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Biosynthesis of mucins (MUC2–6) along the longitudinal axis of the human gastrointestinal tract

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²*Department of Pediatrics, University Hospital Rotterdam, Sophia's Children Hospital, 3015 GJ Rotterdam, The Netherlands; and* ³*Unitat de Biologia Cel·lular i Molecular, Institut Municipal d'Investigació Mèdica, Barcelona 08003, Spain*

Van Klinken, B. Jan-Willem, Jan Dekker, Hans A. Büller, Carme de Bolòs, and Alexandra W. C. Einerhand. Biosynthesis of mucins (MUC2–6) along the longitudinal axis of the human gastrointestinal tract. *Am. J. Physiol.* 273 (*Gastrointest. Liver Physiol.* 36): G296–G302, 1997.— Little is known about the biosynthesis of mucin molecules in humans. Our aim was to examine the mucin biosynthesis (MUC2–6) along the longitudinal axis of the healthy human gastrointestinal tract. Biopsies of human stomach and small and large intestine were metabolically labeled with ³⁵S-labeled amino acids, [³⁵S]sulfate, or [³H]galactose, immunoprecipitated with antibodies against MUC2–6, and analyzed by reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). MUC5AC [apparent molecular weight (*M_r*) 500,000] and MUC6 (apparent *M_r* 400,000) were detected in the stomach but not in the small or large intestine, MUC3 (apparent *M_r* 550,000) was detected in duodenum and jejunum, MUC2 (apparent *M_r* 600,000) was detected throughout the small and large intestine, and MUC4 (apparent *M_r* >900,000) was detected predominantly in the large intestine. Interestingly, some individuals displayed double bands of MUC2 and MUC3 precursors, suggesting allelic variation within the respective genes. Between small and large intestine mature secreted MUC2 showed differences in mobility on SDS-PAGE, suggesting differences in glycosylation. Each of the MUC2, MUC3, MUC4, MUC5AC, and MUC6 precursors could be distinguished electrophoretically, and each showed region-specific expression along the gastrointestinal tract.

biosynthesis; mucins; human; gastrointestinal tract

MUCOSAE OF THE INTESTINE and stomach are covered by a mucus gel layer that protects the epithelium against mechanical damage and chemical irritants (35). Mucus glycoproteins, often referred to as mucins, are synthesized by the mucosal epithelium and form the most important structural component of mucus (12). The class of epithelial mucins can be divided into either secretory or membrane-bound mucins (reviewed in Refs. 29, 40). The central part of the polypeptide backbones of these molecules consists of many tandemly repeated amino acid sequences that are rich in serine and threonine residues; these tandem repeats are unique for each mucin, may vary in number between individuals, and are densely *O*-glycosylated. The NH₂ and COOH terminals of the mucin polypeptides protrude from the heavily *O*-glycosylated central part, do not contain tandem repeats, and, for secretory mucins, contain many cysteine residues forming inter- and intramolecular disulfide bonds. Usually the ter-

minals of the polypeptides have some *N*-glycosylation (29).

Due to diverse *O*-glycosylation, each mucin gene product forms a heterogeneous set of molecules. Several different human epithelial mucin genes have been identified, named *MUC1*–8. *MUC1*, which has been fully sequenced, codes for a membrane-bound mucin (14, 23, 24); *MUC2* and *MUC7* cDNAs have been completely sequenced and code for secretory mucins (2, 15, 17). Only partial cDNA sequences are known for *MUC3*, *MUC4*, *MUC5AC*, *MUC5B*, *MUC6*, and *MUC8* (11, 16, 18, 26–28, 32). Of these, *MUC5AC* codes for a secretory mucin and is expressed at a high level in the human stomach (4, 21, 22). The question of whether the other cDNAs encode secretory mucins has to await their full sequence determination and cell biological assessment of the secretory status of each of these mucins.

Mucin expression is tissue as well as cell type specific. For instance, in the small intestine, *MUC2* and *MUC3* mRNAs are prominently expressed; *MUC2* is confined to goblet cells, whereas *MUC3* is largely confined to enterocytes (6). In the human large intestine *MUC2* is the prominent mucin (34), whereas in the stomach, which also produces a copious mucin layer, *MUC2* is hardly detectable (1, 19). In addition to *MUC2* and *MUC3*, several other mucins, namely *MUC1*, *MUC4*, *MUC5AC*, *MUC5B*, and *MUC6*, are expressed to some extent in the intestine (1, 19, 41; see Refs. 40 and 42 for review). Interestingly, in the glands of the human stomach, *MUC6* protein is also detectable (8), whereas *MUC5AC* protein seems restricted to the superficial epithelium of the stomach (5, 21, 22). Of the other known mucins *MUC8* is not expressed in the small intestine and *MUC7* expression seems restricted to the salivary glands (2, 28).

Alterations in intestinal mucins, especially in glycosylation patterns and expression levels, have been associated with diseases such as inflammatory bowel disease and carcinoma (3, 7, 13, 19, 35, 37, 39). Whether these alterations are the result or the cause of disease is presently unclear.

