IMMUNOHISTOCHEMICAL STUDY OF EXPRESSION OF TROPHOBLASTIC β_1-GLOBULIN IN GASTRIC EPITHELIUM OF A PATIENT WITH STOMACH CANCER

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Trophoblastic β_1-globulin (TBG) is one of the best known oncoplacental antigens, which are regarded as promising tumor markers [7, 8]. TBG is characterized by specificity in relation to the placenta and trophoblastic tumors. Meanwhile the presence of ectopic expression of this antigen in many different epithelial malignant neoplasms has been described [5]. There is evidence of detection of TBG not only in tumor cells, but also in tissue surrounding a neoplasm [6].

The aim of this investigation was to study the characteristics of ectopic expression of TBG in the gastric mucosa in patients with carcinoma of the stomach.

EXPERIMENTAL METHOD

An indirect immunoperoxidase method [4] was used. The reactions were carried out on tissue sections fixed in neutral formalin and embedded in paraffin wax. Altogether 12 patients with chronic gastritis and with foci of intestinal metaplasia and epithelial dysplasia and 41 cases of chronic gastritis with the same characteristics, but with an accompanying gastric carcinoma, were studied.

Sections through the placenta (14-16-week fetus), in which the intensity of the reaction was assessed as strong, were used as the positive control. The reaction was carried out in accordance with the following scheme: 1) removal of the paraffin wax from the sections in three portions of xylol and in a series of alcohols of decreasing strength; 2) inhibition of endogenous peroxidase by treatment (10 min) of the sections with 3% hydrogen peroxide in buffered physiological saline, pH 7.6-7.8, followed by treatment with 0.01% $NaIO_4$ solution (10 min) and 0.01% $NaBH_4$ solution (10 min); 3) blocking of nonspecific sorption of immunoglobulins by application of horse serum in a dilution of 1:20, with the addition of 3-5% of dried human plasma, to the section for 30 min; 4) antibodies to TBG, isolated from monospecific rabbit antisera to this antigen by immunoaffinity chromatography, in a concentration of about 4 μg/ml (incubation for 30 min), were used as primary immunoglobulins; 5) the sections were washed in three changes of buffered physiological saline (BPS) for 5 min each time; 6) peroxidase-labeled antibodies against rabbit immunoglobulins (N. F. Gamaleya Institute of Epidemiology and Microbiology) in a dilution of 1:80 (incubation for 30 min) were used as secondary immunoglobulins; 7) the sections were washed in three changes of BPS for 5 min each time; 8) peroxidase activity was developed by the following substrate: 0.05% solution of diaminobenzidine tetrachloride with the addition of 0.01% hydrogen peroxide and 0.05 M imidazole for 5 min; 9) the sections were counterstained with hematoxylin and eosin and mounted in Canada balsam. Additional immunohistochemical characteristics of the gastric mucosa were obtained by marking the gastric glands with pepsinogen [3], the cervical regions of the glands with MMA antigen [2], and foci of intestinal metaplasia with intestinal antigens [3], and onco-fetal changes by the reaction for carcinoembryonic antigen, using rabbit antibodies against this antigen and a lectin reacting with carcinoembryonic antigen, namely crustacin [1, 3], for this purpose.

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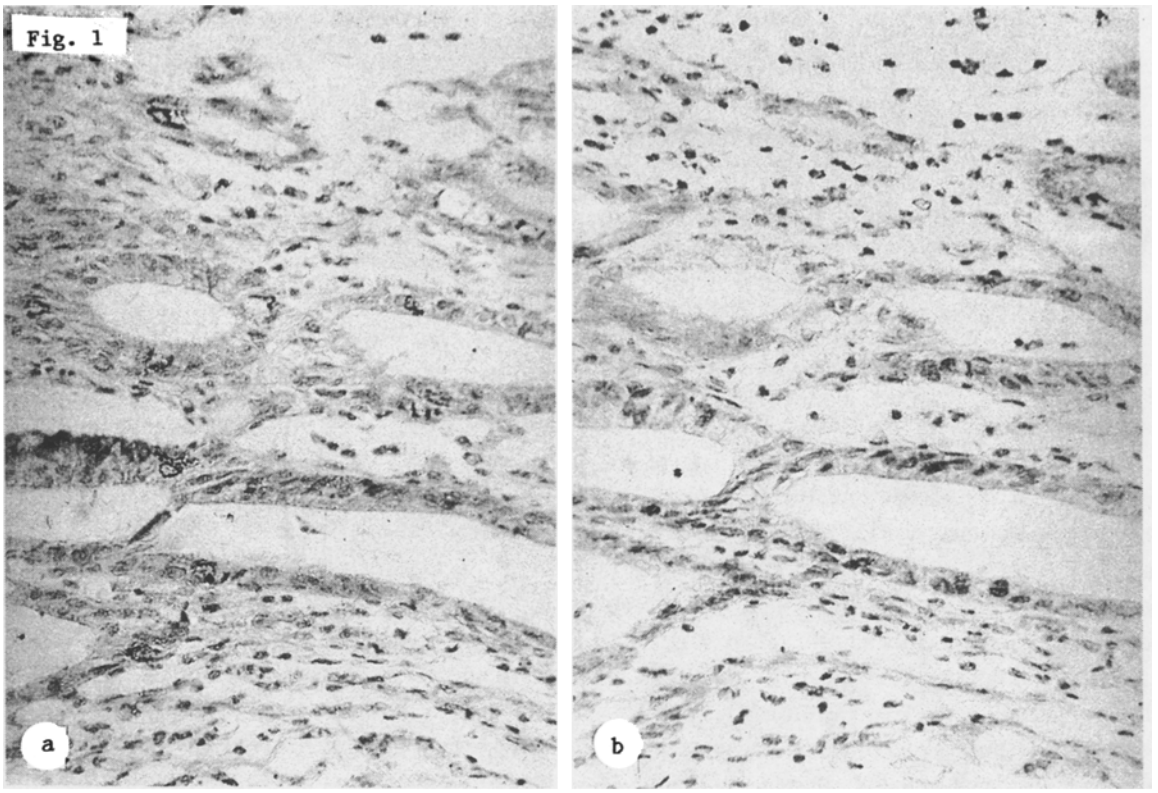

