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Localization of CEA, HCG, Lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotrypsin in gastric cancer and prognosis

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Summary. Carcinoembryonic antigen (CEA), β-human chorionic gonadotropin (HCG), alpha-1-antichymotrypsin (ACT), alpha-1-antitrypsin (AAT) and Lysozyme (LYS) were traced by immunoperoxidase staining in gastric carcinomas. The immunohistological results were evaluated in relation to histological types (WHO and Laurén), stage of disease, grade and survival time. CEA was demonstrated in 96% of the tumours, HCG in 34%, ACT in 78%, AAT in 42%, and LYS in 71%. Comparing the staining patterns of the antigens and the intensity of staining some differences were notable. Except for signet-ring cell carcinomas, all of which were intensively positive, CEA expression decreased significantly with loss of differentiation. This observation was not seen with the other marker substances. None of the tested markers was characteristic for one particular histological type, nor could they be correlated with the tumour stage or grade. The marker positivity of CEA, ACT and LYS was not related to survival time. For HCG only, a correlation between tissue expression and a restricted survival time was established. Patients with AAT positive carcinomas had a significantly better survival probability.

Key words: Tumour related antigens – Gastric cancer – Immunohistochemistry

Introduction

In an attempt to understand the biological behaviour of gastric cancer better, studies of the expression of glycoproteins such as alpha-1-antichymotrypsin (ACT), alpha-1-antitrypsin (AAT) and lysozyme (LYS), normally produced by different parts of the stomach, have been made (Kittas et al. 1982; Tahara et al. 1982; Capella et al. 1984). It has been suggested that the protease

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inhibitors ACT and AAT are related to the ability to exhibit invasive growth of these tumours (Tahara et al. 1984). Gastric carcinomas producing peptide hormones like lysozyme or human chorionic gonadotropin (HCG) were found to have a poor prognosis by Tahara et al. (1982) and Ito and Tahara (1983). Carcinoembryonic antigen (CEA), first described in extracts of colonic adenocarcinomas, has also been described in gastric signet-ring cell carcinomas and later in carcinomas of other histological types (Gold and Freedman 1965; von Kleist and Burtin 1966; Burtin et al. 1973; Denk et al. 1973; Nielsen and Teglebjoerg 1982). The aim of this study was to demonstrate the presence of CEA, HCG, lysozyme, ACT and AAT in gastric cancer and to attempt to correlate the staining patterns with histological types, stage of disease, tumour grade and survival time.

Material and methods

93 gastric carcinoma patients were studied, 70 had had a resectable tumour and from 23 biopsies were available. Patients included in this study were treated at the Dept. of Surgery, University Clinic from 1982–1984. At the end of this study we had data from all patients concerning the extent of their disease.

The formalin fixed and paraffin embedded tissues were sectioned at 5 μm. The histological diagnosis of the tumours was made on haematoxilin eosin (HE) and alcian blue – Schiff's reagent (AB-PAS) stained sections according to the classifications of the World Health Organization (1977) and Laurén (1965). Samples of all tumours were stained for CEA, beta-chain of HCG (HCG), LYS, ACT and AAT. The CEA antiserum was raised in rabbits and prepared as previously described (Wolf et al. 1984). All other antisera were purchased from Dakopatts (Denmark). The staining procedure employed was the indirect immunoperoxidase method. After deparaffination sections were treated with 1% hydrogen peroxide in absolute methanol to block endogenous peroxidase. To reduce background staining the sections were incubated with 5% ovalbumin (Sigma Chemicals, St. Louis, MO, USA) and 10% normal swine serum. Between each incubation step the sections were washed with Tris-buffered saline (TBS), pH 7.4. The incubation with the first and second antiserum was performed for 30 min at room temperature, except for the anti-HCG serum, which was incubated at 37° C. Peroxidase activity was developed by diaminobenzidine tetrahydrochlorid (DAB) with 0.012% hydrogen peroxide. The sections were counterstained in Mayer's haemalaun, dehydrated and mounted in Entellan.

Controls consisted of 1) absorption of the primary antiserum with highly purified CEA, obtained according to Grunert et al. (1983) or to HCG (Boehringer, Mannheim, West-Germany) respectively, 2) replacement of the primary antiserum by nonimmune rabbit serum of the same dilution, 3) replacement of the primary antiserum by the diluent (TBS), 4) treatment of the slides with the DAB/hydrogen peroxide/TBS solution without prior antibody incubation. Staining of control sections was always negative.

