Transport of phagosomes in mouse peritoneal macrophages

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Summary

Mouse macrophages were elicited by the peritoneal injection of chondroitin sulfate solution, harvested and purified, and used as experimental materials. Small and large (diameter: 0.9 μm and 3.0 μm, respectively) polystyrene beads (PB) were used as ingested particles.

When the macrophages were incubated with Hank's solution containing small or large PB for 30 min, the phagosomes containing small or large PB were usually randomly distributed. When the macrophages were further incubated for 45 min in PB-free medium, both small and large phagosomes containing PB accumulated at the perinuclear region. The transport of large phagosomes containing 3.0 μm PB was inhibited by cytochalasin B, but not by vinblastine or podophyllotoxin. Conversely, the transport of small phagosomes containing 0.9 μm PB was not inhibited by cytochalasin B but was inhibited by vinblastine or podophyllotoxin. Immunofluorescence microscopy showed that the small phagosomes appeared to accumulate at the central region of the microtubule network. The large phagosomes, on the other hand, appeared to be surrounded by actin-rich cytoplasm, and in some cells actin filament-like structures could be seen around large phagosomes.

These results suggest that there are two different transport systems of phagosomes in macrophages. Phagosomes smaller than 0.9 μm in diameter are, probably, mainly transported to the perinuclear region by a microtubule-based motility system and those larger than 3.0 μm in diameter by an actin-based mechanism.

It was observed electron-microscopically that accumulated phagosomes containing PB could fuse with each other and form larger phagosomes.

Key words: macrophage, phagocytosis, intracellular transport.

Introduction

Intracellular transport is a ubiquitous function and essential in living cells. By elucidating the structure and function of cytoskeletal systems, the relationships between the intracellular movement of organelles and the cytoskeleton have been analyzed. There are many reports of organelle transport by means of microtubule-based or actin filament-based motility systems (Allen et al. 1985; Koonce & Schiwa, 1985, 1986; Miller & Lasek, 1985; Schnapp et al. 1985; Sheetz & Spudich, 1983; Spudich et al. 1985; Vale et al. 1985a,b).

In addition to the transport of organelles, it is known that transport of pinosomes occurs along microtubule tracks in mammalian cultured cells (Vale, 1987). Pinosomes containing ligands such as low-density lipoprotein or concanavalin A are generally transported to the central region of the cell and accumulated near the nucleus or Golgi complex. Accumulated pinosomes fuse with lysosomes to form pinolysosomes (Herman & Albertini, 1984; Swanson et al. 1987; Vale, 1987).

Pinocytosis is a fundamental function of at least some cells. Phagocytes such as macrophages have the important function of phagocytosis and can ingest particles several μm in diameter, in addition to carrying out pinocytosis. Phagosomes are generally considered to be transported towards the nucleus by the same mechanism as pinosomes are transported (Steinman et al. 1983; Vale, 1987). To date, however, there are few reports on the intracellular transport of phagosomes in macrophages.

The present study aims to reveal the transport mechanisms of phagosomes in mouse peritoneal macrophages.

Materials and methods

Macrophages

Three days after the injection of 0.5 ml of chondroitin sulfate (Fe-collloid) solution (Blutal; Dainippon Pharmacy Co., Ltd, Tokyo) or 5 % chondroitin sulfate A (Sigma, St Louis) solution into the peritoneal cavity of a male mouse (ddy strain), the elicited peritoneal macrophages were harvested and allowed to adhere to tissue-culture coverslips (15 mm in diameter; Lux, Miles Laboratories, Inc., Naperville) by incubation for 30 min at 37°C. Contaminating cells other than macrophages were
Phagocytosis

Polystyrene beads

Two sizes (0.9 µm and 3.0 µm in diameter) of polystyrene beads (Latex beads; Sigma) were used as test particles for ingestion. Polystyrene beads (PB) were washed three times with phosphate-buffered saline containing 1 mM-CaCl₂ and 0.5 mM-MgCl₂ at pH 7.3 (PBS). The suspensions were diluted with Hank's solution to give concentrations for 0.9 µm PB and 3.0 µm PB of 0.05% and 0.025%, respectively.

