ANALYSIS OF MONOCLONAL ANTIBODIES TO DIFFERENT DETERMINANTS OF CARCINOEMBRYONIC ANTIGEN BY MIXED GEL PRECIPITATION

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Gel precipitation (GP) or diffusion is a classical method of immunochemical analysis, which can be used to compare individual antigens without isolating them from mixtures with other antigens. GP is used to detect identical, partly identical, and nonidentical determinants in antigens for comparison [2, 9]. Unlike polyclonal antibodies, monoclonal antibodies (McAb) cannot be used in GP to analyze an antigen, for as a rule they form nonprecipitating soluble complexes with it. However, a method which combines the advantages of GP with the unique specificity of McAb has recently been suggested [3]. In this method McAb are revealed in a mixed precipitate formed by antigen, McAb, and polyclonal antibodies to the given antigen.

In the investigation described below the possibility of using the mixed GP (MGP) method to characterize McAb to carcinoembryonic antigen (CEA), which reveals different determinants of this antigen, was studied.

EXPERIMENTAL METHOD

To obtain McAb to CEA, BALB/c mice were immunized with a perchlorate extract of a strain of carcinoma of the human colon (PES) maintained by passage through nude mice. Cell line X63Ag8.653 of murine myeloma was used for fusion with splenic immune cells. The ratio of spleen cells to myeloma cells was 10:1. Fusion was carried out by the standard method [6].

Antibodies in the incubation medium of the hybridomas were determined by liquid-phase radioimmunoassay using CEA. purified by successive chromatography of sepharose 6B, Sephadex G-200, and concanavalin A-sepharose [7]. CEA was labeled with ^{125}I by the method in [8] in the modification [4]. The complex was precipitated by the use of a rabbit antiserum (AS) to mouse globulin, mixed with polyethylene-glycol [12]. The total value of nonspecific binding and sedimentation of labeled CEA in the absence of the first antibodies usually did not exceed 1-3%.

Of 163 wells with growth of hybridomas five were positive. After cloning, three stable clones producing McAb to CEA were obtained (3C12, 2D10, 2G10). The incubation media containing McAb secreted by these clones were tested in MGP. The incubation media containing McAb secreted by these clones were tested in MGP. Two samples of mouse McAb (35 and 192), gener-

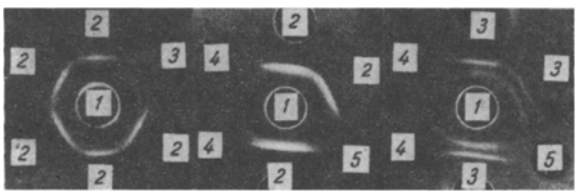

Fig. 1. Test systems to CEA and NCRA. 1) Original rabbit AS containing antibodies to CEA and NCRA; 2) PES; 3) PEC; 4) PEL — antigen of the test system to NCRA; 5) physiological saline.

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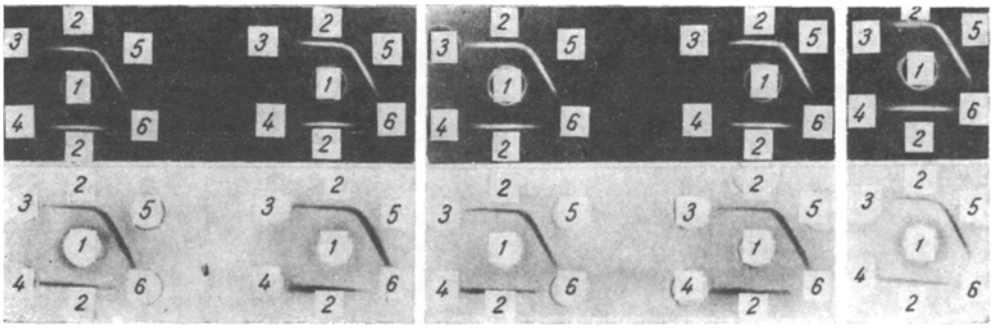


Fig. 2. Inclusion of McAb in monospecific test system for CEA. 1) Mixture of monospecific AS and conjugates; 2) PES — antigen of test system to CEA; 3) PEL — antigen of test system to NCRA; 4) mixture of PEL with test McAb; 5) mixture of PES with test McAb; 6) physiological saline. a) McAb 192; b) McAb 35; c) 2D10; d) 3C12; e) 2G10. Top and bottom rows consist of preparations before and after treatment with substrate, respectively.

ously provided by Dr. J.-P. Mach (Cancer Institute, Lausanne, Switzerland) also were used in the work. McAb 35 recognized a specific determinant on the CEA molecule, whereas McAb 192 react with a common determinant, present on CEA molecules and with a nonspecific cross-reacting antigen (NCRA) [5]. Murine McAb to human alpha-fetoprotein (HAFP) were used as the control for specificity of McAb inclusion in the precipitate.

