RAPID POLYMERIZATION OF *ENTAMOEBA HISTOLYTICA* ACTIN INDUCED BY INTERACTION WITH TARGET CELLS

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The mechanism by which trophozoites of the protozoan parasite, *Entamoeba histolytica* destroy mammalian cells is not clear. A variety of amoeba-associated properties have been correlated with virulence (reviewed in 1 and 2). In vitro studies (3–6) have shown that contact of the trophozoite with a target cell is required. Contact killing has been described (6) as occurring in the sequence: adherence, cytolysis, then phagocytosis (possibly) of the target or its remnants. Dependence of in vivo virulence upon the phagocytic competence of the amoeba has also been reported (7). All steps of the sequence are inhibited by cytochalasins (6, 8, 9), indicating that dynamic participation of the amoeba actin cytoskeleton is required. However, the role of actin in the attack process has not been defined.

By fluorescence microscopy of glutaraldehyde-fixed and rhodamine-phalloidin–stained (10) trophozoites, we have revealed the organization and distribution of polymerized actin in *Entamoeba* involved in a variety of motility-related activities (11, 12), including target cell interactions. Furthermore, by methanol extraction and spectrofluorometric measurement of the bound fluorescence, we have been able to quantitate polymerized actin in trophozoites. In this report, we describe this latter procedure and studies of the organization and quantity of polymerized actin in *E. histolytica* trophozoites before and immediately after challenge with human red blood cells (RBC)[1]. Within 5 s after challenge, polymerized actin appeared at the contact interface with many adherent RBC. A net increase in amoeba polymerized actin content was detectable 1 min after the challenge, and reached a maximum of approximately twice the value in unchallenged cells within 4 min. Latex beads, which were phagocytized by *E. histolytica* trophozoites, neither stimulated an actin response nor were able to diminish the interaction with RBC. RBC, on the other hand, inhibited uptake of latex beads.

These results indicate that the initial interaction of *E. histolytica* trophozoites with target cells is a recognition-specific process that triggers rapid polymerization of amoeba actin at the site of target contact. This actin appears to be involved in phagocytosis of the target cells. The procedure developed to quan-

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; PBS, phosphate-buffered saline; RBC, red blood cell.

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titate polymerized actin may be useful in further studies of the mechanism of target cell attack and other motility-related functions of this pathogen.

Materials and Methods

Entamoeba Cultures. *E. histolytica*, strain HM1-IMSS, was grown axenically in TYI-S33 medium (13).

Amoeba–Target Cell Interactions. Trophozoites in mid–growth phase were centrifuged at 200 *g* for 2 min, and resuspended in their own medium at 10^6 cells/ml. Freshly isolated A^+ human RBC were washed with phosphate-buffered saline (PBS) at pH 6.3, the average pH of growth-phase amoeba cultures, and suspended at 4×10^9 cells/ml in supernatant culture medium left from the above step. 2-ml aliquots of the amoeba suspension were placed in 10 × 75–mm round-bottom plastic tubes. Cells were equilibrated at the experimental temperature for 5 min. After gentle resuspension of the amoebae, 1/10 vol of RBC was added to initiate cell-cell interactions. The suspensions were mixed by inversion, initially, and at 30-s intervals throughout the incubation. Latex beads (6 μm diameter) (Sigma Chemical Co., St. Louis, MO) were washed with PBS and suspended at the same final concentration as RBC in supernatant TYI-S culture medium just before use. Trophozoites were preincubated for 15 min at 37°C with cytochalasin D (final concentrations: 100 μM cytochalasin, 4% vol/vol dimethylsulfoxide solvent) before transfer to the experimental temperature. Controls contained the solvent alone.

Fixation, Detergent Extraction, and Rhodamine-Phalloidin Staining of Cells. Incubations were stopped, and cells were fixed and extracted by addition of an equal volume of 2.5% (vol/vol) glutaraldehyde in PBS, followed, in 20 s, by 1/10 vol of 10% (vol/vol) Triton-X 100. After 15 min, cells were collected and washed a minimum of three times with PBS by centrifugation at 200 *g* for 2 min. >98% of the free RBC were eliminated in the discarded supernatant washes. The washed amoebae were resuspended in 1 ml PBS (pH 7.4) containing 0.1% bovine serum albumin (BSA). After counting in a hemocytometer chamber to determine the concentration of amoebae, and both free and amoeba-associated RBC, the amoeba concentration was adjusted to 10^6 cells/ml by addition of PBS containing BSA. An aliquot of this suspension was stained with 0.3 μM rhodamine-phalloidin (Molecular Probes, Eugene, OR) for a minimum of 1 h at 24°C. Samples of the stained cells were viewed microscopically without further treatment.

