LOCALIZATION OF ORGAN ANTIGENS IN THE HUMAN LARGE INTESTINE

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The localization of soluble organ-specific antigens was studied in the human large intestine. One of the components studied had electrophoretic mobility corresponding to the α-globulins and another to the β-globulins of human serum. Monospecific antisera were obtained against each of the antigens and these were used in the indirect Coons test. The results showed that the first antigen is localized in the cytoplasm of the goblet cells while the second is found mainly in the apical part of the membrane of these same cells. Large quantities of the second component are also present in the cytoplasm.

One of the authors (Rogal'skii) has previously described two organ antigens in neoplasms of the human large intestine [2, 3]. The first of these antigens corresponded in its electrophoretic mobility to human serum α-globulin, the second to β-globulin.

The object of this investigation was to determine the localization of these organ antigens in the human large intestine.

EXPERIMENTAL METHOD AND RESULTS

The fluorescent antibody technique [7] was used. However, difficulties arise when this method is used for work with heterogeneous sera. First, antibodies are required against each of the components. This makes it necessary to add large quantities of extracts of normal organs to the antisera in order to neutralize cross-reacting antibodies. Second, the sera must not contain significant quantities of contaminating tissue antigens. The antisera satisfying the necessary requirements were prepared in two stages.

Two groups of rabbits were immunized initially. The animals of group 1, for the preparation of sera against the intestinal α-antigen, were immunized with native extracts of unchanged intestinal mucous membranes taken along with tumors. The animals of group 2 were to be used for preparing antisera against the organ antigen of the large intestine with β-globulin mobility. Earlier experiments showed that when extracts from malignant tumors are boiled the intestinal α-antigen is destroyed while the β-antigen remains intact [5]. The animals of group 2 were therefore immunized with boiled extracts from neoplasms of the large intestine. The scheme of immunization and the method of absorption of the sera were described previously [2, 4]. By neutralization with lyophilized extracts of human liver, kidney, spleen, lung, and plasma, the sera were made monospecific. They revealed only intestinal β-antigen. However, these sera were unsuitable for the fluorescent antibody test because they were heavily contaminated with antigens added during neutralization. They were very thick and were dark brown in color. The sera of animals immunized with native antigens, after similar absorption, contained antibodies against organ β-antigens.

In the second stage of preparation of the sera against individual antigens of the large intestine the method of immunization by injection of precipitate into the lymph glands [8] was used. To obtain the

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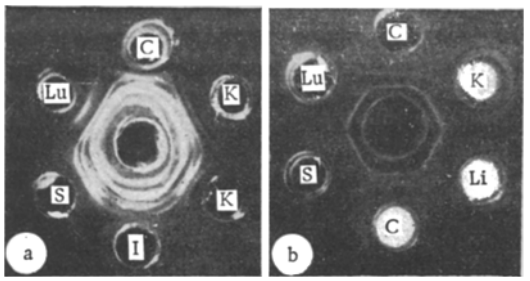

Fig. 1. Cross-reacting antibodies in sera after immunization with extracts (a) and with precipitate into lymph gland (b): a) central well contains antiserum against extracts of carcinoma of the large intestine; b) central well contains serum obtained by immunization with precipitate containing β-antigen into lymph gland. Extracts from normal organs: Li—liver, K—kidney, S—spleen, Lu—lung, C—tumor, I—intestinal mucosa.

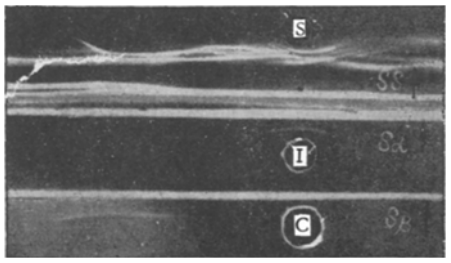

Fig. 2. Monospecificity of sera against intestinal organ antigens. S_α) Antiserum against α-antigen; S_β) antiserum against β-antigen; C) extract from carcinoma; I) extract from intestinal mucous membrane of patient with carcinoma; S) human serum; SS) antiserum against human serum proteins.

precipitate, extracts from the mucous membranes of intestines were treated by electrophoresis in 1% agar made up in veronal-medinal buffer (pH 8.6, ionic strength 0.025, voltage 5.5 V/cm, time 3 h). The antigens were developed with neutralized antiserum against native extracts of the large intestine. The agar blocks after electrophoresis had two precipitation bands with the α- and β-antigens, respectively. They were washed to remove traces of serum and extracts, and the strips were washed in an electric eluter for 2 h at 10 V/cm [1]. The precipitate was then cut out of the washed strips and placed in physiological saline for 1.5 days. The precipitate was ground in a Potter's homogenizer and mixed with Freund's complete adjuvant in the ratio of 1.5 ml precipiate to 0.5 ml physiological saline and 0.8 ml adjuvant. The popliteal lymph glands of the rabbits were exposed at operation and the immunizing mixture prepared as described above was injected into them in a dose of 0.25 ml per gland. The wound was sutured.

