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Spontaneous Leukemia in Germfree AK Mice.* (30446)

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The strain AK mouse was developed, by selective breeding, for high incidence of spontaneous leukemia(1); most of them usually die of lymphatic leukemia by 12 months of age. The nature of the "trigger" which initiates the leukemic disease is unknown; however, a virus has been clearly incriminated in pathogenesis of the disease. A virus recovered from leukemic AK mice has been adapted by passages to other strains of mice and to rats, and is designated Gross strain A leukemia virus(2). The adapted virus induces lymphatic leukemia in mouse strains which under normal circumstances have a low incidence of spontaneous leukemia. Mice, as well as rats, are most receptive to the virus if they are inoculated shortly after birth.

Lymphatic leukemia has been induced by X-irradiation in significant numbers of 6 strains of germfree and of conventional counterpart mice(3,4). They included Swiss-Webster, ICR, C3H, C57 BL, CFW, and Balb/C strains. The incidence of induced leukemia is highest in the Swiss-Webster and lowest in the Balb/C mouse strains. The lesions observed in them were basically the same: enlarged thymus and lymph nodes, and lesser numbers of them with lymphoblastic infiltration of visceral organs and blood. Virus-like inclusions were noted in and around thymus cells of the "normal" and of the leukemic germfree mice by electron microscopy. On this basis, these mice are designated "germfree."

Leukemogenic virus is thought to be disseminated to progeny AK mice by congenital route(s), where it rests in quiescent but observable condition until the spontaneous disease appears. It is likely that a defect develops in the normal homeostatic "cell control system," thereby permitting the disease to become manifest. The biochemical mechanism whereby spontaneous leukemia is initiated and is evolved in AK mice is unknown.

The observations presented here concern spontaneous leukemia in germfree AK mice.

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Methods. A colony of AK mice was developed from conventional stock provided by Dr. John Trentin, Baylor University College of Medicine. Many of the mice died of spontaneous lymphatic leukemia by 12 months of age; the mortality was approximately 80%. Selected females were time-bred and at term their young were delivered by caesarian section into a germfree flexible isolator(5). The young AK mice were foster-nursed on germfree C3Hf mice until weaning age, and thereafter they proceeded to propagate by natural means. The colony of germfree AK mice is now in third generation, and individuals therein are reproducing in excellent fashion. The mice have been examined for microbial flora by standard procedures(6) and the results have been negative. Thus far, *mycoplasma* organisms have not been detected in them.

At intervals, individual mice, sick or healthy-looking, were removed from the germfree isolator for examination. The examinations included blood smears and blood counts. The mice were killed by cervical dislocation and they were autopsied immediately. Specimens of thymus gland and spleen were fixed in osmic acid(7) and processed as ultra-thin sections of epon-embedded cells for examination by electron microscopy(8). Specimens of the following tissues were fixed in Bouin's solution and processed for examination by light microscopy: thymus, lymph nodes, spleen, liver, kidney, lung, and intestine. Leukemic tissues were extracted in physiological saline, the extract was clarified by centrifugation, and cell free filtrates were stored in the dry ice chest for future attempts at induction of leukemia in mice inoculated at birth.

Results. The manifestations of spontaneous leukemia observed in conventional AK mice exemplified the classical lesions as described in the thymus gland, lymph nodes, spleen, liver, kidney, and lung(1,9). In most cases, the visceral organs were infiltrated with lymphoblastic cells, many of which were mitotic. Half of the mice had increased numbers of lymphoblasts in the blood. Virus-like particles (about 100 mμ in diameter) were observed in the cytoplasm of thymoma cells and in intercellular spaces of thymus and spleen tissues.

Ten of the young germfree AK mice of the first 2 generations were undersized; they had diarrhea and prolapsed rectums. In such animals, the thymus was difficult to find, but the lymph nodes were at least 4 times the size of similarly located nodes of other germfree mouse strains.

