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Review

Mucins: A biologically relevant glycan barrier in mucosal protection

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ABSTRACT

Background: The mucins found as components of mucus gel layers at mucosal surfaces throughout the body play roles in protection as part of the defensive barrier on an organ and tissue specific basis.
Scope of the review: The human MUC gene family codes up to 20 known proteins, which can be divided into secreted and membrane-associated forms each with a typical protein domain structure. The secreted mucins are adapted to cross link in order to allow formation of the extended mucin networks found in the secreted mucus gels. The membrane-associated mucins possess membrane specific domains which enable their various biological functions as part of the glycocalyx. All mucins are highly O-glycosylated and this is tissue specific and linked with specific biological functions at these locations. Mucin biology is dynamic and the processes of degradation and turnover are well integrated with biosynthesis to maintain a continuous mucosal protection against all external aggressive forces. Interaction of mucins with microflora plays an important role in normal function. Mucins are modified in a variety of diseases and this may be due to abberant mucin peptide or glycosylation.
Major conclusions: Mucins represent a family of glycoprotein having fundamental roles in mucosal protection and communication with external environment.
General significance: The review emphasises the nature of mucins as glycoproteins and their role in presenting an array of glycan structures at the mucosal cell surface.

1. Introduction

Mucosal surfaces throughout the body require protection against the variety of aggressive agents they encounter during the fulfilment of their normal function [1,2]. The mucins are an integral component in the mucus gel blanket found at these locations [3–15]. The variety of functions include gaseous exchange especially in the respiratory tree, nutrient and cofactor adsorption in the gut, transparency at the ocular surface and general roles such as lubrication, and chemical sensing. In addition, the mucosal surfaces have a close and integrated relationship to both innate and adaptive immune systems and are linked to the systemic circulation. These processes are closely related with the development of diseases at mucosal surfaces. [3,16–22]. Thus, mucosal epithelia throughout the body can be considered to be vital and dynamic entities in regular function and interaction of the body with both internal and external environments encountered on a daily basis. The airways, gastrointestinal and genitourinary tracts, the ocular surface, nasal cavity, mouth and throat are primary sites, while the cornea, cervix and upper gastrointestinal mucosa are accessed via the primary sites. As a result the mucus generated varies between each mucosal surface and requires careful investigation and characterisation in order to allow relevant study of normal and pathological functionality. Furthermore, the general mucoadhesion properties encountered at the mucosal surfaces provide a focus for drug delivery [23–39]. Recently attention has been drawn to the short term changes associated with collected mucus samples and caution is required when extrapolating results to physiological or biological situations [40].

The complexity of the protective mucosal barrier is expected due to the variety of roles and functions carried out at these locations in a continuous and dynamic manner. The normal turnover of the barrier is essential if an ongoing and viable defence is to be maintained [41–43]. This is reflected in the balance between biosynthesis, secretion and degradation. The enzymatic pathways responsible for biosynthesis and degradation are well known and in addition a range of proteins interacting with the glycan units of these glycoconjugates can be found on the CaZY website [44], illustrating the wide array of manipulations available to achieve normal, dynamic function and protection. The presence of these enzymes and proteins across the evolutionary spectrum underlines the fundamental significance of glycans in biology and stresses the need to better understand the intricacies of this system.

The main aim of this review is to present the molecular properties and functional attributes of the mucins in humans from the viewpoint of their glycobiology. This family of O-glycosylated macromolecules represent a glycoarray located on cell surfaces, the extracellular matrix and in the external environment. They have been implicated in many disease processes and a number of examples have been chosen to

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illustrate this point. We are now starting to appreciate the relevance of such glycoarrays at the biological level and recent advances have emphasised their importance [13,45–54].

2. Mucus gels, mucins and the protective mucosal barrier

The mucus layer found at mucosal surfaces throughout the body depends on the mucins or mucus glycoproteins to generate the biophysical and biochemical properties required for optimal mucosal protection. The viscoelastic, rheological and chemical properties of this group of molecules is adapted to physiological requirements dictated by the site of expression in the body. Mucins have evolved [55], leading to the family of over 20 MUC genes, which are shown in Table 1 and are found on a tissue specific basis [2,4,5,7,8,10,56–59].

The mucins are flexible macromolecular polypeptides identified by the characteristic organisation of their monomeric peptide domains (Table 2). Secreted forms appear as networks through the arrangement of monomers in homo-oligomeric structures and are mostly found at mucosal surfaces as viscoelastic gels, while the membrane-associated mucins are typical monomeric, membrane anchored glycoproteins, which do not form gels.

2.1. Secreted mucins

The oligomeric secreted mucins show a characteristic linkage of monomers through disulphide bridges located in cysteine rich, cystine knot and von Willebrand C and D domains at the N- or C-termini of the monomers. These domains flank centrally located variable number tandem repeat sequences which are unique to each MUC gene and which are also proline, threonine, serine (PTS) rich and serve to carry the glycan chains [5,60,61]. Recent work on MUC2 [60,62–65] has established the detail of the peptide domain organisation and its relation to mucin function and gel formation and is shown in Fig. 1. MUC2 has two PTS domains and shows the arrangement:***N-terminus*** von Willebrand D1, D2, D′D3, cystine rich D, *small PTS*, cystine rich D, *large PTS*, C terminal von Willebrand D4, von Willebrand B, von Willebrand C and finally cysteine knot domain ***C-terminus***.

Rapid dimerisation of the translated MUC2 peptide via the cysteine knot (CK) [62,66–68] disulphide bridges, occurs in the endoplasmic reticulum. Subsequent migration to the Golgi apparatus [62,64,68,69] enables glycosylation of the PTS domain serine and threonine residues with mucin type O-linked glycans. In the trans-Golgi network trimer formation takes place [62,64,70] and the macromolecules are concentrated in goblet cell vesicles (Fig. 2). This process is analogous to the oligomerisation and packing of von Willebrand factor and is pH and Ca^{2+} ion concentration dependent [62–64]. The creation of MUC2 trimers is necessary to permit the production of mucus networks at the cell surface and also provides a possible mechanism to account for the dramatic increase in volume seen during mucin secretion [62–64]. The sequence of events from dimerization to secretion is shown in schematic form in Fig. 2. MUC2 is arranged in bundles having an association of N-terminal trimer rings linked at right angles to dimers stabilised by C-terminal CK and von Willebrand domains. On secretion and hydration of the condensed vesicular mucus granules, stacked planar networks are formed with a volume increase of approx 3000 fold relative to the cellular granules [29,60,62–64,71]. The secreted mucins are packed in vesicles where a pH of 5.2, together with a high intragranular Ca^{2+} level is found [63].

The process of MUC5B packing [63] and release [72] is not known. The secretory vesicle pH for MUC5B is much lower than observed for the sites of mucin biosynthesis in the ER (pH 7.2) and trans-Golgi network (pH 6.0). The combination of low pH and high calcium ion concentration allow the packing of the mucin macromolecules in the vesicles and links with the remarkable volume expansion which occurs during secretion. During the process of secretion the divalent calcium ions balancing the negative charges on the mucins are exchanged for monomeric sodium ions. This doubling of counterion concentration causes an increase in osmotic pressure together with swelling of the mucin. It has been proposed that this yields the fully expanded MUC5B mucus gel on secretion [71,73,74], although this remains a controversial issue [75,76]. A role for bicarbonate in the exchange of calcium ions has been demonstrated [77–79] and linked with the CFTR channel [80]. The process of secretion is rapid, taking place in a milli-second to second period [71,74].

The time required to complete the biosynthesis of respiratory MUC5AC mucin is estimated to be approximately 2 h [72] and contrasts with the very rapid secretion and hydration processes as reported for MUC5B noted above [30].

As already implied the ability of mucins to form gels at mucosal surfaces is a crucial property which lies at the heart of the protection

Table 1
The family of mucin (MUC) genes, showing chromosomal location and PTS domain (mucin domain) tandem repeat size.

