Transfer of Zymosan (Yeast Cell Walls) to the Parasitophorous Vacuoles of Macrophages Infected with *Leishmania amazonensis*

By Patricia S. T. Veras, Chantal de Chastellier,* and Michel Rabinovitch

From the Immunoparasitology Unit, Institut Pasteur, 75724 Paris, France; and the "Microbiology Unit, Faculté de Médecine Necker-Enfants Malades, 75730 Paris, France

Summary

*Leishmania* are flagellated protozoan parasites which, in their amastigote stages, survive and multiply within phagolysosome-like parasitophorous vacuoles (PV) of mammalian macrophages (MO). This study develops an earlier ultrastructural, incidental observation that zymosan particles (Z) were transferred to the PV of macrophages infected with *Leishmania amazonensis*. In the present report, a pulse-chase light microscopic assay was used to delineate several features of the Z transfer. The assay reflects both the movement of internalized particles to a position adjoining a PV, and their delivery to the vacuoles. Transfer was selective, in the sense that Z, β-glucan or heat-killed yeast particles were transferred, whereas latex beads, aldehyde-fixed, or immunoglobulin G-coated erythrocytes were not. This selectivity may be related to the high density of carbohydrate ligands displayed on the surface of yeast-derived particles, to ligand resistance to lysosomal degradation or to signals encoded in the cytosolic tails of the receptors involved in particle recognition. A few Z particles could be found within PV after 1 h of incubation with infected MO, but chase periods of several hours at 34°C were required for particle transfer to the PV in a substantial proportion of the MO. Ammonium chloride, chloroquine, or monensin, compounds which increase the pH in acidified compartments, substantially enhanced the transfer of Z particles. Finally, transfer was inhibited by cytochalasin D, but was unaffected by the antitubulin nocodazole. Although it is not yet known if particle transfer occurs by fusion of donor vesicles with PV or by interiorization of the former into the latter, the model described should be useful in the study of the interactions between large phagocytic vesicles and the modulation of those interactions by cellular, parasitic, and environmental signals.

*Leishmania* are unicellular eukaryotic parasites, which in their incompletely flagellated amastigote stage, lodge and multiply within membrane-bound parasitophorous vacuoles (PV) of mammalian macrophages (MO). These vacuoles are assumed to be phagolysosomes because they are acidified, contain lysosomal enzymes, and acquire, presumably by fusion with lysosomes or other endocytic vesicles, electron opaque colloids or molecular ligands taken up by receptor-mediated endocytosis. In addition, lysosomal glycoproteins (110 and 120) and in cryosections, the cation-independent mannose-6 phosphate receptor, a prelysosomal marker (D. G. Russell, personal communication), have been detected in the PV membranes of *Leishmania amazonensis*-infected MO. Most of these studies have been performed with *Leishmania* of the *mexicana* complex, which typically induce the formation of large and rather stable PV, which contain numerous amastigotes.

In the course of experiments in which yeast cell walls (zymosan, Z) were used in induce lysosomal enzyme secretion by *L. amazonensis*-infected MO, transmission electron microscopy observations revealed the presence of Z particles within the PV of the phagocytes (9). This result contrasted with earlier findings that other particulates such as latex beads or glutaraldehyde-fixed red blood cells did not enter PV (10). Both *Leishmania* amastigotes and Z particles are quite stable within host cells, and are easily identified by light and electron microscopy. Furthermore, at low parasite loads the infection is well tolerated by the host cells, as shown by cinematographic observations of dividing infected marrow phagocytes (11). Thus, the vesicular and quantal transfer of the particles to the PV of infected MO offers a useful tool for the study of cellular and biochemical mechanisms which underlie the interactions between large phagocytic vesicles.

In the present report a pulse-chase light microscopic assay
has been used to examine certain features and requirements of the transfer of particles to the PV of L. amazonensis-infected MØ.

Materials and Methods

**Media.** Ca\(^2+\) and Mg\(^2+\)-free PBS, pH 7.3, contained 138 mM NaCl and 6.7 mM potassium phosphate buffer. HBSS and FCS were from Flow Laboratories, Inc. (McLean, VA), and RPMI 1640 was from Gibco Laboratories (Grand Island, NY). HBSS was supplemented with 25 mM Mops [3-(N-morpholino)propane sulfonate] ("HBSS-Mops"). For MØ cultures, RPMI was supplemented with 2 g/l sodium bicarbonate, 10% FCS, 20 mM Hepes, and 20 µg/ml gentamycin (complete medium).

