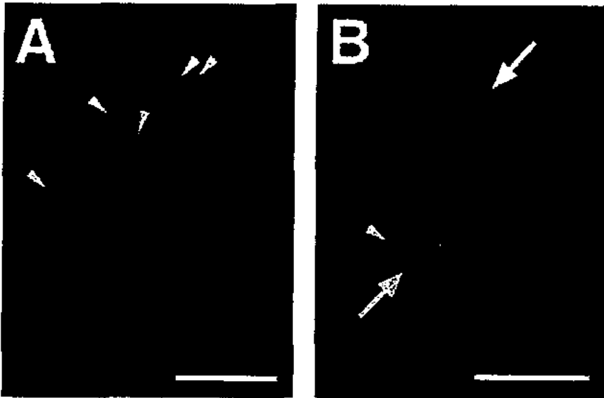

FIG. 4. Immunofluorescent micrographs showing cross-talk between early endosomes and PPV. *A*, the amoebae were pulsed with 2 mg/ml FITC-dextran (*green*) for 10 min, washed with PBS, and then subjected to immunofluorescence assay using anti-HA antibody to probe 3HA-tagged *Eh*Rab5 (*red*). *Yellow arrowheads* indicate endocytosed FITC-dextran. *B*, the amoebae were pulsed with FITC-dextran in the presence of red blood cells for 10 min, and then subjected to immunofluorescence assay. A *yellow* or *white arrow* indicates an *Eh*Rab5-associated PPV that contains or does not contain endocytosed FITC-dextran, respectively. A *yellow arrowhead* depicts the endocytosed FITC-dextran that is not associated with *Eh*Rab5 in the cytoplasm. *Bars*, 10 μm.

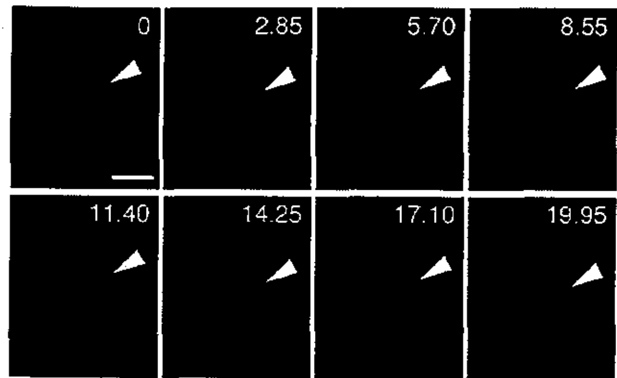

FIG. 5. Time-lapse micrographs of an amoeba expressing GFP-*Eh*Rab5, showing *de novo* formation of PPV. Amoebae were mixed with red blood cells, and then images of a stack of 18 sections along the *z*-axis (every 1.5 μm) were immediately recorded every 2.85 s. From each time point, a representative section showing *Eh*Rab5-associated vesicle or vacuole during the course of PPV formation was chosen to show the *de novo* generation of PPV at a site indicated by the *arrowheads*. Times in seconds are also shown. *Bars*, 10 μm.

nant *Eh*Rab5 and *Eh*Rab7A (Fig. 3, *R-U*; data of *Eh*Rab5 not shown).

EhRab5 Is Not Associated with Endosomes or Lysosomes but Exhibits Cross-talk with These Compartments during Maturation of PPV—To see whether *Eh*Rab5 is associated with endosomes, we examined colocalization of an endocytosed fluid-phase marker, FITC-dextran, and *Eh*Rab5. Amoebae were either incubated with FITC-dextran for 10 min to label the early endosomes or incubated with FITC-dextran for 10 min and further chased without FITC-dextran for 45 min to label the late endosomes, and then subjected to immunofluorescence assay using anti-HA antibody that recognizes *Eh*Rab5 (Fig. 4*A*). Endocytosed FITC-dextran and *Eh*Rab5 did not colocalize at either 10-min pulse (Fig. 4*A*) or at the 10-min pulse followed by a 45-min chase (data not shown). These findings imply that the *Eh*Rab5-positive compartment is neither early nor late endosomes.

When amoebae were simultaneously incubated with red blood cells and FITC-dextran for 10 min, 30% of PPV contained endocytosed FITC-dextran (Fig. 4*B*). When the amoebae were pulsed with FITC-dextran for 10 min and chased for 45 min to label the late endosomes, and further incubated with red blood cells for 10 min, the extent of colocalization of FITC-dextran and PPV was comparable (26%) (data not shown). These results suggest that PPV fuse with both early and late endosomes during maturation.

To assess where and how PPV are formed during phagocytosis, we examined the dynamics of *Eh*Rab5 using the amoeba transformant expressing GFP-*Eh*Rab5 under time-lapse microscopy. Images of 18 planes of the *z*-section with 1.5-μm intervals to cover from the top to the bottom of the cell were recorded at 2.85-s intervals. This allowed us to evaluate the detailed dynamism of PPV formation. After a few minutes of coincubation with red blood cells, an *Eh*Rab5-positive vacuole suddenly emerged in less than 20 s. Neither plasma membrane invagination nor ruffling were observed during this period, suggesting that PPV forms *de novo* (Fig. 5).

We also excluded a possibility that PPVs are micropinosomes or phagosomes. First, the fact that only a minor proportion of PPV contained FITC-dextran at 10 min (Fig. 4*B*) suggests that PPVs are not formed by invagination of the plasma membrane-like macropinosomes, which form by the closure of membrane ruffles and contain a fluid-phase marker (49, 50). Second, PPV is formed in a range of 10 s (Fig. 5), much faster than macropinosomes or phagosomes (49, 51). Membrane closure of macropinosomes and phagosomes was previously shown to occur in 1 and 5 min, respectively. Third, the major Gal/GalNAc lectin on the plasma membrane was abundantly demonstrated in phagosomes by proteomic analysis of phagosome proteins during the course of phagosome maturation (from 0 min to 2 h after ingestion)[2] but was not demonstrated on PPV by immunofluorescence study using a specific monoclonal antibody against heavy or intermediate lectin subunits (data not shown). These results strongly argue against two possibilities: 1) PPV originates from the plasma membrane, and 2) PPV is a remnant of phagosomes.

Acidification of phagosomes has been shown to occur by fusion with late endosomes and lysosomes in mammalian cells (52). We examined by using LysoTracker Red, a membrane-diffusible probe accumulated in acidic organelles (53), whether the PPV and phagosomes of the amoeba are acidified during maturation. Amoebae were pulsed with LysoTracker and then subjected to immunofluorescence assay. At steady state, neither *Eh*Rab5 (Fig. 6*A*, *left*) nor *Eh*Rab7A (Fig. 6*A*, *right*), probed with anti-HA or anti-Myc antibody, respectively, colocalized with LysoTracker. After a 5–10-min incubation with red blood cells when *Eh*Rab7A-positive PPV were formed, only 20–30% of PPV contained LysoTracker, suggesting that PPV were only partially acidified in the early stage (Fig. 6, *B*, *upper panels*, and *C*, data at 5 min not shown). After 30–40 min, a large proportion (50–70%) of PPV became acidified (Fig. 6, *B*, *lower panels*, and *C*).

PPV Are Involved in the Transport of Amoebapore to Phagosomes—We then examined which cargo proteins were transported via PPV. Among several hydrolases and membrane-permeabilizing factors involved in the degradation of internalized host cells and microorganisms, *e.g.* CP (26), amoebapores (27), lysozyme (54), and phospholipases (55), we tested whether amoebapore A and CP2 were transported to phagosomes via PPV. Immunostaining of amoebapore and CP2 using specific antisera showed similar patterns to those obtained with LysoTracker in the absence of red blood cells (Fig. 7*A*, *0*

[2] M. Okada, C. D. Huston, B. J. Mann, W. A. Petri, Jr., K. Kita, and T. Nozaki, submitted for publication.

