Biosynthesis and Secretion of Human Colonic Mucin Glycoproteins

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Abstract

Synthesis and secretion of colonic mucin glycoprotein species were assessed during in vitro culture of colonic mucosal explants. DEAE-cellulose chromatography of endogenously labeled mucin glycoproteins from explant tissue demonstrated the presence of six mucin species (I–VI) similar to those identified earlier in surgical specimens of human colonic tissue. The relative proportions of mucin species I–VI in tissue explants remained constant throughout a 30-h culture period. However, the proportional representation of the various mucin species in media was significantly different from that found in tissue, which suggests that some mucin species (I, II, and III) are differentially secreted, whereas others (IV and V) are retained within intracellular pools. Radiolabeled precursors were incorporated into mucin species I, II, and III at a 2.0–2.6-fold greater rate than their concentration in tissue, supporting the concept that these glycoproteins were both synthesized and secreted at a greater rate than species IV and V.

Colonic mucosal explants from patients with ulcerative colitis showed > 90% reduction of species IV. However, the amount of species IV recovered from culture media of ulcerative colitis explants was comparable to normal controls. It appears that mucin species IV is differentially secreted rather than retained within intracellular pools in mucosa of patients with ulcerative colitis.

Introduction

The mucosal surface of the large intestine is comprised of many constituents including a complex array of high molecular weight glycoproteins (1–3). Studies using lectin and histochemical probes have suggested that alterations of non–reducing termini of mucin glycoprotein oligosaccharide side chains may be found in association with a number of pathologic processes, including colonic cancer and ulcerative colitis (UC)[1] (4–13). Studies in this laboratory have demonstrated the presence of substantial heterogeneity in colonic mucosal mucin glycoproteins (14–15). At least six glycoprotein fractions initially separated by DEAE-cellulose chromatography were shown to represent structurally distinct glycoprotein species (I–VI) (16–19). Whereas the functional significance of this heterogeneity remains unclear, the bi-

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1. *Abbreviations used in this paper:* CD, Crohn's disease; UC, ulcerative colitis.

ologic relevance of these chromatographically distinguished species is underscored by the observation that the colonic mucosal content of one species (IV) is selectively diminished in specific association with UC.

More recent studies utilizing a library of monoclonal antibodies directed against the species I–VI have suggested that there may be a cellular basis for mucin glycoprotein heterogeneity (20). Indirect immunofluorescent staining of colonic mucosa using these reagents indicates that various glycoprotein species are found within different subpopulations of goblet cells that are otherwise morphologically indistinguishable.

A number of investigators have demonstrated the usefulness of mucosal organ-culture techniques for studying metabolic and other processes in colonic epithelium in vitro (21–23). MacDermott et al. (24) utilized these approaches to define the rate of total glycoprotein synthesis and secretion in rabbit and human colonic mucosa through the assessment of the incorporation of a glycoprotein precursor into acid-precipitable material. Interestingly, incorporation and secretion of the radiolabeled precursor, D-glucosamine, was greater in mucosal explants of patients with UC than normal controls. Neutra and co-workers (25) used morphologic techniques in conjunction with in vitro mucosal organ culture to define ultrastructural features of glycoprotein synthesis, transport, and secretion in colonic goblet cells. They demonstrated apparent segregation of newly synthesized glycoprotein within granules of the goblet theca. However, it is unknown whether patterns of glycoprotein packaging and movement within the theca were related to structurally distinguishable populations of mucin glycoproteins.

The manner in which individual colonic mucin glycoprotein species are synthesized and secreted has not been studied previously. It is uncertain whether all glycoprotein species I–VI are synthesized and secreted at equivalent rates, or if individual species, perhaps the products of different goblet cell subpopulations, are produced and secreted in a differential manner. It is also unknown whether the rates of synthesis and mucosal content of the different glycoproteins change in a uniform manner after exposure to secretagogues or other regulatory stimulus, and if ratios of synthesis to secretion of each glycoprotein are constant or vary in response to regulatory factors. In the studies reported here, we have examined the synthesis and secretion of individual colonic mucin glycoprotein species in colonic mucosal explants. We have also examined these processes in mucosal explants from patients with inflammatory bowel disease in an attempt to define the basis for the described alteration in mucosal colonic content of mucin glycoprotein species.

Methods

Patient population and tissue collection. Multiple mucosal pinch biopsies (n = 6–10 patients) from sigmoid colon were obtained for explant culture during routine diagnostic colonoscopy or flexible sigmoidoscopy using standard biopsy forceps. Interbiopsy variation was < 15% by either wet or dry weight. Additional biopsies were obtained for routine histologic

examination. Diagnostic classification was based on the official clinical and endoscopic impression of the staff gastroenterologist as well as the official pathology report of matched specimens taken from the same area as samples used for in vitro studies. Mucosal pinch biopsies were obtained for organ culture from normal controls ($n = 21$), patients with UC ($n = 9$), and patients with Crohn's colitis ($n = 5$). These studies were approved by the Human Studies Committee, Massachusetts General Hospital.

