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An immunocytochemical study of the distribution of lysozyme, a_1-antitrypsin and a_1-antichymotrypsin in the normal and pathological gall bladder

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Summary. We have studied the distribution of lysozyme (Ly), a_1-antitrypsin (a_1AT) and a_1-antichymotrypsin (a_1AChy) in the normal, chronically inflamed and neoplastic gall bladder mucosa using the peroxidase-anti-peroxidase (PAP) method. Ly was absent from the normal mucosa but it was found only in areas of glandular metaplasia of true antral type and in crypts of possible early metaplastic nature in cases of chronic cholecystitis. a_1AT and a_1AChy were also found in such metaplastic areas, but their presence was also observed immunohistochemically in areas of essentially normal and in non-metaplastic, chronically inflamed gall bladder mucosa. The possible local production of these substances by gall bladder epithelial cells is discussed. Ly, a_1AT and a_1AChy were also found in various histological types of adenocarcinoma of the gall bladder in varying degrees of frequency and intensity, unrelated to the histological type and invasiveness of the tumour.

Key words: Gall bladder – Lysozyme – a_1-Antichymotrypsin – a_1-Antitrypsin

The distribution of lysozyme (Ly), a_1-Antitrypsin (a_1AT) and a_1-Antichymotrypsin (a_1AChy) has recently been studied in various parts of the normal adult gastrointestinal tract (Mason and Taylor 1975; Montero and Erlandsen 1978; Geboes et al. 1982; Kittas et al. 1982a). Furthermore, these three markers have been demonstrated immunohistochemically in adenocarcinomas of the gastrointestinal tract (Heitz and Wegmann 1980; Kittas et al. 1982b).

In view of the common endodermal embryological origin of gastric, small intestinal, and gall bladder mucosa (Moore 1974), an immunohistochemical study was undertaken to investigate the presence of Ly, a_1AT

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and a_1AChy in normal, chronically inflamed and neoplastic epithelium of the gall bladder.

To our knowledge, the presence of Ly in neoplastic and a_1AT and a_1AChy in both normal and neoplastic epithelium of the gall bladder has not been previously reported.

Materials and methods

We investigated 10 gall bladders without obvious lesions, 10 cases with changes of chronic cholecystitis, 2 of which showed antral type epithelial metaplasia and 18 cases of various histological types of gall bladder adenocarcinoma. The material was fixed in 10% buffered formalin solution and embedded in paraffin. Sections 5 μm thick were cut and stained with haematoxylin and eosin.

On the basis of their histological characteristics the tumours were classified as a) poorly differentiated, b) well differentiated, c) papillary and d) mucinous types of adenocarcinoma (Table 1).

Immunohistochemical localization of Ly, a_1AT and a_1AChy was accomplished using the peroxidase-antiperoxidase (PAP) method, as previously described (Kittas et al. 1982a, b). Briefly, deparaffinized sections were treated with methanol solution containing 0.3% H_2O_2. Nonspecific background staining was reduced with normal swine serum. The sections were incubated with Ly, a_1AT and a_1AChy rabbit antisera with prior trypsination at a dilution of 1:100. Swine antirabbit serum and PAP complex were subsequently applied. The site of peroxidase localization was made visible by developing slides in freshly made Diaminobenzidine solution (6 mg of DAB with 0.01% H_2O_2 in 10 ml of tris buffer). All preparations were counterstained with haematoxylin. Our antisera were obtained from Dakopatts (Copenhagen, Denmark). Slides obtained through the substitution of nonimmune rabbit serum for the Ly, a_1AT and a_1AChy antisera and a slide treated with DAB reagent alone served as control.