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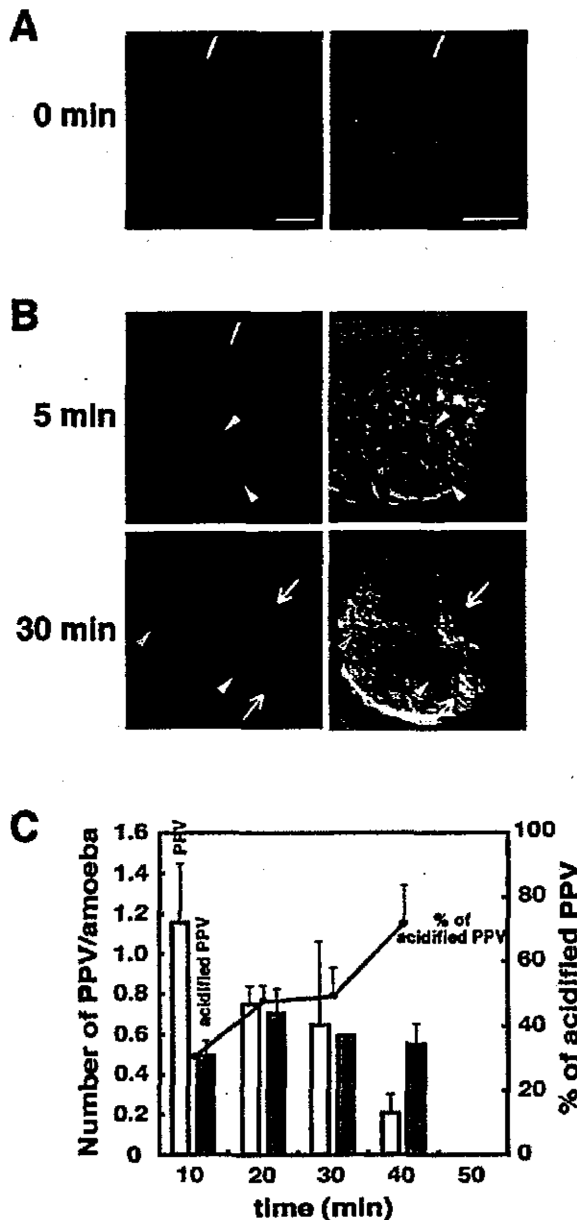


FIG. 6. **Acidification of *Eh*Rab5/*Eh*Rab7A-associated PPV and phagosomes.** *A*, localization of *Eh*Rab5 (*green*) and LysoTracker (*red*) (*left panel*), and *Eh*Rab7A (*green*) and LysoTracker (*red*) (*right panel*) in the absence of red blood cells. Amoebae were pulsed with LysoTracker overnight and subjected to immunofluorescence assay with anti-HA (for *Eh*Rab5) or anti-Myc (for *Eh*Rab7A) monoclonal antibody. *B*, localization of *Eh*Rab7A (*green*) and LysoTracker (*red*) at 5 and 30 min of red blood cell incubation. *White arrowheads* (5 and 30 min) indicate *Eh*Rab7A-positive PPV that do not contain LysoTracker. *Yellow arrowheads* (*30 min*) indicate *Eh*Rab7A-positive PPV containing LysoTracker. *White arrows* indicate phagosomes that contain LysoTracker and are also associated with *Eh*Rab7A. *Yellow arrows* indicate phagosomes containing LysoTracker, but not associated with *Eh*Rab7A. *Bars*, 10 μm. *C*, quantitative analysis of PPV acidification. The number of LysoTracker-associated (*filled bars*) or non-associated PPV (*open bars*) per amoeba is shown together with the percentages of the acidified PPV (*circles* and *a line*). *Error bars* represent S.D. of three independent experiments.

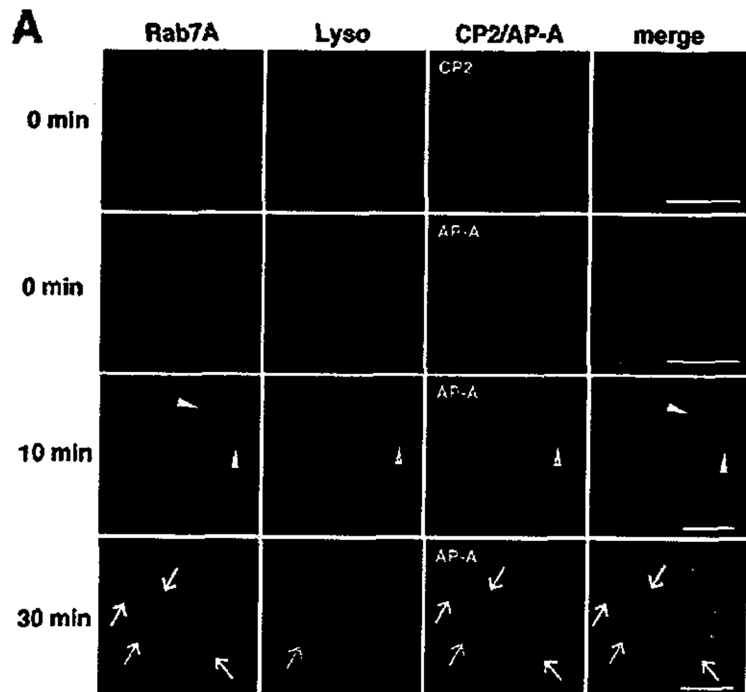
min), suggesting that both amoebapore and CP2 were contained in the lysosomes at steady state. The subcellular localization of both amoebapore and LysoTracker changed during erythrophagocytosis. At 10 min, 80% of acidified *Eh*Rab7A-positive PPV were associated with amoebapore (Fig. 7, *A*, *10 min*, and *C*). At 30 min, all acidified *Eh*Rab7A-positive PPV remained amoebapore-positive (Fig. 7*C*, *30 min*). In contrast, amoebapore and LysoTracker did not perfectly overlap on phagosomes at 30 min; all combinations of amoebapore and LysoTracker positive or negative phagosomes were seen (Fig. 7*A*, *30 min*). As the number of total phagosomes increased during incubation with red blood cells, the number of amoebapore- or LysoTracker-positive phagosomes increased in parallel (Fig. 7*D*). However, the number of *Eh*Rab7A-associated phagosomes did not increase after 30 min; the percentage of *Eh*Rab7A-positive phagosomes transiently increased at 20 min and then decreased (*i.e.* 20, 31, 25, and 19% at 10, 20, 30, and 50 min), consistent with the notion that *Eh*Rab7A was dissociated from phagosomes at this stage. The kinetics of CP2 was indistinguishable from that of amoebapore (data not shown). These results indicate that amoebapore and CP2 were transported from lysosomes to phagosomes via PPV.

We noticed that amoebapore was concentrated in the peripheral part of PPV, and not evenly distributed in the vacuole (*e.g.* Fig. 7*A*, *10 min*). An immunoelectron micrograph using an anti-amoebapore A antibody further documented detailed localization of amoebapore in the PPV (Fig. 7*B*). At the 10-min addition of red blood cells, gold particles were detected on an amorphous structure that partially occupies the lumen. Furthermore, the amoebapore-containing vacuole included membrane structures (Fig. 7*B*, *arrow*). The concentrated localization of amoebapore within PPV was similar to that observed for endocytosed FITC-dextran (Fig. 4*B*).