So far, studies of gastrointestinal mucins have focused on steady-state levels of mRNA and protein by means of *in situ* hybridization, Northern blotting, or immunohistochemistry. Little is known about the biosynthesis of gastrointestinal mucins: only the predominant human colonic and gastric mucins, which were

found to be identical to MUC2 and MUC5AC, respectively, have been studied (21, 22, 34). Our aim was therefore to study mucin biosynthesis along the longitudinal axis of the healthy human gastrointestinal tract by means of metabolic labeling of biopsies, immunoprecipitation, and polyacrylamide gel electrophoresis (PAGE). This information will be valuable in determining which mucin precursors are synthesized in different human gastrointestinal tissues and thus assigning functions to each of the mucins.

MATERIALS AND METHODS

³⁵S-labeled amino acids, [³⁵S]sulfate, [³H]galactose and Amplify were obtained from Amersham International (Buckinghamshire, UK). Eagle's minimum essential medium was from GIBCO-Life Technologies (Breda, The Netherlands); aprotinin, phenylmethylsulfonyl fluoride (PMSF), sodium dodecyl sulfate (SDS), pepstatin A, leupeptin, and iodoacetamide were all obtained from Sigma (St. Louis, MO). High molecular weight markers were obtained from Bio-Rad (Richmond, CA), protein A-Sepharose from Pharmacia (Uppsala, Sweden), Triton X-100 from BDH (Poole, UK), and mouse laminin from Becton Dickinson (Bedford, MA). Films were obtained from Kodak. Chemicals were also obtained from New England Biolabs (Beverly, MA), Merck (Darmstadt, Germany), and Boehringer-Mannheim (Mannheim, Germany).

Antibodies. Rabbit polyclonal antisera were used in all experiments, except for one monoclonal antibody, WE9, which recognizes a peptide epitope in the unique terminals of human MUC2 (36). Anti-human colonic mucin was raised against purified human colonic mucin and recognizes the non-*O*-glycosylated terminals of the polypeptide of human MUC2 (34, 36). Anti-MRP and anti-M3P were raised against synthetic peptides representing the tandemly repeated amino acid sequences of MUC2 and MUC3, respectively (19). Anti-rat gastric mucin was raised against purified rat gastric mucin and recognizes the unique non-*O*-glycosylated terminals of the polypeptides of both rat and human gastric mucin (10, 22). By peptide and cDNA sequencing this human gastric mucin was identified as MUC5AC (21). An independent anti-MUC5AC antibody was raised against a synthetic peptide representing a non-*O*-glycosylated amino acid sequence within the MUC5AC cDNA sequence (4). Anti-MUC6.1 was raised against a synthetic peptide of the tandemly repeated amino acid sequence of MUC6 and purified by peptide affinity chromatography (8); the affinity of anti-MUC6.1 was demonstrated by peptide enzyme-linked immunosorbent assay by de Bolòs et al. (8). Anti-MUC4 was raised against the synthetic peptide with the sequence TSSASTGHATLPVTDTS-SASC, representing the tandemly repeated amino acid sequence of MUC4, and purified with affinity chromatography. This antibody has a high affinity for MUC4 synthetic peptide but not for synthetic peptides representing the tandemly repeated amino acid sequences of MUC2, MUC3, MUC5AC, or MUC6 (not shown). The methods used for MUC4 antibody production, purification, and specificity analysis are similar to those described for the MUC6.1 antibody (C. de Bolòs, A. Lopez-Ferrer, M. Garrido, and F. X. Real, unpublished observations). For a summary of the antibodies see Table 1.

Tissues. All samples were taken from healthy tissues: biopsies from stomach antrum, human duodenum (10–20 cm distal from the pylorus), ascending colon, transverse colon, and sigmoid colon (30 cm proximal to the anus) were obtained by endoscopy. Samples of healthy proximal jejunum (9 individuals) were obtained from patients undergoing a Whipple

Table 1. *Antibodies used to immunoprecipitate gastrointestinal mucins*

Specificity	Name in Study	Original Name	Ref.
Non- <i>O</i> -glycosylated region of MUC2	Anti-MUC2(1)	WE9	36
Non- <i>O</i> -glycosylated region of MUC2	Anti-MUC2(2)	anti-HCM	34, 36
Tandem repeat region of MUC2	Anti-MUC2(3)	anti-MRP	19
Tandem repeat region of MUC3	Anti-MUC3	anti-M3P	19
Tandem repeat region of MUC4	Anti-MUC4		This study
Non- <i>O</i> -glycosylated region of MUC5AC	Anti-MUC5AC(1)	anti-MUC5AC	4
Non- <i>O</i> -glycosylated region of MUC5AC	Anti-MUC5AC(2)	anti-RGM	10, 21, 22
Tandem repeat region of MUC6	Anti-MUC6	anti-MUC6.1	8

HCM, human colonic mucin; RGM, rat gastric mucin.

operation procedure for carcinoma of the pancreas or carcinoma of the papilla Vateri. Biopsies of the ascending colon (4 individuals), colon transversum (1 individual), and sigmoid colon (19 individuals) were taken from healthy tissue after polypectomy for non-polyposis coli, control for colon carcinoma, diverticulosis, irritable bowel syndrome, or constipation. Biopsies of the stomach antrum (3 individuals) and duodenum (17 individuals) were taken from otherwise healthy individuals with unexplained anemia, reflux esophagitis, or Barrett's esophagitis or from individuals after successful eradication of *Helicobacter pylori*. All patients gave informed consent, and use of tissue was approved by the Medical Ethics Committee of the Academic Medical Center.

