

MUCINS IN THE GASTROINTESTINAL TRACT IN HEALTH AND DISEASE

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1. ABSTRACT

Mucins form part of the dynamic, interactive mucosal defensive system active at the mucosal surface of the gastrointestinal tract. They are carbohydrate rich glycoproteins with unique molecular structure and chemical properties. The family of mucin (MUC) genes has 13 members that can be divided into secreted and membrane-associated forms each with characteristic protein domains and tissue specific glycosylation. Biosynthetic pathways have been described for the secreted and

membrane-associated mucins and their eventual degradation and turnover. Mucins are present at all mucosal surfaces throughout the body in typical combinations and relate to the demands of organ function. Patterns of MUC gene expression with gastrointestinal site specific glycosylation are clearly important but are not yet well defined. Mucin production during fetal development shows distinct patterns that may correlate in many cases with neoplastic expression in adult life.

Table 1. Properties of MUC genes expressed in the gastrointestinal tract

Gene	Chromosome	VNTR size	Membrane anchor	EGF-like domains	SEA module	vWF domains	Gel forming
Membrane-associated mucins							
MUC1	1q21	20	yes	no	yes	no	no
MUC3	7q22	17/59/375	yes	yes	yes	no	no
MUC4	3q29	16	yes	yes	yes	no	no
MUC12	7q22	28	yes	yes	yes	no?	no
MUC13	3q13.3	15	yes	yes	yes	no	no
Secreted mucins							
MUC2	11.p15.5	23	no	no	no	yes	yes
MUC5AC	11.p15.5	8	no	no	no	yes	yes
MUC5B	11.p15.5	29	no	no	no	yes	yes
MUC6	11.p15.5	169	no	no	no	yes	yes
MUC11	7.q22	28	?	?	?	?	?

Characteristics of MUC genes in the gut are given. Where data is lacking or incomplete a question mark (?) is shown. The VNTR size is given as the number of amino acid residues. Data is taken from literature quoted in the text.

An increasing number of protective proteins have been identified that appear in the adherent mucus layer at the mucosal surface. These proteins are co-secreted with mucins in some cases, interact with mucins at a molecular level through peptide and carbohydrate sites or benefit from the viscoelastic, aqueous environment afforded by the mucus gel to effect their defensive roles. The mechanism of many of these interaction remains to be elucidated but is clearly part of an integrated innate and adaptive mucosal defensive system relying on the mucins as an integral component to provide a mucus gel.

Recent improvements in the description of MUC gene expression and mature mucin synthesis in the healthy gastrointestinal tract has formed a basis for assessment of mucosal disease at sites throughout the tract. Pathological patterns of mucin expression in disease appear to follow tissue phenotype, so that gastric and intestinal types can be defined and appear in metaplasia in e.g. esophagus and stomach. Adaptation of previous mucin based, histochemical classification of intestinal metaplasia to assess MUC gene expression has proved helpful and promises greater value if reliably combined with mucin linked glycosylation markers. Few changes in MUC gene expression or polymorphism have been detected in inflammatory bowel diseases in contrast to malignant transformation. Glycosylation changes however, are evident in both types of disease and appear to be early events in disease pathogenesis. Review of the major mucosal diseases affecting the gastrointestinal tract in childhood reveals parallel patterns to those found in adult pathology, but with some novel conditions arising through the developmental stages at lactation and weaning. The impact of bacterial colonization and nutrition at these stages of life are important in the evaluation of mucosal responses in pediatric disease.

2. INTRODUCTION

The mucosal surface throughout the gastrointestinal tract must resist the aggressive elements from the external environment present in the diet and encountered during normal function. This defensive system is based on fundamental characteristics shared with the barrier found at other mucosal surfaces. The stable protective barrier enabling exchange between the epithelial cells of the gut lumen for the purposes of nutrition and protection is made up of a layer of secreted mucus and a cell-surface membrane glycocalyx. The mucus defensive

barrier forms the first line of defence to the external environment and contains both innate and adaptive immune elements. This review covers the composition and function of mucus and the rapidly increasing information on the failure of the barrier in gastrointestinal disease. A number of valuable reviews have appeared which add detail to the information presented here. (1-16)

3. THE NORMAL PROTECTIVE MUCUS BARRIER IN THE GASTROINTESTINAL TRACT

3.1. Mucins

Mucins or mucus glycoproteins are a family of polydisperse molecules designed to carry out multiple tasks at the mucosal surface of the gastrointestinal tract. The molecular organization of the individual mucins relates to their many functions. The *secreted* mucins are characterized by their very high molecular weight and size, a high proportion of O-glycosidically linked carbohydrate (usually 50-80%) and their ability to form viscoelastic gels. *Membrane-associated* mucins share many of these basic structural properties, belong to the same family of molecules, but have additional properties as active membrane components. They are integrated into membranes, are monomeric and do not form gels.

The polydisperse properties of the mucins are due to the variation of both peptide domains and oligosaccharide composition. Recent advances in the molecular biology of the MUC genes, described in accompanying reviews in this series, together with improved understanding of the biochemistry of the mature products have now indicated reasons why this polydispersity exists and the biological advantages associated with it.

3.1.1. Genes and peptide structure

The following section draws attention to those MUC genes identified in the gastrointestinal tract. At present 13 members have been identified (14, 17-26) of which ten are found in the gastrointestinal tract. The properties of these genes and their peptides are shown in Table 1.

The MUC genes each contain unique variable number tandem repeat (VNTR) sequences, with high serine, threonine and proline (STP rich regions). (2, 3, 10, 14). Linkage of the carbohydrate side chains through O-glycosidic bonds to serine

and threonine means that these are the major sites of glycosylation in the mucins (10, 27). The unique VNTR regions have been used as markers for the detection of mucins. Antibodies to the VNTR peptide sequences have been prepared as gene specific reagents (28). However, as these sequences are heavily glycosylated the mature mucins may not bind antibody. Only the mucin precursors that have not been fully glycosylated may react with anti-VNTR antibodies. These are normally present in the Golgi apparatus where the peptide is first translated but has not been O-glycosylated. Accordingly, perinuclear - Golgi staining patterns have been reported for these antibodies (29).

The *secreted* mucins are represented by MUC2, MUC5AC, MUC5B and MUC6, all of which are clustered on chromosome 11p15.5. MUC2, MUC5AC and MUC6 show major expression in the epithelia throughout the tract with MUC5AC and MUC6 in the stomach and MUC2 in small and large intestine (30, 31). MUC5B shows major expression in salivary glands (32, 33) and esophageal glands (34, 35) but minor levels in gastric (30, 36) and colorectal epithelia (30, 37). The non-VNTR regions in secretory MUC genes MUC2, MUC5AC, MUC5B and MUC6 contain multiple cysteine rich domains which show homology with the D and C domains of von Willebrand factor and carboxy-terminal, cystine-knot domains (16, 17, 20, 38, 39). These domains are required for dimerisation and oligomerisation of mucins (39, 40) and von Willebrand factor (41) as they are processed through the rough endoplasmic reticulum (ER) and the Golgi network. Analogy of von Willebrand factor domains with a similar molecular organization in the secreted mucins strongly suggests that they are fulfilling similar functions of oligomerisation and storage. Additional support for this argument comes from comparison of the secreted salivary mucin MUC7 with the gel forming mucins. MUC7 has no von Willebrand factor, cysteine rich domains and does not form viscoelastic gels (23).

Five membrane-associated mucins have now been identified (MUC1, 3, 4, 12 and 13). MUC3 and 12 genes have been confirmed in a cluster on Chromosome 7q22 (25, 42, 43). This has attracted attention as it is also a susceptibility locus for inflammatory bowel disease (44). The organization of membrane-associated mucin peptide structure is in keeping with membrane functions. In addition to the typical mucin STP-rich VNTR sequences they contain C-terminal cytoplasmic motifs consistent with signal transduction; SEA modules located in the extracellular region; transmembrane domains, epidermal growth factor (EGF)-like domains (except for MUC1) and cytoplasmic 'tail' domains with sites for serine phosphorylation and tyrosine sulphation (18, 19, 25, 26, 42, 43, 45-47). (Table 1). Signaling roles for these molecules as integral membrane components have been demonstrated. MUC1 cytoplasmic tail contains tyrosine residues that are phosphorylated and are thought to play a role in the regulation of signal transduction (48, 49) and similar tyrosine phosphorylation sites have been identified in MUC3 and 12. The presence of EGF like domains in MUC 3 (43), MUC4 (46, 50), MUC12 (25) and MUC13 (26) has been linked with signaling pathways and supports a role of these molecules in modulation of epithelial cell behavior. These characteristics are in addition to the cell adhesion properties proposed for membrane-associated mucins (1, 15). A further feature of the

membrane-associated mucins is the cleavage of the gene-coded peptide into mucin-like and membrane-associated fragments and appearance of soluble forms as cleavage products or as splice variants without membrane spanning domains. This has been demonstrated biochemically for MUC1 (45, 51), MUC4 (46) and MUC13 (26), while splice variant production of soluble products has been reported for MUC1 (48), MUC3 (43, 47) and MUC4 (50). The functional relevance of these forms is not yet clear.

Most of the MUC genes show polymorphism in the number of tandem repeats present in the VNTR domain (14, 51-54) which result in real size differences in the translated mature mucins as has been shown for colonic MUC2 (55). The only exception is MUC5B, which does not show common allele length variation (52, 54).

3.1.2. Glycosylation

Mucins have a tissue specific glycosylation at each site in the gastrointestinal tract. As carbohydrate constitutes the major part of all mature mucins and is represented by vast array of different oligosaccharide structures the potential for multiple functions related to bulk carbohydrate or individual structures must be examined. Most of the oligosaccharides in mucins are attached by O-links, (10, 56-58). However, a much smaller number of N-linked chains are also present, linked to asparagine residues in the mucin polypeptide through an N-glycosidic bond to N-acetyl-D-glucosamine. The consensus peptide sequence asn-X-ser/thr (NXS/T) is recognized as a potential glycosylation site and these have been detected, mostly outside the VNTR regions (14, 18, 19, 38, 59). N-linked oligosaccharides contain a branched trimannosyl-chitobiose pentasaccharide core attached to the peptide. Further additions to this pentasaccharide core yield a family of oligosaccharides rich in mannose (high mannose) or with D-galactose, N-acetyl-D-glucosamine L-fucose and terminal sialic acids (complex) and mixtures of the two (hybrid) (60). Mannose is found in these chains in contrast to the O-linked oligosaccharides and was at one time taken as a measure of impurity in mucin preparations. The transfer of N-linked oligosaccharides occurs at very early stages of mucin biosynthesis. The N-linked chains have specific functions in subcellular compartment targeting through the Golgi complex and into secretory vesicles, folding of the mucin polypeptides (61) and in recycling pathways for some of the membrane-associated mucins.

The mucin peptide STP rich VNTR regions carry the O-linked oligosaccharides. They are attached through the oxygen of serine and threonine residues to N-acetyl-D-galactosamine (58). No amino-acid consensus sequence for O-linkages has been detected although a predictive system based on sequence context and surface accessibility has been proposed (62). Three structural regions can be identified for O-linked oligosaccharides (63). All chains have a core, some have a backbone and most have peripheral additions. Eight different core units linked to the peptide have been found made up of di- or tri-saccharides all containing N-acetyl-D-galactosamine (58, 64). Core 1-4 are commonly found in mucins throughout the gastrointestinal tract (10, 58, 64). These are shown in Table 2. The salivary and gastric mucins contain all four core structures while the colorectal mucins are limited to core 3 (10). Meconium mucins contain an additional two core structures

Table 2. Common glycosylation motifs in mucin-type O-linked oligosaccharides

Name	Structure	Comment
Core structures		
Core 1	GalNAc-alpha-O-peptide Gal-beta-1-3	Present in salivary, gastric and meconium mucins
Core 2	GlcNAc-beta-1-6 GalNAc-alpha-O-peptide Gal-beta-1-3	Present in salivary, gastric and meconium mucins
Core 3	GalNAc-alpha-O-peptide GlcNAc-beta-1-3	Present as major core in colonic mucins also found at lower level in salivary, gastric and meconium mucins
Core 4	GlcNAc-beta-1-6 GalNAc-alpha-O-peptide GlcNAc-beta-1-3	Present as major core in colonic mucins also found at lower level in salivary, gastric and meconium mucins
Backbone (elongation) structures		
Type 1	Gal-beta-1-3GlcNAc	May be single or multiple (poly N-acetylglucosamine) units
Type 2	Gal-beta-1-4GlcNAc	May be single or multiple (poly N-acetylglucosamine) units
Poly N-acetylglucosamine type 2 (i-antigen,)	(Gal-beta-1-4GlcNAc-beta-1-3) _n	Linear sequence of repeating type 2 units, recognized by anti-blood group I antibodies
I-antigen,	Gal-beta-1-4GlcNAc-beta-1-6 Gal-beta-1 Gal-beta-1-4GlcNAc-beta-1-3	Branched structure recognized by anti-blood group I antibodies
Peripheral structures		
Blood group H Type 1 (Gal-beta-1-3GlcNAc)	Fuc-alpha-1-2Gal-beta-1-3GlcNAc-beta-1-	basic blood group structure, also found on type 2 chains (Galβ1-4GlcNAc)
Blood group A type 1 (Gal-beta-1-3GlcNAc)	GalNAc-alpha-1-3Gal-beta-1-3GlcNAc-beta-1- alpha-1-2 Fuc	terminal alpha linked sugar, also found on type 2 chain (Galβ1-4GlcNAc)
Blood group B type 1 (Gal-beta-1-3GlcNAc)	Gal-alpha-1-3Gal-beta-1-3GlcNAc-beta-1- alpha-1-2 Fuc	terminal alpha linked sugar, also found on type 2 chain (Galβ1-4GlcNAc)
Lewis a	Gal-beta-1-3GlcNAc-beta-1- alpha-1-4 Fuc	Lewis antigen on type 1 chain
Lewis ^b	Fuc-alpha-1-2Gal-beta-1-3GlcNAc-beta-1- alpha-1-4 Fuc	Lewis antigen on type 1 chain
Lewis ^x	Gal-beta-1-4GlcNAc-beta-1- alpha-1-3 Fuc	Lewis antigen on type 2 chain
Lewis ^y	Fuc-alpha-1-2Gal-beta-1-4GlcNAc-beta-1- alpha-1-3 Fuc	Lewis antigen on type 2 chain
Sialyl Lewis ^a	Neu5Ac-alpha-2-3Gal-beta-1-3GlcNAc-beta-1- alpha-1-4 Fuc	sialylated Lewis antigen
Sialyl Lewis ^x	Neu5Ac-alpha-2-3Gal-beta-1-4GlcNAc-beta-1- alpha-1-3 Fuc	sialylated Lewis antigen
Sulfo Lewis ^a	SO ³ -Gal-beta-1-3GlcNAc-beta-1- alpha-1-4 Fuc	sulfated Lewis antigen, also found for Lewis x
Tn antigen	GalNAc-alpha-O-peptide	commonly found as a tumour associated antigen, normally substituted with sialic acid
Sialyl-Tn	Neu5Ac-alpha-2-6GalNAc-alpha-O-peptide	cancer associated antigen
Thomsen Friedenreich T-antigen	Gal-beta-1-3GalNAc-alpha-O-peptide	commonly found as a tumour associated antigen, normally substituted with sialic acid
Sialyl-T-antigen	Neu5Ac-alpha-2-3Gal-beta-1-3GalNAc-alpha-O-peptide Neu5Ac alpha-2-6 Gal-beta-1-3GalNAc-alpha-O-peptide	cancer associated antigen

The structure of non-reducing oligosaccharides commonly found in gastrointestinal mucins are listed under the three main oligosaccharide domains, which are color coded **red** for **Core** - GalNAc-O-peptide; **blue** for **Backbone** Gal-beta-1-3GlcNAc and **green** for **Peripheral** Fuc-alpha-1-2

(10) suggesting that mucin glycosylation has a role in gastrointestinal development.