Expression of EhRab5 Wild Type or Mutants Influences Cell Growth, Ingestion of Red Blood Cells, and Amoebapore Transport to Phagosomes but Not Endocytosis—To further examine the specific role of *Eh*Rab5 and PPV, we introduced a constitutively active GTP form (*Eh*Rab5Q67L) or an inactive GDP form (*Eh*Rab5S22N) mutant of *Eh*Rab5 into wild-type amoeba. Introduction of neither wild-type *Eh*Rab5 nor *Eh*Rab5S22N affected the amoeba growth compared with the vector control independent of coexpression of *Eh*Rab7A (Fig. 8*A*). In contrast, expression of *Eh*Rab5Q67L unexpectedly caused a severe growth defect. This is the first case of a growth defect caused by the expression of a mutant Rab5.

We also studied the effects of expression of wild-type and mutant *Eh*Rab5 on phagocytosis of red blood cells. The number of red blood cells engulfed by the amoebae at 10, 20, or 30 min was counted (Fig. 8*B*). Expression of wild-type *Eh*Rab5 accelerated engulfment of red blood cells by 1.4–2.2-fold, whereas expression of either the *Eh*Rab5Q67L or *Eh*Rab5S22N mutant inhibited the efficiency of phagocytosis by 50–70% compared with the control transformant.

Next, we assessed whether expression of *Eh*Rab5 wild-type or mutants influences the transport of cargo proteins, *e.g.* amoebapore, to phagosomes. Efficiency of the amoebapore transport was evaluated by calculating percentages of phagocytosed red blood cells that colocalized with amoebapore (Fig. 8*C*). In the control transformant cells, 67.0 ± 7.5% of engulfed red blood cells colocalized with amoebapore at 30 min of incubation, whereas 87 ± 2.3% of the ingested red blood cells colocalized with amoebapore in wild-type *Eh*Rab5-expressing cells ($p < 0.01$). In contrast, the expression of *Eh*Rab5Q67L reduced efficiency of the amoebapore transport to 45 ± 3.0% ($p < 0.05$), whereas no significant change was observed in the *Eh*Rab5S22N-expressing transformant (58 ± 2.8%, $p > 0.1$). These data indicate that overexpression of wild-type *Eh*Rab5 or the *Eh*Rab5Q67L mutant increased or interfered with the amoebapore transport to phagosomes, respectively. Neither fluid-phase nor receptor-medicated endocytosis (56), as indi-



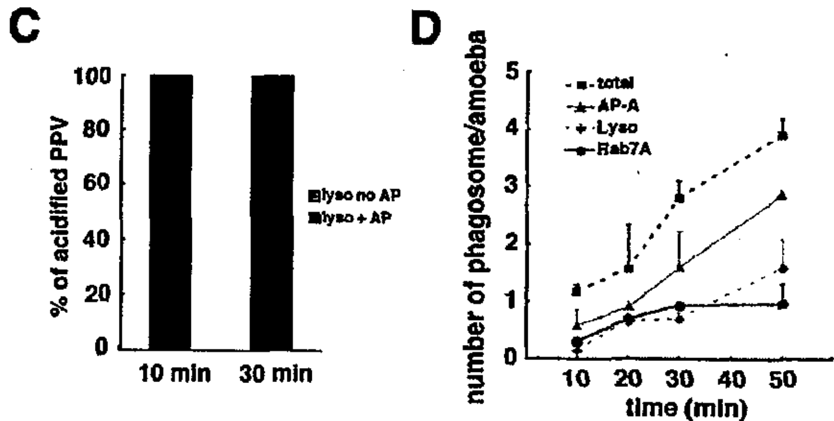


FIG. 7. **Amoebapore A (*AP-A*) and cysteine proteinase 2 (*CP2*) were transported to the red blood cell-containing phagosomes via PPV.** *A*, subcellular localization of *Eh*Rab7A (*green*), LysoTracker (*red*), and CP2 or AP-A (*blue*) in the absence (0 min) or presence of red blood cells (10 and 30 min). *White arrowheads* indicate *Eh*Rab7A-positive PPV that contain neither LysoTracker nor AP-A. *Yellow arrowheads* indicate PPV containing both LysoTracker and AP-A. *White arrows* indicate phagosomes associated with AP-A, but not with LysoTracker. *Yellow arrows* indicate phagosomes that contain both LysoTracker and AP-A. *Bars*, 10 μm. *B*, an immunoelectron micrograph using the amoebapore antibody after a 10-min incubation of red blood cells. Localization of amoebapore indicated by 5-nm of gold particles (*arrow heads*) was partially localized in the vacuole of 2.5 μm in diameter. Luminal membrane structure found in the vacuole was indicated with an *arrow*. *Bar*, 1 μm. *C*, colocalization of LysoTracker and AP-A on PPV. The percentages of PPV that contained both LysoTracker and AP-A (*filled bars*), or LysoTracker only (*gray bars*) at 10 and 30 min of red blood cell incubation are shown. *D*, quantitative analysis of phagosome maturation. The number of total phagosomes, and the AP-A-, LysoTracker-, or *Eh*Rab7A-associated phagosomes is shown at 10–50 min of red blood cell incubation.

cated by FITC-dextran (Fig. 8*D*) or lactoferrin (data not shown) internalization, respectively, was influenced by expression of wild-type or mutant *Eh*Rab5 up to 3 h. Together with the lack of colocalization of *Eh*Rab5 and FITC-dextran shown above (Fig. 4*A*), these results also support the premise that *Eh*Rab5 is unlikely involved in endocytosis. In addition, the efflux of the internalized fluid-phase marker was not affected in *Eh*Rab5 mutants (data not shown).

EhRab5 Plays an Essential Role in the Formation of PPV—Subcellular localization of *Eh*Rab5 mutants was examined to assess possible reasons for a defect in growth, phagocytosis, and amoebapore transport to phagosomes. Confocal immunofluorescence micrographs showed that both *Eh*Rab5Q67L and *Eh*Rab5S22N, probed with anti-*Eh*Rab5 antibody, were localized to small vesicular-like structures that resemble those observed for wild-type *Eh*Rab5 and *Eh*Rab7A at steady state (data not shown). After a 10-min incubation with red blood cells, in contrast to wild-type *Eh*Rab5, *Eh*Rab5Q67L- or *Eh*Rab5S22N-associated vacuoles were not observed; their localization appeared to be identical to that at steady state (Fig.

