Changing pattern of cytokeratin 7 and 20 expression from normal epithelium to intestinal metaplasia of the gastric mucosa and gastroesophageal junction

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Summary. It is currently unclear whether intestinal metaplasia at the esophagogastric junction and in the distal esophagus represent a continuum of the same underlying disease process, i.e., gastroesophageal reflux, or constitute different entities with a different pathogenesis. Biopsies below the Z line might show specialized epithelium in some patients and the question is whether this is another form of short segment Barrett's esophagus or whether it is related to a generalized atrophic process of the stomach. Data from recent studies regarding the expression of cytokeratin CK7 and CK20 in intestinal metaplasia (IM) found at the gastroesophageal junction are conflicting.

Prompted by these data we undertook the present study: a) to evaluate the expression of CK7 and CK20 in IM of the gastric cardia and to compare the findings with those in patients with Barrett's esophagus and IM of the gastric corpus and antrum mucosa; and b) to evaluate the immunophenotype of non-intestinalized cardiac mucosa and to compare it with that of normal gastric epithelium.

We studied the expression of CK7 and CK20 on biopsy specimens from patients with long-segment Barrett's esophagus (n=17) and surgical resection and biopsy specimens of gastric cardia (n=15), corpus (n=14) and antrum (n=22) from patients with histological evidence of IM. Eighty-four biopsy specimens from 42 patients (antrum n=15, corpus n=20, cardia n=7) without evidence of IM were studied as a control group.

We observed an immunophenotype characterised by diffuse moderate to strong CK7 staining on the surface and crypt epithelium combined with strong CK20 staining on the surface and superficial part of the crypts in 94.1% (16/17) of the cases with long-segment Barrett's esophagus, but in none of the 36 cases with IM in distal stomach (antrum and corpus). IM in the gastric cardia expressed the immunophenotype seen in IM of the gastric mucosa in 93.3% (14/15) of the cases. On the other hand, normal cardiac epithelium expressed patchy strong CK7 staining on the surface epithelium and on both, superficial and deep parts of the pits combined with patchy strong CK20 staining on the surface epithelium and superficial pits, a feature permitting distinction of the normal cardiac epithelium from those of the normal gastric antrum and corpus epithelium.

We conclude that the expression of cytokeratins 7 and 20 can be used to distinguish the origin of IM of the gastroesophageal junction. The CK7/20 immunophenotype of IM in the gastric cardia closely resembles that of the IM in the gastric antrum and corpus and is different from IM in long-segment Barrett's esophagus. In contrast, the CK7/20 immunophenotype of the cardiac epithelium is different from that of the gastric antrum and corpus mucosa, suggesting that cardiac epithelium might not be a native normal gastric epithelium but one that is acquired as a consequence of longstanding inflammation. Changing pattern of CK7 and CK20 expression from normal to intestinalized epithelium suggests that IM arising from cardiac epithelium might have distinctive features.

Key words: Cytokeratins, Intestinal metaplasia, Barrett's esophagus, Cardia

Introduction

There is an increasing incidence of adenocarcinoma at the gastro-esophageal junction (Blot et al., 1991; Powell and McConkey, 1992; Pera et al., 1993). For the last three decades the death rate for the carcinoma of the gastroesophageal junction has risen from 1.5 to 3/100 000. Time trends of these carcinomas differ from those of the rest of the stomach and resemble ones rising from

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the Barrett's esophagus (Craanen et al., 1992a). Furthermore, these carcinomas share epidemiological characteristics with carcinomas originating from the Barrett's esophagus (Kalish et al., 1984; Clark et al., 1994; Cameron et al., 1995). Most of these carcinomas arise from the foci of intestinal metaplasia (IM) in the most distant part of the esophagus or just below the gastroesophageal junction. In several studies involving the screening of consecutive endoscopy patients for IM at the gastro-esophageal junction, biopsy specimens obtained immediately below the squamocolumnar junction (SCJ) revealed IM between 5% and 30% of patients without endoscopically apparent Barrett's esophagus (Spechler et al., 1994; Nandurkar et al., 1997; Trudgill et al., 1997; Chen et al., 1998; Goldblum et al., 1998; Hacklersberger et al., 1998). However, it is unclear what condition precedes the development of IM in the gastric cardia. Some investigators support the concept that "carditis" and subsequently IM of the cardiac mucosa are the manifestation of reflux disease and repeated injury of the cardiac epithelium by gastric and perhaps duodenal content (Kauer et al., 1995; Vaezi and Richter, 1995; Oberg et al., 1997; Chen et al., 1998; Oberg et al., 1997). The reason why the process is limited to the cardia in some patients, while in others, it involves esophagus, is due to the competence of the lower esophageal sphincter (Oberg et al., 1997). On the other hand, other investigators have found cardia frequently involved in gastritis associated with *Helicobacter pylori* infection (Genta et al., 1994; Goldblum et al., 1998; Hackelsberger et al., 1997; Morales et al., 1997).