The specificity of the glycosyltransferases predicts the glycosylation pathways and determines that a limited number of oligosaccharide structures can be formed. The presence of a defined core also influences the nature of the final oligosaccharide product (64). Mucin oligosaccharides with elongated chains exhibit backbone regions. They consist of alternating D-galactose and N-acetyl-D-glucosamine residues in either beta-1-3 (type 1) or beta-1-4 (type 2) linkage. Type 2 disaccharide units can be found in linear or branched forms giving rise to the I/i antigen (10, 57) (Table 2).

The peripheral regions often donate characteristic identity to mucins and are also implicated in specific binding properties. They include sialic acids and sulphate esters, which donate negative charge, and the blood group and Lewis antigens, which are present on all mucosal surface glycoproteins in the gastrointestinal tract of secretor positive individuals. These terminal residues have a significant influence on mucin antigenicity, mucin charge (neutral or acidic properties) mucin rheology and in mucin degradation. The sialic acids occur as peripheral residues in alpha-2-3, alpha-2-6 linkage in most oligosaccharides and alpha-2-8 in di-tri- or polysialyl units. In man only N-acetyl-D-neuraminic acid is found while the N-glycolyl form, found in most other mammals, is antigenic and the gene for the hydroxylase which catalyses this conversion is suppressed (65). The sialic acids occur as a family of modified monosaccharides. Most common are the mono- and poly-O-acetylated sialic acids that have a tissue specific distribution in gastrointestinal mucins. The presence of poly-O-acetylated sialic acid in colonic mucins gives these molecules a unique hydrophobic character and resistance to degradation by bacterial enzymes (66, 67). Sulphate esters are found on D-galactose and N-acetyl-D-glucosamine residues in gastrointestinal mucin oligosaccharide chains (68) (Table 2), but only limited information is available and the total scope of sulphated epitopes present has not been examined.

Strong evidence for specific glycosylation of individual mucin genes has been shown in the normal stomach, where the expression of individual fucosyltransferase genes correlates with either MUC5AC or MUC6 (69). However, this example requires direct structural demonstration and is a single example among many possibilities.

A variety of peripheral structures are found which are expressed on mucins as developmental antigens or are induced in response to inflammation. Accordingly the collection of data for mucins in well defined disease conditions has led to the identification of disease related glycosylation pathways and disease related carbohydrate antigens (10, 13, 56) and is considered later in this review.

3.1.3. Biosynthesis and secretion

The biosynthesis of a secreted mucin must ensure translation of the MUC gene; correct folding of the peptide, dimerisation, the appropriate O-glycosylation, polymerization and storage. Separate pathways are responsible for the synthesis of secreted and membrane-associated mucins, dictated by their

location in different cellular compartments. The initial stages of MUC peptide translation include N-glycosylation. The N-linked oligosaccharides direct the precursor peptides to their correct subcellular compartments for dimerisation, subsequent O-glycosylation and oligomerisation (70-72). Furthermore, the ER lectins calnexin and calreticulin recognize N-glycans on MUC2 and appear to modulate synthesis at the stage of folding and oligomerisation in the ER (61). This process ensures the correct alignment for the formation of disulphide bridges in the von Willebrand D-domains, cystine knot and EGF-like domains. Since no interaction of the chaperones with MUC5AC was detected at a similar stage of synthesis, these two structurally similar secretory mucins seem to have different requirements in the ER (61). MUC precursor forms with N-glycans but without O-glycosylation have been shown using specific anti-mucin-peptide antibodies and metabolic labeling (71). The same methods have also been used to follow dimerisation and the movement of the maturing peptides through the rough ER and Golgi apparatus before O-glycosylation. The formation of dimers from mucin peptide precursor monomers in the rough ER is an early stage in the synthesis of secreted mucins. MUC2, MUC5AC, MUC5B and MUC6 undergo this process in the human and rodent gastrointestinal tract (39, 71, 73-75). Reduction sensitive disulfide bridges at the C-terminal domains play a part in this early stage of mucin synthesis. A gene specific system appears to function here as no hetero-dimerization between different MUC subunits has been detected. Monomers and dimers are transferred to the Golgi apparatus and undergo O-glycosylation (71, 73, 74). Multimerisation is thought to take place in different subcellular compartments to dimerisation (40, 71, 73, 74). The von Willebrand D domains at the N-terminus of secreted mucins are required for multimerisation and the cysteine rich motif CGLCG is thought to play a role. These motifs in mucins may facilitate thiol-disulfide interchange reactions during the assembly of disulfide-bridged multimers of mucin (40, 76). In the case of MUC2 additional non-disulphide bond cross linkages occur, but these have not been characterized chemically (55).

The synthesis of O-linked oligosaccharides follows well-defined pathways determined by the substrate specificity of individual glycosyltransferases adding monosaccharides sequentially to the growing oligosaccharide chain along the mucin peptide (10, 56, 57, 60). Activated monosaccharides as their respective nucleotide forms are the donors in these reactions (60, 77). Close regulation of the initiation of the O-linked chains is apparent through the discovery of a family of N-acetylgalactosaminyl transferases which recognize the amino-acid sequence of the VNTR in each mucin gene (78, 79). Different members of this family recognize serine and threonine in various positions in mucin VNTR domains, and are sensitive to the presence of GalNAc already transferred to these sequences (79-81). Some of these enzymes possess a lectin-binding domain, which is active in glycosylated (GalNAc substituted) substrate recognition (82). Extension and completion of the oligosaccharides follows and is determined by the strict substrate specificity and enzyme availability in the respective tissues. The synthesis of a particular pattern of oligosaccharides, characteristic for a defined tissue, relies on the availability of certain glycosyltransferases within particular Golgi subcellular compartments (10, 57, 60, 83). The deletion

or blocking of individual glycosyltransferase reactions has a profound influence on pathways leading to the final oligosaccharide structures and may result in premature termination or elimination in some cases. This is well documented for pathologically abnormal glycoproteins. Together with this type of modulation the induction of novel activities can lead to competition for common substrates on established pathways and lead to altered oligosaccharides which have been identified as disease related carbohydrate antigens (10, 13, 56), see Table 2 for some examples.

As a large proportion of secreted mucins are synthesized and stored in classical secretory cells, the goblet cells, considerable interest has been directed at their mechanisms of secretion. Mucus containing secretory vesicles in goblet cells accumulate at the apical pole and discharge their contents by exocytosis. The plasma and vesicular membranes fuse with the formation of a fusion pore (see 84, 85). The force required to expel the mucins may arise from the hydration of the stored mucins on contact with the external aqueous environment (86). Ion-channel proteins and ATP-dependent proton pumps' located at the base of the granules aid complete secretion by providing a washout secretion (85). Depending on the nature of the secretory stimulus different patterns of morphological change occur during secretion. In some cases rapid and extensive exocytosis may occur leading to typical goblet cell cavitation with loss of cytoplasm and granule membranes (87). Two secretory pathways have been identified in secretory cells. Continuous release of mucus and proteins from vesicles in these cells is mediated by a constitutive pathway which is not subject to the action of receptor mediated secretagogues. The vesicles mature and discharge and show no long term residence at the apical pole of the goblet cells. In contrast, distended vesicles, accumulated at the apical pole of the goblet cells characterize the regulated pathway and which show exocytosis after stimulation of receptors by secretagogues. Many secretagogues have been identified acting through cholinergic, purinergic and neurotensin receptors. Calcium- and protein kinase C mediated signal pathways regulate these events through increased intracellular calcium (84, 85, 88, 89). Protein kinase C mediates increased MUC2 and MUC5AC expression in response to the protein kinase C activator phorbol 12-myristate 13-acetate, suggesting close integration of mucin synthesis and secretion in these cells (90). Intestinal goblet cells operate both constitutive and regulated pathways and thus contain vesicles delivering a maintained secretion and those sensitive to the action of secretagogues. A physiological stimulation-response with regard to the regulated release of mucus from goblet cells in the gastrointestinal tract can be proposed on the basis of the known receptors. Accordingly, many possibilities exist in relation to the wide range of dietary components with potential secretagogue activity, however it is difficult to assess the daily contribution of the diet to this process.

3.1.4. Degradation and turnover

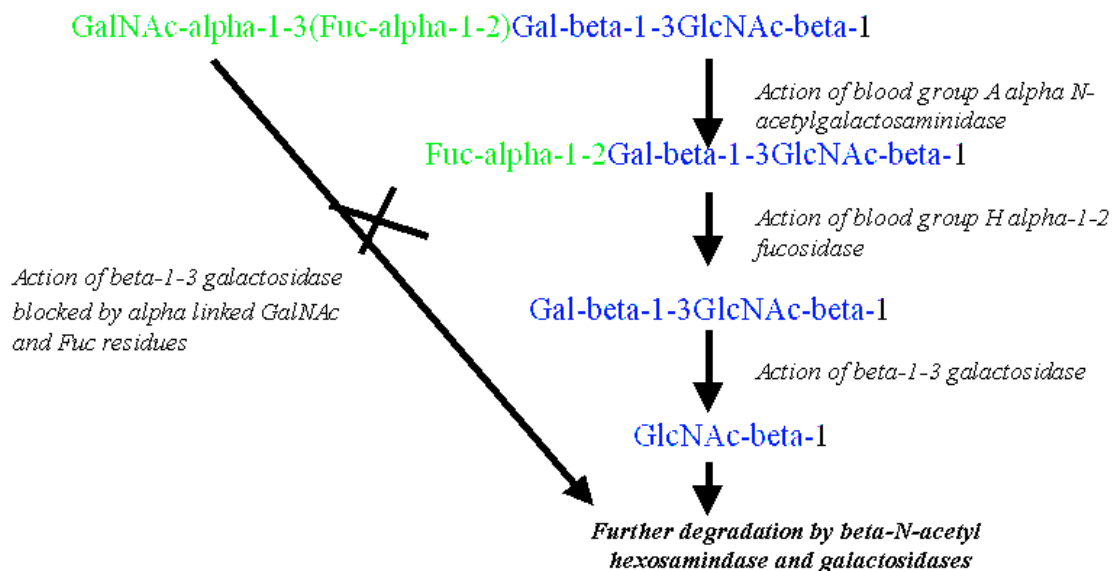
The adherent mucus barrier and the glycocalyx are constantly being turned over as part of their protective functions at the mucosal surface. Thus, degradation of mucus is a normal feature of an equilibrium between mucosal synthesis, secretion and the breakdown of the existing adherent gel. This balance must be regulated to ensure continual mucosal

protection against potentially damaging compounds and organisms entering in the diet. Mucinase activity has been described and is well known from observations of mucin carbohydrate release, sequestration and metabolic conversion by bacteria in the large intestine. A population of mucin oligosaccharide degrading (MOD) bacterial strains has been identified capable of specific and complete degradation of mucins (91). The released carbohydrate breakdown products are utilized for bacterial energy production, resulting in the production of short chain fatty acids, the end products of bacterial metabolism, and potential energy sources and differentiating agents for the host mucosal epithelial cells (92). As the oligosaccharide composition of mucins is diverse the battery of enzymes required for their degradation is also broad (68). Mucinase enzymes act on the mucus gel to reduce it to a viscous fluid, probably through the action of proteinases or peptidases, act further on the accessible peptide backbone, not blocked by oligosaccharide substitution and cleave the individual sugars from the oligosaccharide chains. Integration of these activities occurs in the gastrointestinal tract to effect total turnover as many bacteria do not possess all of the enzymes necessary for complete degradation (68). As these activities exist in secreted, membrane bound and periplasmic forms correlation of substrate specificity with enzyme location and mode of action at the mucosal surface is necessary to understand their physiological relevance. The major site of mucinase activity in the gut is the large intestine. Derived essentially from the enteric gut flora it is acquired at birth and accumulates over the first two years of life to levels established in adulthood (93). Mucinase has been measured in faecal extracts using electrophoretic assessment of mucin degradation (93) and by direct mucinase assays with purified biotinylated mucins (94, 95). Adult mucinase activity contains peptidases, glycosidases, sulphatases and esterases that are present as secreted or membrane bound enzymes in the gut flora (96). Most bacterial strains in the large intestine produce mucin-degrading enzymes in different proportions. The MOD strains possess all hydrolytic enzymes for complete degradation of mucin oligosaccharide chains, but relatively little proteolytic activity (66, 96). The symbiotic relationship between the enteric flora and the gut mucosa is analogous to that observed in the human oral cavity, where different bacterial strains ensure total mucin degradation.

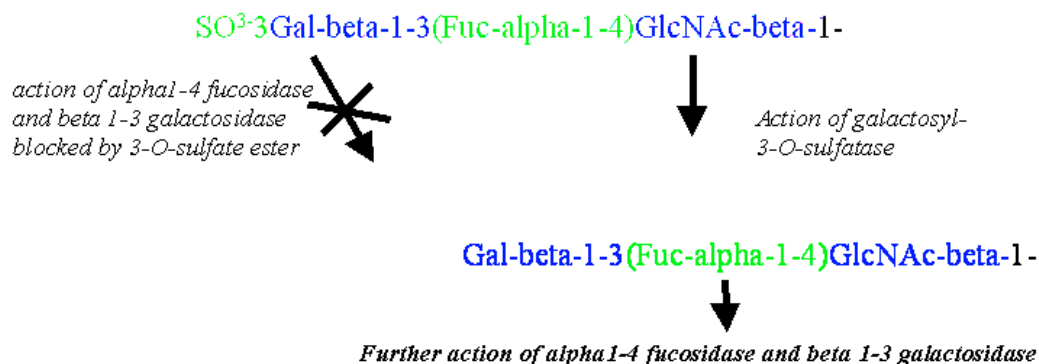
The first stage in mucus degradation is conversion of the mucus gel to a viscous fluid. Faecal bacterial protease activity appears to be the most likely agent responsible for this. A range of cell-associated and secreted protease activities have been identified in the enteric bacterial population which allow optimal interaction with the mucus gel to commence its solubilisation (97). The generation of glycopolypeptides by the action of proteases provides substrates for the glycosidases acting on the carbohydrate chains. Most are exoenzymes removing a single monosaccharide from the non-reducing oligosaccharide terminus. Endoglycosidases act at internal sites in the carbohydrate chains releasing oligosaccharide fragments rather than monosaccharides.

