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Mucin immunohistochemistry of the columnar epithelium of the oesophagus (Barrett's oesophagus)

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Summary. Columnar cell lined lower oesophagus (CELLO), often considered to be a precancerous lesion, is characterized by a glandular mucosa with a predominance of sulphomucins in the specialized epithelium. This histochemical abnormality can be correlated with abnormal differentiation which may also be studied by anti-mucus antibodies (anti-M_1, anti-M_3, anti-SIMA, anti-LIMA). The purpose of this prospective study is to define the mucin profile in a large population of CELLO by immunohistochemistry and to compare it with the results of histochemistry. Biopsies of 79 patients with reflux oesophagitis were included. Thirty-eight had CELLO and 41 had a histologically normal cardia. Six surgical specimens of oesophageal adenocarcinomas were also included. The histochemical methods confirmed the preponderance (57.9%) of type III intestinal metaplasia (IM) found in 57.9% of cases. The immunohistochemical methods showed a similar antigenic profile of type II and III IM with positivity of anti-SIMA and anti-M_3 antibodies in the goblet cells, and positivity of anti-LIMA antibodies in both the goblet and intermediate cells of the specialized epithelium. The mucus secreting cells of the oesophageal adenocarcinomas had the same immunohistochemical profile. These results are similar to those of Filipe et al. in type II and III IM surrounding gastric adenocarcinomas. Immunohistochemical methods allow us to subdivide type II and type III IM into 2 subgroups according to the positivity or negativity of the anti-LIMA antibodies in the intermediate cells. Among the 41 normal cardias in patients with reflux oesophagitis, 10 contain sulphomucin secreting cells positive with anti-LIMA antibodies. We suggest that this anti-LIMA positivity may be a step preceeding type III IM in specialized epithelium.

Key words: Barrett's oesophagus – Mucus – Histochemistry – Immunohistochemistry – Precancerous lesion

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Introduction

Columnar epithelium lined lower oesophagus (CELLO) or Barrett's oesophagus is an acquired condition usually complicating reflux oesophagitis (Spechler and Goyal 1986). CELLO has been classified in two main types: gastric (cardiac or fundic) and specialized type, the latter is the most frequent. This specialized type is singular because of the high frequency of incomplete type of intestinal metaplasia (IM), characterized by intermingled goblet cells and intermediate mucus secreting cells. The unique structure of the specialized type is confirmed by electron microscopic studies (Berenson et al. 1974) which show that columnar intermediate cells have short microvilli and contain a mucin droplet at the top. Histochemical methods show that CELLO often contains sulphomucins, a modification also found in type III IM in the stomach, usually associated with gastric carcinoma. For this reason, it is considered to be a precancerous lesion (Jass and Filipe 1981).

In our previous study (Peuchmaur et al. 1984), we used histochemical methods to demonstrate the frequency of type III IM in CELLO. Our aim in the present study is to use immunohistochemical methods to characterize the mucus secretion in CELLO and to compare the results of the two methods.

Material and methods

The material consisted of biopsy and surgical samples. From biopsies, 79 patients (53 men and 26 women) with symptoms of reflux oesophagitis were included in this prospective study. The endoscopic examination and the biopsies were performed from January 1981 to January 1986 in the same endoscopic center. The patients identified as having CELLO for the study had the diagnosis of Barrett's oesophagus established by identifying columnar epithelium both on endoscopic examination and/or the presence of specialized epithelium in biopsy specimen (Herlihy et al. 1984). The number of biopsy samples taken from each patient ranged from 2 to 12 (mean 3.8). CELLO was present in 38 patients (27 men and 11 women), 35 of specialized type one of cardiac and two of fundic type. In the last three cases, the diagnosis of CELLO was based on the site of biopsies (up to 3 cm above the lower oesophageal sphincter). The mean age was 64.3 years for men (range 42 to 87) and 58.7 for women (range 32 to 82). In the 41 remaining patients (26 men and 15 women), the cardia was histologically normal. In this group, the mean age was 48.7 for men (range 28 to 78) and 56.3 for women (range 18 to 78).

