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Invasion of *Toxoplasma gondii* occurs by active penetration of the host cell

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SUMMARY

***Toxoplasma gondii* is an obligate intracellular parasite that infects a wide variety of vertebrate cells including macrophages. We have used a combination of video microscopy and fluorescence localization to examine the entry of *Toxoplasma* into macrophages and nonphagocytic host cells. *Toxoplasma* actively invaded host cells without inducing host cell membrane ruffling, actin microfilament reorganization, or tyrosine phosphorylation of host proteins. Invasion occurred rapidly and within 25-40 seconds the parasite penetrated into a tight-fitting vacuole formed by invagination of the plasma membrane. In contrast, during phagocytosis of *Toxoplasma*, extensive membrane ruffling captured the parasite in a loose-fitting phagosome that formed over a period of 2-4 minutes. Phagocytosis involved both reorganization of the host cytoskeleton and tyrosine phosphorylation of host proteins. In some cases, parasites that were first internalized by phagocytosis, were able to escape from the phagosome by a process analogous to invasion. These studies reveal that active penetration of the host cell by *Toxoplasma* is fundamentally different from phagocytosis or induced endocytic uptake. The novel ability to penetrate the host cell likely contributes to the capability of *Toxoplasma*-containing vacuoles to avoid endocytic processing.**

Key words: parasite, invasion, phagocytosis, endocytosis

INTRODUCTION

Toxoplasma is a protozoan parasite with an extremely broad host range (Dubey and Beattie, 1988) and unique capacity to invade virtually all nucleated cell types from warm-blooded vertebrate hosts (Werk, 1985). Early cinematographic studies indicate that invasion proceeds rapidly and that during internalization the parasite passes through a visible constriction at the host cell plasma membrane (Hirai et al., 1966; Bommer, 1969; Nguyen and Stadtsbaeder, 1979). Electron microscopy studies have revealed that invasion is an ordered process that initiates with binding of the parasite at its apical end followed by invagination of the host cell membrane to form a vacuole surrounding the parasite (Jones et al., 1972; Aikawa et al., 1977; Nichols and O'Connor, 1981). Despite the efficiency with which *Toxoplasma* enters its host cell, it remains unresolved if invasion occurs by direct penetration of the parasite or by induction of a host-mediated endocytic event.

Toxoplasma is also able to survive in professional phagocytes where it resides within a specialized compartment called the parasitophorous vacuole (PV) that avoids acidification (Sibley et al., 1985b), and lysosome fusion (Jones and Hirsch, 1972). Despite the ability of *Toxoplasma* to efficiently survive in a wide range of cell types, this delicate balance is tipped in favor of the phagocyte when parasites are opsonized with specific antibody. Phagocytosis of antibody-coated parasites by mouse macrophages leads to an elevated respiratory burst (Wilson et al., 1980), rapid acidification (Sibley et al., 1985a), and fusion with endosomes/lysosomes (Sibley et al., 1985a). Internalization of antibody-coated *Toxoplasma* also leads to lysosome fusion in CHO cells transfected with FcRII receptors by a process that it mediated by the FcR cytoplasmic tail (Joiner et al., 1990). The diametrically opposed fates of *Toxoplasma* parasites that are phagocytosed versus those which invade actively, suggests that their respective fates are largely determined by a critical difference at the time of internalization (Jones and Hirsch, 1972; Sibley et al., 1985b; Joiner et al., 1990).

A number of bacterial pathogens gain entry into vertebrate cells by exploiting a normal host cell process for internalization (Falkow et al., 1992). *Salmonella typimurium* induces a process similar to macropinocytosis, thereby enabling the bacterium to enter cells which are normally not phagocytic, such as epithelial cells (Francis et al., 1993; Alpuche-Aranda et al., 1994). To determine if a similar process of induced endocytic uptake might account for the ability of *Toxoplasma* to enter cells, we have used time-lapse video microscopy to examine the entry of *Toxoplasma* into phagocytic and nonphagocytic host cells. These studies reveal important functional differences between phagocytosis and *Toxoplasma* invasion and suggest that intracellular survival is dependent on the active penetration of the host cell by the parasite.

MATERIALS AND METHODS

Reagents

Cell culture reagents were obtained from Gibco BRL (Gaithersburg,

MD) except for FCS which was obtained from Hyclone (Logan, UT). Chemicals were obtained from Sigma (St Louis, MO) unless otherwise noted.

Parasite and cell cultures

RH strain *Toxoplasma* tachyzoites were propagated by serial passage in human fibroblasts (HF) monolayers. HF and CHO cells were cultured in 5% CO_2 at 37°C in DMEM containing 10% FCS, 2 mM glutamine, and 20 μg/ml gentamicin. Bone marrow-derived macrophages (BM) were obtained from the femurs of Balb/c mice and cultured in DMEM supplemented with 10% FCS and 20% conditioned L929 cell medium. BM cells were used in the first or second passage after reaching confluence.

Parasites were isolated from freshly lysed monolayers, separated using 3.0 micron polycarbonate filters, and washed in HBSS containing 1 mM EGTA and 10 mM HEPES. For opsonization experiments, parasites were incubated at room temperature for 30 minutes with monoclonal antibody (mAb) DG52 or rabbit polyclonal antisera to p30 (RPcαp30) followed by extensive washing in PBS.