Morphologically, IM in Barrett's esophagus cannot be distinguished from that in stomach (Toner and Cameron, 1995; Filipe, 1989). Such a distinction may not be crucial, as IM indicates risk of cancer regardless of its precise localization within the gastroesophageal junction. However, the identification of the origin of the IM at the gastroesophageal junction is needed, since different therapeutic, i.e. antibiotics for *Helicobacter pylori* vs. proton pump inhibitors and prokinetics for gastroesophageal reflux disease-GERD (The European *Helicobacter pylori* study group 1997; Moss et al., 1998), and surveillance protocols, i.e. urea-breath test or serology for *Helicobacter pylori* vs. repeated endoscopy and extensive biopsy sampling for Barrett's esophagus, have been established (The European *Helicobacter pylori* study group 1997; Moss et al., 1998).

Recent studies, based on the expression of cytokeratin (CK) 7 and 20 have shown that there is a distinct Barrett's esophagus immunophenotype, clearly different from that seen in IM of the gastric mucosa (Ormsby et al., 1999; Glickman et al., 2001).

However, regarding the IM of the gastric cardia, the origin and its pathogenesis remain unclear, since the results of the aforementioned two studies are contradictory. One study (Glickman et al., 2001) suggests that IM in the gastric cardia has an immunophenotype similar to that seen in Barrett's

esophagus while the other study (Ormsby et al., 1999) reports that the immunophenotype of the cardiac IM is indistinguishable from the "rest" of gastric mucosa.

Furthermore, there is a recently raised question about the existence of normal cardiac epithelium. Indeed, there are studies suggesting that normal cardiac epithelium represents a form of metaplastic epithelium being an early event in pathogenesis of gastroesophageal reflux disease (Oberg et al., 1997; Chadrasoma et al., 2000).

It is well known that different types of human epithelium have various expressions of cytokeratins that can be used to detect the origin of the epithelial cells (Moll et al., 1982). Cytokeratins are constituents of the intermediate filaments of epithelial cells. Phenotypic expression of keratin polypeptides is known to be dependent on various factors, such as epithelial cell type and the degree of differentiation. At least 20 cytokeratins are known, and these are divided into the neutral or basic type II Cytokeratins (numbered 1-8) and the acidic type I (numbered 9-20) (Moll et al., 1982).

Prompted by the aforementioned conflicting data on immunophenotype of the IM of the cardiac epithelium and by the recently raised question regarding the existence of the cardiac epithelium as normal or metaplastic we undertook the present study in order: a) to evaluate the expression of CK7 and CK20 in IM of the cardia and to compare the findings with those of IM in patients with Barrett's esophagus and IM in the gastric corpus and antrum mucosa taking into consideration the type of IM; and b) to evaluate if the immunophenotype of non-intestinalized cardiac epithelium is different from the normal gastric corpus and antrum mucosa.

Material and methods

Patients and tissue samples

All studied cases (n=110) were collected from the files of the Department of Pathology, University Hospital of Heraklion, Crete, Hellas and from the Institute of Digestive Diseases, University Clinical Centre of Serbia, Belgrade, Yugoslavia.

The material included tissue biopsy specimens from n=17 patients representing long-segment Barrett's esophagus, and 25 patients representing intestinal metaplasia of the stomach mucosa (antrum n=11, corpus n=7 and cardia n=7). Esophageal biopsy specimens were obtained from the endoscopically self-evident long-segment Barrett's esophagus (LSBE) at a distance of more than 3 cm proximal to the gastroesophageal junction. Two biopsy specimens from the gastric cardia were taken with the biopsy cup placed across the normal appearing SCJ with the endoscope in anterograde position. In addition, twenty-six (antrum n=11, corpus n=7 and cardia n=8) gastric resection specimens with histological evidence of IM were studied. All gastric resection specimens were obtained from the patients operated for advanced adenocarcinoma of the stomach

and/or gastroesophageal junction from the site without apparent tumour infiltration.

From 42 patients (antrum n=15, corpus n=20 and cardia n=7), 84 biopsy samples without active inflammation, intestinal metaplasia or carcinoma were examined as a control.

Both, surgical and biopsy specimens, with the evidence of dysplasia were excluded from the study.

Immunohistochemistry

Immunostaining was performed on formalin-fixed paraffin wax-embedded tissue sections, using the alkaline phosphatase-antialkaline phosphatase (APAAP) method. Monoclonal antibody directed against CK7 (Dako, dilution 1:25) and CK20 (Dako, dilution 1:50) monoclonal antibodies were used. The bridging rabbit antimouse (Z902, dilution 1:20) and APAAP (D314, dilution 1:30) complex were obtained from Dako.

A step of microwave heating in a solution of sodium citrate (pH 6) was performed prior to incubation with primary antibodies.

Positive control slides were included in all tests and consisted of paraffin wax sections from normal prostatic tissue known to be positive for CK7 and normal colon tissue known to be positive for CK20. Negative control slides were prepared by omitting the primary antibody.