The overall rate of oligosaccharide degradation depends on the exoenzyme degradation of the peripheral residues on oligosaccharides. The enzymes that remove the peripheral sialic acids, sulphate and blood group units in

a) Blood group A



b) Sulfo Lewis^a



c) 7,8,9,tri-O-acetyl-sialyl-Lewis^x

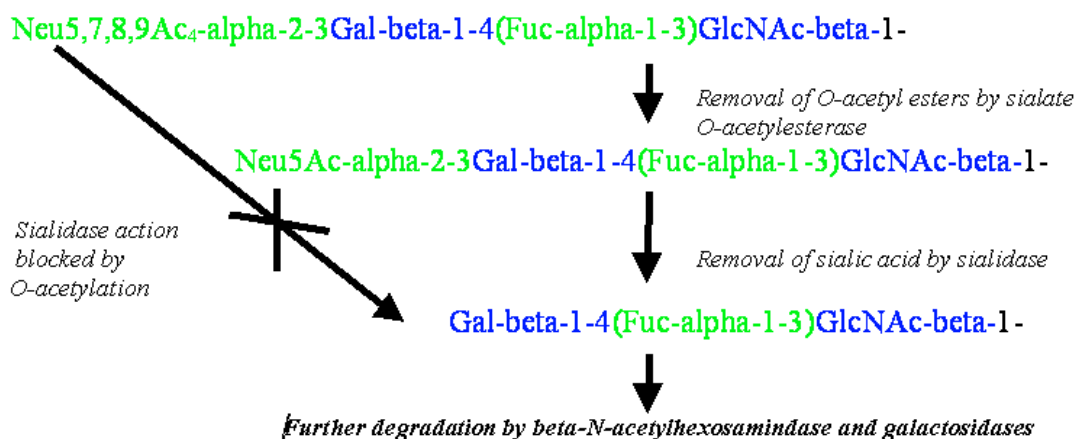


Figure 1. Regulation of mucin oligosaccharide degradation by peripheral residues. Action of enteric bacterial enzymes.

Table 3. Distribution of mucins in the normal gastrointestinal tract

Organ	Location	Cellular identity	MUC gene pattern	Chemical nature of mucins	Literature
Esophagus	Surface epithelium		MUC1, 4	?	34, 35
	Submucosal glands	Acini	MUC5B	mostly sulphated	34, 35
Stomach	Fundus	Ducts	MUC 4	mostly sulphated	34, 35
		Surface epithelium	MUC5B,5AC > 1	neutral with some sialo-sulphomucins	30, 105
	Body and antrum	Glands	MUC6 > 1	neutral with some sialomucins>sulphomucins	30, 105
		Surface foveolar cells	MUC5AC > 1,3	neutral mucins with some sialo-sulphomucins	105, 207
Small intestine	Duodenal gland	Mucous neck cells	MUC6 > 1	neutral mucins, some sialomucins	105, 207
		Acini	MUC6	neutral mucins	7, 30, 105
	Surface/Villi	Goblet cells	MUC2> 3 >, 1, 4	sialomucins	7, 30, 105
		Enterocytes	MUC1,3,4	?	30
	Deep/Crypts	Goblet cells	MUC2 > 1, 4	neutral mucins	7, 30, 105
		Enterocytes	MUC1,4	?	7, 30, 105
Colorectum	Surface/Villi	Goblet cells	MUC2, 4, 11 >12 > 3, 1	Sulphated sialomucin predominant in the right colon, non-sulphated mucin in the left colon	7, 30, 105
		Colonocytes	MUC3, 4 > 1, 12	?	30
	Deep/Crypts	Goblet cells	MUC2, 11 > 4, 12 >5B, 1	Sulphated sialomucin predominant in the left colon, non-sulphated mucin in the right colon	7, 30, 105
		Colonocytes	MUC4 >12 > 1	?	30

The major patterns of mucins detected throughout the gastrointestinal tract are shown. The relative abundance is semi-quantitative based on the use of reagents reported in each study.

oligosaccharide are important as they allow other glycosidases to act on the remainder of the chain. There is evidence that both the sialic acid esterases, sulphatases and blood group specific alpha-galactosidases and N-acetyl- alpha-D-galactosaminidases are rate limiting in mucin oligosaccharide degradation (68) (Figure 1). More than 90% of Europeans and North Americans produce colonic mucins containing C7-C9-O-acetylated sialic acids (98). The O-acetyl esters block the action of sialidases (neuraminidases) present in high levels in many enteric bacteria (66, 68). In human colorectal mucins the release of sialic acids by sialidase is regulated by the action of a specific sialate O-acetyl esterase (66). As this enzyme shows significantly lower activity than the sialidase it acts as a rate limiting step in the removal of sialic acids from mucin oligosaccharide chains (66, 67) (Figure 1c). Sulfate esters on mucin oligosaccharide chains are thought to function in a similar manner (Figure 1b). Sulfated mucins are secreted in regions of the gastrointestinal tract with the greatest bacterial colonization, mainly the large bowel. A family of glyco-sulfatases acts on sulfomucins (99, 100). These enzymes are specific for the sulfated monosaccharide and the position of the sulfate. (99-101), and have been reported in fecal extracts (67, 102) and individual bacterial strains (99-101). The ABH blood group antigens also limit the rate of oligosaccharide degradation (Fig 1a). Mucosal secretions of adult, secretor positive individuals carry the blood group antigens in all regions of the gastrointestinal tract except the distal colon. During development, the complete fetal gut shows blood group antigen expression (103). The glycosidases acting on these structures are the N-acetyl-alpha-galactosaminidases (A activity) and alpha-galactosidases (B activity). Fecal extracts from secretor positive individuals with either A, B or H blood group status acting on mucins with A, B or H blood group activity show the rate limiting activity of these enzymes. Extracts from H individuals were incapable of degrading mucins with A or B activity, while the mucins with H activity are rapidly degraded by extracts from A and B

individuals (104). The degradation of the remaining oligosaccharides exposed after the action of the enzymes releasing the terminal residues is completed by a variety of glycosidases that show specificity for the glycosidic linkage, the neighboring sugar and the region in the chain. High levels of beta-galactosidases and N-acetyl-beta-D-glucosaminidases act on mucin removing the N-acetyl-lactosamine backbone structures where present (68). The action of these glycosidases reduces the oligosaccharides to the core structures and specific O-glycanases remove these disaccharides e.g. Gal-beta-1-3GalNAc (core 1, Table 2) from mucin peptides. These enzymes are found in mucin oligosaccharide degrader and other strains (66, 67). An O-glycanase acting on the common core found in colonic mucins, core 3 (GlcNAc-beta-1-3GalNAc) has not been described.

3.1.5. Sites of production

Mucins have been identified at surface epithelial cells and specialized glands throughout the gastrointestinal tract. Analyses have shown gene product and mRNA for the MUC genes which has backed up the initial histochemical detection of the carbohydrate and sulphation patterns (7, 105) The distribution is summarized in Table 3. The specific functional requirements for mucus at each site are reflected in the appearance of different mucins in the cells at each location. The main population of specially adapted cells producing secreted mucins are the goblet cells, present in varying proportion throughout the gastrointestinal tract (87). The proportion increases through the GI tract with maximal numbers in the rectum.

3.1.6. Mucus barrier thickness and rheology

The supramucosal mucus barrier is made up of the secreted, adherent gel layer and the membrane-associated glycocalyx (1, 15). The composition and thickness of this layer varies throughout the tract due to the nature and extent of secretions from different cells and glands at each location. Thus the characteristics of the mucus barrier at each region of the

Table 4. Mucus layer thickness in the human gastrointestinal tract

Site in gastrointestinal tract	thickness (microns)	literature
Esophagus	Absent	106
	95±12	188
Gastric antrum	144±52	107, 108
Duodenum	16±5	107, 108
Proximal colon	107±48	109
Sigmoid colon	66±47	107, 108
Distal colon	134±68	109
Rectum	155±54	109

tract reflect the physiological demands at that location, e.g. stomach mucus is notably thick due to the need for effective acid resistance. Accordingly, the thickness of secreted mucus layer varies throughout the gastrointestinal tract (Table 4), but remains relatively constant at individual sites suggesting a functionally optimal secretion for each location (106-109). The gastrointestinal glycocalyx also varies throughout the tract and has been linked with a variety of general and specific functions relating to the extension of the extracellular mucin domain at the apical cell surface. This includes a range of interactions with the secreted mucin gels, secreted proteins and effector molecules, bacteria, viruses and other organisms (15, 45, 46, 110).

The viscoelastic properties of the secreted, adherent gel layer are governed by the mucins, they are responsible for the gel-forming properties. The non-mucin components of mucus may influence gel formation or affect gel strength. They are implicated in pathological situations where alterations in the normal composition of mucus are changed resulting in weaker gels and an associated reduction in the efficiency of the physical and biological protection afforded (111, 112). Mucus gels have viscoelastic and plastic properties allowing them to deform and recover under loads experienced at the appropriate mucosal surface. The nature of these forces varies throughout the regions of the gastrointestinal tract. The ability to recover, anneal and flow are fundamental properties required for protection against the mechanical forces experienced during normal gut function (113). Much evidence favors non-covalent, carbohydrate interactions mediating the gel structure (114, 115). Measurement of mucus rheology has been described (113), but is often limited in clinical comparative studies by the size of samples required to obtain reliable results. The use of techniques such as atomic force microscopy, requiring much smaller sample sizes and generating data relevant to mucus gel formation and strength is currently under review (116, 117).

3.1.7. Foetal and neonatal expression

The expression of the mucin genes MUC1-8 during human foetal gastrointestinal development has been characterised largely using mRNA detection (summarised in Table 5). Mucin mRNA transcripts are detectable from the start of the mid-trimester (118, 119). Current data are limited by the small number of foetuses utilised in developmental studies, and the apparent inconsistent nature of mucin gene expression within foetuses of similar gestation. Further work will be required to clearly define the changes in mucin gene expression during foetal development. In particular, data relating to mucin product present within developing foetal gastrointestinal tract is absent.

Expression of mucin genes within the developing foetal gastrointestinal tract has been demonstrated from a gestational age of only 6.5 weeks within the human foetus. However there appears to be a complex spatio-temporal relationship of mucin gene expression. Experiments from different researchers yield conflicting results as to the timing of mucin gene mRNA detection throughout the longitudinal axis of the developing gut (118-122). Recent work (122) has shown the presence of mucin gene mRNA within the foregut derivatives from a gestational age of 8 weeks. The predominant mucin gene profile seen within the developing foregut is similar to that seen in adult tissues (30, 122). MUC5AC has been demonstrated in the surface epithelial mucus secreting cells. MUC5B expression within the surface epithelium of the stomach is present at 8 weeks gestation but is absent by 26 weeks adopting a similar pattern to that seen in adult tissues. MUC6 is expressed within the surface epithelium of the foetal stomach from 8-13 weeks gestation but, as gestation progresses, is found within the crypts and glands. However, there remain important differences in gene expression, particularly at earlier gestational ages. Within the stomach MUC1, MUC4, MUC5AC, MUC5B, MUC6 and occasionally MUC3 can be demonstrated in primitive foetal stomach at 8 weeks of gestation before epithelial cytodifferentiation occurs (122). MUC2 can be demonstrated from 9 weeks gestation. As cells undergo differentiation into epithelial cells or mucous glands, their mucin gene profile changes with MUC1, MUC5AC +/- MUC3 and MUC4 being expressed. Cells destined to become mucous glands are characterised by a different pattern of mucin gene expression i.e. MUC1, MUC2 and MUC6. Expression of mucin genes appears to commence within the antrum of the stomach and progresses outward to the area destined to become the fundus. The primitive pattern of stomach mucin gene expression is similar to the pattern seen within adult neoplastic tissues. Gastric carcinomas are associated with the disappearance of MUC5AC and MUC6 mRNA, the overexpression of MUC2 and the re-appearance MUC5B mRNA (122).

Intestinal MUC2 mRNA could be detected as early as week 9 and correlates well with established patterns of crypt and villous development in the small intestine (119, 120). MUC2 expression adopts an adult pattern of expression at week 23 of gestation in the small intestine and week 27 in the colon (118, 120). A transitory expression of MUC5AC at week 8 in the primitive gut (120) and at week 17 in the colon (119) has been shown. No MUC5AC mRNA was detected after this period and none is found in normal adult intestine. Low levels of MUC6 are expressed from week 13 until week 23 (119) no expression of other mucin genes has been reported. The membrane-associated MUC1, MUC3 and MUC 4 genes show a marked difference in expression to the secreted mucins such as MUC2 (119, 120). MUC3 and MUC4 appear as part of the primitive gut endoderm at a very early gestational age, 6.5 weeks, when the epithelium is stratified and undifferentiated, (120, 122). MUC1 appears in the colon before MUC2, at 18 weeks of gestation, (118). These three membrane-associated mucin genes are found in both mucus secreting and non-mucus secreting epithelial cells of adult intestine and probably reflect a role in cellular behaviour during proliferation and differentiation.

Table 5. Fetal expression of mucin genes in the gastrointestinal tract

Mucin	Stomach	Duodenum	Gall Bladder	Colon
Membrane-associated mucins				
MUC1	Weak homogenous signal from 8 weeks gestation	ND	Weak homogenous signal	Present from 18 weeks gestation
MUC3	Weak/ inconsistently present from 10 weeks gestation	In undifferentiated epithelium 10 weeks gestation	Strongly positive from 18 weeks gestation	Present from 6.5 weeks gestation
MUC4	Weak/Moderate signal from 8 weeks gestation	ND	ND	Present from 6.5 weeks gestation
Secreted mucins				
MUC2	Weak in antral glands from 26 weeks gestation	High levels from 10 weeks gestation	Weak heterogenous signal	Expressed from 9/40 Adult pattern from 27 weeks gestation
MUC5AC	In antrum from 8 weeks gestation	Present in crypts from 18 weeks gestation	Weak homogenous signal	Present transiently at 17 weeks gestation
MUC5B	Weak signal from 8/40 gestation	Present in glands from 18 weeks gestation	Weak homogenous signal	-
MUC6	Weak homogenous signal from 8/40	In crypts from 12 weeks gestation In Brunner's glands from 26 weeks gestation	Weak homogenous signal	-
MUC7	ND	ND	ND	ND

Studies of other foregut derivatives have demonstrated the expression of mucin genes within the developing duodenum, gall bladder, pancreas and liver (121). The pattern of mucin gene expression within the developing duodenum (proximal to the bile duct orifice) is similar to that seen within adult tissues – with MUC2, MUC3, and MUC6 being the predominant mucin genes expressed. MUC1 was not demonstrated in developing duodenum, but is present within the Brunner's glands in adult tissue. The expression of mucin genes within the gall bladder shows strong reaction for MUC3 from 18 weeks of gestation – the predominant mucin gene expressed within the large ducts of the adult tissue. There is also weak homogenous reactivity for MUC5B, MUC6 and MUC1 mRNAs that have been demonstrated within adult tissue. MUC4 and MUC7 were not detected within foetal or normal adult gall bladder tissue, however, MUC4 is the predominant mucin gene expressed in adenocarcinoma of the gall bladder (121).

