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MODEL OF MALIGNANT LYMPHOMA PRODUCED IN RABBITS WITH PRIMATE ONCOGENIC VIRUSES.

PRELIMINARY COMMUNICATION

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UDC 616-006.444-022:578.828]-022.1-092.9

KEY WORDS: oncogenic herpesviruses and retroviruses of primates; rabbits; lymphoma

It was reported previously that lymphoid cell lines producing B-lymphotropic herpesvirus (HVP) had been obtained from baboons [1, 3], and also that an analogous cell culture (MAL-1), producing a similar virus, described as HVMA, had been obtained from peripheral blood lymphocytes of a stumptailed macaque (*M. aretoides*) [4]. Later, the authors obtained a further two lymphoid cell lines MAL-2 and MAL-3, also producing HVMA, from the same species of macaque. Both HVP and HVMA are related to, but not identical with, human B-lymphotropic Epstein–Barr herpesvirus (EBV). Electronmicroscopic studies have shown that cells of some baboons and stumptailed macaques (in particular, MAL-3) produce not only HVP and HVMA, but also C-type retroviruses (Fig. 1).

The aim of this investigation was to obtain a model of a virus-associated malignant lymphoma, investigated by the authors previously in primates, in rabbits. This is an important task at the moment because modeling diseases in primates is complicated by the long incubation period, sometimes measured in years, and other associated difficulties. Rabbits seemed to be promising animals for these purposes because of reports in the literature that rabbit lymphocytes may be transformed in vitro under the influence of human T-lymphotropic virus (HTLV-I) [5] and that a retrovirus (STLV-I), closely related to HTLV-I, has been found in stumptailed macaques [2, 6].

EXPERIMENTAL METHOD

Cells of strains MAL-1, MAL-2, and MAL-3 were injected intramuscularly into the lateral surface of the middle third of the right thigh of gray rabbits weighing 500-600 g (from the nursery of the Research Institute of Experimental Patholgoy and Therapy, Academy of Medical Sciences of the USSR). Two rabbits were inoculated with each strain. The control group was identical (six animals), but the rabbits received injections of cells heated to 56°C for 1 h. Histological, cytogenetic, and electronmicroscopic investigations were carried out by

Research Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 3, pp. 336-338, March, 1987. Original article submitted June 17, 1986.

0007-4888/87/0003-0377$12.50

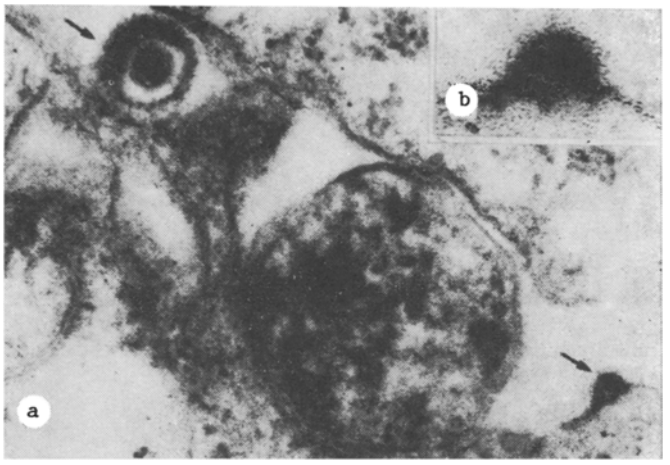

Fig. 1. Herpesvirus virion and budding C-type particle in cells of lymphoid culture from stumptailed macaques (MAL-3). a) Mature herpesvirus virion in intercellular space and C-type particle budding on cytoplasmic membrane. 60,000 ×; b) Fragment of same electron micrograph. 200,000 ×.

the usual methods. The rabbits' sera were tested by the indirect immunofluorescence test on the following target cells: human B-cell strain Raji (not containing virions), human T-cell strain C-91-PL (producing HTLV-I), baboon B-cell strain 594S-F9 (producing HVP, STLV-I, and an endogeneous C-type retrovirus. Rabbit immunoglobulins were identified by the direct immunofluorescence test. The rabbit cells were cultured in vitro in medium RPMI-1640 with the addition of 15% embryonic calf serum.

EXPERIMENTAL RESULTS

All rabbits inoculated with the MAL strains developed generalized undifferentiated non-B-cell malignant lymphomas. The first signs of the disease, which began with hyperplasia of the right popliteal lymph node, were discovered only 20-25 days after inoculation. After 35-40 days the lymphoma was generalized in character. The right popliteal lymph node, pelvic nodes, mesenteric and cervical lymph nodes, and the spleen were most affected, the kidneys, liver, thymus, skin, and bone marrow less frequently. Inoculation of young rabbits with a primary induced tumor led to the development of lymphoma in one of the three inoculated animals. The development of lymphoma also was induced by injection of cell-free filtered culture fluid from cultures of MAL. Culture of a tumor from one rabbit led to the production of a suspension lymphoid cell culture which was called RT-1 (RT = rabbit tumor). This cell line has now gone through 12 passages. Addition of growth factors is not necessary for multiplication of RT-1 cells. Cells of this strain have no surface or cytoplasmic immunoglobulins. Electron-microscopically, herpesviruses and C-type particles could not be found in them. The tumor cells, including those in culture, had the rabbit karyotype.

In the control group no tumor developed in a single case, and this was confirmed by morphological investigations (time of observation 4 months).

Sera of rabbits inoculated primarily with MAL cells and of animals used for transplantation of MAL-induced lymphoma (1st passage) were studied by the indirect immunofluorescence test. Sera from the primarily inoculated rabbits showed marked nonspecific reactivity, and for that reason it is not yet possible to draw even preliminary conclusions regarding the specificity of antibodies in these sera. Investigation of the serum of the rabbit in which passage of the tumor had taken place showed some specificity. This serum did not react with antigens of Raji cells and it revealed cytoplasmic antigens in the 594S-F9 and C-91-PL cells. The serum of this same rabbit, obtained before inoculation, was negative for all three types of target cells. It can be postulated on the basis of these results that this rabbit had antibodies reacting with HTLV-I/STLV-I antigens. However, this hypothesis requires confirmation on a more abundant material, and by methods with greater specificity and resolving power than the indirect immunofluorescence test.

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.

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

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UDC 618.19-008.846.9-008.839.6:[578.828.4:578.224

KEY WORDS: mammary tumor virus; mice; human milk; milk proteins

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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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 3, pp. 338-340, March, 1987. Original article submitted November 13, 1985.

0007-4888/87/0003-0379$12.50