Electron Microscopy of Friend Murine Leukemia Virus in the Mid-Gut of Experimentally Infected Mosquitoes

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Received for publication 18 August 1971

After 30 and 78 hr, Friend murine leukemia virus (FLV) particles were detected by electron microscopy in the mid-gut lumen of the mosquitoes *Aedes aegypti* (Linnaeus) and *Anopheles stephensi* Liston which had fed on leukemia BALB/c mice infected with FLV. Various developmental stages of the virions were observed within and on the surface of ingested blood cells, particularly young erythroblasts, as well as free in the lumen after budding. These preliminary findings indicate that FLV continues to multiply in the mid-gut of these species for at least 3 days despite the action of digestive enzymes. Detailed studies are in progress to determine the fate of FLV in these mosquito species.

The purpose of this investigation was to determine whether a typical C-type virus causing murine leukemia could be detected by electron microscopy in known mosquito vectors of viruses and of malaria, which had fed on a viremic BALB/c donor mouse, and to determine whether the virus would multiply within the vector and ultimately reach the salivary glands for possible transmission. Friend leukemia virus (FLV) was selected as the representative C-type virus. *Aedes aegypti* (Linnaeus), a vector of many kinds of viruses (3), and *Anopheles stephensi* Liston, an important vector of malaria between Saudi Arabia and Burma, were selected for the initial studies. Both species were obtained from the insectary at the U.S. Naval Medical Research Institute, Naval Medical Center, Bethesda, Md.

The mice used were BALB/c obtained from the Charles River Breeding Laboratories, Wilmington, Mass. A standard pool of Friend virus was prepared, following the method of Chirigos et al. (2), and 0.2 ml of this preparation, which had $10^{5.4}$ 50% spleen-enlarging doses (SED₅₀) per 1.0 ml, was inoculated into 6- to 8-week-old mice. Four-day-old *A. aegypti* and 5-day-old *A. stephensi* in separate cages were permitted to take a full blood meal from individual mice 14 days postinoculation, when their spleen weight was 1.1 g. In each case the mouse was strapped upon the nylon netting of the holding cage, ventral side down, and the female mosquitoes rose quickly to commence feeding. After 1 hr the donor mouse was removed, and the holding cages were kept at

30 to 33 C in a humid styrofoam box which functioned as an insectary. Ten fully fed mosquitoes of each species, as well as unfed controls, were dissected after 30 hr (group A) and 78 hr (group B), and their mid-guts, as well as other organs, were prepared for electron microscopy.

The mid-guts were fixed in cold 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for 30 min. After being rinsed in the buffer solution for 30 min, the materials were postfixated with 2% osmium tetroxide in the buffer solution with 5% sucrose at 4 C for 2 hr. They were then dehydrated by passing through a graded series of 50, 75, 90, 95, and 100% ethanol and propylene oxide at 4 C, and embedded in epoxy resin (Epon 812). Polymerization of the epoxy resin was carried out at 45, 50, and 60 C for 12, 24, and 24 hr, respectively. Ultrathin sections were stained with 8% magnesium uranyl acetate for 3 to 5 min at room temperature, followed by 0.4% lead citrate in 0.1 N sodium hydroxide for 3 to 10 min. The sections were then studied with a Siemens Elmiskop I electron microscope, modified to a model IA, at 80 kv.

The blood cells of the donor mouse were seen to have been affected by enzymatic digestion in the mid-guts 30 hr after feeding. They were disintegrated into small cellular debris in the peripheral region of the lumen, along the microvilli of the gut epithelium (Fig. 1), whereas in the inner region of the lumen digestion had taken place to a lesser extent. The ultrastructure of the blood cells was rather well preserved in the inner region al-

FIG. 1. Portion of the mid-gut lumen, close to the microvilli (M) of the gut epithelium of *Anopheles stephensi*, 30 hr after feeding on a leukemic mouse. Note donor blood cells disintegrated by digestion. $\times 7,000$.

FIG. 2. Inner portion of the same mid-gut lumen packed with numerous donor blood cells. Note slightly disintegrated donor blood cells. Arrows indicate a type of blood cell, presumed to be erythroblast, in which FLV particles were observed. $\times 7,000$.

FIG. 3. Donor blood cell in the mid-gut lumen of *A. stephensi*, 30 hr after feeding. Note an FLV particle budding from the surface of the young erythroblast (arrow). $\times 39,600$.