There were 6 resections of the oesophagus for adenocarcinoma: 3 were cardiac adenocarcinomas (2 men and 1 woman), 3 were adenocarcinomas arising in CELLO (3 men). The difference between cardiac and CELLO adenocarcinoma was determined by the presence of columnar epithelium between the carcinoma and either the squamous epithelium or the cardiac line. In addition the adenocarcinoma in CELLO is located at least 75% in the oesophagus. The mean age was 53 years (range from 26 to 80).

All the biopsy samples and surgical specimens were fixed in 3.6% neutral buffered formalin (modified Millonig's phosphate buffer) for 48 h and paraffin embedded. Serial sections, 4 mmu thick, were cut from each sample, and stained by the following: haematoxylin and eosin, AB pH 2.5-PAS to differentiate acid from neutral mucins, HID-AB pH 2.5 to differentiate sulphomucins (Filipe 1983) from sialomucins. The specialized type was classified in type I, II or III IM as previously described (Bogomoletz et al. 1984; Filipe and Fenger 1979).

The antibodies against mucins were anti-M_1 and anti-M_3 from Dr Bara (Bara et al. 1983a; Bara et al. 1983b; Bara et al. 1984a; Bara et al. 1984b), anti-LIMA (large intestin mucus antigen) and anti-SIMA (small intestin mucus antigen) from Dr Mâ.

- Anti-M_1 (Decaens et al. 1983) labels normal gastric mucus cells and fetal large bowel mucus cells; it does not label duodenum, adult small bowel and large bowel mucus cells.
- Anti-M_3 (Decaens et al. 1984) labels all the goblet cells of the small and large intestine, but not the gastric mucus cells.
- Anti-LIMA (Mâ et al. 1982) labels only adult large bowel mucus cells.
- Anti-SIMA (Mâ et al. 1982) labels the normal small bowel mucus cells but not the normal large bowel mucus cells or the gastric mucus cells.

The indirect immunoperoxidase 3 step method (Stein et al. 1982) or PAP method (Sternberger et al. 1970) were used. DAB (Diaminobenzidine Sigma) was used as the chromogen (Graham and Karnovsky 1966). Normal gastric and colonic biopsy samples were used as controls.

Results

Histochemistry

The results of the histochemical study are summarized in Table 1.

Table 1. Histochemical study

1 – Barrett's oesophagus:		
No IM	3 cases (°)	7.9%
Type I–II IM	13 cases	34.2%
Type III IM	22 cases	57.9%
2 – Normal Cardias		
10/41 (24%) with sulfomucins		
3 – Adenocarcinomas		
6/6 (100%) with sulfomucins		

IM: Intestinal Metaplasia
(°) of thoses 3 cases, one cardiac and two fundic type

Table 2. Immunohistochemical study of CELO (38 cases)

	anti-M_1 GC+IC	anti-M_3 GC	anti-LIMA GC+IC	Anti-SIMA GC
Type I (3)	0	1	0	1
Type II (13)	0	13	10	7
Type III (22)	0	22	22	16

GC: Goblet Cells
IC: Intermediate Cells

In 3 cases of CELLO (7.9%), we found no IM (1 cardiac type and 2 fundic type). Type I and type II were present in 34.2%, type III in 57.9%.

Among the 41 normal cases, 31 were similar to the normal cardias of patients without reflux oesophagitis; the mucus cells were Magenta with AB-PAS and always negative with HID-AB. Of remaining 10 cases, some of the mucus cells were stained purple (a mixture of PAS positivity and AB positivity). The same mucus cells in these cases were HID positive, demonstrating the presence of sulphomucins.

In the 6 adenocarcinomas studied, the histochemical results were identical: AB-PAS and HID-AB were positive which indicate the presence of sulphomucins, sialomucins and neutral mucins in all the cases of adenocarcinomas and there was no difference between CELLO's and cardiac adenocarcinomas.