Staining results were evaluated semiquantitatively, taking into account the number of the stained cells ($<10\% = 0$ point, 10–40% = 1 point, 40–70% = 2 points, 70–100% = 3 points) and the staining intensity (negative = 0 point, weakly positive = 1 point, moderately positive = 2 points, strongly positive = 3 points).

The contingency tables were evaluated with the Chi-squared test. Survival analysis and non-parametric-statistic programs were used from the BMDP (University of California) according to Peto et al. (1977).

Results

CEA was demonstrated in 96% of the gastric carcinomas in the cytoplasm of the tumour cells and at the luminal border of carcinomatous glandular

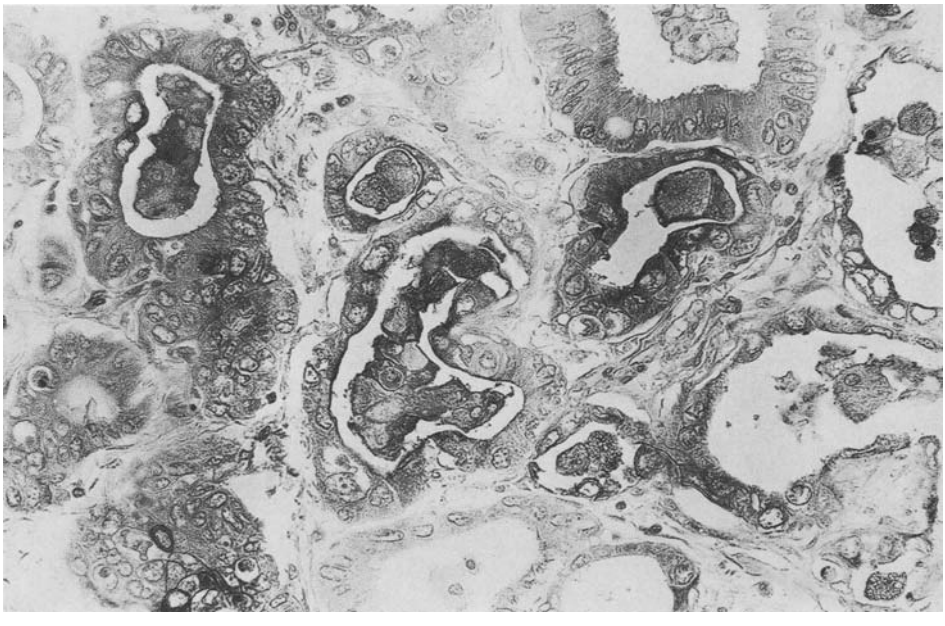

Fig. 1. CEA immunoreactivity in a moderately differentiated tubular adenocarcinoma of the stomach with an accentuated staining at the luminal cell membrane (original magnification ×240)

formations. In highly differentiated tumours CEA positivity was seen predominantly at the cell border, whereas in the less differentiated CEA staining was observed more intracytoplasmatically, in a fine granular pattern. The percentage of CEA positivity of the highly differentiated carcinomas was similar to that of the undifferentiated ones, however, the semiquantitatively estimated amount of staining was significantly higher in the well differentiated carcinomas. In these latter tumours almost all cells were positive, whereas in the second group there was often only a focal positivity. The signet-ring cell carcinomas were in 100% positive for CEA, with a fine granular staining intracytoplasmatically excluding the mucin vacuoles. There was no difference between the central region and the invading parts of these tumours with regard to the CEA staining (Fig. 1).

Faint CEA staining was observed in the cytoplasm of the normal cells of the mucoid surface epithelium of corpus and antral mucosa. Furthermore, a few antral glands were weakly positive.

HCG was seen in 34% of the cases. It was located either in few single cells or in little cell clusters, occurring mainly in the cytoplasm of the tumour cells in fine granules which were sometimes concentrated in the perinuclear region. In the signet-ring cell carcinomas the mucin containing vacuole did not stain for HCG (Fig. 2).

In the normal mucosa adjacent to the carcinomas HCG was demonstrated in few single cells of an occasional gland in different parts of the stomach, often near the pylorus and near the carcinomas (Fig. 3).