Explant organ culture. Organ culture was performed essentially as described earlier (21–24). Sigmoid colonic mucosal biopsies were immediately placed in culture medium (4°C) and promptly transported to the laboratory. Explants were gently washed three times with additional media (1.0 ml) and then transferred onto sterile 1 × 1-cm, 30-gal stainless steel screen squares (two biopsies per screen; mean weight 10.7±12 mg per two biopsies). Each screen was placed in a 35 × 10-mm tissue culture dish containing 1.5 ml of culture medium, warmed to 37°C, and incubated in a humidified, 37°C, CO_2-containing incubation chamber as described (21, 24).

In initial studies, viability was assessed by determination of thymidine incorporation. [³H]Thymidine (10 μCi/ml medium; 20 Ci/mmol sp act, New England Nuclear, Boston, MA) was added to media at initiation of in vitro incubation. At varying lengths of time, biopsies were sonicated briefly and then precipitated in 10 vol 10% TCA (4°C, 30 min). The precipitate was collected by vacuum filtration, washed three times with cold ethanol, and radioactivity was determined. Duplicate tissue explants were prepared for histologic examination and stained with hematoxylin acid and eosin dye using routine procedures. In some studies, endogenous labeling of mucin glycoprotein was obtained by addition of radiolabeled precursors [³H]-D-glucosamine (50 μCi/ml medium; 32.9 Ci/mmol sp act) and [¹⁴C]-L-serine (5 mCi/ml medium; 169 mCi/ mmol sp act). Protein determinations were performed by the method of Lowry et al. (26).

Isolation and characterization of colonic mucin glycoproteins. Soluble mucin glycoproteins from explant tissue and growth medium were obtained and characterized by using techniques described earlier (15). After varying incubation periods, explants were rinsed with new culture media (4°C) to remove adherent mucin, which was processed with the spent culture media as described below. Tissue was subsequently sonicated as described (14, 15), and the supernatant obtained after centrifugation (105,000 g for 60 min) was dialyzed against distilled water (0°–4°C) before lyophilization. Except in those studies utilizing endogenous glycoprotein labeling (see above), mucin glycoproteins were labeled ("exogenously") as described previously (15). Spent culture media was collected at the time of tissue harvest to assess mucin glycoprotein release during in vitro culture. The culture dish and screen were rinsed with ~ 3 vol additional media to remove adherent material that was then combined with the spent culture media. The pooled material was centrifuged (20,000 g for 10 min) to remove cellular debris, dialyzed against water (0°–4°C), and lyophilized.

Lyophilized-labeled glycoproteins from tissue and media were resuspended in 1.0 ml 0.1 M Tris–HCl, pH 8.0. Mucin glycoproteins were separated from other constituents by Sepharose-4B chromatography as described earlier (15). Mucin glycoproteins were dialyzed, lyophilized, and resuspended as before and subsequently chromatographed on DEAE-cellulose that was equilibrated in 0.1 M Tris–HCl, pH 8.0, using an ascending NaCl gradient elution, again as described earlier (15). Glycoprotein species were both quantitated as absolute amounts derived from determination of specific activity or, alternatively, to assess relative representation, individual mucin species were quantitated as counts per minute/10,000 counts per minute total mucin glycoprotein recovered after DEAE-cellulose.

Results

Colonic mucosa obtained by endoscopic pinch biopsy was maintained in explant culture. As shown in Fig. 1, uptake and incorporation of [³H]thymidine from the media into acid-pre-
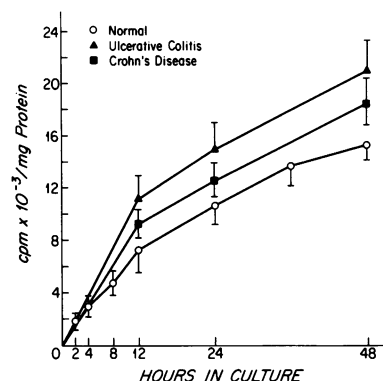

Figure 1. Incorporation of [³H]thymidine by colonic mucosa in vitro. Mucosal explants were rinsed and maintained in culture at 37°C as previously described (21–24). [³H]Thymidine (10 μCi/ml media) was added at the initiation of incubation. Tissue was sonicated, and incorporated thymidine determined in the precipitate that was obtained after addition of 10.0 ml ice-cold 10% TCA.

cipitable material remained linear through 24 h, and active incorporation appeared to continue for at least 48 h. Histological review of explants demonstrated preservation of morphologic features for at least 36 h during routine culture (not shown). These data, which are comparable to the experience of other investigators (21–27), confirm the suitability of mucosal explant techniques for the study of glycoprotein synthesis and turnover.