Measurement of RBC Attachment and Phagocytosis. Internalized RBC were clearly visible in the fixed, detergent-extracted amoebae. The numbers of fully phagocytized and of attached RBC were counted for the first 50 amoebae encountered on a slide prepared from the above cell suspensions. Partially engulfed RBC were scored as attached.

Quantitation of Polymerized Actin. The procedure developed to quantitate polymerized actin in *Entamoeba* by methanol extraction of bound rhodamine-phalloidin was inspired by a similar method introduced by Howard (14) to quantitate polymerized actin in leukocytes. 100-μl aliquots of cell suspensions prepared as above were placed in replicate, conical, 1.5-ml microfuge tubes containing 10 μl of 3.3 μM rhodamine-phalloidin. Staining was carried out for a minimum of 1 h at 24°C on a tube rotator to keep the cells suspended. Thereafter, the cells were pelleted by centrifugation at 12,000 *g* for 2 min, and the supernatant solution, containing unbound rhodamine-phalloidin, was removed by aspiration. 1 ml of methanol was added, the pellet was disrupted by shaking, and rhodamine-phalloidin was extracted for 15 min, with rotation. After centrifugation at 12,000 *g* for 30 s, the first milliliter of methanol was collected, and the pellet was extracted similarly a second time. The fluorescence of the combined methanol extracts was measured in a Perkin-Elmer 650-40 fluorescence spectrophotometer at excitation and emission wavelengths of 540 nm and 565 nm, respectively. For binding studies requiring different concentrations of rhodamine-phalloidin, the solvent of the commercial preparation was evaporated, and the rhodamine-phalloidin was redissolved in PBS (pH 7.4) containing 0.1% BSA. Other details of these assays are described in Results.

Microscopy and Photomicrography. Light microscopy was done using an Olympus Vanox microscope with differential interference contrast (Nomarski) and epifluorescence optics. Fluorescence micrographs were exposed for 32 s on Kodak Tri-X film.

For scanning electron microscopy, amoeba were fixed and washed as above with the omission of Triton-X 100. Washed cells were resuspended in 2.5% glutaraldehyde, and captured by filtration onto polylysine-coated, 5-μm pore size polycarbonate membranes (Nucleopore Corp., Pleasanton, CA). After washing with PBS, the membrane-attached cells were dehydrated with ethanol, critical-point dried, sputter-coated for 5 min with gold-palladium, and viewed at 10 kV in an ETEC Omniscan microscope.

Results

Scanning electron micrographs of suspended amoebae fixed 5 s and 30 s after challenge with RBC at 37°C are shown in Fig. 1. Deformation of the adherent RBC, and the beginnings of phagocytic pseudopodia were evident at the earlier time (Fig. 1 *a*). After 30 s, all stages of phagocytosis were seen. With preparations of live amoebae at 37°C on a warm microscope stage, we observed that complete engulfment could occur within 10 s after initial contact with a target cell (not shown). Often, the advancing front of the engulfing membrane closed before the RBC was completely surrounded. This lead to scission of the target, and release of the external fragment. Such occurrences can be seen in process in the scanning electron micrograph of Fig. 1 *b*, and in the light micrograph of Fig. 2 *e*.

Distribution of Polymerized Actin. The distribution of polymerized actin in trophozoites before and after challenge with target cells is shown in Fig. 2. At 37°C, most unchallenged trophozoites exhibited macropinocytic invaginations of varying size and number, which were outlined by rhodamine-phalloidin–sensitive actin (Fig. 2, *a* and *b*). Within 5 s of challenge, polymerized actin was evident rimming the interface of many target cell contacts, as well as surrounding the pinocytic invaginations (Fig. 2, *c* and *d*). As phagocytosis progressed, pinocytosis was less apparent (Fig. 2 *f*).

