PARANEOPLASTIC PHENOTYPE OF HUMAN GASTRIC GLANDS STUDIED WITH THE AID OF CEA OF THE SPECIFIC LECTIN CRUSTACIN

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With modern methods of investigation the dynamics of changes in the mucous membrane can be studied in sufficient detail during carcinogenesis of the human gastric epithelium [5]. However, it is important to note that not all the details of the morphological and functional state of the mucous membrane can be analyzed equally thoroughly. For instance, virtually no attention has been paid to the study of the phenotype of the specialized gastric glands which remain in foci of intestinal metaplasia of the mucous membrane, with or without the presence of carcinoma or other malignant neoplasms.

The aim of this investigation was to study morphological and functional characteristics of the gastric glands. The main research tool used in the investigation was the lectin crustacin (Cr), which can react specifically with the carbohydrate component of carcinoembryonic antigen (CEA) [1, 3, 4].

EXPERIMENTAL METHOD

Twelve patients with chronic gastritis, associated with enteralization and dysplasia of the epithelium without neoplastic transformation and 41 patients with similar changes, but accompanied by malignant transformation of the gastric epithelium, were studied.

The tissues studied were fixed in neutral formalin and embedded in paraffin wax by the usual method. Sections 4-5 μ thick were used. Histochemical reactions were carried out in the indirect immunoperoxidase variant [8], using Cr as the first layer (the lectin was generously provided by A. F. Pavlenko and A. V. Kurika) in a concentration of 1 μg/ml, or rabbit polyclonal antibodies (AB) to CEA [7] in a concentration of 4-6 μg/ml [3]. The presence of binding of the primary reagents was detected with AB to Cr or AB to rabbit immunoglobulins, labeled beforehand with peroxidase, respectively followed by development of enzyme activity with 0.05% 3,3′-diaminobenzidine tetrachloride solution. The sections were dehydrated and mounted in Canada balsam.

Correlation between the morphological and functional state of the specialized gastric glands studied was confirmed by immunohistochemical determination of pepsinogen in them [2].

EXPERIMENTAL RESULTS

Specialized pepsinogen-positive glands (Fig. 1a) of the mucous membrane in chronic gastritis, unaccompanied by malignant neoplasia, preserved in foci of intestinal metaplasia reacted with the lectin in only one of 12 cases. AB to CEA in these observations did not react with the above-mentioned glands. Meanwhile, in the mucous membrane adjacent to carcinoma

