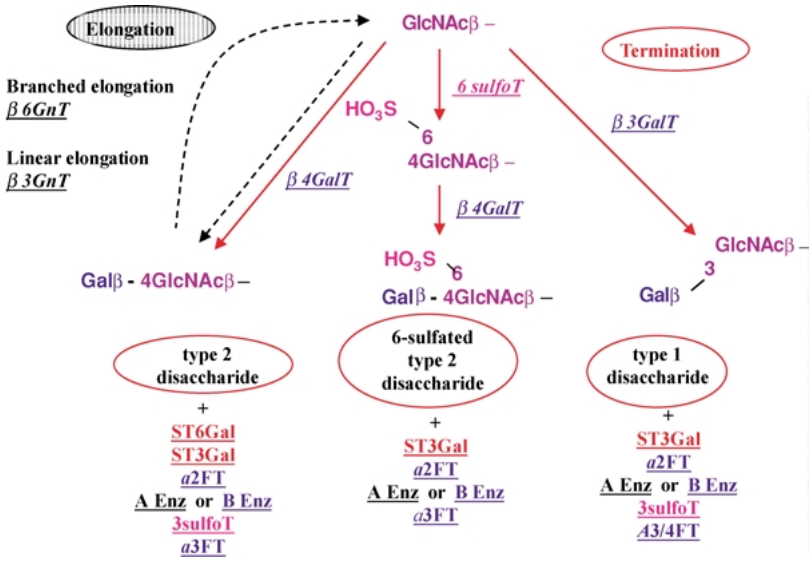

Figure 5. Schematic representation of the different pathways involved in the sequential elongation and the termination of carbohydrate chains of human airway mucins. The enzymes involved in these processes are:

- *for elongation*: β4GalT (= β1,4Gal-transferase), β6GnT (= IGnT or β1,6GlcNAc-transferase) and β3GnT (= iGnT or β1,3GlcNAc-transferase),
- *for termination*: β4GalT, β3GalT (= β1,3Gal-transferase), α2FT (= α1,2-Fuc-transferase), α3FT (= α1,3-Fuc-transferase), 6sulfoT (= GlcNAc-6-*O*-sulfotransferase), 3sulfoT (= Gal-3-*O*-sulfotransferase), ST3Gal (= α2,3-sialyl-transferase), ST6Gal (= α2,6-sialyl-transferase). A and B enzymes are responsible for the biosynthesis of histo-blood group determinants A or B.

UDP-Gal:GlcNAc-β1,4-Gal transferase

The action of a *UDP-Gal:GlcNAc-β1,4-Gal transferase* generates a type 2 peripheral disaccharide (or LacNAc) (Figure 6), a substrate for different transferases which can compete with each other, GDP-Fuc:Gal-α1,2-Fuc transferase, Gal-3-*O*-sulfotransferase, CMP-NeuAc:Galα2,3-NeuAc transferase, CMP-NeuAc:Galα2,6-NeuAc transferase and GDP-Fuc:GlcNAc-α1,3-Fuc transferase (Table 3) to generate a whole series of carbohydrate determinants that have all been identified in human airway mucins (Figure 6).

A GDP-Fuc:Gal-α1,2-Fuc transferase is responsible for the biosynthesis of an H2 antigen (Figure 6). There are two known GDP-Fuc:Gal-α1,2-Fuc transferases : FUT1 and FUT2 (Table 3). Both enzymes are expressed in secondary cultures of human airway cell [126]. FUT2 (or the Secretor enzyme) is a polymorphic enzyme, responsible for the expression of H antigen in mucosae and for the Secretor (Se) or non-secretor (se) status of mucins. FUT1 (or H enzyme) is responsible for the expression of blood group O (H antigen) on blood cells but, in contrast to secondary cultures of airway cells, does not seem to be expressed in the adult human bronchial mucosa (unpublished data), a finding in agreement with the absence of any H antigen in mucins secreted by blood group O and non-secretor individuals [129]. According to the blood group A or B status of each individual, the H2 antigen may be modified by a UDP-GalNAc:Galα1,3-GalNAc transferase (A enzyme) or a UDP-Gal:Galα1,3-Gal transferase (B enzyme) to generate A or B determinants (AH2 or BH2) in airway mucins (Figure 6). The histo-blood groups A, B or O are defined by a polymorphic gene on chromosome 9q.34 responsible for either the expression of the GalNAc-transferase A, or of the Gal-transferase B, or for no activity at all (group O) [127].

In some instances, the penultimate disaccharide unit is fucosylated on the C2 of the galactose residue generating an "internal" H or H2 determinant (Figure 7). The enzyme involved in this process (FUT1, FUT2, or an unknown enzyme) is not yet identified, neither is the substrate of this enzyme (the complete tetrasaccharide, nor the penultimate type 2 disaccharide before completion).

Alternatively, the peripheral LacNAc can be sialylated on the C3 of its galactose residue or, infrequently, on the C6 of this residue, to generate 3-sialyl- or 6-sialyl LacNAc (Figure 6). CMP-NeuAc:Galα 2,3- and 2,6-NeuAc transferases are expressed in the lung (Table 3). Five α2,3-NeuAc transferases are expressed in the lung (ST3Gal-I to -V). However, ST3Gal-V is involved in the biosynthesis of gangliosides. Therefore, only ST3Gal- I to IV may be involved in the biosynthesis of airway mucins. In the mouse, ST3Gal-I and -II showed the highest activity toward core 1 to generate NeuAcα2,3Galβ1-3GalNac- [130]. ST3Gal-III and -IV exhibited the highest activity toward the type 1 and type 2 disaccharides [130].