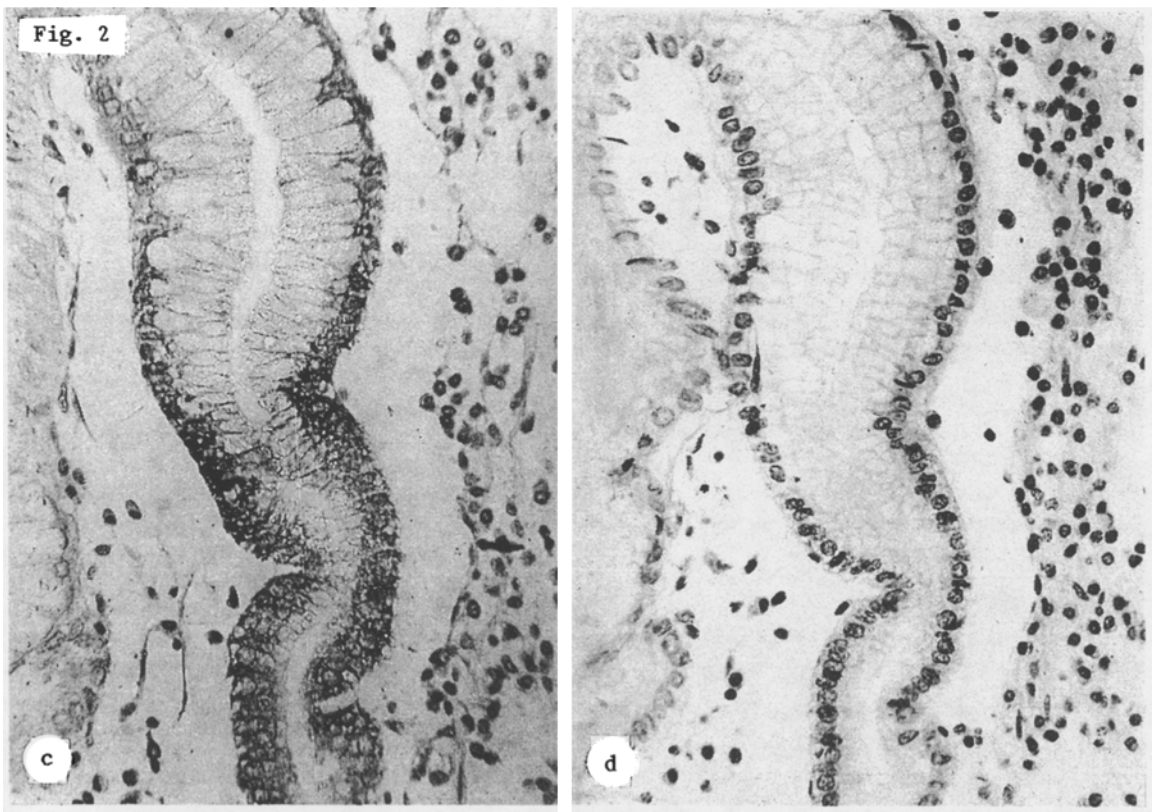

Fig. 1. Gastric mucosa, region of epithelial dysplasia. Immunoperoxidase reaction. Expression of TBG shown by weak reactions in cytoplasm of epithelial cells, in some of which the antigen is more marked: a) experiment, b) control (antibodies neutralized by the TBG preparation). Stained with hematoxylin, 280×.