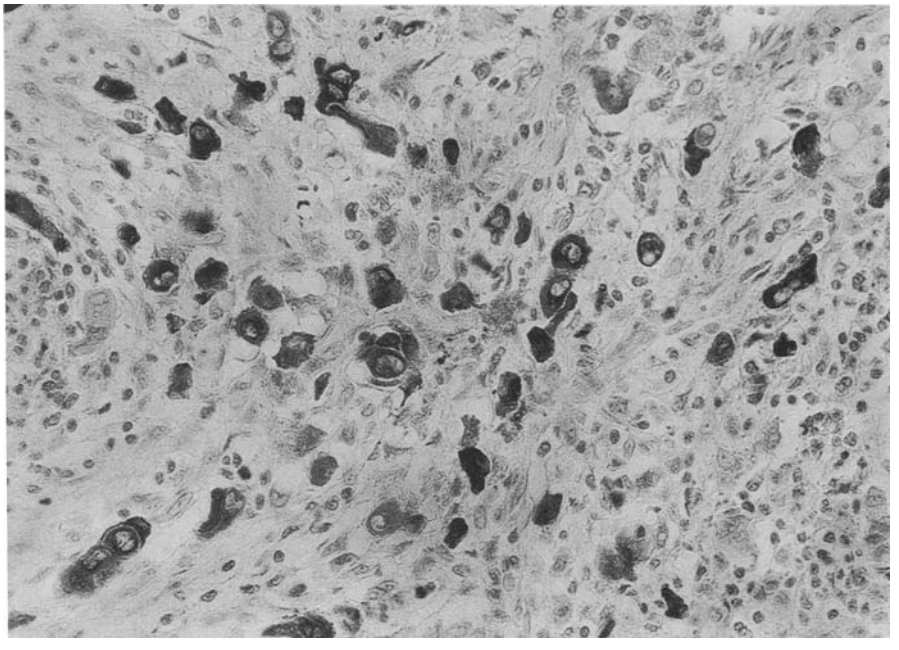

Fig. 2. βHCG immunoreactivity in a lymphnode metastases of a signet ring cell carcinoma after concentrated in the perinuclear region, without staining of the mucus containing vacuoles (original magnification $\times 400$)

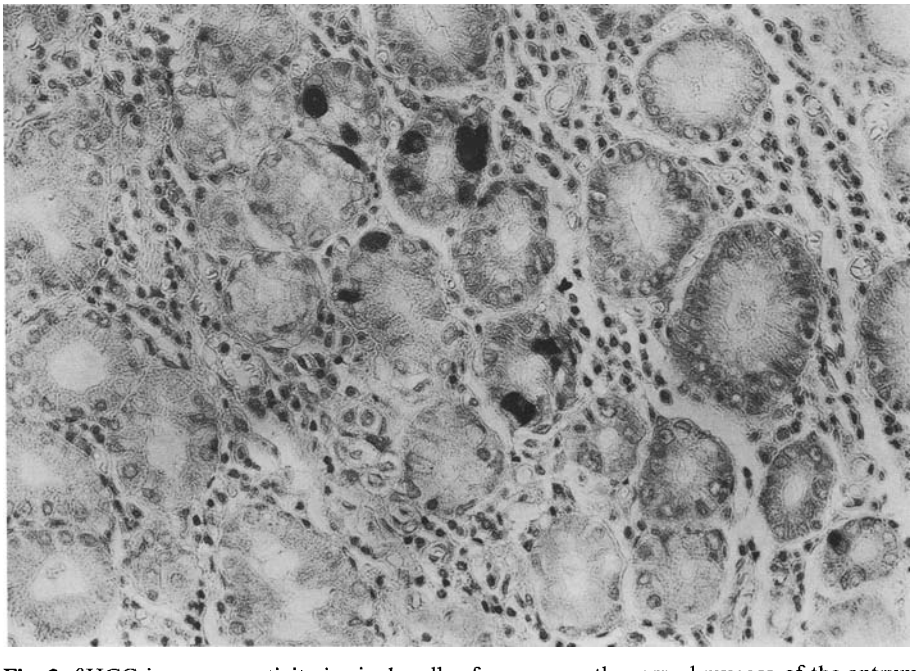

Fig. 3. βHCG immunoreactivity in single cells of an apperently normal mucosa of the antrum at a distance of 2 cm from the tumour (original magnification $\times 320$)