In the human bronchial mucosa, 3-*O*-sulfotransferases acting on the terminal galactose and using PAPS as sulfate donor, induce the synthesis of the 3-*O*-sulfo-*N*-acetyllactosamine [131] (Figure 6). Some evidences for oligosaccharides with terminal 4-*O*-sulfated galactose [52] or 6-*O*-sulfated galactose [37,51,52,55] have been reported but, in contrast to the 3-sulfated galactose, these structures have never been identified using ^{1}H-NMR spectroscopy [50,56,58]. Two 3-*O*-sulfotransferases expressed in the lung, Gal3ST-2, active on type 2 disaccharide, and Gal3ST-4 active on core 1 and type 1 disaccharide, may be involved in the biosynthesis of sulfated mucins (Table 3).

Finally, a GDP-Fuc:GlcNAc-α1,3-Fuc transferase may act directly on the terminal disaccharide, to generate a Lewis x determinant that cannot be further substituted. The polymorphic GDP-Fuc:GlcNAc-α1,3/4-Fuc transferase (Lewis enzyme or FUT3) may act after all the previous transferases to allow the formation of various determinants : 3-sulfo-Lewis, 3-sialyl-Lewis x, Lewis y (Figure 6). It is possible that the same enzyme also acts on blood group A or B determinants to generate A Lewis y or B Lewis y determinants, but these determinants have not yet been isolated in human airway mucins. In Lewis-negative individuals (i.e. not expressing an active FUT3), 3-sialylated Lewis x determinants are nevertheless expressed on human airway mucins: they result from the activity of another α1,3-Fuc transferase, FUT4, or a still unidentified enzyme [129].

UDP-Gal:GlcNAc-β1,3-Gal transferase

The action of a *UDP-Gal:GlcNAc-β 1,3-Gal transferase* on a terminal *N*-acetylglucosamine residue generates a type 1 (Galβ1-3GlcNAc) peripheral disaccharide (Figures 5 and 8), a substrate for different transferases which again can compete with each other, Gal-3-*O*-sulfotransferase, CMP-NeuAc:Galα2,3-NeuAc transferase, GDP-Fuc:Gal-α1,2-Fuc transferase (FUT2) and the GDP-Fuc:GlcNAc-α1,3/4-Fuc transferase (FUT3) (Figure 8). These enzymes enable the synthesis of another series of peripheral determinants, the 3-sialylated and the 3-sulfated derivatives of the type 1 disaccharide, the H1 and Lewis a determinants, which all have been found among the peripheral determinants of human airway mucins (Figure 8).

A unique enzyme, the polymorphic GDP-Fuc:GlcNAc-α1,3/4-Fuc transferase (Lewis enzyme or FUT3) may be responsible for the synthesis of the Lewis a determinant. It is possible that this enzyme also acts on blood group A or B determinants to generate A Lewis b or B Lewis b determinants, but these determinants have not yet been isolated in human airway mucins (Figure 8).

The 3-sialyl Lewis a determinant (and its non-fucosylated precursor) (Figure 8) have not been identified among the shortest mucin chains isolated so far. Nevertheless, it has been identified on whole purified mucins using monoclonal anti-sialyl Lewis a antibodies, suggesting that it is present on longer carbohydrate chains. Interestingly, the reactivity with these antibodies was much higher for airway mucins isolated from non-secretor and Lewis positive individuals than for mucins isolated from secretor individuals [129]. The affinity of murine

