Histochemistry of the colonic epithelial mucins in normal subjects and in patients with ulcerative colitis

A qualitative and histophotometric investigation

V. GRECO, G. LAURO, A. FABBRINI, AND A. TORSOLI¹

From the Institute of Medical Pathology, University of Rome

EDITORIAL COMMENT Distinctive differences are repeated in goblet cells in the sigmoid colon in normals. The histochemical changes associated with mucin production in ulcerative colitis are described.

Histochemical investigations have been carried out on epithelial mucins in the digestive tract of experimental animals (Martin, 1961; Gerard, 1964) and human subjects (Hoskins and Zamcheck, 1963; Schrager, 1964; Lev and Spicer, 1965; Lev, 1965), and different histochemical properties of these mucins have been observed in different parts of the gastrointestinal tract. Preliminary observations in the large bowel have shown the presence of two types of epithelial acid mucins: sialomucins and sulphated mucopolysaccharides (Lev and Spicer, 1965). The other investigations were mostly confined to the gastric mucosa. It was therefore decided to study the goblet cells of the large bowel both in normal subjects and in patients with ulcerative colitis to see if any histochemical differences could be found.

MATERIALS AND METHODS

The mucosa of the large bowel of 13 normal subjects and of 10 patients with ulcerative colitis was studied. The specimens were collected either from surgical material, from rectal biopsies, or by aspiration after end-to-end intubation (Colagrande, Arullani, and Casale, 1966; Torsoli, Arullani, and Casale, 1967).

All specimens were fixed in 10% buffered formaldehyde at pH 7.4 (Millonig, 1964), embedded in paraffin, sectioned at 5 μ , and stained with haematoxylin and eosin.

QUALITATIVE RESULTS Fifteen biopsies from normal subjects and 19 from patients with ulcerative colitis were taken from various regions of the large bowel, as shown in the following table:

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	Caecum	Colon			Sigmoid
		Ascending	Transverse	Descending	
Normal subjects	1	3	1	2	8
Ulcerative colitis	1	4	4	2	8

To visualize acid mucins, sections were generally stained with Alcian blue (Pearse, 1960). Strongly and weakly acid mucins were differentiated with an Alcian blue-Alcian yellow procedure (Ravetto, 1964); neutral and acid mucins with an Alcian blue-P.A.S. sequence (Vialli, 1955).

HISTOPHOTOMETRIC OBSERVATIONS These were carried out on 13 biopsy specimens from normal subjects and on 10 from patients with ulcerative colitis. The tissues were taken from the following regions of the large bowel:

	Caecum	Colon			Sigmoid
		Ascending	Transverse	Descending	
Normal subjects	1	1	1	1	9
Ulcerative colitis	—	2	1	2	5

A Lison histophotometer equipped with a Balzer interference filter (1,671 Å) was used on Alcian-blue-stained sections (Vialli, Zanotti, and Bolognani-Fantini, 1962). A comparison was made between controls and specimens in which the basophilia due to sulphate groups was blocked by methylation (methylation for three hours and saponification for 30 minutes by the method of Fisher and Lillie, 1954). In each section three goblet cells of the neck, the fundus, and the intermediate level of two Lieberkühn crypts were examined. In each cell, 10 central and 10 peripheral cytoplasmic measurements were made. The results were evaluated statistically, when $P = 0.05$.

QUALITATIVE RESULTS

NORMAL SUBJECTS Acid epithelial mucins were revealed in all specimens by Alcian blue. Goblet cells with neutral mucins were demonstrated, except in the sigmoid, in all regions of the large intestine at the bottom and the intermediate level of the crypts of Lieberkühn (Figs. 1 and 2). Sometimes a few goblet cells in the neck of the crypt developed a violet colour with the Alcian blue-P.A.S. procedure. This probably indicated the presence of a mixture of acid and neutral mucins. In the sigmoid occasional goblet cells only showed this histochemical pattern. The Alcian blue-Alcian yellow procedure showed the prevalence of strongly acid mucins (stained blue) at the fundus and the intermediate level of the crypts and the presence of the weakly-acid mucins (stained green-yellow) at the neck and at the surface epithelium (Fig. 3). The mucus was always basophilic, Alcian blue positive.

FIG. 2. Normal sigmoid. All goblets cells are stained with the Alcian blue. (Alcian blue-P.A.S. $\times 56$.)

FIG. 1. Normal caecum, showing the differences in the staining of the goblet cells in the lower two-thirds of the crypts compared with the upper thirds. (Alcian blue-P.A.S. $\times 145$.)

FIG. 3. Normal sigmoid. Progressive fall of affinity for Alcian blue and parallel increase in affinity for Alcian yellow from the bottom to the top of Lieberkühn's crypts. (Alcian blue-Alcian yellow $\times 145$.)

ULCERATIVE COLITIS In these patients we found it useful to divide the results into two groups because of differing histochemical behaviour.

Group I: slight histological changes Lymphocytic and plasmacellular infiltration of the lamina propria with the glandular parenchyma preserved.

In the sigmoid, goblet cells with neutral mucins, usually absent in normal subjects, were found in large quantities (Fig. 4). In the other regions of the large bowel, the neutral goblet cells were not confined only to the lower two-thirds of the glands but appeared to be distributed along the whole length of the gland (Fig. 5). The Alcian blue-Alcian yellow procedure showed an increase of the superficial weakly acid goblet cells. Moreover, with this method, we observed non-uniform staining among a few crypts from the same specimen: some glands, in fact, showed the typical pattern of colour distribution at different levels, common to normal subjects,

FIG. 4. Sigmoid in ulcerative colitis: slight histological changes. Several goblet cells with neutral mucins are seen in the crypts, also basophilic staining of the mucus. (Alcian blue-P.A.S. $\times 145$.)

