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Lymphocytic-Choriomeningitis Virus in Hamster Tumor: Spread to Hamsters and Humans

Abstract. *A passage line of a spontaneous hamster fibrosarcoma is contaminated by the virus of lymphocytic choriomeningitis. Tumors from animals receiving implants when newborn contain high titers of infectious lymphocytic-choriomeningitis virus and complement-fixing antigen, and hamsters receiving implants when weanlings develop high titers of complement-fixing antibody against lymphocytic-choriomeningitis virus. In contrast with the specific reactions of tumorous hamsters to the initiating virus in virus-induced tumors, the development of complement-fixing antibody to lymphocytic-choriomeningitis virus does not depend on the development of tumors. Infant hamsters bearing the tumor have a generalized subclinical infection and seem able to spread virus to other hamsters and to humans.*

During studies to determine whether or not spontaneous hamster tumors can elicit complement-fixing antibody response to specific tumor antigens, comparable with the responses observed with virus-induced tumors (*1*), we found that hamsters bearing transplants of a spontaneous fibrosarcoma developed high-titer complement-fixing antibody to the homologous tumor. However, further study indicated that the reaction was due to lymphocytic-choriomeningitis (LCM) virus, which has been isolated from a number of primary and transplanted tumors of mice (*2*) and guinea pigs (*3*); its occurrence in hamsters, either normal or tumorous, has not been described.

Tissue fragments from the Fortner fibrosarcoma No. 2 (*4, 5*) were transplanted serially in newborn and weanling NIH Syrian hamsters that were free of LCM-virus infection. Serums were obtained from tumor-bearing hamsters that had been implanted as weanlings. Antigens were prepared from tumors that had been transplanted into newborn hamsters. Tumors were collected, and the antigens were prepared and tested in the complement-fixation test (*1*).

The LCM antigen was prepared by inoculation of LCM virus, strain CA 1371, passed through mouse brain (*6*), onto monolayers of kidney cultures from *Cercopithecus* monkeys. Cells and fluids were harvested on the 7th day. The cells were sedimented by centrifugation and resuspended in 5 percent of the original volume of supernatant. Control cells were prepared by a similar technique from the same lot of monkey-kidney cells (*7*). The resulting antigen was standardized in complement-fixation tests with specific guinea pig antiserum to LCM (*8*).

Serums from weanling hamsters bearing the Fortner fibrosarcoma in the first passage in our laboratory contained significant complement-fixing antibody to LCM virus. Sixteen individual serums collected 29 days after transplantation from animals having tumors ranging from 15 to 35 mm in diameter had titers of complement-fixing antibody from 1:20 to greater than 1:160 against both the LCM monkey-kidney antigen and Fortner-tumor antigen (20-percent extract of fibrosarcomas removed from hamsters that had received implants when newborn). Remarkably comparable titers were obtained with both antigens. These same serums gave no reactions when tested with the following groups of antigens: monkey-kidney control, extracts of hamster tumors induced with adenoviruses types 7, 12, and 18; Schmidt-Ruppin strain of Rous-sarcoma virus and simian virus 40; and potent viral antigens for mouse adenovirus, mouse hepatitis virus, Toolan H-1 agent, Kilham rat virus, K virus, polyoma, and simian virus 40.

The Fortner-fibrosarcoma No. 2 antigens from tumors transplanted to newborn hamsters had complement-fixing titers of 1:16 to 1:64 when tested against specific guinea pig antiserums to LCM. None of six serums from normal guinea pigs reacted with the Fortner-tumor antigens. No positive complement-fixing reactions were detected when antigens prepared from seven individual Fortner fibrosarcomas were tested with specific antiserums against the other viral and tumor antigens listed above. No positive reactions with the Fortner-tumor antigen have been detected in 300 serums obtained from normal adult hamsters in the NIH colony (*9*).

Intracerebral inoculation of NIH Swiss mice with extracts from three Fortner fibrosarcomas produced convulsive death, characteristic of LCM infection, in 5 to 10 days. The virus isolated from one of these tumors was designated the Fortner-fibrosarcoma No. 2 LCM virus and was established in serial passage in mouse brains. One tumor extract, from a hamster that had received an implant shortly after birth. had a titer of $10^{6.7}$ mouse median lethal doses per milliliter. Mice inoculated subcutaneously or intraperitoneally with Fortner-tumor antigens or with the Fortner-fibrosarcoma No. 2 LCM virus, passed through mouse brain, uniformly developed complement-fixing antibody titers from 1:40 to greater than 1:80 in 2 weeks; they were immune to intracerebral challenge with a dose of LCM virus, strain CA 1371, that killed all controls in 6 days. Conversely, mice immunized by subcutaneous injection of LCM virus, strain CA 1371, were immune to intracerebral challenge with the Fortner-fibrosarcoma No. 2 LCM virus.

Weanling hamsters inoculated intraperitoneally with cell-free filtrates of Fortner fibrosarcomas developed complement-fixing antibody to LCM mon-

key-kidney antigen and to Fortner-tumor antigen in 2 weeks, indicating that development in hamsters of antibody to LCM is unrelated to the development of tumors.

