Distribution and Significance of Epithelial Types in Columnar-Lined Esophagus

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An abnormal columnar-lined esophagus (CLE) is characterized by the presence of cardiac mucosa (CM), oxynto-cardiac mucosa (OCM), and intestinal metaplastic epithelium (IM) between gastric oxyntic mucosa and esophageal squamous epithelium. Thirty-two patients with CLE measuring 2–16 cm long had 5–37 biopsies per patient that showed CM, OCM, or IM for a total of 424 biopsies. Detailed mapping of the distribution of epithelial types within the CLE showed a distinct zonation of epithelial types; CM was present throughout the CLE, whereas OCM and IM tended to occur in the distal and proximal part of the CLE, respectively. All 32 patients (64 of 68 biopsies) showed IM at the most proximal level, compared with 22 of 32 patients (40 of 102 biopsies) in the most distal level biopsies. The density of goblet cells was highest in the most proximal level. The differences in prevalence and density of goblet cells between most proximal and most distal level biopsies were highly significant. These data suggest that for a given number of biopsies within the CLE, the likelihood of finding IM is greatest when the biopsies are concentrated in the most proximal area of the CLE. We suggest that glandular transformation of squamous epithelium results in CM, which evolves into OCM and IM by development of specialized parietal cells and goblet cells, respectively. The severity and nature of reflux cause these epithelial transformations in a constant and predictable manner. Recognition of these changes permits the development of morphologic definitions of reflux disease and the characterization of the sequence of epithelial changes that represent the reflux–adenocarcinoma sequence.

Key Words: Columnar-lined esophagus—Barrett’s esophagus—Cardiac mucosa—Gastroesophageal reflux disease—Intestinal metaplasia.


The traditional view of the normal histology of the gastroesophageal junction (GEJ) is that cardiac mucosa (CM) lines an undefined part of the proximal stomach and extends into the tubal esophagus to a distance of 2–3 cm. This belief was the original basis for defining Barrett’s esophagus (BE) as the presence of a columnar lining in the distal esophagus exceeding 3 cm. Under this definition, BE was classified into fundic, junctional, and specialized types based on the histology of the epithelial lining. When BE was restricted to patients who had intestinal metaplasia (IM), its definition changed to require IM in a biopsy above the 3-cm limit of “normal” columnar-lined esophagus (CLE). More recently, this definition has changed again to accommodate short segment BE, which is defined as the presence of IM in the distal 3 cm of a CLE. The present definition for BE is the presence of IM in a biopsy taken from any endoscopically visualized CLE.

In a series of recent studies we have provided evidence that the traditional belief concerning normal histology of the GEJ is incorrect and that CM is an acquired abnormal mucosa resulting from reflux-induced damage to esophageal squamous epithelium. Chandrasoma et al. reported that CM was absent in 56% of patients at autopsy. In that study oxynto-cardiac mucosa (OCM), although present in all patients, was absent from a part of the GEJ circumference in 50% of patients. The CM + OCM length was <0.5 cm in the majority of patients. Oberg et al. reported that CM was absent in 56% of patients at autopsy. In that study oxynto-cardiac mucosa (OCM), although present in all patients, was absent from a part of the GEJ circumference in 50% of patients. The CM + OCM length was <0.5 cm in the majority of patients. Oberg et al. in a biopsy study of endoscopically normal patients, reported that CM and OCM were absent in 88 (26%) of 334 patients. The 246 (74%) patients in whom CM or OCM was present had a significantly greater level of acid reflux in a 24-hour pH study. Der et al. reported that CM was present in 179 (38%) of 471 consecutive patients who had biopsy of the GEJ region. When present, CM always showed chronic inflammation and the degree of chronic inflammation had a significant correlation with acid reflux assessed by a 24-hour pH study. Chandrasoma et al. reported that the CM + OCM length significantly correlated with the amount of acid reflux; patients with a CM + OCM length >2 cm had higher acid reflux levels than those with a CM + OCM length of <2 cm.
These studies resulted in a new hypothesis\textsuperscript{5}: the entire esophagus is normally lined by squamous epithelium and transitions to gastric oxyntic mucosa at the GEJ. Gastroesophageal reflux damages the squamous epithelium of the esophagus, resulting in its transformation into glandular epithelium (CLE). Three epithelial types are recognized within the CLE: CM, OCM, and IM. The presence of any of these epithelial types interposed between gastric oxyntic mucosa and squamous epithelium is histologic evidence of gastroesophageal reflux. The length of these epithelial types in serial biopsies permits histologic prediction of severity of acid reflux.\textsuperscript{3,5}