Histochemical technique

Histochemical staining was performed on formalin-fixed-paraffin-wax embedded tissue sections using High Iron Diamine/Alcian blue (Ab/HID) pH 2.5. All specimens with an evidence of IM were categorized as complete (type I) or incomplete (type II or type III) according to the criteria by Jass and Filipe (1981). The complete (type I) IM is characterized by the presence of goblet cells that contain sialomucins or sulphomucins, surface absorptive enterocytes, and occasionally, endocrine and Paneth cells. Type II incomplete IM is characterized by the presence of goblet cells and mucin-secretin epithelial cells that are negative for sulphomucins on High Iron Diamine/Alcian blue (Ab/HID) stain. Type III incomplete IM is characterized by the presence of goblet cells and mucin-secreting epithelial cells that stain positive for sulphomucins.

Normal colonic mucosa served as a positive control for this staining.

Assessment of grading

All studied cases were evaluated without knowledge of the anatomic location of the specimen. The pattern and the intensity of the staining were graded using an empirical semi quantitative system: (-), negative; (+), weak staining throughout or patchy moderate staining; (++), moderate staining throughout the section or patchy intense staining with weaker area; (+++), strong staining throughout the section.

Results

a) Intestinal metaplasia

Three types of CK7/20 immunophenotypes of IM were observed.

Type IM-1: moderate to strong diffuse CK7 staining of the surface epithelium and both, superficial and deep part of the crypts in combination with strong diffuse surface and superficial crypt CK20 staining. (Fig. 1)

Type IM-2: either negative or weak and patchy CK7 staining of the surface and crypt epithelium combined with strong diffuse surface epithelium and patchy crypt CK20 staining. (Fig. 2)

Type IM-3: strong and patchy CK7 staining of the surface and crypt epithelium combined with strong diffuse surface and patchy crypt CK20 staining. (Fig. 3)

b) Normal gastric epithelium

The normal gastric epithelium showed two unique CK7/20 immunophenotypes. Gastric corpus and antrum mucosa was negative for CK7 staining combined with moderate and patchy CK20 staining of the surface epithelium and superficial part of the crypts also referred to as Type N-1. (Fig. 4)

Type N-2 was the unique immunophenotypic feature of nonintestinalized gastric cardia mucosa and corresponded to the biopsy specimens that showed strong and patchy CK7 staining in the surface epithelium and both superficial and deep parts of the pits combined with strong and patchy CK20 staining on the surface

Table 1. The expression of cytokeratin CK7 and CK20 in intestinal metaplasia at different locations.

IMMUNO-PHENOTYPE	LOCATION OF INTESTINAL METAPLASIA			
	Barrett (N=17)	IM cardia (N=15)	IM corpus (N=14)	IM antrum (N=22)
IM-1	94.1 (16/17)	6.7% (1/15)	0	0
IM-2	5.9% (1/17)	80% (12/15)	100% (14/14)	90.9% (20/22)
IM-3	0	13.3% (2/15)	0	9.1% (2/22)

Table 2. Types of intestinal metaplasia at different locations.

INTESTINAL METAPLASIA	LOCATION OF INTESTINAL METAPLASIA			
	Barrett (N=17)	IM cardia (N=15)	IM corpus (N=14)	IM antrum (N=22)
Type I	0 (0/17)	26.7% (4/15)	64.3% (9/14)	68.2% (15/22)
Type IIa	70.6% (12/17)	46.6% (7/15)	35.7% (5/14)	18.2% (4/22)
Type IIb	29.4% (5/17)	26.7% (4/15)	0 (0/14)	13.6% (3/22)

epithelium and superficial parts of the pits. (Fig. 5).

Barrett's esophagus, IM of the cardia, gastric antrum and corpus mucosa

Sixteen of the 17 cases (94.1%) of long segment Barrett's esophagus, expressed the Type IM-1 immunophenotype. In contrast, none of the cases representing IM of the gastric antrum or corpus mucosa expressed this pattern. Only one case (6.7%) of IM in gastric cardia expressed the Type IM-1 immunophenotype. Ninety-three percent (14/15) of the cases with IM in the gastric cardia expressed the Type IM-2 and Type IM-3 immunophenotypes (Table 1).

Immunoreactivity to CK7/20 was neither affected by the type of IM (complete vs. incomplete), nor by the type of tissue samples (resection vs. biopsy specimens) (Table 2). Depth of invasion and lymph nodes

involvement in studied surgical specimens also had no influence on CK7/20 expression (Table 3). In addition, in none of the control cases from any location, CK7/20 immunophenotypes (Type N-1 and Type N-2)

Table 3. Depth of invasion and lymph nodes involvement in studied surgical specimens.

