The Mammary Tumor Virus in Organs of Mice: Normal Distribution and Postinoculation Localization* (33354)

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The presence of the mammary tumor virus (MTV) in normal mammary gland tissue of mice from high mammary cancer strains is well known (1–3), and the wide distribution of MTV throughout extramammary tissues of such mice has been suggested by several investigators (1, 2, 4). There is evidence for the presence of the virus in spleen (1, 4–8), liver (5, 9), placenta (9), testis (10), Harderian gland (11), seminal vesicle (12), contents of the cauda epididymidis (13), male ejaculate (14), and blood (3–5, 10, 15, 16), especially in red blood cells (3).

The data on the presence of MTV in various organs have come from several isolated studies involving several MTV-carrying strains and a variety of test mice. There has been, however, no comprehensive study of many organs from individual donors of a single strain tested in assay mice which were histocompatible with the organ donors. In a recent report (17) it has been shown that red blood cell-borne MTV (R-MTV) can readily infect only H-2 compatible test mice. The relatively low tumor incidence observed my different investigators (1, 9) in test mice receiving transplants or extracts of organs, other than mammary gland, might have been due to histocompatibility differences between the donor and test strains, since it is possible that MTV activity present in many of the non-mammary tissues might be due to their contained red blood cells.

The presence in tissues of red blood cells, carrying MTV activity, has inhibited investigation into the origin of R-MTV. However, in MTV-inoculated BALB/c mice we have recently observed that an interval of several days exists between MTV administration and detection of R-MTV in the blood (18). During this interval, if one could find some preferred organ(s) in which MTV localizes, one might gain some insight into the site of origin of R-MTV.

The present experiments were undertaken to determine whether MTV activity can be found in various organs of MTV-carrying BALB/cfC3H mice and whether MTV, following its inoculation, becomes localized in some particular organ(s) of BALB/c mice.

Materials and Methods. In the two experiments, individual MTV-infected donors provided blood, spleen, liver, thymus, mammary gland, lymph nodes, and bone marrow. For assay purposes the blood and organs of each donor were injected or implanted separately into 3-week-old MTV-free BALB/cCrgl (C—) female mice. In the first experiment, 8-week-old BALB/cfC3H/Crgl female mice served as blood and organ donors. In the second, donors were 8-week-old BALB/cCrgl females that had been inoculated intraperitoneally 3 days before with 25 mg equivalents of a cell-free extract (19) of lactating mammary gland from MTV-infected C3H/Crgl female mice.

Each donor was bled from the tail (3), and 0.1 ml of heparinized whole blood was injected subcutaneously into 3 or 4 C— hosts. The donor was then anesthetized, and the organs excised and implanted into 3 or 4 C— hosts. Hosts received one of the following transplants: one-quarter of a spleen, one-half of a thymus lobe, a piece of liver approximately equal in size to that of the spleen transplant, one mammary fat pad (last thoracic or first inguinal) with its contained parenchyma, two superficial lymph nodes (cervical, brachial, or inguinal), or one-fourth the total amount of bone marrow from the cavities of the femora, tibiae and humeri. The bone marrow was teased gently,

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TABLE I. Nodule Incidences in BALB/c Hosts Receiving Blood or Organs from 8-Week-Old BALB /cfC3H Females.

	No. of hosts	% with nodules	Total no. of nodules
Blood	7	100	337
Spleen	8	100	505
Liver	7	100	411
Thymus	6	100	475
Mammary gland	6	100	310
Lymph node	6	100	346
Bone marrow	6	100	464

suspended in 0.4 ml 0.85% NaCl, and injected intravenously in a volume of 0.1 ml per C— host. All other tissues were transplanted subcutaneously.

The nodule bioassay for MTV(20) was employed to determine whether C— assay hosts were infected with MTV. Two isologous pituitaries, transplanted under the kidney capsule of each test mouse at 4–5 weeks of age, provided the necessary mammary stimulation. Data are reported in terms of the percentage of hosts with nodules and the total number of nodules per group.