Metabolic labeling and immunoprecipitation. Mucin biosynthesis was studied by metabolic labeling with radiolabeled amino acids, sulfate, or galactose. Intracellular methionine/cysteine, sulfate, or galactose was depleted by preincubation for 30 min in Eagle's minimum essential medium containing 1 g/l glucose (for methionine/cysteine and sulfate labeling) or 50 mg/l glucose (for galactose labeling), nonessential amino acids, penicillin, and streptomycin, but without methionine/cysteine, sulfate, or galactose, respectively. Biopsies were cultured at 5% CO₂-95% O₂ at 37°C.

Pulse labeling was performed by adding either ³⁵S-labeled amino acids (Cell Labeling Mix; sp act 1,000 Ci/mmol, containing 65% L-[³⁵S]methionine and 25% L-[³⁵S]cysteine) to label the polypeptides or by adding [³⁵S]sulfate (sp act 1,050 Ci/mmol) or [³H]galactose (sp act 1–20 Ci/mmol) to label mature glycoproteins (9, 10). In experiments with intestinal biopsies, pulse labeling was performed for 30 min ([³⁵S]methionine/cysteine) or 60 min ([³⁵S]sulfate or [³H]galactose) in 100 µCi/100 µl medium per biopsy; stomach biopsies were pulse labeled for 15 or 30 min ([³⁵S]methionine/cysteine) in 50 µCi/100 µl medium per biopsy. For intestinal biopsies only, chase incubations were performed for 4 h by washing the biopsies and then adding 100 µl complete medium per biopsy, containing an excess of unlabeled precursor.

After the respective pulse or chase experiments, explants were washed once with phosphate-buffered saline (PBS) and homogenized at 0°C in buffer containing 50 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.5, 5 mM EDTA, 1% (wt/vol) Triton X-100, as well as 1 mM PMSF, 100 µg/ml pepstatin A, 100 µg/ml leupeptin, 10 mM iodoacetamide, and 0.24 U/ml aprotinin. This buffer was supplemented with 1% (wt/vol) SDS for homogenization of gastric biopsies only. Mucins were

immunoprecipitated from the homogenates overnight at 4°C with the various antibodies. Immunocomplexes were precipitated using Sepharose CL-4B-coupled protein A. Immunoprecipitated mucins were washed, reduced, and analyzed by SDS-PAGE using 3% stacking and 4% running gels as previously described (10, 34). For molecular weight markers, unreduced rat gastric mucin precursors labeled with ^{35}S -labeled amino acids were used, with molecular masses of ~300 kDa for the monomer and 600 kDa for the dimer (9). Prestained high molecular weight markers, with molecular masses between 49.5 and 205 kDa, were used. Additionally, mouse laminin, molecular mass 900 kDa, was used as a marker. Gels were fixed in 10% methanol and 10% acetic acid and incubated for 10 min with Amplify. Radiolabeled mucins were analyzed by fluorography; the films (Biomax MR) were exposed for 1–4 wk at -70°C.

RESULTS

Gastrointestinal mucin precursors are synthesized region specifically and can be distinguished by SDS-PAGE. By pulse labeling with [^{35}S]methionine/cysteine of human gastrointestinal biopsies and immunoprecipitations, we studied the biosynthesis of mucin precursors along the human gastrointestinal tract. For the purpose of this study, we define mucin precursors as mucin proteins that are *N*-glycosylated but not yet *O*-glycosylated and that are present in the rough endoplasmic reticulum, whereas mature mucins are the fully *O*-glycosylated end products of biosynthesis (29). Molecular masses of the mucin precursors that contain *N*-glycans presented in this study are represented as apparent molecular masses. Actual molecular masses may differ from apparent molecular masses on SDS-PAGE, since it is difficult to accurately estimate

the molecular masses of these very large proteins on SDS-PAGE. Moreover, in this very high molecular weight range there are very few molecular weight markers.

The antisera directed against a synthetic peptide of MUC5AC [anti-MUC5AC(1)] or against rat gastric mucin [anti-MUC5AC(2)] both detect a mucin precursor of similar apparent molecular mass in the stomach of the same individual, visible as a band of ~500 kDa on SDS-PAGE (Fig. 1A). Moreover, in the gastric homogenate of the same individual this mucin precursor is easily detected, suggesting that it is a predominant mucin biosynthesized in the stomach (Fig. 1A). In 3 of 3 individuals we detected MUC5AC precursors in the stomach (Table 2). MUC6 precursors were detected in the stomach in 3 of 3 individuals, with apparent molecular mass of 400 kDa (Fig. 1A). In the LS 174T cell line, a colon adenocarcinoma cell line, MUC6 precursors have similar apparent molecular mass as determined by SDS-PAGE (41). No MUC5AC or MUC6 precursors could be detected in the small or large intestine of different individuals (Fig. 1B and Table 2).