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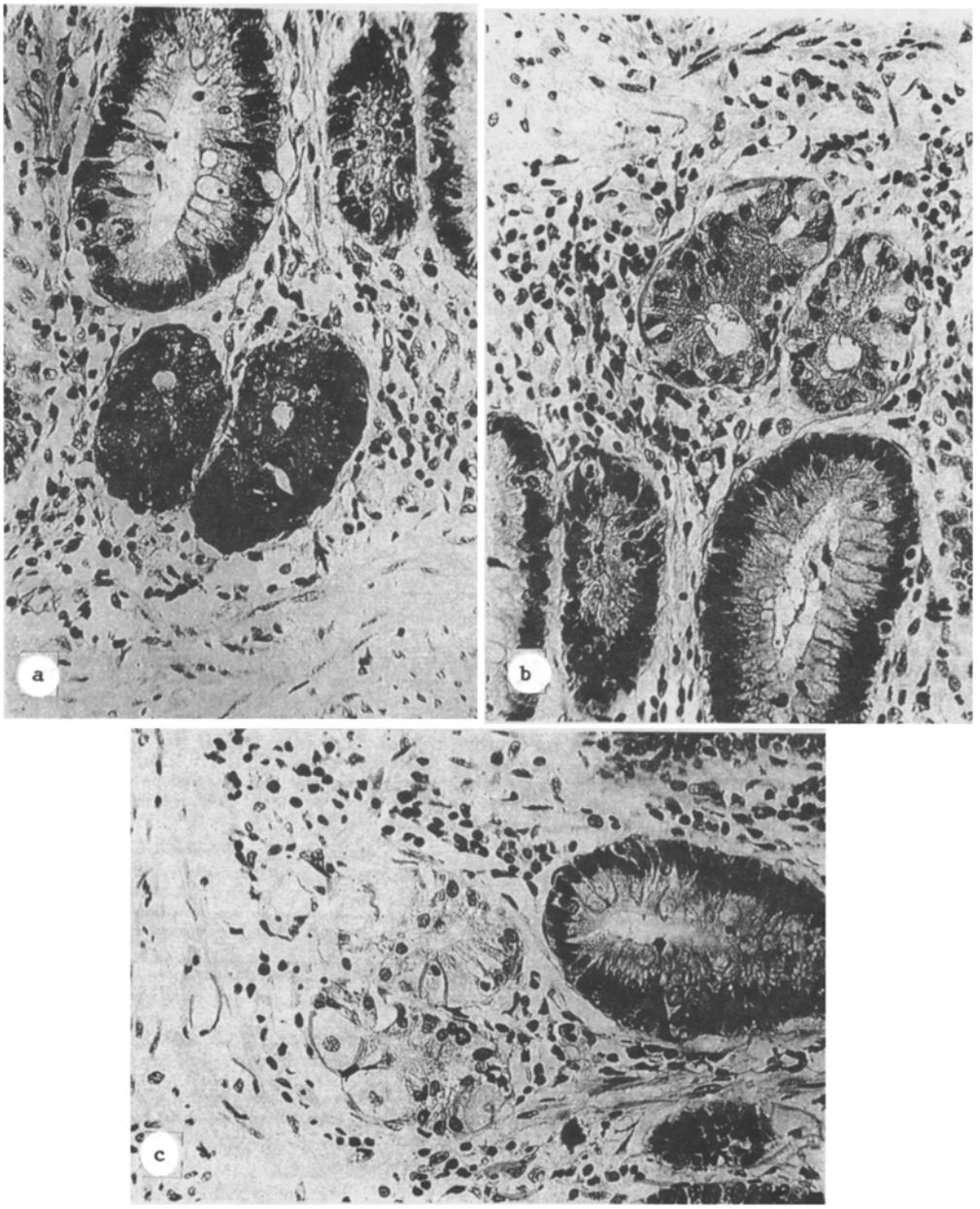

Fig. 1. Area of gastric mucosa adjacent to carcinoma. a) Immunoperoxidase reaction reveals pepsinogen in specialized gastric glands (arrow). Enteralized glands located nearby do not contain this antigen (two arrows); b) immunoperoxidase reaction with the use of Cr reveals CEA in specialized gastric glands (arrow). Enteralized glands negative (arrows); c) reaction control (Cr neutralized by CEA preparation) – no reaction in specialized and enteralized glands. Counterstained with hematoxylin. 300×.

of the stomach a reaction of the lectin was found with the glands in 20 of 41 cases (Fig. 1b), and under these circumstances the use of AB revealed CEA (CEA-AB) in two of 41 cases (Table 1). CEA-AB was found only in glands reacting positively with the lectin. The reaction product revealed with the aid of both AB and Cr was located inside the cytoplasm of pepsinogen-positive zymogen-containing cells with a maximum near the apical border. Compared with AB, the lectin reacted more diffusely within the whole supranuclear zone of the cytoplasm.

The reaction with AB and Cr in the specialized glands was observed only in zones of direct contact with the neoplasm, and was absent at a distance from it.

TABLE 1. Results of Reaction with CEA-AB and Cr in Pepsinogen-Positive Gastric Glands

Observations	Total	Positive		
		CEA-AB	Cr	%
Specialized glands preserved in foci of intestinal metaplasia: no carcinoma in stomach	12	0	1	8,3
Specialized glands preserved in foci of intestinal metaplasia* – carcinoma present in stomach	41	2	20**	48,8

Legend. *) Reaction observed only in zone of mucosa adjacent to carcinoma, **) differences of phenotype of gastric glands in presence or absence of carcinoma are statistically significant: $\chi^2 = 8.1, p < 0.01$.