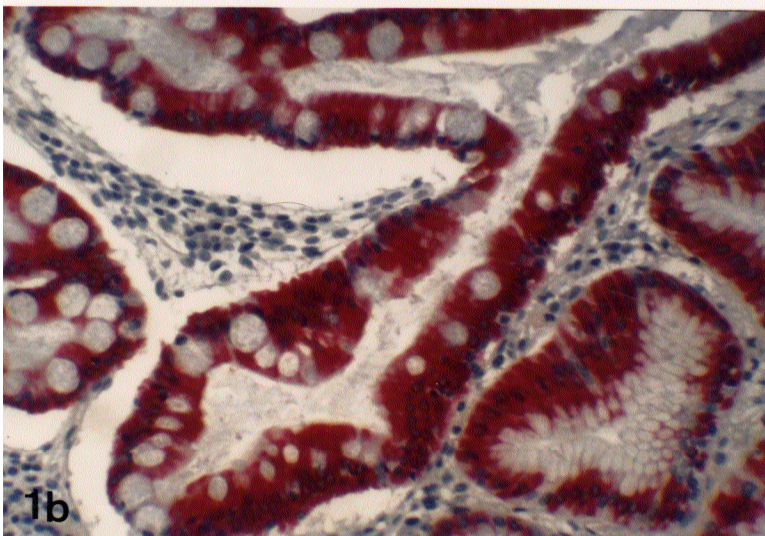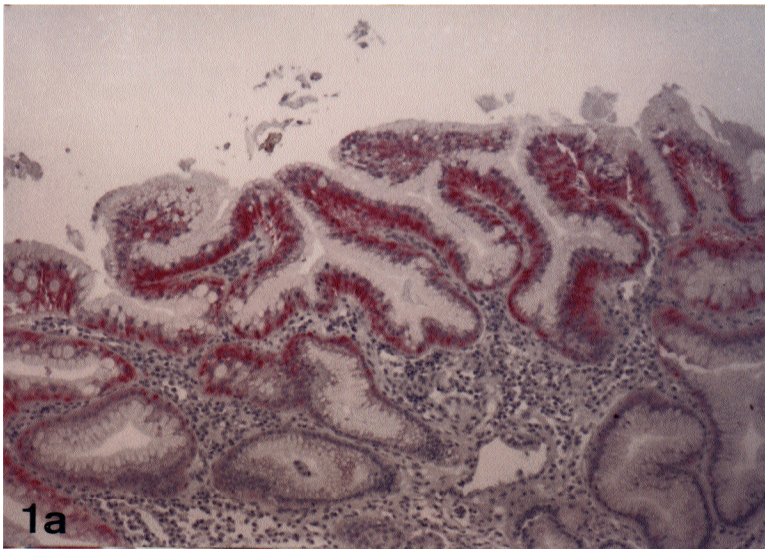|	IM CARDIA (N=8)	IM CORPUS (N=7)	IM ANTRUM (N=11)
Depth			
T1&T2	8	4	7
T3&T4	0	3	4
Lymph nodes			
Negative	1	0	1
positive	7	7	11

Fig. 1. Expression of cytokeratin CK7 (moderate to strong diffuse staining of the surface epithelium and superficial as well as deep part of the crypts, **a**) and CK20 (strong diffuse surface and superficial crypt staining, **b**) in Barrett's esophagus. x 100
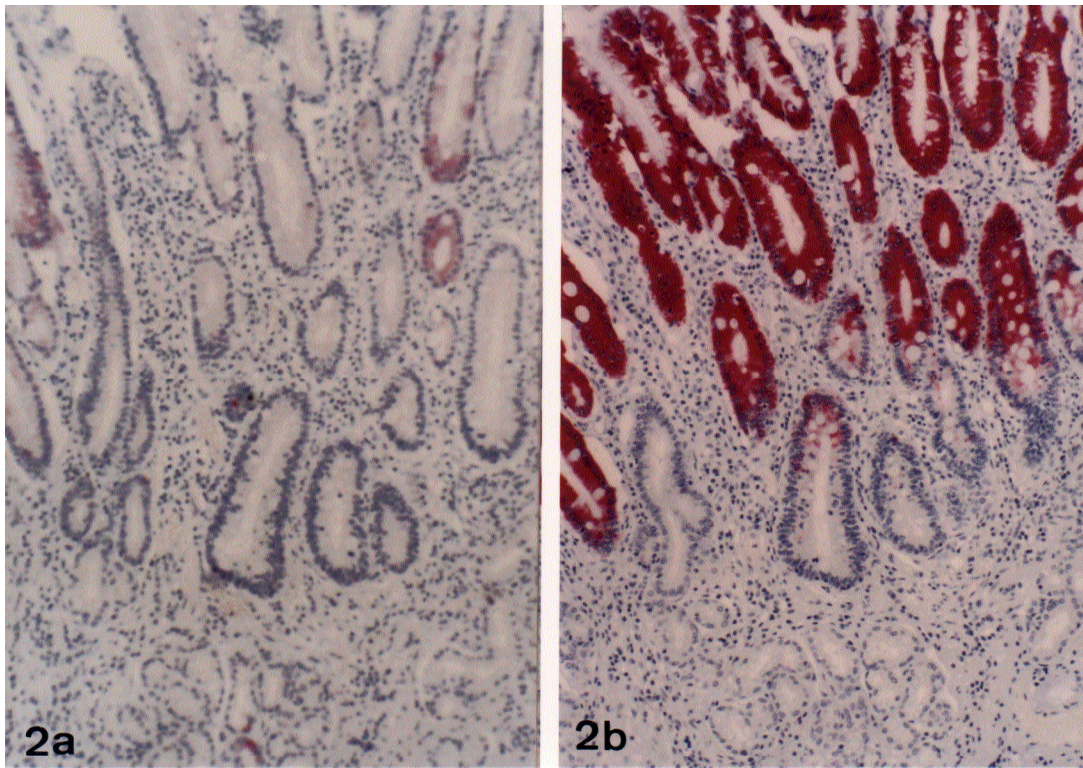
Cytokeratin expression in intestinal metaplasia of gastroesophageal junction