Few studies on the glycosylation patterns of fetal mucins have been performed, and the data are not as extensive as for the MUC genes. Typical colonic glycosylation patterns of sulphation and sialic acid O-acetylation are found in mucins in the foetal colon at week 12. The distribution of these carbohydrate related structures follows the typical developmental pathways progressing to adult patterns (123, 124).

It is important to note that mucin genes are detected before morphogenesis and cytodifferentiation occur within the developing gastrointestinal tract. The complex structural and functional program that determines the development of epithelial organs from primitive intestine may include the expression of most of the mucin genes.

Changes in the pattern of mucin gene expression have been described in preneoplastic and neoplastic tissues. There is evidence that mucins expressed by neoplastic cells play diverse roles in addition to their protective functions. Mucins may be implicated in progression of carcinomas and in the promotion of tumour cell metastases because of alterations in cell growth regulation, cellular adhesion and immune recognition. A role of

mucin glycoproteins in cellular adhesion mechanisms during organogenesis has been proposed (120, 122).

Study of the mucus barrier during the neonatal period is scanty. Nutritional changes feature strongly at this stage of life, and the phases of lactation and weaning, together with the colonisation of the gut with the normal enteric bacterial flora have important roles in the regulation of mucus barrier production. Colonic mucin gene expression in the neonatal period from birth to three months is similar to the adult pattern with strong MUC2 and MUC4 and low to background MUC1 and MUC3 (125). Glycosylation patterns of strong sulphation and O-acetylated sialic acid also appear similar to those found in the adult (125).

3.2. Non-Mucin components

As already noted, study of the mucus barrier demands an understanding of the interaction of mucins in mucus with other components making up the functional mucus barrier. Many of these components support the maintenance of the gel or contribute to the many defensive functions of the barrier. A brief overview of these components is made here as pathological changes in mucus function frequently imply their interaction with secreted or membrane-associated mucins (3, 4, 10).

3.2.1. Electrolytes and lipids

The aqueous environment and electrolytes at the mucosal surface contribute to the status of the whole mucus secretion, having an influence on hydration and rheological properties. Regulation of the secretion and absorption of these components is linked closely with the normal function of mucus at defined mucosal surfaces throughout the gastrointestinal tract. Fluid and electrolyte secretion is coupled with the secretion of mucins through different pathways and is reviewed elsewhere (3, 85).

The gastroduodenal mucosa relies on the secretion of bicarbonate, which interacts with the unstirred mucus layer, to ensure a neutral pH at the mucosal surface, generate a pH gradient across the mucus gel layer and the resist the high acidity of the gastric and duodenal lumen. (4). Gastric lumen H^+

enters the mucus gel but is effectively neutralised by the bicarbonate secreted from the mucosal cells. The mucus gel provides a suitable medium to prevent dissipation of the secreted bicarbonate and allow an efficient mechanism for the neutralization of acid. Constant renewal of the mucus gel layer is crucial to this process. The production of both acid and bicarbonate by the gastric mucosa with the maintenance of pH gradients across the mucus gel may be mediated by viscous fingering. During secretion the acid, a low viscosity fluid, interacts with the highly viscous mucus. The low pH increases the viscosity of the mucus and gives rise to 'viscous fingers' which are maintained as single channels, emerge at the surface and do not reenter the gel (126). The bicarbonate secretion at higher pH does not give rise to such channels and diffuses through the gel layer.

Lipid complexes have been known to form with mucus at the surface of the gastrointestinal mucosa. These complexes have a profound influence on the viscoelastic properties of the gel (127). Significant proportions of lipid have been detected in isolated mucin fractions although the origin may be cell mediated or due to sloughed cells and shed membranes. Typically, phospholipids, ceramides and glyceroglucolipids together with covalently linked fatty acids have been reported in association with mucins. Further support for a role of these lipids in mucosal protection has come from studies of gastric secretagogues which link both phospholipid and mucin secretion (128). Lipids have been linked with mucus resistance to gastric acid attack (127), increased resistance to degradation by bacterial mucinase activity and protection against mucin degradation by free radicals.

3.2.2. Defensive proteins

The mucus barrier also contains defensive proteins that form part of the innate mucosal defensive system. These proteins have different defensive roles at the mucosal surface linked with the need for protection against general and specific threats largely involving bacteria and their products. Some have been known for a considerable time and have a large literature, while other are recent additions to the growing family of protective proteins. The interaction of these proteins with mucus with respect to their protective functions has not been investigated in most cases and the listing of these mucus components serves to draw attention to the need to view mucus as an integrated defensive system. Only brief reference is made to the better known examples, which are reviewed elsewhere in more detail.

3.2.2.1. Lysozyme

Lysozyme is secreted by mucosal cells throughout the gastrointestinal tract where it carries out antibacterial actions (129). Due to its positive charge it is often found complexed with negatively charged secreted mucins, and in some cases is co-secreted with them. The general anti-bacterial properties of this protein make it an important participant in mucosal defence.

3.2.2.2. Lactoferrin

The presence of lactoferrin at mucosal surfaces is linked with its iron-sequestering properties and the limitation of bacterial growth. It has been detected in secreted mucus at most regions of the gastrointestinal tract and is present in the mucosal cells themselves (130). Due to the known protective roles of this protein the benefits of orally administered bovine

lactoferrin have been widely examined as a dietary factor having an influence on bacteriostasis (131) and the immune system (132). Details of the nature and biology of lactoferrin in gastrointestinal defence are discussed elsewhere (130, 133).

3.2.2.3. Secretory IgA

The gastrointestinal mucosa produces and secretes immunoglobulins throughout its length. The major mucosal defence immunoglobulin is secretory IgA (sIgA) is found in mucus lining the whole of the gastrointestinal tract. Human antibodies are products of intestinal mucosa plasma cells and are not obtained to a significant level from the circulation. The innate protection afforded by the efficient prenatal transplacental transport of maternal antibodies, together with post-natal ingestion of milk antibodies during lactation in breastfed neonates, is essential for optimal survival and protection (133).

3.2.2.4. Protease inhibitors

Peptides with anti-protease and peptidase activity have been identified in gastrointestinal tract mucosal cells. The most important of these are alpha 1-antitrypsin inhibitor and pancreatic secretory trypsin inhibitor (PSTI). Evidence for a protective role of PSTI on gastric and colonic mucus has been described and reduction in gastrointestinal disease correlates with depletion of the adherent mucus barrier (134). Secretory leukocyte proteinase inhibitor (SLPI) is a major serine proteinase inhibitor and a potent antibiotic and has been identified in human jejunum and colonic mucosal cells, intestinal lavage fluid and intestinal cell lines (135). The inhibitor is secreted from the apical surface of intestinal epithelial cells, and this is stimulated by TNF-alpha, interleukin-beta and activation of protein kinase C isoenzymes (135). There is no evidence for interaction between SLPI and mucus. It has no influence on epithelial barrier integrity measured by transepithelial conductance or electrogenic ion-transport.

3.2.2.5. Growth factors

A complex relationship has been identified for growth factors and cytokines in the gastrointestinal tract with regard to their individual actions and interactions at the mucosal surface. Many well-known peptide growth factors are found in mucus, each with their own implicated functions. Playford has classified these functions in order to review the type of peptide, their sites of synthesis, their mechanism(s) of action, and their range of concentrations at normal and pathologically affected mucosal locations (136). The peptides are divided into three groups:

- 1) *Mucosal integrity peptides*, present through the entire gastrointestinal tract and functioning to maintain mucosal integrity. Present in this group are transforming growth factor-alpha (TGF-alpha) and PSTI. TGF-alpha shows structural similarity to EGF and is bound by EGF receptors. It is responsible for stimulation of cellular DNA synthesis, initiation of mucosal cell migration and blocking of acid secretion. However, the nature of its interactions with mucus has not been investigated. PSTI is found throughout the gastrointestinal tract and regulates the action of exogenous luminal protease activity on the adherent mucus layer.

- 2) *Luminal surveillance peptides*. The most significant member of this group is EGF. The presence of this

peptide is maintained at all times, but is only shown to act when mucosal damage occurs. Support for this type of role comes from the location of EGF receptors at the basolateral and not apical membranes of mucosal cells. Thus they would only be accessible to luminal EGF when the mucosal surface is disrupted (134). EGF has been shown to stimulate the release of mucus from the jejunum (137) possibly as part of an intestinal cytoprotection mechanism. The importance of EGF binding to gastrointestinal mucus and mucins is not yet clear. Any link with the EGF-like domains identified on MUC3, MUC4, MUC12 and MUC13 and mucosal function is speculative, although at least one of these domains on MUC4 has been shown to act as an intra-membrane ligand for the receptor tyrosine kinase ErbB2 (46).

3) *Rapid response peptides* mediate immediate responses to mucosal injury and promote mucosal repair. The trefoil factor family peptides are examples of this group and are reviewed below.

3.2.2.6. Defensins

Defensins are a family of broad-spectrum, amphipathic antibiotics microbial peptides expressed in epithelial and hematopoietic cells in general but with expression throughout the gastrointestinal tract (138-141). Two peptide families, the alpha- and beta-defensins have been identified (141), of these the beta-defensin hBD1, and the alpha-defensins HD5 and HD6 have been found in the gut. hBD1 transcripts are found at low levels throughout the tract, while HD5 and HD6 are limited to the intestine with highest levels in jejunum and ileum but are absent in the duodenum (142) and are not present in the colon. Most attention has been drawn to Paneth cells (143). Studies in the small intestine have shown localization at the base of the crypts of Lieberkühn in Paneth cell granules. Defensins are thought to act synergistically with the other components of these granules, which include lysozyme, secretory phospholipase A₂, tumor necrosis factor-alpha, cysteine rich intestinal polypeptide, matrilysin and alpha₁-antitrypsin (143). The expression of defensin genes is constitutive and inducible through the action of bacterial lipopolysaccharide and inflammatory mediators. Whether these molecules interact with mucins at any region of the tract has not been determined. The role of defensins is currently seen as part of a broad-based innate host defense mechanism, designed to prevent early colonization by pathogenic microorganisms. Accordingly, their expression is elevated in both infectious (144) and inflammatory conditions (145-147) and is regulated by signaling pathways in shared with other innate immune responses (139).

3.2.2.7. Beta-Galectins

The beta-galectins are a family of 12 or more carbohydrate binding proteins, distinct from the selectins and the calcium dependent carbohydrate binding proteins (C-lectins), and previously known as S-type lectins (148). Galectins-1, 3 and 4 are found in the gastrointestinal tract as secreted proteins, but are also found in mucosal cell apical membranes and in the extracellular matrix (148-151). Both secreted and glycocalyx located gastrointestinal mucins have been shown to act as partners in galectin binding (152-154) and a role in cellular adhesion has been proposed. (155). The galectins bind to poly-N-acetyllactosamine (LacNAc) and

related structures. Galectin-3 binds to terminal, alpha-1-3 linked GalNAc in poly lactosamine chains and related glycotopes in normal colorectal sialo- or asialo-mucins (153) and appears to correlate well with *Griffonia simplicifolia* agglutinin I binding sites (Gal-alpha-1-3Gal- or GalNAc-alpha-termini) (156). Galectins may act as cross-linking binding partners between the oligosaccharide chains of membrane anchored glycocalyx and secreted glycoconjugates. This type of interaction has been proposed for galectins 3 and 4 in the gastrointestinal tract (152, 155). In addition, interactions between extracellular matrix laminin and integrin oligosaccharides, which also carry poly-LacNAc chains, have been demonstrated.

Conflicting data exist for the expression of galectins in gastrointestinal cancer. Immunodetectable galectin-3 levels are higher in high-grade dysplasia and early invasive cancers compared with related adenomatous tissue. Metastases show a higher level of galectin-3 compared with their primary tumors and the strong expression of galectin-3 correlates with poor prognosis for patients (157, 158). In other studies a down-regulation of galectin 3 is found associated with the occurrence of lymph node lesions (159), or in the initial stages of neoplastic progression, with subsequent increases in later phases of tumor progression (160). Galectin 1 is increased in colorectal cancer and is associated with the neoplastic progression (159, 160), although one of these studies also found a decrease in some cases (159). Examination of primary gastric adenocarcinomas revealed slightly higher galectin-3 expression relative to normal controls, but no link between either membrane-bound or cytoplasmic galectin-3 with differentiation parameters or tumor progression was found (161).

Study of the binding properties of tissue specific galectins to both secreted and glycocalyx mucins at each region of the gastrointestinal tract is needed to define normal expression and to identify and clarify the pathologically relevant glycosylation and binding patterns.

3.2.2.8. Trefoil factor peptides

The trefoil factor family peptides (TFF's) are a group of three low molecular weight cysteine rich peptides sharing a characteristic triple loop 'clover leaf' motif, and having considerable resistance to degradation by acid, proteases and heat (162-165). Identification of the peptides as a family occurred sometime after their original discovery and different names were used, e.g. P-domain peptides and trefoil factors. Only recently has a unifying nomenclature been proposed, hence TFF1 is human spasmolytic peptide (hSP), TFF2 is pS2 and TFF3 intestinal trefoil peptide (ITF) (166). These peptides have been identified in a site-specific pattern in the gastrointestinal tract, and also in additional locations where they may serve shared or different, tissue specific functions (162-165). In the human gastrointestinal tract TFF1 is found in the stomach in mucous cells from the level of the neck upwards and including the foveolar epithelial surface cells. Examination of the site of TFF1 location in the gastric mucosa shows a very high level of trefoil peptide in the secreted adherent gel layer (167). TFF2 occurs in the stomach in the fundus mucous neck cells, and in the basal mucous glands of the antrum and pylorus. In addition, TFF2 is found in the Brunner's glands of the duodenum. TFF3 has major expression along the length of the

small and large intestine (162, 163, 165). These are locations that are shared with the secreted mucins and co-localization of these peptides with specific mucin genes has been shown, TFF1 with MUC5AC, TFF2 with MUC6 and TFF3 with MUC2 (36). This study also confirmed the co-localization of all three trefoil peptides with a unique pattern of MUC gene products in the ulcer associated cell lineage. The MUC gene pattern matched the TFF patterns seen in normal mucosa for TFF1/2 and MUC5AC/6 but although TFF3 was strongly expressed no MUC2 was found. Further exceptions to a strict trefoil/MUC gene co-expression have been shown and these suggest broader functional roles for the TFF peptides (36, 164, 165)

The major functional roles of trefoil peptides in the gastrointestinal tract are epithelial protection and mucosal healing (165). As noted above the trefoil peptides can be considered as 'rapid response' agents to mucosal injury, with up-regulation of expression in the early stages of mucosal repair (136). Integration of these functions with mucins has not been shown. A role for trefoil peptides in enhancement of the strength of the adherent mucus gel through mucin cross-linking has been proposed (168), but lacks strong experimental support and requires further study. An interaction between TFF1 and MUC5AC and MUC2 has been demonstrated in the mouse (169). Using the yeast two-hybrid system TFF1 and mucin interaction was detected and localized to the von Willebrand C domain of these two mucins. The nature of these interactions has not been demonstrated in isolated mucus, but it may be speculated that mucin packaging in goblet cell vesicles and subsequent secretion may be regulated through the action of trefoil peptides with these mucins (170).