FIG. 5. Caecum in ulcerative colitis: slight histological changes. The goblet cells with neutral mucins are distributed in the whole length of the crypts. (Alcian blue-P.A.S. $\times 56$.)

FIG. 6. Sigmoid in ulcerative colitis: severe histological changes. In this crypt are present only a few weakly acid goblet cells. (Alcian blue-Alcian yellow $\times 145$.)

while others showed only positive-Alcian blue staining. However, it should be pointed out that this behaviour was also revealed in two specimens with severe histological changes.

Group II: severe histological changes These were abscesses and destruction of the crypts and ulceration of the mucosa. In specimens from all regions of the large intestine a significant decrease and even total disappearance of the neutral goblet cells was observed. The persistence of only goblet cells with a greenish-yellow appearance was evident (Fig. 6).

The mucus was found to be basophilic, was Alcian blue-positive in all specimens, regardless of other histological changes observed.

HISTOPHOTOMETRIC RESULTS

NORMAL SUBJECTS A larger intracellular content of acid mucins was revealed in the goblet cells of the sigmoid colon than in those of the caecum and the other segments of the large bowel. This difference was observed in all levels of the crypts (Fig. 7). No

FIG. 7. Histophotometric results of acid mucins on control specimens and after methylation and saponification.

differences were found in the mucin contents of the central and peripheral cytoplasmic zones of the goblet cells.

Sulphomucins were confirmed in crypts of Lieberkühn by Fisher and Lillie's method. In the goblet cells of the sigmoid a larger amount of sulphomucins was seen in the central cytoplasmic part than at the periphery (Fig. 8).

ULCERATIVE COLITIS The investigations were carried out only on specimens which showed slight histological changes. An increase of the normal content of intracellular acid mucins was observed in the sigmoid and especially in the other parts of the large bowel (Fig. 7). In the sigmoid, both a percentage and an absolute increase of sulphomucins were observed and the distribution of these mucopolysaccharides between the central and peripheral zones of the cells was found to be the same as in normal subjects (Fig. 8).

DISCUSSION

The presence of goblet cells with neutral mucins in the lower two-thirds of the crypts in the caecum and the colon other than the sigmoid probably corresponds to a phase of precocious maturing of these cells. However, they were only occasionally found in the sigmoid. This seems to indicate that, in comparison with the other parts of the large intestine, the goblet cells of the sigmoid have a different biological evolution. These results confirm the different segmental histochemical patterns of the goblet cells of the large bowel seen in animals (Martin, 1961).

Staining with Alcian blue-Alcian yellow has confirmed that the strongly acid mucins are present mainly in the basal and intermediate parts of the crypts, while the weakly acid mucins are in the neck of the glands and in goblet cells of the luminal surface (Lev and Spicer, 1965). Therefore, it should be possible to reconstruct the life cycle of the goblet cells of the colon in three stages. The cells containing neutral mucin first become strongly and then weakly acid. The content of acid mucins appeared to be more raised in the sigmoid of normal subjects than in the other parts of the large bowel, and this was found to be true for all levels of the crypts. Finally in the sigmoid sulphomucins were found in greater amounts in the central part of the cells than in the peripheral part. This finding seems to be in agreement with the present knowledge of the sulphation of mucoprotein in the region of the Golgi's apparatus (Florey, 1962).

In ulcerative colitis a clear-cut alteration of the normal distribution pattern of the goblet cells in the crypts was always observed. The increase of super-

FIG. 8. *Histophotometric determinations of the sulphomucins. The values show the percentage increase in optical transmission in comparison with the control specimens after methylation (three hours) and saponification (30 minutes).*

ficial goblet cells, the appearance of numerous goblet cells with neutral mucin content in the sigmoid, and the altered position of these cells in the crypts of the other regions of large bowel suggest a more rapid turnover of these cells.

The varied staining pattern, showed by the Alcian blue-Alcian yellow procedure, in which the presence in some Lieberkühn glands of only young cells with acid, Alcian blue-positive mucins, seemed to provide further evidence of this alteration of the biological cycle of the goblet cells.

In the advanced stages of ulcerative colitis, the marked reduction, with eventual disappearance of the goblet cells with neutral mucins, and the presence of only the weakly acid goblet cells, possibly in the final stages of the restricted biological cycle, seemed to show a slowing down of cellular turnover, probably due to destruction of the fundus of the crypts (Lumb and Protheroe, 1957). Moreover, this slowing down of cellular multiplication is probably similar to

that observed in carcinoma of the colon (Lipkin, 1965) and may also be related to the high risk of malignant changes in ulcerative colitis (Hinton, 1966).

Finally, in ulcerative colitis, the increase of the intracellular acid mucin content in the caecum and proximal colon probably indicates that these parts of the large bowel have a greater functional capacity and response to stimuli than the sigmoid, due, perhaps, to a lesser production of mucin under basal conditions.

SUMMARY

Histochemical and histophotometric investigations were carried out on the goblet cells of the human large intestine from 13 normal subjects and from 10 patients with ulcerative colitis.

In normal subjects the sigmoid has a different histochemical behaviour from the other parts of the large bowel.

In ulcerative colitis histochemical changes have been observed which differ in slight and severe histological lesions, suggesting that these findings could be the result of a distinctive turnover rate of goblet cells in ulcerative colitis and in the different regions of the large bowel of normal subjects.

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