Results. The results of the assays for MTV of blood and various organs from BALB/cfC3H females are given in Table I. There were nodules in the mammary glands of all 46 hosts receiving blood, spleen, liver, thymus, mammary gland, lymph nodes, or bone marrow.

Table II summarizes the results from the administration to C— hosts of blood and various organs from BALB/c mice inoculated with MTV 3 days previously. No nodules were observed in the mammary glands of hosts that received liver, thymus, or mammary gland. A few of the hosts (4–18%) that received blood, lymph nodes, or bone marrow developed nodules. However, 75% of the hosts that received spleen transplants developed nodules.

TABLE II. Nodule Incidences in BALB/c Hosts Receiving Blood or Organs from Female BALB/c Donors Inoculated 3 Days before with MTV.

	No. of hosts	% with nodules	Total no. of nodules
Blood	23	4	12
Spleen	20	75	630
Liver	18	0	0
Thymus	17	0	0
Mammary gland	18	0	0
Lymph node	17	12	22
Bone marrow	17	18	31

Discussion. In the first experiment high MTV activity was found in the blood and in each BALB/cfC3H organ tested (spleen, liver, thymus, mammary gland, lymph nodes, bone marrow). These were not unexpected results and substantiate the view that MTV is distributed widely throughout the body of the MTV-carrying mouse. It is likely, however, that red blood cell-borne MTV in the BALB/cfC3H organs was partly or wholly responsible for the activity detected.

The wide occurrence of MTV, as in the BALB/cfC3H mice reported here, is probably not unique to this strain. A similar distribution may also occur in other strains (C3H, A, DBA) with blood-borne MTV activity (17), and in fact MTV activity may be demonstrable in all vascularized tissues from infected animals. However, MTV activity may also be associated with certain tissues themselves, such as the mammary tissue of adult mice (3) and the spleen (see below).

When tissues were transplanted from mice 3 days after MTV administration, MTV activity was found to be localized in the spleen. The activity detected in the spleen was not due to the contained blood, since the blood itself did not contain appreciable MTV activity. The presence of high viral activity in the spleen, known to be an erythropoietic organ in the mouse (21), shows it to be a preferred site for MTV localization and supports the suggestion (17) that R-MTV may be produced in erythropoietic tissue. It is also possible that the spleen may only be the site for entrapment of MTV. Experiments are in progress to analyze the mechanism of MTV localization in the spleen and to determine the cell type(s) responsible for this phenomenon.

Summary. Blood and various organs from MTV infected mice were administered separately to histocompatible MTV-free test mice. With tissues from BALB/cfC3H donors MTV activity was found associated with

the blood and with each organ tested. When various organs from MTV-inoculated BALB/c mice were assayed prior to the appearance of MTV activity in their blood, activity was localized primarily in the spleen.

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Preservation of Infectious Cytomegalovirus* (33355)

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Since human cytomegalovirus was first isolated by Smith, Rowe, and Weller in 1956, problems have been encountered in maintaining this infectious agent owing to its lability under variety of experimental conditions (1–3). In the past the strains have either been maintained in serial passage in human embryonic fibroblasts (1, 4) or frozen (with some stabilizing additive) at −70° for relatively short periods of time (5, 6). The purpose of this paper is to report long term stability under rather simple conditions of liquid nitrogen storage.

Materials and Methods. Six strains of cytomegalovirus (obtained through the courtesy of Doctor Robert M. McAlister) were utilized in this study. These strains were initially maintained in serial passage in roller tubes of WI-38 tissue and subsequent stocks of seed virus were made again employing WI-38 tissue. The medium consisted of basal medium Eagle with Earle's balanced salts and 2% calf serum.

The method of infection for seed virus was as follows: cells and supernate from roller tubes in which serial passage had been accomplished were inoculated into 32-oz bottles of WI-38 tissue containing 40 ml of the above media; after 48 hr the media was changed; at 5 days or longer, depending on the strain and the development of full cytopathic effects, the cells were scraped from the bottle and suspended in the media. Initially the virus cell supernate combination was simply placed in "Cryules"[1] in 1-ml aliquots

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[1] Wheaton Glass Company.