MUC2 precursors (apparent molecular mass of ~600 kDa) were immunoprecipitated by different anti-MUC2 antibodies from homogenates of duodenum, jejunum, and sigmoid colon (Fig. 1B). Similar results were obtained by the anti-MUC2 monoclonal antibody, anti-MUC2(1) (data not shown). In fact, MUC2 precursors were detected in both the small and large intestine of each of the individuals examined (Table 2). In some cases, MUC2 precursors could be detected as double bands on SDS-PAGE, for instance, in the jejunum of

Fig. 1. Biosynthesis of mucin precursors along the longitudinal axis of the gastrointestinal (GI) tract. Biopsies of stomach antrum (A) and duodenum, jejunum, and sigmoid colon (B) were pulse labeled for 15 (stomach) or 30 min (all other experiments) with [^{35}S]methionine/cysteine and homogenized. For each region of the GI tract, immunoprecipitations were performed on 1 homogenate of 1 individual and analyzed on 1 gel, except for analysis in jejunum (B), in which immunoprecipitations were performed on homogenates from different individuals. Mucin precursors were immunoprecipitated from homogenates, using antibodies directed against MUC2 [anti-MUC2(2) or anti-MUC2(3)], against MUC5AC [anti-MUC5AC(1) or anti-MUC5AC(2)], or against MUC3, MUC4, or MUC6. Samples were reduced, analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and fluorographed. Arrowheads show borders between 3% stacking and 4% running gels. Molecular mass markers are shown on left, i.e., mouse laminin (900 kDa), unreduced rat gastric mucin precursors (300 and 600 kDa), and prestained high molecular mass marker (205 kDa).

Table 2. Biosynthesis of MUC2–6 mucin precursors in the human gastrointestinal tract

	MUC2	MUC3	MUC4	MUC5AC	MUC6
Stomach antrum	ND	0/3	0/1	3/3	3/3
Duodenum	12/12	9/9	0/4	0/6	0/6
	1*	2*			
Jejunum	8/8	8/8	1/1	0/1	0/1
	1*	3*			
Ascending colon	4/4	0/2	1/3	0/3	0/1
Transverse colon	1/1	ND	0/1	0/1	0/1
Sigmoid colon	13/13	0/3	11/12	0/11	0/2
	1*				

Mucin precursors of MUC2, MUC3, MUC4, MUC5AC, or MUC6 detected in biopsies of stomach antrum, duodenum, jejunum, ascending colon, transverse colon, and sigmoid colon. For each mucin studied in each organ or intestinal region, the number of persons expressing the mucin precursor over the number of persons studied is shown. Values with asterisks indicate number of persons showing double bands for a particular mucin precursor. ND, not determined. For MUC4, MUC5AC, and MUC6, no double bands were found. All analyses were performed on healthy human tissues by metabolic labeling with ^{35}S -labeled amino acids, immunoprecipitation, and reducing SDS-polyacrylamide gel electrophoresis.

one individual (Fig. 1B). In 3 of 38 individuals we detected double bands for MUC2 precursors (Table 2).

In the duodenum of one individual and the jejunum of another, MUC3 precursors were detected as a double and a single band, respectively, at ~550 kDa (Fig. 1B). In the Caco-2 cell line, a colon adenocarcinoma cell line, MUC3 precursors displayed similar apparent molecular mass on SDS-PAGE (41). In the stomach and colon no MUC3 precursors were detected (Table 2). Interestingly, in one individual we found double bands of MUC3 precursors in two organs, the jejunum and the gallbladder (not shown). In 5 of 17 individuals we could show double bands of MUC3 precursors (Table 2). One individual displayed double bands for both MUC2 and MUC3 precursors (not shown).

In jejunum, MUC4 precursors were detectable, migrating halfway in the stacking gel on SDS-PAGE (Fig. 1B). MUC4 precursors were also immunoprecipitated from homogenates of the sigmoid colon but not from the duodenum (Fig. 1B). Electrophoresis was performed for twice the period of time for analysis of MUC4 precursors in the sigmoid colon compared with analysis of MUC4 precursors in the jejunum (Fig. 1B), thus enabling MUC4 precursors to enter the running gel. The apparent molecular mass of MUC4 precursors was estimated at >900 kDa, because the M_r appeared higher than that of mouse laminin (M_r 900,000) on SDS-PAGE. We detected MUC4 precursor in the jejunum of one individual and in the sigmoid colon in 11 of 12 individuals, but not in the stomach or duodenum of different individuals (Table 2). In the ascending colon, however, only 1 of 3 individuals expressed MUC4, and in the transverse colon no MUC4 precursors were detected (Table 2). Strikingly, no double bands were detected on SDS-PAGE for MUC4, MUC5AC, and MUC6 (Table 2).

Mobility on SDS-PAGE suggests that MUC2 is glycosylated differently in the small and large intestine. Intestinal biopsies were pulse labeled with radiola-

beled amino acids, sulfate, or galactose and chase incubated. The biosynthesis of mature MUC3, MUC4, and MUC6 could not be studied, because the available antibodies against the respective mucins were directed against the tandemly repeated amino acid sequences, which become masked upon *O*-glycosylation. The biosynthesis of mature MUC5AC in the stomach has been documented previously (22). After pulse labeling with [^{35}S]methionine/cysteine and immunoprecipitation with anti-MUC2 (2), MUC2 precursors were immunoprecipitated from the homogenate of the duodenum and detected at ~600 kDa on SDS-PAGE (Fig. 2A). This band became fainter after a 4-h chase incubation compared with the band after no chase incubation, due to conversion into mature fully *O*-glycosylated mucin. Mature MUC2 was secreted into the medium, visible as a smear just entering the stacking gel (Fig. 2A, lane m4). This indicates that the mature MUC2 synthesized in the duodenum has a very low mobility on SDS-PAGE. Similar results were obtained for the jejunum (not shown). After pulse labeling with [^{35}S]methionine/cysteine, MUC2 precursor was immunoprecipitated from the sigmoid colon homogenate, visible as a band at 600 kDa on SDS-PAGE (Fig. 2B). After a 4-h chase incubation, a short smear was detectable just below the MUC2 precursor with a mobility of 550 kDa, which represents the mature fully glycosylated MUC2 that was immunoprecipitated from the tissue homogenate. This mature MUC2 was also secreted into the medium, whereas the MUC2 precursor was not secreted (Fig. 2B, lane m4).