Mucin	Chromosome	Tandem repeat size (amino acids)	Main tissue expression
Secreted mucins — gel forming			
MUC2	11p15.5	23	Jejunum, ileum, colon, endometrium
MUC5AC	11p15.5	8	Respiratory tract, stomach, conjunctiva, endocervix, endometrium
MUC5B	11p15.5	29	Respiratory tract, submandibular glands, endocervix
MUC6	11p15.5	169	Stomach, ileum, gall bladder, endocervix, endometrium
MUC19	12q12	19	Evidence for MUC19 protein not reported
Secreted mucins — non-gel forming			
MUC7	4q13–q21	23	Sublingual and submandibular glands
MUC8	12q24.3	13/41	Respiratory tract, uterus, endocervix, endometrium
MUC9	1p13	15	Fallopian tubes
Membrane-associated			
MUC1	1q21	20	Breast, pancreas, duodenum, ileum, colon, trachea, bronchii, cornea, conjunctiva, fallopian tubes, uterus, endometrium, endocervix, ectocervix, vagina
MUC3A/B	7q22	17	Small intestine, colon, gall bladder
MUC4	3q29	16	Breast, respiratory tract, small intestine, colon, conjunctiva, cornea, endocervix, ectocervix, vagina, endometrium
MUC12	7q22	28	Colon, pancreas, prostate, uterus
MUC13	3q21.2	27	Colon, trachea, kidney, small intestine
MUC15	11p14.3	none	Colon, respiratory tract, small intestine, prostate
MUC16	19p13.2	156	Ovary, cornea, conjunctiva, respiratory tract, endometrium
MUC17	7q22	59	Stomach, duodenum, colon
MUC20	3q29	18	Placenta, colon, respiratory tract, prostate, liver
MUC21	6p21	15	Respiratory tract, thymus, colon

Table 2
Peptide domains in the Mucin Gene family. Information taken from; [3,5,7,10,14,61,62,64,165,337].

Peptide domain	Mucin	MUC type	Domain function
PTS — Tandem repeat	All MUCs	Secreted and membrane-associated	Characteristic serine, threonine, proline rich domains carrying the O-linked mucin type glycans
Signal sequence at N-terminus	All MUCs	Secreted and membrane-associated	Targets the insertion to the endoplasmic reticulum and mediates secretion or membrane delivery. Found at N-terminus
Cysteine Rich, CYS domains	MUC2, MUC5AC, MUC5B, MUC19	Secreted	Non-glycosylated multiple copy domains adjacent or interrupting tandem repeat domains. Important for various mucin–mucin interactions.
Cysteine knot	MUC2, MUC5AC, MUC5B, MUC6, MUC19	Secreted	Involved in dimerization. Not found in MUC7
Von Willebrand Factor D (D1, D2, D′, D3)	MUC2, MUC5AC, MUC5B, MUC6, MUC19	Secreted	Mediate oligomerisation located at C-terminus
Von Willebrand Factor D (D4)	MUC2, MUC4, MUC5AC, MUC5B, MUC6	Secreted and membrane-associated	Located N-terminally to the VNTR domains, Contains the GDPH autocatalytic cleavage site. Also present in MUC4 with similar peptide location. MUC19 contains a C3 domain in place of D4
Cytoplasmic tail	MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, MUC17, MUC21	Membrane-associated	Located on the cytoplasmic side of the cell surface membrane. Contains phosphorylation sites involved in signalling. MUC3, MUC12 and MUC17 have PDZ binding motifs
Sperm protein, enterokinase, and agrin SEA domain	MUC1, MUC3, MUC12, MUC13, MUC17, MUC21	Membrane-associated	Protein binding properties. Contains autocatalytic proteolytic cleavage site.
Epidermal growth factor EGF domains	MUC3, MUC4, MUC12, MUC13, MUC17	Membrane-associated	Mediate interactions between mucin subunits and ERBB receptors.
Transmembrane domain	MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, MUC17, MUC20, MUC21	Membrane-associated	Membrane-spanning sequence typical for membrane proteins
GDPH autocatalytic proteolytic site	MUC2, MUC4, MUC5AC	Secreted and membrane-associated	Autocatalytic site cleaving between GD and PH residues
Proteolytic cleavage site	MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, MUC17	Membrane-associated	Found in MUCs with the SEA domain and in MUC16

afforded by the innate mucosal barrier at locations throughout the body. An equilibrium exists between the formation and secretion of fresh mucus gel and its turnover through physical disruption as a result of shear forces at the mucosal surface together with enzymatic cleavage of the glycan chains and polypeptide backbone. MUC2 in the two layer system found in the colon barrier is cross linked through binding to the Fcgbp protein [81]. Other proteins present in mucosal secretions may also contribute to the creation or maintenance of mucus gels through molecular crosslinks, but conclusive evidence remains to be demonstrated in these cases. Examples include the trefoil peptides, gastrokines, transferrin, secretory IgA and others [4,82]. Furthermore a range of mucin-binding proteins have been identified, many of these produced by bacteria, and these also play a role in mucus interaction with the external environment [83]. This is developed further under Section 4 below.

Although there are common features to this process it is adapted to suit the specific needs of individual mucosal barriers at different organ and tissue sites. Mucin glycoforms are found to vary and may originate from the same gland [84] or in neighbouring goblet cells [85]. In addition the stomach shows characteristic secreted mucus gel layers for MUC5AC and MUC6, which are each synthesised at different tissue locations [86]. The evidence for MUC6 is controversial as recent proteomic studies have failed to demonstrate the presence of this mucin [87,88].

2.2. Membrane-associated mucins

Mucins anchored to the apical cell surface form the largest group of mucins (Table 1). Due to their location and size they contribute to the glycocalyx found at mucosal surfaces throughout the body. These mucins are monomeric with characteristic membrane peptide domains (Table 2) and have properties typical of membrane glycoproteins. They do not form gels, in contrast to the secreted mucins. Their surface membrane location ensures that the mucin domains present a glycoarray in the extracellular space, which is available for a wide range of interactions in this external environment. The sequence of events in biosynthesis and transport to the apical cell surface membranes is shown in Fig. 2. Important functional peptide modules include the sea-urchin sperm protein, enterokinase and agrin (SEA) and epidermal growth factor (EGF)-like domains [89]. Autocatalytic peptide cleavage within the SEA module leads to the formation of a non-covalent complex [90] and allows the release of the large extracellular

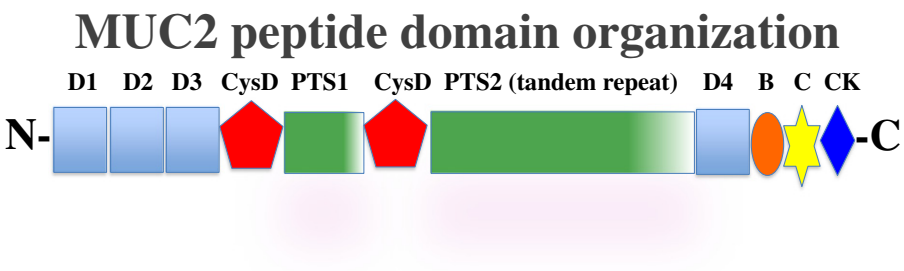


Fig. 1. MUC2 peptide domain organisation. Peptide domains are shown as; von Willebrand D1–4 ; von Willebrand B ; von Willebrand C, ; CysD, ; PTS1 & 2, small and large mucin domains, ; cysteine knot (CK) N- and C-terminae are shown.