More recent demonstration of the influences of trefoil peptides on epithelial cells in the gastrointestinal tract show intracellular responses that relate to the survival of these cells in mucosal restitution. These include ras-dependent MAP kinase activation and activation of epidermal growth factor receptor (171). The activation of these pathways leads to loss of E-cadherin from the cell surface to intracellular complexes with catenins and inactivation of the akt kinase associated with apoptosis. Both the p53 dependent and independent apoptosis are blocked by TFF3 (172). This implies that the trefoil peptides mediate a mechanism that allows cells to detach and migrate without dying. The normal penalty for detachment of cells from a stably anchored situation is apoptosis and trefoil peptides appear to allow cells to detach and survive to play a role in tissue repair (165). These responses appear to be independent of the trefoil-mucin functions.

3.2.2.9. IgG gamma Fc binding protein

Human IgG-gamma-Fc binding protein (Fc-gamma-BP) binds to the Fc region of IgG and is expressed in human small intestinal and colonic mucosa primarily associated with goblet cells and mucins (173). The predicted amino acid sequence contains 12 tandem mucin-like repeats of a 400-amino acid cysteine-rich unit containing the motif CGLCGN which is also found in MUC2 and pre-pro-von Willebrand factor (174). Translated products of >200,000, 140,000, 110,000 and 78,000 Da are detected in colonic mucosa. Amino acid sequences of 450 residues at the N-terminal are necessary and sufficient to confer IgG-Fc binding activity. (173, 174). Fc-gamma-BP is present in the endoplasmic reticulum of colonic

goblet cells, in the cytoplasm between secretory granules of goblet cells, and inside the granules themselves. It appears to be co-secreted with mucus into the intestinal lumen (173). A number of colorectal cancer cell lines have been tested for the expression of Fc-gamma-BP. A selective pattern of binding was found showing that not all mucin producing cell lined expressed the binding protein. HT29-18N2, a mucin-secreting subclone of HT29 cells showed expression while LS 174T had mucin but no Fc-gamma-BP and Colo 205 and LoVo cell lines had neither mucin nor binding protein (175). No examination of the mucin gene expression in these cells was carried out. When tested in HT29-N2 colonic cancer cells TNF-alpha caused a reduction in expression of the Fc-gamma-BP and the proportion of mucin containing cells, without affecting the viability or proliferation. In this system TNF-alpha suppressed Fc-gamma-BP expression and inhibited differentiation to a mucin-producing phenotype (176). In a rodent model of adapting gut an upregulation of Fc-gamma-BP was detected in small bowel and colon goblet cells at the early stages of gut adaptation (177). In addition a regulation of expression during ontogeny could be shown. A role for Fc-gamma-BP in intestinal and colonic goblet cell biology during development and adaptation is implied and deserves further study.

Fc-gamma-BP is thought to contribute to the immunological protection of the intestine through interaction between intestinal mucus IgG and extracellular antigens, including bacteria, or through interference with complement fixation by IgG, and thus reduces complement-mediated tissue damage at the surface of the intestinal mucosa (178). The pattern of Fc-gamma-BP in ulcerative colitis has been linked with a severe goblet cell abnormality with a reduction in the size of mucin granules and an increase in cytoplasm. The distribution of Fc-gamma-BP in these two compartments is altered in favor of the cytoplasm. This pattern was more pronounced with increased inflammation. Electron microscopy showed an increase in Fc-gamma-BP and mucin staining in the endoplasmic reticulum and a reduction in the granules compared with normal controls. These findings point to abnormal targeting of glycoproteins to secretory granules and increased synthesis and turnover of glycoproteins in goblet cells in ulcerative colitis. This is in keeping with the role proposed for Fc-gamma-BP in rodent gut goblet cells in ontogeny and development. The abnormal morphology and distribution of Fc-gamma-BP and mucin seen in UC was not evident in Crohn's disease (178).

3.2.2.10. Heparin and heparan sulfate

Heparin, a highly sulfated glycosaminoglycan is produced exclusively by connective-tissue-type mast cells and stored in the secretory granules as complexes with histamine and various mast-cell proteases. Mast cells are present in mucosae and connective tissue throughout the body including the gastrointestinal tract. Major functions of heparin are concerned with the control of mast cell protease activity and secretory granule integrity (179, 180). However, additional non-mast cell functions for heparin and heparan sulfate have been suggested by the identification of many proteins with heparin binding sites, including growth factors, enzymes, serine protease inhibitors, antimicrobial peptides and extracellular matrix components (see (181) for an overview). Among these proteins are MUC2 and MUC5AC. A common feature of these

Table 6. Expression of major mucin genes in esophageal tissue. Mucin gene expression in normal and pathological esophageal tissues are shown.

Esophageal tissue	MUC1	MUC3	MUC4	MUC2	MUC5AC	MUC5B	MUC6	MUC7
Normal Esophagus	+	-	++	-	-	+++	-	-
	Epi		Epi			Gl		
Gastric Metaplasia	++/+ Col	+/-	+	-	+++ Col	-	++ Gl	-
Intestinal Metaplasia	+	++	+/-	+++ Gob	+++ Gl	-	+++ Gl	-
	Col	Col						
Dysplasia	++/-	+/-	++	+/- Col/G	+/-	-	+ Gl	-
Adenocarcinoma	++/+++	-	++	-	-	-	+/- Col	-
Squamous Cell Carcinoma	+++	-	+++	-	-	-	-	-

The symbols used are - = no expression; + = weak expression; ++ = moderate expression; +++ = strong expression; Gl = glands; Gob = goblet cells; Col= columnar epithelium; Epi= epithelium. Data taken from references34, 35.

mucins is a C-terminal tail motif spanning 14-20 amino-acids rich in positively charged lysine or arginine, which specifically binds heparin. Sequences found in MUC2 and MUC5AC are of the type XBBXBBXXBXXXXX, where B is lysine or arginine and X is an uncharged amino-acid (181). No binding of heparin to purified mucin or mucins from homogenates could be found and no evidence for the presence of the heparin binding sequence beyond the basal perinuclear region in intestinal goblet cells could be seen (181). These results suggest that a cleavage of this domain occurs during post-translational maturation, prior to secretory-mucin packaging in secretory granules (181). Whether heparin or heparan sulfate is present or has a role in the secreted adherent mucus gel or glycocalyx is unknown.

Heparin has been shown to act at the level of tumour cell-platelet interactions mediated by P-selectin and its mucin-like ligands and can abrogate metastasis formation (182). This action is thought to act by inhibition of P-selectin binding to the sialyl-Le^{x/a} and tyrosine-sulphate groups carried on its natural, mucin-like ligands (183).

3.2.2.11. Lipopolysaccharide binding protein

Lipopolysaccharide binding protein (LBP) is an acute phase protein forming part of the immune response to the endotoxins present in the coat of Gram-negative bacteria. The protein sensitizes the immune system to the presence of endotoxin and also enhances the neutralization of exdotoxin by high-density lipoprotein. During inflammation levels of LBP are dramatically increased and this arises through the liver. Recently the synthesis and secretion of LBP in the gut has been demonstrated (184), where it is believed to play a role in mucosal protection against gram-negative organisms. Proinflammatory cytokines including IL1, IL6 and TNF increase the production of LBP in epithelial cells and are present at elevated levels in intestinal inflammation and systemic inflammation. Furthermore, in Caco-2 cells a constitutive apical secretion of LBP occurs and this is enhanced by cytokine stimulation of the apical surface. In contrast basolateral cytokine stimulation leads to secretion of both basolateral and apical LBP (185). LBP is also present in the secreted intestinal mucus in mice and levels increase in response to endotoxin challenge (185). A role for LBP in intestinal mucosal defence is strongly suggested and consideration of the mechanisms of action and interaction in the mucus barrier are due.

3.2.2.12. Serum Amyloid A protein

Serum amyloid A (SAA) is a major acute-phase protein present in the circulation and whose concentration is dramatically elevated in response to injuries including infection, inflammation, neoplasia and trauma. It is found as a group of polymorphic apolipoproteins of 12-14kDa, involved in the binding and routing of high-density lipoproteins to inflammatory cells. Most of this protein is synthesized in the liver, but normal expression is also found in a variety of epithelia including the gastrointestinal tract (186). SAA could be detected in stomach, small and large bowel mucosal cells. SAA is also produced by the intestinal epithelial cell lines Caco2 and Int407, but not by Colo-205 or T84 cells (184). Caco-2 cells have been used to show that the regulation of release is similar to that found in hepatocytes for type 1 acute-phase proteins and is mediated by cytokines (184). Examination of the genes induced by the exposure of germ free mice to normal enteric bacteria showed colon specific expression of SAA (187). Together these data demonstrate that SAA is a part of the normal intestinal mucosal defense system and studies on the integration of this protein with other mucus components should follow.

4. THE MUCUS BARRIER IN GASTROINTESTINAL DISEASE

4.1. Esophageal disease

The esophagus is a highly specialised organ designed for the propulsion of food from the mouth to the stomach. As a consequence of its position in the gastrointestinal tract it may be exposed to a wide variety of noxious stimuli. The mucus barrier is an important factor in the protection of the esophagus from damage during deglutition and episodes of gastro-esophageal reflux (188). However, the existence of an adherent mucus barrier in the normal esophagus is disputed, with reports of a significant secreted layer of 95 microns (188) and complete absence under normal conditions (106). Although there may be technical reasons accounting for the differences observed by these two groups the need to protect the mucosal surface is accepted and the presence of mucins from the saliva or esophageal glands and ducts is not disputed.

The sites and nature of mucin gene expression within the normal adult esophagus has been reported and compared with pathological tissue that has undergone metaplastic or dysplastic change and in Barrett's esophagus (Table 3 and 6) (34, 35). The predominant mucins present in the healthy adult

esophagus are the membrane bound mucins (MUC1 and MUC4) present throughout the epithelial tissue and MUC5B present throughout the submucosal glands in the lower third of the esophagus (34, 35).

Long-term acid exposure as seen in gastro-esophageal reflux may lead to the replacement of squamous columnar epithelium by a columnar epithelium (Barrett's esophagus) which may resemble either gastric or intestinal type (189). The appearance of an adherent secreted mucus layer is associated with Barrett's esophagus, in keeping with the change of epithelium (106). The original observations were based on the periodic acid-Schiff/Alcian Blue and High Iron Diamine/Alcian Blue techniques for histological identification of neutral, sialo- and sulpho mucins in intestinal metaplasia described by Jass (7, 105, 189). The value of histochemistry has been greatly improved by the use of specific antibodies and lectins to detect mucin peptide and glycotopes (7, 105). However, these will continue to remain limited until knowledge of the relevant mucin gene peptide-carbohydrate combination can be assessed. The mucin gene pattern in Barrett's esophagus reflects the gastric and intestinal patterns identified by histochemistry, with MUC5AC and MUC6 prevalent in gastric type while intestinal type shows MUC2 and MUC3 in addition. MUC1 and MUC4 are present in both types at lower levels than normal (34, 35, 190). However, another study detected immunoreactive MUC1, MUC5AC but no MUC2 in Barrett's esophagus (191). The same study showed the presence of the trefoil peptides TFF1 and TFF3 but not TFF2 in normal and pathological samples, TFF1 showing a similar spatial distribution to MUC5AC (191). The presence of Barrett's esophagus requires patients to undergo regular esophagoscopy to detect malignant change early to allow for esophageal resection. The pattern of mucin gene expression may be a useful marker for monitoring disease progression.

The changes seen in Barrett's esophagus are associated with a very high progression to adenocarcinoma of the esophagus, an increasingly common cancer in developed countries. The value of mucin histochemistry in the detection and analysis of esophageal cancer progression has been controversial, (192). This is due in part to variation in the development of dysplasia and the subsequent transition to carcinoma such that knowledge of the risk of developing of carcinoma in low- and high-grade dysplasia is not predictable (192-194). Attention has also been paid to adenocarcinoma at the esophagogastric junction as it is frequently associated with intestinal metaplasia detected using mucin histochemistry (195-197). Acid mucin (sialo- and sulfo-mucin) staining in non-goblet columnar cells at the surface epithelium has been used to characterise this location (197). However, some dispute remains over the high (195) or low (196) frequency of intestinal metaplasia and the predictive value of mucin histochemistry has been questioned (192). Incomplete metaplasia with sulphomucins and aberrant Lewis^a in goblet and columnar cells is present in all patients with esophageal adenocarcinoma (194). The Lewis^(a+b), nonsecretor and blood group A phenotypes, correlate with occurrence of esophageal adenocarcinoma, suggesting that a genetic susceptibility may exist (194). Closer examination of the sulphomucin patterns in the esophagus using immunohistochemistry with antibodies binding to sulfo-Lewis^a has assisted the identification of esophageal adenocarcinoma

and the prediction of premalignant status in Barrett's esophagus (198, 199). Identification of this epitope with a particular mucin gene could not be made (198). Studies with sialomucins have also failed to produce a clear consensus supporting increases in esophageal adenocarcinoma. Measurement of O-acetylated sialic acids as markers of colonic mucins in Barrett's esophagus or adenocarcinoma have not yielded conclusive results (197, 200) and other sialylated epitopes such as sialyl Lewis^x have been detected in normal tissue but not examined in disease (201).