Fig. 2. Gastric mucosa, region of surface epithelium with gastric pits. Immunoperoxidase reaction. Expression of TBG found in some epithelial cells, mainly in basal parts of the cytoplasm. a) Experiment, b) control (antibodies neutralized by TBG preparation). Stained with hematoxylin, 400×.

TABLE 1. Comparison of TBG Expression in Gastric Mucosa and of Crustacin Receptors in Gastric Glands in Carcinoma of the Stomach

Object	Positive reaction for TBG	Negative reaction for TBG
Crustacin-positive glands	13	7
Crustacin-negative glands	10	11

EXPERIMENTAL RESULTS

Investigation of the gastric mucosa from patients with evidence of chronic gastritis but without malignant change in the epithelium revealed no positive reactions for TBG in any of the 12 cases studied.

Meanwhile expression of TBG was discovered in the gastric mucosa adjacent to a malignant neoplasm in 23 of 41 cases studied (the probability that the antigen was present in the gastric mucosa in patients with cancer compared with those without cancer is statistically significant, $p < 0.05$).

Epithelium containing TBG-positive cells was arranged in the form of separate small zones in the immediate vicinity of the malignant focus. Expression of the antigen was observed in various parts of the gastric mucosa: in foci of intestinal metaplasia without signs of dysplasia, in regions of dysplasia (Fig. 1), in the region of the surface epithelium with gastric pits (Fig. 2), and in one case, besides a small zone of enterolyzed epithelium, a positive reaction was observed in the parietal cells of several gastric glands, which remained in a focus of intestinal metaplasia.

On the whole the reaction for TBG in the gastric mucosa was weak or very weak in all cases (Fig. 1). This antigen was localized within the cytoplasm of the cells, and most characteristically TBG was located in the basal portions of the cell (Fig. 2).

In gastric carcinoma cells TBG was found in six of 41 cases. In all these cases the tumor, with a positive reaction for TBG, was surrounded by mucosa with zones of epithelium which also reacted positively for this antigen.

Our investigations thus confirmed that ectopic expression of TBG accompanies malignant transformation of the epithelium of the gastric mucosa. This antigen has previously been found in cells in foci of intestinal metaplasia adjacent to gastric carcinoma in four of 13 cases studied [6]. Analysis of a larger number of cases (41 in the present study) has evidently enabled us to locate ectopic expression of TBG not only in foci of metaplasia, but also in the epithelium of areas of gastric mucosa which have not undergone any structural transformation.

On the whole TBG was found in 56% of cases studied, in which the gastric mucosa adjacent to a carcinoma was investigated, and this is much larger than the number of positive cases (31%) [6]. However, these differences are not statistically significant.

Thus allowing for these data it can be tentatively suggested that expression of TBG is determined not so much by the morphological features of the gastric mucosa surrounding the neoplasm as by factors associated with malignant transformation of the epithelium.

In the present study expression of TBG was found in the cells of six malignant tumors (of 41 studied), which is a much lower figure than given previously in the literature, when this antigen was found in half (nine of 18) [6] or more than half (eight of 12) cases [5]. It must, however, be pointed out that the total number of neoplasms studied in these investigations was less than the number described in the present study. Unfortunately, it is impossible to compare the results we obtained for expression of TBG in gastric carcinoma with the other investigations, for even in reviews dealing with this antigen [7, 8] the only references given are to the above citations.

The problem of where TBG is synthesized is a problem which has not yet been solved, because available information cannot rule out the possibility that a mechanism of selective accumulation of antigen molecules produced externally may not take place in the gastric epithelium.

In a previous communication we reported the appearance of glycoconjugates, reacting with the lectin crustacin [1, 2], in cells of the gastric glands close to a focus of carcinoma, and this was interpreted as a tumor-associated feature of their phenotype. In the present investigation we analyzed two facts relating to the change in phenotype under the influence of the malignant focus, i.e., ectopic expression of TBG in the gastric mucosa and glycoconjugates reacting with the lectin

crustacin in the gastric glands (Table 1). A positive reaction with crustacin in the gastric glands in the presence of carcinoma was observed in 20 of the 41 cases studied, and with antibodies to TBG in 23 of 41 cases. A positive reaction for at least one of these markers or for both of them was present in 30 of the 41 cases (73%). Under these circumstances expression of TBG in the gastric mucosa and of crustacin receptors in the gastric glands (Table 1) are factors statistically independent of each other.

Consequently, these two markers, independently of each other, reflect the existence of a phenomenon whereby a focus of malignant transformation of the epithelium exerts its influence on surrounding tissues, and this is accompanied by the appearance of specific phenotypic features in the epithelium outside the focus of neoplastic transformation.

It can be tentatively suggested that determination of TBG in the gastric juice may prove useful for diagnostic oncology.

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