FIG. 4-9. Various developmental stages of FLV particles budding from the surface of the donor blood cells in the mid-gut lumen of *A. stephensi*, 30 hr after feeding. $\times 84,000$.

FIG. 10-12. Developmental FLV virions, budding from the inner surface of cytoplasmic vacuoles of the donor blood cells in the mid-gut lumen of *A. stephensi*, 30 hr after feeding. $\times 84,000$.

FIG. 13. FLV virions in a cytoplasmic vacuole of the donor blood cell in the mid-gut lumen of *Aedes aegypti*, 30 hr after feeding. $\times 84,000$.

FIG. 14. FLV virion budding from the inner surface of a cytoplasmic vesicle of the donor blood cell in the mid-gut lumen of *A. aegypti*, 30 hr after feeding. $\times 84,000$.

FIG. 15. FLV virion in a small cytoplasmic vesicle of the donor blood cell in the mid-gut lumen of *A. stephensi*, 78 hr after feeding. $\times 84,000$.

FIG. 16. Accumulation of FLV virions in a membrane-bound cytoplasmic inclusion of the donor blood cell in the mid-gut lumen of *A. stephensi*, 30 hr after feeding. $\times 84,000$.

FIG. 17-20. Extracellular FLV virions in immature (Fig. 17) and mature (Fig. 18-20) forms in the mid-gut lumen of *A. stephensi*, 30 hr after feeding. $\times 84,000$.

though slight disintegration of the cells was noticed (Fig. 2).

FLV particles were detected in the mid-gut lumen of both *A. stephensi* and *A. aegypti* after the postfeeding periods of 30 and 78 hr. No significant differences in the morphological appearance of FLV were noticed in the two species. Active virus replication was observed (Fig. 3, arrows) from the cell membrane of young erythroblasts in the center of the blood meal. Various developmental stages of typical C-type virions were seen (Fig. 3-9). In many instances, virus particles appeared in cytoplasmic vacuoles (Fig. 10-13), as well as in small vesicles (Fig. 14). After 78 hr, the mouse blood cells in the mid-gut lumen of both species of mosquito were digested more than those in the 30 hr group; however, a large number of the donor's blood cells in the inner portion of the blood meal still preserved their original ultrastructure to a certain extent. Among these cells, many FLV particles budding from the surface of the erythroblasts were seen. Intracellular, as well as extracellular, virus particles were seen in this region (Fig. 15). Accumulations of the virions within large membrane-bound inclusions consisting of an electron-dense, homogeneous substance were also seen (Fig. 16). Extracellular virions of the immature (Fig. 17) and mature (Fig. 18-20) forms were often seen free in the lumen between blood cells. To date, no virus particles have been detected among the small debris of the ingested blood cells located close to the microvilli of the mid-gut epithelium.

Digestion of a blood meal in *A. aegypti* has been reported to take 48 hr at 29 to 30 C (1), 48 to 52 hr at 25 C (3), and 24 to 72 hr at 25 C (6). Our own findings at 30 to 33 C indicate that digestion was not complete by 78 hr in either *A. aegypti* or *A. stephensi*, as evidenced by the relatively intact appearance of the young erythroblasts from which FLV particles were budding. It is noteworthy that despite the secretion of proteolytic enzymes, detectable within 1 hr after the ingestion of blood (4, 5), enzymes apparently

did not interfere with replication of FLV. Although viral particles were found free in the lumen at 30 hr and at 78 hr, no virus was found in the mid-gut cells. Additional studies are in progress to determine whether any ingested virus passes into the mid-gut wall within the first 24 hr, especially before formation of the peritrophic membrane, which has been described in detail by Bertram and Bird (1). The ultimate fate of FLV in these species of mosquito is also under investigation.

This investigation was conducted under Public Health Service contract PH43-66-8, under the special Virus Cancer Program, National Cancer Institute, National Institutes of Health.

We thank Kazuko Hirumi and Yuko Nagasawa of Boyce Thompson Institute for their capable technical assistance.

ADDENDUM IN PROOF

In a pertinent reference, inadvertently omitted, Edman (Ann. Entomol. Soc. Amer. 63: 1778-1779) reported that in mosquitoes small quantities of blood fractions remained unchanged in the anterior core of the blood pellet until the final stages of digestion.

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