Growth of the phagocytic membrane was accompanied by the appearance of

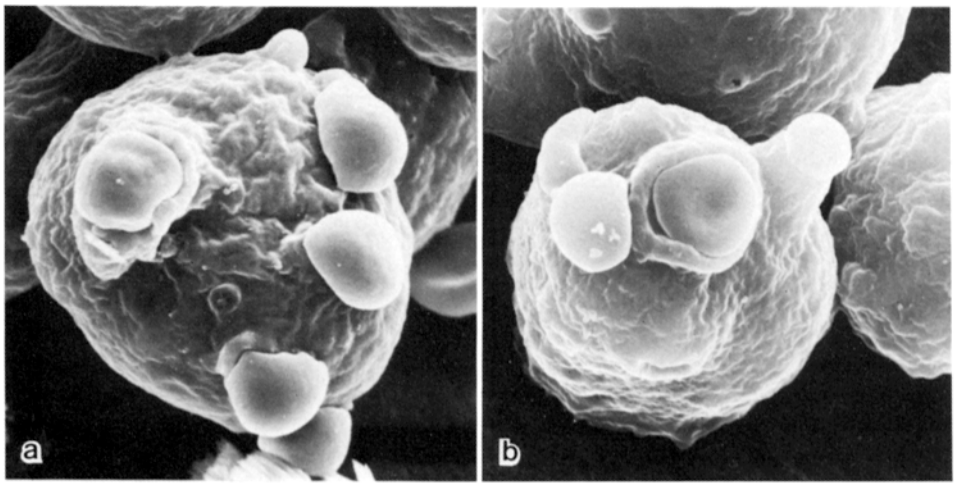

FIGURE 1. Scanning electron micrographs of *E. histolytica* trophozoites fixed 5 (*a*) and 30 s (*b*) after challenge with RBC. At the earlier time, deformation of attached target cells occurred and phagocytic pseudopodia had begun to rise around some of these (upper left). At the later time, all phases of phagocytosis were evident. The partially engulfed RBC at the right in (*b*) is being severed by constriction of the phagocytic mantle. 3,000 ×.