**Chemicals.** Nocodazole (methyl[5-(2-thienylcarbonyl)-1H-benzimidazol-2-y]carbamate), monensin and cytochalasin D were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions of nocodazole (at 1 mM) and of cytochalasin D (at 4 mg/ml) were prepared in dimethylsulfoxide. Monensin was dissolved at 5 mM in ethanol. All stock solutions were stored at -20°C.

**Particles.** Dehydrated baker's yeast (Saccharomyces cerevisiae) and zymosan A were from Sigma Chemical Co. Polystyrene carboxylated beads (2.02 µm) were from Dow Diagnostics (Indianapolis, IN), and polystyrene noncarboxylated beads (1.06 and 3.0 µm) from Sigma Chemical Co. Beads were washed twice (10,000 g, 10 min), resuspended in 5% glucose, and added to the culture medium. Yeast particles were suspended in PBS, autoclaved, washed four times with sterile PBS, resuspended to 1.5 x 10^8 particles/ml, and stored at -20°C. β-glucan particles were prepared from baker's yeast which was autoclaved for 45 min in 1.5 N NaOH, washed, successively extracted with water, 3% acetic acid, water, ethanol, acetone, and dried (12). Glutaraldehyde-fixed sheep and mouse red blood cells (GRC) were prepared as described (13).

**Animals.** Male BALB/c mice, 20–25 g body wt, provided by the Animal Facility of the Pasteur Institute, were used to maintain the infection and as a source of MØ.

**Parasites.** Amastigotes of L. amazonensis LV79 (strain designation MPRO/BR/72/M1841) were obtained from the foot pads of male BALB/c mice as described (14), and suspended in RPMI supplemented with 5% FCS, 50 µg/ml gentamycin, and buffered with 25 mM Mops to pH 7.3. Parasites were kept at 34°C in an air atmosphere and used within 24 h.

**Preparation and Infection of MØ Cultures.** Elicited MØ were obtained from mice 3–5 d after intraperitoneal injection of 1.5 ml of 1% hydroxylated starch (Connaught Diagnostics, Toronto, Canada) in PBS. Cell suspensions in PBS (10^6 MØ) were allowed to adhere onto 12-mm-diameter coverslips for 10–20 min at room temperature. Coverslips were rinsed with HBSS and incubated in complete medium (37°C, 5% CO₂ in air) in 24-well plates. 24 h later, MØ were infected with 2–3 amastigotes per cell and kept in the same medium for 48 h (34°C, 5% CO₂ in air). For electron microscopy, cell suspensions (2 x 10^6 MØ per dish) were allowed to adhere to 35-mm diameter tissue culture plates.

**Pulse-chase Particle Transfer Assay.** Infected MØ cultures on coverslips were washed twice with HBSS-Mops and 0.4 ml HBSS-Mops-10% FCS added per well. Z, β-glucan, or killed yeast particles suspended in 0.1 ml of HBSS-Mops-10% FCS were added per well for 15–60 min at 34°C at multiplicities of 7–30 particles per MØ. Latex beads and GRC were added, respectively, at 50 µg/ml and 25 GRC/MØ. At the end of the pulse, cultures were twice washed to remove noningested particles and chased in complete medium (34°C, 5% CO₂ in air) for the desired time period. When indicated, FCS was omitted from the medium.

Results

**General Observations.** In most experiments Leishmania-infected MØ were pulsed for 1 h at 34°C with Z particles, and washed and chased in complete medium for different time periods before fixation. Fig. 1. A and B show that Z particles could be detected by either phase contrast- or differential interference microscopy within the PV of the infected MØ. This conclusion was supported by transmission electron microscopy (Fig. 2). Fig. 2 A displays an infected MØ containing phagocytized Z particles that have not been transferred to the PV. The absence of unoccupied vesicular space suggests that vesicle membranes were tightly apposed to the particles. In addition, regions of close contact were found between Z-containing vesicles and the PV (arrowheads). Fig. 2 B shows two Z which appear to be free within a PV. Membranes separating the particles from the parasites could not be identified. A vesicular and amorphous material was commonly found within the PV of infected MØ (Fig. 2, A and B).

