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Immunohistochemistry of carcinoembryonic antigen, secretory component and lysozyme in benign and malignant common bile duct tissues

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Summary. An immunoperoxidase technique has been utilized for the localization of carcinoembryonic antigen (CEA), secretory component (SC) and lysozyme (LZ) in normal and cancerous common bile duct tissues. Little or no CEA was found in the non-cancerous common bile duct tissues. SC was found in the surface epithelium and accessory gland epithelium and LZ was demonstrated only in the accessory glands. Some inflammatory cells were also positively stained for LZ. In adenocarcinoma, CEA was always present on the luminar border of the carcinoma cells, occasionally with intercellular and intracellular localization. LZ was absent, or only faintly detected in carcinoma. SC was generally distributed in well-differentiated adenocarcinoma cells, but showed a reduced intensity of staining with progressive dedifferentiation. These findings suggest that CEA, SC and LZ could be useful markers providing valuable information in the pathological diagnosis of bile duct carcinoma.

Key words: Carcinoembryonic antigen – Secretory component – Lysozyme – Immunohistochemistry – Common bile duct

There is an extensive array of tubulo-alveolar accessory glands that communicate with the major biliary tract (McMinn and Kugler 1961; Elias and Sherrick 1969). These glands frequently display reactive hyperplasia or dysplasia induced by inflammation or an adjacent neoplasm, and these changes may be easily mistaken for genuine dysplasia or malignancy. The histological distinction between invasive carcinoma and altered accessory glands may therefore be difficult.

The immunohistochemical localization of carcinoembryonic antigen

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(CEA), secretory component (SC) and lysozyme (LZ) has been reported to be of potential value in the diagnosis of malignent lesions in gastrointestinal tissues (Isaacson and LeVann 1976; O'Brien et al. 1981; Isaacson 1982; Rognum et al. 1982). However, such immunohistochemical observation has, to our knowledge, not been confirmed in the common bile duct. Hence, we aimed to establish the distribution of these epithelial markers, CEA, SC and LZ, in the normal common bile duct, and to study whether the immunohistochemical localization for these markers might be an adjunct for histological diagnosis.

Materials and methods

Tissues. Common bile duct tissues were obtained at the time of surgical removal or diagnostic biopsy from patients operated on for common bile duct carcinoma (12 cases), patients for pancreatic head carcinoma (6 cases), patients with cholelithiasis (9 cases) and patients subjected to Whipple's operation for advanced gastric neoplasm (5 cases). Samples were taken from several sites of the common bile duct of each surgical specimen. The tissues were promptly fixed in periodate-lysine-4% paraformaldehyde (PLP) (McLean and Nakane 1974) or 10% formalin. Tissues fixed in PLP were washed in increasing concentrations of sucrose in phosphate buffered saline (PBS), and finally placed in 20% sucrose in PBS, frozen in OCT-compound (Lab-Tek, Naperville, IL), and sectioned at 6 μm thickness on a cryostat microtome. The other fixed tissue blocks were embedded in paraffin and sectioned at 4 μm thickness on a conventional microtome.

Antisera. Rabbit antisera to human CEA, SC and LZ were purchased from Dako Immunoglobulins (Kyowa Medics, Tokyo). The anti-CEA antiserum was absorbed with crude perchloric acid (PCA) extracts from human spleen and blood group A and B erythrocytes in order to absorb antibodies against non-specific cross-reacting antigen and blood group substances. The specificity of the antisera was tested by immunoelectrophoresis against the purified relevant antigen as previously described (Nagura et al. 1983). Antiserum to rabbit IgG was prepared in goats by weekly injection of rabbit IgG in our laboratory. Antibody-enriched fractions of the antiserum were prepared by affinity chromatography against Sepharose-bound rabbit IgG. Fab' fragments were prepared and conjugated with horseradish peroxidase (HRP) according to the method of Wilson and Nakane (1978). For use in control experiments, the Fab' fragments of nonimmune rabbit gamma globulin were also labeled with HRP.