Table 1. Histological type of gall bladder adenocarcinomas with associated immunohistochemical reaction

Histological type	Ly	a_1AT	a_1AChy[a]
1. Adenocarcinoma poorly differentiated	++++	+	+++
2. Adenocarcinoma well differentiated	−	++	+++
3. Adenocarcinoma well differentiated	+	+	++
4. Adenocarcinoma well differentiated	+	++	+
5. Adenocarcinoma well differentiated	+	−	+
6. Adenocarcinoma well differentiated	−	+	++
7. Adenocarcinoma well differentiated	−	++	++
8. Adenocarcinoma well differentiated	+	−	+
9. Adenocarcinoma well differentiated	+++	++	++
10. Adenocarcinoma well differentiated	−	+	++
11. Adenocarcinoma well differentiated	−	+	++
12. Adenocarcinoma papillary type	−	+	+
13. Adenocarcinoma papillary type	++++	++	+
14. Adenocarcinoma papillary type	−	−	+
15. Adenocarcinoma papillary type	−	+	++
16. Adenocarcinoma papillary type	++	+++	+++
17. Adenocarcinoma mucinous type	−	−	+
18. Adenocarcinoma mucinous type	+	+	+++

[a] (−) negative, (+) slightly, (++) moderately, (+++) strongly and (++++) very strongly positive immunohistochemical reaction

Results

The results of our immunohistochemical study are shown in Table 2.

A moderately positive intracytoplasmic reaction for a_1AT and a_1AChy was found in extensive areas, mainly of the superficial epithelium, in 6 of the 10 normal gall bladders (Fig. 1). There was no positive staining for Ly in the normal epithelial cells of the gall bladder mucosa (Table 2). a_1AT and a_1AChy (Fig. 2) were detected in 5 and 6 cases respectively of chronic cholecystitis with no obvious metaplasia (Table 2). In 3 of the 8 cases of the latter group, occasional epithelial crypts but no superficial cell showed

Table 2. Number of the immunohistochemically positive cases in normal and variably pathological gall bladders for Ly, a_1AT and a_1AChy

	Normal[a]	Chronic Cholecystitis			Adenocarcinoma
		without obvious metaplasia	or	with antral metaplasia	
Ly	0/10 + ve	3/8 + ve		2/2 + ve	9/18 + ve
a_1AT	6/10 + ve	5/8 + ve		2/2 + ve	14/18 + ve
a_1AChy	6/10 + ve	6/8 + ve		2/2 + ve	18/18 + ve

[a] No of positive cases/No of tested cases

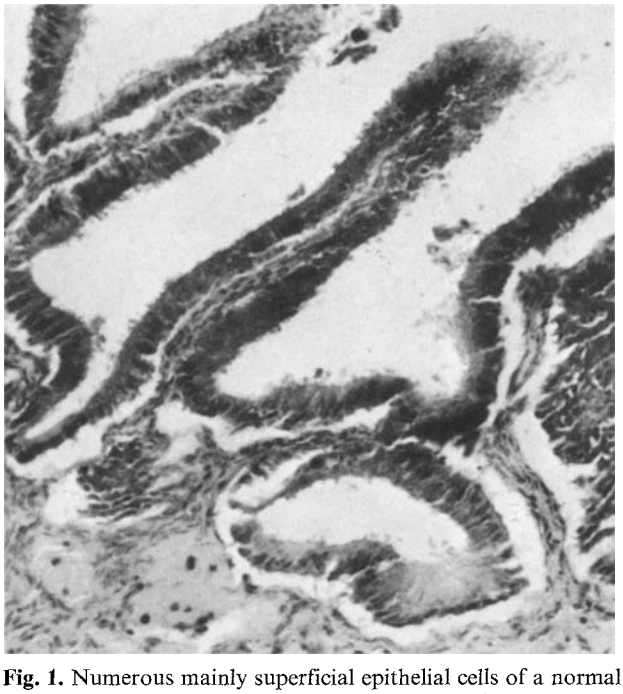

Fig. 1. Numerous mainly superficial epithelial cells of a normal gall bladder showing a moderately a_1AT-positive reaction (PAP-method × 160)

