Spontaneous Mammary Tumors: Decrease of Incidence in Mice Infected with an Enzyme-Elevating Virus

Abstract. *Mice infected with a virus which causes increased activity of lactate dehydrogenase, and of other enzymes in blood plasma, had a significantly lower incidence of spontaneous mammary carcinoma than did controls. When the experiment was terminated at 18 months, the incidence of mammary tumors in controls was 90 percent, and in infected mice, 53 percent.*

The primary factors known to influence the incidence of mammary carcinoma in mice are the Bittner virus, the inherited genetic susceptibility of a specific mouse strain, and the host hormonal influences, particularly as they apply to mammary gland development and activity (*1*). An additional factor which can have a substantial influence on the tumor incidence is the presence of a specific secondary virus. The interfering LDH-elevating virus in question (*2*) is ubiquitously distributed in mouse colonies, although its presence is not generally noticed because it does not cause any known disease or perceptible lesion (*2*). Because of its silent and infectious nature, it could be confused with a genetic trait if asymmetrically distributed in experimental animals (*3*).

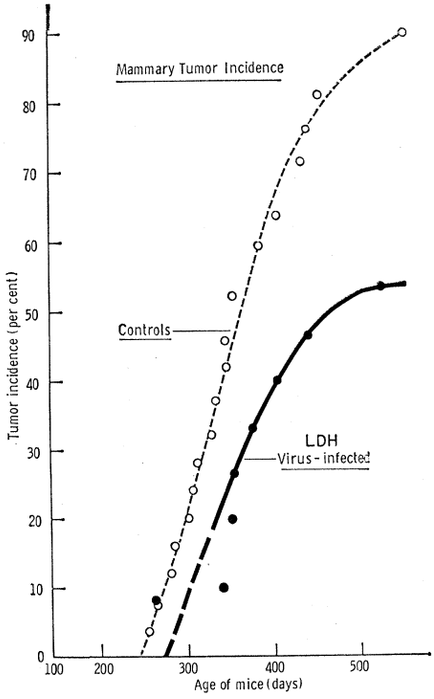

Fig. 1. Effect of the LDH-virus infection on the cumulative incidence of "spontaneous" mammary tumors in non-parous C3H female mice. The percentage of mice with tumors is based on the number of mice at risk at each observation period. The infected group was inoculated intraperitoneally (the mice were 56 days old) with 0.1 ml of a solution containing the LDH-virus, and having a titer of 10^8 or 10^9 ID_{50} unit/ml (*11*).

The relative incidence of mammary carcinoma developing in non-parous C3H/HeJ mice under normal circumstances was compared with an analogous group of females of the same age who were injected with the LDH-virus when they were 56 days old (Fig. 1). The differences in these values are statistically significant. The Bittner virus (*1*) was presumed present from infancy, whereas the LDH-virus (*2*) was not introduced until the mice were 56 days old.

The virus which is the primary etiological agent of "spontaneous" mammary carcinoma in mice has been called "the milk influence," the mammary tumor inciting factor (MTI), the mammary tumor agent or virus (MTV), and the Bittner factor or virus (*1*). Since the natural mode of transmission is through the mother's milk, the infection ordinarily occurs during the initial feeding following birth. The C3H/HeJ strain of mice employed in these experiments not only carries the essential mammary tumor virus, but is also genetically susceptible, so that a 90 to 100 percent mammary tumor incidence is usually observed within 15 to 18 months. The breeding status of the mice may influence the tumor latent periods and the final tumor incidence (*1*).

The LDH virus, also known as the lactate dehydrogenase-elevating agent or virus, the LDH-elevating agent or virus, the LDH-virus (LDV), Riley's enzyme-elevating virus, and the Riley virus (RV) (*2*) causes no recognizable disease in mice and induces no detectable lesions, either grossly or microscopically, in any of the tissues or organs of the host; nor does it induce cytopathic effects either in vivo or in vitro (*2*).

Following injection of the LDH-virus into a normal mouse, the virus titer rises rapidly, reaching a peak of approximately 10^{10} infective units (ID_{50}) per ml of plasma in 12 to 36 hours (*2*). This viremia falls during the next 2 or 3 days with a loss of several logs of activity. After 2 to 3 weeks it becomes stable at a level of approximately 10^5 to 10^6 infectious units per ml of plasma. This persisting viremia suggests either that the mice do not form neutralizing antibodies to the LDH-virus, or, if formed, the antibodies do not completely neutralize the circulating virus in the plasma (*4*). The initial rapid fall in virus titer (*2*) suggests the appearance of circulating interferon. Studies with the LDH-virus (*2*) have demonstrated the presence of viral-inhibiting substances in the plasma of infected mice; this may have some correlation with the observed tumor suppression. There is, however, uncertainty as to how long the LDH-virus interferon may persist, and whether the inhibiting factors detected are typical viral antibodies or some other variety of viral-inhibiting substances (*2, 4*).