To further study the biosynthesis of mature MUC2, we performed metabolic labeling experiments with [^{35}S]sulfate or [^3H]galactose, which are incorporated in the last steps of mucin biosynthesis, enabling the detection of mature mucins (9). Mature MUC2 was immunoprecipitated from the tissue homogenates of

Fig. 2. Biosynthesis and secretion of mature MUC2 in duodenum and sigmoid colon. Biopsies of duodenum (A) and sigmoid colon (B) were pulse labeled for 30 min with [^{35}S]methionine/cysteine and chase incubated with complete unlabeled medium for 4 h. Biopsies were homogenized at indicated chase periods, i.e., 0 and 4 h. Medium was collected after 4-h chase incubation, indicated by m4. MUC2 was immunoprecipitated from tissue and media homogenates using anti-MUC2(2) antiserum and analyzed on reducing SDS-PAGE as in Fig. 1. Chase periods (h) are indicated.

the duodenum: the mature MUC2, labeled with either [35 S]sulfate or [3 H]galactose, just entered the stacking gel and was also detected in the medium, displaying a very similar mobility (Fig. 3A). This indicates that mature secreted MUC2 has the same mobility on SDS-PAGE as was shown for the duodenum in Fig. 2, independent of its mode of labeling. In the ascending colon and sigmoid colon, [35 S]sulfate-labeled mature MUC2 is detectable with a mobility of ~ 600 kDa on SDS-PAGE in both the tissue and media homogenates (Fig. 3, B and C), similar to the mobility of the mature MUC2 in the sigmoid colon after labeling with [35 S]methionine/cysteine, as shown in Fig. 2. On SDS-PAGE mobility of mature MUC2 did not vary between ascending colon, transverse colon, and sigmoid colon (not shown). However, between small and large intestine a clear difference in mobility on SDS-PAGE was observed for mature MUC2 (Fig. 3), most likely due to a different glycosylation pattern. In the immunoprecipitated mature MUC2 from duodenum, an additional band can be observed at ~ 600 kDa (double arrows in Fig. 3A). These could be due to heterogeneity in glycosylation of MUC2 in the duodenum, where the 600-kDa band represents a small subpopulation of mature MUC2 that is similarly glycosylated as the mature MUC2 in the large intestine.

DISCUSSION

In this study we have demonstrated the biosynthesis of mucins in the gastrointestinal tract. With the use of metabolic labeling of gastrointestinal tissue, immunoprecipitation using mucin-specific antibodies, and analysis on SDS-PAGE we were able to discriminate between mucin precursors.

In the stomach, the detection of MUC5AC and MUC6 precursors was likely: high *MUC5AC* and *MUC6* mRNA levels are reported for this organ (1, 5, 8, 20, 21, 32, 41). Klomp et al. (22) showed that antibodies directed against purified rat or human gastric mucin immunoprecipitated mucin precursors with apparent M_r 500,000 from human gastric tissue (22), similar to our findings with antibodies directed against a synthetic peptide of MUC5AC. We conclude that these antibodies all recognize the same mucin, namely, MUC5AC. We could not detect MUC6 precursors in the duodenum. Ho et al. (20) showed, by immunohistochemistry, MUC6 protein

in the Brunner's glands, which are exclusively found in the duodenum. However, de Bolòs and co-workers (8) did not detect MUC6 protein in the duodenum (8). In the present study most biopsies do not contain functional Brunner's glands, possibly impeding the detection of MUC6. In the jejunum and colon no MUC6 precursors were detected, in accordance with immunohistochemical studies (20) and mRNA data (41).

We detected MUC3 precursors in small but not large intestine and MUC2 precursors in both small and large intestine. This was expected, because the *MUC3* mRNA level is high in small intestine but low in large intestine, whereas the *MUC2* mRNA level is high throughout the intestinal tract (1, 6, 19, 34, 41). In the stomach, no immunoprecipitations of MUC2 were performed, but *MUC2* mRNA had previously been shown to be virtually absent in this organ (1, 5, 41). MUC4 precursors were detected in the colon, and studies have suggested that MUC4 is also an abundant colonic mucin: *MUC4* mRNA levels are high in the colon, but low or nil in the jejunum and stomach (1, 37, 41). The mucin precursor MUC4, with apparent molecular mass >900 kDa, has the highest apparent molecular mass thus far identified. The biosynthesis of MUC1 was not studied; MUC1 has been shown to be expressed at low levels throughout the gastrointestinal tract and does not exhibit region-specific expression (5, 41).

Each mucin studied shows a typical pattern of expression in the gastrointestinal tract: MUC2 is expressed in the small and large intestine, whereas MUC3 expression was confined to the small intestine; MUC4 is biosynthesized typically in the distal colon, whereas MUC5AC and MUC6 proteins are only expressed in the stomach. Moreover, these results were consistent in almost all individuals (Table 2). In some individuals a mucin precursor may be deficient or synthesized at a very low level and may therefore escape detection. For instance, MUC4 precursor was not detected in the sigmoid colon of one individual.