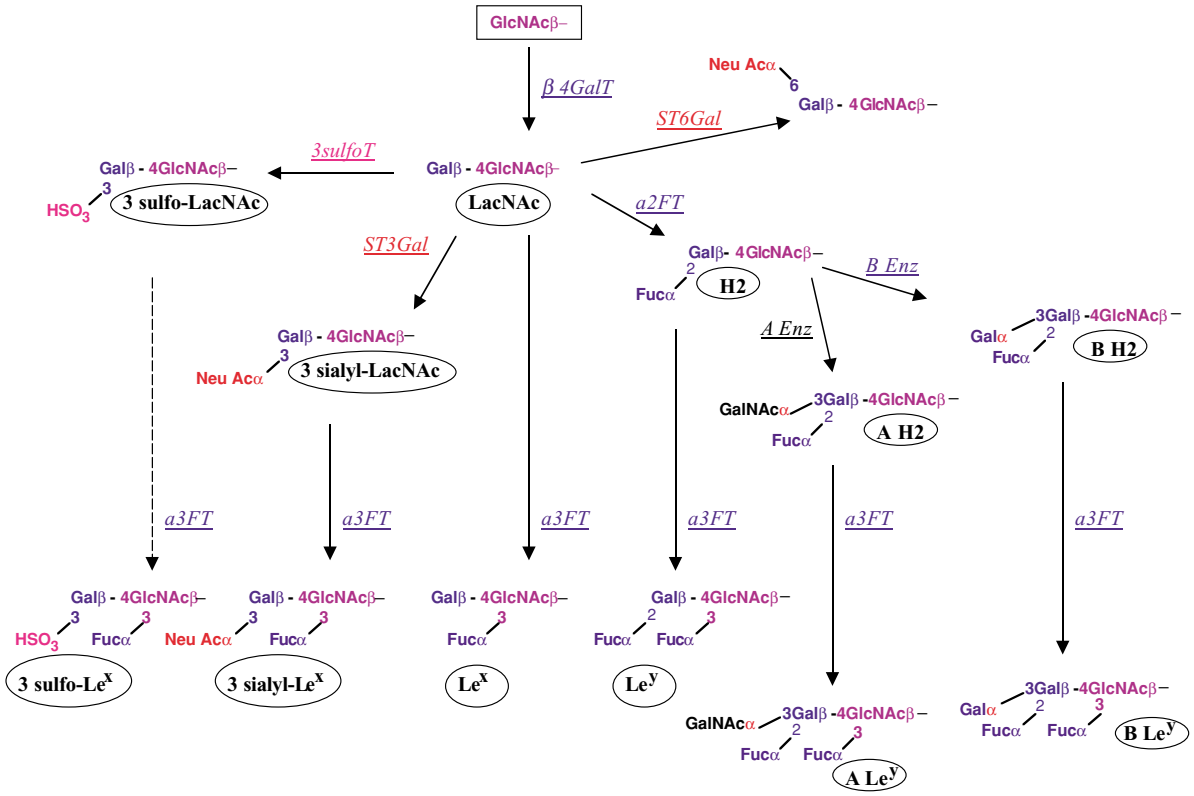

Figure 6. Possible terminal glycosylation reactions occurring on a type 2 peripheral disaccharide. The structural elucidation of these carbohydrate determinants is described in [41,43–47,56–58]. The A and B carbohydrate determinants have been identified with antibodies and the presence of A or B Lewis y is putative. Enzyme abbreviations as in Figure 5.

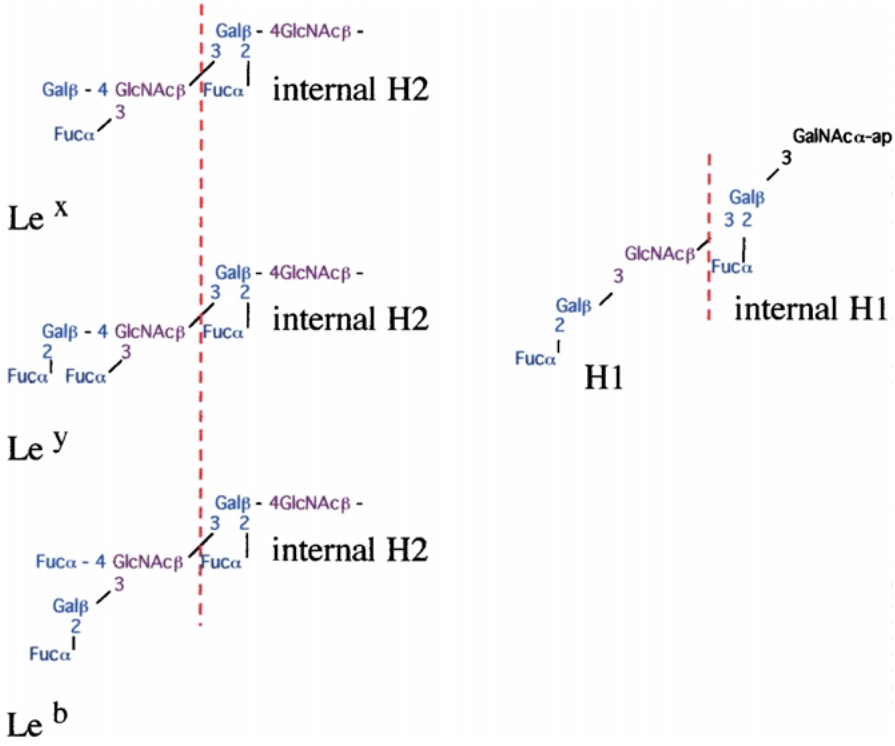

Figure 7. Internal fucosylation of penultimate type 2 disaccharides [47,48].

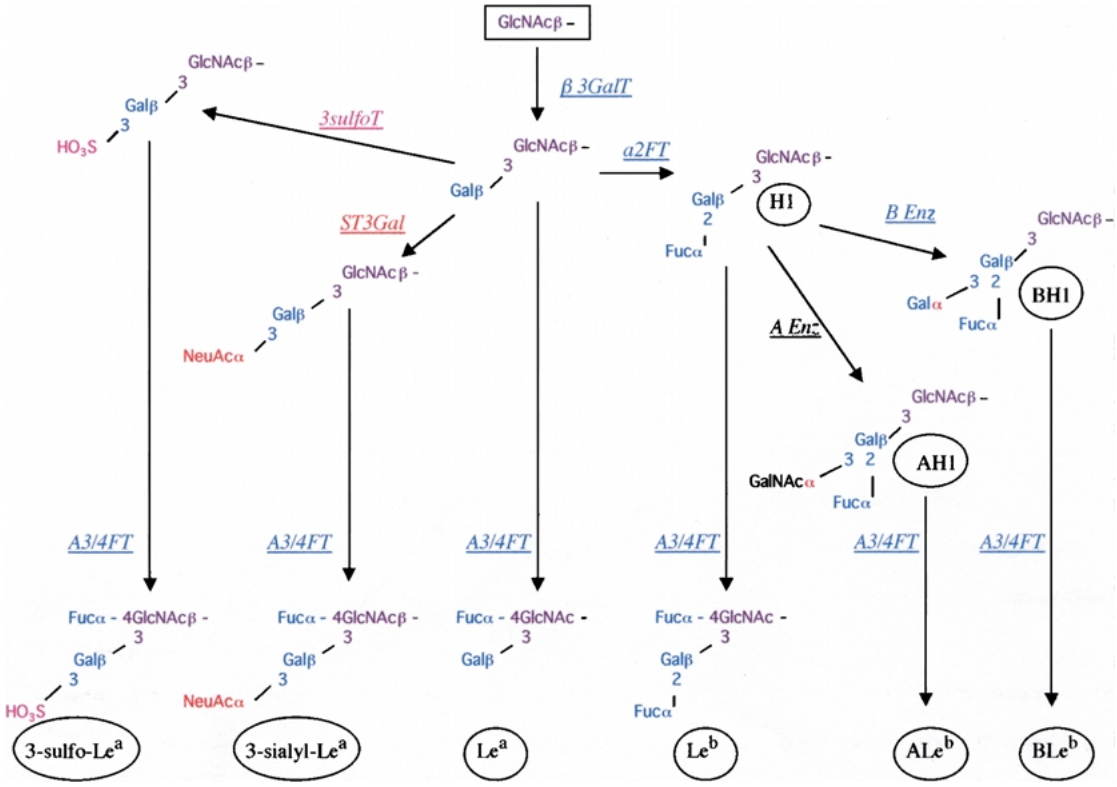

Figure 8. Possible terminal glycosylation reactions occurring on a type 1 peripheral disaccharide. The structural elucidation of these carbohydrate determinants is described in [56]. The A and B carbohydrate determinants have been identified with antibodies and the presence of A or B Lewis b is putative. Enzyme abbreviations as in Figure 5.