The second and subsequent cycles of immunization were given at intervals of 30 days. Preparation and treatment of the precipitate were as in the first cycle, but no adjuvant was added. For each rabbit, 18-20 precipitation strips were prepared. The agar containing the precipitate was homogenized and treated with sterile physiological saline to a volume of 2.5 ml. The immunizing material was injected at 5 points: 0.5 ml intravenously, 0.5 ml intramuscularly into each thigh, and 0.5 ml subcutaneously into the lateral surface of each forelimb. Blood was taken on the 11th day. Sera against the organ β-antigen of the large intestine were obtained in a similar manner.

The sera of rabbits immunized by injection into the lymph glands contained a much lower titer of antibodies against tissue components of normal organs (Fig. 1).

It was comparatively easy to remove cross-reacting antibodies from the sera obtained from rabbits immunized by injection of precipitate into the lymph glands. All that was necessary was to neutralize some of the sera against intestinal β-antigen with the thermostable components of human serum. The rest of the antisera were absorbed by means of an immunosorbent prepared by the method of Avrameas and Ternyck [6]. The immunosorbent contained antigens of normal human organs (liver, kidneys, spleen, plasma). As a result of these procedures, sufficiently pure antisera against each of the organ antigens of the large intestine were obtained (Fig. 2).

To determine the localization of the two components, globulin fractions and eluates of the antibodies were used. The globulin fractions were precipitated by 50% saturation of the sera with ammonium sulfate. The eluates of the antibodies were obtained by the method of Avrameas and Ternynck [6]. The antibodies were adsorbed on the immunosorbent containing extracts of normal mucous membranes and of malignant tumors of the large intestine. The immunosorbent also contained human albumin which was added to the extracts to increase the protein concentration to 50 mg/ml. The antibodies were eluted by the method described above.

The final part of the work was carried out by the indirect Coons method [7]. The localization of the antigens was studied in unchanged mucous membranes of the large intestine removed along with malignant tumors. Sections were cut in a cryostat from tissue frozen to $-70°C$ and fixed with 96° ethanol or acetone. Control sections were treated with nonimmune rabbit globulins. Fluorescence of sections treated with antibodies against intestinal α-antigen and with labeled serum are shown in Fig. 3a. Bright fluorescence

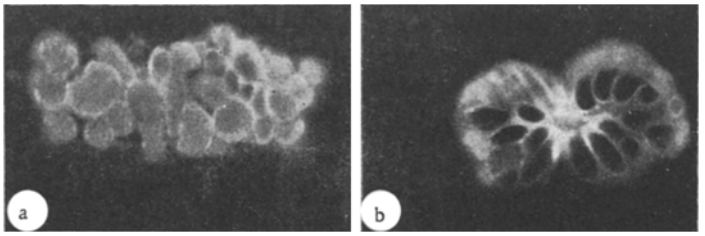

Fig. 3. Localization of organ antigens of the human intestine: a) fluorescence of section of intestine treated with antibodies against α-antigen; b) fluorescence of apical part of membranes and cytoplasm of goblet cells after incubation of intestinal section with immunoglobulins against intestinal β-antigen.

in the form of drops superimposed on each other can be seen. Some of the drops are alone and separated from the remainder by dark zones. Fluorescence was localized inside the goblet cells, with their wide part facing the lumen of the glands. The nuclei and cell membranes of the intestinal epithelium were not fluorescent. Tissue between the intestinal glands gave such weak fluorescence that it was not detectable on the photographs. Weak fluorescence observed uniformly in the stroma of the mucous membrane and in the intestinal epithelium was present in the control sections. The cytoplasm of the goblet cells appeared quite dark.

A different picture was observed after tests for antibodies against β-antigen (Fig. 3b). Brightest fluorescence was observed in the membrane of the apical part of the goblet cells. The cytoplasm of these cells was also distinctly fluorescent, but not so brightly as the membrane. The cell nuclei and the stroma of the mucous membrane, as in the first case, gave only the weak background fluorescence.

Addition of lyophilized extracts of tumors of the large intestine to the solutions of antibodies against intestinal α- and β-antigens led to their complete neutralization.

The investigation thus showed that organ-specific antigen of the large intestine with the electrophoretic mobility of human serum α-globulin is located in the cytoplasm of the goblet cells. The second organ-specific antigen with the mobility of β-globulin is located in the membranes and cytoplasm of these cells.

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