This study is aimed at establishing the distribution of different epithelial types within CLE in a selected group of patients with an endoscopically recognized CLE exceeding 2 cm in length in which IM was demonstrated histologically.

**MATERIALS AND METHODS**

Thirty-two patients with symptoms of reflux who had an endoscopically recognized CLE at least 2 cm in length in whom IM was demonstrated histologically were selected. Endoscopy was performed by faculty of the University of Southern California Foregut Surgery Department using Olympus video endoscopes (Olympus America Inc., Melville, NY), and biopsies were obtained with standard biopsy forceps (microvasive radial jaw 31263–20 or 1597–20). These patients had 2–5 biopsies per level at 1–2 cm measured intervals within the CLE. Biopsies from each level were processed separately, permitting mapping of the different types of glandular epithelium within the CLE. Endoscopically defined CLE was confirmed histologically by the presence of CM, OCM, or IM in biopsies. Oxyntic mucosa, which is normal gastric mucosa, was discounted as representing biopsy error in defining the distal limit of the CLE. An additional retrograde specimen was obtained in 25 patients; this is an unmeasured specimen of 2 biopsies that was usually distal to the most distal measured biopsy and attempted to sample the region near the tops of the gastric rugal folds.

The glandular epithelia found in biopsies with CLE were classified into CM, OCM, and IM.\textsuperscript{1} CM was defined by the presence of only mucus cells in the glands underneath the foveolar region. OCM contained a mixture of mucous cells and parietal cells in the glands. IM was defined by the presence of well-defined goblet cells in the glands, foveolar region, or surface epithelium in hematoxylin and eosin-stained sections. CM, OCM, and IM correspond to the Paull et al. junctional, fundic, and specialized types of CLE.\textsuperscript{10} We did not use the results of Alcian blue stain at pH 2.5 because the frequent variably positive staining of columnar mucous cells in CM (“columnar blue cells”) resulted in overdiagnosis of IM if Alcian blue stain positivity was used to define IM. Restriction of the diagnosis to well-defined goblet cells on hematoxylin and eosin stain had the highest specificity of diagnosis of IM. Differentiation of goblet cells from columnar cells with distended mucin vacuoles (“pseudo-goblet cells”) was based on the following criteria: 1) goblet cells had a single round vacuole, usually containing basophilic material compared with pseudo-goblet cells where the vacuoles were often multiple, clear, and not perfectly round; 2) goblet cell vacuoles were more basal with a band of dense eosinophilic cytoplasm separating the vacuole from the cell surface compared with the apical vacuoles of pseudo-goblet cells; and 3) goblet cells were often separated from one another by nonvacuolated cells with dense eosinophilic cytoplasm compared with pseudo-goblet cells, which were often present as groups of adjacent cells. Pure oxyntic mucosa, defined as a mucosa whose glands contained only parietal and chief cells, and stratified squamous epithelium were the only other epithelia seen in these biopsies; these represent the normal lining of the stomach and esophagus, respectively.

The number of biopsies at each level and their histologic composition in terms of whether they contained CM, OCM, or IM were recorded. When IM was absent, it was recorded as grade 0 (Fig. 1). When IM was present, the density of goblet cells was graded in each biopsy in the following manner: grade 1 = less than one third of the glands contain goblet cells (Fig. 2), grade 2 = one to two thirds of the glands contain goblet cells (Fig. 3), and grade 3 = more than two thirds of the glands contain goblet cells (Fig. 4). An overall IM grade for each level was then given based on the mean IM grade for all biopsies at that level. This permitted an accurate mapping as possible of the density of goblet cells within the CLE.

Statistical analysis used the Fisher exact test and the contingency table $\chi^2$ statistical test for sequenced categories.