In vitro invasion

Unlike receptor mediated phagocytosis, unopsonized *Toxoplasma* cells do not bind to host cells at temperatures below 20°C, consequently all invasion and phagocytosis experiments were conducted at 37°C. For video microscopy, monolayers cultured on coverslips were mounted in Ringer's buffer containing 1% BSA on glass slides supported by lateral strips of vacuum grease (so called Zigmond chambers). Freshly isolated *Toxoplasma* parasites were used to infect these monolayers by infusing Ringer's buffer containing parasites across the coverslip. The slide was incubated upside down at room temperature for 5 minutes to allow the parasites to settle on the monolayer and then re-righted and mounted on the microscope stage which was prewarmed to 37°C. Alternatively, monolayers grown on coverslips were infected at a low multiplicity of infection for 1 hour, washed, and recultured at 37°C overnight in DMEM 10% FCS. Following 18-36 hours of culture, coverslips were mounted on glass slides and examined for lysis of infected cells and invasion of new host cells within the same monolayer.

Time-lapse video microscopy and image analysis

Zigmond chamber slides were maintained at 37°C and examined by phase contrast microscopy using an upright microscope equipped with a 63× NA 1.4 objective lens. Active invasion was strongly inhibited when examined in bright transmitted light. For time-lapse recording, the microscope was equipped with a low-light level silicon-intensified video camera (model C2400, Hamamatsu Photonics, Japan). Video images were averaged for 8 frames with an ARGUS-10 image processor (Hamamatsu) and stored at 1 frame/s on 12″ optical discs using a TQ-3038F recorder (Panasonic, Secaucus, NJ). Images were printed using a UP-870MD video printer (Sony Corp., Japan).

Fluorescence localization of actin and Y-PO_4 residues

Monolayers grown on LabTek chamber slides were infected with freshly isolated *Toxoplasma*, rinsed, and fixed in 2.5% formalin and 0.1% glutaraldehyde in PBS for 10 minutes followed by extraction in cold acetone for 5 minutes. To examine the distribution of actin-rich microfilaments, monolayers were stained with bodipy phalloidin according to manufacturers instructions (Molecular Probes, Eugene, OR). Immunolocalization of actin was also performed on fixed, extracted monolayers using the mAb C4 (Boehringer Mannheim Co., Indianapolis, IN) followed by FITC-conjugated goat anti-mouse IgG. The distribution of phosphotyrosine residues was detected using the mAb PY20 (ICN Biomedicals, Costa Mesa, CA) followed by staining with FITC-conjugated goat anti-mouse IgG. Fluorescently labeled cells were examined using a Axiovert 135 equipped for epifluorescence and Nomarski differential contrast and photographed with TMAX 400 film (Eastman Kodak Co. Rochester, NY).

RESULTS

Time-lapse video microscopy was used to examine the interaction of *Toxoplasma* with host cells in vitro and to analyze the mechanism(s) of cell entry. Internalization of *Toxoplasma* by BM cells in vitro occurred by two distinct processes that differed in kinetics, morphology, and intracellular fate of the vacuole. These two alternative forms of internalization are classified as 'active invasion' and 'phagocytosis' as described in detail below.

Active invasion of *Toxoplasma* into BM and CHO cells

When freshly lysed *Toxoplasma* parasites were added to monolayers of BM cells, many parasites remained loosely adherent to the cell surface without provoking a phagocytic response and without entering the host cell. When invasion did occur, it was typically preceded by helical rotation and gliding motility of the parasite across the substrate or cell surface

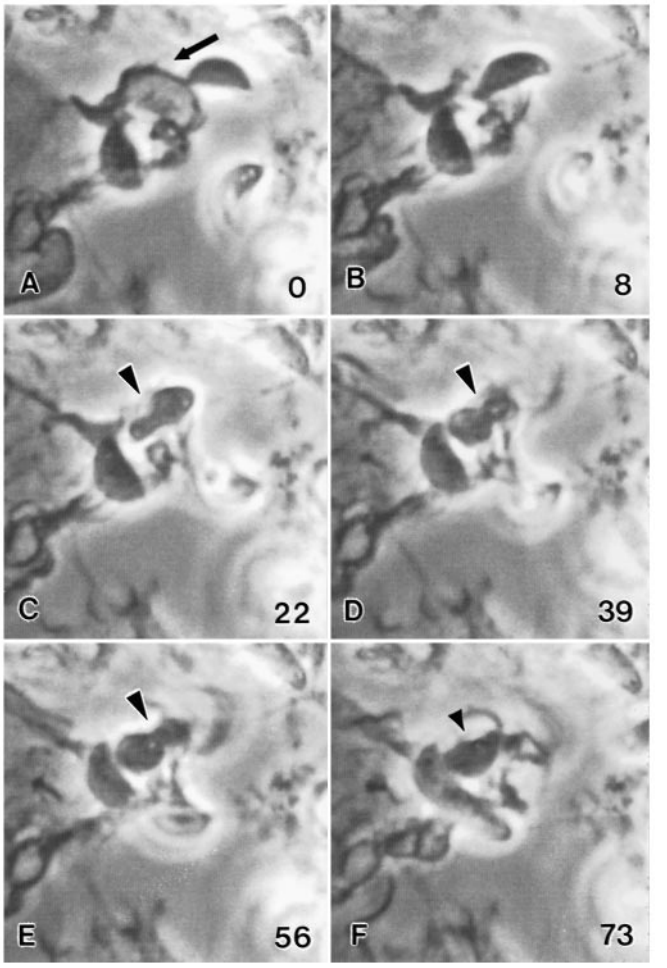


Fig. 1. Active invasion of *Toxoplasma* into BM cells recorded by time-lapse video microscopy. The parasite actively penetrates into a tight-fitting vacuole formed by invagination of the plasma membrane. Arrow in A indicates the direction of migration in preceding frames. Arrowheads (C-E) mark the prominent constriction of the parasite that accompanies invasion. The parasite resides within a tight-fitting vacuole within the cell (F, arrowhead). Elapsed time indicated in seconds in lower right corner.

