IMMUNOHISTOCHEMICAL STUDY OF TROPHOBLASTIC BETA$_1$-GLOBULIN EXPRESSION IN CARCINOMA AND NORMAL MUCOSA OF THE COLON

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The carcinoplacental antigen trophoblastic beta$_1$-globulin (TBG) is nowadays regarded as a promising marker of malignant neoplasms [9]. High TBG concentrations are observed in tissues of the placenta and in cells of trophoblastic tumors. This marker is found in many malignant neoplasms of epithelial nature, such as carcinoma of the stomach, ovaries, breast, lungs, etc. This protein has been found not only in cancer cells, but also in nonmalignant tissues surrounding a focus of cancer. It has been shown, for instance, that TBG expression in epithelium of the gastric mucosa is closely associated with the presence of a focus of neoplastic transformation [1]. Several workers have observed the presence of TBG in carcinoma of the colon, and also in adenomatous polyps of this organ [3, 6, 8]. However, these investigations were carried out on a very limited material, and it was accordingly decided to undertake the investigation described below.

Its aim was to study the characteristics of TBG expression in the mucosa of the normal colon and in patients with carcinoma of the colon.

EXPERIMENTAL METHOD

The indirect immunoperoxidase method was used, by a technique similar to that described in detail previously [1, 2]. Tests were carried out on tissue sections fixed in neutral formalin and embedded in paraffin wax. Altogether 50 patients with carcinoma of the colon were studied: 19 with highly differentiated adenocarcinoma, 26 with moderately differentiated adenocarcinoma, and 5 with undifferentiated adenocarcinoma. In 11 cases a tumor focus and metastasis of the tumor were studied. In 10 cases an adenoma of the colon was studied, in 5 of them a benign tumor was associated with a focus of carcinoma situated nearby. Four patients had nonspecific ulteractive colitis. In all cases of carcinoma of the colon the so-called "transitional mucosa" was studied, and in 32 cases the mucosa of the colon of the proximal and distal margins of the resected segment of the colon. In addition, the following tumors of the colon were investigated: 2 carcinoids, 2 leiomyosarcomas, 1 lymphosarcoma, and 1 squamous-cell carcinoma. Six strains of adenocarcinoma of the colon transplated into nude mice also were studied: RTK-2, RTK-8, RTK-9, RTK-10, RTK-11, and RTK-12. (RTK is an abbreviation of the Russian words signifying carcinoma of the colon). RTK-12 was studied in two versions: a strain obtained from the primary focus and a strain obtained from a metastatic focus. All strains of tumors of the colon were generously provided by E. S. Revazova (Oncologic Scientific Center, Russian Academy of Medical Sciences). In the course of the investigation areas of the mucosa of the stomach (2 cases), duodenum (1 case), and small intestine (2 cases), removed during operations for carcinoma of the colon, also were

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TABLE 1. Results of Demonstration of TBG in Colon and Also in Some Other Tissues

Tissues	Number of observations	Number of TBG-positive observations
Carcinoma of colon	50	19
– highly differentiated adenocarcinoma	19	8
– moderately differentiated adenocarcinoma	26	10
– undifferentiated adenocarcinoma	5	1
Tumor strains	6*	0
Other malignant tumors of the colon	6**	0
Adenoma of the colon	10	0
Nonspecific ulcerative colitis	4	1***
Mucosa:		
– "transitional"	50	0
– proximal and distal margins of segments of colon removed at operation	32	0
– autopsy material from the colon	6	0
– fetal colon	2	0
Other types of mucosa: stomach, duodenum, small intestine, removed at operation for carcinoma of the colon	5	0

Legend: *Strains of adenocarcinoma of the colon transplanted into nude mice: RTK-2, RTK-8, RTK-9, RTK-10, RTK-11, RTK-12; **2 carcinoids, 2 leiomyosarcomas, 1 lymphosarcoma, 1 squamous-cell carcinoma; ***weak reaction in 2 or 3 cells in a focus of dysplasia.

studied. Normal and fetal material was represented by autopsy specimens of mucosa of the large intestine (6 cases) and the intestine of a fetus at 16-28 weeks of development (2 cases).