MUC gene changes in esophageal adenocarcinoma show a progression from those seen in Barrett's esophagus (34, 35, 190, 191). A down regulation or loss of MUC2, MUC3, MUC5AC and MUC6 (34, 35, 190, 191) and upregulation of MUC1 and MUC4 (34, 35) is found in dysplasia, adenocarcinoma and squamous cell carcinoma. In contrast, loss of MUC1 occurs in dysplasia, with re-expression in adenocarcinoma (190) and squamous cell carcinoma (191). A study of esophageal squamous cell carcinomas using anti-MUC1 monoclonal antibodies showed that the expression of MUC1 linked sialyl oligosaccharides is related to poor prognosis (202). The conclusion drawn from these studies is that MUC genes can be considered reliable phenotypic markers of esophageal cell differentiation.

The fetal expression of mucin genes in the esophagus has not been reported. Study of the developmental biology of mucin gene expression may improve understanding of the changes seen as oesophageal tissue progresses to malignant disease.

4.2. Intestinal metaplasia, gastric ulcer and *Helicobacter pylori* infection

Gastric intestinal metaplasia (IM) is associated with gastric ulceration, *Helicobacter pylori* infection and the risk for esophageal and gastric cancer (see also sections 4.1, and 4.3). The mucins may assist in diagnosis of IM and are also implicated in its etiology. Classification of IM into three types has been based on mucin histochemistry, complete (type I), incomplete without sulphomucins (type II) and incomplete with sulphomucins (type III) (7, 105). The histochemical patterns of type I IM correlate with those in diffuse gastric cancers and type III IM with 'intestinal' cancers showing more sulpho- than sialomucins. It has been suggested that the development of gastric cancer involves progression from type I to type III IM (7, 105), although this sequence is not always found, and the appearance and duration of intestinal type III IM is variable (203). O-acetylsialomucins are not seen in type III IM or in tumors, but appear in complete or type I IM (7, 105). A subsequent study reported that O-acetylated sialomucins are much more prevalent in gastric intestinal metaplasia and carcinoma than previously recognized, showing reactivity in type I and type III IM, (204). Screening for the short mucin oligosaccharides Tn, sialyl-Tn, T and sialyl-T show that all IM mucosae express sialyl-Tn while only Tn is present in control mucosa (205).

High levels of MUC2 and MUC3 appear in gastric intestinal metaplasia (122, 206), in contrast to the normal gastric mucosal pattern of MUC1, MUC5AC, and MUC6 (207). Type I (complete form) shows only MUC2 in goblet

cells, while the incomplete forms (types II and III) show MUC1 and MUC5AC in goblet and absorptive cells, MUC2 in goblet cells only and MUC6 in over 60% of cases. One study has identified MUC2 in non-IM tissue and has linked this with regenerative gastric mucosal cells and the appearance of sialyl-Le^x (208). Thus, MUC gene analysis identifies two phenotypes, a small intestinal/colonic pattern and a typical gastric pattern with MUC2 (122, 207).

The colonization of the gastric mucosa by *Helicobacter pylori* (HP) strains leads to the development of gastritis, ulcers and possibly gastric malignancies. After penetration of the gastric mucus layer the bacteria adhere to mucus or epithelial cell targets. Attachment involves recognition of blood group-related carbohydrate antigens, primarily the fucosylated group-O, but also Le^a, sialyl Le^a, Le^b, type 1H, and type 2H (see Table 2 for structures). This has been linked with the higher prevalence of blood group-O individuals with ulcerative disease. Selective binding of *Helicobacter pylori* to incomplete but not intestinal type intestinal metaplasia correlates with expression of the mucin type carbohydrate epitopes in a hybrid epithelium whose cells share characteristics of both gastric surface mucous cells and intestinal metaplastic cells (209). The HP cell wall lipopolysaccharide is implicated as a virulence factor causing weakening of the mucus barrier through interactions with gastric epithelial cell receptors, glycocalyx and secreted mucus, proteins of the extracellular matrix and bioactive peptides. Exposure to HP lipopolysaccharide leads to the histological abnormalities characteristic of acute gastritis and increased epithelial cell apoptosis (210).

Binding of HP to gastric mucin is dependent on glycosylation and sulfation that vary in different gastric compartments. HP infection results in a reversible alteration in glycosylation, which favors attachment of the bacteria (211). Apart from the fucosylated blood group antigens, sialylated and sulphated mucin oligosaccharides are implicated in HP binding to the gastric mucins (212, 213), while specific binding of HP to sulfo-Le^a carried on salivary MUC5B has been demonstrated (214). A range of bacterial adhesins mediating carbohydrate binding has been identified, including sialic acid specific groups (215, 216). In addition this binding is sensitive to both temperature and pH (217). Alterations in mucin glycosylation clearly modify the protective functions of gastric mucins against HP infection. Molecular mimicry between HP LPS and the host, based on Lewis antigen has also been demonstrated indicating an autoimmune mechanism for HP-associated gastritis (218).

Evidence for degradation of gastric mucus by HP by proteases and sulphatases has been produced (219-222) and refuted (223, 224). Others have suggested that the action of urease in creating ammonia causes a rise in pH, which could destabilize the mucus layer (223, 224). Careful examination of the degradation of polymeric mucin showed that this is only a partial process, is not due to enzyme action and could be due to the action of ammonia (108, 224). However, it is not sufficient to cause a collapse of the mucus barrier. Recent reports have shown that colonization by HP partly depends on acid-dependent adherence to gastric mucin by its secreted urease (225, 226). Bound urease shows considerable chemical stability

implying a role for the denatured enzyme in the persistence of the bacterium within the acidified compartment of gastric mucus (227). In addition, HP thioredoxin has been identified as an agent assisting colonization through focal disruption of the oligomeric structure of mucin polymer and inactivating host generated antibodies by catalytic reduction. This molecule can efficiently catalyze the reduction of human immunoglobulins (IgG/IgA/sIgA), and soluble mucin (228).

Agents secreted by or present in HP lysates lead to a decrease in both basal and stimulated secretion of mucus in cultured gastric epithelial cells. HP directly impairs mucin synthesis in the stomach and this response is more profound in cytotoxic cagA positive strains (229). Cultured gastric KATOIII cells show an 80% inhibition of mucin synthesis, determined by metabolic labeling, on incubation with HP, but with no effect on secretion and degradation. Decreased MUC1 and MUC5AC levels are found, but with different kinetics. MUC1 shows a rapid decrease in contrast to a much slower reduction in MUC5AC levels (230). The HP lysate-induced reduction of basal and stimulated mucin secretion in cultured gastric epithelial cells is significantly potentiated by interferon-gamma, further underlining the cytokine mediation of gastric mucin biology by HP (231).

Attempts to identify the type of mucin targeted by HP have shown a striking co-localization with MUC5AC. Bacterial binding to tissue sections was found in the superficial, foveolar region, corresponding to MUC5AC localization, and also in luminal mucus (232). Conflicting results have been reported for the effect of HP colonization on MUC gene expression in the stomach. An increase of MUC6 in surface foveolar mucous cells with an accompanying reduction in MUC5AC has been described (233). This reversal of the normal gastric pattern is corrected on elimination of the infection. In contrast, the absence of any change in the MUC5AC: MUC6 gradient has been found in HP positive patients. Comparison of the gradient before and after eradication of HP shows more pronounced MUC5AC before elimination (234). However, postoperative patients that were HP positive had increased MUC6 in the foveola against a normal background of MUC5AC (235).

4.3. Gastric cancer

The classification systems used to grade gastric cancers, including the WHO and Vienna classifications (236), Lauren (the intestinal and diffuse types) and Goseki, mucin poor (types I and III) and mucin rich (types II and IV) rely on the identification of mucin forms. Detection of mucins relied initially on the histochemical and carbohydrate based methods (156), but has now advanced to include the detection of mucin genes.

The short mucin type oligosaccharide Tn, sialyl-Tn and T antigens (glycotopes) occur in most primary gastric adenocarcinomas (205, 237, 238). Several studies have shown that this link with cancer is not always found in accord with mucin gene patterns and that glycotope expression is related to cell type. Tn is found mainly in columnar cells and sialyl-Tn in goblet cells (239). Also among this group of mucin glycotopes sialyl-Le^a is a strong marker for an unfavorable outcome in all gastric tumors, while sialyl-Tn, Sialyl-Le^a and sialyl-Le^x associate with a poor prognosis in diffuse gastric cancers (240). Sialyl-Tn correlates strongly with small intestinal mucins, is a

marker for small intestinal differentiation in over 60% of gastric cancers (241) and has been proposed as a marker of gastric cancer progression (242). The level of anti-T antibodies in the serum of Le(a-b+) phenotype, gastric cancer patients is decreased compared with normal controls (243), but no explanation for this finding has been put forward. Another Lewis phenotype, Lewis (a+b-) together with nonsecretor phenotypes has a significant positive association with the expression of sulfomucins in gastric cancer populations (244). Although sulfomucins are generally detected by the high iron diamine (HID) stain there very few reagents available for the detection of mucin bound sulphated glycotopes. The best characterized of these, sulpho- Lea, (245, 246) represents only one glycotope among a yet undefined population of sulphated structures. Sulpho- Lea is found with high frequency in gastric cancers (247) although not usually present in normal gastric mucosa (248). The relationship between glycosylation and mucin gene expression has been examined using the association of individual fucosyltransferases with mucin genes found in normal gastric mucosa. Normal stomach shows the presence of FUT1 exclusively with MUC6 and FUT2 with MUC5AC. In gastric cancer this cellular duality is lost together with the differential expression of MUC5AC and MUC6 (see below). These changes occur in intestinal metaplasia and are thus early events in gastric carcinogenesis (69). Additional studies testing the link between mucin apomucin expression and glycosylation using gastric carcinoma cell lines also failed to show an association (249).

Recent attention has focused on the potential use of MUC gene expression as a specific indicator of gastric cancer phenotypes. Work has been focused on the known normal MUC gene patterns and the variations identified in intestinal metaplasia (207) and fetal development (122) as early stage or oncofetal patterns for gastric cancers. Studies on individual MUC genes as markers have given some assistance in identifying patterns of neoplastic development. Using an anti-MUC5AC VNTR peptide antibody, reactive protein appeared in over 60% of gastric cancers, more than 80% of these were diffuse carcinomas while only 59% were intestinal type. The mixed phenotype cancers were in accord with this pattern showing less reactivity in the intestinal regions. Stage specific patterns could be detected with most early cancers strongly positive while the older, more advanced carcinomas showed a loss of reactivity implicating MUC5AC as a marker of gastric differentiation. (250, 251). In the advanced carcinomas the pattern of strong surface expression is reminiscent of the normal gastric mucosal pattern. The pattern of MUC6 in gastric carcinomas has been examined using a novel anti-MUC6 anti-peptide antibody reactive with native and deglycosylated mucin. This work showed the expected reactivity with the pyloric glands of the antrum and mucopeptic cells of the neck zone in the body of normal gastric mucosa (252). Only 30% of gastric carcinomas studied reacted with this antibody and did not correlate with histomorphological type or clinico-pathological features. Some co-expression with MUC5AC (45%) and MUC2 (5%) was detected, but this was also independent of the histomorphological type and stage of the tumors (252).

MUC2 is absent in normal gastric mucosa, but has been detected in gastric tumors where immunoreactivity related best with the histological pattern but not with the patient age or disease outcome (253). The presence of MUC2 alone was seen in 25% of cancers, and those cases with more than 50% positive

cells were classified as mucinous type (252). This is in keeping with the detection of MUC2 in most mucinous carcinomas (254), and correlates with poor prognosis and an advanced stage at diagnosis (255). However, contrasting results have been reported using different anti-MUC2 antibodies where no difference could be found in the classification, stage and lymph node status of tumors and has limited prognostic value (256), or where MUC2 is prognostic for a favorable outcome (257). Immunoreactivity with MUC1 in gastric cancers indicates a poor outcome (253, 257) and predicts development and progression (258). Closer analysis has linked cell invasiveness and poor prognosis with the extracellular cytokine-like receptor sequences in MUC1 (259). Abnormal expression of MUC5B has also been reported in gastric carcinoma and cell lines (260).

As already indicated for intestinal metaplasia, the group analysis of MUC genes gives an improved assessment of disease phenotypes when applied to gastric cancer. An evaluation of MUC1 and MUC2 mucin staining patterns has been used clinically to predict disease outcome. (257). Characteristic patterns for MUC2, MUC3, MUC4, MUC5AC and MUC6 are observed in gastric cancers, showing increasing heterogeneity with advanced cancer stages (206, 261). Typical patterns correspond with intestinal or diffuse type gastric cancer. The gastric type is dominated by MUC5AC and MUC6, especially in early diffuse gastric cancer, while the intestinal type includes significant MUC2 expression against a background of MUC5AC and MUC6. Mixed phenotype tumors present intermediate patterns. (249, 262, 263). One of these studies included the co-expression of the trefoil peptides TFF1 and TFF2 with MUC1, MUC2, MUC5AC and MUC6 to define three phenotypes; complete gastric, incomplete gastric and non-gastric. Diffuse carcinomas showed mainly the complete or incomplete phenotype, while less than 30% of the intestinal carcinomas had a non-gastric phenotype. This work suggests that the majority of gastric carcinomas retain gastric differentiation, particularly in diffuse carcinomas (263). The combination of this analysis with detection of the membrane mucins MUC1, MUC 3 and MUC4 may provide additional refinement.

A rare subgroup of well-differentiated intestinal-type adenocarcinomas has been detected that shows similarity to complete-type intestinal metaplasia. These tumors show complete-type intestinal metaplastic cells that have brush-border features and are positive for sialomucin and MUC2-positive cells. The similarity to complete-type intestinal metaplasia has meant that these tumors were not previously regarded as pre-cancerous condition. (264)

Gastric cancer cell lines have been used in a number of studies in order to provide models for the different phenotypes of gastric cancer (249, 260, 265). Primary cultures of human gastric mucous cells show the same site-specific pattern of MUC1, MUC5AC and MUC6 mRNA expression found in gastric mucosal biopsies. In contrast, all gastric cancer cell lines had aberrant mucin gene expression with the constant feature of MUC2 mRNA and mature protein. The study also shows that gastric mucin gene expression might be regulated by pro-inflammatory cytokines including TNF-alpha (265). A further study of human gastric cancer cell lines shows partial expression of normal MUC1 and MUC5AC, with occasional

aberrant appearance of MUC2, MUC3, MUC4 and MUC5B. However, no MUC6 was found (249). As noted above, this study was unable to demonstrate any relationship between the mucin core proteins and the simple mucin-type or Lewis carbohydrate antigens. The gastric cancer cell lines KATO-III (high-expressing) and AGS (low-expressing) were employed to study the regulation of MUC5B. A highly active distal promoter for MUC5B was identified which is upregulated by protein kinase C, while repression of this gene is controlled by methylation (260).