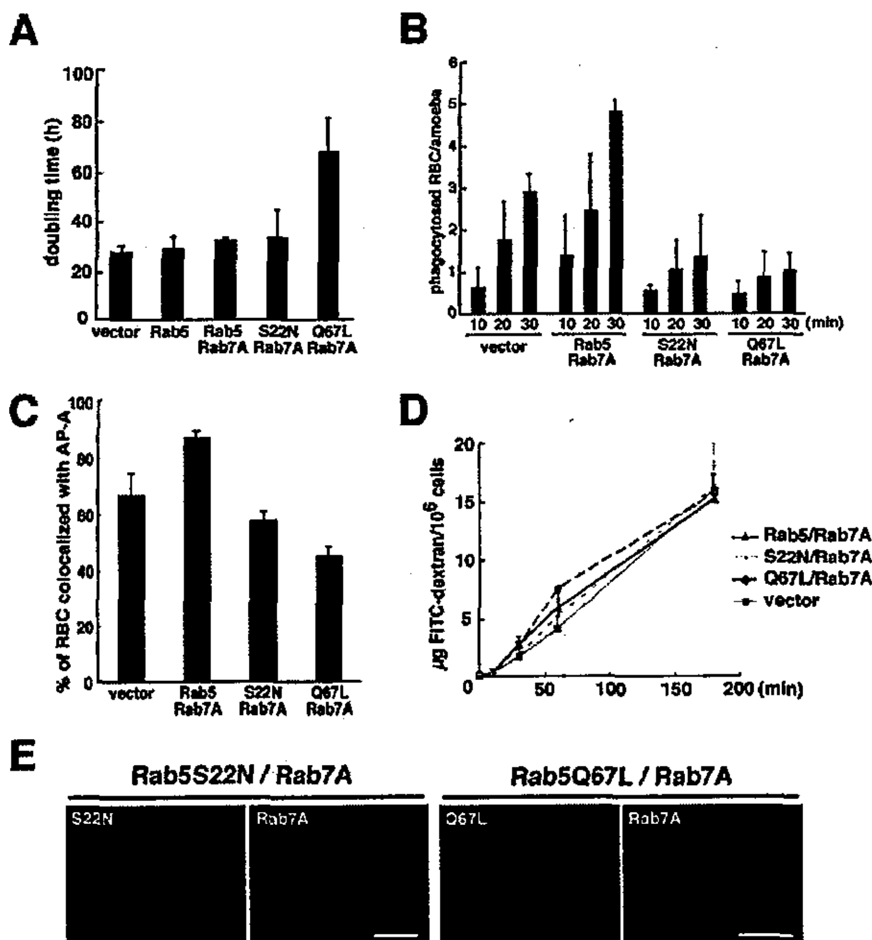


FIG. 8. **Effects of expression of *Eh*Rab5 wild-type and mutants.** *A*, population doubling times of the transformants. *E. histolytica* transformants expressing *Eh*Rab5 (*Rab5*), *Eh*Rab5/*Eh*Rab7A (*Rab5 Rab7A*), *Eh*Rab5S22N/*Eh*Rab7A (*S22N Rab7A*), *Eh*Rab5Q67L/*Eh*Rab7A (*Q67L Rab7A*), and the control transformant with a mock vector (*vector*) were cultured in the presence of 20 μg/ml Geneticin. Doubling times were calculated from three independent experiments performed in triplicate. *B*, overexpression of *Eh*Rab5 wild-type and mutants influenced by phagocytosis of red blood cells. *E. histolytica* transformants overexpressing *Eh*Rab5/*Eh*Rab7A, *Eh*Rab5S22N/*Eh*Rab7A, or *Eh*Rab5Q67L/*Eh*Rab7A and the control transformant were incubated with red blood cells for the indicated times. Phagocytosed red blood cells in these transformants were counted under a microscope. *C*, the transport of AP-A to red blood cell-containing phagosomes was affected by the overexpression of *Eh*Rab5 wild-type and *Eh*Rab5Q67L. The efficiency of AP-A transport was evaluated by calculating percentages of phagocytosed red blood cells colocalizing with AP-A. Two hundred phagosomes containing red blood cells were examined. $p < 0.01$ (Rab5 *versus* control), $p < 0.005$ (Rab5Q67L *versus* control), $p > 0.1$ (Rab5S22N *versus* control), according to Student's t test are shown. *D*, endocytosis of a fluid-phase marker was not affected in the transformants overexpressing *Eh*Rab5 wild-type and mutants. *E. histolytica* transformants were incubated in BI-S-33 medium containing 2 mg/ml FITC-dextran, and endocytosed FITC-dextran was measured using a fluorometer. *Error bars* in *A–D* represent S.D. of three independent experiments. *E*, distribution of *Eh*Rab5S22N, *Eh*Rab5Q67L (*green*), and *Eh*Rab7A (*red*) after a 10-min incubation with red blood cells. *Bars*, 10 μm. Localization of *Eh*Rab5 and *Eh*Rab7A was examined with anti-*Eh*Rab5 and anti-Myc monoclonal antibody, respectively.

8*E*). These results, together with the data shown above, suggest that *Eh*Rab5 is essential for the formation of PPV, which is required for the efficient phagocytosis and transport of the degradative proteins to phagosomes.

DISCUSSION

Although *Eh*Rab5 showed about 50% identity to the mammalian and yeast counterparts and the putative effector domain is very similar between *E. histolytica* and other organisms, the function of the amoebic Rab5 appears to be divergent from that of the mammalian and yeast homologues. First, whereas the mammalian and yeast Rab5/Ypt51p play a role in endocytosis, *Eh*Rab5 is involved exclusively in phagocytosis, but not in endocytosis. This has been shown by the absence of colocalization of *Eh*Rab5 and the endocytosed FITC-dextran (Fig. 4), and also by a lack of augmented uptake of the endocytosis marker by expression of wild-type or the dominant active *Eh*Rab5 mutant (Fig. 8*D*). Second, the localization of *Eh*Rab5 and its association to 3–5-μm translucent PPV, which has not been described in other organisms, are unique to this organism. In the mammalian cells, Rab5 is localized to the early endosomes, and early endosomes directly fuse with primary phagosomes during phagocytosis (4, 7), whereas *Eh*Rab5 is not localized to phagosomes at any stages of phagocytosis in the amoeba (Fig. 3). Instead, *Eh*Rab5 is localized, in conjunction with *Eh*Rab7A, to PPV before these vacuoles fuse with phagosomes containing red blood cells. Third, in contrast to mammals, where similar phenotypes were observed in transformants expressing wild-type and GTP-mutant Rab5 and opposite phenotypes were observed in the GDP-mutant Rab5-expressing transformants (5), expression of *Eh*Rab5 GTP or GDP mutant showed a similar defect in erythrophagocytosis and PPV formation (Fig. 8). This may indicate that requirement of GTP hydrolysis by Rab5 for membrane fusion may differ between the amoeba and other organisms. Fourth,

*Eh*Rab5 does not functionally complement the yeast Δ*ypt51* mutant. This is in good contrast to the yeast and mammalian counterparts, which are virtually interchangeable; Ypt51p expressed in a mammalian cell was properly localized to endosomes and accelerated endocytosis (6). Fifth, compared with other organisms including mammals, plants, yeasts, and a parasitic protist *Trypanosoma brucei*, which have been shown to possess 2–5 Rab5 isotypes with distinct tissue and organelle distribution or developmental stage-specific expression (46, 57–59), *E. histolytica* possesses only a single Rab5 gene based on our thorough search of the genome data base (data not shown). Altogether, *Eh*Rab5 represents a unique Rab5 showing diverse localization and functions.

We have identified and characterized an unprecedented vacuole "PPV," which is coassociated with *Eh*Rab5 and *Eh*Rab7A at the early stage of its formation and becomes dissociated by *Eh*Rab5 during maturation. We have shown that PPV were formed *de novo* in a very short time, and then acidified, in a time-dependent fashion during phagocytosis, by the fusion of lysosomes, which contain at least two independent degradative proteins, *i.e.* CP2 and amoebapore A (Figs. 5–7). We propose that PPV serves as a compartment for the temporal storage, processing, and/or activation of hydrolytic enzymes and lytic peptides before fusion with phagosomes containing ingested host cells and microorganisms. In mammalian cells, a newly formed phagosome is subjected to gradual maturation by continuous exchange of their contents via sequential fusion with the early and late endosomal compartments, leading to the formation of acidified phagolysosome (60). In contrast, in the amoeba, PPV apparently serves as a reservoir of digestive enzymes (Fig. 7) and endosomal content (Fig. 4) prior to fusion with phagosomes (Fig. 7). An immunoelectron micrograph showed that the vacuole containing amoebapore were enclosed by another membrane structure (Fig. 7*B*). Although multivesicular vacuoles have been previously reported in *Entamoeba* (61), this is the first demonstration of a particular protein within these multivesicular vacuoles. In mammalian and yeast cells, multivesicular bodies have been regarded as late endosomes, in which proteins to be transported to lysosomal lumen are selectively packed into internal vesicles (62). These observations imply that some proteins targeted to phagosomes are selectively included in PPV. We have recently identified the homologue of retromer, which functions in retrieval of receptor proteins from late endosomes to the trans-Golgi network (63).[3] The observation that one of retromer components, *Eh*Vps26, is localized on PPV might imply that PPV had a similar function to late endosomes/multivesicular bodies. As far as we are aware, such a "preparatory" organelle has not previously been described and may represent a novel cellular compartment.