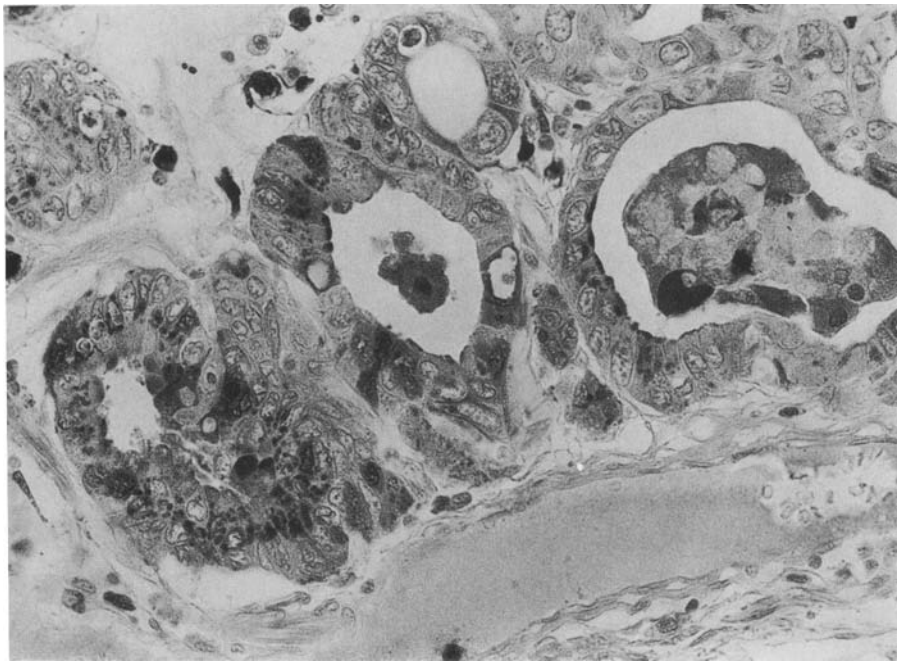

Fig. 4. Lysozyme positivity in a tubular adenocarcinoma of the stomach (same case as Fig. 1) showing a coarse granular intracytoplasmatical staining pattern. Lysozyme positive inflamatory cells are spread through the tumor (original magnification ×400)

Lysozyme was found in 71% of the carcinomas, mostly with the same intensity as CEA. Lysozyme staining was also seen intracytoplasmatically in fine and course granules, often located in the luminal region of the cell and sometimes around the nucleolus (Fig. 4). Lysozyme was also present in normal epithelial cells of the pylorus and cardiac glands, in Paneth' cells, Brunner's glands and in "inflammatory cells" of the normal mucosa. The inflammatory cells were spread throughout the carcinomas in differing numbers.

ACT was stainable in 78% of the cases, again the staining intensity was similar to that of CEA, however, the ACT positive cells were often located in tumour regions where cells did not stain or stained only very weakly for CEA. Areas with tubular or papillar differentiation and signet-ring cell carcinomas were more often positive, with parts of the same tumour being negative. Glands with positive and negative cells side by side were characteristic for ACT (Fig. 5).

The staining pattern of AAT resembled that of ACT, but the frequency of positivity of the latter was much lower, i.e. 42%.

There was no correlation of any marker positivity with the Laurén classification of gastric cancer. The distribution of the markers among the different histological types of WHO classification and the correlation of marker staining behaviour with the stage of disease and tumour grade is shown

