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Research Note

Origin of the Various-Sized Spherical Components with Melanin in Haemolymph of the Mosquito, *Armigeres subalbatus*

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Melanization of filarial nematodes in the haemocoel of mosquitoes is one type of mosquito defense reaction (reviewed by Christensen, 1986). *In vitro,* melanization of *Brugia pahangi* and *B. malayi* microfilariae (Mf) in haemolymph of the mosquito, *Armigeres subalbatus,* results from humoral melanization and from interaction between Mf and various-sized spherical components associated with melanin synthesis in haemolymph (Ogura, 1987b). Both processes of melanization are hypothesized to be mediated by haemagglutinins, and Bacto-Phytohemagglutinin P (PHA-P) (Difco, USA) can be used as a substitute for the intrinsic haemagglutinins (Ogura, 1987c). In this paper, the origin of the various-sized spherical components associated with melanin synthesis was explored *in vitro* using heat-killed Mf of *B. pahangi* (hk-Mf), *Ar. subalbatus* and PHA-P. Results suggested that the spherical components arise from the certain haemocytes.

Each of 8-day-old female adults of *Ar. subalbatus* was injected with 1 μl original solution of PHA-P. Haemolymph and cell-free haemolymph samples from them were prepared as previously described (Ogura, 1987c). Five μl of the haemolymph sample was dropped onto hk-Mf which had been adhered to a glass slide and then the hk-Mf were suspended in the haemolymph sample using a blunt iron needle (Ogura, 1987a). The samples were incubated at 20°C for 3 hrs and examined for deposition of melanin on hk-Mf and spherical component under microscope. Some of the melanized spherical components were fixed by 10% neutral formalin, and embedded into glycol methacrylate (JB-4) (Polyscience, USA). They were sectioned in 3 μm in thickness and observed with or without staining. Stain was toluidine blue (1%) dissolved in borax solution (1%). In order to know whether or not the spherical components with melanin originate from the fat body, an experiment was undertaken as follows: 8-day-old female adults injected with 1 μl PHA-P were dissected in *Aedes* saline (Hayes, 1953), and the lobes of the fat body (approximately 100x200x500 μm) were taken out. The lobes were washed three times with *Aedes* saline. Two lobes of the fat body were placed in 5 μl of cell-free haemolymph sample which had been dropped onto hk-Mf adherent to a glass slide. Then the lobes and hk-Mf were suspended in the cell-free haemolymph sample. Presence or absence of the melanized spherical components in the sample was observed after 3 hrs' incubation.

There were many spherical components in the reaction mixture of the haemolymph sample and hk-Mf. Some of them were intensely melanized and others slightly (Fig. 1-A). On the surface of the faintly melanized components, several patches of melanin were observed (Fig. 1-B). The small spherical

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components sometimes became a cluster and they were heavily melanized (Fig. 1-C).

On the surface of the hk-Mf with melanine granules, translucent spherical components were often observed (Fig. 1-D). Lumps of melanized substances, which sometimes are hemispherical in shape, were also observed to be attached on the surface of the intensely melanized hk-Mf (Fig. 1-E).

In the unstained sections, some spherical components were coated with granular melanin type substance and were in company with both a black spherule and an indeterminate black substance (Fig. 1-F). Another spherical component was surrounded by a black layer and was in company with a black spherule (Fig. 1-G). In a section stained with toluidine blue, some spherical components were hollow, surrounded by well stained substance, and in company with a spherule (Fig. 1-H).

In 3 replications of incubation of fat body and hk-Mf in cell-free haemolymph sample, spherical components liberated from the fat body were not melanized. However, tiny indeterminate lumps of melanin granules and melanized hk-Mf were observed.