EXPERIMENTAL RESULTS

TBG was not found in the epithelium of the mucosa of any of the autopsy specimens of the adult human colon, or in the epithelium of the fetal intestine. Meanwhile, this protein was found in 19 of the 50 patients undergoing surgery for carcinoma of the colon (38%; Table 1). TBG was found in adenocarcinomas with high, moderate, or low levels of differentiation in about equal proportions.

Expression of TBG was observed both in the primary focus of cancer and in metastases. Of 11 cases in which both primary and metastatic foci were studied, TBG was found in the primary focus in 6 cases, in metastases in 4 cases. In 9 cases there was a combined reaction in primary and metastatic foci, i.e., both the primary focus and the metastasis were positive or negative. In two cases absence of expression of TBG was observed in a metastasis whereas the primary focus was TBG-positive.

We did not find expression of TBG in tumor strains of carcinoma of the colon. Negative results also were obtained in a study of carcinoid, leiomyosarcoma, lymphosarcoma, and squamous-cell carcinoma of the colon (Table 1).

TBG was found in adenocarcinoma of the colon as a rule in the form of weak reactions in the cytoplasm of cells of individual glandular structures (Fig. 1). Incidentally, the reaction was found most often in areas of neoplasia in the region of invasion of the intestinal wall by malignant cells, and in some cases, it was associated with secre-

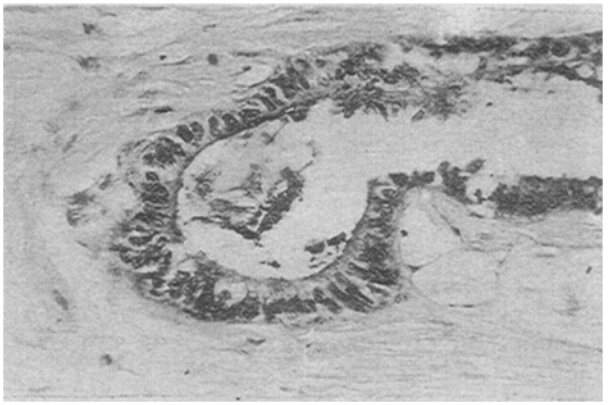

Fig. 1. Adenocarcinoma of the colon. Immunoperoxidase reaction reveals TBG in tumor cells and in mucus in lumen of a glandular structure. TBG localized in basal and apical zones of cytoplasm, strongest reaction observed in apical border of malignant cells. Counterstained with hematoxylin. 300×.

tion and with necrotic masses inside the lumen of the glandular structures. A somewhat unusual fact found during the study of carcinoma of the colon was the discovery of TBG expression in some cases in the cytoplasm of macrophages. These cells, found in the lumen of the glandular structures and in elements of the stroma of carcinomas of the colon, were located both diffusely and in the form of discrete aggregations, characteristically in zones of invasion of the carcinoma. The reason for the appearance of TBG in these cells, located in the tumor tissue, may be synthesis of this protein by the macrophages themselves; this conclusion is supported by the discovery of clusters of TBG-positive mononuclears in the stroma of a TBG-negative metastasis of a carcinoma of the colon (Fig. 2). However, our investigations cannot rule out the presence of TBG due to phagocytosis of material containing this protein. No cells of the macrophagal series, containing TBG, were found in any of the preparations from the mucosa of the gastrointestinal tract outside a focus of carcinoma, whether in mucosa of transitional type or in the mucosa at the margins of the resected segment.

No TBG was found in benign tumors of the colon, including cases in which an adenoma was located close to a focus of carcinoma.

A positive reaction with antibodies against TBG was found in one of four cases of ulcerative colitis studied. This protein was detected literally in 2 or 3 cells in one of several intestinal crypts showing evidence of dysplasia, as a reaction of weak intensity. In other epithelial and stromal cells in this case no TBG could be found.

It can be concluded from the results of this investigation that TBG is an antigenic marker of malignant transformation, taking place in the epithelium of the colon. This conclusion is in some disagreement with the colon (in the present investigation all ten adenomas were TBG-negative), although admittedly with the qualification that this is one of those markers whose expression is enhanced during the conversion of a polyp into a carcinoma of the colon [6, 8]. Meanwhile, our data on the presence of minimal qualities of TBG in epithelium which has undergone dysplasia, in nonspecific ulcerative colitis, may be regarded as confirmation of results cited above, showing that carcinoplacental protein appears in the epithelium of the colon at the stage of precancerous changes.

