Mammary Tumor Virus Activity in Mammary Tissues of Hormone-Stimulated BALB/cfC3H/Crgl Mice* (33833)

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The basis for the ability of short-term hormonal stimulation to increase the frequency of hyperplastic alveolar nodules (HAN) in the mammary gland is unknown. Recently it has been shown that mammary tumor virus (MTV) activity is present in the blood of 1-week-old BALB/cfC3H/Crgl mice and in the blood of 2-week-old BALB/c mice given either whole blood or a cell-free extract of mammary gland from lactation BALB/cf C3H/Crgl mice (1,2). However, MTV, activity does not appear in the primary duct of untreated virgin BALB/cfC3H mice until they are 13 weeks old (1). Moreover, hormonal stimulation of the mammary glands for 12 weeks leads to the appearance of substantial numbers of HAN in 20-week-old BALB/cfC3H mice, although HAN occur only infrequently and late in life in intact virgin mice of this type (Medina, unpublished observation). It was the purpose of the present experiments to determine if hormonal stimulation of the mammary glands leads to increased infectivity of the primary duct as reflected by the presence of MTV activity.

Materials and Methods. BALB/cfC3H primary duct, BALB/c HAN outgrowth [D1 nodule outgrowth, see (3)], and BALB/c lobuloalveolar tissue were assayed for MTV using the noduligenesis test method (4). Tissue was transplanted into the inguinal gland-free fat pads of 3-week-old BALB/c female mice (MTV-free). The recipient mice received pellets of estradiol-17β and deoxycorticosterone acetate (EDCA) which stimulated the host's mammary glands to a state equivalent to midpregnancy. The pellets were changed after 6 weeks and removed after a total of 12 weeks stimulation. The glands were then allowed to regress for a period of 5 weeks, during which time normal alveolar structures regressed whereas HAN remained intact. At the end of this period, the animals were killed and the mammary glands were fixed in 15% formalin for 24 hr, defatted in acetone, and stained with hematoxylin. The wholemounts were examined under the dissecting microscope and the nodules were counted. The presence of nodules indicated the presence of MTV.

Expt. I. Three-week-old BALB/cfC3H female mice were given EDCA pellets subcutaneously. Primary ducts from BALB/cfC3H mice bearing pellets for 4, 6, 8, 10, and 12 weeks were transplanted into BALB/c Simonsen mice and assayed for MTV.

Expt. II. BALB/c HAN outgrowth and normal lobulolaveolar tissue from a midpregnant BALB/c mouse were transplanted into contralateral gland-free inguinal fat pads of 3-week-old BALB/cfC3H mice. At 6 weeks, the mice were given pellets of EDCA. At 3, 5, 7, 9, and 11 weeks after implanting the pellet, both the HAN outgrowth and the normal lobular tissue were assayed for MTV in BALB/c Simonsen mice by the noduligenesis method.

Expt. III. Three-week-old BALB/cfC3H mice were given EDCA pellets subcutaneously. At 10, 20, 30, and 40 days after pellet implantation, the primary ducts from the fourth and fifth mammary glands were assayed for MTV. All recipient mice were 3-week-old BALB/cCrgl female mice. BALB/cCrgl mice were used instead of the BALB/c Simonsen substrain because of the recent finding that some BALB/c Simonsen mice show virus-like particles in their mammary glands 5).

All mice were housed 8–10 to a cage, in temperature- and light-controlled rooms, fed

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TABLE I. MTV[a] Activity in BALB/cfC3H Primary Duct.

Donors			Recipients[b]	
Group	Length of hormonal stimulation (weeks)	Time in MTV + host (weeks)	[No. of hosts w/HAN[c] (%)]/[No. of hosts w/transp.]	Mean no. of HAN/ HAN-bearing mouse
I[d]	—	—	1/12 (8)	79.0
II	4	7	4/5 (80)	13.0
III	6	9	4/4 (100)	11.0
IV	8	11	6/11 (54)	14.0
V	10	13	4/12 (33)	10.2
VI	12	15	4/5 (80)	60.0

[a] Mammary tumor virus.
[b] All assay mice are BALB/c Simonsen.
[c] Hyperplastic alveolar nodules (preneoplastic).
[d] Group I are control BALB/c Simonsen mice that received EDCA pellets for 12 weeks.