A combination of positive reactions for CEA and pepsinogen also was found in glands with cystic changes, but analysis of this characteristic was not included among the aims of this investigation, for only morphologically and functionally preserved glands were examined. We found no correlation between the presence of a reaction with AB and Cr in specialized pepsinogen-positive glands and in the surrounding foci of intestinal metaplasia. In both cases the strongest reactions were found in foci of dysplasia of the enteralized epithelium. They were less marked or absent altogether in other zones of intestinal metaplasia (the reaction was always absent in foci of intestinal metaplasia of the complete type) [2]. Under these circumstances the character of the reactions in the enteralized epithelium was independent of the presence or absence of a carcinoma.

Comparison of the results of the reaction of the lectin in a carcinoma and in the remains of specialized glands in the surrounding mucous membrane yielded evidence more of the independent character of expression of the corresponding determinant in the cells of these structures. For instance, in 12 cases a reaction with Cr was found in the cells and glands of the stomach and of the malignant neoplasm, in seven cases the cells of the carcinoma and glands were negative, in eight cases a reaction was observed in the glands but was absent in the carcinoma, and in 14, on the contrary, the lectin did not react with the gastric glands but did react with the tumor cells. Two cases with a positive reaction with AB to CEA in the gastric glands were combined with a positive reaction to this antigen in cells of a gastric carcinoma.

Thus the histochemical investigation showed that the phenotype of specialized gastric glands adjacent to a carcinoma is characterized by the appearance of subcellular components that are uncharacteristic of it. Expression of CEA in the glands studied can be regarded rather as additional evidence of changes in the phenotype of the cells, taking place as a result of interaction between tumor and surrounding tissue [10]. However, the possibility cannot be ruled out that this phenomenon was of a parallel nature, possibly independent of malignant transformation. Comparison of the antibody and lectin tests for CEA indicates that the leading role in these events is played by changes in carbohydrate complexes which, it can be tentatively suggested, play an independent role.

Regardless of its causes the phenomenon thus discovered is further confirmation of the existence of paraneoplastic phenotypic features, accompanying malignant transformation, in the gastric mucosa adjacent to a carcinoma [9, 11].

The conclusion can evidently be drawn that during the further search for markers capable of indicating, with a high degree of probability, the presence of malignant transformation of the epithelium, lectins may prove to be no less suitable reagents than antibodies [6]. Whatever the case, the histochemical test with the lectin crustacin is a useful addition to the existing antibody tests currently used in pathomorphological investigation.

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EFFECT OF FINOPTIN ON DOXORUBICIN ACCUMULATION IN P-388 LEUKEMIA CELLS WITH INDUCED RESISTANCE TO A COMBINATION OF FINOPTIN AND DOXORUBICIN

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It has recently been shown that the phenomenon of multiple drug resistance (MDR) is connected with active transport of antitumor preparations from cells with the participation of glycoprotein P, which leads to reduction of cytostatic accumulation inside tumor cells. One way of overcoming MDR, in the opinion of many authorities, is to use calcium antagonists and calmodulin inhibitors, blocking glycoprotein P, and thereby causing retention of antitumor agents in the cells and potentiating their cytotoxicity [2, 4, 6]. Despite extensive discussion of this approach to the suppression of MDR, the problem of induction of resistance of tumors to a combination of cytostatics with cell calcium channel blockers and calmodulin inhibitors remains completely unstudied.

The aim of the present investigation was to study induction of resistance of tumor cells of leukemia P-388 to a combination of doxorubicin (Dx) and finoptin (Fp) and also the effect of Fp on Dx accumulation in leukemia P-388 cells sensitive to the antibiotic, leukemia P-388 with induced resistance to Dx, and leukemia P-388 with induced resistance to a combination of Fp + Dx.

EXPERIMENTAL METHOD

Experiments were carried out on male BDF_1(C57BL/6j × DBA) mice aged 2-3 months. Leukemia P-388 cells with induced resistance to Dx (P-388/Dx) were obtained by selection from leukemia P-388 cells (P-388/O, original strain, tumor strain bank, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) during treatment of animals with small doses of the antibiotic. Altogether 35 passages were needed to induce resistance. Leukemia P-388 cells resistant to a combination of Fp + Dx (P-388/Fp + Dx) were obtained from the P-388/Dx strain by selection of cells resistant to this combination. Altogether six passages were needed for leukemia P-388/Dx cells to develop resistance to the combination Fp + Dx. Tumor cells

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