Up to the present 14 germfree AK mice have developed spontaneous leukemia at an average of 8.7 months of age (range 7 to 12 months of age). They developed roughness of the fur, kyphosis, and dyspnea. In all cases, the thymus gland, lymph nodes, and spleen were enlarged at least 8×. The mesenteric lymph node, in most instances, was strikingly enlarged. Ten of the 14 sick animals had increased numbers of lymphoblastic cells in the blood: more than 20,000 per mm^3. Virus-like particles (about 100 mμ in diameter) were observed in cytoplasmic vacuoles of thymoma cells, and in the intercellular spaces of thymus and spleen tissues (Fig. 1).

The lymph nodes of younger symptom- and lesion-free, germfree AK mice were enlarged, and germinal zones in them were small and sparse. The medullary areas contained thick cords and solid sheets of large reticulum cells, and relatively few lymphoid cells. On this basis the tissues of germfree AK mice could be differentiated from the lymph nodes of other germfree mouse strains, in which the cell population contained more lymphocytes. There was no histological evidence of leukemogenesis in the tissues of young AK mice; however, the lesions in diseased, older mice were indistinguishable from those observed in the conventional stock from which they were derived.

All germfree AK mice had enlarged, thin-walled cecums with copious watery contents. This is characteristic of the axenic state.

Discussion. Thus far, leukemia has been observed in 7 strains of "germfree" mice(3, 4). In 6 of them the virus remained quiescent until it was activated by repeated small doses of X-rays. In the seventh strain (AK) the leukemogenic process was initiated spontaneously. The first generation of "germfree"

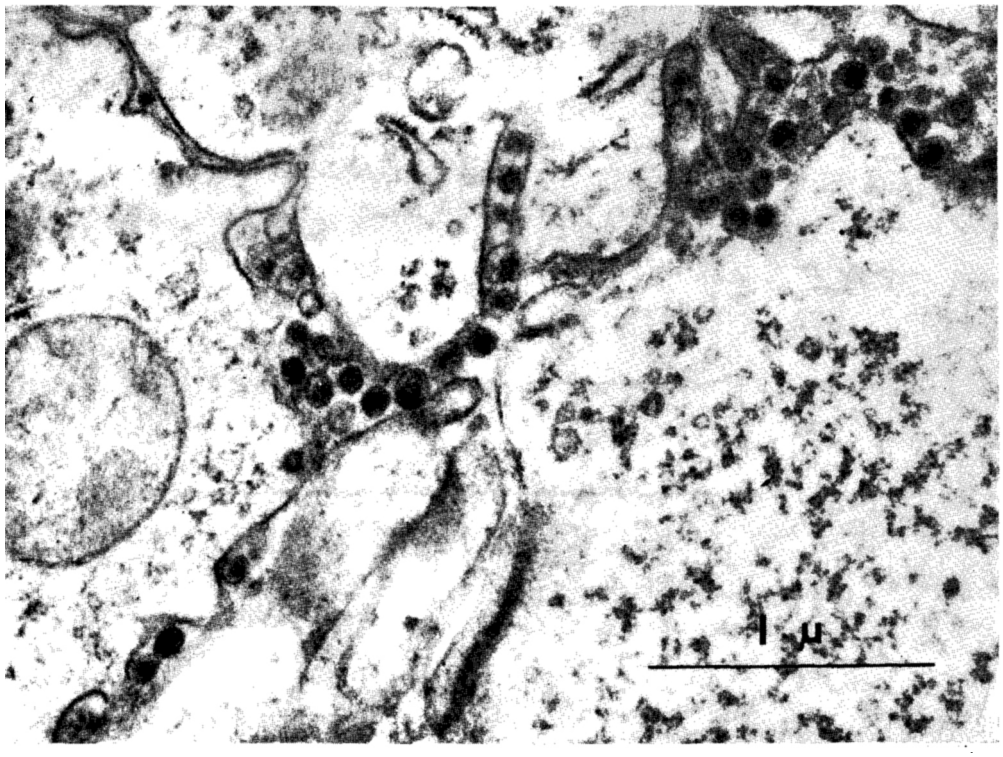

FIG. 1. Virus-like particles in intercellular space of spleen tissue. Magnification: × 40,000.

