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Formation of Virus-like Particles by Bone Cells in Mice with a High Incidence of Spontaneous Leukemia

Abstract. *Bone samples from potentially leukemic and leukemic mice revealed numerous 90- to 110-nanometer particles morphologically identical to murine leukemia virus. Particles were observed budding from plasma membranes of osteocytes and osteoblasts but were most numerous in osteocyte lacunae. Particles were not observed in bone samples from mice which rarely develop leukemia.*

Electron microscopy of lymphopoietic tissues from potentially leukemic and leukemic mice reveals budding and extracellular particles which have been identified as leukemogenic viruses (MLV) (*1*). Similar particles can also be formed by apparently normal epithelial cells (*2*). Budding of virus from connective tissue cells, however, is rare (*3*). During examination of apparently normal bone from leukemic mice, we observed large numbers of particles budding from osteocytes and osteoblasts. Similar production of virus-like particles by bone cells occurs in chickens with osteopetrosis induced by avian leukosis virus (*4*). However, virus production in osteopetrosis was confined to the periosteum and was not observed in osteocytes of the subperiosteal matrix. Virus particles have also been reported in mouse osteosarcomas (*5*).

Ten AKR and ten C3H/Fg female mice, 3- to 36-weeks old were used in this study. These strains have an 80 to 90 percent incidence of spontaneous lymphocytic leukemia which usually develops between 7 and 9 months of age (*6*). Comparable samples from five C57/Bl, five L CS/Fg, five A/Jax, and five Ha/ICR female mice were also examined. Mice of these strains have a low incidence of leukemia (*6*). Portions of skull, sternum, vertebra, metaphyseal bone, bone marrow, thymuses, and spleen were removed under ether anesthesia and prepared by standard procedures for electron microscopic examination following fixation in 6 percent glutaraldehyde (*7*). Bone samples were not decalcified. Samples of the same materials were fixed in 10 percent formaldehyde, and bone samples were decalcified in 10 percent buffered ethylenediaminetetraacetic acid. Paraffin sections stained with hematoxylin and eosin were examined by light microscopy.

Numerous particles morphologically identical to MLV were associated with osteocytes and osteoblasts of every C3H/Fg and AKR mouse (Fig. 1, A and C). More particles were associated with osteocytes than with osteoblasts; they appeared to originate from both cell types by budding from plasma membranes (Fig. 1B). No evidence of virus production by cartilage cells, osteoclasts, or fibroblasts of the periosteum was observed. Particles were not observed in thymuses or spleens of 3- to 5-week-old mice. Although present in small numbers in older mice, large numbers of particles were not observed in lymphopoietic tissues except in obviously leukemic mice. No virus-like particles were observed in bone or lymphopoietic tissues from the four strains of mice having a low incidence of leukemia.

Particles resembling MLV have been reported in newborn and in embryonic AKR mice (*8*), but Dirksen and Cailleau (*9*) did not find similar particles in lymphopoietic tissues of AKR mice less than 10 weeks old. Our study confirms that MLV particles are rare in lymphopoietic tissue of mice of this age and demonstrates that particles morphologically identical to MLV are numerous in bone; this suggests that bone cells contribute significantly to the viremia that precedes the onset of leukemia in these mice. However, we have not determined whether the particles produced by the bone cells are leukemogenic. Although we saw no alterations in bone morphology in this study, female AKR mice, protected from leukemia by thymectomy and surviving beyond 18 months of age, have an 87.5 percent incidence of osteomas. Nonthymectomized AKR mice, which rarely reach this age, exhibit a similar incidence of osteomas (*10*).

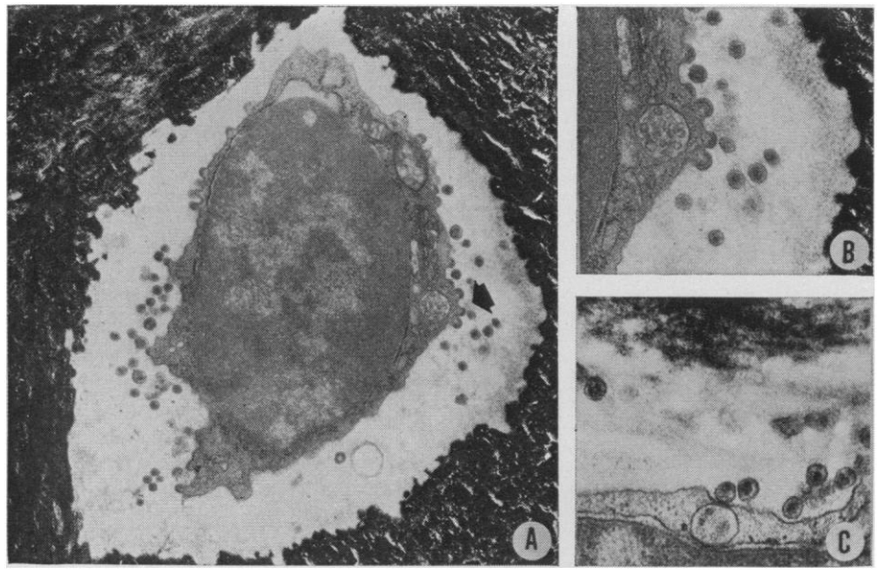

Fig. 1. (A) Osteocyte from undecalcified metaphyseal bone of a 5-week-old AKR mouse showing budding particles (arrow) and particles in the lacuna (× 13,000). (B) Enlargement of budding particles (× 25,000). (C) C-type particles between an osteoblast and the mineralized bone matrix in undecalcified bone of a 6-week-old C3H/Fg mouse (× 31,000).