Fig. 2. Expression of cytokeratin CK7 (negative or weak and patchy staining of the surface and crypt epithelium, **a**) and CK20 (strong diffuse surface epithelium and patchy crypt staining, **b**) in intestinal metaplasia of the gastric antrum mucosa. x 100
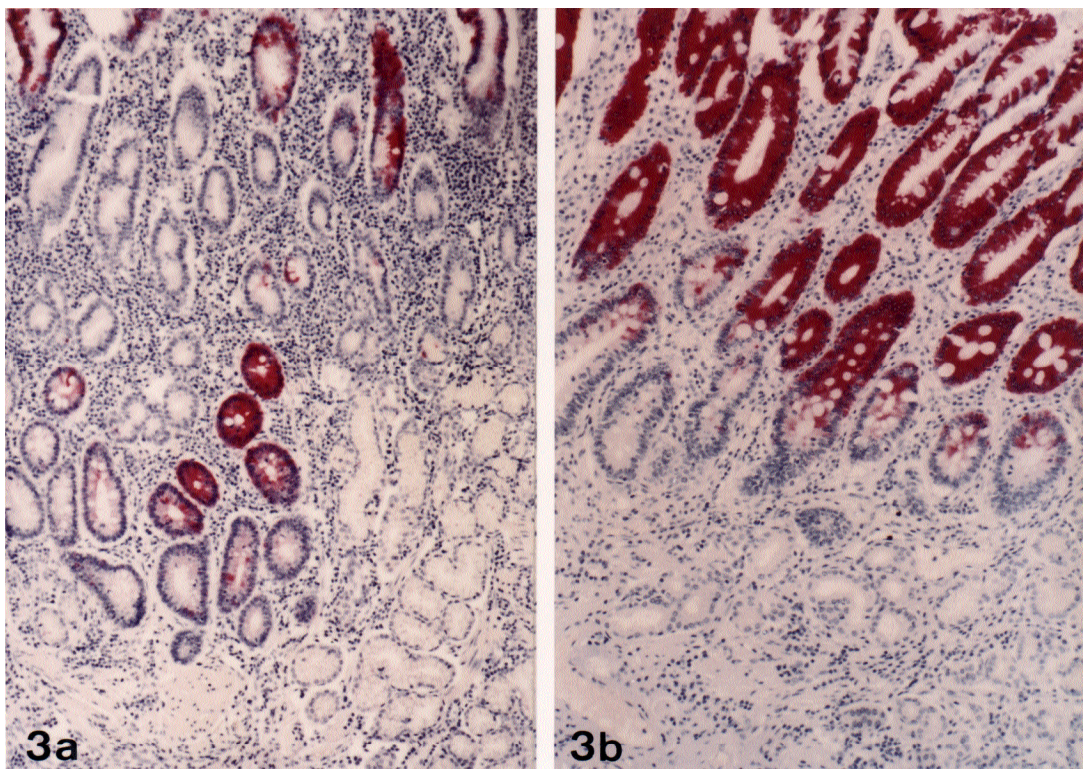

Fig. 3. Expression of cytokeratin CK7 (strong and patchy staining of the surface and crypt epithelium, **a**) and CK20 (strong diffuse surface and patchy crypt staining, **b**) in intestinal metaplasia of the gastric corpus mucosa. x 100

corresponded to any of the previously described patterns for intestinalized epithelium (Table 4).

