Virchows Arch [Pathol Anat] (1983) 399:317–323

Virchows Archiv A

Immunohistochemical demonstration of lysozyme in pseudopyloric glands in chronic cholecystitis*

J.J. van den Oord, K. Geboes, C. de Wolf-Peeters, and V.J. Desmet
Department of Pathology, Laboratorium voor Histo- en Cytochemie, Akademisch Ziekenhuis St. Rafael, B-3000 Leuven, Belgium

Summary. Gallbladders of 12 cases with chronic cholecystitis showing pseudopyloric glands (PPG) and of 18 cases with acute cholecystitis or chronic cholecystitis but without PPG were examined by the peroxidase – antiperoxidase (PAP) method using rabbit antibody against human lysozyme (LM). LM-immunoreactivity was detected in the cytoplasm of PPG and, to a lesser extent, in the pits of epithelial crypts that gave rise to PPG. No LM was found in normal gallbladders; in cases of cholecystitis without PPG, LM-immunoreactivity was restricted to infiltrating inflammatory cells. The presence of LM in PPG suggests that PPG represent functional metaplastic areas, involved in the non-specific defence mechanisms through participation of LM.

Key words: Lysozyme – Enzyme immunohistochemistry – Chronic cholecystitis – Pseudopyloric glands

Lysozyme (LM) is a stabile, low molecular weight enzyme that mediates in non-specific defense mechanisms by attacking the mucopeptides of bacterial cell walls through dissolution of N-acetyl-glucosaminyl-N-acetylmuramic acid linkages (Chipman and Sharon 1969). Using immunohistochemical techniques, LM has been localized in various sites (Mason and Taylor 1975). In the gastrointestinal tract, LM is present in submucosal oesophageal glands and duodenal Brunner's glands (Klockars and Reitamo 1975), small intestinal Paneth cells (Ghoos and Vantrappen 1971) and Kupffer cells of the liver. The normal gallbladder is devoid of LM (Klockars and Reitamo 1975).

However, in chronic cholecystitis mucous glands may be found outside the neck region throughout the gallbladder mucosa (Nicholson 1923; King and McCallum 1931). Since these glands share morphological, histochemical, immunohistochemical and electronmicroscopic features with true glands of

Offprint requests to: V. J. Desmet at the above address

* Presented in part at the World Congresses of Gastroenterology (OMGE), Digestive Endoscopy (OMED) and Colo-proctology, Stockholm, Sweden, June 14–19, 1982

the pyloric antrum, they have been called pseudopyloric glands (PPG) (Häkkinen and Laitio 1970). Despite various investigations on these glands, the function of PPG in chronic cholecystitis remains unclear.

Since the normal glands of the pyloric antrum are known to contain and probably to secrete appreciable amounts of LM (Klockars and Reitamo 1975) we carried out an immunohistochemical study in order to demonstrate the presence of LM within PPG in chronic cholecystitis.

Materials and methods

Materials. Twelve gallbladders showing features of chronic cholecystitis with presence of PPG were studied. In addition, 18 gallbladders without obvious lesions or with features of acute or chronic cholecystitis but without PPG were used as material-controls. All material was obtained during surgery for cholelithiasis. Biopsies, taken from the fundus and corpus of the gallbladders and in 5 cases also from the neck region were fixed in Bouin's solution and stained with H and E. Serial sections were studied with the unlabeled immunoperoxidase method of Sternberger et al. (1970).

Methods. After deparaffinisation and rehydration, 5 μ sections were washed twice for 5 min each time in 0.01 M phosphate-buffered saline (PBS), pH 7.4. In order to reduce background staining, sections were pretreated with Brinase (Astra, Sweden) dissolved in 0.1% $CaCl_2$, pH 7.8, at a dilution of 1 mg/ml, for 30 min at 37° C (personal communication of Dr. R. O'Kennedy, University of Dublin, Ireland to Dr. M.J. Vanstapel, University of Leuven, Belgium). In order to block endogenous peroxidase activity, sections were treated with absolute methanol containing 0.3% H_2O_2 for 30 min. Sections were washed twice for 5 min each time with PBS, pH 7.4 and treated with normal swine serum (1:20 dilution, 7 min). This was followed by sequential application of the following antisera: 1. rabbit antihuman-LM antiserum (Dako, Denmark, 1:2,560 dilution, 30 min), 2. swine antirabbit-IgG antiserum (Dako, Denmark, 1:100 dilution, 30 min) and 3. peroxidase-antiperoxidase complex (Dako, Denmark, 1:100 dilution, 30 min). Each application of antiserum was followed by a 10-min wash in PBS, pH 7.4, twice for 5 min each time. The site of antibody binding was determined by the diaminobenzidine reaction (0.03% 3,3′ diaminobenzidine (DAB), Sigma Chemical Co, St. Louis, MA, USA, in PBS, pH 7.4, containing 0.01% H_2O_2, for 10 min). Sections were counterstained with haematoxylin or Alcian Blue, pH 2.5, in order to demonstrate acid mucosubstances.