ST3Gal-III and - IV for the Galβ1-3GlcNAc disaccharide [130] is higher than that of the secretor enzyme (FUT2) [99]. If this is also true for the human enzymes, and if these sialyltransferases are the only ones involved in the sialylation of the Galβ1-3GlcNAc disaccharide, it would suggest that the expression of FUT2 in the airway is higher than that of the α2,3-NeuAc transferases. Similarly, colon cancer tissues from non-secretor patients exhibit larger amounts of sialyl Lewis a antigens than those from secretor, and this clearly corresponds to a *FUT2* gene dosage effect on the amounts of sialyl Lewis a antigens expressed on the colonic glycoproteins [132].

GlcNAc-6-O-sulfotransferase on a terminal N-acetylglucosamine residue

The action of a *GlcNAc-6-O-sulfotransferase on a terminal N-acetylglucosamine residue*, followed by the action of a β4GalT on this GlcNAc residue, generates a 6-sulfated type 2 (Galβ1-4GlcNAc) peripheral disaccharide [133,134], which is then susceptible to various transferases (GDP-Fuc:Gal-α1,2-Fuc transferase, CMP-NeuAc:Galα2,3-NeuAc transferase and GDP-Fuc:GlcNAc-α1,3-Fuc transferase) (Figure 5 and Table 3), enabling the synthesis of different 6-*O*-sulfated determinants: 6 sulfo-H2, 6 sulfo-3-sialyl-LacNAc, 6-sulfo-Lewis x (Figure 9). The additional effect of a GDP-Fuc:GlcNAc-α1,3-Fuc transferase may generate 6-sulfo-Lewis y or 6-sulfo-sialyl Lewis x (Figure 9). Three GlcNAc-6-*O*-sulfotransferases have been cloned, GST-2, -3 and -4 [135,120] and GST-2 is expressed in the lung (Table 3).

In conclusion, the airway mucin glycosylation represents a combinatorial library of carbohydrate determinants and it is quite probable that only a small part of this combinatory has been identified so far. Each oligosaccharide from the periphery may be substituted in many ways. As an illustration of this diversity, 30 derivatives of the tetrasaccharide Galβ1-4GlcNAcβ1-6[Galβ1-3]GalNAc linked to apomucins have been already identified in human airway mucins (Figure 10). Considering the different backbones that have been described, this suggests that human airway mucins probably contain hundreds of different carbohydrate chains.

This remarkable heterogeneity of carbohydrate chains may result from differences in the glycosyltransferase expression and/or sugar nucleotide availability, from one cell to another. This is for instance true for sialylation in the airways: limulin lectin, which recognizes some sialylated structures but not others, has more affinity for the goblet cells than for the mucous glands [60]. There may be a modification of the expression of glycosyltransferases during cell life. The differences in the peptide sequence of apomucins might generate differences in the synthesis of the different cores and therefore influence the

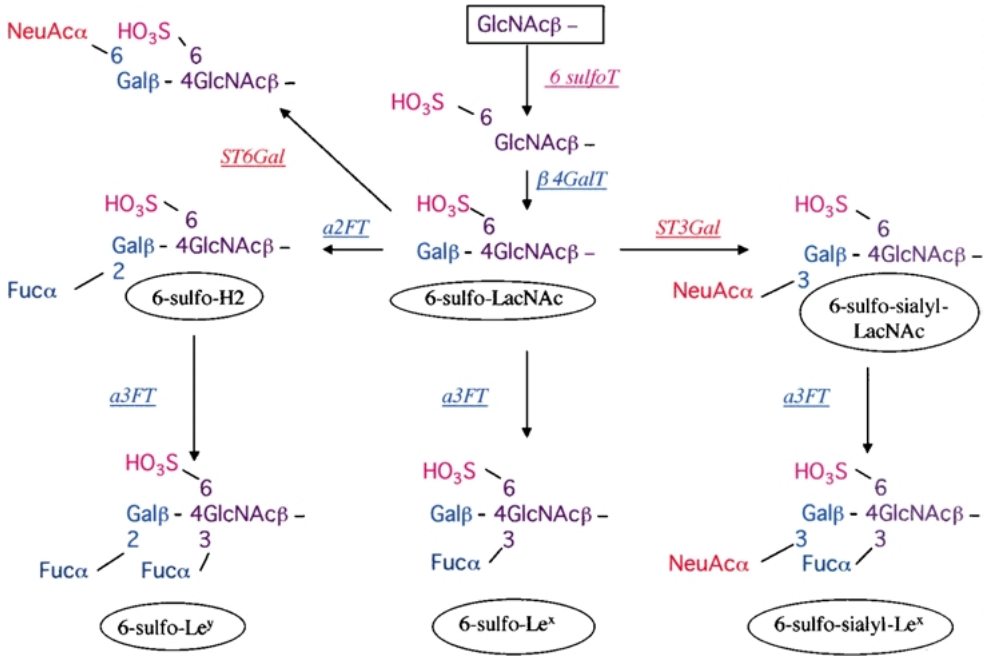

Figure 9. Possible terminal glycosylation reactions occurring after 6-sulfation of a *N*-acetylglucosamine residue. The structural elucidation of these carbohydrate determinants is described in [47,48,58].