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21 January 1970

Antennal Receptors: Reactions to Female Sex Attractant in Periplaneta americana

Abstract. *In* Periplaneta americana, *olfactory receptors on the antennae of male adults and nymphs respond at low threshold to the specific natural odorous attractant produced by virgin females. There are no receptors for that odor on the antennae of female adults or nymphs of any instar.*

An extract of sex attractant of *Periplaneta americana* (L.), claimed to be pure, was reported to stimulate antennal receptors of both males and females and preadult instars (*1*). Our results contradict the above findings. We used an attractant extract which was obtained by the elution of filter paper that had been exposed to a group of virgin females; the extract was purified by distillation and by more rigorous chromatographic procedures than those used previously (*1*). Traces of this extract elicited a vigorous, specific, sexual response in adult males (*2*).

For the electrophysiological tests, the cockroaches were fastened with tape to a piece of cork, and responses from antennal receptors [electroantennograms (EAG's)] were recorded by means of capillary electrodes placed between the base and the tip of the antenna (*1*, *3*). Olfactory stimuli were presented by blowing air (400 liter/hour) through a glass cartridge containing different concentrations of the odorant in 0.2 ml of amyl acetate on filter paper; filter paper alone or impregnated with the solvent served as control. Amyl acetate (0.2 ml) elicits a consistent response from the olfactory receptors in adults of both sexes and in nymphs. Female extract (about 0.1 μg) in the cartridge evoked a response of higher amplitude in the male antenna than 0.2 ml of the amyl acetate (Fig. 1). Even about 0.001 μg of extract in the cartridge elicited a response which was clearly above that of the control. Because one load of 0.001 μg could be used several times as a suprathreshold stimulus, the amount of odor leaving the source per stimulus was but a small fraction of the original dose. However, the number of molecules leaving the source is unknown.

With increasing amounts of odorous material on the filter paper, the amplitude of the EAG rose over a range of at least two orders of odor concentration (Fig. 2). In the EAG test the female extract was effective also in male nymphs, at least in the last preadult instar (Fig. 1). Here the reaction is well above that of the control, but the EAG amplitudes are lower than those elicited by amyl acetate. No response to the female extract could be detected in the antennae of female adults or nymphs (Fig. 1).

Because there is not very much difference in the number of sensilla on the antennae of the two sexes, the difference in response to the attractant extract is presumably due to specificities of the receptor cells as is the case in the silk moth *Bombyx mori* (*3*). Although the smaller number of antennal sensilla in nymphs may be partly responsible for the smaller EAG response in the nymphal stage, the fact that receptors for the attractant become apparent in late instar nymphs suggests that the receptors develop along with sexual development and that their reactivity may possibly depend on hormonal concentrations. The possible connection of the sex attractant receptors with, or their derivation from, receptors for other odors is an open question.

In light of these results and the fact that the 1963 sample had a typical cockroach odor, while our sample is apparently odorless, it is evident that the earlier sample contained a fraction other than that of the sex attractant. Our sample is highly specific for male antennal receptors and also serves as a powerful trigger for sexual behavior.

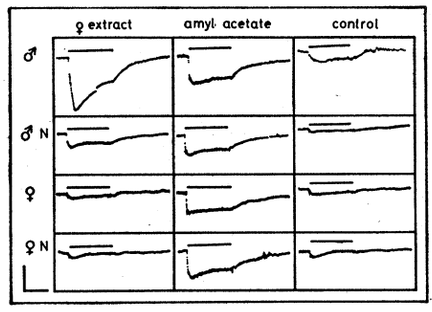

Fig. 1. Electroantennograms recorded from antennae of *Periplaneta americana* adults and nymphs (*N*). About 0.1 μg of female extract and 0.2 ml of amyl acetate on the filter paper were used.

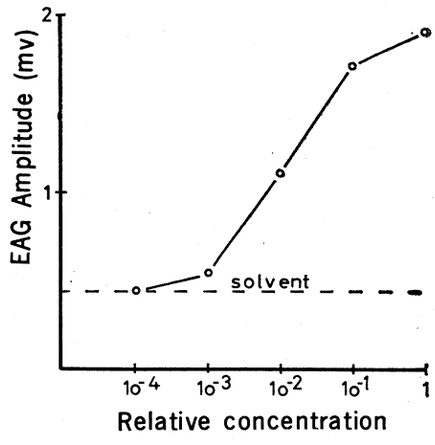

Fig. 2. Amplitude of EAG response of male antennae as a function of the amount of female extract. The relative concentration of the female extract is expressed as the log concentration (1 equals about 0.1 μg).

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