Mucins and mucosal protection in the gastrointestinal tract: new prospects for mucins in the pathology of gastrointestinal disease

The luminal surface of the gastrointestinal tract is covered by a viscoelastic mucous gel layer that acts as a protective barrier against the harsh luminal environment. The structural characteristics of this barrier are primary indicators of its physiological function and changes to its composition have long been identified in gastrointestinal pathologies. During the past decade significant improvements in analytical techniques coupled with detailed knowledge of the genes coding for the mucin proteins have provided exciting new insights into the role of the mucous layer and its relevance to gastrointestinal disease.

The high molecular weight mucins are responsible for the viscoelastic properties of the mucous barrier. They are widely expressed in epithelial tissues and are characterised by variable number tandem repeat peptide sequences rich in serine, threonine, and proline which carry large numbers of O-linked oligosaccharide chains. At present, 12 genes have been described, shown in table 1. Secreted and membrane associated forms have been found based on their function as extracellular viscous secretions or viscoelastic polymer gels or location as membrane anchored molecules in the glycoalyx. Two clusters have been reported, the secretory mucin genes MUC2, MUC5AC, MUC5B, and MUC6 on chromosome 11p15.5, and MUC3, MUC11, and MUC12 on chromosome 7q22.

Histochemical techniques for mucin detection rely on the ability to detect carbohydrate or negative charge and widely were used for classification of changes in disease. The use of lectins and antiacarbohydrate antibodies has greatly improved the specific detection of mucins histochemically and biochemically. A group of mucin oligosaccharide antigens, including Tn, sialyl-Tn, T, Lewis' and Lewis', sia1yl and sulpho-Lewis' and -Lewis', and the blood group ABH antigens, have been identified and which arise from disease related pathways. These carbohydrate antigens appear in relation to disease status, progression, diagnosis, and prognosis but are not limited to the mucins alone and occur on other cellular glycoproteins and glycolipids. Therefore, caution is needed in the interpretation of results in histological analyses where no direct identification of mucin related material is made.

This review focuses on the role of mucins in gastrointestinal disease in the light of the dramatic improvements in specific detection of mucin gene products and sets this against the larger background literature with less informative methods.

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

Mucins are implicated in the aetiology and may assist in the diagnosis of gastric intestinal metaplasia (IM) associated with gastric ulceration, *Helicobacter pylori* infection, and the risk of gastric cancer. The histochemical detection of mucins is used for classification into complete (type I), incomplete without sulphomucins (type II), and incomplete with sulphomucins (type III). The histochemical profiles of type I IM are similar to those in diffuse gastric cancers, and type III IM is similar to “intestinal” cancers with sulphomucins predominating over sialomucins. A progression from type I to type III IM was proposed during the development of gastric cancer. However, this relationship is not always found. Mucins with O-acetylated sialic acids (O-acetylsialomucins) are not seen in type III IM or tumours but are observed in complete or type I IM. However, recent data showing reactivity in types I and III IM suggest that O-acetylated sialomucins are much more prevalent in gastric IM and carcinoma than previously recognised. The truncated mucin oligosaccharides Tn, sialyl-Tn, T, and sialyl-T have been examined and show that all IM mucosae are positive for sialyl-Tn while only Tn is present in control cases.

Normal stomach mucosa is characterised by expression of MUC1, MUC5AC, and MUC6. High levels of MUC2 and MUC3 appear in IM. The complete form (type I) demonstrates only MUC2 in goblet cells. In contrast, incomplete forms (types II and III) exhibit MUC1 and MUC5AC in both goblet and absorptive cells, MUC2 in goblet cells only, and MUC6 in over 60% of cases. This represents two phenotypes, a small intestinal/colonic pattern and a typical gastric pattern with MUC2. MUC3 and MUC4 have not been examined.

*H pylori* infection is associated with the development of gastritis, ulcers, and possibly gastric malignancies. The organism first penetrates the gastric mucous layer and then binds to specific mucus or epithelial cell targets. *H pylori* infection results in a reversible alteration of mucin glycosylation which favours attachment of the bacteria. Fucosylated blood group antigens and sialylated and sulphated oligosaccharides are implicated in *H pylori* binding to the gastric mucins mediated by a variety of bacterial adhesins.