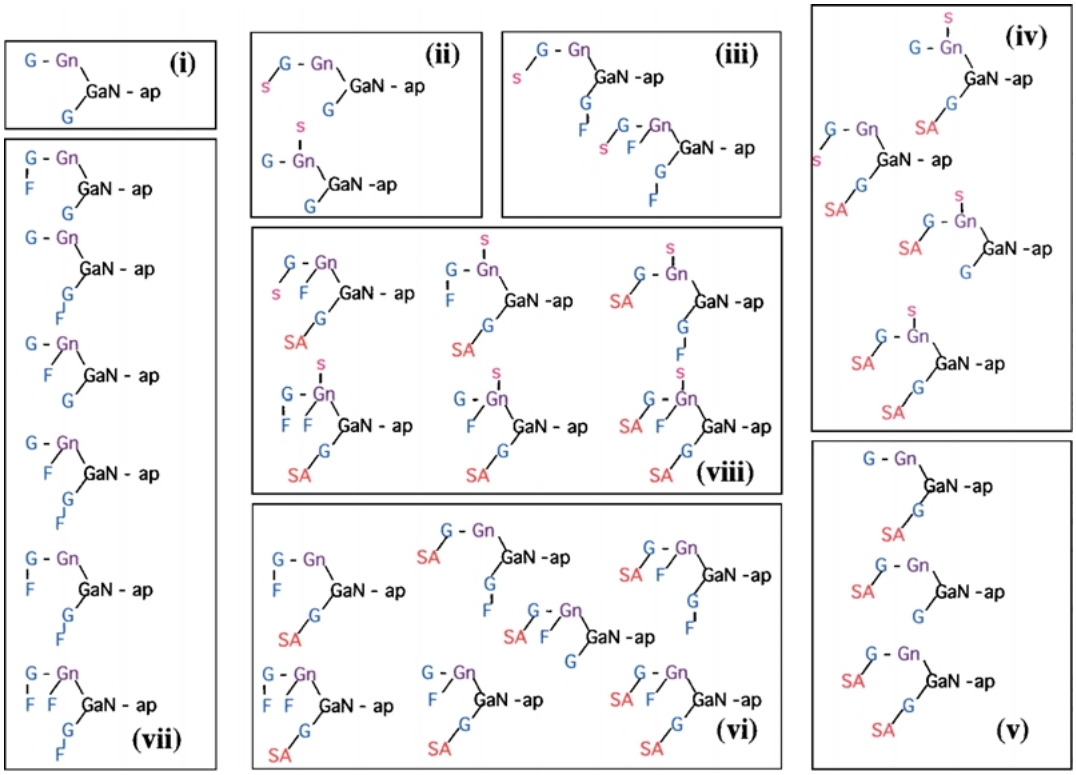

Figure 10. Possible substitutions of a simple tetrasaccharide (i) from human respiratory mucins. This oligosaccharide may be only sulfated (ii), sialylated (v) or fucosylated (vii) It may also be fucosylated and sulfated (iii), sialylated and sulfated (iv), fucosylated and sialylated (vi) or fucosylated, sialylated and sulfated (viii). These oligosaccharides are described in [39,40,43,44,50,56,57]. Sugars are schematized as in Figure 1.

biosynthesis of the rest of the chains. There is also an important need to define the mechanisms regulating the expression of these different transferases.

Functions of human airway mucins

Role in the mucus gel formation

The main features of mucins are their filamentous nature and their extraordinary diversity, at the carbohydrate and peptide levels. MUC 2, MUC5B and 5AC probably dimerize and multimerize, interact with other secreted mucus components (proteins, peptides, lipids, gp340 glycoprotein) [136–139] and, finally are responsible for the visco-elastic properties of mucus. In the airways, mucus and mucins are essential for the efficiency of the muco-ciliary escalator, which traps inhaled particles or microorganisms and allows their removal, thereby keeping the lower airways sterile.

Basic proteins, such as lysozyme [136,137] or mucus protease inhibitor [137], are frequently bound to mucins, although through non-covalent but nevertheless strong interactions. Human neutrophil elastase, a product of the inflammatory response, can be inhibited by human airway mucins [140]. The result of these interactions is probably very important for the rheological properties of the mucus and also for the protection and life-time of some of these molecules.

Interactions with cells

Human airway mucins contain peripheral carbohydrate determinants which are known to interact with different cells present in the airways. This is the case for NeuAcα2-3Galβ1-3GalNAc, a receptor of the macrophage sialoadhesin [141,142], NeuAcα2-3Galβ1-4GlcNAc (sialylated core 1), a receptor of CD22b, a sialic acid-specific lectin from a subset of IGM^+ B cells [143], 3-sialo Lewis x determinant, a receptor for selectins of leukocytes [144,145] and also 3-sialyl-6-sulfo-Lewis x, which is a L-selectin ligand [146].

Interactions with exogenous molecules

In lung diseases, aminoglycosides are sometimes delivered as aerosols, and it has been shown that acidic mucins could bind aminoglycosides and, to a certain extent, block their antibiotic properties on bacteria [147].

Airway mucins and microorganisms

The respiratory mucosa is a complex tissular organization forming a barrier between the *milieu intérieur* and the outside. Through mucus and mucins, mucosae are permanently exposed to a microbial environment, and react to it. Host-microorganism interactions are complex. In the normal lower airways, they lead to an almost complete sterility of the mucosal surface: the mucins trap the inhaled microorganisms, which are subsequently eliminated by the mucociliary escalator.

Airway infection corresponds to an abnormal colonization by a pathogenic strain that overcomes the normal defenses and induces inflammatory alterations of the airway mucosa.

