

ONCOLOGY

A STUDY OF THE ANTIGENIC DIFFERENCES BETWEEN TISSUES OF THE NORMAL RECTUM AND OF RECTAL CARCINOMA

(UDC 616.351-006.6-018-097-07+611.351-018:612.017.1-08)

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Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 58, No. 10,
pp. 82-84, October, 1964

Original article submitted August 10, 1963

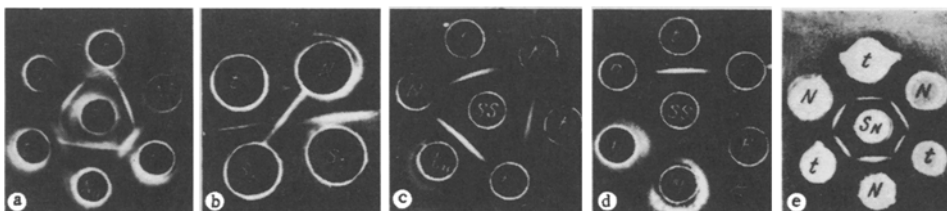
A comparative study of the antigens of normal rectal tissue and of malignant tissue from a rectal tumor has been reported [5]. The authors concerned, using the method of fluorescent antibodies, found that the tumor had lost its organ-specific antigen. The object of the present investigation was to study the antigenic differences between normal and malignant rectal tissue. The intestinal epithelium from which tumors develop is homogeneous, so that it is possible to talk of antigenic changes (when these exist), not at the tissue, but at the cellular level, and the production of monospecific sera is made easier.

EXPERIMENTAL METHOD

Thirteen malignant tumors of the rectum were studied. As controls, the mucous membranes of the rectum and sigmoid colon taken from persons dying from trauma were used, and were obtained between 2 and 24 h after death. Altogether 10 specimens of normal mucous membranes were used in the investigation. Saline extracts of the normal and tumor tissues were prepared by the usual method [3]. The antigens used in the reactions were prepared in veronal-medinal buffer solution (pH 8.6) in a dilution of 2:1. The nitrogen in the antigen solution was determined by Kjeldahl's method. The extracts of malignant and normal tissues were made equal in their nitrogen content. To obtain immune sera, rabbits were given 5 injections of antigen solution in increasing doses at 3 day intervals. One month later, the animals were reimmunized with 3 injections of antigen solution. In order to stimulate antibody production, each injection of antigen was accompanied by injection of the rabbit's own blood, taken from its auricular vein in a volume of 1-2 ml and given subcutaneously into the abdominal wall. Blood used for preparing immune sera was taken on the 7th-10th day after the last injection of antigen. Sera obtained after the 2nd and 3rd cycles of immunization were used in the investigation. In order to obtain specific sera, they were absorbed with antigens from human serum, kidney, spleen, lung, and liver until reactions with these antigens completely disappeared, after which they were concentrated 7-10 times by the alcohol method [6]. In the present investigation 2 sera to malignant tissue and 2 sera to normal intestinal mucous membrane were used. The technique followed by the investigation was that of precipitation in agar, in a semimicro-modification [2].

EXPERIMENTAL RESULTS

After absorption of the sera to tumor tissue, they reacted both with extracts of tumor tissue and with extracts of normal mucous membrane, although additional lines were formed opposite the well containing malignant antigens (see figure, a). When the reaction was carried out in the same way with the organ-specific serum, no such lines were found (see figure, e). During the crossed reaction by Abelev's method [1], lines appeared between the serum to the malignant tissue (see figure, St) and the malignant antigen (t), their ends reaching both the well with intestinal antigen and the well with serum to normal mucous membrane (see figure, b). After determination of the limiting dilution of the test system, the character of the line remained as before. Experiments were next carried out in which serum



Reaction of antisera to malignant and normal tissues with corresponding antigens. a) Reaction of antiserum to tumor tissue, absorbed with antigens of human serum, kidney, spleen, lung, and liver, with extracts of normal and tumor tissue; b) crossed reaction between antisera to tumor and intestinal tissue, absorbed with normal antigens; c) detection of "specific" tumor antigen by monospecific serum against tumor tissue; d) monospecificity of antiserum to tumor tissue; e) reaction between organ-specific antiserum to intestinal tissue and extracts of tumor and normal tissues; N, N_I, N_{II}) extracts of normal intestinal mucous membranes; t, t_I, t_{II}) tumor extracts; St) immune serum to carcinoma of the rectum, absorbed by antigens of human serum, kidney, spleen, lung, and liver; SS) monospecific serum against tumor tissue; SN) antiserum to intestinal tissue, absorbed with normal antigens; P) lung extract; L) spleen extract; H) liver extract; R) kidney extract).

against tumor tissue was neutralized with antigens from normal mucous membrane. The neutralization was carried out both in tubes and by impregnation of agar [4]. In both cases the results were the same. The monospecific sera obtained in this manner reacted only with antigens from the tumor, with which they gave only one line (see figure, c). "Specific" antigen was found in extracts from all 13 tumors, but the extracts from 2 tumors with extensive destruction gave weak reactions. Both sera reacted in the same way. These results indicate an antigenic difference between the extracts from tumor tissue and those from the corresponding normal tissue. Antiserum to intestinal tissue, after neutralization with antigen of normal mucous membrane, reacted neither with intestinal nor with tumor antigen. However, these results might be attributable to absence of the whole spectrum of antibodies to normal antigens in the antisera to intestinal tissue, and to its presence at the same time in the antisera to tumor tissue. To test this hypothesis, antisera to tumor and intestinal tissue were investigated in a reaction with extract from normal mucous membrane, but no differences were found in the spectrum of antibodies to normal antigens. The presence of antibodies to endotoxins in the monospecific sera might have an important influence on the experimental results, and, accordingly, a further series of control experiments was carried out with endotoxins of microorganisms cultivated from the tumors and intestine. Seedings were made in carbohydrate broth, and on the 4th day agar slopes were inoculated. After incubation of the culture for 14 days smears were taken and tested in the precipitation reaction with antisera to tumor and intestinal tissue. The reaction was negative.

Hence, an antigenic difference was demonstrated between the tumor tissue and tissue of the normal rectal mucous membrane, and in the conditions of the precipitation reaction this difference appeared to be qualitative.

As mentioned above, antiserum to tumor tissue, when absorbed by antigens from human serum, kidney, spleen, lung, and liver, reacted with extracts from normal mucous membrane (see figure, a), indicating the presence of antibodies to intestinal organ antigen in antiserum to tumor tissue. On the other hand, the organ-specific serum to intestinal mucous membrane gave a clear reaction with extracts of tumors (see figure, e), from which it follows that extracts of the malignant tumors contained organ-specific intestinal antigen. The reactions described above were observed constantly. Both sera against intestinal mucous membrane reacted in the same way. The phenomena described above were especially pronounced when the crossed version of the reaction was carried out (see figure, b). The organ-specific antiserum against the intestinal mucous membrane (see figure, SN) gave a distinct reaction with the tumor extract (t); in turn, antiserum to tumor tissue (St), when absorbed by antigens of serum, kidney, spleen, lung, and liver, reacted with extract from normal mucous membrane (N).

Hence, no loss of organ-specific antigen from the tumor tissue could be detected.

SUMMARY

Antigenic differences between the malignant and normal rectal tissue were studied by precipitation test in agar. Antitumor sera and sera against normal mucous were used in the investigation. An antigen not detectable in

the extracts from the normal mucous was found in the tumor tissue extracts. The tumor tissue contained an organ-specific intestinal antigen tumor.

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