**Selectivity of Particle Transfer.** Heat-killed yeast and β-glucan particles were also transferred to PV. Heat-killed yeasts were larger, more refractile than Z particles, and often presented a distinct cell wall. Once within MØ, their refractility was reduced, presumably by lysosomal degradation (Fig. 1 C). β-glucan particles were smaller, less refractile than Z, and tended to form aggregates within PV (Fig. 1, D and E). In contrast, and confirming earlier preliminary results (10),...
Relationship of Transfer to Cell Size and Shape. Transfer of Z was more commonly found in smaller cells, which contained a single large PV surrounded by a rim of cytoplasm, and ingested few particles (Fig. 1, A and B). Transfer was less frequent in larger, elongated, or polygonal cells which took up more particles and displayed several PV. The reason(s) for these differences need further study, but one explanation is that the path from pseudopod to PV is shorter in the smaller than in the larger cells.

Relationship of Z Transfer to PV Size. Transfer of Z to small vacuoles, defined as those with a diameter less than the length...
Zymosan Transfer to Leishmania Parasitophorous Vacuoles in Macrophages
of two amastigotes, was rarely found. It was intermediate in medium-sized vacuoles, of diameter equivalent to two to five amastigotes (Fig. 1, C–E), and most common in large vacuoles (diameter greater than five amastigotes, Fig. 1, A and B).

**Time Course.** For long-term kinetics, infected cultures were pulsed with 6 Z particles per cell, washed and chased for different time periods. Under these conditions, each Mϕ took up an average of three to four particles, and little transfer to PV was detected at the end of the pulse. Thereafter, transfer increased linearly for up to 24 h, when, in the experiment shown, about 35% of the Mϕ contained one or more Z in at least one PV (Fig. 3). Higher numbers of Z per cell (30:1) were used to evaluate the early kinetics of transfer. Fig. 4 A summarizes such an experiment in which infected Mϕ cultures were fixed at different times after coincubation with Z particles. Transfer was rare after 15 min and reached 16% of the Mϕ after 1 h. During this time, the number of Z particles taken up by the Mϕ with transfer increased from 2.5 at 15 min to about 12 at 60 min (Fig. 4 B). In the same experiment, at each time period parallel cultures were rinsed free medium before fixation. The percentage of Mϕ containing at least one Z in one PV (□) and the total number of Z transferred per 100 Mϕ (△) were determined as in Materials and Methods. In this and in the following figures, each point gives the average of three coverslips. At least 200 cells were scored per coverslip and SE are indicated by vertical lines.

**Transfer at Different Z Loads.** Infected Mϕ were pulsed for 1 h with an estimated multiplicity of 2.5, 5.0, or 10 Z per cell, and washed and fixed after 4 h chase. These particle inputs resulted in average cell uptakes of 2.1, 3.4, and 6 Z in the Mϕ with transfer (Fig. 5 A). Fig. 5 B shows that the percentage of Mϕ with transfer was linearly related to the numbers of Z taken up per phagocyte. However, as shown in Fig. 5 C, the proportion of Z transferred, a measure of the efficiency of the process, decreased as particle loads increased.

**Temperature Requirement.** In the previous experiments, both pulse and chase were performed at 34°C. To examine temperature dependence of transfer, Mϕ were loaded for 45 min at 20°C, (a temperature at which particle uptake does occur, albeit less effectively), rinsed, and either fixed immediately or chased at 20 or 34°C for 2 or 4 h. In a typical experiment with 20 Z/Mϕ, no transfer was detected at the end of the pulse. After periods of chase of 2 and 4 h at 20°C, the percentages of Mϕ with transfer were, respectively, 4.8 ± 0.7 and 6.0 ± 1.1 (mean ± SE of triplicate coverslips). At 34°C, the corresponding figures were, respectively, 8.8 ± 1.7 and 19.2 ± 1.8. Thus, although some transfer was obtained at 20°C, transfer was clearly higher at 34°C.