Immunohistochemistry. CEA, SC and LZ were localized in tissues according to techniques previously described for studies on gastrointestinal mucosa (Nagura et al. 1983). In order to inhibit endogeneous peroxidase, cryostat sections or deparaffinized sections were treated successively with 5 mM periodic acid and 3 mM sodium borohydride before immunological reaction (Isobe et al. 1977). In cryostat sections, the direct HRP-labeled antibody method was used. The sections were reacted with HRP-labeled Fab' fragments of the antisera or non-immune rabbit serum for two hours at room temperature. In paraffin-embedded sections, the indirect HRP-labeled antibody method was used. The first layer reagent was either IgG of the antisera or non-immune rabbit serum, and the second layer reagent was HRP-labeled Fab' fragments of anti-rabbit IgG. In addition, control sections were reacted with the antisera absorbed with the relevant antigens. After immunological reactions, the sections were incubated with 0.025% diaminobenzidine solution containing 10 mM hydrogen peroxide and 10 mM sodium azide, and then counterstained with methyl green (pH 4.0).

Results

The results of this study are summerized in Table 1. Control staining for SC, CEA and LZ was uniformly negative.

Table 1. Immunocytochemical staining of common bile duct

	SC	CEA	LZ
Normal mucosa			
surface epithelium	+	−	−
accessory gland	+	−	+
Hyperplasia			
surface epithelium	+	−	±[a]
accessory gland	+	−	+
Adenocarcinoma	+~±[b]	+	±~−

[a] Weakly positive in the surface epithelium and positive in goblet cells
[b] Positive staining in well-differentiated adenocarcinoma, fading to negative with progressive dedifferentiation – see text

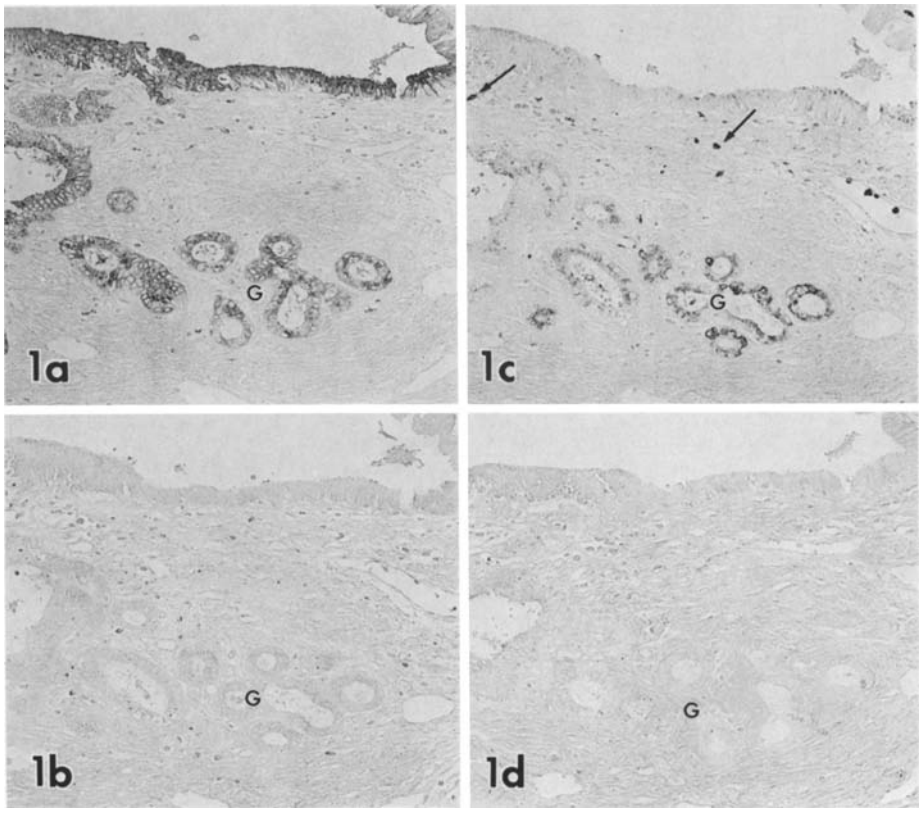

Fig. 1a–d. Histologically normal common bile duct showing (**a**) positive staining for SC in the surface epithelium and accessory glands, (**b**) no staining for CEA, and (**c**) positive staining for LZ in accessory glands. LZ shows weaker or absent staining in the surface epithelium, and an intense staining in some inflammatory cells. (**d**) The corresponding field from an adjacent section as a control shows no staining. (× 150) (G: accessory glands, →: inflammatory cells)