Degradation of gastric mucins by *H pylori* by proteases and sulphotases has been proposed and refuted. The action of urease generating ammonia increases pH which destabilses the mucous layer. This action, however, is not sufficient to cause collapse of the mucous barrier and together with bacterial binding to mucus favours survival of *H pylori* in the mucous layer. Adherence of *H pylori* to the gastric epithelial cells results in more serious damage and is mediated by blood group related carbohydrate antigens, for example, Le'1, sialyl Le', Le', type 1H, and type 2H and in particular fucosylated blood group antigens associated with blood group O phenotype. This explains the higher prevalence of ulcerative disease in individuals with this blood group.

*H pylori* positive patients exhibit an increase in MUC6 in surface mucous cells with a reduction in MUC5AC. Reversal of the normal gastric pattern is corrected on elimination of infection. The advantage of such a reversal

**Abbreviations used in this paper:** IM, intestinal metaplasia; UC, ulcerative colitis; CD, Crohn’s disease; VNTR, variable number tandem repeat.
in sulphation detected by metabolic labelling and a slight reduction detected by metabolic labelling. These data suggest a difference in mucin sulphation between mild and severe UC and CD. Sialylation is increased in UC in agreement with lectin binding studies suggesting that the grade of inflammation also plays a role in the glycosylation patterns seen in the mucins. Expression of sialyl-GalNAc (sialyl-Tn) antigen on mucins in the non-dysplastic colonic mucosa of longstanding UC patients identifies an increased cancer risk. South Asian colitics have different mucin sialylation compared with their European counterparts. South Asians in Britain have a high incidence of UC but a low incidence of UC associated colorectal carcinoma relative to European patients. No comparable studies with CD have been done.

Depletion of mucin sulphation and sialic acid O-acetylation are important factors affecting mucin degradation by the enteric bacterial flora. They have been linked with a reduced protective function of the mucous barrier in UC.

MUC2 is the major secreted mucin gene in normal and UC colonic mucosa and levels of MUC2 mRNA are similar to controls in UC patients with active and quiescent disease. No alteration in the normal pattern of high MUC2 and MUC4 and low MUC1 and MUC3 occurs in European colitic patients with severe disease or in South Asian colitics. Susceptibility genes for inflammatory bowel disease include 7q22, the locus of MUC3, MUC11, and MUC12. Analysis of polymorphisms of variable number tandem repeats (VNTRs) within the intestinal MUC3 gene suggests that rare alleles of the MUC3 gene may confer genetic predisposition to UC.

In CD, MUC1 is reduced only when healthy and involved ileal mucosa from the same patients are compared. However, comparison of healthy mucosa from CD patients with normal controls shows reductions in MUC3 and MUC4, both major expressed genes in ileum, and also of MUC5B, present in low levels in normal intestinal mucosa. No changes were found for MUC1 or MUC2 in healthy intestinal mucosa from CD patients. The decrease in expression of MUC3 and MUC4 in both healthy and involved ileal mucosa suggests a primary or very early mucosal defect of these genes in CD.
colorectal mucosa from CD patients no changes in MUC2 or MUC3 mRNA were detected.\textsuperscript{35} In contrast with the mRNA results, a significant decrease in MUC2 precursor biosynthesis and total MUC2 levels occurs in UC patients with active inflammation while normal levels are detected during remission of inflammation.\textsuperscript{36} However, an increase in MUC2 reactivity using VNTR antibody detection has been reported in both UC and CD due to post-transcriptional abnormalities.\textsuperscript{37} In view of the differing reactivity of VNTR and non-VNTR anti-MUC2 antibodies (for example, see Aksoy and colleagues\textsuperscript{38}), careful reassessment of these results is required. However, these studies show that colonic biosynthesis and total levels of MUC2 vary according to the activity of the disease and implicate a role for inflammation.