Purina breeder chow, and given water *ad libitum*.

Results. The data presented clearly indicate substantial MTV activity in the primary ducts of 6-week-old BALB/cfC3H/Crgl mice, provided the mammary glands had been under the influence of hormonal stimulation. In the first experiment, all groups assayed showed MTV activity in BALB/cfC3H primary ducts (Table I). After only 4 weeks of hormonal stimulation, substantial MTV activity was present in 7-week-old BALB/cfC3H primary duct. We have no explanation for the low activity in groups IV and V, but do not consider the difference of consequence. In an attempt to find the time of initial appearance of MTV activity, primary ducts after 10, 20, 30, 40, and 50 days of hormonal stimulation were assayed for MTV. Whereas hormonal stimulation for 10 days gave uniformly negative results, MTV activity was present after 20 days of stimulation (Table III).

Like BALB/cfC3H primary duct, both BALB/c normal lobules and BALB/c HAN (Table II) outgrowths show MTV activity when placed in MTV-bearing hosts and subjected to only 3 weeks of hormonal stimula-

TABLE II. MTV Activity in BALB/c Normal Lobules and BALB/c Nodule Outgrowths.

Donors				Recipients[a]	
Group	Tissue	Length of hormonal stimulation (weeks)	Time in MTV + host (weeks)	[No. of hosts w/HAN (%)]/[No. of hosts w/transp.]	Mean no. of HAN/ HAN-bearing mouse
I	Normal lobule	3	6	9/20 (45)	26.0
II		5	8	6/6 (100)	35.7
III		7	10	6/10 (60)	23.0
IV		9	12	7/9 (78)	32.0
V		11	14	10/11 (91)	17.4
VI	HAN outgrowth	3	6	15/21 (72)	15.0
VII		5	8	7/7 (100)	22.6
VIII		7	10	4/6 (67)	28.8
IX		9	12	8/12 (67)	31.0

[a] All assay mice were BALB/c Simonsen except Groups I and VI which were BALB/cCrgl.

TABLE III. MTV Activity in BALB/cfC3H Primary Duct.

Donors			Recipients[a]	
Group	Length of hormonal stimulation (days)	Time in MTV + hosts (days)	[No. of hosts w/HAN (%)]/[No. of hosts w/transp.]	Mean no. of HAN/ HAN-bearing host
I	10	31	0/25 (0)	—
II	20	41	5/9 (55)	45
III	30	51	5/10 (50)	45
IV	40	61	5/6 (83)	12[b]

[a] All assay mice were BALB/cCrgl.

[b] Two mice developed mammary tumors in their own glands during the course of the experiment; nodules were not counted in these two mice; therefore $n = 3$ here.

tion. No apparent difference in susceptibility to MTV existed between normal lobules and hyperplastic nodule outgrowth.

Discussion. One of the principal effects of short-term hormonal stimulation might be the early appearance of MTV activity in the mammary glands of BALB/cfC3H female mice. Substantial MTV activity occurs early in the primary ducts of hormone-stimulated BALB/cfC3H mice. Previously, without hormonal stimulation, substantial MTV activity was not found in mammary tissues before 13 weeks of age. Thus, the early noduligenesis in MTV-positive mice following hormonal stimulation may be the result of the early occurrence of MTV activity in the mammary tissues.

These data agree well with the observations of Nakayama (6) on the appearance of virus-like particles associated with MTV activity. She treated BALB/cfC3H female mice with estrogen and progesterone for 9 days, then cultured the whole mammary gland in hormone-supplemented media for 5 days, before looking for A- and B-particles with the electron microscope. These particles first occurred in mammary glands of 6-week-old BALB/cfC3H mice, but were not seen in mammary glands of 4-week-old mice.