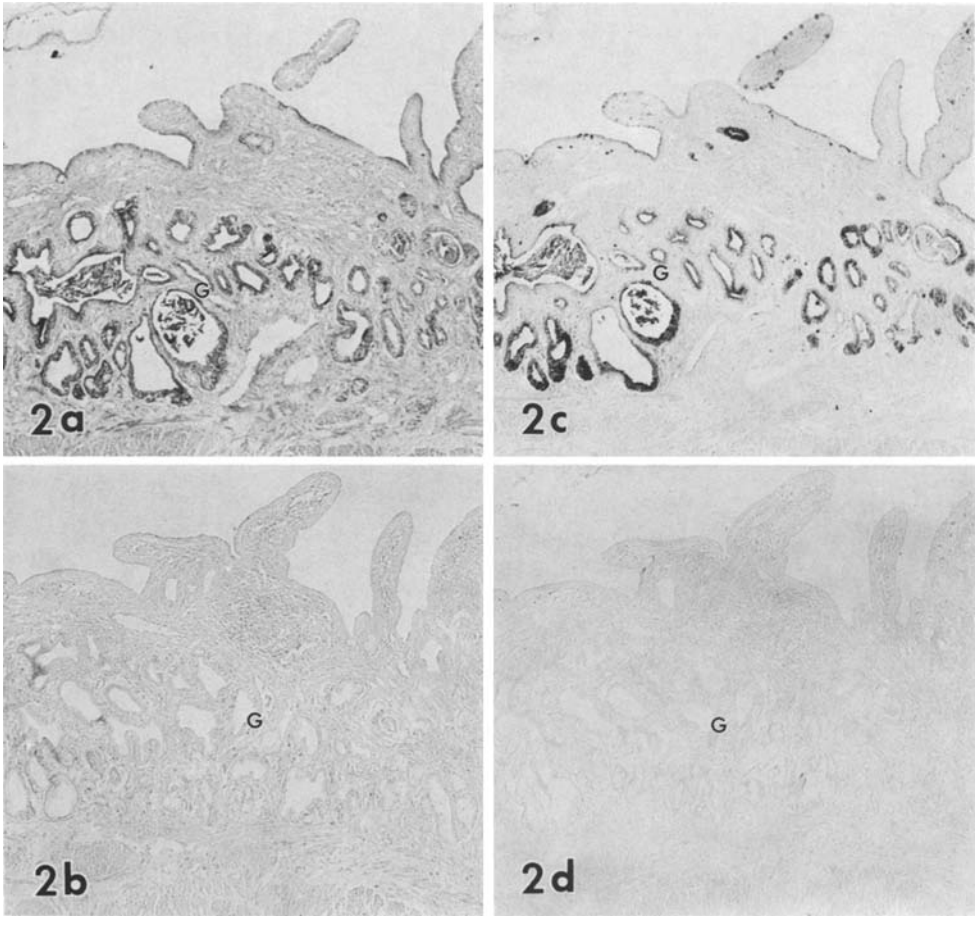

Fig. 2a–d. Common bile duct having hyperplasia of the accessory gland in a case of cholelithiasis. (**a**) Both surface and accessory gland epithelial cells show a positive staining for SC, but (**b**) no staining for CEA. (**c**) Accessory glands and mucous cells in the surface epithelial lining are strongly positive for LZ. The surface epithelium is weakly positive for LZ. (**d**) Control section shows no staining. (× 60) (G: accessory glands)

Histologically normal tissues. Numerous tubulo-alveolar glands were found at irregular intervals in the wall of histologically normal common bile duct. SC was demonstrated in columnar epithelial cells of both surface and accessory glands, usually with apical intensification (Fig. 1a). Surface and accessory gland epithelial cells were all devoid of CEA (Fig. 1b). LZ was present in the cytoplasm of accessory gland cells, whereas only very weak LZ staining was noted in the surface epithelial cells (Fig. 1c). Some inflammatory cells were stained intensely for LZ (Fig. 1c). Figure 1d shows the absence of any specific staining after the primary antiserum was replaced by non-immune rabbit serum.