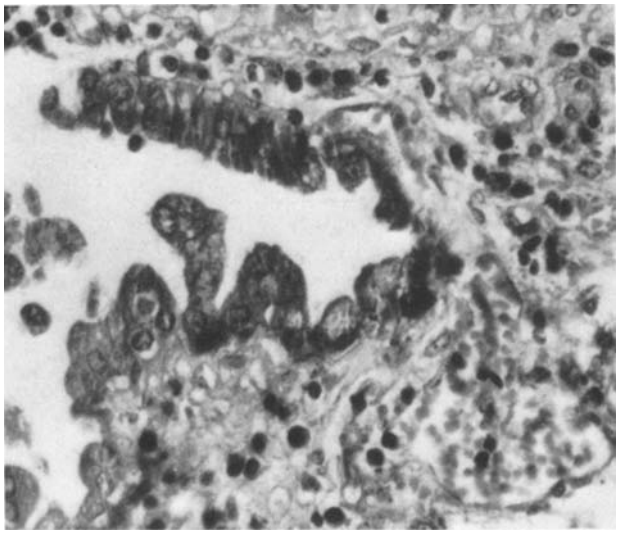

Fig. 2. A case of chronic cholecystitis with numerous moderately a_1AChy-positive epithelial cells. The inflammatory cells of the lamina propria are also strongly positive (PAP-method $\times 300$)

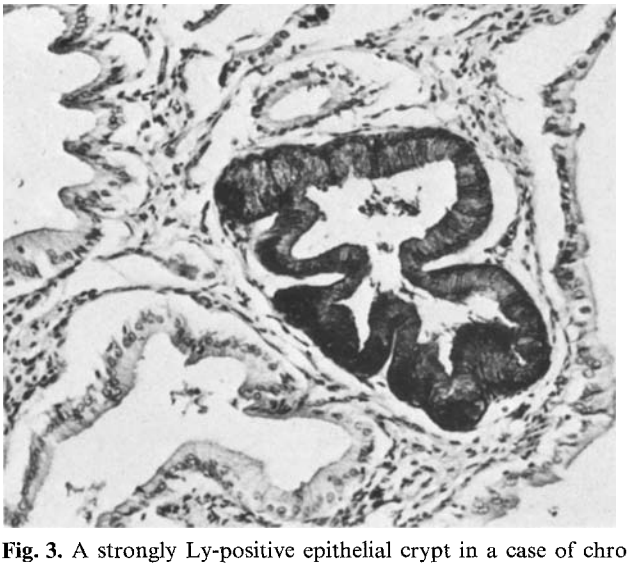

Fig. 3. A strongly Ly-positive epithelial crypt in a case of chronic cholecystitis (PAP-method $\times 125$)

slightly to strongly positive immunohistochemical reaction for Ly (Fig. 3 and Table 2). A slight to moderate positivity was obtained for all three enzymes in the cytoplasm of metaplastic epithelial cells in both cases of chronic cholecystitis with antral type glandular metaplasia (Fig. 4 and Table 2).

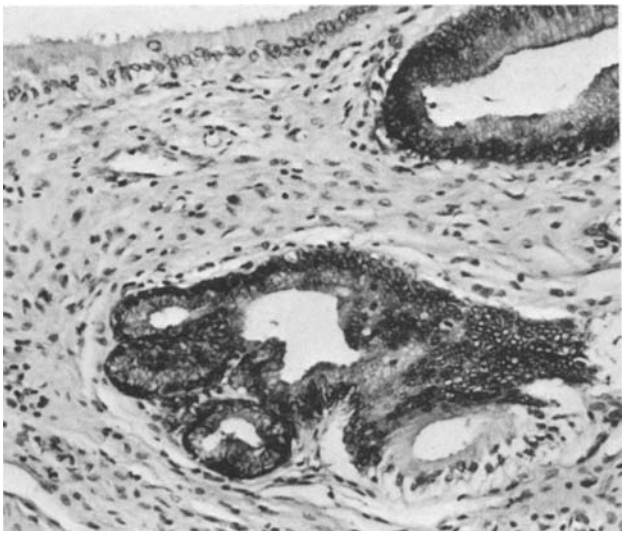

Fig. 4. Moderate Ly-positive epithelial cells in area of antral metaplasia of the gall bladder mucosa. The superficial normal epithelium is completely negative (PAP-method ×125)