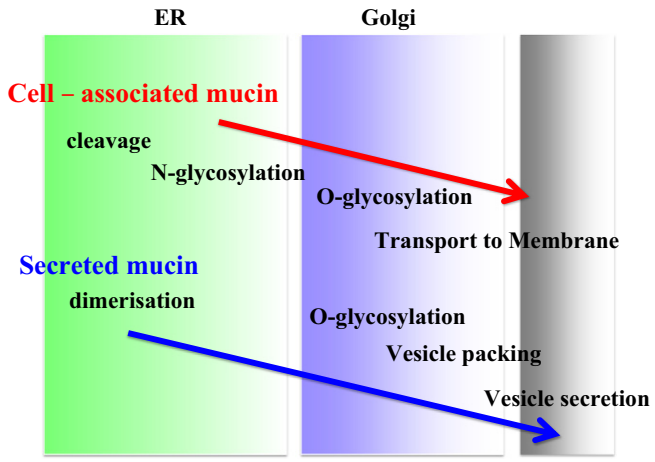


Fig. 2. Sequence of events in the biosynthesis of secreted and membrane-associated mucins. The principal events in the biosynthesis and final targeting of membrane-associated and secreted mucins are represented in diagrammatic form.

mucin component into the mucus gel layer, while the membrane-specific domain is retained in the membrane [91–93]. The presence of the scaffold protein PDZ has been identified as a sequence at the C-terminal of MUC3, MUC12 and MUC17 [94]. This peptide domain is responsible for binding of these mucins to the apical surface membrane in the small intestine. In addition a small number of soluble mucins have been identified, which are formed due to alternative splicing of the MUC1 gene [95]. These unusual molecules have no transmembrane or cytoplasmic sequences [96,97]. As these mucins are smaller than the typical mucin glycoproteins they must be detected and isolated using different methods. Table 1 shows that this group of mucins includes MUC1, MUC3A/B, MUC4, MUC7, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20 and MUC21. At present MUC1 remains the only example where alternative splicing has been shown.

Other, mucin-like molecules have been demonstrated which are generally smaller, but have some of the characteristics of mucins including tandem repeat peptides and high glycosylation, these include endomucins [98–100], Glycam-1, CD34, PSGL-1, MAdACM-1, [3] and DMBT1 [101,102]. Further characterisation of these glycoproteins is required before they can be included or excluded from the Mucin Gene Family. This should include comparison of recognised PTS rich domains with high O-glycosylation, Cys domains, von Willebrand factor B, C and D and cysteine knot (CK) domains. The membrane-associated mucins will also share the PTS rich and Cys domains but also have an N-terminal signal sequence, transmembrane and cytoplasmic tail sequences and may contain SEA, nidogen homology sequence (NIDO), other proteins (AMOP) and epidermal growth factor (EGF)-like regions

2.3. Mucin degradation and turnover

A fundamental feature of the surface mucus gel is its turnover in a dynamic manner, in response to the continual need for mucosal protection. The degradation of mucus is balanced against synthesis and gel formation as described above. The disruption of the mucus gel would appear to be a prerequisite for efficient proteolytic attack of the mucin. Degradation of the mucus networks and monomers occurs through the action of enzymes which have been termed mucinase and which cleave or dissociate the mucin. A number potential covalent mucin cross-link of targets have been described including disulphide bridges in dimers and trimers, Cys D domain crosslinks, autocatalytic VWD4 domain cleavage sites and non reducible peptide sites [62]. On disruption of the gel structure for gel forming mucins and in general for the membrane-associated mucins, a series of enzymes which degrade both glycan and peptide moieties of mucins have been found and lead to the formation of glycopeptides and ultimately complete degradation of the mucins [4,41,42,103–106]. This is essentially the catabolic aspect of the mucin network synthesis described above under secreted mucins. In vivo studies on the turnover of mucus in the gastrointestinal tract have given an indication of the time required for the inner layer, about 60 min [107,108] and 24 h for the whole cycle [96,109–112]. The preparation and analysis of mucins has demanded the development of methods that reliably generate mucin fragments that can be biochemically analysed and related to the biologically mature mucins. A significant proportion of the mature mucin peptide is protected against proteolytic action due to the presence of the glycan chains, especially in the PTS domains. Proteolytic cleavage through peptidases or proteases is therefore limited and common proteolytic enzymes such as trypsin have been used to generate high molecular weight glycopeptides, or T-domains, for structural studies as the glycosylated domains remain resistant to enzymatic degradation [110–112]. Alternatively, chemical agents can be used. Solubilisation of the mucus gel using chaotropic chemicals such as guanidine hydrochloride or urea is then followed by the reduction of the disulphide bridges using dithiothreitol, and alkylation of the liberated sulphydryl groups with iodoacetamide, to prevent reformation of the disulphide bridges Examples of this strategy can be found in the work initiated by Ingemar Carlstedt and continued together with John Sheehan ([110–112]).

In vivo mucin processing and degradation at mucosal surfaces relies on the availability of proteases and glycosidases, either within the mucosal cells themselves, secreted by other mucosal cells or present in the extracellular microflora. A specific protease, which is involved in the processing of mucins, namely the conversion of the inner adherent mucus layer in the colon and ileum to the looser, expanded outer layer has been demonstrated [81,113]. This endogenous activity acts on the extracellular, secreted mucins. The autocatalytic cleavage taking place in the SEA domain in membrane-associated mucins occurs in the membrane and is a different event.

The glycan chains carried by the peptide are degraded by a range of glycosidases. These are specific for each sugar in the chain and act either as exoenzymes, cleaving the terminal monosaccharide alone, or as endoglycosidases removing a complete glycan chain. In mammalian systems these enzymes are integrated into the lysosomal and subcellular compartments responsible for degradation and recycling [114,115] and linked to biological events such as development, organogenesis and tissue homeostasis [116–118].

However in microorganisms and parasites, a wider range of enzymes are found. These are selective for each organism and adapted to the environmental niche where they are active. [119–122]. A number of bacterial strains have been shown to interact with the mucus biofilm as part of the normal and pathological dynamic processes [123,124]. This crosstalk has been shown for mucin degrading strains e.g. *Akkermansia muciniphila*, [125], *Campylobacter jejuni*, [119] *Clostridium difficile* [126] and *Bacteroides cellulosilyticus* [120]. The composition of the microbiome at mucosal surfaces reflects microorganism interaction with the glycan composition of the cell surface glycoconjugates. Local function at these sites is also important, nutritional aspects have been well documented [127–139]. Many additional examples have been reported at other mucosal surfaces.

The glycosidases relevant to the release of mucin glycans include N-acetyl-D-glucosaminidase, N-acetyl-D-galactosaminidase, L-fucosidase, D-galactosidase, D-mannosidase and sialidase (D-neuraminidase). Each of these enzymes may have either alpha- or beta-glycosidic specificity acting on the prevalent glycosidic linkages found in the naturally occurring glycan chains. In addition, further modification of the individual monosaccharides including acetylation, acylation, phosphorylation and sulphation is accommodated by specific glycoesterases, glycophosphatases and glycosulphatases [140–151] and the sialic acids are degraded to N-acetyl-D-mannosamine by the action of N-acetyl-D-neuraminate lyase [152].

All of these enzymes are catalogued in the CaZy database which lists all proteins and enzymes which interact with glycans [44].

3. Glycosylation of mucins and glycobiology

The mucins are glycoproteins rich in O-linked, "mucin type" glycans. The characteristic mucin domains rich in proline/threonine/serine motifs (PTS domains) are the primary site of this glycosylation. The serine and threonine residues are the linkage amino acids, in each case through N-acetyl-D-galactosamine. The peptide attachment site for the O-glycans is recognised by the family of UDP-N-acetylgalactosamine: polypeptide N-acetylgalactosaminyltransferases (ppGaNTases) [154,155] and is in common with the tripeptide sequon identified for the attachment of N-glycan chains [11,43,82,153,156–159]. The structure of the mucin type glycans is built up as three regions; the linkage of N-acetyl-D-galactosamine to peptide serine/threonines to yield a series of eight different core structures (Table 3); a backbone, extended region, when found (Table 4); and a peripheral, terminal region, which shows considerable variety and is the main source of individual mucin glycoarray properties (Table 5). Variety is found through the density of chains per amino acid, the degree of distribution with other glycan types, and the size and range of the glycan chains. The O-glycans are built up stepwise through well-known pathways based on the action of the glycosyltransferases (see Fig. 3) [160], which are detailed in the CaZy database [44].