The formation of PPV was induced most efficiently by interaction with red blood cells. A membrane ghost, but not a soluble fraction, of red blood cells also induced the formation (data not shown). However, latex beads, yeasts, and *Escherichia coli* cells induced the formation of PPV to a much lesser extent (data not shown). Thus, Rab5 recruitment to PPV in this parasite may occur predominantly in a case of the engulfment of red blood cells. PPV were also observed in cells that did not initiate engulfment of red blood cells (Fig. 3, *E–L*). These findings suggest that interaction with red blood cells, but not engulfment *per se*, is sufficient for the induction of PPV formation. One intriguing hypothesis to explain why PPV formation is specifically induced by red blood cells is that PPV is required for the degradation and/or detoxification of the content of red blood cells. It was previously reported that amoebae recognizes surface glycans with Galβ1–4GlcNAc terminal glycosphingolipid on red blood cells (64) by a Gal/GalNAc-inhibitable lectin (21, 22). The Galβ1–4GlcNAc terminal glycosphingolipid is absent on the surface of latex beads, yeast, and *E. coli* (65, 66). It has also been demonstrated in macrophages that the phagocytosis-induced response also depends on receptors (67). For example, phagocytosis via the Fc receptor lead to the production of proinflammatory molecules such as reactive oxygen intermediates, whereas phagocytosis involving mannose receptor produced proinflammatory cytokines including interleukin-1β and tumor necrosis factor-α (68). In contrast, phagocytosis via the complement receptor did not elicit release of inflammatory mediators (69). Actin and microtubles were shown to be important for the complement receptor system, whereas two regulatory proteins of actin cytoskeleton, vinculin and proxilin, were not necessary for the mannose receptor system, indicating diversity of the receptor-response relationship during phagocytosis (70).

In view of the signals necessary for PPV formation, we also noted that a phosphatidylinositol 3-kinase inhibitor, wortmannin at 100 nM, abolished both ingestion, as previously reported (71), and PPV formation (data not shown). This finding also supports a tight correlation between ingestion of red blood cells and formation of PPV. The fact that expression of wild-type or GTP mutant *Eh*Rab5 resulted in augmented or diminished ingestion of red blood cells, respectively, also supports the premise that signal transduces from PPV to an initial site of engulfment. However, whether a phagosome-associated phosphatidylinositol 3-kinase is present in the amoeba, as shown in mammals (Vps34) (12) and what effector proteins (*e.g.* EEA1 in mammals) (72, 73) are recruited to the phagosomes of the amoeba in a phosphatidylinositol 3-kinase-dependent manner remain unknown.

We have shown detailed quantitative data on how the maturation of PPV and phagosomes occur during erythrophagocytosis (Figs. 3*Q*, 6*C*, and 7, *C* and *D*). In contrast to the gradual and continuous acidification of PPV, which occurs in parallel with recruitment of digestive enzymes to PPV, acidification of phagosomes appears to be interrupted or, more likely, reversed by neutralization, which synchronizes with the dissociation of *Eh*Rab7A from phagosomes (Fig. 7*D*). A few lines of evidence suggest that neutralization of phagosomes takes place soon after the content of PPV is transported to phagosomes. First, the percentage of acidified phagosomes remained unchanged between 20 and 50 min after ingestion (*e.g.* 43 and 38%, at 20 and 50 min, respectively). Second, the percentage of acidified phagosomes was significantly lower than that of amoebapore-containing phagosomes at all time points (*e.g.* 58 and 71% at 20 and 50 min).

We propose here a model by which *Eh*Rab5 and *Eh*Rab7A coordinately regulate membrane fusion during phagocytosis. Upon the interaction of red blood cells with the amoeba plasma membrane, independent *Eh*Rab5- or *Eh*Rab7A-associated vesicles receive a signal, in a phosphatidylinositol 3-phosphate-dependent manner, presumably from the Gal/GalNAc-specific surface lectin or a not yet identified plasma membrane receptor, which initiates subsequent sorting and reorganization of these compartments. *Eh*Rab5 vesicles start to heterotypically fuse with *Eh*Rab7A-associated vesicles, and then form PPV. PPV simultaneously fuse with lysosomes containing amoebapore and hydrolases. *Eh*Rab5 is then dissociated from PPV before the content of *Eh*Rab7A-associated PPV is targeted to phagosomes. Because the size of phagosomes did not increase after *Eh*Rab7A was transported from PPV to phagosomes, the direct fusion between PPV and phagosome likely does not oc-

[3] K. Nakada-Tsukui, Y. Saito-Nakano, V. Ali, and T. Nozaki, manuscript in preparation.

cur. We propose that transfer of the content of PPV involves vesicular trafficking, *i.e.* budding from PPV followed by fusion of these vesicles to phagosomes. Once the content of PPV is transferred to phagosomes, *Eh*Rab7A is dissociated from phagosomes, whereas digestive proteins remain in phagosomes. Neutralization of phagosomes also takes place in close timing with *Eh*Rab7A dissociation. After degradation of internalized materials, membrane recycling from phagosomes also likely occurs via the budding of recycling vesicles (74). Finally, the molecular dissection of a unique function of *Eh*Rab5 and a novel *Eh*Rab5-associated compartment in this parasite may shed light on the *Entamoeba*-specific phagocytic mechanisms closely related to its virulence competence.