Controls. Method-controls consisted of 1. omission of primary antiserum and substitution by PBS; 2. preabsorption of primary antiserum with purified human LM, kindly provided by Prof. Dr. G. Vantrappen, University of Leuven (20 μg LM per ml antiserum) and 3. use of DAB alone without primary or secondary antibodies to detect endogenous peroxidase-activity.

Results

The presence of LM was indicated by a brown reactionproduct. All polymorphonuclear granulocytes and macrophages disclosed intense staining.

The epithelial crypts that gave rise to PPG showed variable staining for LM, mostly confined to their pits. Either a diffuse cytoplasmic or basally located, subnuclear staining was observed (Fig. 1). In general the surface epithelium was negative.

In most, but not all PPG, immunoreactivity was found either throughout the cytoplasm in the form of a net-like staining or restricted to the basal

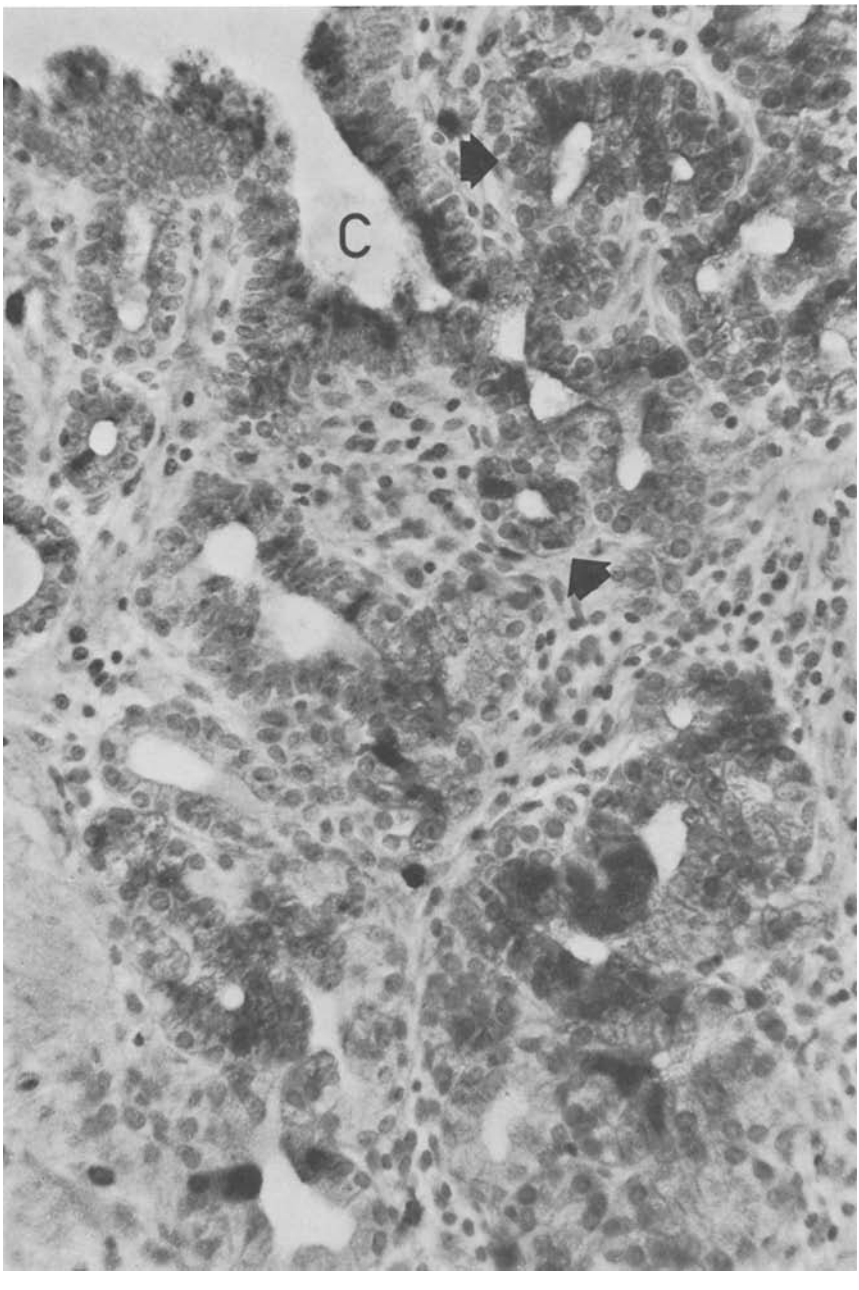

Fig. 1. Chronic cholecystitis. Branching epithelial crypt (*C*) surrounded by pseudopyloric glands (PPG) (*arrow*). LM-immunoreactivity is present in the pit of the epithelial crypt and in PPG (PAP-method for LM, counterstained with haematoxylin, ×400)

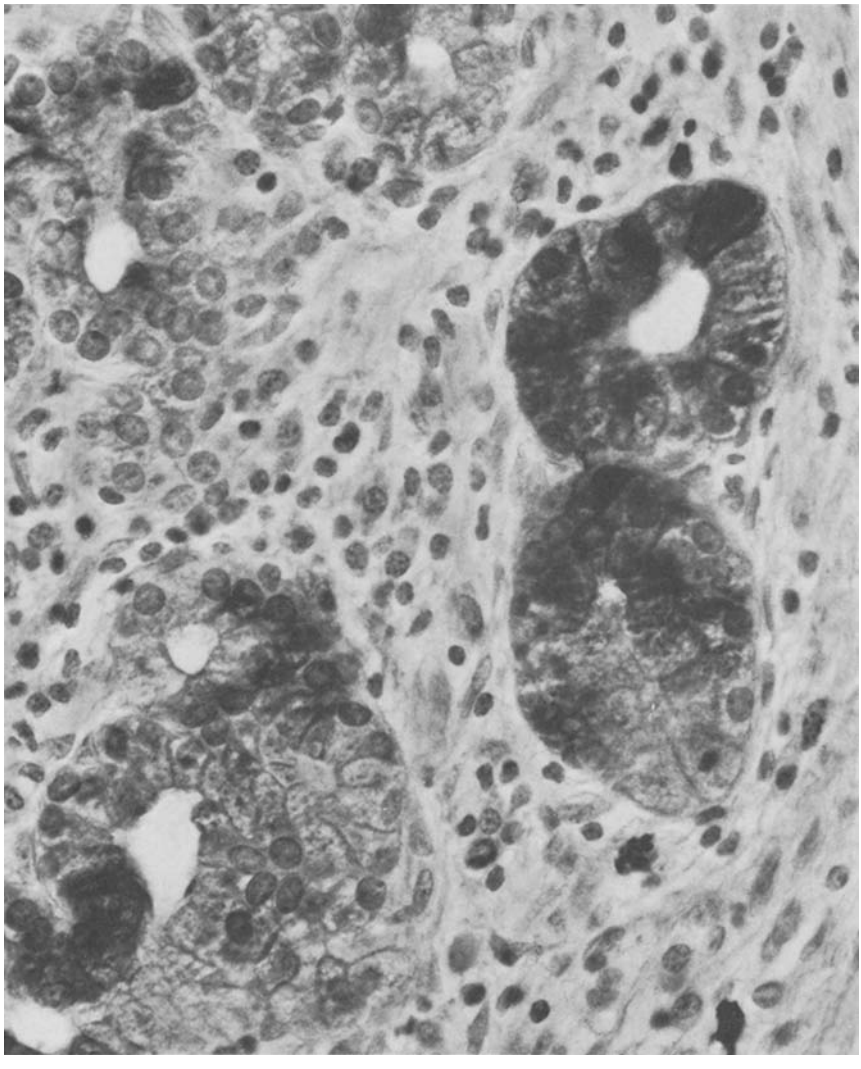

Fig. 2. PPG show net-like immunoreactivity for LM. Some inflammatory cells are also LM-positive (PAP-method for LM, counterstained with haematoxylin, × 640)

part of the lining cells (Fig. 2). In sections, counterstained with Alcian Blue, no immunoreactivity was found in those lining cells that contained acid mucosubstances.