Examination of the frequency of short MUC1 alleles in Portuguese gastric cancer patients showed this to be higher than the normal population (266). However, comparison of the distribution of short MUC1 alleles in Portuguese and Danish populations revealed higher incidence in the Danish group. This refutes the hypothesis that a relatively higher frequency of the small MUC1 alleles is responsible for the high incidence of gastric carcinoma in Portugal, compared to Denmark (267). MUC6 gene polymorphism was also examined for involvement in individual susceptibility to gastric cancer development. Significant differences in frequencies were observed for 4 of the 10 MUC6 alleles in gastric cancer patients compared with normal control (blood donors). In agreement with the MUC1 study the largest allele was more frequent in controls while the shorter alleles were more common in cancer patients. This supports a role for MUC6 gene polymorphism in the development of a predisposition to gastric carcinoma.

4.4. Inflammatory bowel disease

4.4.1. Ulcerative colitis and Crohn's disease

The inflammatory bowel diseases are largely covered by two distinct conditions, ulcerative colitis (UC) and Crohn's disease (CD). These are diseases with a poorly defined etiology and are widely thought to arise due to a combination of multiple disorders e.g. (268). Although there are differences in the location and morphology of UC and CD (7, 269), much of the research has run in parallel and it is convenient to compare their disease patterns of mucus biology.

In UC the adherent mucus gel in the colorectum is reduced in thickness compared with either normal or CD mucosa (109). The causes of this loss in UC have been associated with an overall depletion of goblet cells in the colorectum, where the disease is confined (270). A pattern of increased sialomucin, with variable loss of sialic acid O-acetylation and reduced sulphomucins has been reported for the histochemical analysis of UC. The severity of these changes correlates with an increased inflammatory index (7, 105). In CD the loss of sulphation is not seen and increased O-acetylation occurs in the terminal ileum (7, 105). The alterations in sialic acid O-acetylation may come within the range expected (approx. 10%) for non-O-acetylators individuals in the normal population (98). The demonstration of a sub-population of O-acetylated sialic acid containing epitopes within the total O-acetylated mucin species in the colorectum has been reported using a monoclonal antibody (271). A selective tissue expression occurs in the normal colon with an increasing gradient from caecum to rectum. A reversion of this pattern occurs in UC. This sub-population of O-acetylated sialic acids is lost when the faecal flow is removed e.g. as a result of surgical isolation of the colon, and is thought to be under the control of factors produced by the faecal bacterial flora (272).

Biochemical analysis of colorectal mucins using ion-exchange chromatography reveals up to 6 sub-fractions (273), one of which is lost in UC but retained in CD (274, 275). This may represent a general depletion of mucin in UC (276). The identity of the subfraction as a MUC gene product has not been made. Recent chemical and metabolic labeling analyses of colonic mucins have shown a loss of MUC2 sulfation in severe UC (277-279). They suggest that although MUC2 sulphation is reduced, a preferential secretion of sulphated MUC2 takes place in the active phase of the disease and this mechanism maintains a constant level of secreted, sulphated MUC2 (279). In contrast to these studies, largely with European colitis patients, no reduction of colonic mucin sulphation could be found in a South Asian colitic population with histochemical and biochemical techniques (280). In addition, CD patients show no loss of histochemical sulphation and only slight reduction detected by metabolic labeling (278). Thus, a difference in mucin sulphation exists between mild and severe UC and CD.

An increase in mucin sialylation has been shown in UC using metabolic labeling (281) and lectin binding studies (282) and this correlates with an increased inflammatory index. South Asian colitics in Britain have different mucin sialylation compared with their European counterparts (283). Mucin sialylation and sulfation patterns in this group of patients is consistent with a low incidence of UC associated colorectal carcinoma when compared with European patients (284). Among the many sialylated epitopes present in colorectal mucins the sialyl-GalNAc (sialyl-Tn) antigen is a marker for an increased cancer risk in the non-dysplastic colonic mucosa of long-standing ulcerative colitis patients (285). No comparable data for CD are available. As mucin sulphation and sialic acid O-acetylation play a role in the rate of mucin oligosaccharide degradation by the enteric bacterial flora alterations in their substitution on disease derived mucins are expected to have a detrimental effect on protective function of the mucus barrier (68).

Several studies have shown that no major changes in MUC genes occur in UC. The pattern of high MUC2 and MUC4 and low MUC1 and MUC3 is seen in the colorectal mucosa of European colitic patients with severe disease as in normal tissue occurs in (286, 287) and is also found in South Asian colitics. MUC2 mRNA levels do not vary in active, quiescent or control groups and are independent of disease activity, implying post-transcriptional regulatory mechanisms (288, 289). Quantitative examination of MUC2 using metabolic labeling and antibody techniques shows a significant decrease in MUC2 precursor biosynthesis and total MUC2 levels in active disease, but were no different to controls and quiescent disease with a reduced inflammatory index (288, 289). Hanski and co-workers reported increased MUC2 levels in both UC and CD using a VNTR antibody and also suggest post-transcriptional abnormalities (290). In view of the differing reactivity of VNTR and non-VNTR anti-MUC2 antibodies with precursor and mature MUC2 e.g. (291) cautious interpretation of these results is necessary. However, these studies demonstrate that MUC2 levels vary according to the degree of inflammation at any stage of the disease. A role for MUC2 polymorphism in UC appears unlikely as no preferred allele length has been identified in the disease (292). Increased levels

of anti-colonic mucin and anti-MUC1 antibodies have been detected in the serum of UC patients (293, 294). These findings suggest an involvement of mucins in the disease process through destruction of colonic cells by anti-MUC1 antibody-dependent cell-mediated cytotoxicity (293), and persistence of colonic mucosal inflammation (294).

The participation of cytokines in the mediation of MUC gene expression has been implicated in UC through analogy to results obtained with cultured human colorectal cell lines. Exposure of LS180 cells to IL1 gave a transient increase in MUC2 and MUC5AC mRNA, IL6 led to an early response with MUC2, MUC5B and MUC6 and TNF-alpha increased MUC2 and MUC5B mRNA, but did not affect on MUC 5AC and MUC6. The increases in MUC2 and MUC5AC were confirmed at the protein level for TNF-alpha action and all cytokines resulted in reduced and altered glycosylation (295). Confirmation of these results in UC patients is still outstanding.

Examination of ileal tissue from the 'skip lesions' characteristic of Crohn's disease has allowed a comparison of disease and apparently normal intestinal tissue in individual patients. A reduction of MUC1 is observed when healthy and involved ileal mucosa from the same patients is compared (296). Reductions in MUC3, MUC4 and MUC5B mRNA are seen in the ileum of CD patients compared with normal controls (296). No changes in MUC1 or MUC2 are found in the healthy intestinal mucosa of CD patients. The decrease of MUC3 and MUC4 mRNA in both healthy and involved ileal mucosa suggests a primary or very early mucosal defect of these genes in CD (296). In colorectal mucosa from CD patients no changes in MUC2 or MUC3 mRNA are detected. (287, 290).

Among different loci identified for IBD susceptibility genes is 7q22 (297), which is also the locus of MUC3, MUC11 and MUC12 (25). Analysis of MUC3 gene VNTR polymorphisms in the human intestine shows that some rare alleles of this gene may confer a genetic predisposition to UC (44). Other studies on IBD susceptibility have shown a requirement for both internal and environmental conditions. Mucin abnormalities have been identified among the internal factors (298).

4.4.2. Diversion colitis

Diversion colitis is an iatrogenic condition, which arises in the colorectum as a result of the elimination of faecal flow to the large bowel by surgical intervention for a variety of reasons including inflammatory bowel disease (299, 300). The luminal short chain fatty acids derived from the enteric bacterial flora are vital for the nutrition of the colorectal mucosa (301). Their loss in the defunctioned colorectum leads to the micro- and macroscopic inflammatory change seen in diversion colitis (302). The precise pathogenesis of diversion colitis is unexplained and the role of the protective mucus barrier in this process only partly elucidated. Changes in mucin sulfation and sialic acid O-acetylation have been identified. Metabolic labeling, chemo- and immuno-histology served to show a reduction in sulfation in diverted Crohn's disease compared with UC and non-IBD diverted groups. Increased sulfation is seen in the UC group independent of inflammatory status. However, inflammation is a major factor in mucin sulfation in IBD as noted in section 4.4.1. The loss of a

subpopulation of O-acetylated sialic acid epitopes is found for all diverted groups (272) in line with previous reports in UC (271). These findings further strengthen support for the participation of bacteria in the regulation of host mucosal defence.

4.5. Ileoanal pouch

Ileoanal pouches are formed from distal ileal tissue, function as faecal reservoirs and are usually constructed in patients where a risk of colonic neoplasia exists and where the colon is resected. After formation and restoration of the fecal flow the mucosa adapts morphologically histologically and biochemically to show a more colonic phenotype (303, 304). The adaptive process in the ileoanal pouch can be followed by monitoring mucin synthesis. Low levels of sulphated and O-acetylated sialylated mucins are typical of the normal ileum but are strongly expressed in the colon. Histochemical, immuno-histochemical and metabolic labeling techniques have been used to illustrate the changes from the initial ileal pattern to a more colorectal type within the first 6-9 months after pouch formation (304, 305). Continuing assessment of pouch mucosal properties, including mucin characteristics has shown that a complete adaptation to a colonic mucosa does not occur. A limited change in reservoir morphology from villous to partial villous type suggested that adaptation beyond 12 months is not only incomplete, but may involve reversion to a more ileal phenotype. These conclusions are drawn from sulphomucin staining with HID/AB and anti-sulpho-Lewis^a antibody and O-acetyl sialic acid staining with the mild PAS stain and monoclonal antibodies to mucin specific O-acetylated epitopes. Metabolic labeling also supports these findings (306). Analysis of the villous and partial villous cases in older pouches shows increased sulphation, but at a level remaining within the ileal range, and lower than the early response. Thus, a reversion to a more ileal phenotype occurs with increasing pouch age beyond 12 months.

MUC gene expression in ileoanal pouches should reflect relatively higher levels of MUC3 normally present in the ileum. No detectable change in MUC1-4 mRNA could be found, but significant reductions in immuno-detectable MUC1 and MUC3 do appear in the pouch compared with ileal controls (306). Assessment of the changes in MUC1 and MUC3 and the persistence of ileal patterns for MUC2 and MUC4, confirm that the adaptive process shows only limited progress to a more colonic type mucin gene expression (306). Sporadic expression of MUC5AC and MUC6 is found in a small number of pouches and may be relevant to potential neoplastic development as noted below for colorectal cancer. However, examples of dysplasia in the pouch are very rare and these changes need to be further assessed with regard to inflammation and pouchitis.

4.6. Colorectal cancer

The pathways of colorectal carcinogenesis have received wide attention and continue to be discussed, however, most colorectal cancers have been divided into hereditary non-polyposis colorectal cancer, sporadic colorectal cancer, familial adenomatous polyposis and flat adenomas. The evolution of colorectal adenoma is thought to occur through quite distinct genetic pathways in addition to the well-known adenoma-carcinoma sequence. The definition and of these pathways has been reviewed e.g. (307-310). The increasing knowledge of

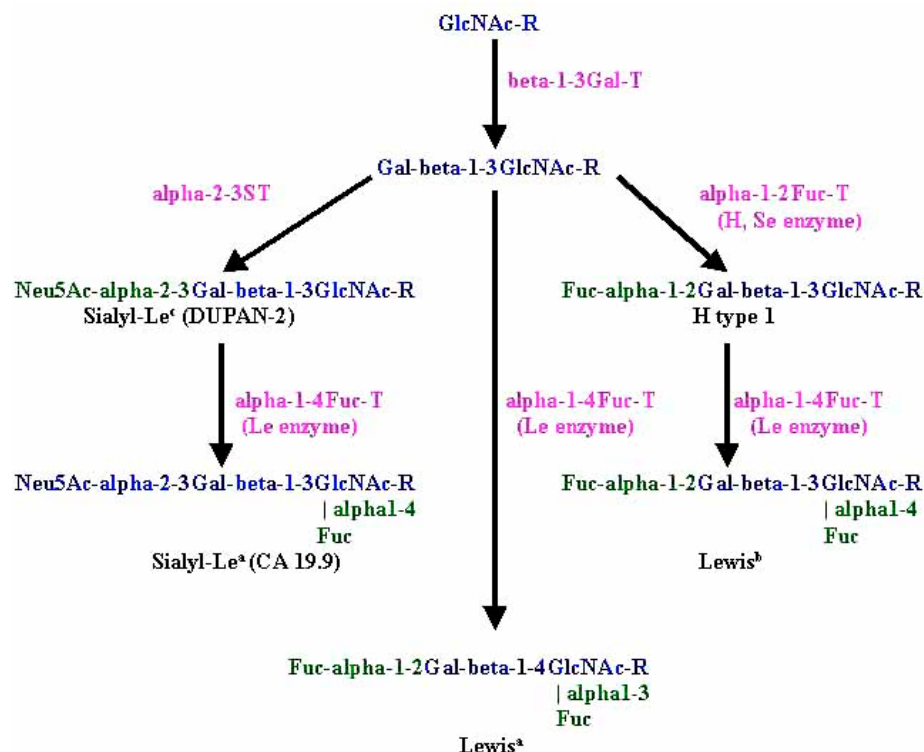


Figure 2. Biosynthesis of Lewis^b and type 1 Lewis blood group antigens in normal colonic mucosa and colorectal cancer. Action of H and Se enzymes

mucin and MUC genes in the colorectal epithelium provides excellent opportunities to understand their relevance in the pathways of colorectal carcinogenesis.

It is well known that the amount and nature of mucus is altered as a result of colorectal cancer (13). The majority of these tumors are adenocarcinomas (~80%), with a mucinous carcinoma phenotype making up most of the remaining cases. Properties identified in adenocarcinomas include a reduction in total mucus output (7), loss of sulfation and sialic acid O-acetylation and an increase in sialylated colorectal mucin (13). In contrast, mucinous carcinomas are hypersecretory for mucus, with modification of sialic acid composition and reduction in O-acetylation (254, 311), but show no loss of sulfation.

A feature of cancer associated mucins is the appearance of short oligosaccharide chains, in contrast to the longer branched chains found in normal colorectal mucins (13). These structures include the truncated structures Tn, T, sialyl-Tn, the Lewis blood group antigens and their sialylated and sulphated derivatives (13, 56) (see Table 2 for structures). The short oligosaccharides are found *de novo* in the majority of colorectal tumors (312, 313), and in colorectal polyps, where they correlate with size, histological type and level of dysplasia. Moderately and well-differentiated colorectal adenocarcinomas are rich in T antigen, while poorly differentiated tumors exhibit Tn antigen (314). A number of colorectal cancer related glycotopes antigens have been identified, which are carried on mucins and other glycoconjugates and are the basis of screening kits (315). They include sialylated Lewis^a (Ca 19.9) which is increased in most colorectal cancers in addition to hyperplastic and adenomatous polyps (316). However,

detection is independent of site, differentiation, the disease stage or the prognosis. Study of individual adenocarcinomas and cultured cell lines has shown that sialic acid O-acetylation is lost at an early stage in the adenoma – carcinoma sequence (317). Furthermore, O-acetylation masks both sialyl-Tn (318) and sialyl-Le^x (311) in the normal colon and implies that the appearance of these antigens in neoplasia (314, 319) is due to loss of O-acetylation.