(J. H. Morisaki, J. E. Heuser and L. D. Sibley, unpublished). Following initial contact at its apical end, the parasite rapidly penetrated the host cell, narrowing at the point of passage through the plasma membrane and entering a tight-fitting vacuole (Fig. 1). Disappearance of the normally phase-bright ring surrounding the parasite was used to differentiate extracellular from intracellular parasites. Invasion was not accompanied by membrane ruffling or other detectable reorganization of the host cell plasma membrane.

Active invasion of live *Toxoplasma* into CHO cells was also characterized by the rapid penetration of the parasite into the host cell without induction of membrane ruffling (Fig. 2). A noticeable constriction of the parasite cell at the junction of the host cell plasma membrane was again observed in most cases, and intracellular parasites ended up in tight-fitting vacuoles (Fig. 2). In both BM and CHO cells, adjacent parasite-containing vacuoles in the same cell remained segregated and did not undergo detectable fusion with each other or with other endocytic vesicles such as macropinosomes.

Phagocytosis of effete *Toxoplasma* by BM cells

When extracellular *Toxoplasma* were incubated for >2 hours in PBS at 37°C, their ability to actively invade host cells was greatly decreased. When these effete parasites were added to monolayers of BM cells, the majority (>90%) of parasites taken up were internalized by phagocytosis. Phagocytosis was characterized by extensive, localized membrane ruffling that captured the parasite in a loose-fitting compartment (Fig. 3). Macropinosomes were often formed in the same region of the cell as a consequence of extensive membrane ruffling. These large phase-bright compartments typically fused with the phagosome soon thereafter, further increasing its fluid volume (Fig. 3E,F). Adjacent parasite-containing phagosomes in the same cell were also observed to coalesce into a single large compartment (not shown). In the subsequent 30 minutes after formation, phagosomes characteristically moved to the cell center and the clear space around the parasite decreased in size, indicating a progressive reduction in the fluid volume.

Phagocytosis of antibody-coated *Toxoplasma* by BM cells

To examine the uptake of opsonized parasites, *Toxoplasma* cells were coated with antibodies specific to the major surface protein p30. *Toxoplasma* cells labeled with either mAb DG52 or RPcαp30 exhibited a uniform surface staining that was not capped during incubation with host cells, as verified by staining of fixed cells with secondary antisera conjugated to FITC (not shown). When live antibody-coated *Toxoplasma* were added to BM cells, they were internalized almost exclusively by phagocytosis as described above. Antibody-coated parasites were able to invade CHO cells normally and did so at the same frequency as uncoated parasites.

Redistribution of host-cell microfilaments during invasion versus phagocytosis

In order to directly examine the redistribution of the cytoskeleton during invasion versus phagocytosis, host actin microfilaments were stained with fluorescently conjugated phalloidin which does not bind to *Toxoplasma* under the condition used here. During phagocytosis of antibody-coated (DG52) parasites by BM cells, extensive reorganization of host cell microfilaments occurred, forming an actin-rich phagocytic cup around the entering parasite (Fig. 4A,B). In contrast, during invasion of live *Toxoplasma* into BM (Fig. 4C,D) or CHO (Fig. 5C,D) cells, there was no apparent reorganization of the host cell microfilaments. Antibody-coated (DG52) *Toxoplasma* were able to normally invade CHO cells and did so without causing reorganization of the host cell cytoskeleton (Fig 5A,B). Similar results were obtained using mAb C4 to localize actin by immunofluorescence labeling (not shown).

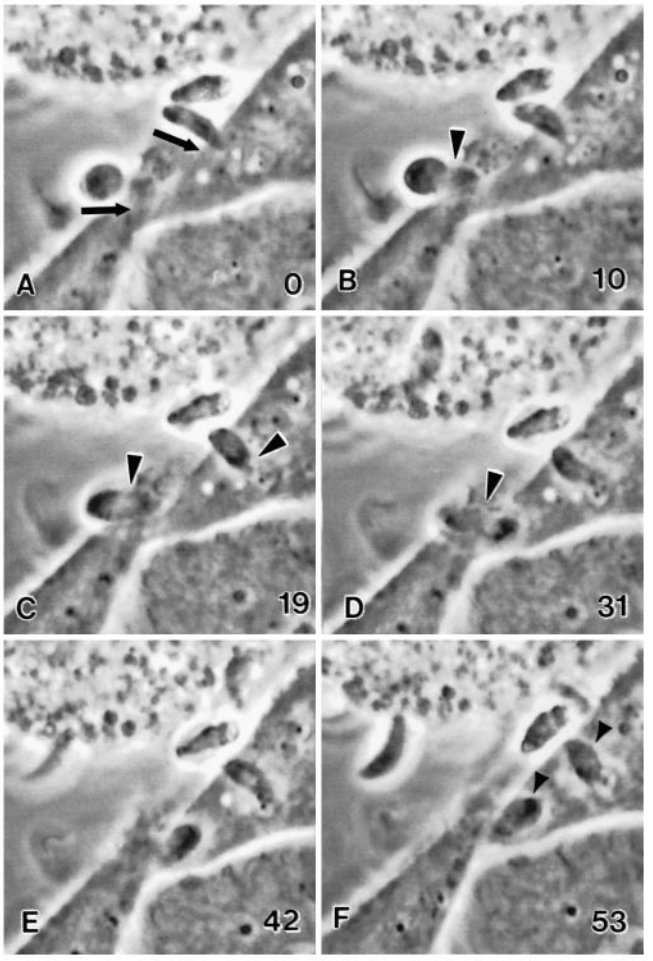


Fig. 2. Active invasion of *Toxoplasma* into CHO cells. Invasion is normally associated with a prominent constriction in the parasite at the site of invasion (arrowheads in B-D). Arrows in A indicate the direction of migration in preceding frames. Within the host cell, the parasites reside within tight-fitting vacuoles (F, arrowheads). Elapsed time indicated in seconds in lower right corner.