**Effect of Agents Known to Induce Vacuolar Albinization.** The percentage of Mϕ with transfer was increased by monensin, ammonium chloride, and chloroquine (CQ) (Figs. 6–8). These agents, particularly CQ, increased the size of PV and induced Mϕ vacuolization. The latter was more noticeable in noninfected than in infected cells. Monensin and ammonium chloride did not increase the numbers of Z transferred per Mϕ, and did not appear to damage intracellular amastigotes. In contrast, CQ increased the number of Z transferred per Mϕ and killed many parasites when used at a concentration of 50 μM for 2 or 4 h. To examine if the effect of CQ continues after the drug is removed from the medium, the following
Figure 6. Effect of monensin on the transfer of Z to the PV. MØ cultures pulsed with 6 Z/MØ, washed and chased for 60 min in the presence of the concentrations of monensin indicated. Values corrected for transfer of Z in cultures fixed at the end of the pulse.

Figure 7. Effect of ammonium chloride on Z transfer to PV. MØ cultures pulsed with 7 Z/cell and chased with different concentrations of NH₄Cl for the time periods indicated. Values corrected as Fig. 6.

Figure 8. Effect of CQ on the transfer of Z to PV. MØ cultures pulsed with 7 Z/cell for 45 min, chased for 15 min to allow for internalization of bound particles, and incubated for the times indicated with different concentrations of CQ. Values corrected as in Fig. 6.

Figure 9. Effect of removal of CQ from the medium on the transfer of Z to PV. MØ cultures pulsed for 45 min with 15 Z/cell were fixed after a 2- or 4-h chase in the presence of 20 μM CQ (-- , ○) or in plain medium (O). Other cultures were chased for 2 h in the presence of CQ washed and chased again for 2 h in plain medium (-----). SE were close in size to the symbols.

Figure 10. Effect of cytochalasin D on the transfer of Z particles to PV. MØ pulsed with 15 Z/cell, and chased for 4 h in complete medium alone, or containing the indicated concentrations of cytochalasin D.

experiment was performed. MØ cultures were pulsed with Z particles and chased for 2 or 4 h in medium alone or with the addition of 20 μM CQ. Other cultures were incubated with CQ for 2 h, and washed and chased for another 2 h in the absence of the drug. The results, summarized in Fig. 9, show that removal of the drug reduced the rate of transfer of Z into the PV.

Requirement for Actin Polymerization. Transfer was reproducibly inhibited by cytochalasin D given during chase (Fig. 10), suggesting that actin polymerization is directly or indirectly required for the translational movement of Z before they reach the PV. In contrast, transfer of Z was unaffected by treatment with the antitubulin nocodazole which was given at 1 μM for 18 h and was continued throughout the pulse and chase periods.

Discussion

We have confirmed an earlier incidental observation that Z particles can be transferred to the PV of Leishmania-infected MØ (9). We have also established a light microscopic procedure to quantify the transfer, and delineated several features and requirements of the process.

Transfer Assays. In most experiments, infected MØ cultures were pulsed with Z particles for 60 min, washed, chased for different time periods in particle-free medium, and fixed for microscopic counts. This design permits the separation of phagocytosis, which takes place during the pulse, from particle transfer to PV, which mainly occurs during the chase period. However, since transfer requires that donor vesicles and recipient PV be close to each other, translational movement of the former should be a necessary component of the overall process. This consideration is relevant to the interpretation of several of the experiments reported here. For instance, the kinetics of transfer (Figs. 3 and 4) may not mainly reflect the time needed for actual particle transfer, but rather that required for translocation of donor vesicles. Similarly, the inhibition by cytochalasin D of the transfer of Z to PV (Fig. 10) may be explained by the involvement of an actin motor in the translocation of Z vesicles, rather than in the Z transfer event (16, 17).