Total mucin content in explant tissue and media was assessed by quantitation of high molecular weight glycoprotein labeled through exogenous procedures. Total mucin glycoprotein content, measured as exogenously labeled glycoprotein eluting in the void volume after application to Sepharose-4B columns, remained constant throughout a 24-h culture period (Fig. 2 *A*). In contrast, mucin glycoprotein content in the media increased in a linear fashion throughout the incubation period. To determine whether the appearance of glycoprotein in media reflects ongoing production and secretion of mucin rather than release or loss from non–viable cells, incorporation of precursors into high molecular weight glycoprotein and their subsequent appearance in media was examined. As shown in Fig. 2 *B*, incorporation of the amino acid precursor, serine, and carbohydrate precursor, D-glucosamine, into tissue mucin glycoprotein remained linear throughout the 24-h incubation. Endogenously labeled mucin glycoproteins remained low in media for the 1st 12 h of maintenance in vitro, but increased in a linear manner during further incubation. Insofar as the tissue content of mucin glycoprotein remains constant during actual synthesis, these data suggest that the colonic mucosal explant tissue secretes mucin glycoproteins at a rate equivalent to that of synthesis, which results in the maintenance of constant overall mucosal mucin content.

In subsequent experiments, the relative proportions of individual mucin glycoprotein species were determined in tissue and culture media after varying culture intervals by DEAE-cellulose chromatography of exogenously labeled mucin glycoproteins (14, 15). Representative elution profiles of material recovered from explanted normal colonic tissue and culture media after in vitro maintenance for 12 h are depicted in Fig. 3. Mucin species content in the explanted tissue was comparable to that found in mucosal biopsies in earlier studies encompassing six species, I–VI, with a preponderance of mucin species III, IV, and V, lesser amounts of mucin species VI, and small quantities of species I and II. In contrast, mucin glycoproteins found in the media demonstrated a distinctly different profile. As shown in Fig. 3, mucin glycoproteins recovered from the media were
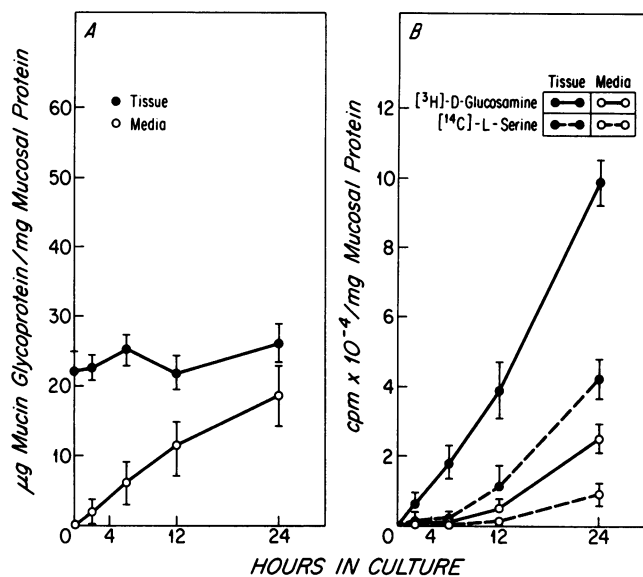
Figure 2. Mucin glycoprotein content in colonic mucosa in vitro. (*A*) Total mucin glycoprotein content. After varying intervals, media and tissue were harvested, and lyophilized soluble glycoproteins were prepared as detailed in text. Glycoproteins were labeled at non-reducing termini by sequential treatment with sodium metaperiodate, galactose oxidase, and $NaB[^3H]_4$, with final specific activity of tissue and media glycoproteins $9.2\pm0.6 \times 10^6$ and $2.1\pm0.3 \times 10^6$ cpm/mg, respectively. Mucin glycoprotein were quantitated as the labeled glycoproteins eluting in void volume after Sepharose-4B chromatography performed as described (15). (*B*) Endogenous incorporation of precursor into total mucin glycoprotein. [^3H]-D-Glucosamine (50 μCi/ml media) and [^{14}C]-L-Serine (5 μCi/ml) were added to culture media at the initiation of incubation. Mucin glycoproteins were isolated and quantitated as described above, except that exogenous-labeling procedures were omitted.

Figure 3. DEAE-cellulose chromatography of mucin glycoprotein of human colon mucosa in vitro. Exogenously labeled mucin glycoprotein were prepared from tissue (*top*) and media (*bottom*) after 12 h in vitro culture as described in legend to Fig. 2 and text. Mucin glycoproteins recovered from Sepharose-4B chromatography were dialyzed, lyophilized, and resuspended in 0.5 ml 0.1 M Tris–HCl, pH 8.0, then applied to a 0.9 × 30-cm column containing DEAE-cellulose equilibrated in the application buffer. Elution was accomplished with an ascending NaCl gradient as previously described (15), and radiolabeled mucin glycoprotein within each fraction was determined. Roman numerals indicate elution position of human colonic mucin glycoproteins I–VI as previously defined (15).

substantially enriched for mucin species I and II relative to the proportional representation of these species in the tissue-derived glycoproteins. Species III was also found in somewhat greater abundance in the media than the tissue. Also, there appears to be a parallel reduction in the proportional representation of mucin species IV in the culture media. The disparities in tissue and media content of individual mucin species were apparent within 6 h after initial incubation as demonstrated in Fig. 4, which details the recovery of individual mucin species in tissue and media at different intervals. The differences in the relative concentration of the various glycoprotein species in media and explant tissue became increasingly marked with continued maintenance in culture.

