METHODS OF OBTAINING ANTISERA AGAINST ANTIGENS OF MAMMARY GLAND CARCINOMA VIRUS

I. N. Kryukova, G. G. Miller, and T. Yu. Frolova

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Various methods of obtaining virus-containing preparations from mouse mammary gland tumors in order to prepare rabbit antisera against antigens of mammary gland carcinoma virus were tested. The best results were obtained by precipitating the virus from the culture fluid of tumors grown in vitro after preliminary hormonal stimulation of virus production and subsequent purification of the virus in a sucrose density gradient.

KEY WORDS: mouse mammary gland carcinoma; oncogenic viruses.

The agent of mouse mammary gland carcinoma (MGCV), "milk factor," or Bitner's virus has been known since 1936 [5]. However, research on this virus based on the only test available at that time (biological activity) was made extremely difficult by the need for prolonged observation on the infected mice. With the discovery of nucleoid soluble antigens in MGCV [1, 4, 5], similar to the soluble nucleoid group-specific antigens of the sarcoma–leukemia complex of viruses, and also after the obtaining of antibodies against the corpuscular antigens of MGCV [6], serological tests for MGCV became possible and the interest in its study grew.

Despite the numerous papers on the study of the various properties of MGCV, the production of highly specific sera against its antigens still remains a difficult probelem. Various methods of obtaining them have been described: 1) The most trustworthy method is by precipitating MGCV from the milk of high-cancer strains of mice, purifying it in a density gradient of sucrose, ficoll, or urographin, and immunizing rabbits with the purified virus [4, 6, 7, 15]; 2) immunization of rabbits or rats with mammary gland (MG) tissue homogenates from high-cancer or noninbred pregnant or lactating mice, followed by absorption of the resulting sera with normal tissue homogenates and the sera and milk of mice of low-cancer strains [3, 4]; 3) immunization of rabbits with fractions with a buoyant density of 1.16 g/cm^3, isolated from homogenates of mammary gland carcinoma (MGC) and MG of high-cancer strains of mice in a sucrose density gradient [17]; 4) immunization of rabbits with MGCV precipitated from the culture medium of MGC growing in vitro and purified in a density gradient of sucrose or urographin. Liberation of MGCV into the medium was stimulated by treatment of the growing cultures with cortisone or insulin.

The first method is rarely used because of the difficulty of obtaining adequate quantities of mouse milk at any one time (up to 20 g).

Methods 2 and 4 were used; the MGCV in preparations obtained by method 3 was tested with the sera obtained.

The tissues used were MGs and spontaneous and transplanted MGCs from C3H/He and C3H/Sn mice. The mice were obtained from the Laboratory of Inbred Animals of the N. F. Gamaleya Institute of Epidemiology and Microbiology. To obtain virus-containing preparations by methods 2 and 3, homogenates were made from MGC and MGs of pregnant mice in dilutions of 1:3 in Hank's solution, clarified at 5000 g, and

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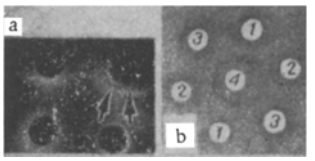

Fig. 1. Precipitation test in gel with various test systems for internal soluble antigen of MGCV. Autoradiograph. a) On the left, precipitation lines between antiserum against MGCV from Dmochowski (above) and milk antigen of mice of strain RIII (below); on the right, two precipitation lines between serum No. 11 against MGC homogenate (above) and MGC homogenate (below) (indicated by arrows); b) precipitation reaction showing identity of immune sera from Moore (1), Muller (2), and No. 65 (3), obtained against MGCV from culture fluid, purified in a sucrose density gradient; in center (4) milk of strain RIII mice.

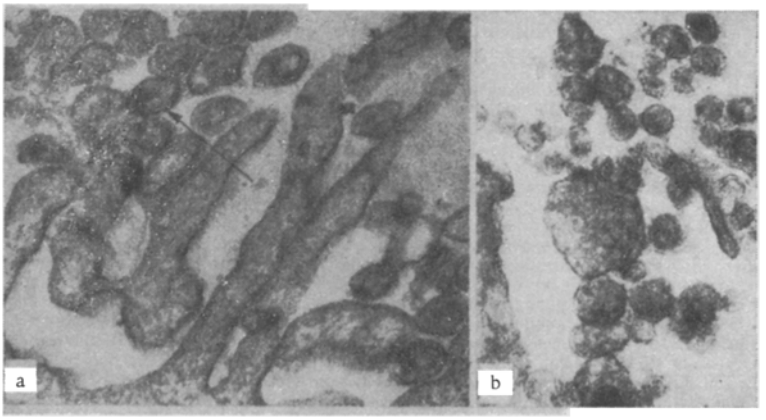

Fig. 2. Electron micrographs of ultrathin sections through cells of MGC tissue culture (method of treatment, see in text) (magnification 250,000 ×): a) general survey photomicrograph demonstrating cluster of type C virus particles (arrow); b) cluster of small particles 20-50 nm in diameter in MGC preparation, constantly accompanying production of type C particles.

used to immunize rabbits, leaving some of the homogenate for further purification of the virus in a sucrose density gradient.

