POSSIBLE ROLE OF ENDOGENOUS MMTV VIRUS IN CHEMICAL MAMMARY GLAND CARCINOGENESIS IN MICE

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Many investigators have shown [9-11] that normal cells of various mouse tissues contain sequences of the integrated genome of MMTV — murine mammary gland tumor virus (endogenous virus). The distribution of endogenous MMTV viruses among mice is evidently ubiquitous. Infection of mice with exogenous MMTV virus is known to lead to mammary gland tumor development in the animals. Tumors also arise in mice free from exogenous virus, but in a much smaller proportion of cases and later. The percentage of tumors can be increased by the action of hormones and also of chemical carcinogens. Whether endogenous virus plays any role in this case in the development of mammary gland tumors is not yet certain. In hormonally induced mammary gland carcinogenesis the onset of preneoplastic changes in the gland (hyperplastic alveolar nodes) correlates with increased expression of virus-specific RNA of the endogenous virus [6, 7]. It is an interesting fact that in the viral mRNA population isolated from hyperplastic alveolar nodes and also from mammary gland tumors, there is a disproportionate number of transcripts from provirus DNA, with a higher content of sequences with 3'-terminal viral specificity and, evidently, responsible for synthesis of the end-product [4, 7]. In chemical carcinogenesis, increased expression of virus-specific mRNA, mainly with 3'-terminal virus specificity, also takes place, but to a lesser degree than in hormonally induced carcinogenesis [8].

To determine whether endogenous MMTV virus participates in chemically induced mammary gland carcinogenesis in mice, it was decided to study the effect of immunization with an inactivated MMTV preparation on the appearance of mammary gland tumors in mice free from exogenous virus (line BALB/c) after exposure to the repeated action of dimethylbenzanthracene (DMBA).

There was no intention to analyze the dynamics of MMTV expression during chemical carcinogenesis and immunization, but the aim was to study the degree of its expression in the final stage of carcinogenesis — in mammary gland tumors.

EXPERIMENTAL METHODS

Experiments were carried out on BALB/c mice from the "Stolbovaya" Nursery. The frequency of spontaneous mammary gland tumors in this line does not exceed 5%.

Females aged 2 months were given DMBA, in a dose of 1 mg dissolved in 0.2 ml of sunflower oil, weekly for 1.5 months. The BALB/c mice were immunized intraperitoneally with 10 μg (0.1 ml) of formalinized virus 1 week after the end of DMBA administration. The virus suspension was mixed with formalin solution until the final formalin concentration was 1:4000, and it was then kept for 2 days at room temperature with Freund's complete adjuvant (0.1 ml). The mice were tested twice a week for the presence of tumors 2 months after the beginning of the experiment.

To immunize animals of the experimental groups an MMTV concentrate was used, and mice of the control group were immunized with a concentrate of Moloney murine leukemia virus [MuLV (Mo)] obtained under the arrangements of the Soviet-American agreement.

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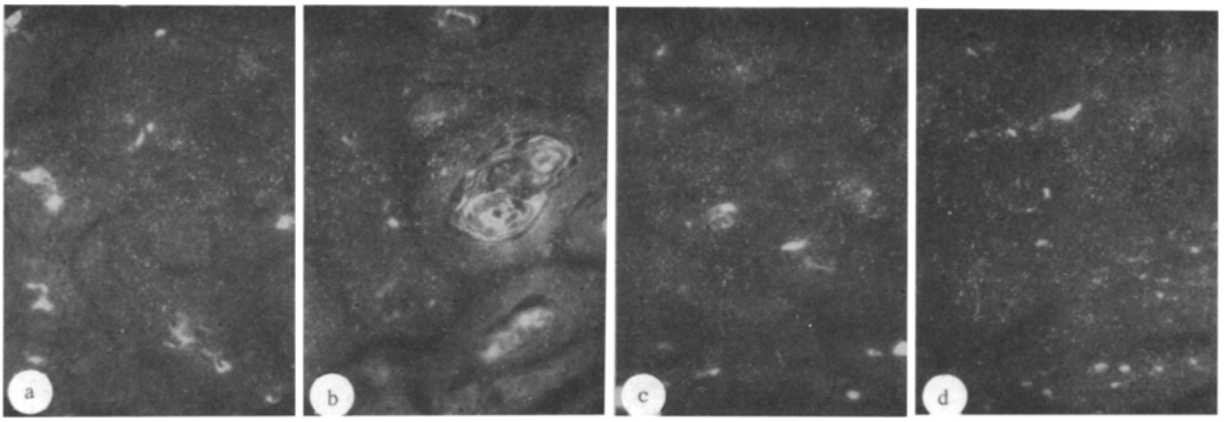

Fig. 1. Indirect fluorescence in paraffin sections. a, b) Mammary gland tumor arising after administration of DMBA and immunization with MMTV; a) antiserum against p27; b) antiserum against gp52; c, d) mammary gland tumor arising after administration of DMBA and immunization with MuLV (Mo): c) antiserum against p27; d) antiserum against gp52. 100 ×.