Phagocytes

A 1.5 ml sample of Hank's solution containing 0.05% or 0.025% was added to a plastic vessel (Linbro; 15 mm x 10 mm; Flow Laboratories, Inc., Virginia) in which a coverslip with adherent macrophages was placed, and incubated for 30 min at 37°C. After the incubation, non-ingested PB were removed by washing with Hank's solution. Macrophages that ingested PB were observed and photographed by phase-contrast microscopy. Then, the macrophages were incubated in PB-free Hank's solution for 45 min at 37°C, and the distribution of PB was observed microscopically. By comparing with the first micrographs the intracellular transport of PB was observed.

The effects of cytoskeletal inhibitors (25 µM-vinblastine (Sigma), 25 µM-podophyllotoxin (0.5% ethanol; Sigma) and 6µM-cytosolitin B (0.5% dimethyl sulfoxide; Sigma)) on the intracellular transport process were examined. Namely, macrophages were incubated in the medium containing small or large PB for 30 min, washed, and then incubated in the PB-free medium in the presence or absence of an inhibitor for 45 min.

Immunofluorescence

After incubation to permit PB transport, the macrophages were processed for indirect immunofluorescence microscopy. The cells were fixed for 20 min with 3% formaldehyde-PBS, washed five times with PBS, treated with 0.1% Triton X-PBS for 7 min and washed five times with PBS at room temperature. Then the coverslip was applied cell-side-down onto 20-40 µm PB of 0.05% and 0.025% respectively (Latex beads; Sigma) were used as test particles for ingestion. Two sizes (0.9 µm and 3.0 µm in diameter) of polystyrene beads (Latex beads; Sigma) were used as test particles for ingestion. Polystyrene beads (PB) were washed three times with phosphate-buffered saline containing 1 mM-CaCl₂ and 0.5 mM-MgCl₂ at pH 7.3 (PBS). The suspensions were diluted with Hank's solution to give concentrations for 0.9 µm PB and 3.0 µm PB of 0.05% and 0.025%, respectively.

Macrophages incubated in Hank's solution containing 0-9 µm PB for 30 min at 37°C contained 20-50 beads per cell. These appeared to be randomly distributed (Fig. 1A). With the larger (3-0 µm) PB, each cell contained several; these were also randomly distributed (Fig. 1B). Many macrophages that had ingested large beads appeared to elongate (Fig. 1B; arrow). A typical scanning electron micrograph of such a macrophage revealed that there were many phagosomes containing 0-9 µm PB in the broad pseudopod and central region of the cell (Fig. 2A). PB in the phagosomes were partially dissolved by the preparation process for scanning electron microscopy, and large phagosomes containing 3-0 µm PB were particularly collapsed. The large phagosomes were also confirmed by scanning electron microscopy to be distributed at random (Fig. 2B).

Following the ingestion of PB, the macrophages were incubated in PB-free Hank's solution for 45 min at 37°C for observation of phagosome transport. Both large and small phagosomes accumulated near the nucleus (Fig. 3A,B). This was confirmed by scanning electron microscopy (Fig. 4A,B). Comparison of Figures 1 and 2 with Figures 3 and 4 shows that almost all of the small and large phagosomes are transported to a position near the nucleus.

Effect of cytoskeletal inhibitors on transport of phagosomes

Macrophages were incubated in Hank's solution containing small or large PB for 30 min, washed, and then incubated in the PB-free solution in the presence or absence of a cytoskeletal inhibitor for 45 min at 37°C. When the macrophages were incubated in the presence of 25 µM-vinblastine, the transport of small phagosomes containing 0-9 µm PB was inhibited severely; they remained randomly distributed (Fig. 5A). However, the transport of large phagosomes containing 3-0 µm PB was not inhibited significantly by vinblastine (Fig. 5B).