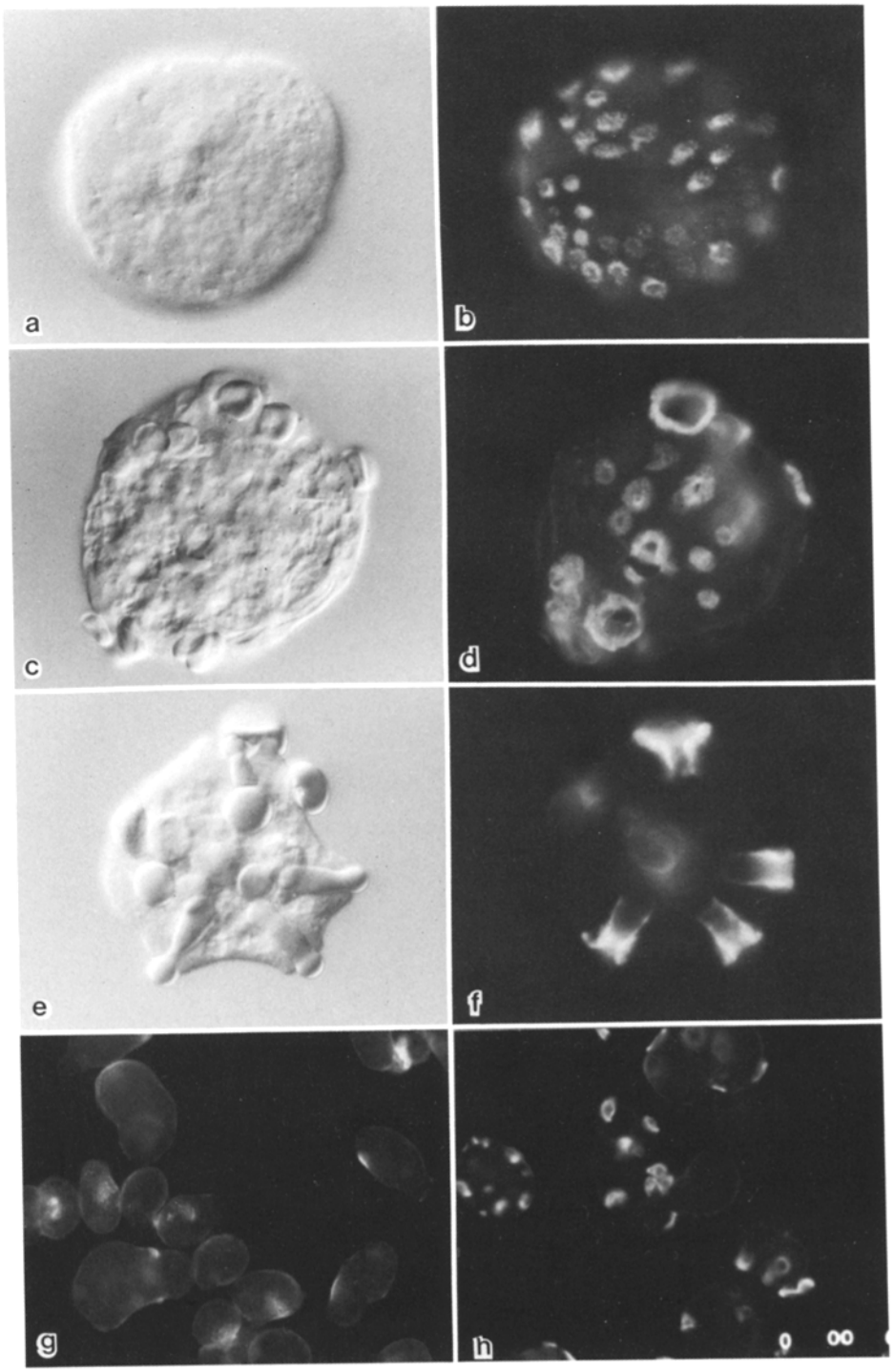

FIGURE 2. Differential interference contrast (Nomarski) and corresponding fluorescence micrographs of glutaraldehyde-fixed, Triton-X 100–extracted, and rhodamine-phalloidin–stained *E. histolytica* trophozoites before and after challenge with RBC. (*a* and *b*), Unchallenged trophozoites at 37°C exhibited actin-lined macropinocytotic invaginations of varying size and number. (*c* and *d*), 5 s after challenge with RBC, many attached target cells were rimmed by polymerized actin. Pincytotic invaginations were still evident. (*e* and *f*), 30 s after challenge, attached, partially engulfed, and fully engulfed RBC could be seen. Constriction of the phagocytic mantle and fragmentation of partially engulfed targets often occurred. Evidence of polymerized actin around target cells disappeared as they entered the trophozoite interior. (*g*), At 25°C, the polymerized actin associated with pinocytosis in unchallenged trophozoites was diminished. However, (*h*) challenge with RBC still triggered rapid formation of polymerized actin at sites of contact and phagocytosis (1.5 min after challenge). *a–f*, 1,500 ×; *g* and *h*, 500 ×.

polymerized actin along the interface with the target cell (Fig. 2, *e* and *f*). The RBC appeared both stretched and squeezed as it entered the trophozoite. The fluorescent sheath disappeared, as did the evidence of target constriction, when the RBC passed into the trophozoite interior. In experiments not described here, in which *E. histolytica* trophozoites were challenged with erythroleukemia (Friend) cells, we observed analogous rapid changes in target cell morphology and amoeba actin organization, indicating that these responses by *Entamoeba* were not restricted to RBC.

Fluorescence associated with macropinocytosis was greatly diminished at 25°C (Fig. 2*g*). However, attachment and phagocytosis of RBC, with the accompanying appearance of polymerized actin, still occurred (Fig. 2*h*). For this reason, we chose the lower temperature for experiments to detect changes in actin content following challenge with target cells.

Quantitation of Polymerized Actin. In preliminary experiments, we determined that: (*a*) the rate of dissociation of bound rhodamine-phalloidin was sufficiently rapid in PBS to preclude washing of stained cells before methanol extraction; (*b*) rhodamine-phalloidin binding reached equilibrium within 30 min, and (*c*) fluorescence was extracted maximally by shaking with two 1-ml aliquots of methanol, for as little as 10 min each. Addition of a 150-fold excess of unlabeled phalloidin blocked all microscopically distinguishable staining of organized actin by rhodamine-phalloidin in RBC-challenged trophozoites. The extracted fluorescence in these controls amounted to ~10% of the added amount when 10^5 amoebae were stained. Experimental values were corrected for this nonspecific fluorescence.

RBC were chosen as the target cell for this study because they have a low content of rhodamine-phalloidin–sensitive actin; rhodamine-phalloidin fluorescence associated with RBC was not detectable microscopically. The amount of fluorescence extracted from twice the maximum number of RBC encountered in amoeba suspensions was no greater in the presence than in the absence of an excess of nonfluorescent phalloidin, indicating that, with this number of cells, specific binding of rhodamine-phalloidin to RBC actin was undetectable. Furthermore, addition of fixed RBC, in the numbers encountered in challenged amoeba suspensions, to assays of unchallenged amoebae did not increase the level of extracted fluorescence above that detected in the absence of added RBC. Thus, fluorescence contributed by rhodamine-phalloidin–stained RBC actin was not a source of error in the measurement of amoeba-bound fluorescence.

Using amoebae that had been challenged with RBC for 3 min at 37°C before fixation and extraction, equilibrium amounts of bound and free rhodamine-phalloidin were determined over a range of 3 nM to 0.5 μM initial ligand concentration in two experiments. Plots of bound vs. free rhodamine-phalloidin were hyperbolic, and plots of bound vs. log free were sigmoidal (15). Scatchard plots were linear, with correlation coefficients of 0.99 and 0.93, and yielded dissociation constants (K_d) for rhodamine-phalloidin of 57 and 61 nM. Assuming 59 nM as the K_d, the cellular actin available for binding was ~80% saturated by 0.3 μM initial rhodamine-phalloidin, the concentration used routinely in our assays.