The most interesting discovery in the present investigation is evidently the fact that TBG was found in cells of the macrophagal series even when it was absent in the epithelium of the malignant tumor. Several investigations have demonstrated the possibility of finding TBG expression in normal cells in experiments on cultures of fibroblasts and also in polymorphonuclear neutrophils [5, 7]. However, neither in the present study nor in a previous study of

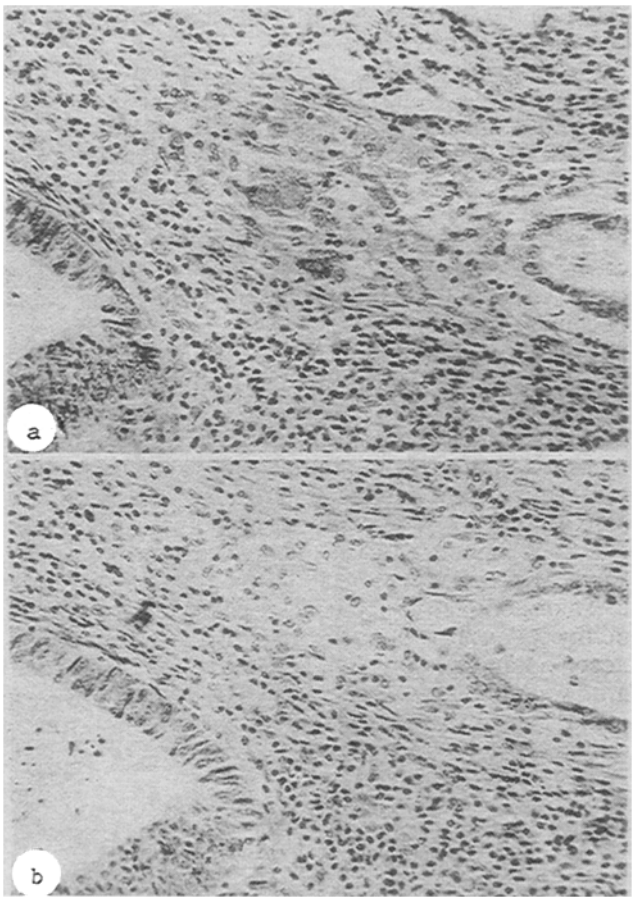

Fig. 2. Metastasis of adenocarcinoma of colon in a lymph node. a) Immunoperoxidase reaction reveals TBG in a group of macrophages. No TBG found in epithelium of carcinoma; b) the same area of tumor. Control reaction – antibodies to TBG were neutralized by a preparation of this antigen. Counterstained with hematoxylin. 250×.

the stomach [1], did we find cells of this type containing TBG either in cancer or in the case of noncancerous pathology or in the normal state. The exceptions were embryonic and fetal tissues, where in several cases we found TBG in different types of cells and, in particular, in neutrophils.

It can be tentatively suggested that TBG expression in macrophages in carcinoma of the colon is a phenomenon associated with the presence of a focus of malignant change. TBG expression in such cells can be interpreted as a manifestation of strengthening of embryonic determination, brought about by factors in the microenvironment of the tumor, if only because TBG-positive cells of this kind could not be found outside the focus of malignant transformation.

This discovery can also be regarded from the standpoint of confirmation of the similarity of certain properties of trophoblastic cells, neoplastic cells, and certain mononuclears. This fact is interesting also in connection with the characteristic localization of TBG-positive macrophages in the region of active growth and invasion of the tumor. The in our view paradoxical conclusion that, in principle, it is possible to detect an epithelial neoplasm by a reaction

in mesenchymal cells, which is in full agreement with experimental results described above, may probably also be suggested for examination.

It can thus be concluded from these results that TBG may be useful as a tumor marker and, in particular, to increase the accuracy of immunohistochemical diagnosis of malignant neoplasms of the colon.

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