Precipitating test systems to human CEA and NCRA were used. Rabbit AS were obtained by immunization in the lymph nodes with perchlorate extract of carcinoma of the human colon (PEC), purified beforehand on an antibody immunosorbent [1]. The immunosorbent was prepared from the serum of a rabbit immunized with perchlorate extract of normal human lung (PEL). Either PES or PEC, obtained from tumors from several patients, was used as the antigen of the test system for CEA. PEL was used as the antigen of the test system for NCRA. Monospecific serum to CEA was obtained by exhaustion of AS in the equivalence zone with the PEL preparation.

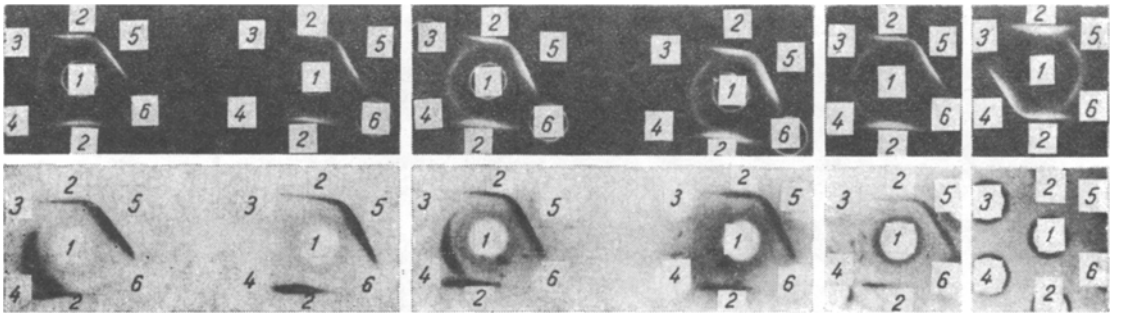
To reveal McAb in the composition of the precipitate, a conjugate of a preparation of Fab'-fragments of antibodies to mouse IgG with horseradish peroxidase (type VI RZ-3.0, from "Sigma," USA), obtained by the periodate method, was used [11]. Antibodies to mouse IgG were isolated by means of an immunosorbent from rabbit antiserum to the globulin fraction of mouse serum, obtained from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). The immunosorbent consisted of IgG, isolated from adult mouse serum by triple reprecipitation with ammonium sulfate, covalently bound with sepharose CNBr-4B ("Pharmacia," Sweden).

The mixed precipitation reaction was carried out in 2% agar made up in physiological saline [3]. Incubation media of clones 3C12, 2D10, and 2G10, and McAb 35 and 192 were mixed with antigens of the test systems, and peroxidase-labeled Fab'-fragments were added to the rabbit AS (the dilution of the labeled conjugate was chosen in preliminary experiments). McAb 35 and 192 were used in a concentration of 10 μg/ml. The mixtures thus prepared were added to the corresponding wells. After incubation in a humid chamber and after washing with buffered physiological saline to remove nonreacting substances, the plates were transferred into a substrate of 3,3'-diaminobenzidine tetrachloride ("Sigma").

EXPERIMENTAL RESULTS

Two test systems used to analyze inclusion of McAb in the precipitate are shown in Fig. 1. The original AS to CEA formed as a rule one precipitation band with PES, which separated distinctly into two lines when PEC was used. The outer precipitation band corresponded to CEA, the inner to NCRA of the tumor, which was immumologically identical to MCRA or normal human lung. After exhaustion of AS with the PEL preparation only one precipitation line was found both with PES and with PEC, and there was no reaction with PEL (Fig. 2).