The core structures, shown in Table 3, are for the most part represented in nature by members 1–4, whereas the remainder are relatively uncommon. A biological distribution, with typical tissue-specific design is found related to the function of the mucins at these locations. The core structures dictate the final, complete O-glycan and this is evident from the glycosylation pathways (Fig. 3). The initial addition of GalNAc to the peptide is important as it is part of the system ensuring optimal glycan occupancy at serine/threonine attachment sites and regulating the subsequent extension of the glycan chains. The glycosyltransfer step is catalysed by a family of enzymes, the UDP-N-acetylgalactosamine: polypeptide N-acetylgalactosaminyltransferases (ppGaNTases) [155,161,162]. There are at least 19 members in this family, further emphasising the significance of this glycosylation step [116,163,164]. The individual isoenzymes show a tissue specific distribution and high evolutionary conservation. They exhibit substrate specificity recognising the serine or threonine residue and whether neighbouring residues are already substituted by a GalNAc. In addition, the lectin binding site in these glycosyltransferases has also been shown to participate in the glycosyltranfer reaction [154]. This order of action demands the co-ordinated participation of different isoenzymes to achieve optimum glycosylation.

The existence of branching, through cores 2 and 4, increases the range of structures found. Larger glycans are formed through the extension of the core structures by backbone repeat sequences. These include type 1 and 2 N-acetyl-lactosamine units, Galβ1–3/4GlcNAc-; i-antigen Galβ1–4GlcNAcβ1–3- and I-antigen Galβ1–4GlcNAβ1–3(Galβ1–4β1–6)GalNAc-. Decoration of the core and backbone sequences by fucosylation, sialylation, sulphation, acetylation and methylation leads to the abundance of O-glycans seen in nature [11,43,82,153,156–158]. The O-glycans make up the bulk of the glycan units found in mucins, but there are also other types of glycan chains, each of which have a particular role in the biological functions of these complex molecules.

The N-glycans occur mainly in membrane-associated mucins and show distinct patterns for MUC1, MUC4 and MUC16. MUC1 shows some N-glycans in the PTS domains but also in the SEA domains, while in MUC4 these are in the EGF region away from the PTS domain. In MUC16 N-glycans are present in the PTF repeat domain [7].

In common with many other glycoproteins the N-glycans function in the processing of the peptide during biosynthesis [2,165,166].

A further post translational modification has been reported in the mucin CysD domains and concerns the covalent C – C linkage between α-mannopyranosyl units and the tryptophan indole C2 carbon atom in mucin peptide WXXW motifs [167]. These units are thought to act in protein folding, sub-cellular localisation and trafficking in the endoplasmatic reticulum–Golgi membranes [60,168]. C-mannosylation of Cys domains is necessary to allow normal maturation and secretion of mucins. The number of these varies, with two in MUC2, seven in MUC5B and nine in MUC5AC. Faulty C-mannosylation leads to the induction of ER stress as the mucins do not exit the ER [61]. Further work is needed to corroborate these initial findings on C-mannosylation.

Table 3
Mucin core structures.

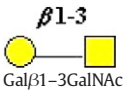
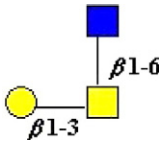
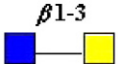
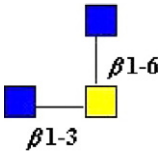
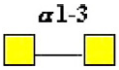
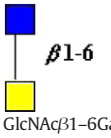
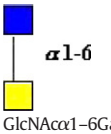
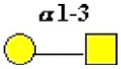
Core type	Structure
1	 Galβ1–3GalNAc
2	 Galβ1–3(GlcNAcβ1–6)GalNAc
3	 GlcNAcβ1–3GalNAc
4	 GlcNAcβ1–3(GlcNAcβ1–6)GalNAc
5	 GalNAcα1–3GalNAc
6	 GlcNAcβ1–6GalNAc
7	 GlcNAcα1–6GalNAc
8	 Galα1–3GalNAc

Table 4
Backbone repeat glycans.

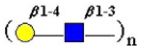
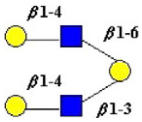
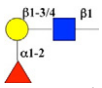
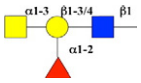
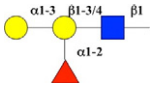
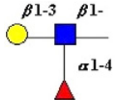
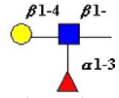
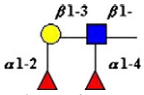
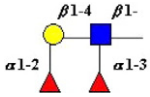
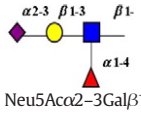
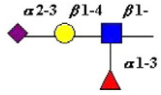
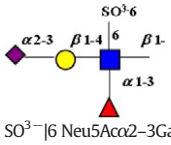
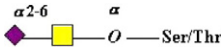
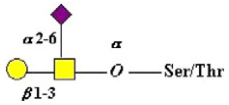
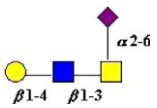
Backbone repeat	Structure	Name
Type 1	 Galβ1–3GlcNAc	
Type 2	 Galβ1–4GlcNAc	N-acetyllactosamine
Poly N-acetyllactosamine type 2	 (Galβ1–4GlcNAcβ1–3-)$_n$	i antigen
Branched N-acetyllactosamine type 2	 Galβ1–4GlcNAcβ1–6 Galβ1 Galβ1–4GlcNAcβ1–3	I antigen

Table 5
Examples of peripheral sequences found on O-glycans.

Type of Glycan	Structure
Blood group H	 Fucα1–2Galβ1–3/4GlcNAcβ1-
Blood group A	 GalNAcα1–3Galβ1–3/4GlcNAcβ1-| α 1–2 Fuc
Blood group B	 Galα1–3Galβ1–3/4GlcNAcβ1- | α1–2 Fuc
Lewis[a]	 Galβ1–3GlcNAcβ1-| α1–4 Fuc
Lewis[x]	 Galβ1–4GlcNAcβ1- | α1–3 Fuc
Lewis[b]	 Galβ1–3GlcNAcβ1- | α1–2 | α1–4 Fuc Fuc
Lewis[y]	 Galβ1–4GlcNAcβ1- | α1–2 | α1–3 Fuc Fuc
Sialyl Lewis[a]	 Neu5Acα2–3Galβ1–3GlcNAcβ1- | α1–4 Fuc
Sialyl Lewis[x]	 Neu5Acα2–3Galβ1–4GlcNAcβ1- | α1–3 Fuc
6′Sulfo sialyl Lewis[x]	 SO^{3-}|6 Neu5Acα2–3Galβ1–3GlcNAcβ1- | α1–3 Fuc–
Sialyl-Tn	 Neu5Acα2–6GalNAc-α-O-Ser/Thr
Sialyl-T-antigen	 Neu5Acα2–3Galβ1–3GalNAc-α-O-Ser/Thr
	 Neu5Ac | α2–6 Galβ1–3GalNAc-α-O-Ser/Thr
Monosialylated core 3	 Neu5Ac | α2–6 Galβ1–3GalNAc-α-O-Ser/Thr
Sd[a] antigen Type 1 & 2 chains	 Neu5Ac | α2–3 GalNAcβ1–4Gaḷβ1–3/4GlcNAc ,β1–3GalNAc

The natural variation of glycosylation occurring between organs, tissues and cells reflects the specific functions associated with these molecules. Studies have shown that the expression of the glycobiome is adapted to functional aspects of the mucosal surfaces throughout the body and that distinct and discrete patterns of glycosylation exist. Examples of this phenomenon have been identified in the oral cavity [84]; the stomach [82,169] large and small intestine and rectum [4,62, 170,171]; pancreas [172]; trachea and bronchus in the respiratory tract [111,112,173–176]; the ocular surface and conjunctiva [177,178]; and the female reproductive tract [49,56]. This variation is reproducible and can be detected in individual mucins. MUC2 glycosylation in the human gastrointestinal tract is a good example with regional glycosylation, largely due to sialic acids and glycosulphate esters, occurring from the small intestine to the rectum [170,171]. The glycosylation in the human sigmoid colon has been found to be constant in over 50 individuals [179]. Abberant glycosylation was detected during the active phase of ulcerative colitis and this reverted to normal patterns on remission, emphasising the inflammatory processes associated IBD [51].