TABLE 1. Appearance of Mammary Gland Tumors in BALB/c Mice after Administration of DMBA Followed by Immunization with MMTV and MuLV

Immunization in latent period	No. of mice in experiment	No. of tumors appearing	Latent period of appearance of tumor, days
MMTV	106	23 (21%)	119±11
MuLV (Mo)	40	16 (39%)	88±7
Without immunization	33	13 (40%)	82±9

Legend. Difference between percentage of tumors arising in mice of control and experimental groups significant by χ^2 test ($P < 0.01$); difference between durations of latent period in control and experiment significant by Student's t-test ($P < 0.05$).

The protein concentration was determined by Lowry's standard method. The tumors were studied by indirect immunofluorescence on paraffin sections. To obtain sections 3 μ thick, pieces of tumor were fixed with a mixture of 3 parts of 0.1 M lysine, with sodium metaperiodate dissolved in it (21 mg/10 ml fixative) and 1 part of 8% paraform for 4 h in the cold. The sections were washed overnight in 0.05 M phosphate buffer, then taken through 3 portions of acetone in the cold and petroleum benzin, for 1 h in each case, and embedded in histoplast. The straightened and glued sections were taken through a battery of xylols and alcohols with decreasing concentration and placed in phosphate buffer medium. Immune sera were then applied to them for 20 min and, after rising, they were treated with fluorescent sera for the same time. After final washing the sections were mounted in buffered glycerol and examined under the microscope.

The following antisera were used: 1) anti-gp52 (rabbit), obtained by immunizing rabbits with purified gp52 of MMTV [1], 2) anti-p27 (goat), obtained from the USA in accordance with the Soviet-American agreement, 3) antirabbit fluorescent antiserum, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, and 4) antigoat fluorescent antiserum, obtained from the USA in accordance with the Soviet-American agreement.

EXPERIMENTAL RESULTS

Each female received a total of 6 mg DMBA. As Table 1 shows, the yield of mammary gland tumors after treatment with the carcinogen was about 40% (tumors only of the mammary gland

were counted, after histological investigation). This figure is almost the same as the percentage of tumors arising in animals of the control group, immunized with the MuLV (Mo) preparation (10 μg per mouse). After a single immunization of the mice with formalinized MMTV virus suspension, in a dose of 10 μg per mouse (50 days after the beginning of the experiment), the number of tumors arising in the BALB/c mice was reduced by almost half compared with the control (21%).

The mean latent period of appearance of tumors in the experimental animals was 119 days, much longer than in the control group (88 days).

Expression of virus antigens — membrane (gp52) and nucleoid (p27) — was investigated in sections of tumors arising in animals of the control and experimental groups by the indirect fluorescence method in paraffin sections (Fig. 1). Both in the control and in the experiment virus antigens p27 and gp52 were found to be expressed. The tumors arising in the experimental animals could evidently overcome the immunologic barrier formed after a single immunization with the MMTV preparation.

Fluorescence due to the membrane antigen was brighter than that due to the nucleoid. The gp52 antigen (product of the env gene) is most probably synthesized in larger quantities than p27.

No gene resembling the src transforming gene in type C viruses could be found in the system of type B viruses, although it has been suggested that there is a mam gene, the product of which transforms mammary gland cells [5]. It can be tentatively suggested that induction of synthesis of virus-specific mRNA [4, 6-8] and its products (gp52), which is especially intensive during hormonal stimulation, is the crucial moment of the neoplastic process in the mouse mammary gland during hormonal and chemical carcinogenesis. However, no convincing proof of the transforming role of products of the env gene has yet been obtained.

Immunization of mice against mammary gland tumors may either inhibit or stimulate tumor growth. The effect depends, first, on dose: Low doses of vaccine usually weaken tumor growth whereas high doses strengthen it [2]. The reason is evidently the appearance of blocking factors in the serum, which interfere with the cellular immune response to MMTV. This cellular factor is most probably an antigen—antibody complex. Second, the result of the immune response depends on the line of mice and strain of virus used for immunization, on the time of immunization, and on many other factors [3].

In the present experiments, with a single immunization with MMTV antigens, in the latent period of appearance of tumors after administration of DMBA the percentage of tumors developing fell and the latent period was considerably lengthened. Immunization with virus antigens probably activated the factors of humoral and cellular immunity, which inhibited the tumor process. This may be evidence in support of a role of endogenous virus in chemically induced mammary gland carcinogenesis.

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