To obtain immune sera by method 4, MGCs of generations II-VII in vivo were trypsinized and seeded in Povitskaya's flasks on medium containing lactalbumin hydrolyzate with 15% bovine or calf serum and antibiotics in the usual proportions. The cultures were maintained and hormonal stimulation of the liberation of virus carried out in the usual way [8, 12].

The residue equivalent to 2 liters of culture medium was collected from the three series of cultures, reprecipitated, and resuspended to a final concentration of 500:1. The presence of MGCV in the preparation was verified by the crossed reaction with a test system for soluble MGCV antigen obtained from Dmochowski and Moore (USA) and with the aid of a serum obtained from Muller (West Germany). The presence of biologically active virus in the preparations was verified by induction of hyperplastic nodules (HNs) in the MG of female BALB/c mice receiving estradiol (1 mg per mouse) and deoxycorticosterone acetate (500 μg per mouse) for 75 days, on alternate days, after injection of the ultraprecipitates [14].

Attempts were made by electron microscopy of ultrathin sections to determine the presence of particles of the B-type in the cells of the growing cultures. For this purpose cultures of growing MGCs were fixed with 1% glutaraldehyde solution in cacodylate buffer, pH 7.4, and then postfixed with chrome-osmium by Dalton's method [9]. After dehydration, the preparations were embedded in Epon–Araldite by Mollenhauer's

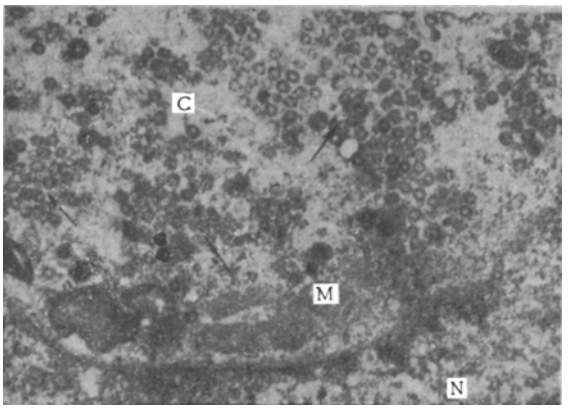

Fig. 3. Survey photomicrograph of MGC culture from generation XV in mice (35,000 ×). Collection of intracytoplasmic type A particles is demonstrated (indicated by arrows). C) cytoplasm; M) mitochondrion; N) nucleus.

method [13]. Ultrathin sections were cut on the LKB-4800 ultratome (Sweden) and examined in the JEM-100V electron microscope with intrumental magnifications of 35,000 and 50,000 ×.

Further purification of virus from the culture fluid and homogenates of MGCs was carried out in a linear sucrose density gradient of 20-60%. The zone with a density of 1.16 g/cm^3 was collected and the virus was reprecipitated and the precipitate was resuspended up to a final concentration of 2000:1. Immunization was carried out into the popliteal lymph glands [1]. The sera were tested by the gel precipitation test by the method of Gusev and Tsvetkov [2].

Immunization with MG and MGC Homogenates. Four immune sera were obtained. However, after exhaustion with homogenates of the internal organs, with MG tissue, and with milk and serum from mice of several low-cancer strains, the activity of the sera fell so much that even the globulin fraction, isolated from them by 50% saturation with ammonium sulfate, gave hardly visible precipitation lines. Work with these sera was possible only by the immunoautoradiographic method.

Besides soluble MGCV antigen, the fourth serum also revealed an antigen not identical with MGCV in the MGs of mice of certain low-cancer strains, especially after injection of urethane, Rauscher leukemia virus, and Rous virus into them (Fig. 1a). This antigen was not identical with the group-specific antigen (GSA) of mouse leukemias, as was shown by the absence of a crossed reaction with a test system for GSA obtained from O. M. Lezhneva [4]. The nature of the antigen thus discovered still awaits explanation.

Electron-microscopic investigation of cultures from generations II-V in mice revealed numerous virus particles of the C type in the extracellular space (Fig. 2a). Tiny particles, varying from 20-30 to 50 nm in size, were associated with the type C virus particles. They contained an electron-optically loosely packed nucleoid, surrounded by a single membrane (Fig. 2b). No type B virus particles could be found in these preparations. However, in cultures seeded from later generations, many MGCV particles could be found (Fig. 3).