Discussion

Intestinal metaplasia was found in several studies in the peritumorous mucosa of the gastric cardia tumors (Hamilton and Smith, 1987; Cameron et al., 1995; Spechler and Goyal, 1996). Therefore, it is believed that intestinal metaplasia in the gastric cardia also may have a neoplastic potential. While there is strong evidence that specialized columnar epithelium in classical Barrett's esophagus is related to the reflux disease and represents the precursor lesion for the development of adenocarcinoma of the esophagus (Hamilton and Smith, 1987; Haggitt, 1994; Cameron et al., 1995; Spechler and Goyal, 1996), so far, it has been difficult to assess the causes and the malignant potential of intestinal metaplasia in the gastric cardia. Furthermore, it is currently unclear whether specialized columnar epithelium of the short segment of Barrett's esophagus (SSBE) (Schell et al., 1992), measuring less than the traditionally accepted 3 cm in length, and intestinal metaplasia in the gastric cardia are manifestations of the same disorder (i.e., gastroesophageal reflux), or

Table 4. The expression of cytokeratin CK7 and CK20 in cardiac and normal gastric antrum and corpus epithelium.

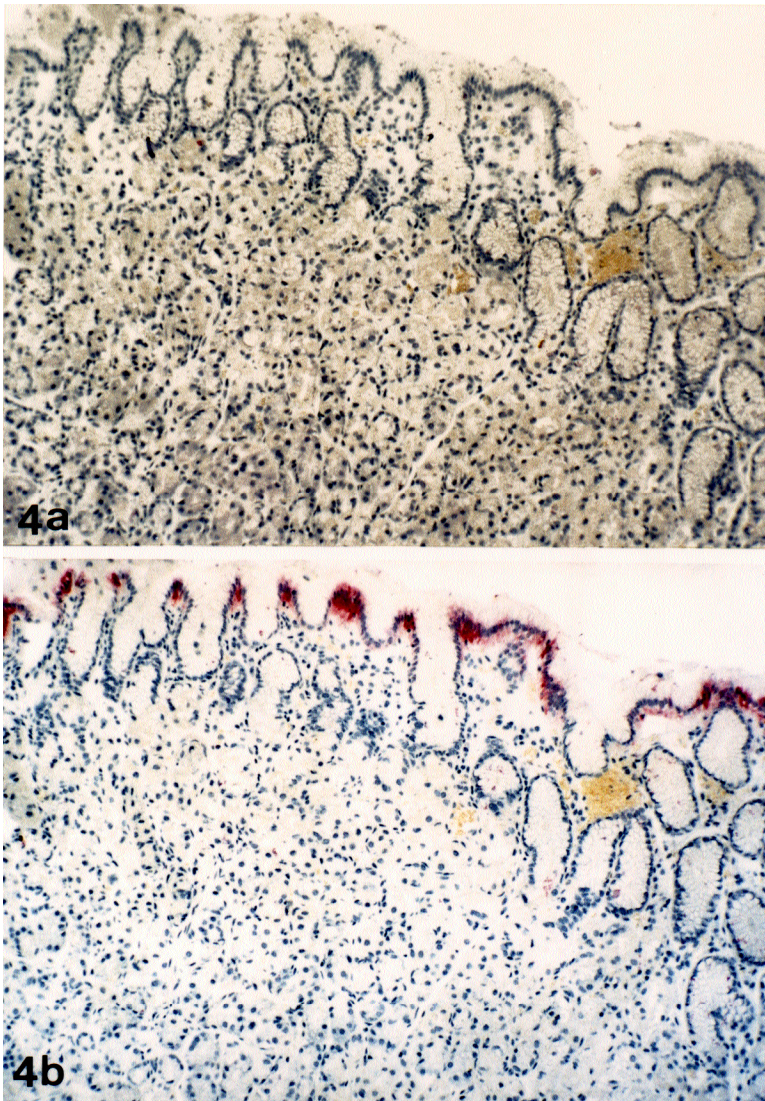| IMMUNO-PHENOTYPE	GASTRIC SITE		
	Normal antrum (N=15)	Normal corpus (N=20)	Cardia (N=7)
N-1	100% (15/15)	100% (20/20)	0
N-2	0% (0/15)	0% (0/20)	100% (7/7)

4a

4b

Fig. 4. Expression of cytokeratin CK7 (negative, **a**) and CK20 (moderate and patchy staining of the surface epithelium and superficial part of the crypts, **b**) in the normal gastric corpus mucosa. x 100

constitute different entities with different pathogenesis.

Recently it was suggested that phenotypic expression of cytokeratin CK7 and CK20 polypeptides can be used to identify patients with specialized intestinal metaplasia originating from the esophagus as opposed to the stomach (Ormsby et al., 1999). In that study (Ormsby et al., 1999) the authors described a very specific immunoreactivity to CK7/20 of Barrett's esophagus characterized by strong CK7 staining of both superficial and deep glands in combination with strong CK20 staining of the superficial epithelium and superficial part of the crypts. This specific pattern was described in 94% of the surgical specimens and 100% of the biopsy specimens. In their study, none of the either biopsy or surgical specimens representing intestinal metaplasia of the gastric cardia expressed this pattern. Another group of investigators recently reported similar findings regarding the CK7 and CK20 expression in Barrett's esophagus (Glickman et al., 2001).