Not much is known about the specific functions of each of the epithelial mucins. Mucins are generally thought to play a role in protecting the underlying epithelium from luminal noxae and mechanical stress (12, 29). However, the regional distribution of MUC2–6 in the gastrointestinal tract indicates specific functions for these mucins. For instance, the gastric mucins

Fig. 3. Biosynthesis of MUC2 in duodenum (A), ascending colon (B), and sigmoid colon (C). Biopsies were pulse labeled for 1 h with [35 S]sulfate ([35 S] SO_4) or [3 H]galactose ([3 H]Gal) and chase incubated with complete medium for 4 h and homogenized after 0- or 4-h chase incubation. In all experiments media were collected and homogenized after 4-h chase incubation, indicated by m4. Mucin was immunoprecipitated using anti-MUC2(2) antiserum. Analysis was on reducing SDS-PAGE, as in Fig. 1. Chase periods (h) are indicated. Double arrows are explained in text.

MUC5AC and MUC6 could be particularly beneficial in protecting against the low pH in the stomach. Also, the mucins with the largest polypeptides, MUC4 and MUC2, are typically highly expressed in the distal colon and may form very large hydrated polymers, functioning as a lubricant to enable the passage of stool and/or as a protective layer against pathogenic organisms.

We have shown that at least two intestinal mucins, MUC2 and MUC3, displayed double precursor bands in some individuals, whereas no double bands for MUC4, MUC5AC, and MUC6 precursors were detected. A possible explanation could be that these double bands result from allelic variation of the respective genes. Due to the variable number of repeated amino acid sequences of mucins, allelic variation among mucins is common and was demonstrated at the genomic level for the *MUC1* and *MUC2* genes (30, 31) and at the protein level for rat gastric mucin and rat and human MUC2 (10, 33, 39). The frequencies of these double bands may be underestimated, because small variations in the sizes of the proteins encoded by the different alleles may not be distinguished by SDS-PAGE. Side-by-side analysis of mucin precursors of different individuals is another experimental alternative. This side-by-side analysis was previously performed for MUC2, showing MUC2 protein products with different apparent molecular mass and occasionally double bands on SDS-PAGE (39).

Using antibodies that are directed against the non-*O*-glycosylated epitopes of MUC2, we were able to detect precursor and mature MUC2, and we were able to show intracellular processing of this mucin. This will also be possible for other mucins, such as MUC3, MUC4, and MUC6, when antibodies against the non-*O*-glycosylated regions become available. Previous studies have shown that mature MUC2 is synthesized and migrates at a position around 550–600 kDa on SDS-PAGE in both the sigmoid colon (34) and in LS 174T and LS 180 cells, two colon adenocarcinoma cell lines (25, 41). We showed that in small intestine the mobility on SDS-PAGE of mature secreted MUC2 is markedly different from that found in large intestine. In fact, the mobility of mature mucins is known to be aberrant on SDS-PAGE and depends largely on intrinsic negative charge, which is determined by terminally attached sialic acid and sulfate residues (38). Therefore, MUC2 may be glycosylated differently in small compared with large intestine. However, more evidence of differences in *O*-glycosylation could be obtained by structural analysis of MUC2 oligosaccharides after isolation of MUC2 from small and large intestine. Variation in glycosylation between different regions of the intestine is likely, because the glycosyltransferases responsible for *O*-glycosylation show tissue and cell-type-specific distributions (12, 29). Glycosylation is important for the function of mucins, for instance, in bacterial interaction with mucus, because some bacteria can bind to mucin oligosaccharides, facilitating colonization by commensals or removal of pathogenic organisms (12, 35). Therefore, differences in the *O*-glycan structure of mucins between small and large intestine may par-

tially account for differences in bacterial flora and bacterial pathogenicity. In addition, *O*-glycans are important for gel formation (29).

In conclusion, we have shown that mucin precursors of MUC2, MUC3, MUC4, MUC5AC, and MUC6 can be distinguished electrophoretically and exhibit region-specific expression in the gastrointestinal tract. Moreover, it seems likely that some forms of allelic variation of MUC2 and MUC3 can be detected through expression of double bands of precursors in metabolic labeling experiments. Differences in mobility of mature secreted MUC2 on SDS-PAGE between small and large intestine suggest differences in glycosylation of this mucin between these regions of the intestine.