Particle Selectivity. The pioneering studies of Oates and Touster (18, 19) on Acanthamoeba demonstrated and quantified in intact cells and in cell homogenates the nonselective fusion of particle-containing phagocytic vesicles. In the present experiments, transfer of the same particulate markers to MØ PV was apparently selective, since yeast-derived particles were transferred, whereas latex beads or aldehyde-fixed erythrocytes were not. The mechanisms that account for this selectivity are not understood. Yeast-related particles display a high density of carbohydrate ligands, and thus can be expected to induce receptor aggregation, known to be important in the transfer of molecular or particulate ligands to lysosomes (20). Furthermore, the relative resistance of yeast polysaccharides to lysosomal degradation may extend the life of the ligand-receptor complexes. A second possibility is that transfer is dictated by the nature of the receptors that recognize the particles. Different receptors may account for the direct recognition of yeast particles by MØ. They are the mannose-fucose receptors (21, 22), the lectin-binding site of the α chain of CR3 (CD11b-CD18) (23), and the β-glucan receptor (24, 25). It is possible that the cytosolic tails of one or more of these receptors contain signals that trigger the interaction of donor vesicles with PV (26).

Lastly, selectivity of transfer may depend on different rates of progression of the particles along the endocytic pathway, i.e., whether particles are contained in phagosomes, in phagolysosomes, or in more complex organelles (27). Although Z, as well as reduced and alkylated, nonopsonized or opsonized yeast particles are competent inducers of phagosome lysosome fusion (28, 29), MØ infected with L. amazonensis are depleted of cytochemically...
or ultrastructurally identified lysosomes (30). It is thus possible that the kinetics of phagosome-lysosome fusion may be slower in infected than in control \( \text{MO} \). This could make more phagosomes available for interaction with PV.

**Mechanisms of Transfer.** We have as yet no information on the mechanisms of delivery of yeast-derived particles to PV. Such delivery may involve fusion of PV with the donor vesicles, but a mechanism analogous to phagocytosis, involving attachment and invagination of donor vesicles into PV cannot be excluded. Indeed, since internalized membranes could be digested during the chase periods, both mechanisms could result in similar ultrastructural features (18). The second mechanism is compatible with earlier cinematographic observations that the large PV are easily deformable (11), and with the present finding that Z particles were more frequently transferred to large than to small PV. Furthermore, light microscopic observations of “herniation” of Z particles into PV, and of, albeit infrequent, “mesentery-like” phase dense connections between the Z particles and the inner face of the PV, may be best explained by a vesicle invagination mechanism (P. Veras and M. Rabinovitch, work in progress).

**Enhancement of Z Transfer by Compounds Known to Increase the pH of Acidified Organelles.** The ionophore monensin, and the weak bases NH\(_4\)Cl and CQ enhanced in time- and concentration-dependent fashion the transfer of Z to PV (Figs. 6–9). Ionophores and acidotropic compounds have been shown to reduce both the intracellular dissociation of receptor–ligand complexes, and the recycling of certain receptors to the plasma membrane, and to inhibit the delivery of the complexes to lysosomes (31). It is thus possible that alkalinization increased the number of receptors associated with the donor vesicles, and therefore the postulated signals associated with the cytosolic tails. The possibility that vacuolar swelling alone promoted or enhanced the interaction of PV with donor vesicles is not compatible with the observations that: (a) CQ did not induce the transfer of latex beads or of aldehyde-treated erythrocytes to PV (not shown); (b) addition of 50 mM sucrose 24 h before and during the chase period induced \( \text{MO} \) vacuolization, but did not enhance transfer of Z to PV (not shown); and (c) studies in progress have shown that D-Leu-O\(_{\text{Me}}\), an amino acid ester which is only slowly hydrolyzed in lysosomes and does not induce detectable vacuolization of macrophages, markedly enhanced the transfer of Z particles to PV.

It was recently reported that ammonium chloride, which suppresses phagosome-lysosome fusion, increased the fusion of endosomes containing Lucifer yellow or cationized ferritin, to yeast-containing phagosomes of \( \text{MO} \) (32). Culture conditions were different from those reported here and live yeasts were used as test particles. These findings raise the possibility that in our experiments NH\(_4\)Cl induced the accumulation of Z-containing phagosomes particularly prone to fuse with PV. However, in Hart and Young’s experiments, CQ did not increase the fusion of yeast phagosomes with endosomes.

Recent studies documented phagosome-endosome fusion in vivo and in isolated vesicle preparations (33) and endosome–lysosome interactions have been reconstituted in a cell-free system (34). Several requirements for phagosome-endosome fusion such as for ATP, GTP, cytosolic factors, and GTP binding proteins, have now been defined (35, 36). The transfer of particles to the *Leishmania* PV in \( \text{MO} \) offers a model which may permit the study of the mechanisms involved in the movement and interactions of large endocytic vesicles, and of their modulation by host-cell or parasite-derived signals, as well as by exogenous effector molecules and pharmacological agents.