Localization of Y-PO residues during invasion vs phagocytosis

During phagocytosis of antibody-coated (RPcαp30) *Toxoplasma* by BM cells, extensive phosphorylation of tyrosine residues on host cell proteins was detected in the vicinity of the phagosome as revealed by staining with anti-Y-PO_4 mAb (Fig. 6A,B). In contrast, Y-PO_4 staining revealed no appreciable change in phosphorylation of tyrosine residues during invasion of normal *Toxoplasma* into BM cells (Fig. 6C,D). Invasion of both normal and antibody-coated (RPcαp30) *Toxoplasma* into CHO cells did not result in a detectable change

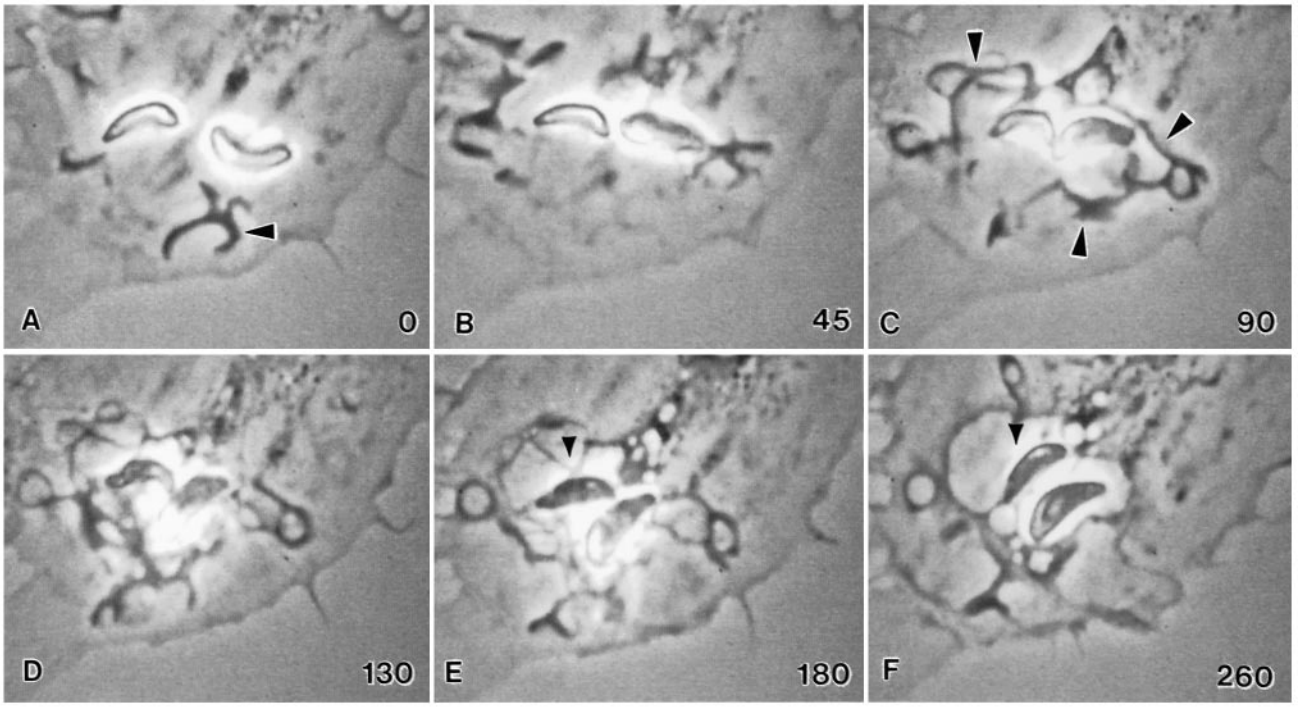
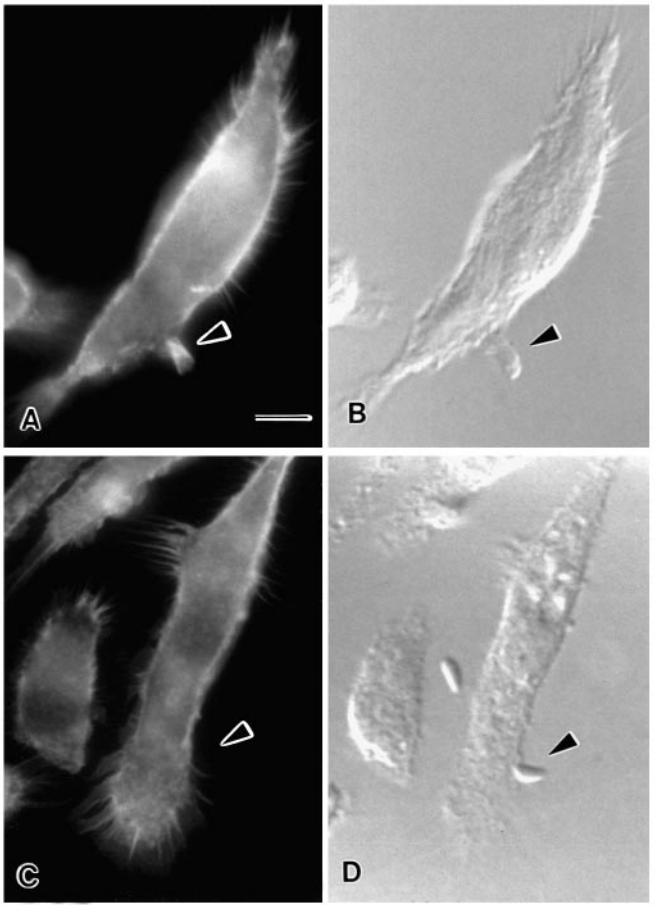