It would be incorrect to assume that primary duct *per se* is infected. After 30 days of hormone stimulation, some lobule formation has occurred in the mammary gland accompanied by the release of B-particles from the mammary cells (6). The MTV activity found in primary ducts could be due to such B-particles accumulating in their lumens. B-particles were not seen budding from primary or secondary duct cells, but only in tertiary ducts and alveoli (6).

The BALB/c normal lobules are as susceptible to MTV as are BALB/c HAN outgrowths, inasmuch as MTV activity occurs after only 3 weeks of stimulation in both tissues. Apparently, the nodule state *per se* does not confer any special susceptibility to MTV. The MTV activity measured in lobule and HAN tissues could conceivably be due to the MTV activity present in the blood of BALB/cfC3H at this time. However, the small size of the transplant and the minute amount of contained blood would presumably be too small to account for such high activity. Furthermore, the absence of MTV activity from the group treated for hormones for 10 days indicates that the transferred blood is insufficient to account for ultimate MTV infection.

These data indicate that there is a minimal response time required for this tumor virus to be detectable by the assay method used. A relationship appears to exist betwen the "conditioning" influence of the hormonal background and the appearance of infective tumor virus.

Summary. Hormonal stimulation leads to early MTV activity in the mammary glands of BALB/cfC3H female mice. Whereas substantial MTV activity does not appear until 13 weeks in the intact virgin BALB/cfC3H

mammary gland, 3 weeks of hormonal stimulation leads to MTV activity in mammary ducts of 6-week-old BALB/cfC3H female mice. In addition, both BALB/c normal lobules and BALB/c hyperplastic nodule outgrowths possess MTV activity after 6 weeks in a MTV-positive host, following 3 or more weeks of hormonal stimulation.

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Congenital Lipoprotein Lipase Deficiency and Hyperlipemia in the Young Puppy* (33834)

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In man, exogenous hyperlipemia is characterized by large amounts of chylomicrons in plasma in the presence of a normal fat-containing diet, and their disappearance when dietary fat is withdrawn. The disorder results from a defect in the removal of chylomicrons and of triglyceride-rich lipoprotein associated with a deficiency of lipoprotein lipase, the enzyme located predominately in adipose tissue capillaries and presumed to be important in the regulation of particulate triglyceride removal from the circulation. A congenital deficiency of this enzyme appears to be the basis for the familial form of exogenous lipemia (1). A low plasma lipolytic activity after heparin injection (2) closely reflects reduced tissue lipoprotein lipase activity (3). In the present study hyperlipemia was found in a young dog in association with deficient lipoprotein lipase activity, as manifested by reduced postheparin plasma lipolytic activity. Although "essential hyperlipemia" has been described in a 2-year-old dog (4), we know of no canine case of hyperlipemia in whom a deficiency of lipoprotein lipase or postheparin lipolytic activity has been demonstrated.

Methods, Materials, and Results. The subject of this report is a male puppy who was the product of an uncomplicated gestation to a seemingly normal mongrel bitch. Nothing is known of its father. This puppy was the smallest of the litter of three, and grew more slowly than the others. At 28 days of age, the unweaned animal was taken from his mother to be used in an experiment, and was found to weigh 950 g (1210 ± 280 g; mean ± SD in 19 N mongrel puppies, 27–29 days of age). He was irritable and weak. The liver was palpably enlarged. A blood sample obtained from a femoral artery cannula looked like "cream-of-tomato soup." After cold centrifugation of a blood sample collected in a heparinized tube, a lactescent zone was visible in the upper half of the plasma layer. The triglyceride concentration (5) of this plasma was 830 mg/100 ml, the cholesterol concentration (6) was 312 mg/100 ml, and the glucose concentration (7) was 126 mg/100 ml (122 ± 23 mg/100 ml; mean ± SD in 19 normal mongrel puppies). Unfortunately, the plasma was frozen and therefore unsuit-

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