The results of immunohistochemical studies of CELLO are summarized in Table 2 where they are compared with the results of the histochemistry in CELLO. Anti-M_1 was negative in all the specialized mucosas, neither the goblet nor the intermediate cells. Anti-M_3 was positive in most of the goblet cells of the 35 out of 38 cases, negative in all the intermediate cells (Fig. 1). Anti-LIMA was positive in the goblet and the intermediate cells (Fig. 2) in 32 cases associated with Types II and

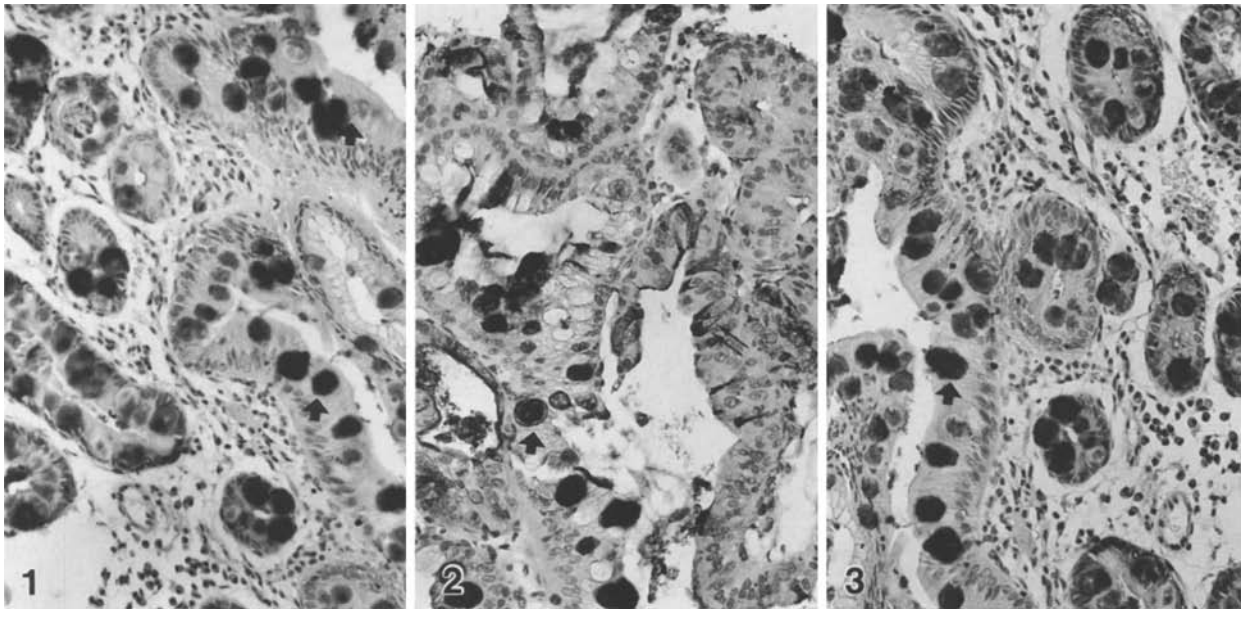

Fig. 1. Labelling with anti-M3. The goblet cells are strongly marked (←), the intermediate cells are negative, in a specialized mucosa. ×400 (reduced to 90%)

Fig. 2. Labelling with anti-LIMA. The goblet cells (→) and the intermediate cells (↑↑) are positive, in a specialized mucosa. ×400 (reduced to 90%)

Fig. 3. Labelling with anti-SIMA. Most of the goblet cells are positive (→), the intermediate cells are negative, in a specialized mucosa. ×400 (reduced to 90%)

Table 3. Immunohistochemical study of normal cardias (41 cases)

	Positive	Negative
Anti-M_1	41	0
Anti-M_3	0	41
Anti-LIMA	10	31
Anti-SIMA	0	41

III IM, but negative in type I IM. Anti-SIMA was positive in the goblet cells in 24 cases but always negative in the intermediate cells (Fig. 3).

The results of immunohistochemical study of 41 cases of normal cardias are summarized in Table 3. The mucus cells were positive in all 41 cases with anti-M_1, negative in 41 cases with anti-M_3 and anti-SIMA, positive in 10 cases out of 41 with anti-LIMA; these 10 cases were the same ones showing HID positivity with HID-AB method.

Cardiac adenocarcinomas showed anti-M_3 positivity in the 3 cases, anti-LIMA and anti-SIMA positivity in 2. CELLO's adenocarcinoma were anti-M_3 and anti-SIMA positive in 2 cases, anti-LIMA positive in the 3 cases.