Fig. 3. Phagocytosis of *Toxoplasma* by BM cells. The parasites are engulfed by large membrane ruffles and internalized into large, fluid-filled compartments. Arrowheads in A and C mark host cell membrane ruffles. Macropinosomes and adjacent vacuoles often fuse into a single large compartment (arrowheads in E and F). Elapsed time indicated in seconds in lower right corner.



in phosphorylation of tyrosine residues at the site of cell penetration (not shown).

Phagosome escape and antibody-thwarted escape

Although the majority of live *Toxoplasma* parasites that entered BM cells did so by active invasion, approximately 10-20% were internalized by phagocytosis. The majority of these phagocytic compartments underwent subsequent fusion with other cytoplasmic vacuoles. However, in approximately 20% of these cases, the parasite was able to escape from the phagosome by a process that resembled invasion at the plasma membrane. Typically, the parasite completed this escape in a single step, leaving the loose-fitting phagosome and passing through a prominent constriction as it entered a second compartment where the membrane was tightly opposed to the parasite. The example shown in Fig. 7 depicts a double-escape event where the first attempt to exit the phagosome resulted in formation of another loose-fitting compartment (Fig. 7A-F). The parasite rapidly initiated a second escape, ultimately ending up in a tight-fitting vacuole (Fig. 7G-L). During both escape events, the parasite passes through a prominent constriction as it leaves the first vacuole and enters the second (arrows in Fig. 7D,I). During the

Fig. 4. Bodipy-phalloidin staining of actin-rich microfilaments during phagocytosis (A,B) versus invasion (C,D) of *Toxoplasma* into BM cells. During phagocytosis of antibody-coated parasites (A,B), host cell microfilaments are reorganized into an extensive meshwork around the forming phagosome. In contrast, during active invasion of uncoated parasites, no rearrangement of host cell microfilaments is detected. Arrowhead indicates presence of parasite in each frame. Bar, 5 μm.

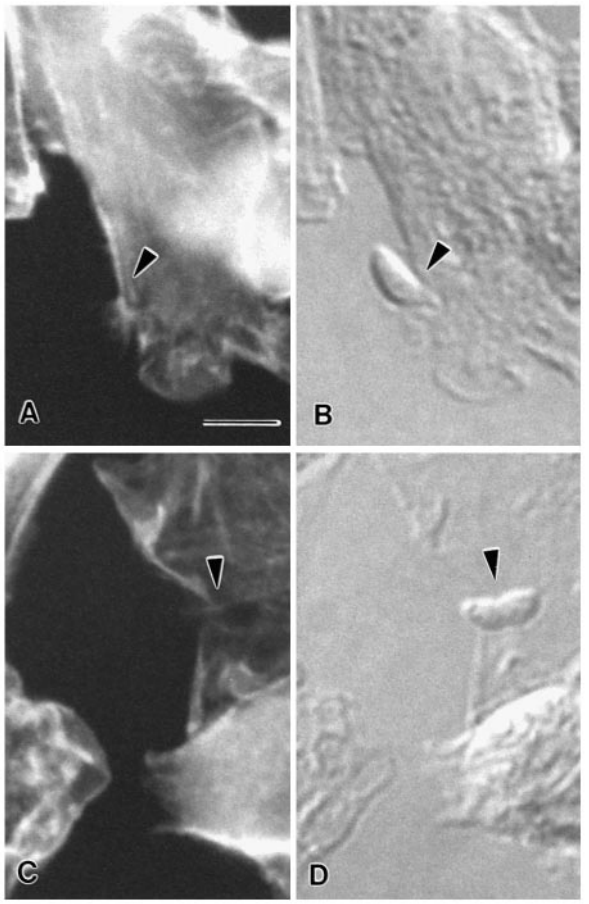


Fig. 5. Bodipy-phalloidin staining of actin-rich microfilaments in CHO cells during invasion of *Toxoplasma*. There is no detectable rearrangement of host-cell microfilaments during invasion of either antibody-coated (A,B) or uncoated parasites (C,D). Arrowhead indicates presence of parasite in each frame. Bar, 5 μm.