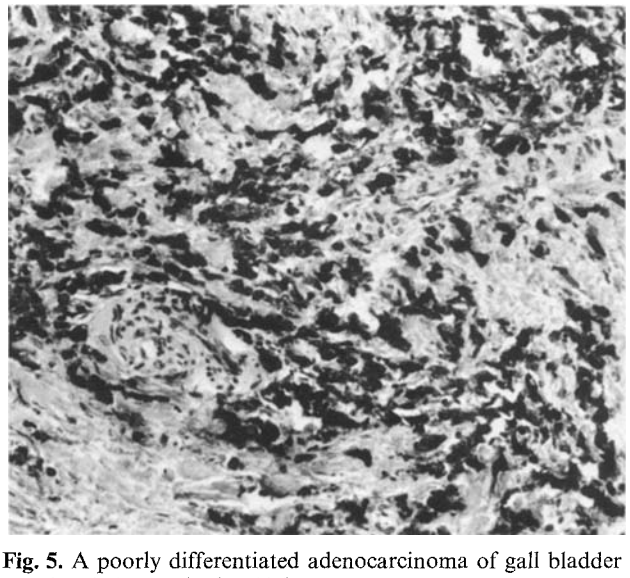

Fig. 5. A poorly differentiated adenocarcinoma of gall bladder with very strongly Ly-positive reaction (PAP-method ×125)

The most impressive results were found in the 18 cases of adenocarcinoma, as given in Table 1. The intensity of the reaction was estimated using the following scale: negative (−), slightly positive (+), moderately positive (++), strongly positive (+++) and very strongly positive (++++). There was no obvious correlation between the histological type and invasive-

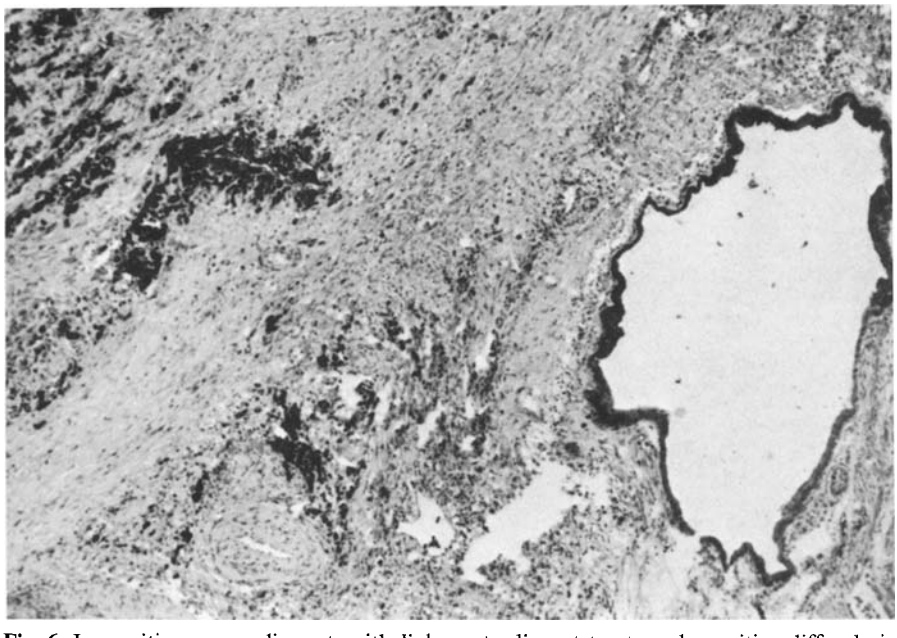

Fig. 6. Ly-positive non-malignant epithelial crypt adjacent to strongly positive diffusely infiltrated malignant cells (PAP-method ×40)