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REFERENCES

1. Audie, J.-P., A. Janin, N. Porchet, M. C. Copin, B. Gosselin, and J.-P. Aubert. Expression of human mucin genes in respiratory, digestive and reproductive tracts ascertained by *in situ* hybridization. *J. Histochem. Cytochem.* 41: 1479–1485, 1993.
2. Bobek, L. A., H. Tsai, R. A. Biesbrock, and M. J. Levine. Molecular cloning, sequence and specificity of expression of the gene encoding the low molecular weight human salivary mucin (MUC7). *J. Biol. Chem.* 268: 20563–20569, 1993.
3. Buisine, M.-P., A. Janin, V. Maunoury, J.-P. Audie, M.-P. Delescaut, M.-C. Copin, J.-F. Colombel, P. Degand, J.-P. Aubert, and N. Porchet. Aberrant expression of a human mucin gene (MUC5AC) in rectosigmoid villous adenoma. *Gastroenterology* 110: 84–91, 1996.
4. Carlstedt, I., A. Herrmann, H. Hovenberg, G. Lindell, H. Nordman, C. Wickström, and J. R. Davies. "Soluble" and "insoluble" mucins—identification of distinct populations. *Biochem. Soc. Trans.* 23: 845–851, 1995.
5. Carrato, C., C. Balague, C. de Bolòs, E. Gonzalez, G. Gambus, J. Planas, J. M. Perini, D. Andreu, and F. X. Real. Differential apomucin expression in normal and neoplastic human gastrointestinal tissues. *Gastroenterology* 107: 160–172, 1994.
6. Chang, S. K., A. F. Dohrman, C. B. Basbaum, S. B. Ho, T. Tsuda, N. W. Toribara, J. R. Gum, and Y. S. Kim. Localization of mucin (MUC2 and MUC3) messenger RNA and peptide expression in human normal intestine and colon cancer. *Gastroenterology* 107: 28–36, 1994.
7. Corfield, A., N. Myerscough, M. Gough, I. Brockhausen, R. Schauer, and C. Paraskeva. Glycosylation patterns of mucins in colonic disease. *Biochem. Soc. Trans.* 23: 840–845, 1995.
8. De Bolòs, C., M. Garrido, and F. X. Real. MUC6 apomucin shows a distinct normal tissue distribution which correlates with Lewis antigen expression in the stomach. *Gastroenterology* 109: 723–734, 1995.