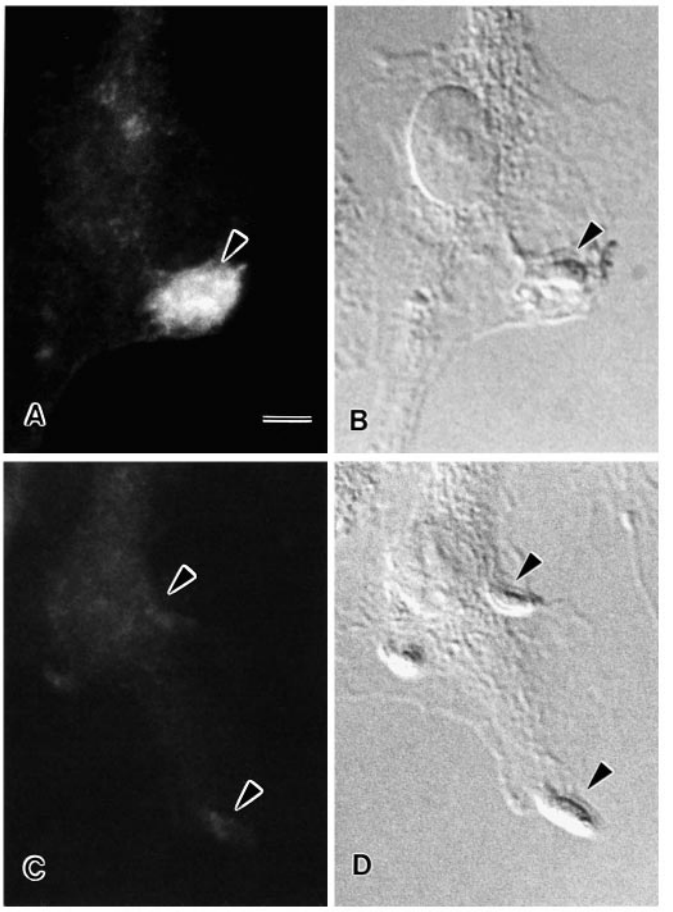


Fig. 6. Localization of phosphorylated tyrosine residues during phagocytosis versus invasion of *Toxoplasma* into BM cells. During phagocytosis of antibody-coated parasites (A,B), extensive phosphorylation of tyrosine residues on host cell proteins occurs in the region of the forming phagosome. In contrast, during invasion of uncoated parasites (C,D), there is no detectable increase in tyrosine phosphorylation. Arrowheads indicate presence of parasites in each frame. Bar, 5 μm.

formation of the second vacuole, the membrane of the first vacuole is consumed, leaving no visible remnant. When antibody-coated *Toxoplasma* cells were phagocytosed by BM cells, they also occasionally attempted to escape from the phagosome; however, these escape events were typically unsuccessful and left the parasite entrapped within the phagosome (not shown).

Kinetics of invasion, phagocytosis and escape

In addition to the profound morphological differences between active invasion and passive uptake by phagocytosis, these two processes occurred at very different rates. The average times for formation of phagocytic (loose-fitting) versus invasion (tight-fitting) vacuoles were analyzed from a series of separate events recorded by time-lapse video microscopy (Table 1). To establish the kinetics of uptake, events were scored beginning from the first indication of membrane ruffling or penetration until the parasite was fully internalized, as judged by the loss of a phase-bright halo. Invasion into both BM and CHO cells occurred 3-4 times more rapidly than phagocytosis. Phagocytosis of effete and antibody-coated parasites was similar and is therefore presented as a single category in Table 1. Phagosome escape mirrored the rapid kinetics of invasion.

DISCUSSION

The present studies reveal fundamental differences between *Toxoplasma* invasion and phagocytosis that are evident in the pattern of entry, the kinetics of vacuole formation, morphological and biochemical changes in the host cell, and the ultimate fate of the vacuole within the host cell.

Active invasion by *Toxoplasma*

Continuous recording by time-lapse video microscopy revealed that *Toxoplasma* invasion occurred approximately 3-4 times more rapidly than phagocytic uptake and did not involve host cell membrane ruffling or pseudopod formation. Consistent with this, host cell microfilaments were not reorganized and tyrosine phosphorylation of host proteins was not detected during *Toxoplasma* invasion. The absence of events normally associated with phagocytosis during *Toxoplasma* invasion suggests that the host cell plays a passive role in parasite entry.

In contrast to the active invasion of *Toxoplasma*, host cell entry by *Salmonella* is achieved by induction of an endocytic