In our study we were able to confirm previously described CK7/20 Barrett's pattern in 94.1% of our cases with 100% specificity, since none of the cases (0/36) representing IM of the antrum and corpus mucosa expressed this CK7/20 immunophenotype. Ninety-four percent of gastric antrum (20/22 cases) and corpus (14/14 cases) resection and biopsy specimens with the evidence of IM expressed the Type IM-2 immunophenotype irrespectively of the type of intestinal metaplasia. These findings were partially different from those described by Ormsby, who observed the aforementioned pattern only in the areas of complete IM (Ormsby et al., 1999). In our study absence of CK7 staining was not related to the presence of foci of incomplete IM. Furthermore, diffuse and strong CK20 staining of the surface epithelium and superficial part of the crypts with patchy involvement of the deep part of crypts was observed in our study irrespectively to the type of tissue specimens (resection vs. biopsy) and type of IM.

Focusing on the IM of the gastric cardia, we were able to observe that intestinalized epithelium of the gastric cardia expresses CK7/20 immunoreactivity identical to those described in intestinalized epithelium of gastric antrum and corpus (Type IM-2 and Type IM-3) in 93.3% (14/15) of the cases with the predominance of the Type IM-2 immunophenotype. Although our results concerning the IM of the gastric cardia are consistent with previous findings of Ormsby (Ormsby et al., 1999) our observations regarding the immunoreactivity of the normal control gastric mucosa were quite different. This prompted us to raise questions about the existence and origin of cardiac mucosa.

All cases of normal gastric antrum and corpus mucosa in our study were negative for CK7 expression in all layers of the pits. On the contrary, non-intestinalized cardiac epithelium uniformly expressed strong and patchy CK7 immunoreactivity in all layers of the epithelium, a feature permitting distinction of the cardiac epithelium from the epithelium of the rest of the stomach.
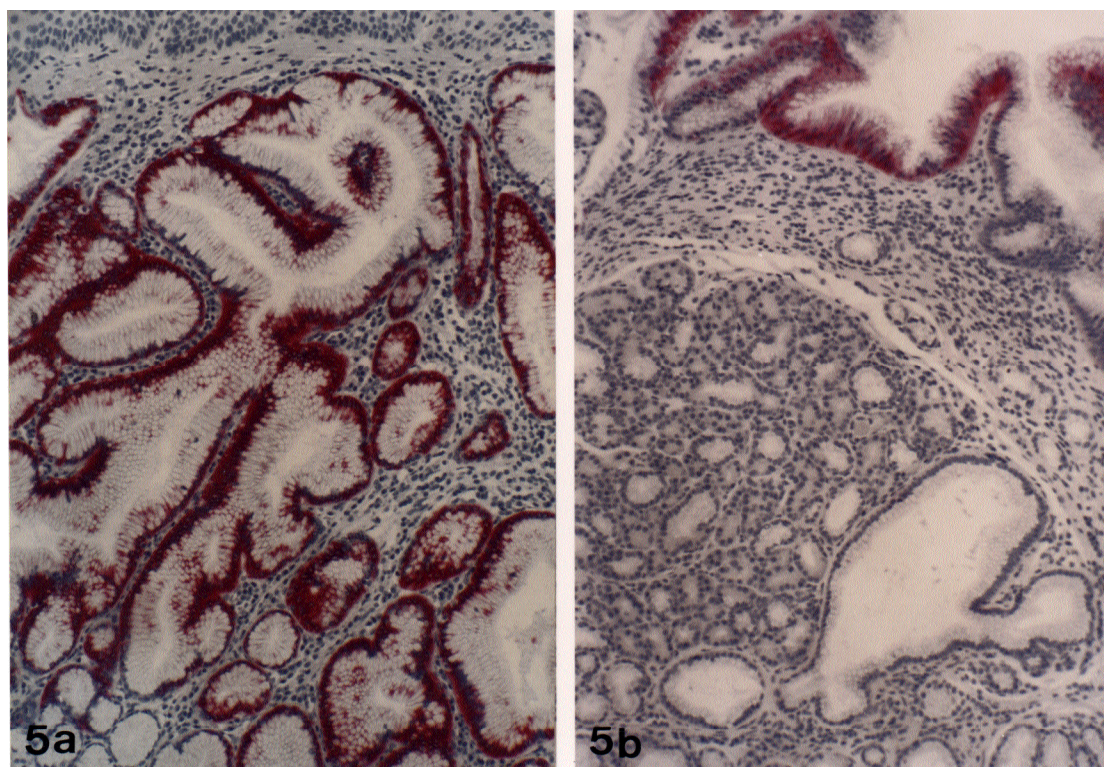

Fig. 5. Expression of cytokeratin CK7 (strong and patchy staining in the surface epithelium and both superficial and deep parts of the pits, **a**) and CK20 (strong and patchy staining on the surface epithelium and superficial parts of the pits, **b**) in the cardiac epithelium. x 100