A number of factors are involved in host-microorganism interactions. Using different assays, microtiter plates adhesion assays [148,149] and liquid phase adhesion assays [150], different strains, such as *Pseudomonas aeruginosa, Burkholderia cepacia, Staphylococcus aureus*, non-typeable *Hemophilus influenzae* and viruses have been found to bind airway mucins *in vitro* [148–159]. They may express adhesins or lectins, in addition to LPS which binds to asialo-GM1 [160] and pilin which binds specifically to the carbohydrate sequence GalNAcβ1-4Galβ- found in glycosphingolipids like asialo-GM1 and asialo-GM2 [161]. *Pseudomonas aeruginosa* expresses non-pilus proteins which bind to human airway mucins [162–164]. Fli-D, the flagellar cap protein, has been shown to play an important role in this binding [165,166]. By using various neoglycoconjuates, different carbohydrate determinants, in particular Lewis x or acidic derivatives of Lewis x epitopes, may be recognized *in vitro*, by different components of *Pseudomonas aeruginosa* [167–172].

The binding of *influenza* viruses to airway cells is mediated by viral hemagglutinins, which recognize cell surface glycoconjugates containing terminal sialic acid residues. The hemagglutinins from different strains may differ in their ability to recognize different ligands containing sialic acids [173]. Hemagglutinins from human strains of *influenza* A viruses preferentially recognize receptors with the terminal NeuAcα2-6Gal sequence, whereas hemagglutinins from avian strains preferentially recognize receptors with the terminal NeuAcα2-3Gal sequence [174,175]. These two types of ligands are distributed differently in the tracheal epithelial cells, the NeuAcα2-6Gal motifs being primarily expressed on ciliated cells and the NeuAcα2-3Gal structure being mainly expressed in the goblet cells [159] as well as in their secreted products, the airway mucins [41,43]. As a matter of fact, human bronchial mucins are potent binding-inhibitors for viral strains of the avian type recognizing the NeuAcα2-3Gal motifs, but not for strains of the human type recognizing the NeuAcα2-6Gal motifs [159]. Therefore, the predominance of the NeuAcα2-6Gal sequence on ciliated cells and of the NeuAcα2-3Gal sequence on human respiratory mucins may combine to select for the receptor specificity of human *influenza* A virus strains [159]. Type C *influenza* virus C has not been associated with as severe a disease as types A and B. Unlike *influenza* viruses A and B, which recognize sequences containing *N*-acetylneuraminic acid, type C influenza virus recognizes 9-*O*-acetyl-*N*-acetylneuraminic acid [176], a sialic acid which has never been described so far in human airway mucins.

Thus, the wide diversity of carbohydrate epitopes encountered in airway mucins may be envisaged as a mosaic, or a combinatory, of carbohydrate chains that are possible sites of attachment for microbes and thereby protecting the underlying mucosae.

Human airway mucins and cystic fibrosis

Cystic fibrosis (CF) is the most common severe genetic disease among Caucasians. It affects the exocrine glands and, in its most typical form, the main symptoms are a chronic pulmonary disease, a pancreatic insufficiency and elevated sweat electrolytes (chloride and sodium). In cystic fibrosis, there is a mucus hypersecretion as in chronic bronchitis. Unlike chronic bronchitis, the CF lung infection is very peculiar and characterized by the predominance of *Staphylococcus aureus* in early life and, rapidly if not directly, of *Pseudomonas aeruginosa*. The latter is almost impossible to eradicate and is responsible for most of the morbidity and mortality of the disease [177].

Cystic fibrosis is due to mutations of a gene localized on chromosome 7, *Cftr*, encoding for a *N*-glycosylated membrane glycoprotein, CFTR (cystic fibrosis transmembrane conductance regulator) [178]. CFTR is a chloride channel of low conductance activated by protein kinase A, which influences other ion channels and has probably additional unknown functions [179].

Nearly 1000 mutations of the CF gene have been observed so far [180]. However, in the American and Northern European populations, one mutation, ΔF508, is found in about 70% of the CF chromosomes and more than 90% of the CF patients have at least one ΔF508 allele [178]. This ΔF508 mutation generates an endoplasmic reticulum storage disease, since most of the mutated CFTR fails to be processed past its immature high-mannose state and is subsequently degraded [181].

A major problem in the pathophysiology of CF is to relate these abnormalities to lung infection by *P. aeruginosa*, the main pathogen encountered in this disease.

CF respiratory cells have defective chloride secretion and elevated sodium absorption [182], and lung infection may result, at least in part, from an alteration of the airway mucociliary clearance. It has been suggested that the fluid layer covering the airway surface was normally hypotonic and that the fluid layer of CF patients had an increased salt concentration, leading to alterations in the defense mechanisms of the mucosa: in this hypothesis, the decreased activity of peptides such as defensins would be due to abnormal ion and water movements across the airway epithelium [183,184]. However, the ion concentration of airway surface liquid of patients suffering from cystic fibrosis has been controversial [185]. This is also the case for the fluid of xenograft models of CF airway mucosa [186,187] and the most recent studies tend to support the idea that both fluids from normal individuals or CF patients are isotonic [188].

Recently, much attention has been paid to the precocity of lung inflammation in CF patients, which might occur before bacterial colonization [189–192]. The data obtained with CF mice have been disappointing because, in contrast to the human disease, there was no spontaneous infection of the airways by *Pseudomonas aeruginosa*. However, van Heeckeren et al. have reported elevated levels of inflammatory cytokines corresponding to an excessive inflammatory response of the airways of these mice, when they are challenged with *Pseudomonas aeruginosa* [193]. Moreover, Sajjan et al. have also shown that $Cftr^{-/-}$ knockout mice exposed to repeated instillation of *Burkholderia cepacia* demonstrate an enhanced inflammatory response but apparently less effective than the $Cftr^{+/+}$ [194].