The transport of the small phagosomes was not inhibited by 6 µM-cytosolitin B (Fig. 5C). Conversely, the transport of large phagosomes containing 3-0 µm PB was inhibited by cytosolitin B. The large phagosomes were very seldom transported to the central region of the cells, and remained widely distributed (Fig. 5).

Results

Intracellular transport of PB

Macrophages incubated in Hank's solution containing 0-9 µm PB for 30 min at 37°C contained 20–50 beads per cell. These appeared to be randomly distributed (Fig. 1A). With the larger (3-0 µm) PB, each cell contained several; these were also randomly distributed (Fig. 1B). Many macrophages that had ingested large beads appeared to elongate (Fig. 1B; arrow). A typical scanning electron micrograph of such a macrophage revealed that there were many phagosomes containing 0-9 µm PB in the broad pseudopod and central region of the cell (Fig. 2A). PB in the phagosomes were partially dissolved by the preparation process for scanning electron microscopy, and large phagosomes containing 3-0 µm PB were particularly collapsed. The large phagosomes were also confirmed by scanning electron microscopy to be distributed at random (Fig. 2B).

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Fig. 1. Macrophages were incubated in Hank's solution contained PB for 30 min. Cells with ingested 0.9 μm PB (A) and ingested 3.0 μm PB (B) are shown. The shape of cells changed to elongated in many cells during their ingestion of 3.0 μm PB (B; arrow). Both types of PB are randomly distributed in the cells. Bar, 50 μm.

Table 1 shows the effect of cytoskeletal inhibitors on the transport process of phagosomes containing 0.9 μm PB or 3.0 μm PB. The effect of 25 μM-podophyllotoxin on the phagosome transport of the macrophages was similar to that of vinblastine.

**Immunofluorescence**

After ingestion of PB, the macrophages were incubated for 45 min in the PB-free solution, treated with the tubulin antibody and then stained with FITC-labelled secondary antibody. Figure 6A and C shows fluorescence micrographs of the stained macrophages, and Figure 6B and D shows light micrographs of the same field as the fluorescence micrographs. Microtubules were observed in the pseudopods of the cells (Fig. 6A). Small phagosomes containing 0.9 μm PB were transported to a position near the nucleus that appears to coincide with the central region of the microtubule network (Fig. 6A,B; arrows). Small phagosomes containing small PB appear to be attached to microtubules (Fig. 6A,B, arrow a). On the contrary, microtubules were scarcely ever observed in the case of large phagosomes containing large PB (Fig. 6C). The large phagosomes accumulated near the nucleus and the site of accumulation did not appear to be related to the microtubule network (Fig. 6C,D).

Furthermore, in macrophages treated with actin antibody and stained with FITC-labelled secondary antibody the general cytoplasm of those that had many small phagosomes was hardly stained, and their broad pseudopods were slightly stained (Fig. 7A,B). On the other hand, in macrophages that had accumulated large phagosomes there was strong staining in the cytoplasm enveloping the large phagosomes and filaments extending from the central region of the cell into the pseudopods (Fig. 7C,D).

**Ultrastructure**

Figure 8A shows a macrophage with small phagosomes. Seven small phagosomes can be observed. Five of them were aggregated, and their membranes appear to be partially fused (Fig. 8A; arrow). Figure 8B shows a macrophage with large phagosomes. Two large phagosomes containing 0.9 μm PB were transported to a position near the nucleus that appears to coincide with the central region of the microtubule network (Fig. 8A,B; arrows). Small phagosomes containing small PB appear to be attached to microtubules (Fig. 8A,B, arrow a). On the contrary, microtubules were scarcely ever observed in the case of large phagosomes containing large PB (Fig. 8C). The large phagosomes accumulated near the nucleus and the site of accumulation did not appear to be related to the microtubule network (Fig. 8C,D).