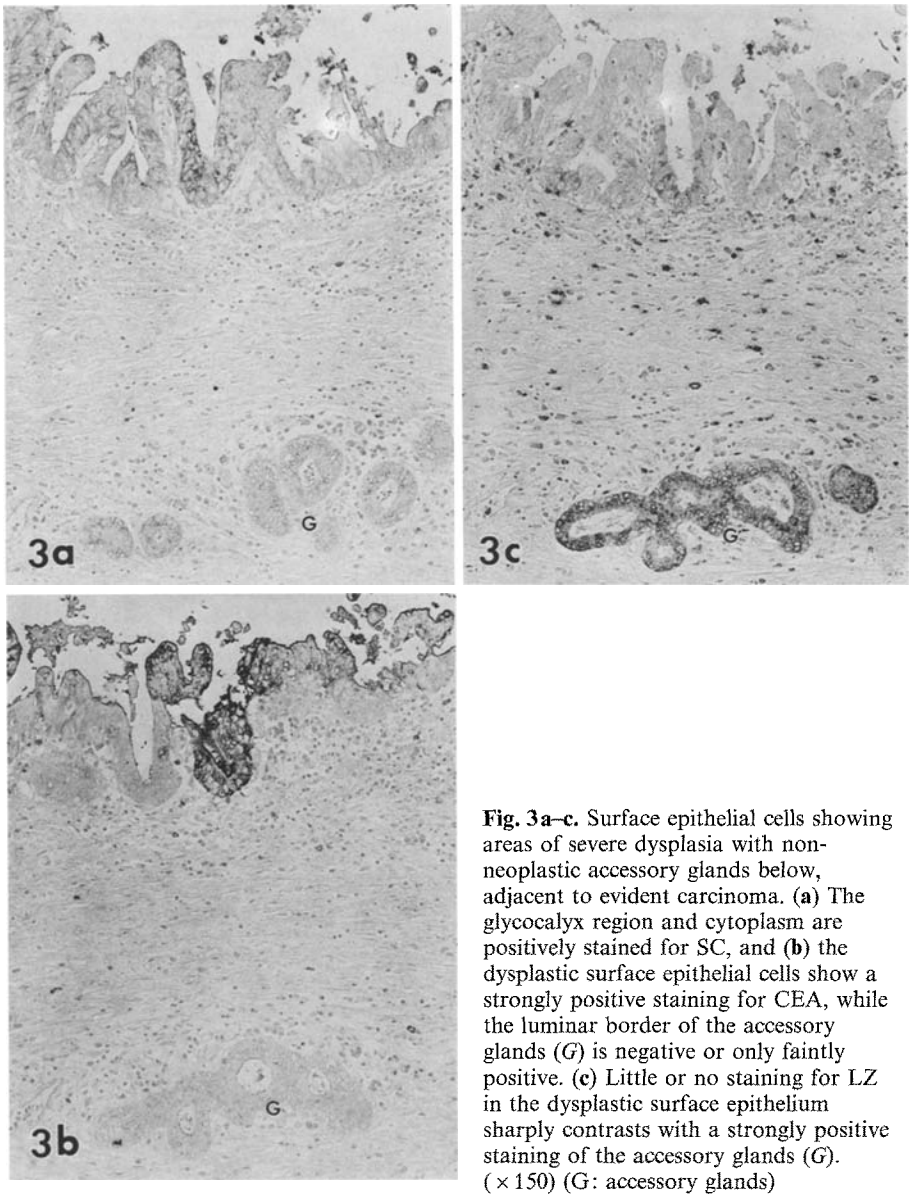

Fig. 3a–c. Surface epithelial cells showing areas of severe dysplasia with non-neoplastic accessory glands below, adjacent to evident carcinoma. (**a**) The glycocalyx region and cytoplasm are positively stained for SC, and (**b**) the dysplastic surface epithelial cells show a strongly positive staining for CEA, while the luminar border of the accessory glands (*G*) is negative or only faintly positive. (**c**) Little or no staining for LZ in the dysplastic surface epithelium sharply contrasts with a strongly positive staining of the accessory glands (*G*). (×150) (G: accessory glands)

Glandular hyperplasia. The localization of SC, CEA and LZ in almost all the samples showing glandular hyperplasia were generally identical to the histologically normal common bile duct (Fig. 2a–c). However, the surface epithelial lining was often weakly stained by the anti-LZ antibody, and mucous cells amongst the surface and accessory gland epithelial cells, if present, showed strongly stained intracytoplasmic granules of LZ (Fig. 2c).