These data suggest that mucin glycoprotein species appear in the media in a differential fashion. Insofar as the amount of each mucin glycoprotein species in explant tissue remains constant during in vitro maintenance, these data also suggest that various glycoprotein species are selectively and differentially secreted. These impressions are supported by the determination of the ratios of media-to-tissue content of individual mucin species during in vitro culture (Fig. 5). Not surprisingly, the amounts of all mucin glycoprotein species recovered from the media at 4 h are low relative to the amounts of these species in tissue, with ratios < 1.2. However, note that even at this period species I and II appeared to be accumulating in the media in greater relative concentrations than their representation in tissue. During
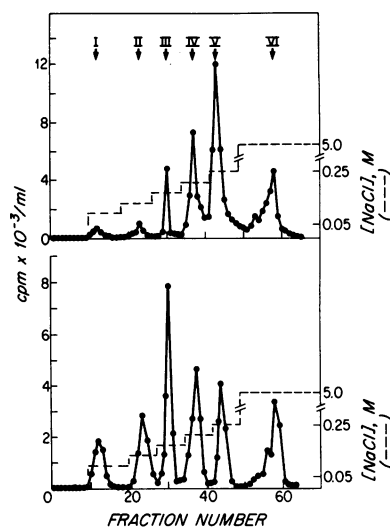
continued culture, the media-to-tissue ratios of species IV and V remained < 1.0, which indicates that these glycoproteins are being released into the media very slowly (medium-to-tissue content; $P < 0.001$ for V, $P < 0.005$ for IV). In contrast, concentrations of other mucin species, most notably I, II, and III, are rapidly increased in the media achieving medium-to-tissue ratios > 3.0 within 24-h incubation period ($P < 0.01$ and $P < 0.005$, respectively).

Because of the constant tissue content of these mucin species, increasing media-to-tissue ratios likely reflect selective secretion of these glycoprotein species. These conclusions are supported by the results of endogenous-labeling experiments in which the incorporation of radiolabeled precursors D-glucosamine and L-serine into individual mucin glycoprotein species in both tissue
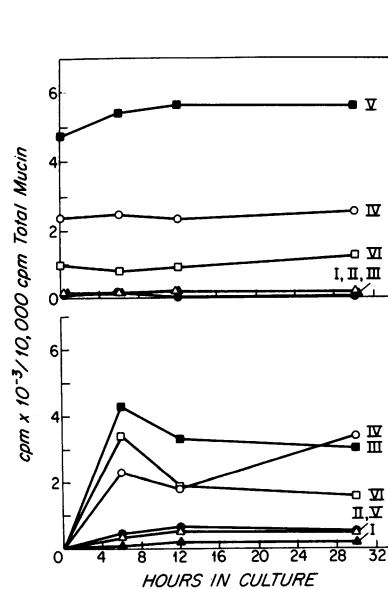

Figure 4. Mucin glycoprotein species content of human colonic mucosa in vitro. Mucin glycoprotein species in explant tissue (*top*) and culture media (*bottom*) were determined after in vitro culture as described in legend to Fig. 3 and text. Recovery of individual species was normalized to total 10^4 cpm to facilitate comparison. Total mucin glycoprotein species were determined from recovery of mucin glycoprotein after DEAE-cellulose chromatography (recovery was 79±6%).
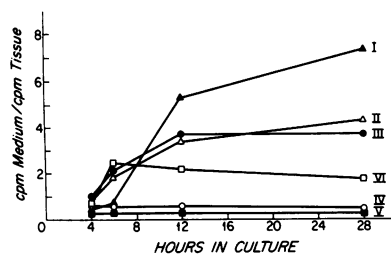
Figure 5. Media-to-Tissue ratio of human colonic mucin glycoprotein species during in vitro culture. Content of individual mucin species in media and tissue were determined as described in text and legend to Fig. 3. Ratios were determined on the basis of actual content rather than normalized proportions depicted in Fig. 4.

and media were examined. As shown in Table I, both precursors were incorporated into all mucin glycoprotein species. However, the proportional incorporation of precursors into species I, II, and III by explant tissue was substantially greater than the absolute content of these species in the tissue. The disproportionate incorporation of labeled precursors into glycoprotein species I, II, and III is demonstrated by the increase in specific activity of labeling of these species (calculated as the amount of label incorporated per total amount of individual mucin species determined by exogenous labeling of duplicate samples). The increasing specific activity of I, II, and III suggests that these components were indeed turning over at a greater rate than other constituents.