No evidence of LCM has been observed in any hamsters bearing the Fortner fibrosarcomas. Newborn hamsters inoculated with cell transplants shortly after birth develop visible tumors in 7 to 10 days; the tumors enlarge rapidly and most animals die with massive tumors (25- to 50-mm) in 14 to 31 days. Weanlings develop palpable tumors within 10 to 12 days after receiving implants; none died after 2 months of progressive growth of tumors.

The data indicate that the Fortner fibrosarcoma No. 2 is contaminated with LCM virus and that presence of this virus in tumor transplants is responsible for the antibody response observed in tumor-bearing hamsters. Preliminary studies reveal that serum and organs (brains, lungs, liver, spleen, and kidney) from tumor-bearing hamsters that received implants during the first 24 hours of life also contain LCM virus. Consequently transplantation of tumor fragments from hamster to hamster results in a generalized infection and is simply a mode of inoculating virus. Development of high-titer complement-fixing antibody in weanling hamsters within 2 weeks of inoculation with cell-free tumor extracts corroborates this point.

Weanling hamsters bearing transplanted tumors induced by the Schmidt-Ruppin strain of Rous-sarcoma virus and an avian adenovirus–like agent (chicken-embryo lethal-orphan virus, CELO) (*10*) were housed in the same room with the animals bearing Fortner-fibrosarcoma No. 2 tumors. A number of the weanlings developed LCM-antibody titers of 1:80 or greater. Both the Rous and the CELO tumors were known to be free of LCM virus in earlier passages. One CELO-tumor antigen reacted at a 1:32 dilution with specific hamster antiserum to LCM. Also a number of hamsters bearing various primary or transplanted tumors (free of LCM in earlier passages), and housed in nearby facilities serviced by the same personnel, have developed complement-fixing antibody to LCM. Such data suggest that infected hamsters shed LCM virus and that a colony of susceptible hamsters may become contaminated with LCM by exposure to fomites.

Several weeks after the hamsters bearing Fortner-fibrosarcoma No. 2 tumors were introduced into the animal colony, personnel working with these animals developed influenza-like illnesses and serological evidence of LCM infection. This is further evidence of environmental contamination and indicates hazards that may attend work with transplantable rodent tumors, even when such transplanted tumors are derived from spontaneous or chemically induced neoplasms. The Fortner fibrosarcoma No. 2 had been contaminated by LCM while it was carried in certain other laboratories working on LCM infection in mice. Tumor samples received from one such laboratory were free of LCM virus and antigen.

Sabin (*11*) has shown that serological reactions to isoantigens in transplanted tumors may resemble the virus-specific reactions described by Huebner *et al.* (*1*). Our results demonstrate that a contaminating virus also may be a source of misleading results. Awareness of possible contamination of hamster tumors by LCM is also important for the protection of personnel. Presence of LCM virus in tumors has one possibly useful aspect: since the titer of LCM-complement-fixing antigen is superior to that obtained in other systems, the tumor extracts may be useful as potent and cheap diagnostic reagents.

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Rabbit Muscle Lactate Dehydrogenase 5; A Regulatory Enzyme

Abstract. *Lactate dehydrogenase isozyme 5 from rabbit skeletal muscle is activated by citrate,* cis*-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, aspartate, and glutamate. In the presence of these activators the shape of the pyruvate saturation curve is changed from sigmoid to hyperbolic. Lactate dehydrogenase isozyme 1 from rabbit heart gives a hyperbolic pyruvate saturation curve and is not activated by these compounds. Oxalacetate is a competitive inhibitor of both isozyme 5 and isozyme 1 but at low concentration it activates the former. These results indicate that lactate dehydrogenase isozyme 5 from rabbit skeletal muscle is an allosteric protein and a regulatory enzyme, while lactate dehydrogenase isozyme 1 from rabbit heart is apparently neither.*

A regulatory enzyme is one which takes part in the intracellular control of metabolic pathways. Evidence is accumulating that such enzymes have a number of characteristics in common; for example, (i) they are composed of subunits; (ii) the substrate saturation curves under certain conditions are sigmoid shaped; and (iii) they undergo conformational changes when exposed to "effectors" (*1*). The effectors may be activators or inhibitors and are bound to the enzyme at a site distinctly different from the substrate site. This type of molecular alteration has been termed an allosteric transition (*2*) and, as pointed out by Umbarger (*3*), is a special case of the "induced-fit" hypothesis of Koshland (*4*). Aspartate transcarbamylase is a classic example of a regulatory enzyme (*5–7*).

The subunit nature of various lactate dehydrogenases has been well documented (*8*). This report will present evidence that lactate dehydrogenase isozyme 5 (LDH 5) from rabbit skeletal muscle has a sigmoid-shaped substrate saturation curve which becomes hyperbolic in the presence of a number of effectors. The results suggest that this isozyme can be classified as a regulatory enzyme.

Twice recrystallized rabbit muscle LDH, which contains all five isozymes, was purchased from Worthington Biochemical Corporation. Pure LDH 5 was obtained from this prepa-