PRESENCE OF ANTIBODIES REACTING SPECIFICALLY WITH STRUCTURAL PROTEINS OF MOUSE MAMMARY TUMOR VIRUS (MMTV) AMONG ANTIBODIES COMPOSING CIRCULATING IMMUNE COMPLEXES IN BREAST CANCER PATIENTS

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Antigens immunologically related to structural proteins of mouse mammary tumor virus are found in the tumors of some patients with breast cancer (BC) [2, 3, 5, 8], and antibodies reacting specifically with MMTV proteins are found in these patients' sera [1, 4, 9, 10]. A group of workers headed by Keydar [8] found that MMTV-related antigens are produced in the culture fluid by human T47D BC cells. Previously [1] the present writers described a phenomenon of a lower incidence of antibodies to MMTV in patients with BC in advanced stages compared with early stages. Since the tumor can produce MMTV-related antigens, this phenomenon could be explained, besides by other causes, by the formation of immune complexes, in a similar way to the phenomenon known for mice with MMTV-induced tumors [7].

The aim of this investigation was to study preparations of circulating immune complexes (CIC) isolated from sera of BC patients to look for antibodies reacting with MMTV proteins.

EXPERIMENTAL METHODS

Isolation of CIC. Immune complexes were sedimented by the following method: to 3 ml of freshly prepared serum the following reagents were added: aprotinin (Sigma, USA) up to 1%, 0.5 ml of borate buffer, pH 8.3 (0.2 M), 0.5 ml of a 12.5% solution of polyethyleneglycol (PEG) (mol. wt. 6,000 daltons) in the same buffer, and 1 ml of 0.2 M EDTA (pH 7.5); the precipitate of complexes found at 4°C overnight was sedimented at 5,000 g and washed with 10 ml of a 2.5% solution of PEG in borate buffer. The CIC preparation, isolated from a pool of 15 sera from BC patients (70 ml) was dissociated in a buffer of 0.15 M NaCl, 0.2 M glycine (pH 3.5), and fractionated by chromatography on Sephadex G-200 in the presence of the same buffer. The fraction containing immunoglobulins (Ig) was determined by the dot blocking method. This fraction was dialyzed against PBS and clarified by centrifugation.

Of 22 sera from BC patients, CIC preparations were obtained individually. Preparations of Fab'-fragments present in the immunoglobulin complexes were obtained by treatment with trypsin in 0.1 M sodium acetate buffer, pH 4.5, overnight at 37°C (ratio of protein/enzyme 100:1). The pH of the preparations was adjusted to 8.0 with 1 M Tris solution, after which they were clarified by centrifugation and used in the work.

Enzyme Immunoassay (ELISA). An MMTV preparation from C3H mice (from the culture medium of MM5/mt cells), obtained from the National Cancer Institute (Bethesda, Md., USA), was disintegrated by repeated freezing and thawing and introduced into wells of a panel for ELISA in a concentration of 5 μg/ml and in a volume of 150 μl. The virus preparation was made up in 0.01 M sodium-carbonate buffer (pH 9.6). Sorption of the antigen took place overnight at 4°C. The test preparation of antibodies or Fab'-fragments in a dilution of 1:20-1:100 in PBS with 0.05% Triton X-100 was introduced into the washed panel. To set up the blocking reaction the preparation of antibodies or Fab'-fragments was preincubated with the virus preparation or another protein for 2 h at 37°C. The panel with antibodies or Fab'-fragments was incubated overnight at 4°C. To determine bound antibodies a conjugate

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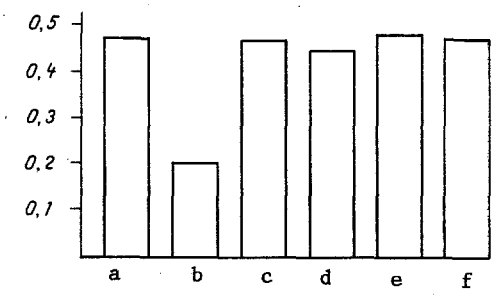

Fig. 1. Blocking the reaction of an Fab'-fragment preparation from CIC of serum from BC patients with MMTV preparation by the MMTV preparation itself and by control preparations. a) Without blocking; b-f) blocking reaction by MMTV preparation: b) 1 μg, c) 10 μg RaLV; d) 10 μg MPMV; e) 10 μg of C57Bl mouse milk preparation; f) 40 μg of preparation of normal mammary gland proteins of BALB/c mice. Ordinate, intensity of reaction (in optical units).

of goat IgG against human IgG with peroxidase (1000 U/mg; Serva, West Germany), prepared by periodate oxidation [6], was used. After each operation the panel was washed with PBS containing 0.05% Triton X-100. The substrate was 5-aminosalicylic acid [8]. The intensity of the reaction was assessed by the MR 590 Minireader (Dynateck, USA) at 450 nm.

