The results of this investigation thus demonstrated the high oncogenicity of viruses produced by B-cell cultures from stumptailed macaques for rabbits. It is not yet clear whether the oncogenic activity of these cultures is associated with herpesvirus (which is unlikely) or with a C-type retrovirus (which is most likely). The possibility cannot be ruled out that the oncogenicity of cultures of the MAL series is determined by the combined action of both types of viruses producing them. The answer to these questions will be given by research currently in progress.

LITERATURE CITED

1. B. A. Lapin, L. A. Yakovleva, V. Z. Agrba, et al., Hemoblastoses of Primates and the Role of Viruses in Their Origin [in Russian], Moscow (1979).
2. A. Komura, T. Watanabe, I. Miyoshi, et al., Virology, 138, 373 (1984).
3. B. A. Lapin, Advances in Biosciences. Vol. 50. Pathomorphological Aspects of Cancer Epidemiology (G. Mathe, ed.), Oxford (1985), p. 163.
4. B. A. Lapin, V. V. Timanovskaya, and L. A. Yakovleva, Hematology and Blood Transfusion. Vol. 29. Modern Trends in Human Leukemia, Berlin (1985), p. 312.
5. I. Miyoshi, S. Yoshimoto, H. Taguchi, et al., Gann, 74, 1 (1983).
6. T. Watanabe, M. Seiki, H. Tsujimoto, et al., Virology, 144, 59 (1985).

PROTEIN RELATED TO THE MAIN CORE PROTEIN OF MOUSE MAMMARY TUMOR VIRUS IN VIRUS-LIKE DENSITY FRACTIONS FROM HUMAN MILK

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Antibodies reacting with structural proteins of mouse mammary tumor virus (MMTV) have been demonstrated in man [2, 7, 12, 14]. Proteins immunologically related to products of the env and gag genes of this retrovirus have been found in mammary gland tumors [5, 6, 8, 13]. Should these MMTV-related antigens belong to a human type V retrovirus the possibility cannot be ruled out that virus particles are produced in milk, in a similar way to the phenomenon known for MMTV [13]. In fact, particles similar in morphology to MMTV have been found in milk and cells from the milk of certain women [1, 9, 11]. According to data in the literature [4], a major protein with mol. wt. of 27 kilodaltons (kD) is present in the fraction of human milk that corresponds in density to the cores of retrovirus particles, but its immunologic kinship with MMTV proteins has not been demonstrated. Meanwhile, the presence of antigens interacting with antibodies against an MMTV preparation was discovered by the agar diffusion test in this fraction [15].

In the present investigation the aim was to discover whether a protein related to the MMTV core proteins is present in human milk and, if so, to determine whether it is the antigen against which antibodies of human serum interacting with p27 MMTV are directed.

EXPERIMENTAL METHOD

Individual 50-ml samples of human milk from six healthy women, lactating for 50-70 h, were decanted and then quickly diluted with an equal volume of 0.01 M Tris-HCl buffer, pH 7.6, containing 0.15 M NaCl, 0.125 mM EDTA, and 0.1% aprotinin (Sigma, USA). Further fractionation of the milk was carried out as described previously [15], with slight modifications: cells and large membrane fragments were first sedimented (1000g, 10 min), after which the serum was separated from fat and the fraction of fat globule membranes (12,000g, 30 min); the ultraresidue from the serum was sedimented by centrifugation at 27,000 rpm for 90 min (SW-27