These patterns implicate fundamental biological functions and some examples can be drawn from the interactions of the gastrointestinal tract mucosal mucin glycoarray with the microflora as outlined below in Section 4.

4. Interaction of the mucin glycoarray with the microflora

The mucosal mucin glycoarray at all sites throughout the body interacts with the native microflora and is a major emphasis of attention. Improvements in genomic screening and mapping technology have led to the documentation of numerous microbiomes which have played a significant part in current understanding of host microflora relationships [118–121,123,131,180,181]. The formation of a mucosal gel layer has been detailed at a number of mucosal surfaces [2,6,8,29, 64,65,73,86,87,182]. A two-layer mucus gel has been identified in the gastrointestinal tract [88,183–185]. The inner, adherent mucus — adjacent to the epithelial cells provides a bacteria-free environment [183], while the outer layer harbours bacterial populations, is less viscous and represents the external partially degraded layer where the action of bacterial hydrolytic enzymes contribute to the turnover [88, 184,185]. The mucus barrier is crucial in preventing the colonisation of the epithelium by the enteric microflora and pathogens. It is the glycoarray presented by the mucins, largely MUC2 in the gastrointestinal tract, which serve as targets for the attachment of the microflora. This is clearly a selective event with strain specific bacterial adhesins exhibiting glycan sequence recognition on the mucin glycoarray [49, 83,186–189]. Accordingly, abberations in the glycosylation of the mucins, as noted above for the MUC2 in ulcerative colitis [51], constitute a basis for disease and inflammation [14,190–193]. This is further discussed in Section 5 below.

Typical patterns of microflora are associated with the main regions of the gastrointestinal tract. The oral cavity is characterized by Streptococcus, Prevotella, Porphyromonas and Fusobacterium species [194–196], the stomach is rich in Streptococcus, Lactobacillus, Staphylococcus and Peptostreptococcus [197], while the small intestine and the colon host over 400 different species which are anaerobic, gram negative bacteria and gram positive rods [95,138,198,199]. Furthermore, there are normal developmental adaptations that relate to the neonate, lactation, weaning, infantile, juvenile and adulthood age ranges [127,199–205].

As suggested above, any analysis of the molecular elements that mediate the nature of these interactions requires consideration of the range of mucin glycoarray glycans expressed by the host and the capacity of the microflora to access and exploit them. In line with the need for a dynamic and continuous mucosal protective barrier and in order to resist infection by potential pathogens and maintain a mutualistic–symbiotic relationship with the microflora the diversity of the mucin glycoarray is essential and has arisen as a result of these combined and selective processes [206–209]. Manipulation of the mucin glycoarray by the

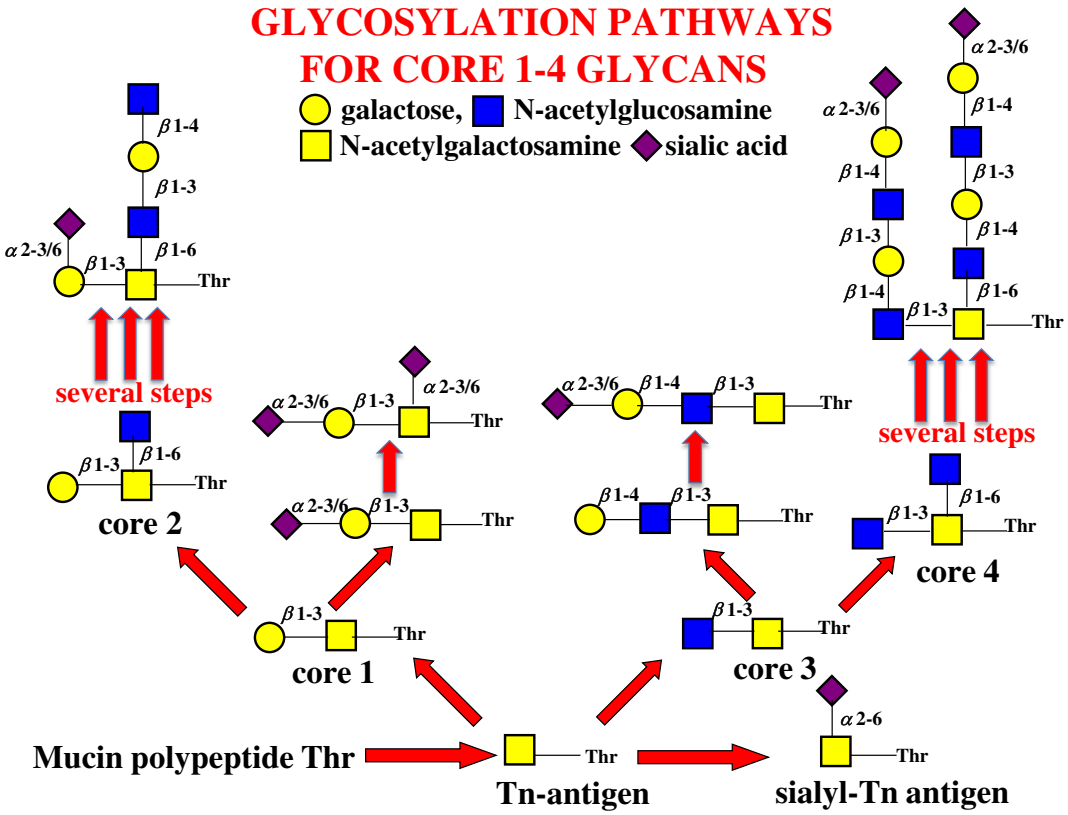


Fig. 3. Glycosylation pathways for core 1 to core 4 glycans. The diagram shows the formation of core 1, core 2, core 3 and core 4 structures and some of the extended glycans formed from these precursors.

microflora exists at all mucosal surfaces and includes the appropriate enzymes and proteins necessary for such management at each site (see also Section 2 above). This manipulation involves release of monosaccharides and their subsequent recovery and metabolic usage. Among these proteins responsible for these processes are the glycosidases, sugar transporters and permeases and a variety of monosaccharide intermediary metabolic enzymes. The employment of these proteins is important to accommodate the dynamic fluctuation and composition of the microflora. Proof of such dynamism at the glycan recognition level exists for the enteric microflora of individuals with different blood groups. In order to degrade and turn over the mucus throughout the gut each secretor positive individual possesses bacterial glycohydrolases associated with their own blood group. Experimentation with isolated human mucin oligosaccharide degrader (MOD) faecal bacterial strains, from individuals with different blood groups shows typical enzymatic activity for each blood group [106,143,150,210,211]. These data imply that transfer of faecal microflora between individuals with different blood groups is incompatible and would lead to destruction of the mucus barrier, [143,144,212] but in vivo experiments have not been reported.

The mucins play an important part in the general metabolic support for the intestinal microflora and are integrated into the dynamic nature of these interactions encountered at the mucosal surface. The mutualism exhibited by the gut microflora is derived from the genetic diversity, high population density and capacity for rapid growth. These features have led to the evolution of an operative, dynamic host-microflora association [121,130,134,213].

