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Observation of the Early Processes of Phagocytosis by the Granular Cells of the Silkworm, *Bombyx mori*¹

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It is well known that insect hemocytes phagocytose smaller particles such as bacteria or India ink, and encapsulate larger ones such as nylon thread, foreign tissues or araldite (SALT, 1970; SCOTT, 1971; AKAI and SATO, 1973, 1976; RATCLIFFE and ROWLEY, 1974; SCHMIT and RATOLIFFE, 1977, 1978; LACKIE, 1979). In these two cellular reactions, the granular cells are mostly involved and plasmatocytes are also responsible for the encapsulation of foreign body. In *Bombyx mori*, the granular cells function as phagocytes (AKAI and SATO, 1973, 1976; WAGO and ICHIKAWA, 1979a, b), and they constitute the first phase of encapsulation (SATO et al., 1976), as reported in other insects (SCHMIT and RATCLIFFE, 1977, 1978). Although the fine structure of encapsulation has been discussed by many workers (e.g. GRIMSTONE et al., 1967; POINAR et al., 1968; SCHMIT and RATCLIFFE, 1977, 1978), little is known of the surface morphology of phagocytosis by the granular cells. Moreover, it is still unknown whether the granular cells are capable of discriminating the foreignness size, thus either phagocytosis or encapsulation occurs. The aims of this report are, therefore, to add some informations on the early phase of phagocytosis

by the granular cells employing the scanning electron microscopy, and to determine the size of foreignness which the granular cells are able to internalize.

The larvae of *B. mori* (J 131 × C 131) were reared on an artificial diet under a photoperiod of 9 hr light and 15 hr dark and under a specific-pathogen-free condition at 25±1°C. Larvae 3 hr after final moult were used in this experiment. Latex particles (Serva Dow-latex) which were 0.81 and 5.7 µm in diameter were washed twice in insect saline and their final concentration was adjusted to 25% with saline, respectively. 20 µl of latex suspension was injected into the hemocoel of larva by inserting the needle into the lateral surface between the 3rd and the 4th abdominal segments. 30 min after injection when the initial cellular attachment to foreignness was observed at the light microscope level (WAGO and ICHIKAWA, 1979b), the blood was directly collected into 2.5% glutaraldehyde in 0.1 M cacodylate buffer and fixed for 30 min at 4°C. Then, hemocytes were rinsed in 8% sucrose solution in the same buffer, and post-fixed in 1% osmium tetroxide for 60 min at 4°C. After fixation, hemocytes were dehydrated in a graded series of ethanol taking a repeated centrifugation at 40G for 5 min, and then transferred to isoamyl acetate. Finally, the cell pellets were placed on a glass coverslip (9×9) which was kept in an aluminium plate with 2 mm frame around so as to prevent the loss of hemocytes. Then, critical point drying was performed in liquid carbon dioxide with HITACHI critical point dryer HCP-1, and the specimens were coated with gold. Employing the scanning electron microscope (SEM: Nihondenshi JEM-100B with EM-ASID) with an accelerating vol-

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Fig. 1. SEM micrographs of attachment (A) and internalization (B and C) of $0.81\ \mu\text{m}$ latex particle to and by one granular cell. F: filopodia; SL: small latex particle; VMP: veil-like membrane process. A: $\times 5000$; B: $\times 3000$; C: a higher magnification of Fig. 2B, $\times 9000$.

Fig. 2. SEM micrographs of attachment (A) and internalization (B) of $5.7\ \mu\text{m}$ latex particle to and by one granular cell. F: filopodia; LL: large latex particle; VMP: veil-like membrane process. A and B: $\times 4000$.

tage of 20 kV, the phagocytic process by the granular cell was observed.

Figure 1A depicts representative granular cell to which $0.81\ \mu\text{m}$ latex particles have adhered. In general, the granular cell was rough and villous with elongated filopodia which emanated from the cell surface as reported before (e.g. AKAI and SATO, 1976; WAGO, 1980), and these small latex particles (SLP) were mostly attached to the cell surface. The actual internalization of one SLP by the small granular cell (about $4\ \mu\text{m}$ in diameter) was also found in many specimens. In this case, the veil-like membrane process (VMP) was observed to start enclosing the SLP as depicted in Figs. 1B and 1C. This method of particle uptake appeared to occur by spreading of thin plasma membrane around the SLP, and internalization was carried out by creeping up over the particle. No filopodia were seen to be involved in SLP-internalization.

Figure 2 shows the attachment and internalization of $5.7\ \mu\text{m}$ large latex particle (LLP) to one granular cell. One LLP was observed to be caught by long filopodia (F) of the granular cell

(Fig. 2A). Therefore, unlike the SLP-attachment the elongated filopodia appeared to play an important role in LLP-attachment. Moreover, it was also seen that the veil-like membrane process enclosed the surface of one LLP (Fig. 2B). This was the same as SLP-internalization.

The results in this study showed that the granular cells were phagocytic and the attached granular cell to LLP which size is almost the same as that of the granular cell could advance the subsequent process of phagocytosis, internalization. When the granular cell internalizes either small or large latex particle, the veil-like membrane process was involved in enclosing the particle. Accordingly, it is likely to consider that when the granular cell phagocytoses the foreignness which size is at least smaller than that of the granular cell, the veil-like membrane process will be needed to enclose the foreignness. This process observed was different from the surface structure of filopodia, and seemed to function as the plasma membrane to enclose the particle. However, it is unclear how the granular cell reacts to the foreignness less than $0.81\ \mu\text{m}$ in size. RATCLIFFE and ROWLEY

(1974) reported that bacterial phagocytosis by the plasmatocytes of *Galleria mellonella* occurred by pseudopod formation and that the uptake of larger particles such as cell debris was accomplished by invagination of the cell membrane. Thus, the manner of phagocytosis by the granular cells in *B. mori* is probably different from that by the plasmatocytes in *G. mellonella*.

As reported in another paper (WAGO, 1980), the granular cells cultured *in vitro* in the presence of hemolymph were attached to the glass surface with radiated filopodia which were full of variety. The filopodia observed in this experiment were of long and thin type. In addition, since filopodia of the granular cell were actually engaged in attachment to LLP, they seemed to play an essential role in foreignness-attachment as described in another report (WAGO, 1980). However, it was not observed that filopodia were involved in SLP-attachment. This reason may depend upon the centrifugation procedure, during which the attached SLP to the filopodia is probably fallen down from the filopodia.

In encapsulation, it is known that lysing granular cells constitute the first phase and release some chemotactic factor which attracts plasmatocytes to the implant (SATO et al., 1976; SCHMIT and RATCLIFFE, 1977, 1978). If the granular cells release chemotactic factor as a result of distinguishing the foreignness size and the encapsulation occurs, the releasing mechanism of chemotactic factor may be dependent upon the size of contact region between the granular cells and the foreignness. One possible reason for this is present in the observation that a relatively large granular cell phagocytosed one goose erythrocyte and a smaller one constituted the first phase of encapsulation, an aggregation of the granular cells around it (WAGO and ICHIKAWA, 1979b; WAGO, 1980). If there should be the critical size of

foreignness to determine the release of chemotactic factor from the granular cells, it will be at least larger than the size of the attached granular cell, because the granular cells could internalize the same size of foreignness as described in this paper.

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