Endogenous glycoprotein labeling of the mucin glycoproteins species in tissue was paralleled by the sequence of appearance of endogenously labeled glycoprotein species in the culture medium. Mucin species I, II, and III were the first endogenously labeled species to appear in the medium during in vitro culture and continued to represent a disproportionate fraction of the labeled glycoprotein secreted throughout the culture period as compared with the absolute levels of these mucin species in tissue explants. Not surprisingly, endogenously labeled glycoproteins were found in media only subsequent to their appearance in tissue explants with little discernible secreted labeled glycoproteins earlier than 12 h (Fig. 2, Table I).

It should also be noted that the relative extent of labeling of individual glycoprotein species by the two precursors glucosamine and serine varied with time. During the 1st 6 h of culture, glucosamine was almost the exclusive source of radiolabel found in the complete mucin glycoproteins. Subsequently, increasing amounts of serine were present in the high molecular weight mucin glycoproteins. Early labeling of the large molecular weight mucin glycoproteins by glucosamine presumably reflects completion of glycosylation of mucin glycoproteins that were already partially synthesized within goblet cells at the time of addition of the radiolabeled precursors.

Because of earlier studies suggesting a distinctive alteration in the mixture of mucin glycoprotein species in colonic mucosa from patients with UC, we examined mucin glycoprotein species profiles in explant cultures from this patient group as well as patients with Crohn's colitis. Colonic mucosal explants from UC and Crohn's disease (CD) also remained viable for 24–48 h in vitro as judged by thymidine incorporation and preservation

Table I. Incorporation of Precursors into Mucin Species by Human Colon Mucosa In Vitro

Precursor	Mucin species					
	I	II	III	IV	V	VI
	*cpm × 10⁻⁴/μg mucin species±SD**					
Tissue[‡]						
6 h						
[³H]Gln	1.2±0.3	1.5±0.5	1.8±0.7	0.4±0.1	0.4±0.2	0.6±0.3
[¹⁴C]Ser	0.3±0.1	0.4±0.2	0.6±0.3	0.2±0.1	0.1±0.1	0.4±0.2
12 h						
[³H]Gln	2.4±0.4	3.1±0.6	4.2±0.7	0.6±0.2	0.5±0.3	1.0±0.4
[¹⁴C]Ser	0.8±0.3	1.8±0.5	2.0±0.8	0.4±0.2	0.3±0.2	0.6±0.3
24 h						
[³H]Gln	5.6±0.7	7.2±1.5	7.6±1.4	0.8±0.3	1.7±0.8	1.9±0.9
[¹⁴C]Ser	3.7±0.8	2.9±0.7	3.0±0.9	0.9±0.2	0.8±0.5	1.0±0.5
Medium[‡]						
6 h						
[³H]Gln	0.2±0.1	0.3±0.2	0.7±0.3	<0.1	0.2±0.2	0.7±0.3
[¹⁴C]Ser	0.1±0.1	0.2±0.1	0.2±0.1	<0.1	0.1±0.1	0.2±0.1
12 h						
[³H]Gln	0.4±0.2	2.0±0.6	2.1±0.5	0.7±0.1	0.8±0.3	0.9±0.4
[¹⁴C]Ser	0.2±0.1	0.8±0.3	0.9±0.3	0.1±0.1	0.6±0.2	0.3±1.2
24 h						
[³H]Gln	6.7±1.5	8.2±1.3	5.4±1.1	0.9±0.4	1.5±0.6	1.8±0.7
[¹⁴C]Ser	3.5±0.9	3.3±1.0	2.1±0.9	0.6±0.3	1.0±0.3	1.9±0.4

* Individual mucin species were isolated from total mucin glycoprotein by DEAE-cellulose chromatography and expressed as counts per minute per microgram of that individual species, which was determined as previously described (14, 15). ‡ Mucosal explants were grown in presence of [³H]-D-glucosamine and [¹⁴C]-L-serine as detailed in text. Tissue and medium collected after indicated intervals for isolation of mucin glycoproteins. Two biopsies per individual per time point (n = 7).

of histologic structural features (Fig. 1). Mean incorporation of thymidine in UC was higher than that found in normal tissue, although statistically significant only at the level of $P = 0.05$ (Fig. 1). Whereas CD tissue also appeared to incorporate thymidine at an increased rate, this rate was not significantly different from control (Fig. 1).

Overall, incorporation of radiolabeled precursors into mucin glycoproteins was somewhat increased in both UC explant tissue and to a lesser extent, tissue from CD (Table II). However, these overall differences in tissue content were not significant. The apparent increase in labeled mucin glycoprotein in media from UC cultures achieved marginal statistical significance after 24 h ($P = 0.05$), whereas the amount of total mucin glycoproteins in media of CD remained statistically indistinguishable from normal controls.