Ileoanal pouch
The characteristic differences in ileal and colonic mucin sulphation and O-acetylation are a good indicator of the adaptation of ileoanal pouch mucosa and indicate that these changes are established within the first 6–9 months after pouch formation.\textsuperscript{39} Low levels of sulphated and O-acetylated siaiylated mucins are present in normal ileum and in the new pouch mucosa, and this increases in 50–72\% of established pouches to show a more typical colonic type pattern.\textsuperscript{40} The mucins synthesised by ileoanal pouches have increased sulphation relative to normal ileum.\textsuperscript{40} However, complete adaptation to a colonic mucosa is not seen, even in pouches established for over five years. Recent investigation of MUC gene expression in ileoanal pouches showed no changes in detectable MUC1–4 mRNA but significant reductions in immunodetectable MUC1 and MUC3 compared with ileal controls.\textsuperscript{41}

Barrett’s oesophagus and oesophageal adenocarcinoma
Barrett’s oesophagus may precede the development of oesophageal adenocarcinoma. In reflux oesophagitis replacement of squamous epithelium by columnar epithelium (Barrett’s oesophagus) resembles either gastric or intestinal mucosa.\textsuperscript{42} Mucin histochemistry has been used to characterise the malignant progression and its value in cancer prediction. However, no clear consensus has formed supporting an increase in sulphomucin or loss of cancer prediction. However, no clear consensus has formed to characterise the malignant progression and its value in intestinal mucosa.\textsuperscript{39} Mucin histochemistry has been placed of squamous epithelium by columnar epithelium. Barrett’s oesophagus may precede the development of adenocarcinoma.\textsuperscript{43}

Gastric cancer
Detection of mucin features in the classification systems used to grade gastric cancers, including the WHO classification, Lauren; intestinal and diffuse types, and Goseki, mucin poor (types I and III), and mucin rich (types II and IV).

The mucin oligosaccharides Tn, sialyl-Tn, and T antigens are found in more than 90\% of primary gastric adenocarcinomas.\textsuperscript{44} However, the association with cancer is not always clear, for example, antigen expression is related to cell type, with Tn predominantly in columnar cells and sialyl-Tn in goblet cells.\textsuperscript{45} Further, sialyl-Le\textsubscript{a} shows a strong association with an unfavourable outcome if all gastric tumours are grouped, while sialyl-Tn, sialyl-Le\textsubscript{a}, and sialyl-Le\textsubscript{b} are linked with a worsening prognosis in the subgroup of diffuse gastric cancers.\textsuperscript{46} Sialyl-Tn is an excellent marker of small intestinal mucins and is indicative of small intestinal-type differentiation in two thirds of gastric cancers.\textsuperscript{48} It is a marker of gastric cancer progression suggesting that cancer associated mucins play a role in the malignant behaviour of these tumours.\textsuperscript{49} Lewis (a+/b−) and non-secretor phenotypes have a significant association with expression of sulphomucins in gastric cancer populations\textsuperscript{47} and sulpho-Le\textsubscript{a} is found with high frequency in gastric cancers compared with normal gastric mucosa.\textsuperscript{47} It is clear from these studies that the significance of these carbohydrate antigens in mucins remains to be explained. As already emphasised, they are not exclusive to mucins and are present in cell membranes and cytoplasm.

Patterns of MUC gene expression appear to be more promising as specific indicators of gastric cancer phenotypes. Immunohistological detection of MUC1 in gastric cancers correlates with gastric cancer development and progression,\textsuperscript{50} poor outcome,\textsuperscript{51} cell invasiveness, and poor prognosis.\textsuperscript{51} MUC2 is not normally found in the healthy gastric mucosa but is present in tumours where immunoreactivity relates best with the histological pattern but not with patient age or disease outcome.\textsuperscript{51} Detection in gastric carcinoma is prognostic for a favourable outcome.\textsuperscript{51} However, a different anti-MUC2 VNTR antibody shows no significant difference between tumours according to their classification, stage, and lymph node status and has limited prognostic value,\textsuperscript{52} underlining the importance of antibody design in relation to the expected population of mucins detected.