It has long been known that mucin glycan can be released and utilised as part of the microflora energy requirement [105,120,128, 130,134,191,213–224]. In addition, mucin oligosaccharide degrader (MOD) strains have been identified and assessed for enzymatic mucin degradating activities and support the glycan foraging and glycan legislation (Fig. 4) premises [106,143,150,210,211]. The utilisation of host-derived glycans as an energy source represents an ecological benefit for reproducible, natural function and the balance of the normal microflora in healthcare issues [104,117,118,120,134,203,213–215,217,221, 222,225–228]. The process termed glycan legislation [117] embodies the basic mode of action necessary for the dynamic environment in the gut (Fig. 4). Evidence for the same process in man has also been proposed [43,213].

Regulation of intestinal angiogenesis through the enteric microflora has also been demonstrated [118,131,213,226,229,230]. In addition, there are many indications for significant roles of the enteric microflora in the innate and adaptive immune systems [132,231–237], but this is a large topic and is beyond the scope of this review.

Thus the evidence presented above demonstrates a role for mucins as part of the protective barrier at mucosal surfaces interacting and responding to the enteric microflora. It provides a dynamic, adaptive and continuous organisation in the face of potential mucosal damage. It maintains the two layer system, vital for normal microflora function in the gastrointestinal tract. Through the provision of an interactive mucin glycoarray mucosal integrity is maintained. The analysis of mucin diversity at protein and glycan levels and its manipulation forms a basis for routine review of normal and pathological events at mucosal surfaces.

These aspects donate further levels of sophistication to the overall integration of functions in host–microflora interactions at the gastrointestinal mucosal surface.

5. The role of mucins and glycogenes in cancer and mucosal diseases

The impact of mucins and their glycoarrays in disease is a very large topic and has been reviewed widely to include both secreted and membrane-associated forms [165,169,238–245]. Changes in glycosylation will influence all glycoconjugates, including the mucins. In this section a few well defined examples have been chosen to illustrate

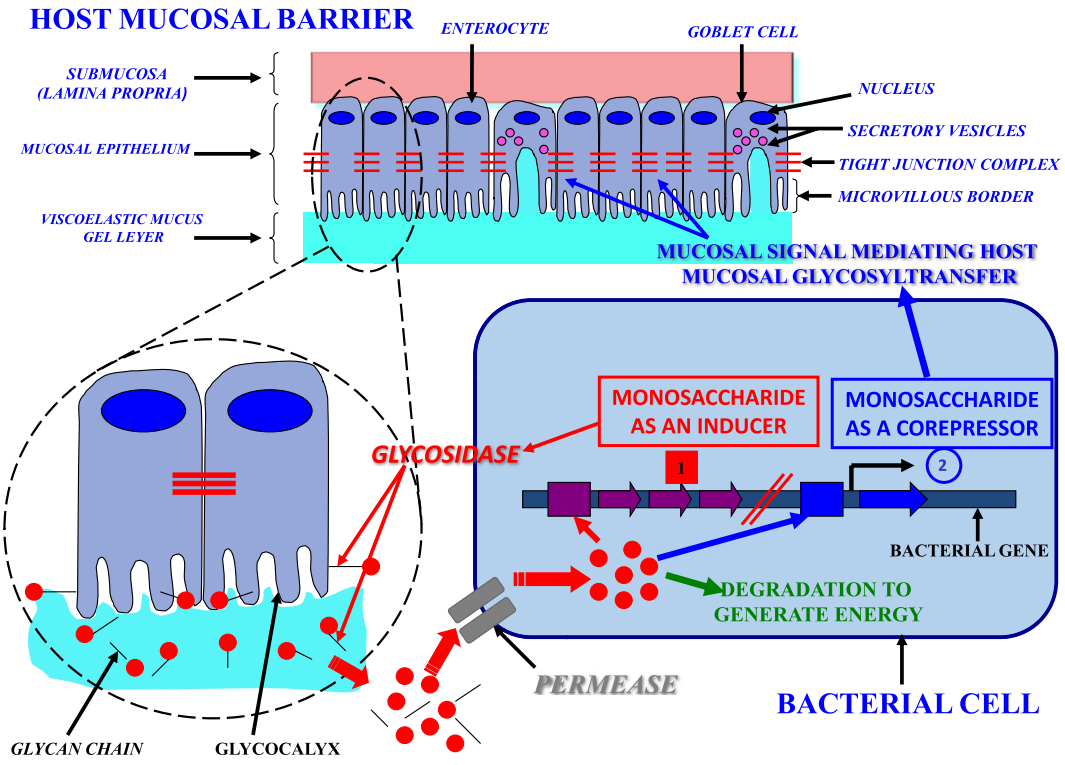


Fig. 4. Glycan legislation. The cartoon shows the relationships at the host mucosal surface with the enteric bacterial population in the colorectum.

this phenomenon. In some cases the major focus has been non-mucin glycoconjugates, but the identified pathological glycosylation patterns will apply to the mucins too.

Major focuses of attention in cancer have been in the female breast; the intestinal tract for gastric and colon cancers; the liver for hepatocellular carcinomas; pancreatic cancer; the respiratory tract for lung cancer and the male and female reproductive tracts for prostate, cervical and ovarian cancers. Together with the mucins the impact of glycosylation in disease pathology has been identified and screened as an important biomarker sharing many glycan structures found in mucins and other glycoconjugates. It has been adopted as a targeted approach in a variety of diseases [11,13,246–264].

Similarly, changes in bacterial flora are well established in association with disease conditions where mucins are also implicated [139, 265,266]. Well described examples include Diabetes, [267,268]; *Helicobacter pylori* infection in the stomach [269–272]; Inflammatory Bowel Disease [190,273] as Ulcerative colitis [274,275] and Crohns' Disease [276], Irritable Bowel Syndrome [277]; Bacterial Vaginosis in the Female reproductive tract [278–280]. Detail of these diseases will not be presented in this review.

In this section a few examples have been chosen to illustrate the pathological roles of mucins, mucin type O-glycans and glycosylation as biomarkers for disease.

5.1. Mucin modulation in cancer

Changes in mucin expression in cancer have been identified at mucosal surfaces throughout the body and generally lead to decreased mucosal protection, loss of normal function and modified interaction with bacterial populations in normal and pathological contexts. The disease mucins formed arise through abberant synthesis of the mucin peptide and the subsequent enzymatic glycosylation events, which may be by incomplete- or neosynthesis and are catalysed by the transformed tissue and cells. The resultant tumour cells thus possess a broad range of potential ligands which may interact with receptors at the cell surface. Consequently these cells express novel, pathological combinations of different mucins which modifies their behaviour and survival during invasion and metastatic events. As this is a large topic covered by many reports, a few selected examples have been chosen for this review.

5.2. Breast cancer

Breast cancer is the most common cancer diagnosed and is the leading cause of death in women [281]. Immunohistological examination of mucin expression in invasive ductal carcinomas of the breast demonstrated an elevated level of MUC1 also associated with abnormal glycosylation [282] and a range of different anti-MUC1 have been employed to detect the mucin in normal breast tissues and tumours. Over 90% of all tumours showed overexpression which correlated with reduced tumour size, a lower grade, a high oestrogen receptor positive status and no evidence for metastasis at distant sites or of regional recurrence. Cytoplasmic, nuclear and membrane location deviating from the normal apical expression is also a feature as detected by immunohistochemistry [283,284].

Vaccine design has been based on the PTS domain amino acid variations observed and the glycosylation patterns observed in cancer, with typical truncated O-glycan structures such as core 1, Galβ1–3GalNAc-Ser/Thr, the Tn antigen GalNAc-Ser/Thr and sialyl-Tn-antigen Neu5Acα2–6GalNAc-Ser/Thr [282]. Initial use of peptide and glycopeptide antigens were not effective and recently the use of a tripartite, abberantly glycosylated MUC1 vaccine has proven to be particularly effective [20].