Evaluation of mucin glycoprotein species heterogeneity in the UC tissue explants during in vitro culture revealed elution profiles comparable to those found in colonic biopsy specimens in earlier studies (14, 15). As shown in the representative profile of material isolated after 12 h culture, UC tissue was selectively deficient in mucin IV, whereas content of other species was preserved (Fig. 6). However, the pattern of mucin species recovered from media of UC explants after 12 h contrasted markedly with that found in the tissue. Levels of mucin species IV in culture

Figure 6. DEAE-cellulose chromatography of colonic mucin glycoproteins from UC in vitro. Exogenously labeled mucin glycoprotein were prepared from tissue (top) and media (bottom) of UC explant culture after 12 h in vitro, and mucin glycoprotein species were evaluated on DEAE-cellulose as described in legend to Fig. 3 and text.

media were equivalent to that found in normal controls (Fig. 6). Other mucin species in media of UC explants were comparable to normal controls. In contrast, the chromatographic profiles of both tissue- and media-derived, exogenously labeled mucin glycoprotein from explants of patients with CD were comparable to controls, with proportional representation of all mucin glycoprotein species (data not shown). Mucin species IV assessed by exogenous-labeling methods was initially detectable in media of UC explant cultures within 6 h and increased steadily throughout more extended culture periods (Fig. 7).

Despite the accumulation of species IV in media during the culture intervals, the total tissue content of this glycoprotein remained consistently low, which suggests that the glycoprotein found in the media represented an apparent failure to retain mucin species IV within intracellular pools despite ongoing biosynthesis. These conclusions are reinforced by assessment of the endogenous incorporation of labeled precursor into media and tissue mucin glycoprotein species by UC explants (Fig. 8). When UC explants were grown in the presence of [³H]-D-glucosamine and [¹⁴C]-L-serine, the radiolabeled precursors were incorporated into newly synthesized mucin species as indicated by the appearance of labeled, species IV–like material in the media of UC cultures. However, there was no proportional increase in species IV concentration of the tissue itself. The total amount of apparent mucin species IV in UC media appears comparable

Table II. Incorporation of Precursors into Total Colonic Mucin Glycoprotein In Vitro

Sample	Time	Precursor	Precursor incorporation into mucin glycoprotein	
			Tissue	Media
	h		*cpm × 10⁻⁴/µg mucosa protein±SD**	
Normal[‡]	6	[³H]Gln	1.6±0.8	<0.2
		[¹⁴C]Ser	0.2±0.2	<0.2
	12	[³H]Gln	3.8±1.6	0.5±0.2
		[¹⁴C]Ser	1.2±0.8	0.2±0.1
	24	[³H]Gln	9.8±1.8	2.5±0.5
		[¹⁴C]Ser	4.4±1.2	0.9±0.3
UC[‡]	6	[³H]Gln	2.0±0.6	0.2±0.1
		[¹⁴C]Ser	0.4±0.2	<0.2
	12	[³H]Gln	4.5±1.4	1.4±0.6
		[¹⁴C]Ser	2.2±0.9	0.5±0.2
	24	[³H]Gln	12.8±3.4	3.8±1.3
		[¹⁴C]Ser	5.7±1.1	1.6±0.7
CD[‡]	6	[³H]Gln	1.8±0.6	<0.2
		[¹⁴C]Ser	0.4±0.2	<0.2
	12	[³H]Gln	3.5±0.9	0.4±0.2
		[¹⁴C]Ser	1.0±0.4	0.3±0.2
	24	[³H]Gln	8.8±1.7	2.4±0.8
		[¹⁴C]Ser	4.6±0.9	1.1±0.5

* Mucin glycoproteins were isolated by Sepharose-4B chromatography, as described in text, and expressed as mean counts per minute per microgram protein in initial mucosal biopsy, which was determined by method of Lowry et al. (26).

[‡] Mucosal explants from normal controls and patients with UC and CD were grown in presence of [³H]-D-glucosamine and [¹⁴C]-L-serine; media and tissue were harvested after varying culture intervals.
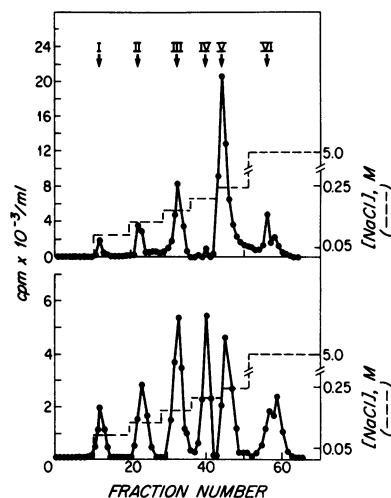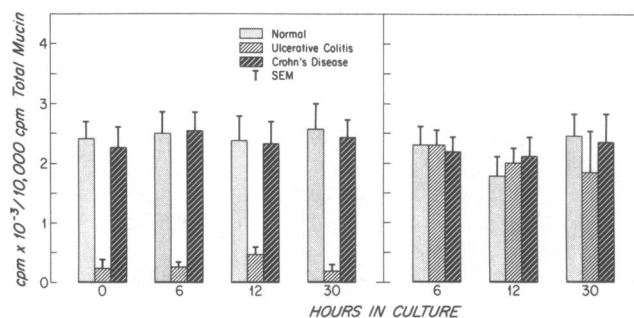