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REFERENCES

1. Tjelle, T. E., Lovdal, T., and Berg, T. (2000) *Bioessays* **22,** 255–263
2. Greenberg, S., and Grinstein, S. (2002) *Curr. Opin. Immunol.* **14,** 136–145
3. Novick, P., and Zerial, M. (1997) *Curr. Opin. Cell Biol.* **9,** 496–504
4. Desjardins, M., Celis, J. E., van Meer, G., Dieplinger, H., Jahraus, A., Griffiths, G., and Huber, L. A. (1994) *J. Biol. Chem.* **269,** 32194–32200
5. Stenmark, H., Parton, R. G., Steele-Mortimer, O., Lutcke, A., Gruenberg, J., and Zerial, M. (1994) *EMBO J.* **13,** 1287–1296
6. Singer-Kruger, B., Stenmark, H., and Zerial, M. (1995) *J. Cell Sci.* **108,** 3509–3521
7. Jahraus, A., Tjelle, T. E., Berg, T., Habermann, A., Storrie, B., Ullrich, O., and Griffiths, G. (1998) *J. Biol. Chem.* **273,** 30379–30390
8. Duclos, S., Diez, R., Garin, J., Papadopoulou, B., Descoteaux, A., Stenmark, H., and Desjardins, M. (2000) *J. Cell Sci.* **113,** 3531–3541
9. Alvarez-Dominguez, C., and Stahl, P. D. (1999) *J. Biol. Chem.* **274,** 11459–11462
10. Stenmark, H., Vitale, G., Ullrich, O., and Zerial, M. (1995) *Cell* **83,** 423–432
11. Christoforidis, S., and Zerial, M. (2000) *Methods* **20,** 403–410
12. Vieira, O. V., Botelho, R. J., Rameh, L., Brachmann, S. M., Matsuo, T., Davidson, H. W., Schreiber, A., Backer, J. M., Cantley, L. C., and Grinstein, S. (2001) *J. Cell Biol.* **155,** 19–25
13. Vieira, O. V., Bucci, C., Harrison, R. E., Trimble, W. S., Lanzetti, L., Gruenberg, J., Schreiber, A. D., Stahl, P. D., and Grinstein, S. (2003) *Mol. Cell. Biol.* **23,** 2501–2514
14. Wichmann, H., Hengst, L., and Gallwitz, D. (1992) *Cell* **71,** 1131–1142
15. Via, L. E., Deretic, D., Ulmer, R. J., Hibler, N. S., Huber, L. A., and Deretic, V. (1997) *J. Biol. Chem.* **272,** 13326–13331
16. Hashim, S., Mukherjee, K., Raje, M., Basu, S. K., and Mukhopadhyay, A. (2000) *J. Biol. Chem.* **275,** 16281–16288
17. Harrison, R. E., Bucci, C., Vieira, O. V., Schroer, T. A., and Grinstein, S. (2003) *Mol. Cell. Biol.* **23,** 6494–6506
18. Petri, W. A., Jr. (2002) *Curr. Opin. Microbiol.* **5,** 443–447
19. W.H.O./PAHO/UNESCO (1997) *Epidemiol. Bull.* **18,** 13–14
20. Orozco, E., Guarneros, G., Martinez-Palomo, A., and Sanchez, T. (1983) *J. Exp. Med.* **158,** 1511–1521
21. Vines, R. R., Ramakrishnan, G., Rogers, J. B., Lockhart, L. A., Mann, B. J., and Petri, W. A., Jr. (1998) *Mol. Biol. Cell* **9,** 2069–2079
22. Cheng, X. J., Tsukamoto, H., Kaneda, Y., and Tachibana, H. (1998) *Parasitol. Res.* **84,** 632–639
23. Voigt, H., Olivo, J. C., Sansonetti, P., and Guillen, N. (1999) *J. Cell Sci.* **112,** 1191–1201
24. Guillen, N., Boquet, P., and Sansonetti, P. (1998) *J. Cell Sci.* **111,** 1729–1739
25. Labruyere, E., Zimmer, C., Galy, V., Olivo-Marin, J. C., and Guillen, N. (2003) *J. Cell Sci.* **116,** 61–71
26. Que, X., and Reed, S. L. (2000) *Clin. Microbiol. Rev.* **13,** 196–206
27. Leippe, M. (1999) *Dev. Comp. Immunol.* **23,** 267–279
28. Saito-Nakano, Y., Nakazawa, M., Shigeta, Y., Takeuchi, T., and Nozaki, T. (2001) *Mol. Biochem. Parasitol.* **116,** 219–222
29. Rodriguez, M. A., Garcia-Perez, R. M., Garcia-Rivera, G., Lopez-Reyes, I., Mendoza, L., Ortiz-Navarrete, V., and Orozco, E. (2000) *Mol. Biochem. Parasitol.* **108,** 199–206
30. Temesvari, L. A., Harris, E. N., Stanley, S. L., Jr., and Cardelli, J. A. (1999) *Mol. Biochem. Parasitol.* **103,** 225–241
31. Juarez, P., Sanchez-Lopez, R., Stock, R. P., Olvera, A., Ramos, M. A., and Alagon, A. (2001) *Mol. Biochem. Parasitol.* **116,** 223–228
32. Diamond, L. S., Mattern, C. F., and Bartgis, I. L. (1972) *J. Virol.* **9,** 326–341
33. Diamond, L. S., Harlow, D. R., and Cunnick, C. C. (1978) *Trans. R. Soc. Trop. Med. Hyg.* **72,** 431–432
34. Tanaka, T., Tanaka, M., and Mitsui, Y. (1997) *Biochem. Biophys. Res. Commun.* **236,** 611–615
35. Nozaki, T., Asai, T., Kobayashi, S., Ikegami, F., Noji, M., Saito, K., and Takeuchi, T. (1998) *Mol. Biochem. Parasitol.* **97,** 33–44
36. Hamann, L., Buss, H., and Tannich, E. (1997) *Mol. Biochem. Parasitol.* **84,** 83–91
37. Landt, O., Grunert, H. P., and Hahn, U. (1990) *Gene (Amst.)* **96,** 125–128
38. Ramakrishnan, G., Rogers, J., Mann, B. J., and Petri, W. A., Jr. (2001) *Parasitol. Int.* **50,** 47–50
39. Nozaki, T., Asai, T., Sanchez, L. B., Kobayashi, S., Nakazawa, M., and Takeuchi, T. (1999) *J. Biol. Chem.* **274,** 32445–32452
40. Hellberg, A., Leippe, M., and Bruchhaus, I. (2000) *Mol. Biochem. Parasitol.* **105,** 305–309
41. Tomishige, M., Sako, Y., and Kusumi, A. (1998) *J. Cell Biol.* **142,** 989–1000
42. Leippe, M., Ebel, S., Schoenberger, O. L., Horstmann, R. D., and Muller-Eberhard, H. J. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 7659–7663
43. Novikoff, A. B., Novikoff, P. M., Davis, C., and Quintana, N. (1972) *J. Histochem. Cytochem.* **20,** 1006–1023
44. Jaunin, F., Burns, K., Tschopp, J., Martin, T. E., and Fakan, S. (1998) *Exp. Cell Res.* **243,** 67–75
45. Stenmark, H., and Olkkonen, V. M. (2001) *Genome Biol.* **2,** Reviews 3007
46. Singer-Kruger, B., Stenmark, H., Dusterhoft, A., Philippsen, P., Yoo, J. S., Gallwitz, D., and Zerial, M. (1994) *J. Cell Biol.* **125,** 283–298
47. Horazdovsky, B. F., Busch, G. R., and Emr, S. D. (1994) *EMBO J.* **13,** 1297–1309
48. Jay, D. G. (1996) *Cell* **86,** 853–854
49. Rupper, A., Grove, B., and Cardelli, J. (2001) *J. Cell Sci.* **114,** 2449–2460
50. Araki, N., Hatae, T., Furukawa, A., and Swanson, J. A. (2003) *J. Cell Sci.* **116,** 247–257
51. Henry, R. M., Hoppe, A. D., Joshi, N., and Swanson, J. A. (2004) *J. Cell Biol.* **164,** 185–194
52. Hackam, D. J., Rotstein, O. D., Zhang, W. J., Demaurex, N., Woodside, M., Tsai, O., and Grinstein, S. (1997) *J. Biol. Chem.* **272,** 29810–29820
53. Bucci, C., Thomsen, P., Nicoziani, P., McCarthy, J., and van Deurs, B. (2000) *Mol. Biol. Cell* **11,** 467–480
54. Nickel, R., Jacobs, T., and Leippe, M. (1998) *FEBS Lett.* **437,** 153–157
55. Vargas-Villarreal, J., Olvera-Rodriguez, A., Mata-Cardenas, B. D., Martinez-Rodriguez, H. G., Said-Fernandez, S., and Alagon-Cano, A. (1998) *Parasitol. Res.* **84,** 310–314
56. Batista, E. J., de Menezes Feitosa, L. F., and de Souza, W. (2000) *Parasitol. Res.* **86,** 881–890
57. Ueda, T., and Nakano, A. (2002) *Curr. Opin. Plant Biol.* **5,** 513–517
58. Field, H., Farjah, M., Pal, A., Gull, K., and Field, M. C. (1998) *J. Biol. Chem.* **273,** 32102–32110
59. Bucci, C., Lutcke, A., Steele-Mortimer, O., Olkkonen, V. M., Dupree, P., Chiariello, M., Bruni, C. B., Simons, K., and Zerial, M. (1995) *FEBS Lett.* **366,** 65–71
60. Desjardins, M. (1995) *Trends Cell Biol.* **5,** 183–186
61. Mazzuco, A., Benchimol, M., and De Souza, W. (1997) *Micron* **28,** 241–247
62. Odorizzi, G., Babst, M., and Emr, S. D. (1998) *Cell* **95,** 847–858
63. Seaman, M. N., McCaffery, J. M., and Emr, S. D. (1998) *J. Cell Biol.* **142,** 665–681
64. Bailey, G. B., Nudelman, E. D., Day, D. B., Harper, C. F., and Gilmour, J. R. (1990) *Infect. Immunol.* **58,** 43–47
65. Klis, F. M., Mol, P., Hellingwerf, K., and Brul, S. (2002) *FEMS Microbiol. Rev.* **26,** 239–256
66. Silver, L. L. (2003) *Curr. Opin. Microbiol.* **6,** 431–438
67. Aderem, A., and Underhill, D. M. (1999) *Annu. Rev. Immunol.* **17,** 593–623
68. Ravetch, J. V., and Clynes, R. A. (1998) *Annu. Rev. Immunol.* **16,** 421–432
69. Pommier, C. G., Inada, S., Fries, L. F., Takahashi, T., Frank, M. M., and Brown, E. J. (1983) *J. Exp. Med.* **157,** 1844–1854
70. Allen, L. A., and Aderem, A. (1996) *J. Exp. Med.* **184,** 627–637
71. Ghosh, S. K., and Samuelson, J. (1997) *Infect. Immunol.* **65,** 4243–4249
72. Fratti, R. A., Backer, J. M., Gruenberg, J., Corvera, S., and Deretic, V. (2001) *J. Cell Biol.* **154,** 631–644
73. Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J. M., Brech, A., Callaghan, J., Toh, B. H., Murphy, C., Zerial, M., and Stenmark, H. (1998) *Nature* **394,** 494–498
74. Damiani, M. T., and Colombo, M. I. (2003) *Exp. Cell Res.* **289,** 152–161