There are several possible explanations for such differences. First, we collected the cardiac mucosa samples with the biopsy cup placed across the Z-line while other investigators (Ormsby et al., 1999) considered cardiac mucosa present at the distance "within 5 mm from normal-appearing squamocolumnar junction". Biopsy specimens obtained arbitrarily at a distance (i.e. 5 mm) below the squamocolumnar junction may be gastric but not "cardiac" in location, since precise distal extent of the cardia could not be studied. Our decision was supported by the results of a previous study in which it was shown that gastric cardia is only 1 to 4 mm in length and increases slightly with age (Kilgore et al., 2000). This provided us precise information on exact cardia location and its relation to the squamocolumnar junction as well as to the fundic mucosa. Secondly, there is a possibility that although cardia anatomically and histologically belongs to the stomach, it may have a distinct cytoskeleton structure from the rest of the stomach representing specific transitional zone between columnar and squamous epithelium. Recently, it was hypothesised that cardiac mucosa actually does not exist as a native structure, but rather arises as a metaplastic phenomenon in the distal esophagus secondary to gastroesophageal reflux (Oberg et al., 1997; Chandrasoma et al., 2000). Although the results of our study concerning the CK7/20 immunophenotype of intestinal metaplasia at these three locations do not support this hypothesis, we cannot rule out the possibility that biological features of the cardiac epithelium are different from the rest of the stomach. Our study shows that there is no significant alteration of CK7/20 immunophenotype of the normal cardiac mucosa in comparison to the intestinal metaplasia in this region, findings that are different from the rest of the gastric mucosa. Although during the metaplastic changes, CK7/20 immunophenotypes of IM in these three locations become similar, our results that the normal cardiac epithelium has a distinct CK7/20 immunophenotypic pattern from the normal epithelium of the gastric antrum and corpus could suggest that cardiac epithelium may already be present as a metaplastic one (i.e. first detectable changes in the pathogenesis of gastroesophageal reflux disease). Despite the fact that in the present study only the combination of two markers were used, there are several lines of evidence to support this hypothesis. The infection rate for *Helicobacter pylori* infection is declining world-wide as well the incidence of gastric cancer (EHPSG, 1993; Parsonnet, 1995) while there is an opposing time trend for esophageal adenocarcinoma (Craanen et al., 1992a). If we assume, based on the CK7/20 expression, that metaplastic changes of the cardiac epithelium and of the rest of the stomach share common pathogenetic pathways, the question is why the incidence of cardiac carcinomas is rising when gastric adenocarcinoma is falling (Blot et al., 1991; Craanen, 1992a; Powell and McConkey, 1992; Pera et al., 1993). Even though the expression of cytokeratins seems to be

useful in the diagnosis of Barrett's esophagus and the origin of IM in the cardia, based on these markers we are not able to conclude if IM of the cardia is related to the pathological processes affecting gastric mucosa. The etiology and pathological relevance of the inflammation and intestinal metaplasia in the cardia remains unclear. Factors that have been proposed to cause the "carditis" and consequently IM of the cardiac epithelium are *Helicobacter pylori* infection and related gastritis (Genta et al., 1994; Hackelsberger et al., 1997; Hirota et al., 1997; Morales et al., 1997; Goldblum et al., 1998) and gastroesophageal reflux disease (Kauer et al., 1995; Vaezi and Richter, 1995; Oberg et al., 1997; Chandrasoma et al., 2000).

If we accept the concept that intestinal metaplasia arising from the esophagus has a different clinical importance than intestinal metaplasia of the stomach (Matsukura et al., 1980; Jass and Filipe, 1981; Trier, 1985; Zwas et al., 1986; Craanen et al., 1992b; Das et al., 1994) then a clear understanding of intestinal metaplasia of the cardiac epithelium and its pathogenesis is important.

Histologically, although gastric antrum and corpus epithelium exhibit different morphological and architectural features, they still have the same immunophenotype concerning the expression of cytokeratins 7 and 20, in normal and metaplastic epithelium. On the other hand, histologically and functionally, antrum and cardia share a similar epithelium (Toner and Cameron, 1995) with mucus secreting cells and similar local pH but there is a clearly observed difference in immunophenotype of these two sites.

The described differences and changes in pattern of CK7/20 expression from normal to intestinalized epithelium in cardia and gastric antrum and corpus mucosa support the hypothesis that cardiac epithelium is either metaplastic in origin or that the IM arising from cardiac epithelium has unique features. The IM in the cardiac epithelium may be an early marker for gastroesophageal reflux disease but its presence should be interpreted with caution since we cannot base on the single marker to ascertain if the risk of dysplastic changes is lower in IM of gastric cardia as opposed to Barrett's esophagus. More studies are needed to elucidate the biological meaning of IM in the cardia with special emphasis on its neoplastic potential. Therefore, large scale prospective studies, including minute diagnosis of reflux disease (pHmetry, manometry etc) versus *Helicobacter pylori* associated "carditis" are required. Changes in pattern of cytokeratin expression along with other markers may be helpful for defining the patients with intestinal metaplasia in the gastric cardia at high risk of cancer development.

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