**RESULTS**

The 32 patients had CLE lengths composed of OCM, CM, and IM ranging from 2 to 16 cm (mean 5.7 cm, median 5 cm). The number of biopsies containing OCM, CM, or IM was 424 for the 32 patients and ranged from 5 to 37 (mean 13.3) per patient. Of these biopsies, 311 (73.3\%) showed IM. In only two patients was IM present in all the biopsies taken; in six patients <50\% of the total biopsies taken showed IM. The density of goblet cells also varied considerably in the individual biopsies showing IM. In 202 of 311 biopsies (65\%) showing IM, the goblet cell density was grade 3, in 55 (17.6\%) it was grade 2, and in 54 (17.4\%) it was grade 1.

The biopsies taken at the most proximal level of the CLE, which was frequently associated with squamous
epithelium, were positive for IM in all 32 patients (Table 1). There were a total of 68 biopsies containing glandular mucosa at the most proximal level in these 32 patients; of these, 64 (94%) were positive for IM. Only four biopsies at the most proximal level were negative for IM. No patient had more than one biopsy at the most proximal level that was negative for IM. This contrasted with a much lower frequency of IM in the biopsies from the most distal level of the CLE that contained CM, OCM, and IM (biopsies that showed pure oxyntic mucosa were excluded because of the probability that they represented gastric mucosa rather than mucosa from the CLE). At the most distal level IM was found in 22 of 32 (69%) patients. Of a total of 102 biopsies at the most distal level, only 40 (39%) were positive for IM. The difference in IM frequency between the most proximal and most distal levels was highly significant.

The goblet cell density, expressed as IM grade, was also greater in the biopsies at the more proximal levels when compared with the more distal levels (Table 2). The IM grade in the most proximal level was greater than that in the most distal level in 26 patients; in 5 patients it was equal, and in 1 patient the grade in the most distal level was greater. At the most proximal level, IM was grade 3 in 21 patients, grade 2 in 6 patients, and grade 1 in 5 patients. At the most distal level IM was grade 3 in 1 patient, grade 2 in 13 patients, grade 1 in 8 patients, and grade 0 in 10 patients. The difference in goblet cell density between the most proximal level and most distal level was highly significant. We observed that there was a progressive decline in goblet cell frequency and density from the proximal to distal end of the CLE that was very consistent in these patients.

IM occurred only in CM. The presence of a single definite goblet cell in CM satisfies diagnostic criteria for IM, although the number of goblet cells seen is usually numerous. IM did not occur in OCM, i.e., parietal cells and goblet cells did not coexist in any gland, although different areas at the same level sometimes showed IM, CM, and OCM.

CM was present at all levels in all patients, commonly coexisting with IM in the more proximal CLE and with
OMC in the more distal CLE. OCM was absent at the most proximal level in all patients; it was present at the most distal level in 16 patients and in the retrograde biopsy in an additional 3 patients. We observed that the distribution of OCM was the exact reverse of IM; the number of parietal cells was maximal at the distal end and progressively decreased more proximally in the CLE.

IM was present in the retrograde biopsy in only 6 of 25 (24%) patients. In all these cases IM was also present in a more proximal level biopsy. The six patients with IM in the retrograde biopsy also showed CM (all six cases) and OCM (two cases). In 16 patients the retrograde biopsies showed oxyntic mucosa, most likely representing biopsies from the proximal stomach rather than CLE; OCM was present in six of these, and CM was present in four. Two patients had only CM and OCM in the retrograde biopsy and one patient showed only CM.

DISCUSSION

Acceptance of the hypothesis that the only normal epithelial types are esophageal squamous epithelium and gastric oxyntic mucosa has a profound impact on the understanding of the pathology of gastroesophageal reflux. There is evidence supporting the concept that CM is an acquired mucosa. CM has been shown to develop above the anatomic line in patients after esophagogastrectomy with gastric pull-up. In a recent study Glickman et al. showed that CM frequently had a staining profile for cytokeratin 7, cytokeratin 20, 45 M1, and Das-1 that was different from gastric oxyntic and antral mucosa and similar to BE.