Compared to invasive ductal carcinomas, mucinous or colloid breast carcinomas showed increased levels of the secreted mucins MUC2 and MUC6, while MUC1 was reduced [285,286].

Abnormal O-glycosylation is also associated with breast cancer [287], typically with increased sialylation [288] leading to truncation of the normal core 2 polylactosaminoglycans and lower levels of core 2 [289]. This is due to a reduced activity of C2GnT1 and higher levels of ST3Gal-I [290]. The action of the sialytransferase generates a terminal structure which cannot be extended, in contrast to the core 2 glycan

which gives rise to extended and branched structures. Modification of N-glycans in breast cancer arose through the action of N-acetylglucosaminyltransferase Va (GnT-Va) leading to increased β1–6-branched N-linked glycan structures on glycoproteins [12].

5.3. Gastric cancer

In gastric cancer a wide range of immunohistological, biochemical and molecular biological studies have identified the involvement of MUC1-MUC6 expression [291] and their glycosylation [292], largely as core 1, sialyl-Tn, sialyl-Lewis^a and sialyl-Lewis^x. Antibody probing for MUC1 revealed an association with increased expression and disease staging [293]. MUC2 correlated with improved survival, which was narrowed to intestinal type carcinomas [293]. Strong MUC5AC levels could be associated with a good prognosis [294], although contradictory findings have been reported [295]. The mucin peptide glycan marker Galβ1–3GalNAc-Ser/Thr (core 1 or Thomsen-Friedenreich (TF) antigen) relates to a poor outcome independent of tumour stage and is also a prognostic marker in stage I tumours [296]. Sialyl-Tn correlates with a reduced survival time [103], while sialyl-Le^a showed prognostic marker properties [293].

5.4. Colorectal cancer

MUC5AC has been shown to be an early oncofetal marker of colon carcinogenesis [297,298]. The de novo expression of MUC5AC, together with low levels of MUC6 during the adenoma–carcinoma sequence in colorectal cancer represent additions to the normal pattern of High MUC2 and low MUC5B expression. These studies used immunohistological detection, supplemented by biochemical and molecular biological methods [299–303]. The increase in MUC5AC and MUC6 is also seen in colorectal polyps [304] and is differentially regulated in adenomas [304]. MUC1 also shows increased expression during colorectal neoplasia [305] and loss of MUC3 also occurs in adenomas [306].

A number of glycobiomarkers correlate with dysplasia, histologic type and polyp size in dysplastic adenomas and include TF antigen [254], sialyl-Tn [242], Lewis^x and Lewis^y on elongated Galβ1–4GlcNAc, type 2 chains, α1–3 fucosylated polylactosaminoglycans [307] and sialyl-Lewis^x [308].

The enhanced formation of these disease associated glycotopes by glycosyltransferases may arise by either incomplete or neosynthesis relative to the normal patterns [160,257,258,309]. The enhanced expression of sialyl-Lewis^x can arise by the impairment of GlcNAc 6 sulphation on Galβ1–3GlcNAc, type 1 chains and α2–6 sialylation on Galβ1–4GlcNAc, type 2 chains [310]. A further example leading to increased sialyl-Lewis^x concerns the deletion of Sd^a synthase [311,312] (Fig. 5). This is a β1–4N-acetylgalactosaminyltransferase B4GNT2, which generates the Sd^a antigen, Neu5Acα2–3(GalNAcβ1–4)Galβ1–3/4GlcNAc- from either sialylα2–3 N-acetyllacto N-biose1amine Neu5Acα2–3Galβ1–3GlcNAc- or sialylα2–3 N-acetyllactosamine Neu5Acα2–3Galβ1–4GlcNAc- [311]. Under normal conditions sialylα2–3 N-acetyllactosamine is a substrate for Sd^a, sialyl-Lewis^a and sialyl-Lewis^x and as the activity of Sd^a synthase is greater than the α1–3 and α1–4 fucosyltransferases forming sialyl-Lewis^a and sialyl-Lewis^x, lower amounts of the sialyl-Lewis structures occur relative to the Sd^a antigen. In colorectal cancer the Sd^a synthase is greatly diminished and the fucosyltransferase activities lead to increased expression of sialyl-Lewis^a and sialyl-Lewis^x [312]. Further deletions found in colorectal and gastric cancer are the core 3 synthase transferring GlcNAc in β1–3 linkage to GalNAc [313,314] and the sialic acid O-acetyltransferase, OAT, which acetylates sialic acids on colorectal mucins [148]. The loss or downregulation of any of these three enzymes individually leads to the development of colorectal cancer.

5.5. Pancreatic cancer

Normal pancreas expresses MUC1 as the main membrane-associated mucin in the cytoplasm of acinar cells and on the apical membranes in ductal cells. MUC5B is the principal secreted mucin, found in both acinar

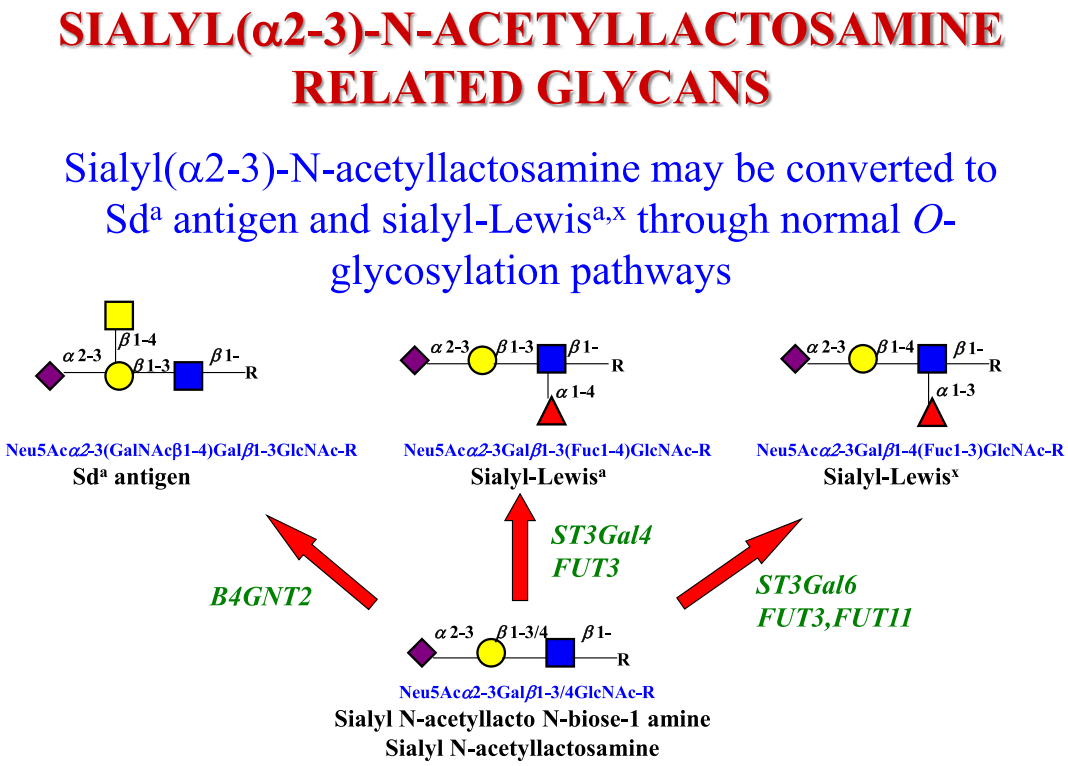


Fig. 5. Sialyl N-acetyllactosamine metabolism. Sialyl (α2–3) N-acetyllactosamine is a branch point in glycosylation pathways leading to the formation of the Sd^a antigen and sialyl-Lewis^a and sialyl-Lewis^y. The glycosyltransferases responsible for these steps, Sd^a synthase, B4GNT2, α1–3/4 fucosyltransferases FUT3 and FUT11 and the sialyltransferases ST3Gal4 and ST3Gal6 are shown.