Immunoreactivity with an anti-MUC5AC VNTR peptide antibody shows positive binding in over 60\% of gastric cancers with >80\% of diffuse carcinomas but only 59\% of the intestinal type. Mixed phenotype cancers fitted this pattern with less reactivity in the intestinal regions. All early cancers are positive while advanced carcinomas lose reactivity suggesting that MUC5AC expression may be used as a marker of gastric differentiation.\textsuperscript{53} A pattern of strong surface expression and weak expression in deeper areas of advanced carcinomas reflects the normal pattern in gastric mucosa.

Improved assessment of mucin phenotypes in gastric cancer has come from analysis of groups of MUC genes. Combined evaluation of MUC1 and MUC2 mucin staining has been proposed as a clinically useful test to predict disease outcome.\textsuperscript{54} Characteristic patterns for MUC2, MUC3, MUC4, MUC5AC, and MUC6 are found in gastric cancers with increasing heterogeneity in advanced cancer stages.\textsuperscript{2} Two patterns relate to intestinal- or diffuse-type gastric cancer. These are the gastric-type dominated by MUC5AC and MUC6, especially in early diffuse gastric cancer, and the intestinal-type with significant mucin expression against a background of MUC5AC and MUC6. Mixed phenotype tumours present intermediate patterns. Levels of the membrane mucins MUC1, MUC3,
Colorectal cancer

Both qualitative and quantitative changes occur in the mucins produced in colorectal neoplasia. In adenocarcinomas these included reduction in total mucus output, reduction in sulphation, and sialic acid O-acetylation, but an increase in sialylated mucin. Mucinous carcinomas show a different phenotype with hypersecretion of mucin, no significant loss of sulphation but with changes in sialic acid content and O-acetylation. The oligosaccharide chains of mucins from colorectal cancers are shorter, resulting in new antigenicities. Most attention has focused on the truncated structures Tn, T, sialyl-Tn, the Lewis blood group antigens, and their sialylated and sulphated derivatives. Tn, T, and sialyl-Tn are expressed in over 90% of colorectal cancers and are absent in normal colonic mucosa. T antigen is found in moderately and well differentiated colorectal adenocarcinomas, in contrast with Tn, common in poorly differentiated tumours. All three antigens are present in colorectal polyps and correlate with size, histological type, and level of dysplasia when found. Modified forms of carbohydrate chains found in normal colorectal mucins include sialylated Lewis’ (Ga 19.9) and sialyl-Le”. Sialyl-Lewis’ is increased in most colorectal cancers, and in hyperplastic and adenomatous polyps, although its appearance is independent of location, differentiation, stage of disease, or prognosis in colorectal cancer. Sialic acid O-acetylation is deleted early in the adenoma-carcinoma sequence and has been shown to mask the detection of both sialyl-Tn and sialyl-Le” in the normal colon, suggesting that the appearance of these antigens in neoplasia may be due to loss of O-acetylation. Sulphomucin depletion occurs in colorectal adenocarcinoma but not mucinous carcinoma. Sulphomucins on colorectal mucins shows a progressive decrease from early to advanced adenocarcinomas. Improved detection of total sulphated epitopes in sulphomucins is required to evaluate this phenomenon.

Normal expression of the A, B, H, Le”, and Le” blood group antigens is found in the fetal gut but is deleted in the normal distal colon after birth. A high proportion of colorectal cancers show abnormal re-expression at this site together with incompatible blood group antigen in colon cancers. More extensive reviews of the changes in glycosylation and their associated mechanisms occurring in colorectal cancers have been presented (see Kim and colleagues).

Levels of MUC1, 2, and 3 mRNA are decreased in colorectal cancer and premalignant polyps but mucin peptide detected with antibodies against deglycosylated mucins showed increased reactivity. These results may suggest abnormal apomucin processing, as fully glycosylated mucin is not detected by these antibodies. A dual nature for mucins in colorectal cancer based on MUC1 and MUC2 expression defines absorptive and goblet cell phenotypes, respectively.

