Mammary Tumor Development in Transplanted Hyperplastic Alveolar Nodules of the Mouse.* (26917)

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Hyperplastic alveolar nodules are commonly found in the mammary glands of old females from strains of mice, such as the mammary tumor virus (MTV)-infected C3H/Crgl, which have high incidences of mammary tumors. These nodules have been shown to be preneoplastic; when transplanted into the gland-free mammary fat pads of young virgin females, they develop mammary tumors more frequently and in less time than do samples of normal mammary tissue similarly transplanted(1,2).

Nodules are also found in large numbers in the mammary glands of old multiparous females from C3H sublines which do not possess demonstrable biologically active MTV (MTV-free). The nodules of the MTV-infected and the MTV-free sublines of C3H are similar in morphology, in ability to form hyperactive outgrowths following transplantation into gland-free mammary fat pads, and in the presence within their cells and lumina of virus-like particles as seen in the electron microscope(3). However, few females from the MTV-free C3H sublines develop mammary tumors.

Previous studies(4,5) have shown that cell populations derived from individual C3H/Crgl nodules have stable, inherent potentials of tumor development, and that these potentials may be either high or low. The occurrence in the C3H/Crgl females of some nodules with low potentials for mammary tumor development suggested that a higher proportion of such nodules in the MTV-free sublines could account for the observed low incidence of tumors. The present experiment was designed to test this possibility.

Materials and methods. Hyperplastic alveolar nodules from one strain (C3H/Crgl) and 2 sublines (C3Hf/Crgl and C3H/Crgl/2) were studied. The C3H/Crgl strain, which carries the MTV, is characterized by almost 100% tumor incidence in breeding females. In the C3Hf/Crgl subline, which was freed of biologically active MTV by foster-nursing on MTV-free females, approximately 20% of the breeding females develop mammary tumors at a much later average age. Another subline, C3H/Crgl/2, arose spontaneously in the C3H/Crgl strain, has no biologically active MTV, and is also characterized by a low incidence of mammary tumors at an advanced age.

Nodules from the mammary glands of nonpregnant, non-lactating, multiparous, tumor-bearing females of the C3H/Crgl strain were transplanted into the right and left mammary-gland-free inguinal mammary fat pads of 3-week-old C3H/Crgl females. Similarly, nodules from C3Hf/Crgl females were transplanted into C3Hf/Crgl hosts, and nodules from C3H/Crgl/2 females were transplanted into C3H/Crgl/2 hosts. Also, samples of adult normal C3H/Crgl mammary gland were transplanted into 3-week-old C3H/Crgl hosts. All hosts were maintained as virgins and were sacrificed 36-62 weeks after transplantation, or at prior tumor development. Some C3H/Crgl nodules were terminated without tumor development at ages earlier than 36 weeks when the nodule transplant in the host's other inguinal fat pad developed a tumor. At sacrifice, each inguinal fat pad containing a nodule transplant and any outgrowth was removed from the host, fixed in 10% formalin, stained with iron hematoxylin, and examined in methyl salicylate. Nodules from which no transplants were recovered were excluded from the data.

Results and discussion. Termination in weeks after transplantation for each nodule or sample of normal tissue is recorded in Table I. Twenty of 29 of the recovered C3H/Crgl nodules (69%) developed mammary tumors. However, only 2 of 15 C3Hf/

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TABLE I. Distribution in Time of the Appearance of Tumors in C3H/Crgl Normal Adult Mammary Gland and in C3H/Crgl, C3Hf/Crgl, and C3H/Crgl/2 Nodules Transplanted Respectively into C3H/Crgl, C3Hf/Crgl, and C3H/Crgl/2 3-Week-Old Females. Tumor development, or termination without tumor development, for each nodule or sample of normal tissue is recorded in weeks after transplantation.

Tissue and strain of origin		Weeks after transplantation					
		9–15	16–22	23–29	30–36	37–43	44 & over
C3H/Crgl nodules	No. with tumor	6	4	10	—	—	—
	No. without tumor	2	1	3	1	—	2
C3Hf/Crgl nodules	No. with tumor	1	—	—	—	—	1
	No. without tumor	—	—	4	1	—	8
C3H/Crgl/2 nodules	No. with tumor	—	—	1	—	—	—
	No. without tumor	—	—	2	4	4	6
C3H/Crgl normal mammary gland	No. with tumor	1	—	—	2		
	No. without tumor	—	3	9	20		

Crgl nodules (13%) and only one of 17 C3H/Crgl/2 nodules (6%) developed mammary tumors. Three of 32 transplants of normal C3H/Crgl mammary tissue (9%) developed mammary tumors within 36 weeks. Thus, more of the C3H/Crgl nodules developed mammary tumors in less time than either the C3Hf/Crgl or the C3H/Crgl/2 nodules. In fact, the nodules from the 2 MTV-free sublines developed no more tumors than did samples of normal C3H/Crgl mammary tissue.