and ductal cells while MUC6 is found in ducts. Only low levels of MUC5AC have been detected [13,172,315,316]. Pancreatic carcinogenesis progresses through several different stages; pancreatic intraepithelial neoplasia (PanIN1 2 and 3), intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) leading to pancreatic adenocarcinoma. Mucin expression changes with the progression. PanIN shows increased MUC1 and neoexpression of MUC4, while strong MUC1, MUC4 and MUC3 are seen in PanIN3 [315]. A gradual increase of MUC4 accumulates in carcinogenesis [317]. MUC17 is also overexpressed in tumours relative to normal tissue [318]. MUC5AC is a strong marker, being absent in normal pancreas, but appearing at the early PanIN1 stage. MUC6 is at high levels in PanIN1 and subsequently diminishes in the later stages. Intraductal papillary mucinous neoplasm with MUC1 has a short survival rate and a poor prognosis while those with MUC5AC are slow growing adenomas with a good prognosis. Most IPMN show MUC2 [319]. Little data is available for MCN as they are uncommon. Invasive MCN show MUC1 [320], while MUC2, 3 5 AC and 6 are also expressed [321].

The major glycosylation pattern found in normal pancreas includes the blood group Lewis^a (Galβ1–3(Fucα1–4)GlcNAc-), sialyl-Lewis^a (Neu5Acα2–3Galβ1–3(Fucα1–4)GlcNAc- often referred to as CA 19.9) and Lewis^b (Fucα1–2Galβ1–3(Fucα1–4)GlcNAc-) antigens, showing a type 1 (Galβ1–3GlcNAc-) base and, in addition, the H2 (Fucα1–2Galβ1–4GlcNAc-) and Lewis^y (Fucα1–2Galβ1–4(Fucα1–3)GlcNAc-) antigens with a type 2 (Galβ1–4GlcNAc-) base. Lewis^y has also been reported in pancreatic cancers [322–325].

Pancreatic tumours show an increase in sialylated blood group antigens, predominantly sialyl-Lewis^a and sialyl-Lewis^x(Neu5Acα2–3Galβ1–4(Fucα1–3)GlcNAc-), but also Lewis^x structures that have been extended and sialylated [322,326–331]. More recently it was shown that depletion or loss of core 3 synthase (β3GnT-6) occurs in pancreatic cancer, in common with both gastric and colorectal cancer [332]. This downregulation results in the biosynthesis of truncated, mucin type O-glycans, a common feature of cancer cell glycobiology. Mucins, cell surface and extracellular glycoconjugates all show modified glycosylation as a result. Re-expression of core 3 synthase in the pancreatic cancer cell lines Capan-2 and FG led to lower in vitro proliferation, migration, invasion and metastasis demonstrating that changes in mucin type O-glycosylation modulate cancer progression [332].

5.6. Respiratory diseases and lung cancer

The impact of mucus and mucin biology in the field of respiratory health is considerable. In addition to lung cancer, cystic fibrosis (CF), asthma and COPD all contribute to significant healthcare concerns at a global level. There is great interest in understanding the disease mechanisms leading to the characteristic mucus hypersecretion, pulmonary obstruction, reduced mucociliary clearance and subsequent infection observed. Mucus and mucins play a fundamental role in these processes.

The expression of at least nine mucin genes has been reported in the human respiratory tract. These are MUC 1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC16 and MUC19 [7,10,333,334]. The data for MUC19 remain controversial as no evidence for a MUC19 protein has been shown experimentally [335–337]. The most abundant mucins here are MUC5B and MUC5AC, both located in goblet cells. MUC5AC is found in the nasal septum and nasopharynx and in the superficial epithelium of the trachea and bronchii. MUC5B shows high expression in the nasal glands and in airway submucosal glands at all locations in the lung. Animal models have been used to demonstrate the mucus phenotype of CFTR loss in CF [80]. Secretion of the two major mucin products generates a continuous viscoelastic gel at mucosal surfaces throughout the respiratory tree. The thickness of this gel layer is normally a few tens of micrometres, thinner than the barriers found in the gastrointestinal and female reproductive tracts [58,333].

The production of sputum in respiratory diseases has been linked with the hypersecretion of the two main mucin genes MUC5B and MUC5AC [338]. Changes in the glycosylation of these mucins have been described and the combination of secreted mucins and extracellular DNA leads to highly viscoelastic sputum, which causes the respiratory obstruction [10]. The increase of mucin secretion is the basic mechanism leading to pulmonary obstruction.

Respiratory tract infection and inflammation both generate modifications in mucin O-glycosylation. During the inflammatory response the induction of TNFα results in altered tracheal sialylation [339] and both α2–3 sialylation and fucosylation in the bronchii [340]. Cystic fibrosis (CF) and chronic bronchitis are both associated with multiple glycosylation changes, increased glycan sulphation is seen in CF. Investigation of bronchial mucin glycosylation in CF shows increased sulphation, sialylation and fucosylation [174,176,341,342]. Structural features include increases in the levels of the sialyl-Lewis^x epitope, GlcNAc-6-sulphate, Gal-6-sulphate and Gal-3-sulphate in common with changes seen in other respiratory diseases [173]. There is some evidence that the loss of the CFTR in CF has a differential effect on glycosylation and sulphation of bronchial mucins. Membrane bound glycoconjugates show decreased sialylation in CF patients in contrast to the increase seen for secreted mucins [175,343,344]. The increased expression of sialyl-Lewis^x and 6-sulfo-sialyl-Lewis^x epitopes is in good agreement with the infection of sputum by *Pseudomonas aeruginosa* which is known to bind to these glycotopes [345].

5.7. IgA nephropathy

The inclusion of this renal based disease against a background of mucosal pathologies serves to illustrate the impact of shared glycosylation aberrations. Abnormal IgA1 in the circulation leads to mesangial aggregation of polymeric IgA with complement resulting in the glomerular inflammation seen in IgA nephropathy. Normal circulating IgA1 is monomeric while polymeric IgA may be associated with the secretory component and common to sIgA found at mucosal surfaces. The abnormal nature of the polymeric IgA is related to its glycosylation. O-glycosylation is located exclusively in the Fc hinge with up to six sites and consists of short O-glycans, GalNAc, Galβ1–3GalNAc- and some sialylated forms of these neutral structures [346–348]. Changes in O-glycosylation of the Fc hinge region have been proposed as a possible mechanism leading to the disease [349]. Reduction of sialylated O-glycans and increased levels of Galβ1–3GalNAc-(TF antigen) were found associated with nephropathy [349]. Reduction of sIgA1 sialylation through the action of a bacterial sialidase has been demonstrated for bacterial vaginosis and proposed as a potential mechanism for reduced efficiency of sIgA1 in this infection [350].

The N-glycans linked to the heavy chains have been analysed [348]. Evidence for variation of heavy chain N-glycosylation between monomeric and polymeric IgA has been shown, with greater levels of high mannose N-glycans in polymeric IgA [351]. Stronger binding of the polymeric IgA by *Helix aspers*a, a GalNAc specific lectin, to mannose binding lectin and to mesangial cells could be shown [351].

6. Concluding remarks

This review draws attention to the variety of mucin genes and their glycosylation that are known to play roles at mucosal surfaces throughout the body. The scope and adaptation of this family of molecules to their function is enormous and attracts constant reconsideration in the light of the plethora of biological events where they are implicated. The abundance of mucin literature available has meant that some aspects have not been covered in full, however the focus presented here will give ample food for though in the future.

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