Therefore, an altered inflammatory response of CF airway mucosa, paving the way for subsequent bacterial colonization, is an interesting hypothesis.

Sulfation, glycosylation and cystic fibrosis

In addition to its role on ion movements, CFTR may influence directly or indirectly the processing of different glycoproteins [195], and more specifically, alterations of the CF gene may affect the biosynthesis of glycoproteins in the CF airway mucosa.

Various abnormalities of secreted or membrane-bound glycoconjugates have been described in CF.

As far as sulfation is concerned, respiratory and salivary mucins from CF patients are more sulfated than those from other individuals [37,196–199]. Similar abnormalities have been observed for glycoconjugates from CF cells in culture [200–203]. Airway mucins, secreted by a xenograft model of CF airway mucosa, are also hypersulfated [204]. Since there is no bacterial infection in the xenograft model, a link between hypersulfation of CF mucins and the primary defect of the disease has been envisaged.

With regard to glycosylation, various abnormalities of membrane-bound glycoproteins from CF cells and of respiratory mucins secreted by CF patients have been described. An increased fucosylation of glycoconjugates has been observed in CF cells in culture that was reversible, when these cells were transfected with wild type *Cftr* [205]. A decreased sialylation of glycoproteins from CF cells has also been reported in various CF cells derived from the respiratory mucosa of CF patients [206,207]. Also, the undersialylation is reversible when these cells are transfected with wild type *Cftr* [205].

Several hypotheses have been postulated to explain these abnormalities. It has been suggested that in CF cells, defective acidification of the trans-Golgi network may modify the activity [208,209] or the cellular localization [209] of several transferases. However, this issue of defective acidification of the *trans*-Golgi network is controversial [210] and it has recently been suggested that there was no defect in acidification but rather an hyperacidification of endosomal organelles in CF lung epithelial cells [211].

It has also been suggested that the concentration of PAPS, the sulfate donor, is regulated in part by CFTR [212], and therefore may influence the sulfation process. The wild-type CFTR would tend to lower the PAPS concentration in the Golgi lumen by letting PAPS leak out of the Golgi, whereas the lack of normal CFTR in CF would increase PAPS concentration in the Golgi and therefore favor sulfation reactions.

In contrast to the observations made with CF airway cells, an increased sialylation has been observed for airway mucins secreted by CF patients. Airway mucins secreted by 39 patients suffering either from cystic fibrosis or from chronic bronchitis,

with or without a severe infection, have been compared for their sialic acid and sulfate contents, as well as for the sialyl-Lewis x expression [129]. In mucins from severely infected patients, hypersialylation and overexpression of the sialyl-Lewis x epitope were observed. With regard to these discrepancies, several comments have to be made:

(i) CFTR is highly expressed in non-ciliated epithelial cells, in some duct cells and in serous cells of the tubular glands [213]. In contrast, the expression of CFTR in cells synthesizing mucins (goblet cells and mucous glands of the acinar cells) is very low, if any [214]. Therefore, the effect of the CFTR defect on mucin glycosylation may not be primary but secondary.
(ii) *In vivo*, there are differences in the sialylation pattern of the different cells of the bronchial epithelium: the sialylation of the terminal galactose residues of mucins contained in goblet cells occurs mostly on the 3-OH of these residues, whereas the sialylation of membrane-bound glycoproteins of the other bronchial cells occurs predominantly on the 6-OH of the terminal galactose residues [159]. This last observation is also in agreement with all the structural works performed on carbohydrate chains of secreted human bronchial mucins, showing very little sialylation of bronchial mucins on the 6-OH of a terminal galactose.
(iii) The phenotype of airway cell lines that express CFTR is not always well characterized. Some cell lines may have a serous or sero-mucous phenotype: they may express some mucin genes but do not synthesize high molecular mass mucins [215], and the expression of some glycosyltransferases may vary according to culture conditions [216].

One may therefore wonder if the glycosylation machinery and its regulatory mechanisms in cells synthesizing mucins *in vivo* are different from those of cells in culture expressing CFTR and having a different serous or sero-mucous phenotype, and, importantly, one may question the influence of inflammation on the glycosylation process of the airway mucins.

Inflammation and airway mucins in CF

Inflammation as such may modify the synthesis and glycosylation of certain glycoproteins. The increased secretion of acute phase glycoproteins synthesized in the liver in relation to inflammation is well known, but several recent reports have indicated possible glycosylation modifications of acute phase glycoproteins such as an increased expression of sialyl-Lewis x epitopes in relation to the secretion of cytokines [217,218]. TNFα may induce the expression of sialyl Lewis x [219,220], and many carbohydrate chains containing the sialyl-Lewis x epitope have been isolated from airway mucins secreted by patients suffering either from cystic fibrosis or from chronic bronchitis [41,43,46,54]. Therefore, one may raise the question of the influence of inflammation on the glycosylation process in the airway mucosa of severely infected patients.

As already mentioned, mucins secreted by patients suffering either from cystic fibrosis or from chronic bronchitis, with or without a severe infection, were compared for their sialic acid and sulfate contents, as well as the sialyl-Lewis x expression. This study confirmed the higher sulfation already reported in cystic fibrosis [37,196–200]. However, the sulfate content of the mucins from the infected patients was also higher than that of the mucins from the non-infected patients and this raises the question of a possible influence of severe inflammation on the sulfation process.

The sialic acid level of bronchial mucins from both severely infected CF and chronic bronchitic patients was significantly higher than that of the mucins secreted by non-infected patients suffering from chronic bronchitis. The increased sialylation of mucins from infected patients is in agreement with a previous report showing an increased sialylation of salivary mucin glycopeptides from CF patients as compared to normal individuals [199].