MUC gene expression in the adenoma-carcinoma sequence is characterised by switch on of MUC5AC and MUC6. These genes are detected at the mRNA and peptide levels. In addition, upregulation of MUC2 mRNA occurs in adenomas with increasing villosity and is reduced in adenocarcinomas, although this was not found by Ho and colleagues. Increased MUC2 peptide is detected with increasing villous architecture and polyp size. In contrast, flat and polypoid tubular adenomas show reduced MUC2 and MUC4 mucin in high grade atypia using VNTR antibodies. These changes indicate the appearance of the gastric-type MUC gene phenotype against a background of colonic mucin (MUC2, MUC3, and MUC4). The significance of increased anti-VNTR antibody reactivity may be related to premature apomucin or abnormal processing and requires further study. MUC2 gene suppression in colorectal cancer is linked with methylation of the promoter region and may represent a major regulatory mechanism.

Mucinous carcinomas represent a separate group of colorectal cancers characterised by over expression of MUC2, a common genetic lesion in these tumours. Increased membrane mucin MUC1 levels correlate with progression of colorectal cancer and metastasis and location of MUC1 in the deep invasive region of tumours is prognostic. Detection of MUC1 in serum is a predictor of colorectal and other cancers. MUC1 expressed in colorectal cancer shows abnormal glycosylation, in particular increased levels of sialyl-Le”. The high expression of MUC1 and increased exposure of the tandem repeat sequence through aberrant glycosylation has led to the development of anti-MUC1 vaccine cancer therapies.

Two new MUC genes, MUC11 and 12 (table 1), with major colorectal expression, both downregulated in colorectal cancer, have been identified recently. The presence of epidermal growth factor-like domains in MUC12 shows sequence homology with epidermal growth factor receptor-binding growth factors and with similar domains on MUC3 and MUC4. It has been proposed that these regions are important for the function of these mucins as growth regulators in the colon and their downregulation in colorectal cancer an important stage in colorectal carcinogenesis.

Paediatric disease

Most studies of mucosal paediatric disease have been concerned with Hirschprung’s disease and associated enterocolitis. The production of a viable mucous barrier in the colorectal mucosa in patients with Hirschprung’s disease has been questioned due to the increased susceptibility of these patients to enterocolitis. Histological examination of the mucus present in the mucosa from these patients appeared to be normal, the colonic mucins being primarily O-acetylated and sulphated. Mucus turnover determined by metabolic labelling showed a reduction in both Hirschprung’s disease (aganglionic) colon segments and in adjacent ganglionic bowel, which is retained at the definitive pull through operation. MUC gene expression was similar in patients and controls. MUC2 and 4 were strongly expressed, MUC3, and 5B had moderate to weak expression, and MUC 5AB, 6, 7, and 8 had baseline expression. Thus expression of mucin genes and the quality of mucins is similar to normal controls.

The abnormal mucus turnover will result in a weakening of the protective function of the mucous barrier and may be an aetiological factor in the pathogenesis of enterocolitis of Hirschprung’s disease. In addition, demonstration of the same defect in adjacent ganglionic bowel used to repair the lesion suggests that a reassessment of the surgical procedure may be required and the extent of the modified mucus turnover in ganglionic bowel for these patients should be assessed. A mechanism for reduced turnover in these patients remains to be determined.

A significant alteration in mucins with an increase in neutral mucins and a decrease in acidic-sulphomucins occurs in Hirschprung’s disease associated enterocolitis.
and although mucus depletion is strongly implicated here and in necrotising enterocolitis, there are few data indicating the precise nature and cause.

Conclusions and summary
Recent advances in the detection of the growing family of MUC genes using nucleic acid probes and both VNTR and non-VNTR antibodies are making a significant impact on the ability to detect patterns of expression in gastrointestinal disease. This progress suggests that it may be possible to identify disease phenotypes more readily than with previous glycosylation based detection. The clinical impact of these developments will be in improved diagnosis and prognosis in disease screening and expansion of the current antimucin vaccine trials with MUC1 and sialyl-Tn to other mucin genes implicated in gastrointestinal disease. Sharing of carbohydrate antigens with non-mucin glycoconjugates remains a significant limitation in this respect. The secretory and membrane associated nature of the MUC gene products and their cellular function suggest significant, and regulation of these genes is only starting to be investigated. Future development will be aimed at identification of MUC gene protein glycosylation in diseases related to the protective functions at the mucosal surface, their role in cell and matrix adhesion, and metastasis.

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