Discussion

The histochemical study of this biopsy series confirms the high percentage of type III IM in CELLO. We have found 57.9% (22/38) of CELLO producing sulphated mucins (type III IM) versus 53% in our previous study (Peuchmaur et al. 1984). In the literature, many authors (Jass 1981; Zwas et al. 1986; Lee 1984) have found comparable results (43–45% of type III IM). The higher frequency (74%) found by Rothery et al. (1986) is explained by the interpretation of the positivity in both goblet and intermediate cells. One hypothesis is that this mucin modification could be a precancerous marker, because sulphomucins can be detected in both type II IM and oesophageal adenocarcinomas. However, the frequency of type III IM is too high to discriminate a high risk group in the CELLO population.

The immunohistochemical study shows a very close antigenic profile of type II and type III IM in CELLO (Table 2). It is characterized by anti-M_3 and anti-SIMA positivity in the goblet cells, and by anti-LIMA positivity in both the goblet and the intermediate cells. But it is interesting to note that the positivity of the anti-LIMA is slightly dif-

ferent in type II and in type III IM: in type II IM, there is an 83% positivity versus a 100% in type III IM. Our results should be compared with the results of the study of Filipe et al. (1988) in the stomach. In this study, which was performed with the same anti-LIMA and anti-SIMA to label the IM surrounding gastric carcinomas and dysplasia, anti-LIMA is positive in the intermediate cells in 85% of the type II IM, and in 100% of the type III IM. In case of dysplasia, the anti-LIMA is always positive. In contrast, anti-LIMA is negative in type I and type II IM surrounding benign lesions of control stomachs. These results show that in the stomach, the authors find a correlation between LIMA-positivity, dysplastic lesions and gastric carcinomas. Our data does not support the same conclusion in CELLO: LIMA-positivity in type II and III IM is too high to be interpreted as a precancerous marker in CELLO. It would be interesting to do the same study in CELLO on dysplastic lesions, but we have not had the opportunity to explore this aspect. Anti-M_3 labels the goblet cells in all the types of IM; we have never had any positivity in intermediate cells.

We have compared (in a large number of biopsies) the results of histochemical and immunohistochemical methods in CELLO. The latter does not reproduce the results of the histochemical methods exactly. Our immunohistochemical results allow us to divide types II and III IM into two subgroups: the first is characterized by anti-LIMA positivity in the goblet and the intermediate cells. It includes type III IM and a proportion of the type II IM. The second is characterized by the negativity of intermediate cells and the positivity of goblet cells. It includes the remaining part of the type II IM.

For comparison with the 38 CELLO patients, we included in this study 6 adenocarcinomas, of which 3 were cardiac, and 41 biopsy series of the apparently normal cardiac region of patients with gastroesophageal reflux:

– It is interesting to note that CELLO's adenocarcinomas always contain sulfomucins and are positive for anti-LIMA antibody. Even though the number of carcinomas in our study is too small to draw conclusions, identical histochemical and immunohistochemical patterns are observed constantly in adenocarcinomas and in type III IM.

– Finally, in histologically normal cardias of patients with gastroesophageal reflux, histochemical study shows a normal profile, similar to the normal gastric mucosa (Filipe 1979): neutral mucins, anti-M_1 positivity, anti-M_3, anti-SIMA and anti-LIMA negativity. Nevertheless, in 10 normal cardias (24%), though the histological profile appears normal without IM, the histochemical study reveals the presence of sulphomucins and a positivity with anti-LIMA antibodies. Sulphomucins are quite common in the cardiac glands, but are not present in the mucus surface cells (Filipe 1979). However, the distribution of LIMA positivity in the gastro-oesophageal region has not been investigated in large series of patients without reflux oesophagitis. Nevertheless, the presence of sulphomucins and the LIMA-positivity is observed in the same surface mucus cells on serial sections: this suggests a modification of the mucus secretion in the surface mucus cells, possibly a step preceeding IM in patients with gastroesophageal reflux. Careful reevaluation of the HE slides shows that the surface epithelium in these cases contains some cells with a "ball shaped" droplet of mucus, intermediate between normal gastric mucus cells and goblet cells. This particular type is characterized by the shape of the drop of mucus, the purple color with AB-PAS method, the presence of sulphomucins with HID-AB and the anti-LIMA positivity. The term "modified cardiac mucosa" could be proposed for this type of columnar epithelium at the cardia, because the pattern of the epithelium is intermediate between the intestinal metaplasia and the normal cardia. This histochemical alteration is probably the first sign of an alteration of the cellular differentiation and could be considered to be an early stage of the metaplastic process.

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