The sialyl-Lewis x epitope is also overexpressed in mucins from infected patients, CF or not CF [129]. This overexpression of sialyl-Lewis x in mucins from severely infected patients may correspond (i) to an increased expression of an α2,3-sialyltransferase (ST3Gal), competing with the fucosyltransferase FUT2 for the same substrate, the terminal Galβ1-4GlcNAc chains (type 2 chains) (Figure 5), and (ii) to an increased expression of an α1,3-fucosyltransferase activity.

TNFα is an important factor of airway mucosa inflammation, acting as an initial inflammatory cytokine that subsequently regulates both early neutrophil infiltration and eosinophil recruitment into the lung and airspace [221]. TNFα, as other cytokines, is found in the airways of patients suffering from bronchial diseases such as chronic bronchitis or cystic fibrosis [222,223]. In order to investigate the role of cytokines on mucin sulfation and glycosylation, explants of human airway mucosa have been exposed to TNFα [224]. TNFα, increases the expression of α2,3-sialyltransferases (*ST3-GalIII* and *ST3-GalIV*), α1,3-fucosyltransferases (*FUT3* and *FUT4*), galactose-3-*O*-sulfotransferase and *N*-acetylglucosamine-6-*O*-sulfotransferase, which are involved in the biosynthesis of Lewis x, sialyl-Lewis x, sulfo-Lewis x and sulfo-sialyl-Lewis x determinants by the human bronchial mucosa, but it does not influence the α1,2-fucosyltransferases [224]. These Lewis x derivatives are abundant in CF aiway mucins [56].

Carbohydrate receptors for *Pseudomonas aeruginosa*

In cystic fibrosis, the airway colonization by *Pseudomonas aeruginosa* is probably a multifactorial phenomenon but the adherence the bacteria to mucus and mucins might represent an important factor in the pathogenesis of lung infection.

An increased affinity of *Pseudomonas aeruginosa* for different mucins from patients with cystic fibrosis has been described. Devaraj et al. have shown an increased affinity of *Pseudomonas aeruginosa* for respiratory mucins in cystic fibrosis [150]. Carnoy et al. have made similar conclusions for

CF salivary mucins [199]. Several adhesins localized on the flagella and the outer membrane of *Pseudomonas aeruginosa* are able to bind to the carbohydrate part of respiratory mucins [162,165,166], but it is not known if they have an increased affinity for CF mucins or if their expression is increased in the mucus lining the airways of CF patients.

In CF airways where hypersecretion or possible fluid abnormalities alter the mucociliary clearance, mucin sulfation or/and glycosylation alterations might also favor colonization by *Pseudomonas aeruginosa*.

The different modifications of secreted CF mucins have raised the question of their relationship with the colonization by *Pseudomonas aeruginosa*. As already mentioned, respiratory and salivary mucins from CF patients have a higher affinity for *Pseudomonas aeruginosa* than most mucins from non-CF subjects [150,199]. Several mucin-type carbohydrate epitopes, sialylated or neutral, are recognized *in vitro* by this microorganism. There are also data concerning the involvement of sialic acid in the aggregation of *Pseudomonas aeruginosa* by CF saliva: an increased aggregation of *Pseudomonas aeruginosa* mediated by saliva from patients with CF has been observed and seems to be directly related to the sialic acid content [225].

In recent studies, the affinity of different strains of *P. aeruginosa* for various carbohydrate determinants (Lewis x, sialyl-Lewis x, 3-sulfo-Lewis x, 6-sulfo- sialyl-Lewis x, blood group A...) present on airway mucins was compared using flow cytometry and polyacrylamide based fluorescent glycoconjugates. For four strains isolated from CF patients, the best ligands were sialyl-Lewis x and 6-sulfo-sialyl-Lewis x [169–171], which are well expressed on mucins from CF patients [56].

In conclusion, bronchial mucins from CF patients severely infected by *Pseudomonas aeruginosa* undergo at least two modifications in their biosynthesis: (i) oversulfation, (ii) hypersialylation and overexpression of sialyl-Lewis x. These features are not specific for CF and may be observed in some cases of adult patients suffering from severe bronchiectasis. They may correspond to a strong inflammatory response of the respiratory mucosa paving the way for colonization by *Pseudomonas aerugin osa*.

Conclusions

Airway mucins represent a very broad family of polydisperse high molecular mass glycoproteins encoded by at least 6 different genes. The numerous carbohydrate chains that cover the apomucin may be extremely diverse, adding to the complexity of these molecules. Very little is known concerning the relation between a given apomucin molecule and its glycosylation, except for the addition of GalNAc, the first sugar. Consequently there is still a lot of work to be done on mucin genes, carbohydrate structure elucidation, correlation between carbohydrate and peptide, biosynthesis as well as secretion and regulation.

Due to their wide structural diversity forming a combinatory of carbohydrate determinants as well as their location at the surface of the airways, mucins are involved in multiple interactions with microorganisms, and are very important in the protection of the underlying mucosae.

Airway mucins are oversulfated in CF. They also appear to be highly sialylated, a feature which is not specific for the disease but may reflect the mucosal inflammation.

Airway mucins may somehow react to local inflammation, as acute phase proteins do in the liver to systemic inflammation. Airway inflammation may simultaneously induce the expression of mucin genes and also influence the expression of several glycosyl- and sulfo-transferases, thereby modifying the combinatory glycosylation of the mucins. As a matter of fact, TNFα is able to induce the expression of at least one mucin gene, *MUC2*, and to increase the expression of glycosyl- and sulfo-transferases able to generate carbohydrate epitopes with a strong affinity for *Pseudomonas aeruginosa*.

In the airways of patients suffering from cystic fibrosis, the mucin alterations due to a strong inflammatory reaction may be a major factor in the lung colonization by *Pseudomonas aeruginosa*.

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