EXPERIMENTAL RESULTS

CIC were isolated from the pool of sera from BC patients and the antibodies composing them were purified by chromatography under complex-dissociating conditions. Preparations of Fab'-fragments also were isoalted by digestion with pepsin from 22 individual CIC preparations also sedimented from sera of BC patients. Antibodies and Fab'-fragment preparations were investigated by the indirect immunoenzyme method for activity directed against epitopes of structural proteins of MMTV virus.

The pool of antibodies from the patients' CIC contained antibodies reacting with MMTV. Among individual Fab'-fragment preparations, activity of this kind was found in only one of the 22 tested. The specificity of binding of antibodies or Fab'-fragments with structural proteins of MMTV itself was verified by blocking the reaction with the MMTV preparation itself and with control preparations: type C (RaLV) and type D (MPMV) viruses, C57Bl mouse milk, and extract or normal mammary gland of BALB/c mice. An example of depression of the response by the MMTV preparation itself, but not by control preparations for a positively reacting Fab'-fragment preparation, is illustrated in Fig. 1. This excludes a reaction to the sugar residues of the glycosylated MMTV proteins or a reaction to mouse mammary gland proteins present in the virus preparation.

Thus some of the CIC from sera of BC patients contain antibodies which react with MMTV structural proteins. Evidently the prevalence of complexes containing such antibodies exceeds 1/22, for we were also able to test them in the antibody fraction from the serum pool. In our view the fact that antibodies reacting with MMTV may be components of CIC (which was demonstrated by this investigation) is important.

Production by the tumor of an antigen or antigens having common epitopes with MMTV structural proteins, and its release into the blood stream, is analogous with the phenomenon observed in mice with virus-induced mammary gland tumors [7] and can explain the fall of the titer of antibodies to MMTV in the late stages of BC.

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ANTIDOTAL AND ANTITUMOR PROPERTIES OF COPPER SULFATE

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The antitumor agent cisplatin, which is a complex compound of platinum, is known to give rise to undesirable side effects. In particular, it disturbs renal function, inhibits hematopoiesis, and induces nausea and vomiting. Copper sulfate, injected subcutaneously into mice with Ehrlich's tumor, has been shown to reduce the nephrotoxicity of cisplatin, without reducing its antitumor activity [4]. It was therefore interesting to study the antidotal properties of copper sulfate when given internally, for this therapeutic agent cannot be given subcutaneously [1].

To discover whether copper sulfate can be used in clinical practice the antitumor activity of copper sulfate given internally, and its action on the antitumor effect and various side effects of cisplatin were investigated.

EXPERIMENTAL METHODS

Chemotherapeutic experiments were carried out on 50 C57Bl/6 mice, 90 (CBA × C57Bl)F_1 hybrid mice, 32 BALB/c mice, 40 (DBA × C57Bl)BDF_1 mice, and 50 SHK mice. The antidotal properties were studied on 50 noninbred rats and 125 F_1 hybrid mice. An aqueous solution of copper sulfate in 0.1-0.5% concentration was given internally in a single dose or 5 daily doses, within the dose range from 10 to 120 mg/kg (in doses of 60-120 mg/kg copper sulfate was given with milk to reduce its toxicity). Cisplatin was injected intraperitoneally in single doses of 8 mg/kg for rats and 6, 14, and 16 mg/kg for mice. The antitumor activity was studied against transplantable strains of tumors: Lewis epidermoid lung carcinoma (LLC) adenocarcinoma of the colon (ACACOL), mammary gland adenocarcinoma Ca 755, and Ehrlich's ascites tumor (EAT). The tumors were transplanted by the standard method [3]. Antitumor activity was estimated relative to two parameters for solid tumors: the percentage inhibition of tumor growth by volume and the increase in survival period of the animals in percent relative to the control, and for the ascites tumor, by the increase in duration of survival in per cent of the control. In toxicologic tests, the state of the animals was inspected, the number of animals which died and at what times was noted, the animals were weighted, and the leukocyte and erythrocyte counts determined in the peripheral blood of the rats on the 3rd and 5th days after injection of cisplatin. The protein concentration in the urine was determined by the sulfosalicylic acid test. The creatinine and urea concentrations

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