Table 1. Effect of cytoskeletal inhibitors on the intracellular transport of phagosomes

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Concentrations (μM)</th>
<th>Diameters (μm) of PB</th>
<th>Non-accumulation frequency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>3.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>25</td>
<td>89.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Podophyllotoxin</td>
<td>25</td>
<td>87.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Cytochalasin B</td>
<td>6</td>
<td>2.3</td>
<td>87.5</td>
</tr>
</tbody>
</table>

*Each percentage shows the frequency of the preparation of macrophages in which phagosomes remained randomly distributed.
Fig. 2. Typical scanning electron micrographs of macrophages that were incubated in Hank's solution containing PB for 30 min are shown. Small phagosomes containing 0.9 μm PB are observed to be dispersed in a cell (A). Large phagosomes containing 3.0 μm PB are also observed to be dispersed in a cell (B). PB, especially in large phagosomes, appear to be dissolved during the preparation of specimens. Bar, 5 μm.

Phagosomes are in contact with each other, and the membranes of the phagosomes appear to fuse at the contact region. Numerous lysosomes were observed near the phagosomes (Fig. 8B). These electron micrographs imply that the membranes of phagosomes fuse to form a phagolysosome.
Fig. 3. Macrophages that had ingested PB for 30 min were then incubated in PB-free Hank's solution for 45 min. Small phagosomes containing 0.9 μm PB (A) and large phagosomes containing 3.0 μm PB (B) in the cells are shown. Both of the phagosomes accumulated near the nucleus. Bar, 50 μm.

Discussion

It is known that two kinds of cytoskeletal systems are associated with the intracellular transport of organelles in several kinds of cells. In a squid giant axon the axoplasmic organelles are transported by the microtubule-based system (Allen et al., 1985; Gilbert & Sloboda, 1984; Miller & Lasek, 1985; Schnapp et al., 1985) and by an actin filament-based mechanism (Brady et al., 1984; Goldberg et al., 1980). A giant freshwater ameba (Reticulomyxa filosa) has a cytoskeletal framework of colinear microtubules and actin filaments, and organelles are transported bidirectionally along these (Koonce & Schliwa, 1986, 1985). As well as the bidirectional organelle transport along microtubules, myosin-coated beads were observed to move unidirectionally along actin filament arrays in Nitella (Sheetz & Spudich, 1983) and Chara (Shimmen & Yano, 1984); these were also observed to move on oriented filaments that were reconstituted from purified actin (Spudich et al., 1985).

The intracellular transport system of small phagosomes in the macrophage is considered to be similar to the unidirectional transport of dynein-coated pinosomes in mammalian cultured cells (Vale, 1987). However, the present study suggests that large phagosomes are transported by an actin-based motility system. In macrophages showing active phagocytosis stretch fibers of actin have not been observed. It is suggested that actin filaments in macrophages are not merely associated with cell adhesion or cell extension, but function as a kind of cell movement system (Stossel & Hartwig, 1976). The ingestion and intracellular transport of particles larger than 3.0 μm in diameter can be considered to be similar to cell movement in the macrophage.

It is reported that several lysosomotropic chemical agents inhibit the saltatory movement of organelles in macrophages (Hart et al., 1983), and organelles or microinjected 0.26 μm PB in mammalian cultured cells exhibit saltatory movements along microtubules (Beckerle, 1984; Hayden & Allen, 1984). The bidirectional movement is considered to be generated by the conformational change in dynein, which forms cross-bridges with microtubules, and the direction of conformational change probably determines the retrograde or anterograde transport of organelles (Miller & Lasek, 1985).