---

Eduardo Cesar Santos Lima, then a medical student at the University of Bahia (Salvador, BA, Brazil), participated in the first light microscopic studies in December 1990.

P. Veras is the recipient of a fellowship from Conselho Nacional de Pesquisas, sponsored by the Graduate Program of the Instituto Oswaldo Cruz (Rio de Janeiro, Brazil). The authors thank Mr. J. C. Benichou and Mr. J. H. Panaud of the Pasteur Institute for printing the photographs. This work was supported by the Centre National de la Recherche Scientifique, Institut Pasteur, and by the Commission of the European Communities Program “Science and Technology for Development” (Contract TR2-0110-F). C. D. Chastellier is supported by INSERM (CJF 90-04).

Address correspondence to Dr. M. Rabinovitch, Unité d’Immunoparasitologie, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France.

Received for publication 13 April 1992.

**References**

1. Chang, K.P., G. Chaudhuri, and D. Fong. 1990. Molecular determinants of *Leishmania* virulence. *Annu. Rev. Microbiol.* 44:499.

2. Russell, D.G., E. Medina-Acosta, and A. Golubev. 1991. The interface between the *Leishmania*-infected macrophage and the host’s immune system. *Behring Inst. Mitt.* 80:68.

3. Antoine, J.C., E. Prina, C. Jouanne, and P. Bongrand. 1990.

4. Prina, E., J.C. Antoine, B. Wiederanders, and H. Kirschke. 1990. Localization and activity of various lysosomal proteases in *Leishmania amazonensis*-infected macrophages. *Infect. Immun.* 58:779.

5. Berman, J.D., T.B. Fioretti, and D.M. Dwyer. 1981. *In vivo*...
10. Dedet, J.P., E. Brunet, G. Topper, and M. Rabinovitch. 1981. Receptor-mediated entry of β-glucuronidase into the parasitophorous vacuoles of macrophages infected with Leishmania mexicana amazonensis. J. Exp. Med. 157:1471.

7. Rabinovitch, M., G. Topper, P. Cristello, and A. Rich. 1985. Receptor-mediated entry of peroxidases into the parasitophorous vacuoles of macrophages infected with Leishmania mexicana amazonensis. J. Leukocyte Biol. 37:247.

8. Antoine, J.C., C. Jouanne, T. Lang, E. Prina, C. de Chastellier, and C. Freheli. 1991. Localization of major histocompatibility complex class II molecules in phagolysosomes of murine macrophages infected with Leishmania amazonensis. Infect. Immun. 59:764.

9. Rabinovitch, M., V.L. Shepherd, and P.D. Stahl. 1985. Secretion of lysosomal enzymes by control- and Leishmania-infected macrophage. Fed. Proc. 44:7549 (Abstr.)

10. Dedet, J.P., E. Brunet, G. Topper, and M. Rabinovitch. 1981. Localization of exogenous markers in the parasitophorous vacuoles of macrophages infected with Leishmania mexicana amazonensis. J. Cell Biol. 91:2424 (Abstr.)

11. Rabinovitch, M., J.P. Dedet, A. Ryter, R. Robineux, G. Topper, and E. Brunet. 1982. Destruction of Leishmania mexicana amazonensis within macrophages in culture by phenazine methosulfate and other electron carriers. J. Exp. Med. 155:415.

12. Czop, J.K., M.F. Gurish, and J.L. Kadish. 1990. Production and isolation of rabbit anti-idiotypic antibodies directed against the human monocyte receptor for yeast β-glucans. J. Immunol. 145:995.

13. Rabinovitch, M. 1967. The dissociation of the attachment and ingestion phases of phagocytosis by macrophages. Exp. Cell. Res. 46:19.

14. Rabinovitch, M., V. Zilberfarb, and C. Ramazellines. 1986. Destruction of Leishmania mexicana amazonensis by lysosomotropic amino acid esters. J. Exp. Med. 163:520.