Changes in Polymerized Actin Content in Trophozoites Challenged with RBC. The relative content of polymerized actin and the corresponding extent of erythro-

phagocytosis by trophozoites at selected times after challenge with RBC at 37°C and at 25°C are shown in Fig. 3. The data show that interaction with the target cells triggered net polymerization of amoeba actin. We presume that the fluorescence appearing at the contact interface immediately after adherence (Fig. 2*d*), and surrounding RBC during phagocytosis (Fig. 2*f*), represented most of the newly assembled polymer. The drop in polymerized actin content at the later times reflects the decline in phagocytic activity indicated by the decreasing slope of the phagocytosis curve. As predicted, the maximum relative increase in polymerized actin after challenge was greater at 25°C than at 37°C. Thus, even though phagocytic activity and total polymerized actin content was greater at 37°C, detection of changes was more sensitive at the lower temperature.

Chilling of trophozoites to 4°C for 15 min totally blocked phagocytosis following challenge with RBC, and decreased the polymerized actin content of both challenged and unchallenged amoebae ~30% compared to unchallenged trophozoites kept at 25°C (data not shown). However, adherence of RBC to the chilled amoebae was not prevented, as has been shown previously (6).

Specificity of Interactions with RBC. Latex beads of the same diameter, and presented at the same concentration as RBC were phagocytized by *E. histolytica* trophozoites at 37°C, but to a lesser extent than RBC in the same time period (Table I). When RBC and beads were presented together to trophozoites, the extent of RBC phagocytosis was the same as in the absence of the beads, while latex bead uptake was diminished significantly ($p < 0.001$). Challenge of trophozoites at 25°C with latex beads for 4 min (the time at which the maximum increase in polymerized actin was observed with RBC) caused little or no creation of polymerized actin at the sites of bead attachment (Fig. 4, *a* and *b*) and no

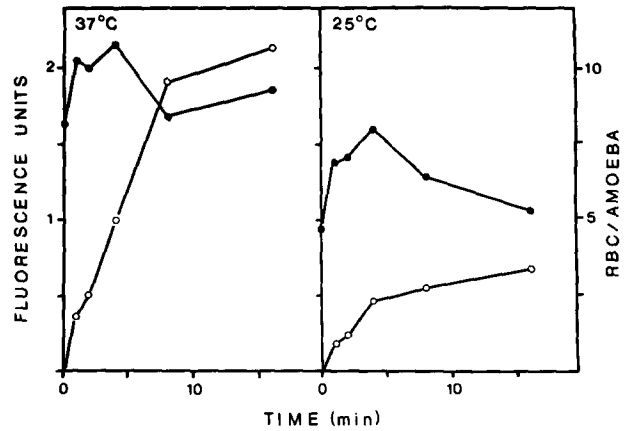

FIGURE 3. Actin polymerization and erythrophagocytosis by *E. histolytica* trophozoites after challenge with RBC at 37°C and 25°C. RBC were added (4×10^8 cells/ml) to suspensions of trophozoites (10^6 cells/ml). At the indicated times, samples were fixed and processed for quantitation of rhodamine-phalloidin binding to polymerized actin (●) (relative fluorescence units bound per 10^5 trophozoites) and for microscopic counting of the number of engulfed RBC (○) (average number of RBC per trophozoite, estimated from counts of 50 cells). The 0-min sample was fixed before addition of RBC. The plotted points are averages of the values obtained from duplicate samples. These results are representative of those obtained in two experiments conducted at 37°C and three at 25°C.

TABLE I

Relative Specificity of Phagocytosis of RBC and Latex Beads by E. histolytica

Target	Phagocytosis*	
	RBC per amoeba	Beads per amoeba
RBC	6.6 ± 0.19‡	—
Latex beads	—	2.50 ± 0.53
RBC + latex beads	6.5 ± 0.35	0.91 ± 0.18

*Phagocytosis was allowed to proceed for 30 min.
‡ Values are means ± SEM of results obtained in three separate experiments.