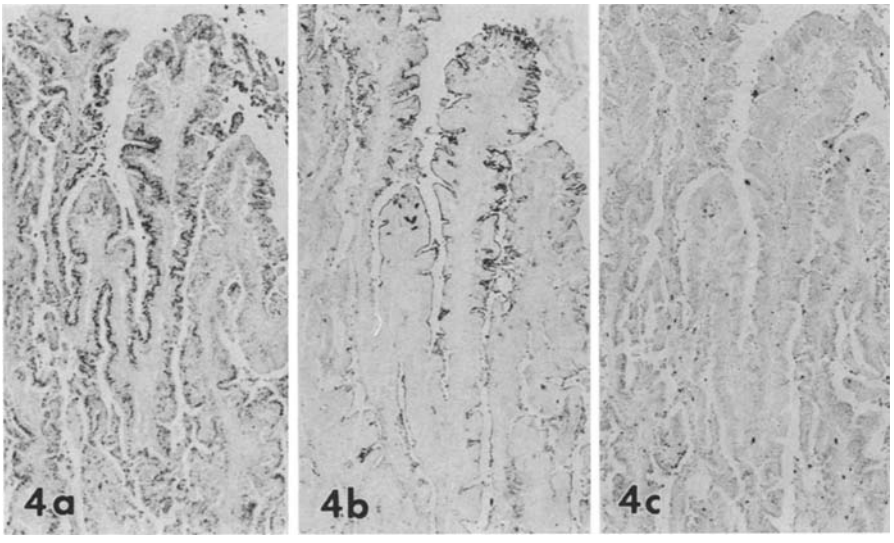

Fig. 4a–c. Papillary adenocarcinoma showing (**a**) positive cytoplasmic staining for SC, (**b**) positive staining for CEA mainly restricted to an apical rim, and (**c**) little or no staining for LZ. (× 60)

Adenocarcinoma. The severely dysplastic surface epithelium with a villiform structure adjacent to evident carcinomatous tissues, being putatively malignant, showed a positive CEA staining as a thick luminar rim. CEA was also occasionally found intracellularly. The CEA staining was often discontinuous and varied considerably in degree within a single section of the specimen. However, accessory glands in the duct wall were negative or only faintly positive for CEA on the luminal border (Fig. 3b). The intensity of the immunohistochemical reaction for CEA was related to the severity of dysplasia in the specimens studied. In the adjacent section, LZ was observed in the cytoplasm of the accessory gland epithelium, whereas little or no staining was found in the surface dysplastic epithelia (Fig. 3c). SC was present in the glycocalix region and partly in the cytoplasm of the dysplastic epithelial cells, but the intensity of the staining reactions was unrelated to the degree of dysplasia (Fig. 3a).

In the case of well-differentiated papillary adenocarcinoma, SC was ubiquitously present in the apical cytoplasm (Fig. 4a) whereas CEA appeared as a thick border in the glycocalyx region of the carcinoma cells. A weak cytoplasmic staining of CEA sometimes occurred (Fig. 4b). LZ was absent or faintly detected in the apical cytoplasm (Fig. 4c).

Almost all samples of infiltrating carcinoma cells in the bile duct wall where a tubular arrangement or more poorly differentiated pattern predominated over papillary one showed a negative or weak staining for SC and LZ (Fig. 5a, c) in contrast with a strongly positive staining for CEA (Fig. 5b). Membrane and cytoplasmic stainings of CEA were observed in the invasive carcinoma cells. The accessory glands adjacent to the carcinoma cells were generally identical to non-cancerous bile duct (Fig. 5b, c).