In sections, taken from the neck region, no immunoreactive LM was found in the cytoplasm of cells constituting the neck-glands.

Cases of acute cholecystitis and chronic cholecystitis without PPG showed only immunoreactive inflammatory cells. In normal gallbladders no LM could be detected. No detectable staining was found in method-controls.

Discussion

PPG were first described by Ashoff (1905) and Schridde (1909). They are formed by branching off from the mucosal crypt epithelium, mostly close to the pits, followed by secondary branching of this glandular bud with subsequent formation of glandular aggregates (Laitio 1975). The occurrence of PPG correlates well with the presence of inflammation and lithiasis (Järvi and Meurman 1964).

In the findings presented above, we have demonstrated the presence of immunoreactive LM in the lining cells of PPG in chronic cholecystitis by use of the two-step immunoperoxidase method of Sternberger et al. (1970). Histochemically, like normal pyloric glands, PPG contain non-sulphated acid or, more often neutral mucins (Laitio 1975). In man, it has been found that LM in secretory cells is predominantly associated with the presence of neutral mucosubstances (Klockars and Reitamo 1975). The present demonstration of immunoreactive LM within most PPG and its absence from Alcian Blue-positive, acid mucuscontaining cells is in agreement with these data.

By immunofluorescence, using antisera directed against epithelial glycoproteins of true pyloric glands, Häkkinen and Laitio (1970) found that the pyloric-type mucosal areas of the inflamed gallbladder show similar antigenic properties to normal pyloric mucosa. Our findings suggest that the presence of LM in PPG and in normal pyloric glands may in part be responsible for this cross-reactivity.

We demonstrated the presence of LM in granulocytes and macrophages; these served as built-in method-controls since these cells have been found to contain LM (McClelland and van Furth 1975).

Earlier authors (Bolek and Machnik 1979) stated that the increase in mucous glands in chronic cholecystitis is due to a proliferation of glands in the neck region and subsequent extension of these glands throughout the gallbladder mucosa. In comparison with PPG however, neck glands differ in morphology, histochemistry and electron-microscopy (Laitio and Nevalainen 1975). Morphologically neck glands have wider lumina, bordered by cuboidal epithelium containing a centrally located, large round or oval nucleus. Mucin droplets are scarce. PPG however are composed of mucous cells with basally located, flattened nuclei and abundant cytoplasm, filled with mucin droplets. Histochemically, neck glands contain sulphated mucins whereas mucins in PPG are predominantly neutral (Laitio 1975). Ultrastructurally, neck glands contain few or no secretory granules and a poorly developed Golgi apparatus, whereas PPG resemble true pyloric glands by the presence of membrane-limited secretory granules and a prominent Golgi-apparatus (Laitio and Nevalainen 1975). The present finding of LM within PPG and some epithelial crypts and its absence from neck glands further indicates the difference between both types of glands and supports the assumption that PPG originate from the surface epithelium as a metaplastic phenomenon.

PPG are not restricted to the inflamed gallbladder. Roberts (1974) dis-

cussed pyloric gland metaplasia in chronic cholecystitis and showed these PPG histochemically to resemble both normal pyloric glands and metaplastic mucous glands in the small bowel and gallbladder mucosa. Recently, we demonstrated the presence of immunoreactive LM in metaplastic pyloric glands in chronic pancreatitis (van den Oord et al. 1982). Moreover, in cases of Crohn's disease PPG are a well established feature (Liber 1951) and we demonstrated the presence of immunoreactive LM in the neutral mucin-containing PPG of the terminal ileum in patients suffering from Crohn's disease [Geboes et al. (1982) Immunohistochemical identification of lysozyme in pseudopyloric gland metaplasia in Crohn's disease (submitted for publication)].

Therefore, pyloric gland metaplasia seems to be a common, functional reaction of the pancreato-biliary and small intestinal mucosa in response to chronic inflammation.