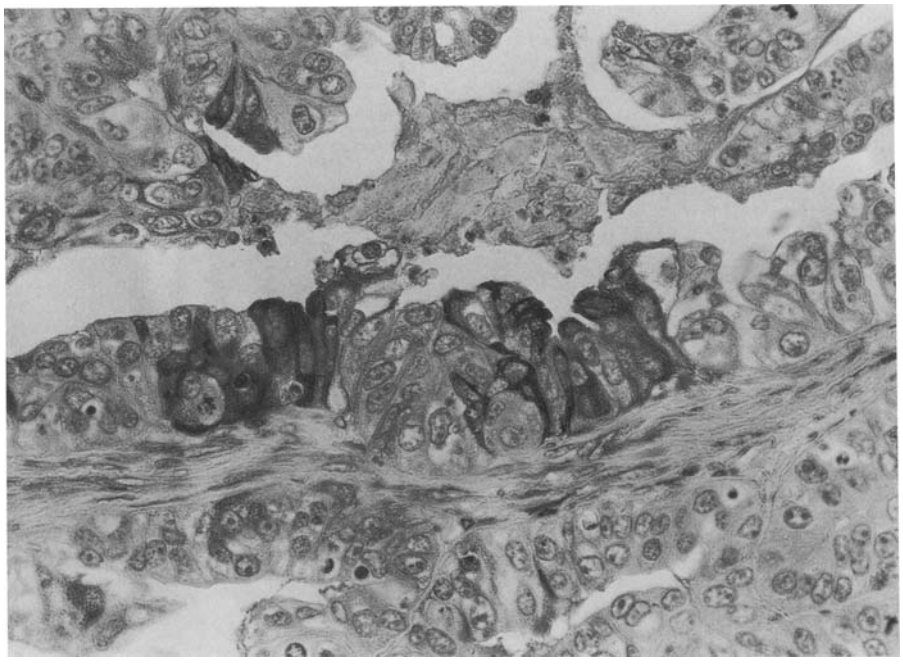

Fig. 5. α_1 Antichymotrypsin immunoreactivity in a tubular adenocarcinoma of the stomach showing positive and negative cells side by side in a fine granular staining pattern (original magnification × 500)

Table 1. Occurrence of marker substances and histological type (WHO) of gastric carcinomas

Histological type	Number of cases positive					Total No. of cases
	CEA	HCG	LYS	ACT	AAT	
Adenocarcinoma						
papillary	14	4	10	10	5	15
tubular	29	10	23	25	17	31
mucinous	4	0	1	2	2	4
Undifferentiated ca.	12	2	7	12	6	13
Signet-ring cell ca.	30	16	25	24	9	30
	89 (96%)	32 (34%)	66 (71%)	73 (78%)	39 (42%)	93 (100%)

in Tables 1–4. None of the tested markers was characteristic for one particular histological type, nor could they be correlated with the stage or the tumour grade. The only tendency observed was that HCG and lysozyme were more often seen in signet-ring cell carcinomas, while ACT and AAT were more often expressed by undifferentiated carcinomas. The marker positivity of CEA and ACT was not related to survival time. Patients with

Table 2. Occurrence of marker substances and histological type (Laurén) of gastric carcinomas

Histological type	Number of cases positive (%)					Total No. of cases
	CEA	HCG	LYS	ACT	AAT	
Intestinal type	41 (93%)	13 (30%)	29 (66%)	32 (73%)	21 (48%)	44
Diffuse type	48 (98%)	19 (39%)	37 (76%)	41 (84%)	18 (37%)	49
	49 (96%)	32 (34%)	66 (71%)	73 (78%)	39 (42%)	93 (100%)

Table 3. Occurence of marker substances in different stages of gastric cancer

Stage	No. of cases positive (%)					Total No. of cases
	CEA	HCG	LYS	ACT	AAT	
I	7 (100%)	2 (29%)	5 (71%)	5 (71%)	2 (29%)	7
II	11 (100%)	3 (27%)	8 (73%)	9 (82%)	3 (27%)	11
III	41 (98%)	20 (48%)	34 (81%)	39 (93%)	29 (69%)	42
IV	30 (91%)	7 (21%)	19 (58%)	20 (61%)	5 (15%)	33
	89 (96%)	32 (34%)	66 (71%)	73 (78%)	39 (42%)	93 (100%)

Table 4. Occurrence of marker substances and histological grade of gastric carcinomas

Histological grade	No. of cases positive					Total No. of cases
	CEA	HCG	LYS	ACT	AAT	
Grade 1	7	1	6	4	2	8
Grade 2	23	10	15	18	9	24
Grade 3	17	3	13	15	13	18
Undifferentiated ca.	12	2	7	12	6	13
Signet-ring cell ca.	30	16	25	24	9	30
	89 (96%)	32 (34%)	66 (71%)	73 (78%)	39 (42%)	93 (100%)

HCG positive tumours and patients with lysozyme negative tumours had a tendency to have shorter survival times (Breslow-Test $P<0.19$ and $P<0.17$, respectively). However, patients with AAT negative carcinomas had a significantly shorter survival time ($P<0.04$).

Discussion

The CEA production of tumour cells is a phenomenon frequently observed in carcinomas of the stomach and was noted in 96% of these cases. However, this finding could not be correlated with the histological type of carcinoma although undifferentiated carcinomas did not contain much of this antigen

in contrast to the signet-ring cell carcinomas. These were always intensively stained, with almost all tumour cells being positive. CEA was demonstrated not only at all stages of disease but also in tumours of different grades. Accordingly, it had no value in predicting survival times. The high CEA positivity rate corresponds to the findings of Tahara et al. (1982). However, in respect to HCG we could not confirm their results (19% positive cases) as our HCG positivity rate was much higher at 34%. We found no correlation of this antigen with the tumour grade. The difference in the percentage of HCG positivity is probably due to the fact that we investigated several parts of one tumour (sometimes more than 10 tissue samples) and HCG was often located in only a few single cells or cell clusters. The HCG positivity did not differ in the various histological types, although the antigen was observed more often in undifferentiated or poorly differentiated areas within a given tumour.