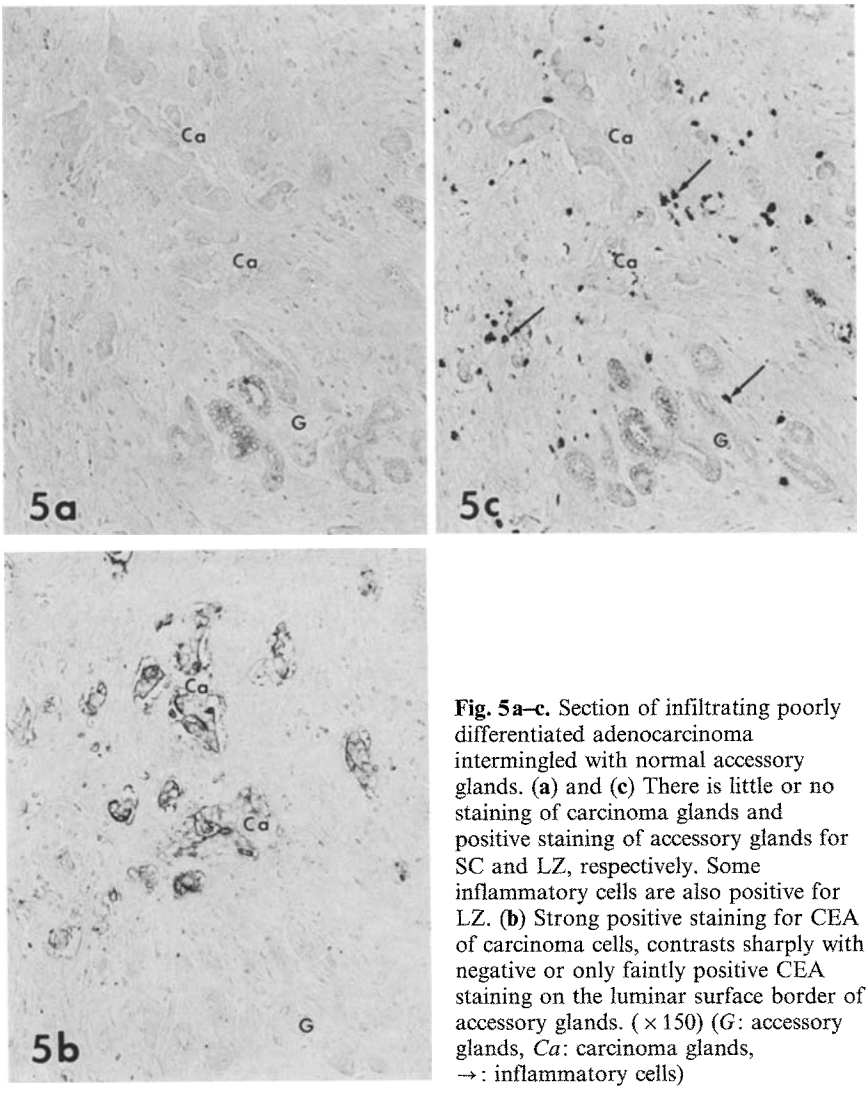

Fig. 5a–c. Section of infiltrating poorly differentiated adenocarcinoma intermingled with normal accessory glands. (**a**) and (**c**) There is little or no staining of carcinoma glands and positive staining of accessory glands for SC and LZ, respectively. Some inflammatory cells are also positive for LZ. (**b**) Strong positive staining for CEA of carcinoma cells, contrasts sharply with negative or only faintly positive CEA staining on the luminar surface border of accessory glands. ($\times 150$) (*G*: accessory glands, *Ca*: carcinoma glands, →: inflammatory cells)

It is noteworthy that the choice of fixatives and tissue preparation did not influence the stainability for these antigens; that is, formalin-fixed paraffin-embedded specimens were suitable for demonstration of CEA, SC and LZ.

Discussion

The results of the immunohistochemical analysis reported here have indicated that the normal common bile duct tissues of this study lack a stainable level of CEA, but all cases of common bile duct cancer give positive staining the antigen. However, LZ is found in the accessory glands, but hardly

ever in carcinoma glands. SC is generally distributed in well-differentiated papillary neoplasms, as in normal common bile ducts, but shows a substantially reduced intensity of the staining in poorly differentiated adenocarcinomas when compared with the normal epithelium. Thus, it would appear that the immunohistochemical localization of CEA, SC and LZ adds some objectivity to the histological diagnosis of common bile duct cancer.

In addition, two different fixatives and tissue preparations, PLP-fixed cryostat sections and formalin-fixed paraffin-embedded sections, were applied to the given specimens for the study of these antigenic marker substances. Both fixatives and embedding techniques employed in this study conserved the antigenic reactivity, thus making the immunoperoxidase method applicable for use with sections from bile duct tissues conventionally prepared for light microscopy, as shown in gastrointestinal tissues (Isaacson et al. 1976; O'Brien et al. 1981; Isaacson 1982). The localization of CEA, SC and LZ was studied extensively in colorectal and gastric mucosa, and in a few gallbladders (Tsutsumi et al. 1982; Albores-Saavedra et al. 1983; van den Oord 1983). Until the present time, however, systemic studies on these markers in normal and cancerous human common bile duct by the immunohistochemical method have not been reported.