感染症の診断・治療ガイドライン2004

監修　日本医師会感染症危機管理対策室
厚生労働省健康局結核感染症課
編集　感染症の診断・治療ガイドライン編集委員会

JMA 日本医師会

五類感染症●全数把握／7日以内に届け出

好発時期：①②③④⑤⑥⑦⑧⑨⑩⑪⑫月（通年）

アメーバ赤痢

amoebiasis, amebiasis

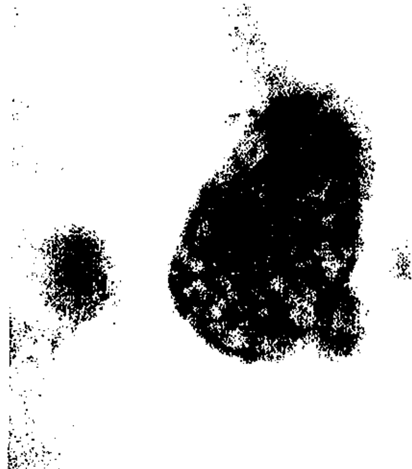


図1　赤痢アメーバの栄養型

病原体	赤痢アメーバ *Entamoeba histolytica*
好発年齢	成人
性差	地域差はあるが一般に男性に多い
分布	世界的に分布

◉感染経路
- 患者糞便の成熟嚢子の経口感染

◉潜伏期間
- 数日〜数カ月（平均2〜4週間）

◉伝播可能期間
- 糞便中に病原性のあるアメーバ（*E. histolytica*）の嚢子を排出している期間，治療しない場合には数年にわたるとされる

◉症状
- 腸アメーバ症と腸外アメーバ症に分類される．腸アメーバ症はさらにアメーバ赤痢とアメーバ性大腸炎に分けることができる
- 腸アメーバ症：比較的ゆるやかな発症で，アメーバ赤痢では粘血便を伴ういわゆるイチゴゼリー状の下痢，腹痛が主症状．テネスムスはあっても軽度．大腸炎では種々の性状の下痢と腹痛．粘血便をみることはない
- 腸外アメーバ症：肝膿瘍が最多．肝腫大，同部の重圧感，右肩に放散する自発痛，圧痛，嘔気，嘔吐．腸管症状はないことが多い

◉オーダーする検査
- 糞便検査
- ゲル内沈降反応，間接蛍光抗体法（IFA），ラテックス凝集法，ELISAなどの血清学的検査，サンドイッチELISAなどによる糞便中などの特異抗原検出，PCR
- 内視鏡，肝膿瘍の場合には画像診断

◉確定診断のポイント
- 開発途上国からの帰国者，男性同性愛者，施設等の利用者など
- 粘血便を伴う下痢
- 糞便検査の繰り返し実施
- 肝膿瘍での高い血清抗体価
- 大腸内視鏡検査と生検
- 従来，腸アメーバ症の鑑別診断の対象は細菌性赤痢だったが，近年は潰瘍性大腸炎，大腸癌が重要

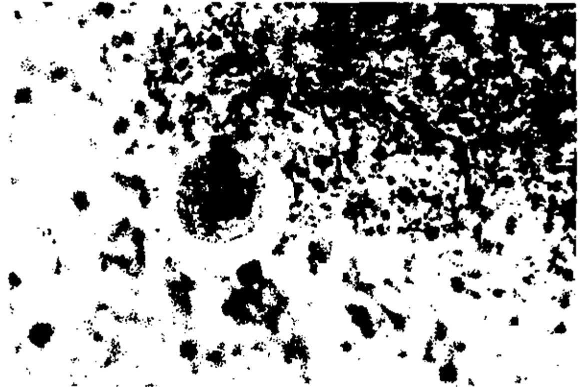
◉治療のポイント
- 病型にかかわらず，化学療法が基本．第1選択薬は，メトロニダゾール．重症赤痢または大きな肝膿瘍の症例にはメトロニダゾール点滴用製剤（薬価未収載）を用いる．肝膿瘍では外科的にドレナージを行うこともある．

感染症法

◉報告の基準

- 診断した医師の判断により，症状や所見から当該疾患が疑われ，かつ，以下のいずれかの方法によって病原体診断や血清学的診断がなされたもの．
- 病原体の検出：[例]糞便からの赤痢アメーバ栄養体の検出，病変部位（組織切片または膿瘍液）からの本原虫の検出など．
- 病原体の遺伝子の検出：[例]赤痢アメーバに特有な遺伝子配列の検出（PCR 法等）など．
- 病原体に対する抗体の検出：[例]患者血清からの赤痢アメーバに対する特異抗体の検出など．

図 2　赤痢アメーバ嚢子（鉄ヘマトキシリン染色）



未成熟の嚢子で両端が鈍円状の類染色質体をみる．

アメーバ赤痢の背景

■疫学状況

- 世界人口のうち，約 5 億人が従来赤痢アメーバとされた原虫（*Entamoeba histolytica/E. dispar*）に感染し，そのうち約 4,000 万人が病原種である *E. histolytica* によって赤痢，大腸炎や肝膿瘍を発症し，毎年 4 万～11 万人が死亡している．*E. dispar* は光顕レベルでは鑑別困難で病原性を有しない．
- 赤痢アメーバは世界各地に分布している．
- 海外渡航者が感染することが多いといわれていたが，近年では国内感染による発症例が多く，福祉施設などでの集団感染も報告されている．
- 男性同性愛者にも多くみられ，性感染症（STD）の 1 つと考えられる．最近異性間での性行為によって感染したと想定される例も報告されている．