This selected group of patients with a >2-cm length of CLE represents those at highest risk for BE. The likelihood of finding IM increases progressively with increasing length of the CLE, approaching 100% with a >3-cm length of CLE. Patients with a shorter CLE have a lower frequency of IM; in patients without IM the CLE is composed of CM and OCM. These findings strongly suggest that CM and OCM precede the development of IM in patients with gastroesophageal reflux. This is supported by the finding that the prevalence of IM is low in children with reflux and a long segment of CLE compared with adults.

The present study suggests that there is a constant anatomic zonation of the different epithelial types within the CLE. CM is present throughout the CLE segment, but IM tends to favor the proximal end and OCM the distal end. The amount of IM present varies greatly within these patients. In patients with small amounts of IM, only the most proximal biopsies have IM; with increasing amounts of IM, goblet cells are found in the more distal part of the CLE. IM appears to begin with the development of goblet cells at the most proximal extent of the CLE immediately distal to the squamocolumnar junction. The further development of goblet cells then appears to extend distally in the CLE in a continuous manner, with little tendency to skip areas. In one patient IM was restricted to one gland immediately distal to the squamocolumnar junction (Fig. 2). The reverse is true for OCM, which tends to favor the distal part of the CLE. This constant zonation was previously observed by Paull et al. in their classic article on the histology of CLE.

The observed distribution of CM, OCM, and IM within the CLE provides insight into the pathogenesis of reflux-induced epithelial damage in the esophagus. Acid-induced damage of the squamous epithelium results in an increase in the intercellular space diameter and an increase in paracellular permeability, permitting acid to penetrate the squamous epithelium. When the acid reaches the germinative cells in the basal layer of the squamous epithelium, it may be hypothesized that these cells are stimulated to differentiate into glandular epithelium. The germinative cells in the basal layer of the squamous epithelium very likely retain the full differentiating capability of foregut epithelium. We have postulated that the original differentiation in early life of undifferentiated mucous cells only, which is the definition of CM. We have observed epithelia in patients with reflux that show transitional features between squamous and glandular epithelium, which supports the glandular transformation of squamous epithelium.

### TABLE 1. Distribution of intestinal metaplasia in 424 biopsies within CLE in 32 patients at the most proximal and most distal levels of the columnar lined segment

<table>
<thead>
<tr>
<th>Level</th>
<th>No. of patients with CLE</th>
<th>No. of biopsies with CLE</th>
<th>No. of biopsies with IM</th>
<th>No. of biopsies without IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>32</td>
<td>424</td>
<td>311 (73%)</td>
<td>113 (27%)</td>
</tr>
<tr>
<td>Most proximal level</td>
<td>32 (100%)*</td>
<td>68</td>
<td>64 (94%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Most distal level</td>
<td>22 (69%)*</td>
<td>102</td>
<td>40 (39%)</td>
<td>62 (61%)</td>
</tr>
</tbody>
</table>

* p <0.001 by Fisher’s exact test.
CLE, columnar-lined esophagus; IM, intestinal metaplasia.

### TABLE 2. Mean density of goblet cells in biopsies at the most proximal and most distal level biopsies of the columnar lined segment

<table>
<thead>
<tr>
<th>Level</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most proximal level</td>
<td>0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Most distal level</td>
<td>10</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

p <0.0001 by contingency table chi-square statistical test for sequenced categories.
CM appears to be susceptible to damage by refluxed gastric acid. It invariably shows chronic inflammation and features of reactive epithelial proliferation such as foveolar hyperplasia and lamina propria smooth muscle proliferation.\textsuperscript{1,7,18} Der at al.\textsuperscript{7} reported a significant correlation between the degree of chronic inflammation in CM and the level of acid reflux by a 24-hour pH study, suggesting that the chronic inflammation in CM was caused by acid. If CM is the glandular mucosa that first develops from squamous epithelium, OCM and IM represent an evolution of CM by the development of more specialized cells, parietal cells in the case of OCM and goblet cells in the case of IM. Other specialized cells such as Paneth cells and pancreatic cells have also been shown to develop in CM.