AK baby mice was foster-nursed on "germfree" C3H mothers. The leukemia manifested in them was of the spontaneous AK type, not of the radiation-dependent type as encountered in C3H mice. This implies that a genetically-controlled mechanism renders the AK mouse susceptible to disease after attainment of maturity. While the AK host-virus interaction may be gene-mediated, the disease induced in C3H mice by AK virus is also a manifestation of host control. C3H mice must be inoculated at or near time of birth, otherwise the disease fails to develop(2).

The AK leukemia virus penetrated the barrier erected by germfree methodology; and very likely it was transmitted vertically, by congenital route(s). Virus-like particles, presumably leukemia virus, have been observed in the germ plasma and in fetal tissues of conventional AK mice(10). The disease was not modified by the bacteria-free status of the host, nor by habitation in the "germfree" environment. The virus-like particles observed in thymus cells, and in spleen tissue, may represent leukemia virus, plus others which as yet await identification.

The morphological characteristics and pathogenesis of lymphatic leukemia—the spontaneous form in AK mice, and the radiation-induced form in the others—are indistinguishable. This applies also to the morphology and location of virus-like particles, as viewed by electron microscopy. We may assume from this, therefore, that the mechanism(s) of lymphoid leukemogenesis in the various strains of mice are closely related. Therefore, identification of the X-ray "trigger" which initiates disease in one strain may be relevant to the others, and this may apply also to the spontaneous disease.

Summary. Lymphatic leukemia developed spontaneously in "germfree" AK mice. The characteristics of disease were the same as those observed in the conventional control AK mice. The data indicate that the leukemia agent was transmitted to progeny by congenital route(s).

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Progestin Levels in the Ovaries and Ovarian Effluent Blood in Pregnant Rabbits.* (30447)

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The mechanisms controlling the secretory activity of the corpus luteum have been attracting increasing attention in recent years. Progesterone protects the fetus during pregnancy from excessive myometrial activity and influences the onset of parturition(1). In the rabbit, the corpus luteum is essential for maintenance of the entire gestation period of 30 days. The effect of progesterone on the myometrium declines on the 29th day(2). However, the hormonal requirement of implantation varies from that required for fetal survival and prenatal development(3).

The ovarian vein blood of non-pregnant rabbits contains progesterone (preg-4-ene-3, 20-dione) and Δ^4-3-ketopregnen-20 α-ol (20 α-hydroxy-pregn-4-ene-3-one)(4,5). Progestin levels were determined in the peripheral blood serum of rabbits by the Hooker-Forbes technique(6). A rapid increase was seen during the first 12 days of pregnancy and slower rise from the 12th day until term; maximal concentration of 10 μg/ml of serum was noted on the day before expected parturition. In the ovarian vein blood the concentration of progesterone was found to rise gradually to a peak of 2.36 μg/ml at mid-pregnancy, followed by a gradual decline in the second half of pregnancy, with consistent low values of about 0.41 μg/ml 2 days prior to parturition (7).

In the present study ovarian progestin levels were estimated during pregnancy, pseudopregnancy and superovulation in the rabbit. Data were gathered simultaneously on progestin levels in ovarian effluent blood.

Materials and methods. Fifty-four adult New Zealand White rabbits were randomly assigned to one of 3 groups: pregnant, pseudopregnant, or superovulated. Pregnancy was achieved by breeding twice to fertile bucks followed by an intravenous injection of 20 IU of luteinizing hormone (HCG, Upjohn). Pseudopregnancy was achieved by breeding to a vasectomized buck. Superovulation was induced by 3 successive injections of 50 IU of pregnant mare serum (PMS, Ayerst) and one intravenous injection of 20 IU of HCG. The animals were laparotomized at different stages of pregnancy or pseudopregnancy. The number of corpora lutea and number of implantations were recorded. The animals were injected with nembutal and 10 mg heparin.

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