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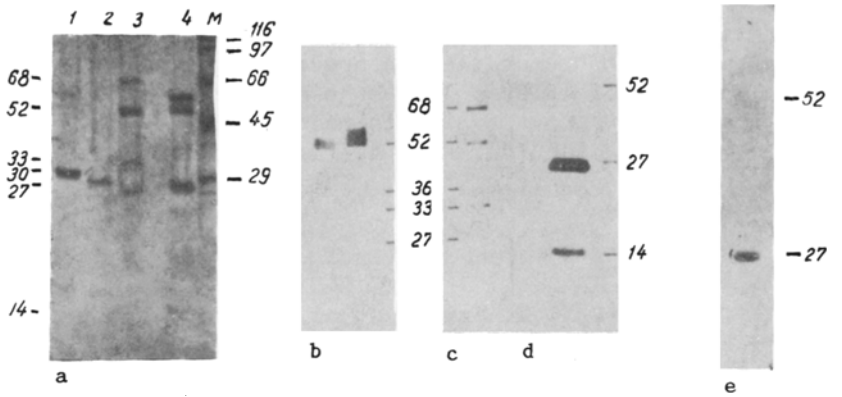

Fig. 1. Virus preparations and specificity of sera against them. a) Electrophoresis of virus preparations: M7 (1), SSV (2), MMTV RIII (3) and MMTV C3H (4); b, c, d, e) interaction of goat serum against gp52, rabbit serum against gp52, p27 goat serum, and serum No. 206 with MMTV proteins, respectively (immunoblotting). Here and Figs. 2 and 3: M denotes markers of molecular weight.

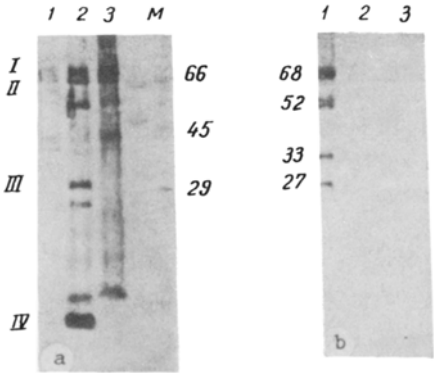

Fig. 2. Search for MMTV-related antigens among milk proteins. a) Preparations of fat globule membranes (1), milk serum (2), and cell membranes from milk (3); I) lactogerrin, II) serum albumin, III) caseins, IV) lactalbumin. b) Immunoblotting with these proteins and with serum against MMTV proteins.

rotor, Sorvall, France). The resulting pellet was either purified by centrifugation through a "cushion" of 30% sucrose or separated in a 20-68% sucrose density gradient. The sucrose solutions were made up in STE buffer. All operations were conducted at 4°C.

The following sera were used: rabbit serum against MMTV protein, rabbit serum against gp52 MMTV, goat sera against gp52 and p27 MMTV; the serum of a healthy woman, containing antibodies to p27 MMTV (No. 206). Rabbit antisera against human and goat globulins and donkey sera against rabbit globulins were obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. The immunoglobulin fraction was isolated from all sera by precipitation with ammonium sulfate (up to 40%) and purified on DEAE-sepharose. Conjugation with peroxidase (1000 U/mg, from Serva, West Germany) was done by the method in [10].

For the immunoblotting test the protein preparations were fractionated in 15% polyacrylamide gel and transferred to BA85 nitrocellulose (Schleicher und Schüll, West Germany) in an electric field with intensity of 10 V/cm (2 h). The filter with transferred proteins was incubated overnight in a 3% solution of ovalbumin in PBS (0.2 M phosphate buffer, 0.15 M NaCl) to inactivate the free binding valencies of the nictocellulose. The filter was then treated for 2-4 h with specific serum in a dilution of 1:300-1:400 at room temperature and, after washing, with the conjugate in dilutions of 1:300-1:600 (2 h). Dilutions of serum and