The reason why IM occurs in CM in the most proximal area of the CLE that is at the greatest distance from the source of refluxed gastric contents is uncertain. There is evidence that at least some of the time IM represents a genetic mutational change in CLE. Alternate explanations for the development of IM, such as an injury reaction, exist. Aneuploidy and genetic abnormalities have been shown to be present in nondysplastic BE.\textsuperscript{17} If IM is a mutational abnormality, any factor that increases the cell turnover rate in CLE will increase the probability of development of IM. Recent studies using cell cultures of BE have shown that the cell proliferative rate is much higher with pulse exposure to both bile salts\textsuperscript{15} and acid\textsuperscript{8} than to continuous exposure to bile salts or acid. These experiments may provide an insight to the occurrence of IM at the most proximal part of the CLE. It is likely that the most proximal part of the CLE is exposed to a more pulse exposure to acid and bile salt containing gastric refluxate than the more distal part. The higher proliferative rate that is associated with pulse exposure increases the probability of mutation and IM. This would also explain the increasing risk of developing IM as the total length of CLE increases; the greater the distance from the stomach, the higher the likelihood of pulse exposure to refluxate. In contrast, the distal region of the CLE is more likely to be exposed to a more continuous exposure of gastric juice, resulting in lower proliferative rates within the CLE. This appears to favor the development of parietal cells in the mucous glands in the CLE, essentially converting CM to OCM.

This hypothesis establishes the entire reflux–adenocarcinoma sequence. Reflux damage of the squamous epithelium resulting in transformation to CM is the first step in the sequence. Patients with CM who are subject to mutational changes progress through IM and increasing dysplasia to adenocarcinoma. The presence of CM in a biopsy indicates that the patient has entered the reflux–adenocarcinoma sequence. We have suggested that the presence of CM in a biopsy should be used to define gastroesophageal reflux disease histologically.\textsuperscript{1} This morphologic definition of reflux disease permits the recognition of a subpopulation of the population that is at risk for IM and adenocarcinoma at the time of the study.

Another important finding of the study is that parietal cells never coexist with goblet cells in the same gland. This observation was also made in the Paull et al. study.\textsuperscript{16} We have observed that when CM and OCM coexist, OCM always shows a lesser degree of inflammation and reactive epithelial change than CM. This suggests that OCM is less susceptible to damage by refluxed acid and does not undergo mutational changes leading to IM and adenocarcinoma. The finding of OCM as the only abnormal glandular epithelium without CM and IM was the commonest finding in our autopsy study of the general population.\textsuperscript{2} The complete conversion of CM to OCM removes the patient from the reflux–adenocarcinoma sequence.\textsuperscript{5} Such an occurrence is only seen in patients with very short segments of CLE and was not observed in any patient in this study, where the selection criteria excluded patients with a CLE <2 cm long.

The data from this study have practical value. In a patient who has a visible segment of CLE of any length at endoscopy, the histologic finding of IM establishes the diagnosis of BE. BE is an indication for long-term endoscopic surveillance to prevent esophageal adenocarcinoma. This study indicates that there is a slight risk of a false-negative biopsy because of inadequate sampling, even in this group of patients with the highest prevalence of IM. Only 2 of 32 patients had IM in all their biopsies and 6 of 32 patients had IM in <50% of their biopsies. Of a total of 424 biopsies done in these 32 patients, 311 (73.3%) were positive for IM. Even in the biopsies that had IM, the density of goblet cells varied considerably.

Because of the significant variation of finding goblet cells within the CLE, it is crucial to establish a biopsy protocol that will maximize the likelihood of finding IM during the initial endoscopy. It is generally recommended by academic gastroenterologists that patients with a visible segment of CLE should have four quadrant biopsies taken at 1–2-cm intervals within the CLE. However, many gastroenterologists take a smaller number of random unmeasured biopsies within the CLE. The present study suggests that for a given number of biopsies within the CLE, the likelihood of finding IM is greatest when the biopsies are concentrated in the most proximal area of the CLE immediately distal to the squamocolumnar junction.

\section*{REFERENCES}
\begin{enumerate}
\item Chandrasoma PT, Der R, Ma Y, et al. Histology of the gastro-
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