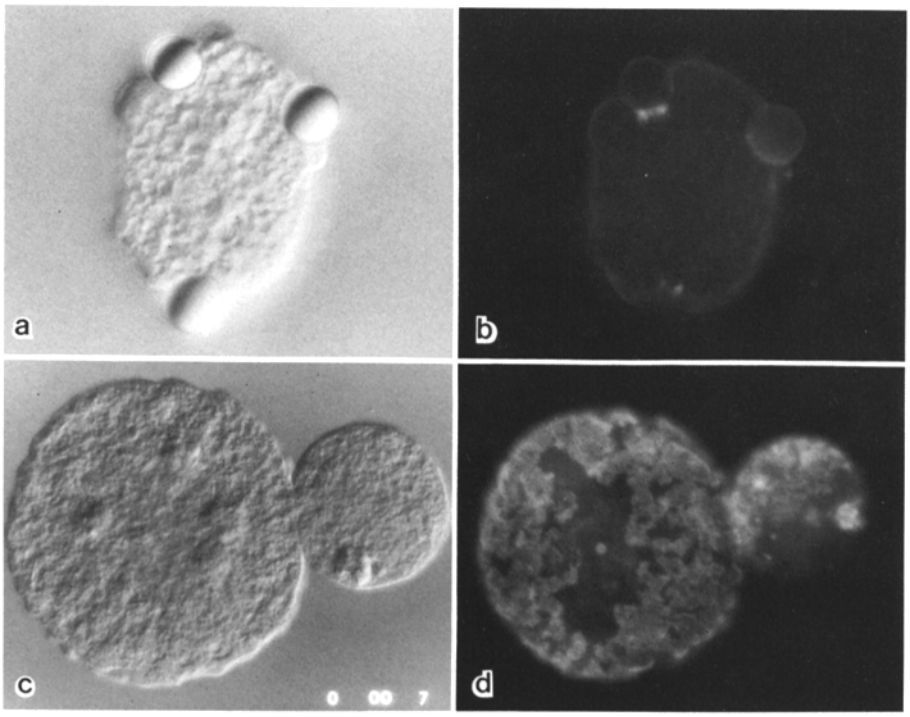

FIGURE 4. Nomarski and corresponding fluorescence micrographs of glutaraldehyde-fixed, Triton-X 100–extracted, and rhodamine-phalloidin–stained *E. histolytica* trophozoites after challenge with latex beads (*a* and *b*) or treatment with cytochalasin D (*c* and *d*). Polymerized actin was less evident at the sites of contact and engulfment of latex beads (*b*) than of rbc (compare with Fig. 2*f*). Cytochalasin-treated cells were all spherical, and displayed a bright, mottled pattern of peripheral fluorescence (*d*). 1,500 ×.

measurable increase in extractable fluorescence above that in the unchallenged controls (Table II). The same results were obtained when the challenge time was extended until internalization of beads at 25°C was measurable (20–30 min). Thus, although the nonbiological particles were internalized, this appeared to be accomplished by a less specific mechanism, which did not trigger actin polymerization as RBC did.

Effects of Cytochalasin D. Several studies (6, 8, 9) have shown that concentrations of cytochalasin as low as 10 μM will prevent adherence, cytolysis, and

TABLE II

Effects of Various Treatments on E. histolytica Polymerized Actin Content, RBC Adherence, and Phagocytosis

Conditions of challenge*	Polymerized actin content‡	Adherence§	Phagocytosis§
Unchallenged	0.69 ± 0.07‖	—	—
RBC	1.25 ± 0.28	3.98 ± 0.69	2.43 ± 0.49
Latex beads	0.72 ± 0.10	0.64 ± 0.08	0.0
Unchallenged	0.58 ± 0.09	—	—
Cytochalasin D	1.45 ± 0.18	—	—
RBC	1.08 ± 0.05	3.74 ± 0.27	2.15 ± 0.27
RBC + Cytochalasin D	1.72 ± 0.20	2.23 ± 0.47	0.04 ± 0.04

* Interaction with targets was allowed to proceed for 4 min.
‡ Relative fluorescence units per 10^5 amoebae.
§ RBC per amoeba.
‖ Values are means ± SEM of results of a minimum of three experiments.

phagocytosis of target cells, including RBC, by *E. histolytica*. We observed total loss of phagocytotic ability and reduction of RBC adherence at 25°C after preincubation of trophozoites at 37°C with cytochalasin D (Table II). However, the effect of the toxin treatment on fluorescent staining of rhodamine-phalloidin, even at concentrations as high as 100 μM, was unexpected. The cytochalasin-treated trophozoites were spherical, indicating a loss of cytoskeleton control of cell shape, but contained a bright, mottled fluorescence pattern (Fig. 4*b*). The amount of extractable fluorescence in the cytochalasin-treated cells, whether challenged with RBC or not, was significantly greater than that in the untreated unchallenged controls (Table II). The fluorescence was not due to cytochalasin per se, since cells did not fluoresce before staining with rhodamine-phalloidin. Possibly only partial disassembly of polymerized actin occurred in the presence of cytochalasin D, sufficient to prevent cytoskeleton function, but actually exposing more polymer for phalloidin binding.