Myosin-coated beads (0.7 μm in diameter) form cross-bridges with actin filaments and are transported along them in Nitella (Sheetz & Spudich, 1983). After the microinjection of PB into cultured cells (BS-C-1 and PtK1), the largest size of PB showing saltatory movement was 0.26 μm in diameter (Beckerle, 1984). PB larger than 1.0 μm in diameter are not ingested and not transported by most cells except phagocytes. Phagocytes such as macrophages can ingest and transport PB larger than 1.0 μm in diameter to a position near the nucleus. Considering that the volume or weight of 3.0 μm PB is 37 times larger than that of a 0.9 μm PB, it is probable that transport of the large phagosomes is carried out by an
Fig. 4. Typical scanning electron micrographs of macrophages that had ingested PB for 30 min previously and were incubated in PB-free Hank's solution for 45 min, are shown. Small phagosomes containing 0.9 μm PB accumulated at the perinuclear region (A). Large phagosomes containing 3.0 μm PB accumulated at the perinuclear region (B). PB in the phagosomes appear to be dissolved during the preparation of specimens. Bar, 5 μm.

actin-based mechanism similar to that of cell movement in macrophages.

Phagocytosis has been generally distinguished from pinocytosis on the basis of the size of ingested particles. Mammalian cultured cells carry out pinocytosis but not phagocytosis. Phagocytes such as a macrophage can
Fig. 5. After ingestion of PB, the macrophages were incubated in PB-free Hank's solution in the presence of 25 μM-vinblastine for 45 min (A, B). The transport of small phagosomes containing 0.9 μm PB is inhibited (A), but that of large phagosomes containing 3.0 μm PB is not inhibited by vinblastine (B). After the ingestion of PB, macrophages were incubated in PB-free Hank's solution in the presence of 6 μM-cytochalasin B for 45 min (C, D). Small phagosomes containing 0.9 μm PB accumulate at the perinuclear region (C), but the accumulation of large phagosomes containing 3.0 μm PB is inhibited by cytochalasin B (D). Bar, 50 μm.

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Fig. 6. After ingestion of PB, the macrophages were incubated in PB-free Hank's solution for 45 min and then stained with
tubulin primary and FITC-labelled secondary antibodies. Stained cells (A, C) and light micrographs of the same field (B, D),
respectively, are shown. The accumulation of small phagosomes containing 0.9 μm PB is observed, and the region of the
accumulation appears to coincide with the central region of microtubules (A, B; arrows). A phagosome appears to be in contact
with a microtubule (A, B; arrow a). In the case of large phagosomes containing 3.0 μm PB, the cytoplasm appears to be vague,
with fluorescence staining and large phagosomes accumulated at the perinuclear region (C, D). Bar, 50 μm.
Fig. 7. After ingestion of PB, the macrophages were incubated in PB-free Hank's solution for 45 min and then stained with actin primary and FITC-labelled secondary antibodies. Stained cells (A, C) and light micrographs of the same field (A, D), respectively, are shown. In the case of small phagosomes containing 0.9 μm PB, the cytoplasm appears to be vague, with fluorescence staining and small phagosomes accumulated at the perinuclear region (A, B). In macrophages with accumulated large phagosomes containing 3-0 μm PB there is strong staining in the cytoplasm enveloping the large phagosomes and filaments (C, D). Bar, 50 μm.
Fig. 8. Typical transmission electron micrographs of macrophages that had ingested small or large PB for 30 min in the presence of 90-7 μm latex beads, were washed and then incubated in PB-free Hank's solution for 45 min with 90-7 μm latex beads. Five phagosomes containing 0·9 μm PB are aggregated and their membranes disappear partially (A; arrow). Two large phagosomes containing 3·0 μm PB appear to fuse with each other and form a large phagolysosome (B). N, nucleus; m, mitochondria; j, lysosome; PB1, 0·9 μm PB; PB2, 3·0 μm PB. Bar, 2 μm.
ingest various sizes of particle (e.g., proteins or bacteria). The present study implies that there are two different intracellular transport systems of phagosomes in mouse macrophages: small phagosomes are mainly transported by a microtubule-based mechanism, and those larger than 3-0 μm in diameter are transported by an actin-based system.

References


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