CEA was first described by Gold and Freedman (1965) as a glycoprotein present exclusively in adenocarcinoma of the digestive tract and in the digestive organs of fetuses. However, published reports show a considerable variation of CEA localization in normal and cancerous tissues. The diversity between reported findings may be caused by differing specificity of the CEA antibodies used and immunohistochemical technique (Rognum et al. 1980; Ahnen et al. 1982; Nap et al. 1983). Some antisera to CEA, for example, are known to crossreact with other glycoproteins. CEA prepared by perchloric acid extraction from liver metastases of human colon cancer is reported to crossreact with several related glycoproteins found in the normal and neoplastic tissues including normal colon, lung, spleen and blood group glycoproteins (Holburn et al. 1974; Burtin 1978; Nap et al. 1983). In addition, Svenberg (1976) identified a CEA-like glycoprotein from bile and demonstrated it in the biliary tract tissue. Therefore, the anti-CEA serum used here was absorbed with AB0 blood groups and perchloric acid extracts of human spleen. Consequently it gave only one immunodiffusion precipitin line of β-mobility with perchloric acid extracts of bile duct cancer tissues and colonic cancer tissues (Nagura et al. 1983). All pancreatic cancer samples were positively stained with both non-absorbed and absorbed anti-CEA antisera with an occasional decrease of the positive area by the absorption procedure, while noncancerous pancreatic duct epithelial cells were often stained by the non-absorbed serum but not by the absorbed serum (Tsutsumi et al. 1984). The recent observation of CEA in the normal gallbladder epithelium by Albores-Saavedra et al. (1983) would be explained by this heterogeneity of anti-CEA sera.

Numbers of previous studies also have documented the distribution of SC in the normal and abnormal stomach and intestine (Poger et al. 1976; Nagura et al. 1983), but the common bile duct has received little attention.

SC, a glycoprotein receptor for dimetric IgA (Crago et al. 1978; Nagura et al. 1979), is synthesized by epithelial cells of various mucous membranes (Tourville et al. 1969). It has been reported that SC immunoreactivity is often diminished in neoplastic colonic cells corresponding to increasing degrees of dysplasia (Poger et al. 1976). An ultrastructural study has proved that malignant change of the gastric mucosal cells is accompanied by alteration in the cells' surface distribution of SC (Nagura et al. 1983). These immunohistochemical studies are compatible with the present findings in the common bile duct tissues.

The cationic antibacterial enzyme, LZ or muramidase, has been identified by immunohistochemical technique in a variety of human cells and tissues including neutrophilic granulocytes, mononuclear phagocytes, cells of pyloric gland, mucous neck cells of the gastric fundus, Paneth cells of small intestine, Brunner's glands of the duodenum and salivary and bronchial glands (Klockards and Reitamo 1975; Mason and Taylor 1975). Although normal gallbladder is devoid of LZ, which is restricted to infiltrating inflammatory cells (Klockards and Reitamo 1975), LZ-immunoreactivity appears to be present in the cytoplasm of pseudopyloric glands in the case of cholecystitis (Tsutsumi et al. 1982; van den Oord et al. 1983). In this study, the presence of immunoreactive LZ has been demonstrated in accessory glands of the common bile duct as in pseudopyloric glands of the gallbladder and in Brunner's glands of the duodenum, all of which contain a mucosubstance positive for paradoxical concanvalin A staining after Katsuyama and Spicer (1978) (Tsutsumi et al. 1982). Namely, the presence of LZ might be related to a certain mucous component. Positive staining for LZ in well-differentiated gastric adenocarcinoma (Isaacson 1982) is in contrast with the analogous biliary tract lesion in which little or no LZ is found in the present study. Even in the gastric adenocarcinoma, however, positive staining for LZ in the well-differentiated lesion fades to negative with progressive dedifferentiation. Although there seems to be no logical explanation for the disappearance of LZ in the cancerous lesions of the common bile duct, LZ, like CEA, is a good marker for the discrimination between carcinoma glands and non-cancerous accessory glands in the wall of the common bile duct.

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