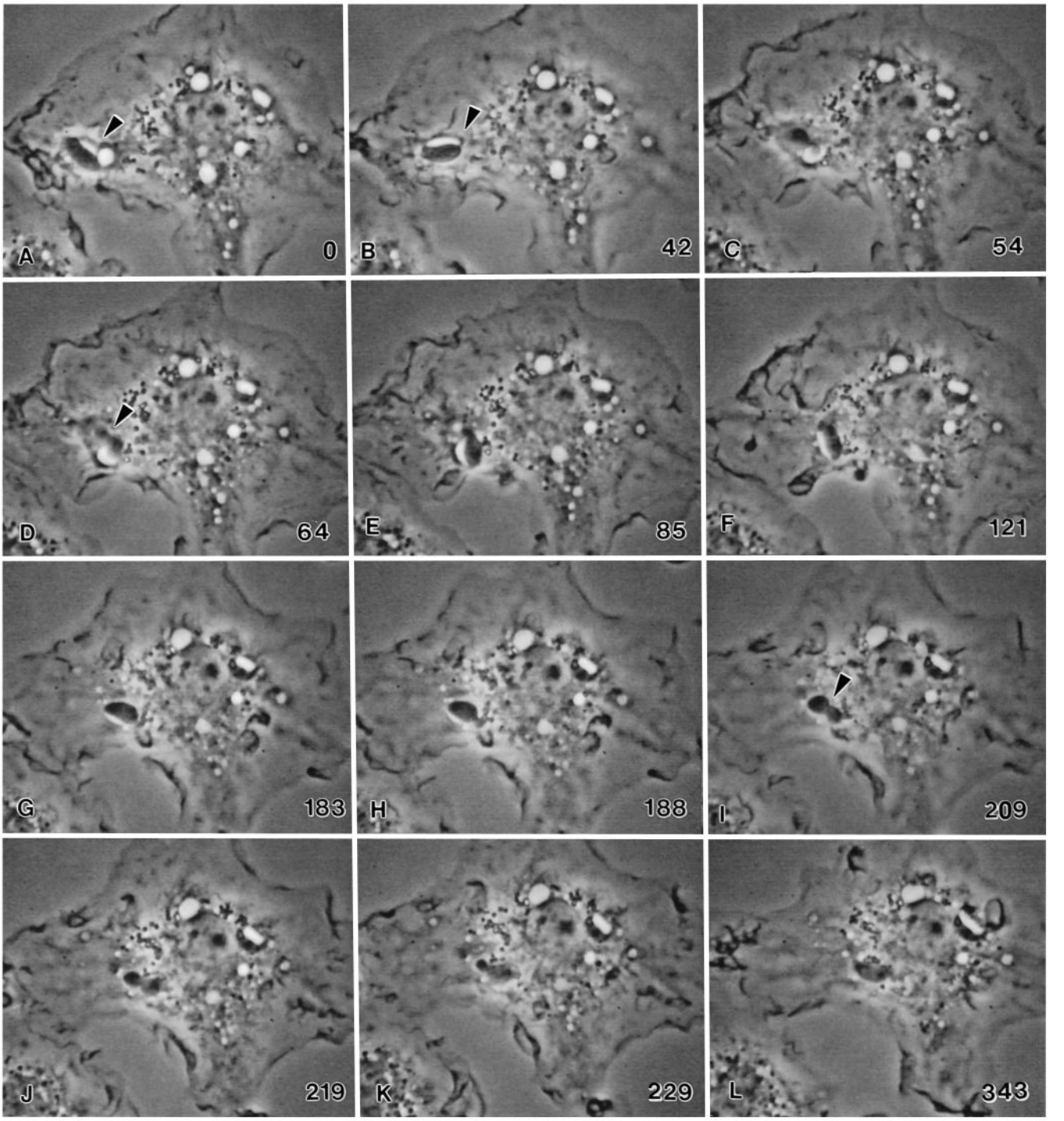


Fig. 7. Phagosome escape by sequential invasion of *Toxoplasma* out of the phagocytic vacuole in a BM cell. The parasite was initially engulfed into a loose-fitting phagocytic compartment which fuses with an adjacent macropinosome (arrowheads in A,B). The parasite undergoes two sequential invasion-like events to escape from the phagosome. In the first invasion-like event, the parasite passes through a prominent constriction (arrowhead in D) and enters a new compartment which is loose-fitting like the original vacuole (F). The parasite undergoes a second invasion-like event, again passing through a prominent constriction (arrowhead in I) and entering a tight fitting compartment (L). Elapsed time indicated in seconds in lower right corner.

event similar to macropinocytosis (Alpuche-Aranda et al., 1994). Contact of the bacterium with epithelial host cells induces a series of profound changes that include extensive membrane ruffling (Francis et al., 1993), host cell cytoskeletal rearrangements (Finlay et al., 1991), and tyrosine phosphorylation of host cell proteins (Bliska et al., 1993; Rosenshine et al., 1994). During internalization by macrophages, a similar process is triggered and the bacterium is captured in a spacious phagosome that undergoes fusion with other host cell endocytic vacuoles (Alpuche-Aranda et al., 1994).

Table 1. Kinetics of invasion, phagocytosis and phagosome escape during Toxoplasma infection of BM and CHO cells in vitro

Cell type	Elapsed time*	Parasite constriction†	Host-cell ruffling†
BM			
Phagocytosis	160.0±29.1 (9)	0/9	9/9
Invasion	36.2±8.9 (18)	15/18	2/18‡
Phagosome escape	39.0±14.3 (4)	4/4	0/4
CHO			
Invasion	47.0±9.5 (6)	5/6	0/6

*BM or CHO cell monolayers were challenged with freshly isolated *Toxoplasma* and the internalization of parasites was recorded continuously at 1 frame/second. Sequences were analyzed for the average time for formation of phagosomes (spacious vacuoles) vs vacuoles formed by invasion (tightly fitting vacuoles) and are reported as mean elapsed time ± s.d. (*n*). The kinetics of phagocytosis of antibody-coated and uncoated parasites by BM cells were similar and are grouped together.

†Sequences were also analyzed for the presence of membrane ruffling and constriction of the parasite during entry and are indicated as the number of times observed/number of separate events scored.

‡In two cases, parasites invaded at the site of extensive membrane ruffling. These events were characterized by an obvious constriction in the parasite and entry into a tight-fitting vacuole and are thus scored as invasions.

Phagocytosis of *Toxoplasma*

Phagocytosis of *Toxoplasma* by BM involved extensive membrane ruffling and reorganization of host cell actin microfilaments, events that are characteristic of receptor-mediated phagocytosis (Greenberg et al., 1990). During phagocytosis of antibody-opsonized *Toxoplasma* by BM cells, there was extensive phosphorylation of host cell protein tyrosine residues. This result is consistent with studies on phagocytosis of antibody-opsonized RBCs showing that several host cell proteins including FcR are phosphorylated during phagocytosis (Greenberg et al., 1994). Tyrosine phosphorylation is evidently essential as phagocytosis is blocked by inhibitors of tyrosine kinases (Greenberg et al., 1993).