Content of Polymerized Actin in E. histolytica Trophozoites. Actin polymers bind approximately one molecule of phalloidin per actin monomer (16). Assuming that all actin polymers in *Entamoeba* are available for phalloidin binding (which may not be the case in view of the results obtained with cytochalasin D treatment), we calculated that *E. histolytica* trophozoites contained an average of ~20 pg/cell polymerized actin, minimally (4°C, unchallenged), and as much as 150 pg when maximally stimulated (4 min after challenge with RBC at 37°C). From total protein determinations (measured by the method of Bradford [17]) with whole sonicates of trophozoites, we estimated that this represents a range of 0.7–5% of total cellular protein.

Discussion

Phagocytic Response, and Induction of Actin Polymerization. Others (18, 19) have described the dynamics of erythrocyte phagocytosis by *E. histolytica*, and the phagocytic attack of trophozoites upon larger mammalian cells has been illustrated dramatically by Martinez-Palomo (20) in scanning electron micro-

graphs. Herein, using rhodamine-phalloidin, which has been shown to bind specifically to polymerized actin (16), we have further characterized the interaction between *E. histolytica* trophozoites and RBC. Within 5 s of challenge, adhesion and deformation of target cells occur, polymerized actin appears at the interfaces with many targets, and phagocytosis commences immediately at these sites. RBC, many of which are elongated and constricted, are surrounded by a sheath of polymerized actin as they pass through the cortical cytoplasm of the trophozoite. Concomitant with these events is a net increase in the average polymerized actin content of the amoebae. This latter response, presumably reflecting polymerized actin involved in target attachment and phagocytosis, is one of the earliest quantifiable events in the in vitro attack sequence of this pathogen.

Involvement of the actin cytoskeleton in phagocytosis is well-documented (21, 22). One recent study (23) showed that phagocytosis by neutrophil leukocytes involves transient increases in polymerized actin. The actin response was initiated within 10 s of recognition of the phagocytic substrate. Studies (24) with *Acanthamoeba* have demonstrated the speed of phagocytosis; once a decision to phagocytize was made, complete engulfment of yeast occurred within 32 s. The responses of *Entamoeba* appear similar to those of other phagocytes in these regards.

There are several reasons to conclude that the cytoskeletal changes at the site of target cell attachment to the *Entamoeba* surface are induced by specific interactions occurring upon contact (i.e., are receptor mediated) and are not the consequence of an unstimulated mechanism, or of one that responds to contact with any foreign particle: (*a*) The dimensions of the initial polymerized actin ring at the contact interface with a target cell were greater than those of most of the spontaneously arising pinocytotic stomata on the same amoeba (Fig. 2*d*), and fit the shape of the attached RBC. (*b*) Recruitment of actin occurred at target cell contact sites at 25°C, at which temperature the pinocytotic activity of unchallenged cells was greatly reduced. (*c*) RBC prevented phagocytosis of latex beads, whereas the noncellular particles had no effect on RBC phagocytosis. Finally, latex particles themselves stimulated little actin polymerization at the site of contact, and no detectable increase in total cell polymerized actin when used as the challenge.

Given the rapidity of actin polymerization and extension of a phagocytic membrane following target cell contact, and the induced nature of these responses, we suggest, as have others (7), that phagocytic action is part of the primary attack strategy used by *E. histolytica* in the destruction of mammalian cells. If, during the initial stages of phagocytic attack, the molecular elements responsible for target lysis acted upon the target contact surface, then loss of target cell membrane integrity might occur before complete engulfment, as has been noted (5). In this view, the mechanisms of cytolysis and phagocytosis would be coincident and interdependent, and the requirement for actin action in both would be explained.

Quantitation of Polymerized Actin. The DNase I inhibition assay (25), which has been used most commonly to quantitate nonmuscle cellular actin, is not applicable to *Entamoeba* because *Entamoeba* actin does not inhibit this enzyme

(26, 27). Howard (14) recently introduced the method involving methanol extraction of bound fluorescent phalloidin as a means to quantitate polymerized actin in neutrophils. This method, like the DNase inhibition assay, provides an average actin content per cell, but unlike the enzyme assay, does not allow determination of total cellular actin. Applied to *Entamoeba*, we find this assay to be simple, sensitive, precise, and to require easily obtainable numbers of cells.