In addition to the modulation of sialic acids on colorectal cancer mucins sulfation is also significantly depleted (320). This could be confirmed, using metabolic labeling and sulfotransferase activity, in cultured colorectal cancer cell lines (321) and individual tumors (322). Monoclonal antibody detection of sulfo-Le^a in colorectal cancer mucins supports these results with an accumulative loss from early to advanced adenocarcinomas (323). No loss of sulfation has been detected in mucinous carcinomas, underlining the different phenotype of this group of colorectal tumors. (324). The nature of mucin sulfation in colorectal cancer is limited due to the lack of reagents for the detection of individual sulfoglycotopes within the total sulphomucin population.

The ABH and Lewis blood group antigens, Le^a and Le^b have a characteristic pattern of expression in the normal colon. They are all present in the fetal gut, but are repressed in the distal colon at birth, with the exception of Le^a which shows pan-colonic distribution in both secretor and non-secretor individuals. Colorectal cancer mucins exhibit re-expression and/or inappropriate blood group at this site for A, B, H and Le^b. The molecular mechanism of the Le^b and type I Lewis blood group antigen expression in normal and colorectal cancer

tissue depends on the action of the H and Se enzymes in normal and pathological tissue (Figure 2) (325). A decreasing gradient of Se activity is found from proximal to distal colon, matching immuno-detectable Le^b. In distal colonic cancers both H and Se enzymes are upregulated relative to adjacent non-neoplastic mucosa, while only the H enzyme is found in proximal colorectal tumors. The overall level of the Se enzyme appears to govern the expression of Le^a and Le^b and their sialyl antigens in both normal and pathological colonic tissues, and this occurs through competition with alpha-2-3sialyltransferases and the Le enzyme for type I acceptors (Figure 2) (325).

Study of the initial glycosyltransfer to mucin tandem repeat peptide by the action of UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferases (GalNAc transferases) has been carried out using anti-enzyme polyclonal antibodies to T1 and T2, two of the isoenzymes. In colorectal cancer tissue positive staining for T1 and T2 was significantly higher than that in normal epithelium although individual levels varied between patients (326). Interpretation of these results requires consideration of the tissue specific complement of GalNAc transferase isoenzymes and their individual action in initial glycosylation of serine and threonine acceptors in the tandem repeat domain of each mucin. Measurement of the glycosyltransferases contributing to colonic mucin oligosaccharide formation reveals a loss of key enzymes in the formation of the characteristic colon type core 3, core 4 and branched structures. This leads to the synthesis of less abundant, sialylated, fucosylated and extended, unbranched core 1 structures in malignant colorectal cancer cells (321) and colonic mucosal cells from patients with colorectal adenocarcinomas (322). Examination of mucin related glycosyltransferase mRNA in cells from colorectal tumors and their metastases further confirms the importance of mucin glycosylation in malignant transformation in the colorectum (327). Detailed reviews of the modulation of glycosylation in cancer have been presented elsewhere (13, 56).

The study of mucin genes in colorectal cancer has highlighted the need for improved understanding of the regulation of mRNA and subsequent translation into mature product and the need for anti-MUC reagents that detect secreted or membrane associated mucins in the cells. Initial studies with MUC1-3 show a decrease in mRNA in colorectal cancer and premalignant polyps, while immunodetection with antibodies to deglycosylated mucin is increased (287, 328). These results focus attention on the processing of the precursor forms of these MUC genes as the fully glycosylated mucins are not detected.

The staining patterns for MUC1 and MUC2 have been used to define absorptive and goblet cell phenotypes in colorectal cancer (329). This reveals goblet cell type with MUC2, non-O-acetylated sialic acid, sialosyl Tn, Tn (Golgi region) and sialyl-Lex, and columnar cells with MUC1, Lex, sialyl-Lex, Tn (cytoplasm) and Ley. It also confirms two mucin types, secretory mucin linked to cells of goblet lineage and up-regulated membrane-associated mucin of presumed columnar cell origin (329).

Progression through the adenoma-carcinoma sequence in the colorectum involves *de novo* synthesis of MUC5AC and MUC6. Both mRNA (330-332) and mucin peptide (331-333) can be detected. Upregulation of MUC2

mRNA is found in adenomas with increasing villosity but is reduced in adenocarcinomas (330), and higher levels of MUC2 peptide are detected with increasing villous architecture and polyp size (331). However, flat and polypoid tubular adenomas show reduced MUC2 and MUC4 mucin in high-grade atypia using VNTR antibodies (333, 334). Patterns described in these studies show the appearance of a gastric type MUC gene phenotype in the presence of typical colonic MUC2, MUC3 and MUC4. Increased anti-VNTR antibody reactivity may be related to detection of premature apomucin by these antibodies (331), or to abnormal processing. Non-VNTR antibodies are required to identify the nature, location and amount of the mature mucins. MUC2 gene suppression in colorectal cancer may be achieved through methylation of elements in the promoter region and could represent a significant regulatory mechanism (335).

MUC1 levels are increased in the adenoma-carcinoma sequence and are elevated in associated metastases (336) and expression in the deep invasive region of colorectal tumors indicative for poor prognosis (337). Furthermore, an increased serum concentration of MUC1 is a predictor of colorectal in addition to other cancers (338).

Demonstration of the difficulties due to the large number of anti-MUC1 antibodies available and the tissue specific nature of glycosylation and peptide processing is evident in a study assessing MUC1 in tissue and stool of colorectal patients. Three different anti-MUC1 antibodies were employed, one for tissue screening and the other two for fecal samples. Colorectal mucosa was strong for MUC1 staining in adenocarcinomas, moderate in adenomas, weak in adjacent mucosa and absent in distant mucosa. Stool testing showed no differences between colorectal cancer cases and controls. Based on these results, MUC1 appears to be a functional tumor biomarker in colorectal tissue but not in stool (339). Such differences may arise due to extracellular degradation of MUC1, or variation in antibody binding properties. More detailed knowledge of the molecular processing of MUC1 in individual tissues is needed to resolve this question. Changes in the glycosylation of MUC1 in colorectal cancer have been reported (51, 340). Considerable attention has been paid to increased levels of sialyl-Le^x and T-antigen (341-343). The combination of high level MUC1 synthesis and increased access to the tandem repeat peptide sequence as a result of shortened oligosaccharide structures has promoted vaccine cancer therapies based on MUC1 (344).

Two novel MUC genes, MUC11 and 12 were detected by differential display, both down regulated in colorectal cancer (25). These two genes have major colorectal expression and are localized on chromosome 7q22 close to MUC3. MUC12 shows EGF-like domains in common MUC3, MUC4 and MUC13. These EGF-like domains are thought to be related to growth regulator function in the intestine (25, 26).

Mucinous carcinomas of the colon constitute a further phenotype of colorectal cancers where survival is poorer than adenocarcinomas (345). A common feature of mucinous carcinomas in the colorectum is overproduction of MUC2, in contrast to the reduction of this gene in adenocarcinomas (346). Retention of MUC2 appears to be a general property of

mucinous carcinomas in the colon (347) and is seen as a *de novo* expression in these tumors in other organs (254). Examination of MUC2 levels in malignant cultured colorectal cell lines with mucinous phenotype shows a significant increase in the synthesis and secretion of MUC2 precursors and mature mucin (324). Furthermore, the tumor-associated MUC2 from patients with mucinous carcinomas has also been shown to be a potential target antigen for cytotoxic T cells (348). MUC1 expression in mucinous carcinomas is weak in comparison with non-mucinous cancers (349). The strong association of MUC1 with T antigen found in non-mucinous colorectal cancers could not be confirmed for the mucinous phenotype and may be expressed on both MUC1 and MUC2.

A study of MUC1, MUC2, MUC4, and MUC5AC distribution in sporadic colorectal cancers classified according to levels of DNA microsatellite instability (MSI) identified three groups of patients with high, low or stable MSI. MUC2 and MUC5AC immunoreactivity is present in a higher proportion of the high MSI group relative to the other two groups. MUC1 and MUC4 show no correlation with these groups. Thus increased expression of secretory MUC2 and MUC5AC is found in sporadic high microsatellite instability cancers (350). The same mucin changes and DNA microsatellite instability is found in serrated epithelial polyps of the colon, which show a high frequency of DNA instability and arise through the suppressor and mutator pathways, suggesting that they may be precursors of high microsatellite instability cancers (350).

The use of RT-PCR to detect the presence of MUC2 transcripts in the lymph nodes of histologically negative colorectal cancer patients has been taken as evidence for micrometastatic disease. The method has been proposed as a sensitive and specific marker for occult micrometastases and has the potential to identify those patients at risk for early cancer recurrence (351).

4.7. Paediatric disease

The paediatric patient offers a unique opportunity to study mucin gene expression in an evolving gastrointestinal environment. The massive physiological changes that occur shortly after birth serve to prepare the body for its first contact with the external environment. The introduction of diet is associated with changes in gut function and in the acquisition of a gastrointestinal bacterial flora (93, 352). However, the changes in mucin production within the gastrointestinal tract remain poorly understood as tissue acquisition in this period of life is uncommon. The study of gastrointestinal mucins in childhood has been restricted to pathological conditions where there is a requirement for surgery and access to resected tissue. The production of a viable mucus barrier in the colorectal mucosa in patients with Hirschsprung's disease has been questioned due to its association with subsequent enterocolitis. Mucins are deficient in the colonic mucosa of patients with Hirschsprung's disease (353). Detailed immuno-histochemical analysis of mucins in the colonic mucosa demonstrates no qualitative differences, the colonic mucins being primarily O-acetylated and sulphated (125). Qualitative mucin gene expression was not affected by the lesion with strong MUC2 and 4 expression, moderate to weak MUC1, 3, and 5B expression and no MUC5AC, 6, 7, and 8. This is the pattern

observed in normal controls (125). However a reduction in mucin turnover was found using metabolic labelling. This was seen in aganglionic Hirschsprung disease segments, and in adjacent ganglionic bowel which is retained at the pull-through operation (353). These changes are independent of the bowel innervation status and may account for the increased incidence of enterocolitis in these patients following corrective surgery (353). The reduced mucus turnover will limit the protective function of the mucus barrier and a defective mucus barrier may be an etiological factor in the pathogenesis of Hirschsprung's enterocolitis. The occurrence of a similar abnormality in the adjacent ganglionic bowel used to repair the lesion requires further investigation to determine the extent of the defect. Once this is known a reassessment of surgical procedure can be undertaken. The mechanism responsible for reduced turnover in these patients is not yet known.

Data regarding changes in qualitative and quantitative mucin production seen during colitis in children is scanty. Of particular interest are the changes that occur in necrotizing enterocolitis. This is a severe form of colitis of unknown etiology seen usually in premature infants. This condition has an increasing incidence in the UK and is associated with significant morbidity and mortality. Histochemical evidence of increased neutral mucins and reduction in acidic-sulphomucins has been found in Hirschsprung's disease associated enterocolitis (354). A significant loss of the mucus barrier is strongly implicated here and in necrotising enterocolitis (355). The role of mucins in predicting disease susceptibility and as markers for disease severity remains unknown.

5. SUMMARY AND PERSPECTIVE

This review covers many areas that relate to the physiological appearance and function of the mucus barrier at the mucosal surface throughout the gastrointestinal tract. New developments in these areas are combining to bring together an improved overview of the interactions necessary to provide a continuous and effective defensive barrier.

The improved understanding of the structure of the mucins at genetic and translated levels has given rise to the interest in mucin peptide fragmentation and processing during biosynthesis. The maturation of the precursor peptides in sub-cellular compartments appears to involve both peptide cleavage and glycosylation events ensuring that the respective secreted and membrane-associated molecules arrive at their appropriate locations. An understanding of the relationship between individual mucin-gene peptide expression and glycosylation is actively being sought. Tissue specific patterns have been demonstrated and linked to developmental and disease related processes, however, the manner in which these two processes are integrated remains unclear and the object of current research.

A more direct examination of the patterns of individual MUC genes during gastrointestinal pathology has revealed phenotypes that may have value in the classification and staging of disease. This is currently of great interest with a view to improved screening and will benefit from the increasing number of studies using anti-mucin peptide antibodies to the non-VNTR domains of the MUC genes. As the understanding of mucin peptide fragmentation/processing

improves the value of these reagents in detecting distinct pathological events will become clear.

Parallel to the improved knowledge of mucin structure/function the number of mucosally synthesized and secreted protective proteins has increased. The nature of potential interactions between the protective proteins and mucins has not yet been examined in many cases. This has implications for the general protective functions of the proteins in the mucus gel and the structural stability of the gels. In addition, the multiple roles of these proteins impinge on mucosal cell behavior (e.g. apoptosis, differentiation and proliferation) and add a further dimension to normal mucosal function affecting the protective mucus barrier.

The gastrointestinal tract has a significant interaction with the enteric microflora and much work suggests that there is a continuous regulation of host mucosal cell behavior through the resident bacteria in the mucus barrier. The increasing number of studies on the role of the microflora in normal mucosal defence through probiotic and other nutritional interventions reflects the need to define the host-bacteria symbiotic process at a mucus barrier - mucosal defence level.

Identification of monosaccharide sensor systems allowing enteric bacteria to influence the glycosylation of the host mucosal cells is a first step in defining the molecular processes at play in these regulatory events. It provides a link with the well-known degradative action of enteric bacterial glycosidases. Knowledge of such systems will pave the way for a closer examination of the dynamic adaptive events taking place during colonization of the neonatal gut and the continual maintenance of a normal mucus barrier throughout later life. The mucins are major molecular targets in enteric microbiology and are now becoming accessible to more precise study.

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Abbreviations: CD: Crohn's disease; EGF: epidermal growth factor; ER: endoplasmic reticulum; Fc-gamma-BP: IgG-gamma-Fc binding protein; HP: *Helicobacter pylori*; HID: high iron diamine; IL: interleukin; LPS: lipopolysaccharide; MOD: mucin oligosaccharide degrading; MSI: microsatellite instability; MUC: mucin; PSTI: pancreatic secretory trypsin inhibitor; SLPI: secretory leukocyte proteinase inhibitor; STP: serine, threonine, proline; TGF-alpha: transforming growth factor-alpha; TNF-alpha: tumor necrosis factor-alpha; UC: ulcerative colitis; VNTR: variable number tandem repeat.

Key Words: Mucin, Gastrointestinal, Glycosylation, Mucinae, Enteric Bacteria, Mucosal Defense, Mucus Barrier, Esophagus, Gastric Cancer, Colorectal Cancer, Inflammatory Bowel Disease, Fetal Development, Pediatric Disease, Intestinal Metaplasia, Review

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