■病原体・毒素

- *E. histolytica* の生活環は栄養型（図 1）と嚢子（図 2）で構成される．感染能力があるのは成熟嚢子のみ．

■感染経路

- 無症状の嚢子保有者が感染源として重要．
- 感染にかかわる要因は前述のとおりであるが，いずれも成熟嚢子の経口摂取による．

■潜伏期

- 明確な潜伏期間の基準は示せないが，次のようにして発症に至る．
- アメーバが大腸粘膜に侵入し，潰瘍を形成すれば腸アメーバ症が成立する．一般的に緩徐な発症．腸管腔のみに存在すれば無症状の嚢子保有者として推移．
- アメーバ性肝膿瘍は大腸粘膜に侵入した原虫が経門脈的に肝に達し，そこで微小膿瘍を形成し，やがて肝膿瘍に進展する．

診断と治療

■臨床症状

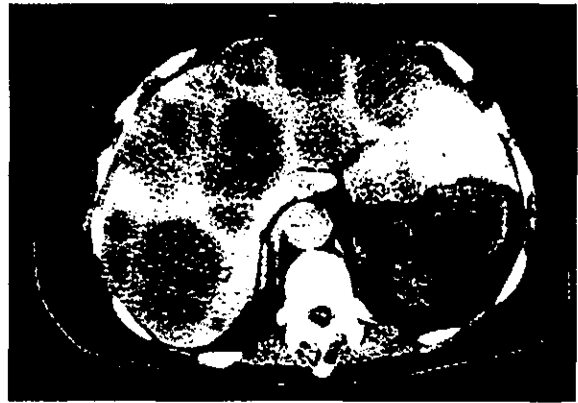
◉病型

- 病型は腸アメーバ症と腸外アメーバ症に大別される．
- 腸外アメーバ症は大腸組織から血行性にアメーバが他臓器に転移して形成された病変を称し，肝膿瘍が最も高頻度である．膿瘍が 2 次的に転移したり直接進展すると横隔膜下，心外膜，肺，脳，脾，皮膚に膿瘍が形成されることがある．肝膿瘍が破裂すると腹膜炎を併発する．

図3　腸アメーバ症の大腸病理組織図

病変部の壊死が強く，潰瘍底部に多数の赤痢アメーバ栄養型虫体がみられる.

図4　アメーバ性肝膿瘍のCT像



(都立北療育医療センター・増田剛太博士のご好意による)

◉腸アメーバ症

• 下痢，粘血便，鼓腸，排便時の下腹部痛，あるいは不快感などの症状を伴う慢性腸管感染症であり，アメーバ赤痢，アメーバ性大腸炎などの病型を含む.

• 典型的なアメーバ赤痢ではイチゴゼリー状の粘血便を排泄するが，数週間程度の間隔で症状発現と寛解を繰り返すことが多い．次第にこの間隔が延長し，慢性化していく.

• アメーバ性大腸炎の場合はやはり腹痛と下痢を主症状とするが，下痢の性状は血便，粘液便など多様である.

• アメーバによる潰瘍の好発部位は盲腸から上行結腸にかけてとS状結腸から直腸にかけての大腸である.

• まれに肉芽腫性病変が形成されたり，潰瘍部が拡大し，穿孔することもある.

◉腸外アメーバ症

• 肝膿瘍が最も多い.

• 肝膿瘍では発熱(38〜40°C)，右季肋部痛，同部圧痛，肝腫大，嘔気，嘔吐，体重減少，寝汗，全身倦怠などを伴う.

■検査所見

• 糞便の顕微鏡所見：通常，下痢(粘血便)を伴う症例では *E. histolytica* の栄養型が，軽症例またはキャリアの有形便では嚢子(シスト)が証明される.

• 検便は原則として1回の検査にとどめず，連続3日間程度の集中検査で検出精度を高める措置が求められる.

• 大腸炎では白血球数正常．肝膿瘍を合併すれば白血球数増多(10,000/mm^3以上)し，時に血漿プロトロンビン値減少，血清コレステロール値低下.

• 血清アメーバ抗体価：大腸炎では必ずしも上昇しない．肝膿瘍では陽性率95%.

• 糞便中の特異抗原検出も信頼性は高い.

• 大腸内視鏡：大腸粘膜面にタコイボ状潰瘍．粘膜生検組織のアメーバ検出率は50〜70%程度(図3).

• CTやエコーで肝に膿瘍性病変を検出(図4).

■診断・鑑別診断

◉確定診断

• 下痢(粘血便，他)，腹痛，肝腫大など.

• 糞便または大腸組織内に *E. histolytica* を

証明．下痢便中の栄養型虫体で赤血球を捕食しているものが見出されれば*E. histolytica* である可能性は高い．生検組織内に栄養型虫体を見出したときも同様．

- 内視鏡で大腸粘膜にアメーバによる潰瘍を証明．
- 血清抗アメーバ抗体価上昇．
- 糞便内のアメーバ特異抗原検出．
- PCR．特に糞便内嚢子の DNA を標的にした PCR は，*E. histolytica* と *E. dispar* の鑑別に有用．

◉**鑑別診断**

- 細菌性腸炎：① 臨床症状が急性，② 抗菌薬投与歴を聴取．細菌培養を行う．
- 寄生虫症：① 生活歴を聴取，② 糞便からの虫体または虫卵の直接証明．または血清抗体による間接証明を行う．

■**治療**

- 第1選択薬はメトロニダゾール．作用は殺アメーバ的で，大腸症状は多くの場合，速やかに反応して治療開始数日後から粘血便などの消失と有形便化をみる．しかしメトロニダゾールの腸管吸収が速いため，腸管内の虫体を完全に殺滅できないことがある．そのためしばしばテトラサイクリンなど，あるいはディロキサンド(熱帯病に対するオーファンドラッグ開発研究班より供与可能)を併用する．
- 重症赤痢などではメトロニダゾール点滴用製剤(前記研究班より供与可能)を用いる．
- 肝膿瘍の場合で，その直径が 3～5 cm 以上の巨大な膿瘍には肝ドレナージを併用することもある．2～3 週間で膿瘍腔の縮小が認められる．ドレーン抜去後，膿瘍陰影が消失するのに数カ月を要することもある．
- 赤痢症状が強いときは食物の経口摂取制限が必要．
- 非病原性である *E. dispar* のみの感染であれば治療は不要．
- 無症状で *E. histolytica* か *E. dispar* かの同定ができない場合は，治療せず経過観察とする．

■**経過・予後・治療効果判定**

- アメーバ性大腸炎では，投薬による治癒後も糞便中に嚢子が認められ，感染源となることがあるので経過観察が必要．
- 家族内での感染の有無に関して観察する．

■**合併症・続発症とその対応**

- 腸外アメーバ症：わが国では腸アメーバ症例の約 20～40% が肝膿瘍を合併する．
- 特に同性愛者の場合，梅毒や HIV 感染の合併も考えて治療を進める．

■**2 次感染予防・感染の管理**

- 衛生の徹底．
- 患者の届け出と，必要に応じて接触者および患者家族での感染の有無の確認．
- ワクチンは存在しない．

(竹内　勤)