It was suggested by Capella et al. (1984) that lysozyme in gastric cancer cells might be restricted to neoplastic Paneth' cells, but we also found lysozyme positivity in other histological types of carcinomas, since lysozyme was shown in 71% of all cases with the highest incidence in signet-ring cell carcinoma. The overall frequency was higher than reported by other authors but this might be due to the fact that lysozyme positive cells can easily be missed, since they are often present in only small areas of the carcinomas (Tahara et al. 1982; Capella et al. 1984).

The presence of lysozyme in gastric carcinomas did not correlate with the histological type, tumour stage or -grade. The observation of Tahara et al. (1982) that patients with lysozyme positive carcinomas have a poor prognosis could not be confirmed. On the contrary, in this study patients with gastric cancers positive for lysozyme had a tendency to have better survival times.

ACT and AAT were demonstrated in 78% and 42% of cases respectively, ACT being positive in 92 of undifferentiated carcinomas, while AAT was more often detected in tubular carcinomas. In general, parts of tumours which expressed AAT were also positive for ACT, the latter substance being more intensively stained. Borchard (1983) reported an ACT positivity of 68% and an AAT positivity of only 18%. Tahara et al. (1984) found their AAT positivity mainly in low-stage well differentiated adenocarcinomas. We could not confirm this observation. In our material there was an almost significant prevalence of AAT staining in stage III carcinomas. However, we agree with Tahara et al. (1984) that immunohistological staining of lysozyme, AAT and ACT did not differ by the site of origin of tumours as has been postulated by Kittas et al. (1982). In the former work and in our study, carcinomas arising in the fundus had numerous AAT and ACT positive cells.

The presence of lysozyme and protease inhibitors in cells of gastric carcinomas raises two provocative questions: 1) concerning the origin of these substances and the 2) in respect to their effect on tumour growth and metastasis. Concerning the first question two possibilities have been taken into consideration. Lysozyme, AAT and ACT in neoplastic cells might originate

in surrounding inflammatory cells, however, our own results as well as those of Tahara et al. (1984) do not support such an origin, since the immunoreactivity of all three substances was not associated with the degree of inflammatory cell infiltration. Capella et al. (1984) postulated that the production of lysozyme appeared to be related to neoplastic Paneth' cells. This speculation is in accordance with the findings of Heitz and Wegmann (1979), who identified lysozyme positive cells in a well differentiated adenocarcinoma of the stomach as neoplastic Paneth' cells by electron microscopy. In our study the characteristic lysozyme staining pattern, with positive cells in close proximity to negative ones, provides further support to the theory that lysozyme is, indeed, produced by neoplastic cells. However, we could not identify the positive staining cells as neoplastic Paneth' cells. Montero and Erlandsen (1978) described the presence of lysozyme in mucus-producing tumour cells. Based on the above mentioned observations one can speculate that a malignant stem cell may be able to differentiate towards pyloric type glands and mucus neck cells of gastric fundus, all of which have been shown to contain lysozyme by both this and by previous studies (Klockars and Reitamo 1975; Mason and Taylor 1975; Reitamo et al. 1981; Isaacson 1982).

The same mechanisms of differentiation may account for the production of AAT and ACT in neoplasms of the stomach. Never-the-less one must take into consideration that these substances might be produced by the tumour cells, due to a change in the expression of differentiation.

Concerning the second question (the effect of AAT- and ACT production on tumour growth and metastasis), Naito et al. (1983) reported that cultured human gastric tumour cells produce plasminogen activator as well as tissue thromboplastin. Therefore focal increases in AAT- and ACT-containing tumours may be considered to be a protective phenomenon against high levels of thromboplastic or fibrinolytic and proteolytic enzymes in gastric carcinomas and from surrounding inflammatory cells. On that basis the significantly better survival probability of our patients with AAT positive tumours could be explained. Tahara et al. (1984) explained their finding of a bad prognosis for patients with AAT rich carcinomas by an immunosuppressive effect of AAT (Arora and Miller 1978).

Further investigations are required to determine the biological significance of marker production in stomach cancers and to elucidate the prognostic information of AAT tissue positivity observed in this study.

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