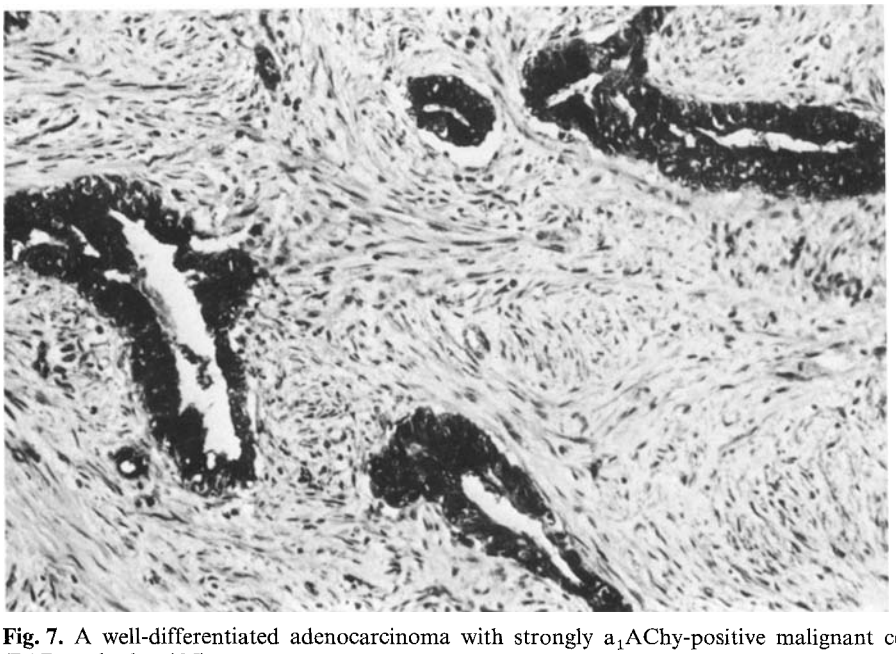

Fig. 7. A well-differentiated adenocarcinoma with strongly a_1AChy-positive malignant cells (PAP-method ×125)

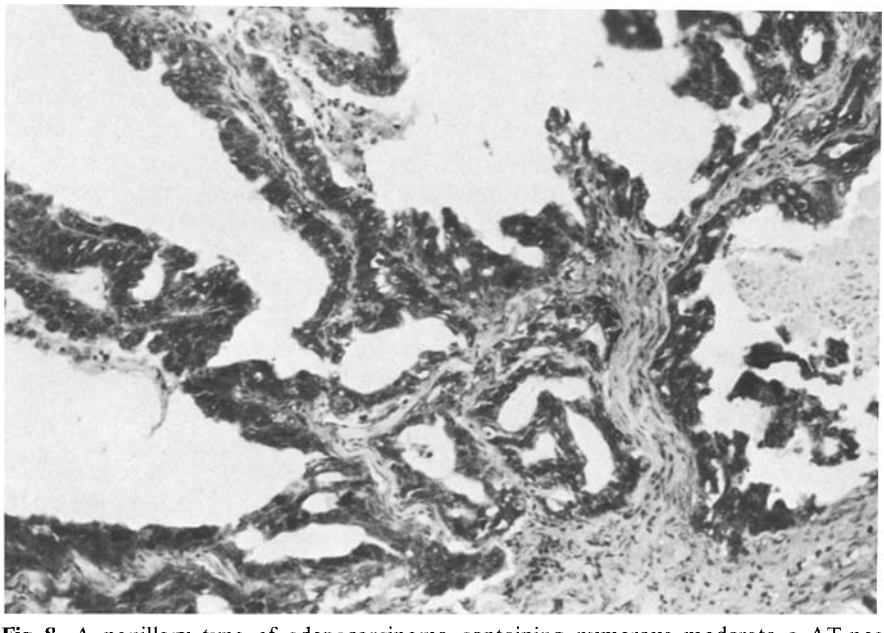

Fig. 8. A papillary type of adenocarcinoma containing numerous moderate a_1AT-positive malignant cells (PAP-method × 125)

ness of adenocarcinomas and their immunohistochemical profile. The Ly reaction was slightly to very strongly positive (Fig. 5) in nine cases, while in the remainder it was completely negative. In several cases a few epithelial crypts without features of malignant or dysplastic changes produced strongly Ly-positive immuno-reactivity, identical to that of the adjacent adenocarcinoma (Fig. 6). a_1AChy was slightly to strongly positive (Fig. 7) in malignant cells of all cases investigated while a positive reaction for a_1AT of variable intensity was seen in all but four gall bladder adenocarcinomas (Fig. 8 and Table 1). In one mucinous type of adenocarcinoma (No. 18 of Table 1) an intestinal type of glandular metaplasia was observed in the adjacent mucosa, containing Ly-positive non-malignant Paneth cells, and a_1AT-, a_1AChy-positive bening epithelial absorptive lining cells. Two adenocarcinomas of the gall bladder (Nos. 10 and 11 of Table 1) had metastasized to the liver. The primary and metastatic malignant cells showed identical immunohistological profiles having Ly-negative and a_1AT-, a_1AChy-positive immunohistochemical reaction.