Figure 7. Content of human colonic mucin glycoprotein species IV in vitro. Mucin species IV content in media (right) and tissue (left) of normal, UC, and CD explants were determined after DEAE-cellulose chromatography of exogenously labeled glycoprotein prepared and isolated as described in legends to Figs. 2 and 3 and text.
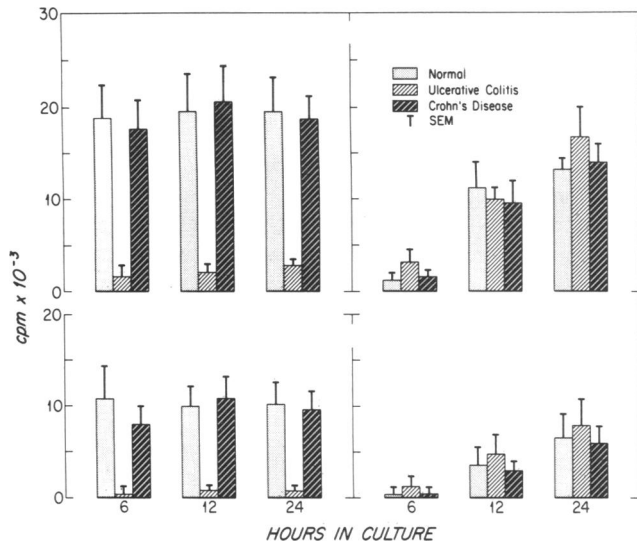
Figure 8. Incorporation of precursors into mucin species IV by colonic mucosa in vitro. Mucin species IV content was assessed by DEAE-cellulose chromatography of the mucin glycoprotein isolated from media (*right*) and tissue (*left*) of normal, UC, and CD explants maintained in media that was supplemented with [^{3}H]-D-glucosamine (*top*) (50 μCi/ml media) and [^{14}C]-L-serine (*bottom*) (5 μCi/ml media).

to that found in normal tissues and media. It is interesting to note that endogenously labeled, species IV–like material appears in the media of UC cultures somewhat earlier than that observed in normal explant cultures (6 h vs. 12 h). In contrast, the extent and pattern of endogenous labeling of tissue and media species IV in patients with CD was indistinguishable from that found in normal controls (Figs. 7 and 8). Indeed, the tissue and media content of all mucin species in CD explant cultures were comparable to normal controls throughout in vitro culture maintenance (Fig. 9).

Discussion

The colonic mucosa contains a great abundance of high molecular weight mucin glycoproteins (1–3). These constituents are both highly complex and heterogenous structures. Studies using
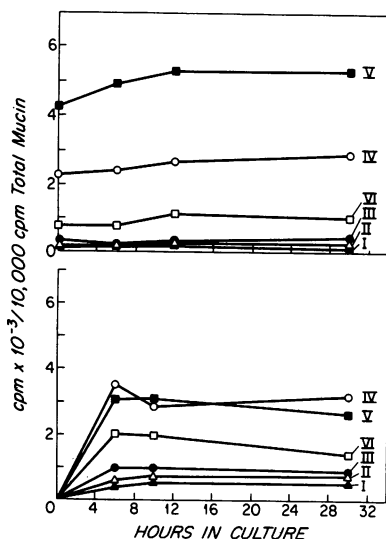

Figure 9. Mucin glycoprotein species content of CD colonic mucosa in vitro. Mucin glycoprotein species content of explant tissue (*top*) and culture media (*bottom*) of CD explant culture was determined as described in legend to Figs. 3 and 4 and text.

histochemical- and lectin-staining techniques support the presence of significant variations in the nature of non–reducing termini of the oligosaccharide side chains of glycoconjugates within colonic mucosa (4–13). The extent of human colonic mucin glycoprotein heterogeneity has been partially clarified by structural analysis of purified mucin glycoproteins (14, 16, 17). These studies demonstrated the presence of at least six distinct colonic mucin glycoproteins. Although the nature of the protein backbones of the different species and their relatedness remains unknown, these species contain distinctive mixtures of a large number of varied oligosaccharides.

The development of a library of monoclonal antibodies with defined, anti–mucin species specificity has provided some insight into the cellular basis of colonic mucin glycoprotein heterogeneity. Studies using indirect immunofluorescent-staining techniques suggest that there may be a previously unappreciated diversity within the large population of morphologically indistinguishable goblet cells in colonic mucosa (18, 20). Staining of colonic goblet cells using monoclonal antibodies that recognize different mucin glycoprotein species indicate that particular combinations of the glycoprotein species are found in distinct subpopulations of goblet cells. The distribution of some of the different mucin species appears to be limited to mutually exclusive goblet-cell subsets.

The studies presented in this paper demonstrate that various colonic mucin glycoprotein species are synthesized at different rates. This conclusion is supported by the results of endogenous-labeling studies in which the rate of incorporation of precursors into individual mucin glycoproteins cannot be correlated with the relative steady state abundance of each species in the colonic mucosa. Thus, the appearance and subsequent increase in specific activity of labeled amino acid and monosaccharide precursors were most rapid in species I, II, and III despite their wide variation in proportional tissue concentration. Furthermore, secretory rates (i.e., appearance of mucin glycoprotein in media) appeared to vary widely. These studies suggest that some mucin glycoprotein species may contribute to baseline mucin secretion, whereas others (notably, species IV and V) are retained within intracellular pools. It is possible that the latter constituents may require specific stimuli to prompt secretion and/or altered rates of synthesis.