On closer examination of the time-lapse recordings, it was evident that live parasites often remained loosely adherent to host cells without invading and without inducing phagocytosis, sometimes for many minutes. When extracellular parasites were incubated in PBS, their ability to invade cells rapidly declined leading to an increase in uptake by phagocytosis. The avoidance of phagocytosis by live parasites indicates *Toxoplasma* may actively prevent phagocytosis, possibly by altering host cell signaling. Other microrganisms such as *Yersinia* are able to prevent phagocytosis by production of a protein tyrosine phosphatase that acts on the host cell (Bliska et al., 1993).

Phagocytosis of *Toxoplasma* was chaotic rather than a highly ordered event. Extensive membrane ruffling of the entire area surrounding the parasite resulted in capture by closure of membrane folds over the parasite. Surprisingly, this event was morphologically identical for both effete and live, antibody-coated parasites indicating that while Fc receptors contributed to increased efficiency of phagocytosis, they did not change the mechanism of uptake. Phagosomes engulfing *Toxoplasma* typically underwent fusion with other host cell endocytic vacuoles that formed simultaneously in the adjacent area contributing to the loose-fitting profile of the vacuole. These observations are consistent with previous studies showing these compartments rapidly undergo acidification (Sibley et al., 1985b) and fusion with endocytic vacuoles and lysosomes (Sibley et al., 1985a; Joiner et al., 1990).

Phagocytosis of opsonized RBCs by macrophages proceeds by gradual extension of pseudopods to surround the particle (Alpuche-Aranda et al., 1994). This ordered process of engulfment is consistent with the 'zipper model' of phagocytosis that is based on studies showing additional ligand-receptor interactions, outside the zone of initial binding, are necessary for particle uptake (Griffin et al., 1975). Our observations indicate that once triggered, phagocytic uptake can proceed by capture of the particle within a loose-fitting compartment formed by localized membrane ruffling. A possible explanation for this difference is that *Toxoplasma* may provide an additional signal(s) to the host cell that increases cortical ruffling during phagocytosis. Alternatively, our results may reflect an enhanced appetite for phagocytosis by BM cells cultured with colony-stimulating factors which have been shown to stimulate phagocytosis (Collins and Bancroft, 1992) and macropinocytosis (Racoosin and Swanson, 1993). Consistent with this, BM cells also engulf *Listeria* and *E. coli* by a process involving localized membrane ruffling that captures the organism in a loose-fitting phagosome (J. E. Heuser and J. H. Morisaki, unpublished results).

Mechanism of active cell penetration by *Toxoplasma*

One of the most distinctive features of *Toxoplasma* invasion is the prominent constriction that occurs in the parasite at the site of penetration. This constriction is similar to the 'moving junction' that forms between the parasite and the red cell membrane during the invasion of malaria (Aikawa et al., 1978). In the case of *Toxoplasma* invasion, the constriction may result from physical limitations imposed by the rigid host cell cytoskeleton surrounding the point of invasion. The use of time-lapse video microscopy clearly reveals that this hourglass constriction remains fixed as the parasite is propelled forward and enters the host cell. The specific components that contribute to the moving junction have not been identified; however, it likely forms by interaction between the anterior end of the parasite and some component on the host cell membrane. By capping this attachment zone from anterior to posterior, the parasite moves forward into a vacuole formed by invagination of the host cell plasma membrane.

The significance of the moving junction may lie in modulation of the contents of the parasitophorous vacuole that is formed by invasion. During invasion of malaria, red cell surface proteins are excluded from the forming vacuole (Aikawa et al., 1981; Ward et al., 1993). Similarly it has been reported the PV surrounding *Toxoplasma* contains fewer intramembranous particles than the plasma membrane (Porchet-Hennere and Torpier, 1983). More direct analysis indicates that several abundant host cell proteins are internalized during *Toxoplasma* invasion, but that these components are rapidly removed and remain absent from mature PVs (Sibley et al., 1994; de Carvalho and de Souza, 1989). It is possible that the exclusion of key host cell molecules during formation or their removal shortly thereafter, is responsible for the resistance of these compartments to endocytic fusion.

Toxoplasma cells do not have specialized organelles for motility such as cilia or flagella, yet they are able to move along surfaces by gliding. Both gliding and invasion are blocked by cytochalasins, providing evidence that motility is based on a microfilament system (Ryning and Remington, 1978;

Schwartzman and Pfefferkorn, 1983). Using a combination of host cell versus parasite mutants, we have recently shown that the invasion blocking activity of cytochalasins is due to their direct action on the parasite (Sibley et al., 1994). Consequently, the active penetration of the host cell is likely to be driven by a microfilament-based motility mechanism within the parasite, the exact mechanism of which remains to be determined.

Phagosome-escape and intracellular survival

The present studies demonstrate that *Toxoplasma* actively invades the host cell to form a parasitophorous vacuole that is distinct at the outset from those formed by phagocytic/endocytic uptake. While the parasitophorous vacuole resists fusion, parasites engulfed into phagosomes rapidly undergo fusion with endosomes/lysosomes and are digested. Remarkably, some parasites were able to avoid this fate and escaped from newly formed phagosomes by rapidly invading into a secondary, tight-fitting vacuole. Presumably, such escape dramatically alters the composition of the vacuole membrane, thus recreating the fusion-resistant state characteristic of the parasitophorous vacuole. Phagosome escape provides a novel mechanism for intracellular survival of *Toxoplasma* that occurs by a mechanism similar to the active invasion of the host cell. The intracellular survival of *Toxoplasma* is evidently based on this unique ability to penetrate the host cell.

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