The data illustrated in Fig. 1 were obtained from a uniform group of 55 non-parous C3H females (*5*). These mice were observed for about 18 months, and in that time there was a 90 percent mammary tumor incidence in the controls, in contrast to 53 percent in analogous animals which had been injected secondarily with LDH-virus ($P < .01$). The time required for 50 percent of the infected animals to produce mammary tumors was 484 days; that required by the controls was 353 days. At 340 days, the time of the median tumor latent period for the controls, there was approximately a 40 percent tumor incidence in the controls, compared with 10 percent in the LDH-virus-infected animals. There was no observable difference in mouse weights between control and infected mice during the period prior to the development of the tumors. This eliminates caloric restriction as having any significant influence on the difference in tumor incidence.

In a separate experiment we found that intensive breeding lessened the ultimate protective effect of the LDH-virus observed in the non-parous animals; there was a final tumor incidence of 100 percent in both infected and control parous animals at 526 days (Table 1). However, there was a statistically significant difference in the tumor distribution as a function of mouse age between the control and infected groups ($P < .001$). When the mice were 294 days old, the tumor incidence was 83 percent in the controls compared with 36 percent in the in-

Table 1. Effect of infection with the LDH-virus on "spontaneous" mammary tumor incidence in C3H parous mice.

Latency period (age of mouse, days)	Control mice			Mice infected with LDH-virus		
	Tumors (No.)	Tumors (No.) Mice (No.)	Tumors (%)	Tumors (No.)	Tumors (No.) Mice (No.)	Tumors (%)
215	1	1/17	6			
216	2	2/17	12	1	1/19	5
226				2	2/19	10
235	3	3/16	19			
236	4	4/16	25			
256	5	5/15	33			
257				3	3/16	19
262	6	6/15	40			
272	7	7/12	58			
275	8	8/12	67			
276	9	9/12	75			
280				4	4/14	29
294	10	10/12	83	5	5/14	36
303				6	6/14	43
306				7	7/14	50
314				8	8/14	57
356				9	9/14	64
361				10	10/14	71
384	11	11/12	92			
385				11	11/14	78
386				12	12/14	86
396				13	13/14	93
449	12	12/12	100			
526				14	14/14	100

fected animals. Also, a highly significant difference existed between the tumor latent periods ($P < .005$) since the control mice developed tumors earlier, with a median latency period of 267 days compared with 310 days for the infected animals. Most of the tumors arose in the control mice relatively early when they were between 215 and 276 days old, while in the infected mice, most of the tumors occurred after this period (Table 1).

The widespread distribution of the LDH-virus in experimental mice (particularly those bearing transplanted tumors) may explain some of the discrepancies reported in the literature on mammary tumors. However, of more immediate concern is the necessity for testing mice for the LDH-virus when it is known that the mice are intended for study of mammary tumors or assay of the Bittner virus. The presence or absence of the LDH-virus in these mice can be established readily by plasma lactate dehydrogenase (LDH) determinations (*6*). These findings emphasize the general need for better characterization of inapparent infectious entities and point up the need for germfree and virusfree animal stock (*7*) for use in studies of mixed virus infections, nucleic acid interchange with resulting hybrid viruses, and for detecting and clarifying both synergistic and inhibitory microbiological phenomena (*8*).

The reduction in the occurrence of mammary tumors in mice infected with the LDH-virus would seem to occur through two possible routes: suppression of the Bittner virus or impairment of the incipient tumor cells induced by the virus. The following are possible mechanisms. (i) Direct inhibition of the Bittner virus by the LDH-virus through competition for available attachment sites at the surface of host cells, or by an intracellular competition at the nucleic acid or protein level. In this connection, it may be pertinent that the LDH-virus nucleic acid is of the RNA type (*9*), the same as that reported to be associated with the Bittner mammary tumor virus (*10*). (ii) Suppression of the Bittner virus as a consequence of the production of interferon or other virus inhibitors by the LDH-virus. Evidence for the chronic production of viral inhibitors or interferon by the persisting high titers of the benign LDH-virus is being examined further. (iii) Suppression of Bittner virus tumor cells at an early stage by the LDH-virus, either through its inhibitory products, or by metabolic alterations which the LDH-virus causes in the host (*6*). The LDH-virus alters some aspects of the host metabolism (as shown by enzyme changes) (*2*), and may also be capable of altering the permeability of host cells. Thus it is conceivable that tumor inhibition could be accomplished through injury or destruction of the incipient tumor cells. However, no evidence of tumor suppression by the LDH-virus was observed when tumors were induced with chemical carcinogens. On the other hand, the development of spontaneous leukemia in AKR mice, presumably incited by the Gross virus, was slightly suppressed in mice inoculated as adults with the LDH-virus. These and related observations again raise the obvious question of the potential utility of specific living benign viruses, or their products, as useful antagonists for replicating oncogenic agents.

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5. The four groups of mice used for these experiments were not littermates, although the control and experimental groups were carefully matched in terms of age and weight. There is no evidence that the LDH-virus has any influence on the estrous cycle or the course of pregnancy.
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11. To test the effects of different dosages of LDH-virus, half of the mice in each experimental group were inoculated with undiluted infectious mouse plasma and the other half with a 10^{-3} dilution of the same preparation. There was a suggestive virus-protective dose effect with animals surviving to the end of the experiment. Ony two of the six mice that had received undiluted plasma developed tumors, whereas tumors developed in six of the eight mice inoculated with the plasma diluted 1 to 1000. These two groups were combined for the analyses expressed in Fig. 1 and Table 1.
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