15. Swanson, J. 1989. Fluorescent labeling of endocytic compartments. Meth. Cell Biol. 29:137.

16. Toyohara, A., and K. Inaba. 1989. Transport of phagosomes in mouse peritoneal macrophages. J. Cell Sci. 94:143.

17. Vasselon, T., J. Mounier, M.C. Prevost, R. Hellyo, and P. Santonetti. 1991. Stress fiber-based movement of Shigella flexneri within cells. Infect. Immun. 59:1723.

18. Oates, P.J., and O. Touster. 1976. In vitro fusion of Acanthamoeba phagolysosomes. I. Demonstration and quantitation of vacuole fusion in Acanthamoeba homogenates. J. Cell Biol. 68:319.

19. Oates, P.J., and O. Touster. 1978. In vitro fusion of Acanthamoeba phagolysosomes. II. Quantitative characterization of in vitro vacuole fusion by improved electron microscope and new light microscope techniques. J. Cell Biol. 79:217.

20. Ukkonen, P., V. Lewis, M. Marsh, A. Helenius, and I. Mellman. 1986. Transport of macrophage Fc receptors and Fc receptor-bound ligands to lysosomes. J. Exp. Med. 163:952.

21. Sung, S.J., R.S. Nelson, and S.C. Silverstein. 1983. Yeast mannans inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages. J. Cell Biol. 96:160.

22. Taylor, M.E., J.T. Conary, M.R. Lennartz, P.D. Stahl, and K. Drickamer. 1990. Primary structure of the mannose receptor contains multiple motifs resembling carbohydrate-recognition domains. J. Biol. Chem. 265:12156.

23. Ross, G.D., J.A. Cain, B.L. Myones, S.L. Newman, and P.J. Lachman. 1987. Specificity of membrane complement receptor type three (CR3) for β-glucans. Complement. 4:61.

24. Goldman, R. 1988. Characteristics of the β-glucan receptor of murine macrophages. Exp. Cell Res. 174:481.

25. Czop, J.K., and J. Kay. 1991. Isolation and characterization of β-glucan receptors on human mononuclear phagocyte. J. Exp. Med. 173:1511.

26. Joiner, K.A., S.A. Fuhrman, H.M. Miettinen, L.H. Kasper, and I. Mellman. 1990. Toxoplasma gondii: fusion competence of parasitophorous vacuoles in Fc receptor-transfected fibroblasts. Science (Wash. DC). 249:641.

27. Rabinowitz, S., H. Horstmann, S. Gordon, and G. Griffiths. 1992. Immunocytochemical characterization of the endocytic and phagolysosomal compartments in peritoneal macrophages. J. Cell Biol. 117:95.

28. Coln, Z.A., and E. Wiener. 1963. The particulate hydrolases of macrophages. II. Biochemical and morphological response to particle ingestion. J. Exp. Med. 118:1009.

29. Kielian, M.C., and Z.A. Coln. 1980. Phagosome-lysosome fusion. Characterization of intracellular membrane fusion in mouse macrophages. J. Cell Biol. 85:754.

30. Barbieri, C.L., K. Brown, and M. Rabinovitch. 1985. Deposition of secondary lysosomes in mouse macrophages infected with Leishmania mexicana amazonensis: a cytochemical study. Z. Parasitenk De. 71:159.

31. Mellman, I., R. Fuchs, and A. Helenius. 1986. Acidification of the endocytic and exocytic pathways. Annu. Rev. Biochem. 55:663.

32. Hart, P. D'Arcy, and M.R. Young. 1991. Ammonium chloride, an inhibitor of phagosome-lysosome fusion in macrophages, concurrently induces phagosome-endosome fusion, and opens a novel pathway: studies of a pathogenic Mycobacterium and a nonpathogenic yeast. J. Exp. Med. 174:881.

33. Mayorga, L.S., F. Bertini, and P.D. Stahl. 1991. Fusion of newly formed phagosomes with endosomes in intact cells and in a cell-free system. J. Biol. Chem. 266:6511.

34. Wilson, D.W., S.W. Whiteheart, L. Orci, and J.E. Rothman. 1991. Intracellular membrane fusion. Trends Biochem. Sci. 16:334.

35. Pitt, A., L.S. Mayorga, A.L. Schwartz, and P.D. Stahl. 1992. Transport of phagosomes components to an endosomal compartment. J. Biol. Chem. 267:126.