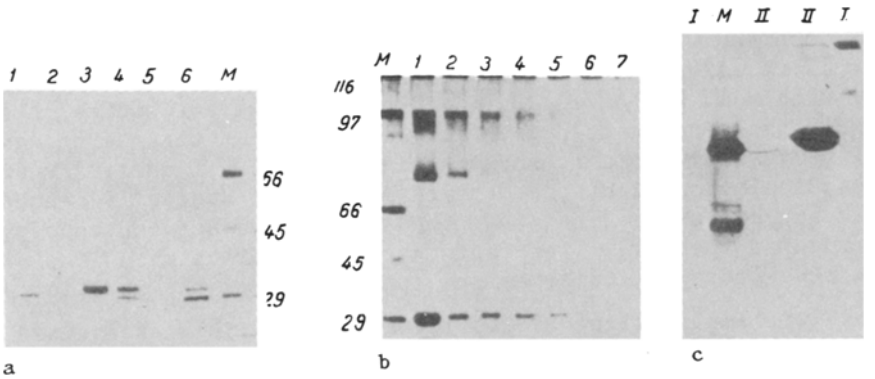

Fig. 3. Protein related to p27 MMTV and ultraresidue from milk. a) Electrophoresis of different ultraresidues (tracks 1-6), b) most typical distribution of proteins of ultraresidue in sucrose density gradient: electrophoresis of different sections of gradient. Buoyant density of particles forming preparation: 1) 1.12, 2) 1.17, 3) 1.18, 4) 1.19, 5) 1.2, 6) 1.24, 7) 1.28 g/ml; c) immunoblotting with serum against p27 MMTV. On left: I) fat globule membranes, II) ultraresidue; M denotes MMTV; the same protein preparations, stained with Amido black, are shown on the right.

conjugate were made up in buffer: PBS with 0.05% Triton X-100 and 1% ovalbumin. After each incubation the filter was washed in PBS with 0.05% Triton X-100 in 10 changes of buffer in the course of 2 h at room temperature. A solution of diaminobenzidine tetrahydrochloride (DAB) (0.5 mg/ml in 0.05 M Tris-HCl, pH 7.5) was used as the chromogen. To 1 ml of the freshly prepared solution of DAB 2 µl of 33% hydrogen peroxide was added. The reaction was stopped by elution of the substrate.

EXPERIMENTAL RESULTS

At the beginning of the work the specificity of the sera against the various MMTV proteins was verified by the immunoblotting test. The results of electrophoresis of MMTV preparations from RIII and C3H mice and control viruses are illustrated in Fig. 1a. The sera used in the work did not react with preparations of other retroviruses, and specific goat sera were found to be the most convenient for the work: anti-gp52 reacted only with proteins that were the product of the env gene, anti-p27 only with proteins that were products of the gag gene, whereas rabbit sera (against gp52 and MMTV) reacted also with p68, which is a protein of nonviral origin present in the virion. The results of immunoblotting with all specific sera are given in Fig. 1b, c, d, and the reaction of serum No. 206 with p27 MMTV in Fig. 1e. In these experiments the incubation conditions were worked out and the sensitivity of the indirect immunoperoxidase reaction determined under these conditions: for all specific antiviral sera about 30 mg of antigen (protein) per strip could be clearly revealed.

As a result of fraction of the milk, besides the ultraresidue from the serum, three other protein fractions of different components of the milk were obtained: a preparation of serum proteins after ultracentrifugation, milk cell membranes, and fat globule membranes. By means of the immunoblotting test with all these preparations it was found that the sera used did not contain antibodies reacting with the main proteins of the serum, fat globule membranes, and milk cell membranes, and that these fractions did not contain antigens related to MMTV in quantities which could be detected, given the sensitivity of the method (Fig. 2).

Five ultraresidues were isolated from the serum, purified through a "cushion" of 30% sucrose, and yet another residue was fractionated in a sucrose density gradient. It will be clear from Fig. 3, which shows the results of electrophoresis of preparations of the ultraresidues and the various fractions from the sucrose gradient, that most of the preparation consisted of caseins, the spectra of which can evidently vary considerably from one donor to another. Judging from the character of distribution of the caseins in a sucrose gradient, casein micelles differ in their buoyant density, and one difference is that they are present also in density zones characteristic of retrovirus particles. If a small quantity of the ultraresidue preparation was applied to the track, no reaction of sera against

MMTV proteins was observed with any of the proteins of the preparation. However, if a large quantity of ultraresidue preparation was applied to the track, a protein migrating in front of caseins, with mol. wt. of 27 kD, was observed, and antibodies of serum against products of the MMTV gag gene reacted with it (Fig. 3c). If this serum was replaced by anti-gp52, normal goat serum, or goat serum against p10 MPMV (generously provided by A. V. Morozov, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) this protein could not be detected, so that the reaction of antibodies of the anti-p27 MMTV serum can be regarded as specific. The fact that of all proteins of the preparation only the minor p27 was revealed also indicates the specificity of the reaction.