The extent to which in vitro organ culture inherently perturbs normal secretory patterns is unknown. Whereas no known secretagogues were added to the culture media, it is possible that organ culture conditions or the absence of an inhibitory influence present in vivo may lead to distortion of baseline secretory patterns. The maintenance of steady state proportions of individual mucin glycoprotein species as well as overall mucin glycoprotein content throughout the incubation does suggest the persistance of feedback or other regulatory mechanisms that maintain constant levels of these substances in the mucosal tissue in vitro.

Regardless of whether in vitro culture alters inherent secretory patterns, the present studies demonstrate that some mucin species may be synthesized and secreted at rates independent of other mucin glycoprotein species. However, more detailed information will be needed to understand the biosynthetic basis of these disparities. It is unclear whether the production of each mucin glycoprotein is controlled as an integrated whole, or if specific and selective steps in the biosynthetic pathways are regulated, such as the initiation of translation, of glycosylation and/or oligosaccharide–side chain elongation, and completion.

Whereas the metabolic dynamics of mucin glycoprotein

synthesis and secretion will require further clarification, insight into these processes may provide a basis for defining relevant secretory stimuli and their functional significance. LaMont and Ventola (27) as well as Neutra and colleagues (25) have suggested that secretagogues can effect non-uniform release of mucin glycoprotein from colonic mucosa. Both Neutra and colleagues (28–31) as well as Brady and co-workers (32) have identified at least some of the factors that may stimulate colonic goblet-cell secretion. Phillips et al. (30) suggest that crypt cells may differentially respond to acetylcholine and perhaps other secretagogues when compared with crypt and surface goblet cells. The present studies suggest that individual glycoprotein species are produced and secreted in a varied manner and therefore could be responsive to different secretory stimuli or, alternatively, respond differentially to the same secretagogue. It will be important to define the effects of potential secretagogues on the synthesis and secretion of the different glycoprotein species. The demonstration of subpopulations of colonic goblet cells containing distinctive combinations of glycoprotein species raises the possibility that a cellular basis for differential secretory regulation could exist. It is unclear whether the rates of synthesis and/or secretion of different glycoproteins within a single cell may be dissociated.

The present studies using UC explants are consistent with the earlier findings of MacDermott et al. (24) and suggest that there may be an overall increase in colonic glycoprotein turnover in mucosa of patients with UC. Whereas total tissue-mucin content differed only modestly from controls, incorporation of radiolabeled mucin glycoprotein precursors was somewhat greater in UC than control explants. However, CD explants also exhibited signs of accelerated mucin turnover, although to a lesser extent than that found in UC. It is possible that increased levels of overall mucin glycoprotein turnover may be related nonspecifically to inflammation. It is possible inflammatory mediators may be present within explant samples tissue at the time of biopsy that are released or activated in the process of harvesting the tissue and initiating in vitro cultures. Conceivably the presence of such substance could contribute to an "artifactual" increase in mucin glycoprotein turnover. Modest increases in thymidine incorporation were also present, a finding similar to that noted by other workers (33–36). It is therefore possible that any general alteration in total glycoprotein production and secretion may be related in part to overall changes in the rate of cellular turnover.

Although overall mucin glycoprotein turnover is preserved or increased in UC explants, the present studies reaffirm the presence of a selective deficiency of mucin species IV in mucosa of patients with UC. The markedly low levels of species IV in UC tissue were present throughout the culture period. However, mucin glycoprotein, chromatographically indistinguishable from mucin species IV, was found in great abundance in the media. The levels of the presumed species IV in the media of UC explants equals or exceeds that found in normal and CD controls. Further work will be needed to determine whether the mucin species secreted by UC explant is structurally the same as species IV found in normal explants. These findings suggest that the basis of the observed decrease in species IV in mucin of UC patients may be related to the abnormal secretion of a constituent that is usually preferentially retained within intracellular pools in colonic mucosa. If this is indeed the case, the inappropriate secretion could reflect alteration in the mucin glycoprotein itself or, alternatively, an abnormality in the goblet cells from which it derives. Careful structural analysis of the species IV secreted

by UC explants in conjunction with efforts to define the structure of the protein backbone of the normal species IV may reveal the basis of the seemingly inappropriate secretion of this substance in UC.

The functional consequences of an alteration in the structure of species IV or its metabolism by the colonic mucosa remain unknown. Further clarification of the basis of this finding may provide insight into the normal functional role of species IV as well as the other mucin glycoproteins in maintaining the integrity of the colonic mucosa. Insight into these processes may in turn clarify the manner in which an alteration in species IV can predispose to at least one form of colonic mucosal injury.

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