To characterize McAb both test systems were used. McAb 35 and 192 were included in the test system for CEA when both exhausted and original AS were used (Fig. 2). However, when



a b c d e f

Fig. 3. Reaction of McAb to CEA with lung NCRA. 1) Polyclonal original AS + conjugate; 2-6) a-e: the same as Fig. 2; f) McAb to HAFP. Top and bottom rows comprised preparations before and after treatment with substrate, respectively.

the original AS and PEL were used (test system for NCRA) only McAb 192 was included in the precipitate with NCRA. In both cases mouse NCAB and HAFP, which is not a specific antigen for these test systems, were not included in the precipitate (Fig. 3), although they were included in the precipitate of the test system for HAFP (data not given).

Inclusion of McAb 192 and noninclusion of McAb 35 in the precipitate with NCRA are in full agreement with their epitopic specificity [5], which shows that this method can be used to characterize other McAb to CEA.

The analogous analysis of the chosen McAb (3C12, 2D10, and 2G10) showed that all three McAb bind CEA (Fig. 2). In addition, McAb 2D10 also bind NCRA (Fig. 3), i.e., they are aimed at a determinant which is common for CEA and NCRA. McAb 3C12 and 2G10 were not included in the precipitation line between PEL and AS (Fig. 3), evidence that these McAb differ in epitopic specificity from McAb 2D10.

The results thus showed that the MGP method is very useful and informative for the identification of McAb to CEA and the analysis of their epitopic specificity. The five McAb studied by the use of the test system for NCRA were divided into two groups. McAb 2D10 and 192 are aimed at a determinant or determinants common for CEA and NCRA. Conversely, McAb 3C12, 2G10, and 35 did not react with NCRA of the nurmal human lung. It is not clear from these data whether the McAb within each group differ in their own epitopic specificity. There is reason to suppose that McAb 3C12 and 2G10 are different. The explanation of this problem is currently being sought. Considering that several antigens closely related to CEA and having common antigenic determinants with them [10] exist, it would be advantageous to have a panel of test systems for these antigens for the more detailed characterization of McAb. This would allow McAb of the necessary specificity to be selected.

It must be emphasized that the method does not require purified preparations of antigens, which are absolutely essential for the study of these problems by the generally accepted methods — radioimmunoassay or enzyme immunoassay. The MGP method can be used for primary screening and analysis of the specificity of McAb for any precipitating antigens.

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DISTRIBUTION OF CYTOPLASMIC FERROPROTEINS AND IRON IN TUMORS OF THE HUMAN KIDNEY

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The existence of an association between disturbance of iron metabolism and the development of carcinoma of the kidney was postulated by the authors previously [6]. The carcinogenic effect of iron for the kidneys was subsequently confirmed experimentally [7, 15]. Interest in the study of ferroprotein and iron metabolism in carcinoma of the kidney also is due to the functional characteristics of the kidney as the main organ of iron excretion [11]. Transport of iron to the cells is effected by transferrin, whereas ferritin biosynthesis depends directly on the intracellular iron concentration [14].

The aim of this investigation was to study the distribution of cytoplasmic ferroproteins and iron in different regions of tumors of the human kidney.

EXPERIMENTAL METHOD

Adenocarcinoma of the kidney develops from the epithelium of the renal tubules. A characteristic feature of this tumor is that the primary lesion is separated from the renal parenchyma by a quite dense fibrous capsule. In the latter stages of development pairs appear in the capsule and the tumor infiltrates without any definite borders [3]. These morphological features lie at the basis of the subdivision of the kidneys by tumors, with the distinction between the renal parenchyma, the primary node, and tumor tissue infiltrating beyond the capsule of the primary node.

Altogether 19 kidneys with tumors, and 11 definitive and 9 embyonic kidneys were studied, with definitive, adenomatous, and carcinomatous tissues of the prostate gland as the control. Extracts were prepared in Tris-glycine buffer, pH 8.3, under standard conditions: the ratio of tissue to buffer was 1:2 (w/v). Extraction was done once, for repeated extraction gave only a negligible yield of the test proteins. The previously minced tissue was washed thoroughly with physiological saline. Besides the parenchyma and primary node, tumor tissue infiltrating beyond the capsule of the primary node was isolated from 7 of the 19 kidneys with tumors. Standard solutions containing 50 mg/ml of dry substances were prepared from the clarified and freeze-dried extracts: the excess of dry substances (60 mg/ml) was dissolved in the same buffer, incubated for 1 h at 37°C and for 24 h in a refrigerator, and the insoluble residue was separated by centrifugation and its protein concentration determined by Lowry's method, after which the solutions were diluted to a concentration of 50 mg/ml (quantity of soluble protein per gram wet weight of tissue).

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