In vitro, various-sized spherical components with melanin appeared in haemolymph samples taken from *Ar. subalbatus* (Ogura, 1987b, c). Spherical components in haemolymph are usually the haemocytes or the fat body cells. The former are scarcely distinguishable from

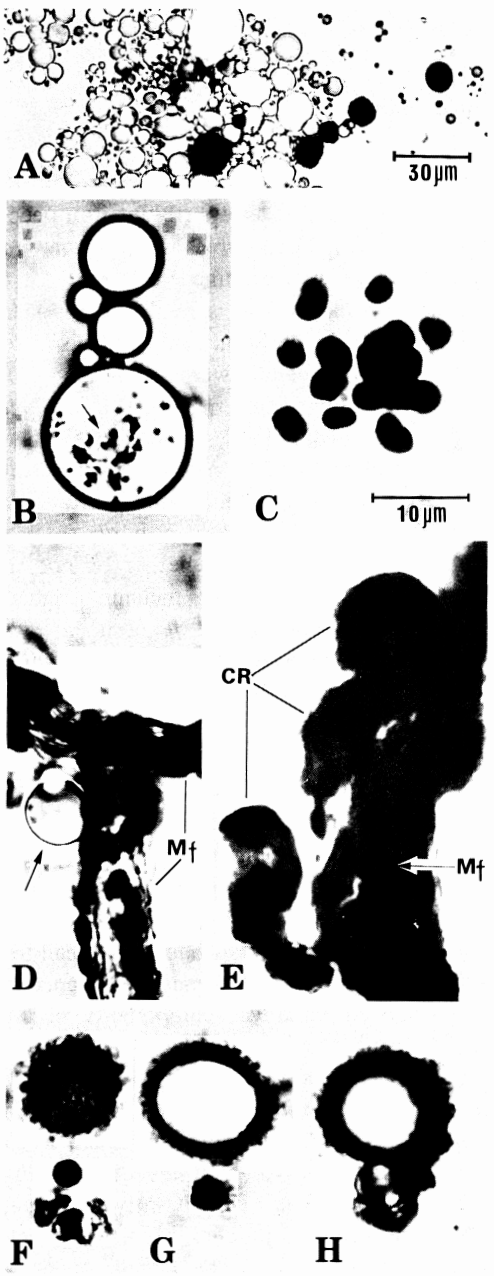

Fig. 1. Various components and heat-killed microfilariae (hk-Mf) of *Brugia pahangi* in the haemolymph sample taken from the female adults of *Armigeres subalbatus* injected with Bacto-Phytohemagglutinin P.
A: Various-sized spherical components in the haemolymph.
B: Melanin formation (arrow) on the surface of spherical component.
C: A cluster of small spherical components which were melanized heavily.
D: Translucent spherical component (arrow) adherent to hk-Mf melanized in spotty lumps.
E: Heavily melanized substances including hemispherical substances adherent to intensely melanized hk-Mf. Such substances may be cell-remnants (CR).
F-G: Unstained sections of spherical components with melanin. Spherical components are surrounded with indeterminate prominences and are in company with a spherule.
H: Toluidine blue stained section of spherical component with melanin. Hollow spherical components is surrounded with well-stained envelope.
B to H are the same scale.

the latter in appearance (Wigglesworth, 1972). Present results suggest that the spherical components associated with melanin synthesis are derived from the certain haemocytes, since melanized spherical components were not observed in the cell-free haemolymph samples where hk-Mf and spherules released from ruptured fat body were coincubated. *In vitro,* melanized haemocyte-remnants adhered to the melanized Mf of *B. pahangi* in the haemolymph samples of *Anopheles quadrimaculatus* (Chen and Laurence, 1987a, b). Ultrastructural study on the haemocyte of *Tipula paludosa* (Order: Diptera) (Carter and Green, 1987) presents the following observation: a vast number of fragments of cells, that appear to be derived from the granular cells, are present in the collected haemolymph, and such fragments may be concerned with melanization and/or coagulation of the haemolymph. *In vivo,* ultrastructural study on melanization of *Dirofilaria immitis* Mf in *Aedes aegypti* illustrated melanized haemocyte-remnants near the surface of the Mf (Christensen and Forton, 1986). Therefore, various-sized spherical components arising from certain haemocytes are possibly involved in melanization of filarial larvae occurring in the haemocoel of *Ar. subalbatus.* Ultrastructural study will elucidate this point. Moreover, it remains to be solved whether haemagglutinins adherent to filarial larvae activate prophenoloxidase to form melanin or only assist in binding prophenoloxidase activating system including β-1, 3-glucan and peptidoglycan receptors (reviewed by Söderhall and Smith, 1986) to the larvae in haemocoele of *Ar. subalbatus.*

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