Discussion

Our results confirm previous observations concerning the absence of Ly from the epithelial cells of normal gall bladder (Klockars and Reitamo 1975; Van den Oord et al. 1983) and its presence in antral type metaplastic glands in cases of chronic cholecystitis (Van den Oord et al. 1983). Moreover, we have shown that these metaplastic glands are also positive for

a_1AT and a_1AChy, having a close resemblance to normal pyloric glands of the gastric antrum which have been found to contain all these enzymes (Kittas et al. 1982a). Such a resemblance has been previously suggested by Hakkinen and Laition (1970), who studied the epithelial glycoproteins of the human gall bladder. We have, however, also demonstrated Ly-positive epithelial cells in occasional crypts in three cases of chronic cholecystitis or in several cases adjacent to adenocarcinomas which do not have the appearance typical of any type of glandular metaplasia. These epithelial crypts may represent an early stage of metaplastic change.

Ly is also found in malignant cells in half of the cases of adenocarcinoma in our series. The absence of Ly from normal gall bladder mucosa and its presence in metaplastic and neoplastic epithelial cells of this organ support the previously reported suggestion that a relation exists between metaplasia and adenocarcinoma of the gall bladder (Azabeh and Parai 1980; De Boer et al. 1981).

a_1AT and a_1AChy show a slight to moderate positive intracytoplasmic reaction in numerous mainly superficial epithelial cells in more than half of the essentially normal gall bladders tested. Since the liver was originally considered as being the main source of production of a_1AT (Talamo 1971; Kyaw-Myint et al. 1975), which subsequently may well be partly secreted in the bile (Kyaw-Myint et al. 1975), one may argue that the presence of a_1AT in the normal gall bladder is due to absorption rather than to local cellular production. We have recently shown that some types of gastric and intestinal epithelial cells may serve as additional sources of a_1AT (Kittas et al. 1982a). It is possible that the epithelial cells of the normal gall bladder constitute another area of a_1AT synthesis, since the liver, gall bladder, stomach and small intestine have a common endodermal origin (Moore 1974).

a_1AChy has been less extensively studied and its presence in the bile has not been reported. However, we have previously found a_1AChy in other parts of the gastrointestinal tract (Kittas et al. 1982a). We do believe that a_1AChy is also locally produced by the epithelial cells of normal gall bladder mucosa, in a way analogous to that in which a_1AT is produced.

Since a_1AT and a_1AChy are seen in normal gall bladder mucosa, their presence in non-metaplastic epithelial cells in cases with chronic cholecystitis is not unexpected. Moreover, positive immunoreactivity for these enzymes in most cases of adenocarcinoma of the gall bladder, even in deeply invasive malignant cells, strongly supports the possibility of local epithelial cell production rather than that of absorption from the bile content. It is of interest to note that the capacity to produce a_1AT and a_1AChy appears to be maintained in numerous malignant cells of primary and metastatic lesions. In particular, in our two cases of gall bladder adenocarcinoma with hepatic metastasis the immunohistochemical reactions of the latter were identical to those of the primary tumour, being both Ly-negative and a_1AT-, a_1AChy-positive. It is obvious that the presence of these enzymes in metastatic cells lends further support to their being produced locally.

At present, no correlation between the immunohistochemical profile and the histological type and invasiveness of adenocarcinoma of the gall bladder can be established. Certainly, more

extensive studies, including a follow-up of the patients, need to be made for the clarification of the prognostic value of the presence of Ly, a_1AT and a_1AChy in gall bladder adenocarcinoma.

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