Thus a protein with mol. wt. of 27 kD, revealed by antibodies against MMTV core proteins, is present in the ultraresidue fraction of human milk. This protein was found by the writers in five of the six ultraresidues studied. It may perhaps correspond to MMTV-related antigens described for this fraction [15]. Although the serum which we used also contained antibodies to p14 MMTV, it is interesting to compare the protein now described with p27 with respect to molecular weight. It is now known whether this problem is in fact a component of retrovirus particles or associated with casein micelles, but there is no doubt that it is structurally organized in a certain way and is not found in other milk fractions in determinable quantities.

To establish the degree of immunologic kinship between this protein and products of the gag gene of MMTV, a series of experiments was carried out involving absorption of serum against p27 MMTV and serum No. 206. The ultraresidue fraction containing the p27 which we described eliminated activity of the serum against p27 MMTV against p27 MMTV itself, evidence of the sufficiently close kinship of p27 MMTV and the protein now being described.

Because the women's serum contained large amounts of antibodies to normal milk proteins we could not use serum No. 206 in the immunoblotting test; however, after its exhaustion with positive ultraresidues serum No. 206 did not react with p27 MMTV in the immunoblotting method. This is evidence of the high probability that the protein which we described is that same antigen to which antibodies reacting with the basic core protein of MMTV arise in man. This also confirms that the p27 which we described is related to p27 MMTV and not to p14. However, the problem of the viral nature of this protein requires further study.

LITERATURE CITED

1. G. G. Miller, I. N. Kryukova, N. N. Mazurenko, and V. N. Zhdanov, Vopr. Virusol., No. 4, 429 (1975).
2. N. Day, S. Witkin, N. Sarkar, et al., Proc. Natl. Acad. Sci. USA, 78, 2438 (1981).
3. D. Du Bois and R. Rossen, J. Immunol. Meth., 63, 7 (1983).
4. P. Furmanski, C. P. Loeckner, C. Longley, et al., Cancer Res., 36, 4001 (1976).
5. I. N. Kryukova, E. Komarova, S. Zotter, and H. Grossman, Arch. Geschwulstforsch., 51, 517 (1981).
6. P. Levine, R. Mesa-Tejada, I. Keydar, et al., Int. J. Cancer, 33, 305 (1984).
7. S. V. Litvinov, L. V. Remennik, and I. N. Kryukova, Bull. Exp. Biol. Med. 97, 649 (1984).
8. R. Mesa-Tejada, I. Keydar, M. Ramanaraganan, et al., J. Histochem. Cytochem., 26, 532 (1978).
9. D. H. Moore, Cancer Res., 34, 2322 (1974).
10. P. Nakane and A. Kawaoi, J. Histochem. Cytochem., 22, 1084 (1974).
11. N. Sarkar and D. H. Moore, Nature, 236, 1003 (1972).
12. M. Tomana, A. Kajdos, W. Niedermeier, et al., Cancer (Philadelphia), 47, 2696 (1981).
13. N. S. Yang, H. D. Soule, and C. M. McGrath, J. Natl. Cancer, Inst., 59, 1357 (1977).
14. S. Zotter, C. Kemmer, and M. Muller, Exp. Pathol., Suppl. 6 (1981).
15. S. Zotter, C. Kemmer, A. Lossnitzer, et al., Eur. J. Cancer, 16, 455 (1980).