The presence of LM in the secretory cells of PPG in chronic cholecystitis is probably related to an extracellular antibacterial action. This bactericidal activity may require the interaction of LM and IgA since it has been shown that LM discloses considerable bactericidal activity in vitro in conjunction with IgA and complement (Bladen et al. 1973; Hill and Porter 1974). Green and Fox (1972) demonstrated that IgA is the predominant type of immunoglobulin, secreted by lymphoid cells in the inflamed gallbladder. Moreover, it has been reported that IgA is the major immunoglobulin present in bile (Dive 1970).

To conclude, the present results show the presence of immunoreactive LM in PPG, demonstrating a further analogy with true pyloric glands. The presence of LM also suggests that PPG represent functional metaplastic areas, involved in the non-specific defence mechanisms of the host against environmental pathogenic and non-pathogenic microorganisms through participation of LM.

References

Ashoff L (1905) Bemerkungen zur pathologischen Anatomie der Cholelithiasis und Cholecystitis. Verh Dtsch Ges Pathol 9:41–48

Bladen H, Hageage G, Harr T, Pollock F (1973) Lysis of certain organisms by the synergistic action of complement and lysozyme. J Dent Res 52:371–376

Bolek F, Machnik G (1979) Spezielle pathologische Anatomie, Bd 10, Leber und Gallenwege. W Doerr, G Seifert, E Uehlinger (eds). Springer, Berlin Heidelberg New York, p 735

Chipman DM, Sharon N (1969) Mechanism of lysozyme action. Science 165:454–465

Dive C (1970) Les protéines de la bile, leur composition et leur origine. Thesis, Brussels

Ghoos Y, Vantrappen G (1971) The cytochemical localization of lysozyme in Paneth cell granules. Histochem J 3:175–178

Green FHY, Fox H (1972) An immunofluorescent study of the distribution of immunoglobulin containing cells in the normal and inflamed gallbladder. Gut 13:379–384

Häkkinen I, Laitio M (1970) Epithelial glycoproteins of Human Gallbladder. Arch Pathol 90:137–142

Hill IR, Porter P (1974) Studies of bactericidal activity to Escherichia Coli of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. Immunology 26:1230–1250

Järvi O, Meurman L (1964) Heterotopic gastric mucosa and pancreas in the gallbladder with reference to the question of heterotopias in general. Ann Acad Scient Fennicae 106:1–42
King ESJ, McCallum P (1931) Cholecystitis glandularis proliferans (cystica). Br J Surg 19:310–323
Klockars M, Reitamo S (1975) Tissue distribution of lysozyme in man. J Histochem Cytochem 23:932–940
Laitio M (1975) Goblet Cells, Enterochromaffin Cells, Superficial Gastric-Type Epithelium and Antral-Type Glands in the Gallbladder. Beitr Pathol 156:343–358
Laitio M, Nevalainen T (1975) Gland ultrastructure in human gallbladder. J Anat 120:105–112
Liber AF (1951) Aberrant pyloric glands in regional ileitis. Arch Pathol 51:205–212
Mason DY, Taylor CR (1975) The distribution of muramidase (lysozyme) in human tissues. J Clin Pathol 28:124–132
McClelland DBL, Furth R van (1975) In vitro synthesis of lysozyme by human and mouse leucocytes and tissues. Immunology 28:1099–1114
Nicholson GW (1923) Heteromorphoses (metaplasia) of the alimentary tract. J Pathol 26:399–417
Peeters T, Vantrappen G (1975) The Paneth cell: a source of intestinal lysozyme. Gut 16:553–558
Roberts PF (1974) Pyloric gland metaplasia of the human pancreas. Arch Pathol Lab Med 97:92–95
Schridde H (1909) Die ortsfremde Epithelgewebe des Menschen. Untersuchungen und Betrachtungen. Sammlung anatomischer und physiologischer Vorträge und Aufsätze. Gaupp E, Nagel W (eds). Gustav Fischer, Jena
Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG (1970) The unlabelled antibody enzyme method for immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochaetes. J Histochem Cytochem 18:315–333
Van den Oord JJ, Geboes K, Desmet VJ (1982) Immunohistochemical demonstration of lysozyme in chronic pancreatitis. Scand J Gastrenterol (in press)

Accepted January 10, 1983