Outgrowths from several of the C3Hf/Crgl and C3H/Crgl/2 nodules have been retransplanted, and are being maintained serially. For 3 generations the C3Hf/Crgl outgrowth which developed a mammary tumor within 15 weeks (Table I) has continued to show a high tumor potential. In addition, 5 C3Hf/Crgl and 6 C3H/Crgl/2 nodules which did not produce tumors in the original experiment have retained that characteristic following retransplantation of the outgrowths. Thus, in the C3Hf/Crgl and C3H/Crgl/2 sublines, as in the C3H/Crgl strain(4,5), the potential for tumor development is an inherent character of the cell populations comprising nodules.

Preliminary data from an experiment in progress indicate that simple exposure to the MTV (achieved by transplanting outgrowths from C3Hf/Crgl and C3H/Crgl/2 nodules into MTV-infected hosts) is not sufficient to create a tumor potential in these nodule outgrowths similar to that seen in most C3H/Crgl nodules. The 13 non-tumor-producing C3Hf/Crgl nodules (Table I) were retransplanted into 3-week-old C3H/Crgl females. The nodule outgrowths have been maintained in these MTV-infected hosts for at least 31 weeks, and 11 of them have developed no mammary tumors. None of the outgrowths from 11 C3H/Crgl/2 nodules similarly retransplanted into C3H/Crgl females have developed mammary tumors after at least 25 weeks. This is in contrast with the development of mammary tumors in 69% of the C3H/Crgl nodules within 29 weeks in the original experiment.

Although the hyperplastic alveolar nodules from the MTV-infected C3H/Crgl strain and the MTV-free C3H sublines are similar in the several characteristics mentioned above, the population of nodules created in the presence of the MTV contains more nodules with a high capacity for tumor development than do the nodule populations created in the absence of the MTV.

Summary. Nodules from adult C3H/Crgl females produce a high incidence of mammary tumors following transplantation into young females. On the other hand, nodules from two C3H sublines which lack demonstrable biologically active mammary tumor virus (MTV) rarely develop mammary tumors when similarly transplanted. Simple exposure to MTV (achieved by transplanting nodular outgrowth from the MTV-free sublines into MTV-infected C3H/Crgl hosts) does not create tumor potentials in these outgrowths equivalent to those seen in the C3H/Crgl population of nodules. It is concluded that a different population of nodules (with

regard to tumor potential) is formed in the absence of the MTV than in its presence.

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Blockade of Gonadotrophin Release for Ovulation in the Hen following Stimulation with Stainless Steel Electrodes. (26918)

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The release of pituitary gonadotrophin for ovulation following electrical stimulation of certain regions of the brain has been demonstrated in several mammalian species(1). Similar responses appear not to have been described in birds. The experiments reported here were undertaken to ascertain the possible effectiveness of stimulation in the induction of ovulation prematurely in the hen. Our procedures proved of no value in this respect, but unexpectedly resulted in apparent postponement of ovulation for some hours beyond the time of its normal occurrence in a substantial proportion of treated birds. These experiments, some results of which have been reported in abstract(2) are described here. Evidence is presented also for the conclusion that the observed delays in ovulation are dependent upon delay in, or blockade of, release of the ovulation-inducing hormone (OIH) in quantities adequate to assure ovulation.

Procedures. All experiments were carried out with White Leghorn hens in first or second years of production, individually caged in batteries under electric lights from 6:00 a.m. to 8:00 p.m., and with free access to feed and water. Hourly records of lay, routinely maintained from 8:00 a. m. through 4:00 p. m., served to identify hens regularly ovulating sequences of two or three members and failing to ovulate on but a single day between repeated sequences(3). Under conditions prevailing in this Laboratory, ovulation of the first or C_1 follicle in such sequences takes place at about 6:00 a.m. The administration of exogenous luteinizing preparations or of progesterone at around 4:00 p. m. of the day preceding ovulation of the C_1 follicle (the day of "missed" ovulation between sequences) forces its ovulation some 7 to 8 hours thereafter, or 6 to 7 hours prematurely. On the basis of these well-documented normal and experimental relationships(3), electrical stimulation was applied between 3:00 and 5:00 p. m. for effect on C_1 follicles normally destined to ovulate at about 6:00 a.m. of the following day.

The hour of ovulation was estimated by routine digital palpation of the oviducal egg (4), supplemented by observations at autopsy. Two orders of delayed ovulations were recognized; (i) presumptively delayed ovulations, completed before autopsy; and (ii) definitively delayed ovulations, based on presence of the intact C_1 follicle and complete absence of follicular atresia at time of autopsy.

Using the stereotactic procedures described by Ralph(5), bipolar stainless steel electrodes were placed bilaterally in the diencephalon, 1.0 mm on either side of the midline. The electrodes consisted of 2 parallel lengths of 29 gauge wire, each completely insulated except for a 0.5 mm ring at the tip, with the exposed ends separated by 0.5 mm.

The standard stimulus consisted of biphasic 1 msc pulse pairs, 100 cps, applied during