9. Dekker, J., and G. J. Strous. Covalent oligomerization of rat gastric mucins occurs in the rough endoplasmic reticulum, is *N*-glycosylation dependent, and precedes *O*-glycosylation. *J. Biol. Chem.* 265: 18116–18122, 1990.
10. Dekker, J., W. M. O. Van Beurden-Lamers, and G. J. Strous. Biosynthesis of gastric mucus glycoprotein of the rat. *J. Biol. Chem.* 264: 10431–10437, 1989.
11. Dufosse, J., N. Porchet, J.-P. Audie, V. Guyonnet-Duperat, A. Laine, I. Van Seuning, S. Marrakchi, and J. P. Aubert. Degenerate 87-base-pair tandem repeats create hydrophilic/hydrophobic alternating domains in human mucin peptides mapped to 11p15. *Biochem. J.* 293: 329–337, 1993.
12. Forstner, J. F., and G. G. Forstner. Gastrointestinal mucus. In: *Physiology of the Gastrointestinal Tract* (3rd ed.), edited by L. R. Johnson. New York: Raven, 1994, p. 1255–1283.
13. Gambús, G., C. de Bolòs, D. Andreu, C. Francí, G. Egea, and F. X. Real. Detection of the MUC2 apomucin tandem repeat with a mouse monoclonal antibody. *Gastroenterology* 104: 93–102, 1993.
14. Gendler, S. J., C. Lancaster, J. Taylor-Papadimitriou, T. Duhig, N. Peat, J. Burchell, L. Pemberton, E. N. Lalani, and D. Wilson. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J. Biol. Chem.* 265: 15285–15293, 1990.
15. Gum, J. R., J. C. Byrd, J. W. Hicks, N. W. Toribara, D. T. A. Lampion, and Y. S. Kim. Molecular cloning of human intestinal mucin cDNAs. *J. Biol. Chem.* 264: 6480–6487, 1989.
16. Gum, J. R., J. W. Hicks, D. M. Swallow, R. L. Laglace, J. C. Byrd, D. T. A. Lampion, B. Siddiki, and Y. S. Kim. Molecular cloning of cDNAs derived from a novel human intestinal mucin gene. *Biochem. Biophys. Res. Commun.* 171: 407–415, 1990.
17. Gum, J. R., Jr., J. W. Hicks, N. W. Toribara, B. Siddiki, and Y. S. Kim. Molecular cloning of human intestinal mucin (MUC2) cDNA. *J. Biol. Chem.* 269: 2440–2446, 1994.
18. Guyonnet-Duperat, V., J.-P. Audie, V. Debailleul, A. Laine, M.-P. Buisine, S. Galiegue-Zouitina, P. Pigny, P. Degand, J.-P. Aubert, and N. Porchet. Characterization of the human mucin gene MUC5AC: a consensus cysteine-rich domain for 11p15 mucin genes? *Biochem. J.* 305: 211–219, 1995.
19. Ho, S. B., G. A. Niehans, C. Lyftogt, P. S. Yan, D. L. Cherwitz, E. T. Gum, R. Dahiya, and Y. S. Kim. Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res.* 53: 641–651, 1993.
20. Ho, S. B., A. M. Robertson, L. L. Shekels, C. L. Lyftogt, G. A. Niehans, and N. W. Toribara. Expression cloning of gastric mucin complementary DNA and localization of mucin gene expression. *Gastroenterology* 109: 735–747, 1995.
21. Klomp, L. W. J., L. Van Rens, and G. J. Strous. Cloning and analysis of human gastric cDNA reveals two types of conserved cysteine-rich domains. *Biochem. J.* 308: 831–838, 1995.
22. Klomp, L. W. J., L. Van Rens, and G. J. Strous. Identification of a human gastric mucin precursor; *N*-linked glycosylation and oligomerization. *Biochem. J.* 304: 693–698, 1994.
23. Lan, M. S., S. K. Batra, W.-N. Qi, R. S. Metzgar, and M. A. Hollingsworth. Cloning and sequencing of a human pancreatic tumor mucin cDNA. *J. Biol. Chem.* 265: 15294–15299, 1990.
24. Ligtenberg, M. J. L., H. L. Vos, A. M. C. Gennissen, and J. Hilken. Episialin, a carcinoma-associated mucin, is generated by a polymorphic gene encoding splice variants with alternative amino-termini. *J. Biol. Chem.* 265: 5573–5578, 1990.
25. McCool, D. J., J. F. Forstner, and G. G. Forstner. Synthesis and secretion of mucin by the human colonic tumor cell line LS 180. *Biochem. J.* 302: 111–118, 1994.
26. Meerzaman, D. P., P. Charles, E. Daskal, M. H. Polymeropoulos, B. M. Martin, and M. C. Rose. Cloning and analysis of cDNA encoding a major airway glycoprotein, human tracheobronchial mucin (MUC5). *J. Biol. Chem.* 269: 12932–12939, 1994.
27. Porchet, N., J. Ngyen Van Cong, J. Dufosse, J.-P. Audie, V. Guyonnet-Duperat, M. S. Gross, C. Denis, P. Degand, A. Bernheim, and J.-P. Aubert. Molecular cloning and chromosomal localization of a novel human tracheo-bronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. *Biochem. Biophys. Res. Commun.* 175: 414–422, 1991.
28. Shankar, V., M. S. Gilmore, R. C. Elkins, and G. P. Sachdev. A novel human airway mucin cDNA encodes a protein with unique tandem-repeat organization. *Biochem. J.* 300: 295–298, 1994.
29. Strous, G. J., and J. Dekker. Mucin-type glycoproteins. *Crit. Rev. Biochem. Mol. Biol.* 27: 57–92, 1992.
30. Swallow, D. M., S. Gendler, B. Griffiths, G. Corney, J. Taylor-Papadimitriou, and M. E. Bramwell. The human tumour-associated epithelial mucins are coded by an expressed hypervariable gene locus PUM. *Nature* 328: 82–84, 1987.
31. Toribara, N. W., J. Gum, P. J. Culhane, R. E. Lagace, J. W. Hicks, G. M. Petersen, and Y. S. Kim. MUC2 human small intestinal gene structure. Repeated arrays and polymorphism. *J. Clin. Invest.* 88: 1005–1013, 1991.
32. Toribara, N. W., A. M. Robertson, S. B. Ho, W.-L. Kuo, E. Gum, J. W. Hicks, J. R. Gum, J. C. Byrd, B. Siddiki, and Y. S. Kim. Human gastric mucin. Identification of a unique species by expression cloning. *J. Biol. Chem.* 268: 5879–5885, 1993.
33. Tytgat, K. M. A. J., F. J. Boveland, H. A. Büller, and J. Dekker. Biosynthesis of rat colonic mucin: the major mucin is analogous to human MUC2. *Biochem. J.* 309: 221–229, 1995.
34. Tytgat, K. M. A. J., H. A. Büller, F. J. M. Opdam, Y. S. Kim, A. W. C. Einerhand, and J. Dekker. Biosynthesis of human colonic mucin: MUC2 is the prominent secretory mucin. *Gastroenterology* 107: 1352–1363, 1994.
35. Tytgat, K. M. A. J., J. Dekker, and H. A. Büller. Mucins in inflammatory bowel disease. *Eur. J. Gastroenterol. Hepatol.* 5: 119–127, 1993.
36. Tytgat, K. M. A. J., L. W. J. Klomp, F. J. Boveland, F. J. M. Opdam, A. Van der Wurff, A. W. C. Einerhand, H. A. Büller, G. J. Strous, and J. Dekker. Preparation of anti-mucin polypeptide antisera to study mucin-biosynthesis. *Anal. Biochem.* 226: 331–341, 1995.
37. Tytgat, K. M. A. J., F. J. M. Opdam, A. W. C. Einerhand, H. A. Büller, and J. Dekker. MUC2 is the prominent colonic mucin expressed in ulcerative colitis. *Gut* 38: 554–563, 1996.
38. Tytgat, K. M. A. J., D. M. Swallow, B. J. W. Van Klinken, H. A. Büller, A. W. C. Einerhand, and J. Dekker. Unpredictable behaviour of mucins in SDS/polyacrylamide-gel electrophoresis. *Biochem. J.* 103: 1053–1054, 1995.
39. Tytgat, K. M. A. J., J. W. G. Van der Wal, A. W. C. Einerhand, H. A. Büller, and J. Dekker. Quantitative analysis of MUC2 synthesis in ulcerative colitis. *Biochem. Biophys. Res. Commun.* 224: 397–405, 1996.
40. Van Klinken, B. J.-W., J. Dekker, H. A. Büller, and A. W. C. Einerhand. Mucin gene structure and expression: protection vs. adhesion. *Am. J. Physiol.* 269 (Gastrointest. Liver Physiol. 32): G613–G627, 1995.
41. Van Klinken, B. J.-W., E. Oussoren, J.-J. Weenink, G. J. Strous, H. A. Büller, J. Dekker, and A. W. C. Einerhand. The human intestinal cell lines Caco-2 and LS 174T as models to study cell-type specific mucin expression. *Glycoconj. J.* 13: 757–768, 1996.
42. Van Klinken, B. J.-W., K. M. A. J. Tytgat, H. A. Büller, A. W. C. Einerhand, and J. Dekker. Biosynthesis of intestinal mucins: MUC1, MUC2, MUC3 and more. *Biochem. Soc. Trans.* 23: 814–818, 1995.