The appearance of HNs in the hormonally stimulated BALB/c females in response to injection of ultraprecipitates of the culture fluid was found in all 14 MGs in a number of 1-2 HNs per mouse. In 19 MGs of control hormonally stimulated mice no HN could be found.

Immunization of rabbits by ultraprecipitates of culture fluid purified in a sucrose density gradient led to definite production of antibodies against MGCV antigen in one of two rabbits. After removal of contaminating antibodies against normal mouse tissue and serum antigens and bovine serum, an essential component of the growth culture medium, the antiserum gave a single clear precipitation line identical with the sera of Moore and Muller (Fig. 1b). No antibodies against GSA of leukemic viruses could be found in the immune serum. The serum did not react with the milk or tissue antigens of low-cancer strains of mice. Of the two methods of obtaining antisera against MGCV antigens that were tested, method 4 was thus more effective. Preparations of virus precipitated from MGC homogenates and purified in a sucrose density gradient

also gave only one clear precipitation line with the resulting antiserum; consequently, the method suggested by Muller can be used perfectly well for the above purpose, more especially because it is simpler than the method used by the writers.

In work with MGC tissue cultures the possible presence of leukemic viruses in their cells must be borne in mind; this has been demonstrated not only by us but also by other workers [10, 11, 16]. The fact that the type C virus found in these experiments is a leukemia virus was demonstrated by the writers previously. Preparations of precipitated virus and antiserum against it must therefore be carefully tested in a reaction with a test system for GSA of mouse leukemic viruses. Tiny virus-like particles were frequently found in other systems producing associations of A- and C- or A- and B-types of particles and, although the nature of these particles has not been explained, their presence must be taken into account in immunologic experiments.

LITERATURE CITED

1. A. I. Gusev and V. S. Tsvetkov, "Technique of the microprecipitation test in agar," Lab. Delo, No. 2, 43 (1961).
2. A. I. Gusev and A. K. Yazova, "An effective method of obtaining antisera against human and animal embryonic α-globulins," Byull. Éksperim. Biol. i Med., No. 4, 120 (1970).
3. E. R. Karamova, "Specific soluble antigen in normal and malignant tissues containing mammary gland carcinoma virus," Vopr. Virusol., No. 5, 605 (1968).
4. O. M. Lezhneva, "A comparative study of the antigenic structure of lactating mammary glands of high- and low-cancer strains of mice," Vopr. Onkol., 7, 62 (1961).
5. J. Bittner, "Some possible effects of nursing on the mammary gland tumor incidence in mice," Science, 84, 162 (1936).
6. P. Blair, "Immunology of the murine mammary tumor virus. A qualitative in vitro assay for MTV," Nature, 208, 165 (1965).
7. P. Blair, "Immunology of the mouse mammary tumor virus: comparison of the antigenicity of MTV obtained from several strains of mice," Cancer. Res., 30, 605 (1970).
8. R. Cardiff, P. Blair, and K. De Ome, "In vitro cultivation of the MTV: replication of MTV in tissue culture," Virology, 36, 313 (1968).
9. A. Dalton, "A chrome-osmium fixative for electron microscopy," Anat. Rec., 121, 281 (1955).
10. L. Dmochowski, P. Langford, W. Williams, et al., "Electron microscope and bioassay studies of milk from mice of high and low mammary cancer and high and low leukemia strains," J. Nat. Cancer Inst., 40, 1339 (1968).
11. E. Lasfargues, B. Kramarsky, N. Sarcar, et al., "Stimulation of mammary tumor virus production in a mouse mammary tumor cell line," Cancer Res., 30, 1109 (1970).
12. C. McGrath, "Replication of mammary tumor virus in tumor cell cultures: dependence on hormone-induced cellular organization," J. Nat. Cancer Inst., 47, 455 (1971).
13. H. Mollenhauser, "Plastic embedding for use in electron microscopy," Stain Technol., 39, 111 (1964).
14. S. Nandy, "New method for detection of mouse mammary tumor virus. II. Effect of administration of lactating mammary tissue extracts on incidence of hyperplastic mammary nodules in BALB/c Crgl mice," J. Nat. Cancer Inst., 31, 75 (1963).
15. R. Nowinski, L. Old, D. Moore, et al., "A soluble antigen of the mammary tumor virus," Virology, 31, 1 (1967).
16. T. Yumoto, L. Young, W. Williams, et al., "Studies on spontaneous mammary tumors in high-leukemia strain mice and on mammary tumors induced by mouse leukemia virus in low-leukemia strain mice," in: Proceedings of the Twenty-Fifth Annual Meeting of the Electron Microscope Society of America, Chicago (1967), pp. 108-109.
17. S. Von Zotter, C. Kemmer, M. Muller, et al., "Immunologische Kreuzreaktionen zwische virusproduzierenden und einem nicht virusproduzierenden murinen Mammacarcinom," Arch. Geschwulstforsch., 40, 23 (1972).