The average K_d (59 nM) we determined for binding of rhodamine-phalloidin to polymerized actin in glutaradehyde-fixed *Entamoeba* trophozoites is between the value found for unmodified phalloidin (36 nM) (28) and that found for fluorescein-labelled phalloidin (270 nM) (10) binding to native eucaryotic actin in vitro. It is considerably lower than the value (15 μM) reported (27) for fluorescein-phalloidin binding to purified *E. histolytica* actin. We do not know the reason for the difference between our value and the other found for *E. histolytica*. However, it should be remembered that our measurements were conducted on glutaraldehyde-fixed whole cells, and the others were made using isolated native actin.

Assuming unit binding of rhodamine-phalloidin to actin monomers in polymerized actin, we calculated that polymerized actin represents an average of from 0.7% (in chilled unchallenged trophozoites) to 5% (in challenged trophozoites at 37°C) of total cell protein. By fluorescent microscopy, we observed considerable variation between individual cells in the extent to which actin polymerization occurred after challenge with RBC (Fig. 2 *h*). Some cells had no adherent RBC or no fluorescence at the contact interface, while others had numerous attached target cells and sites where extensive polymerization was evident from the associated fluorescence. We have attempted to adjust neither our calculations of the percent increase of polymerized actin content after challenge, nor our estimates of the average cellular concentration of polymerized actin in trophozoites for this variability. If we had, the percent increase in polymerized actin content and the percent of total protein represented by polymerized actin in the responding cells would have been much greater than that inferred by the average per-cell values.

We were surprised to find that, although all motility-related functions in addition to phagocytosis and adherence of RBC were inhibited by treatment with cytochalasin D, rhodamine-phalloidin staining was dramatically enhanced, whether or not the amoebae had been challenged with target cells. Microscopic observations of these cells (Fig. 4, *c* and *d*) revealed a mottled pattern of bright fluorescent aggregations, but no distinct staining at the few sites where RBC adhered. Other studies (29) have shown similar disruption and aggregation of intracellular actin by cytochalasins, but the change in polymerized actin content was not quantitated. Cytochalasins appear to block actin polymerization in vitro by binding to the fast-growing end of polymerization nuclei or of fractured actin filaments, but do not cause depolymerization of actin directly (30 and references therein). Thus, we expected that cytochalasin treatment would have either prevented a change or caused a decrease in the measurable polymerized actin content of cells. Possibly cytochalasin, by fragmenting the cortical actin mesh, renders more polymer accessible to phalloidin. Further experimentation will be required to explain this observation.

In conclusion, we have demonstrated that *Entamoeba* trophozoites respond to contact with mammalian target cells with a rapid and quantifiable polymerization of actin at the site of contact. This response appears to be receptor mediated, and to signal the start of a phagocytic attack upon the attached target. We believe that this physical assault should not be ignored as a possible essential component of the mechanism by which this pathogen initiates the destruction of mammalian cells. The ability to quantitate the cytoskeleton response of the parasite may prove useful in the further study of this mechanism.

Summary

Within 5 s of challenge of *Entamoeba histolytic* trophozoites with red blood cells (RBC), attachment and deformation of target cells occurred at multiple sites on the amoeba surface. Many trophozoite–target interfaces were outlined with a ring of polymerized amoeba actin, revealed by rhodamine-phalloidin staining of glutaraldehyde-fixed and Triton-X 100–extracted cells. The beginnings of phagocytic pseudopods rimmed many targets. The phagocytic membrane and underlying actin network grew uniformly about a target cell, which became dramatically elongated and constricted, sometimes severed, as it entered the amoeba. Total engulfment of RBC targets occurred within 10 s.

By methanol extraction and spectrofluorimetric measurement of bound rhodamine-phalloidin we were able to quantitate polymerized actin in amoebae. Interaction with target cells was accompanied by a net increase of up to twofold in the average polymerized actin content of trophozoites. This reached a maximum during the period of most active phagocytosis (4 min after challenge at 25°C), and declined as phagocytic activity diminished (8–16 min).

Challenge with latex beads of similar size and number, which *E. histolytica* phagocytized more slowly than RBC, induced neither a detectable increase in polymerized actin content nor appearance of polymerized actin at the contact interface. RBC inhibited phagocytosis of latex beads, but the reverse did not occur.

The results demonstrate a rapid, recognition-specific stimulation of reorganization of the actin cytoskeleton of *E. histolytica* induced by binding to target cells. Vigorous phagocytic activity is frequently an immediate consequence of cell-cell contact, which emphasizes the importance of this process in the contact-mediated attack mechanism of this pathogen. The quantitative assay of polymerized actin may be useful in further studies of this mechanism.

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