Entamoeba histolytica-Induced Dephosphorylation in Host Cells

José E. Teixeira and Barbara J. Mann

Departments of Internal Medicine and Microbiology, University of Virginia, School of Medicine, Charlottesville, Virginia 22908

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Activation of host cell protein tyrosine phosphatases (PTPases) and protein dephosphorylation is an important mechanism used by various microorganisms to deactivate or kill host defense cells. To determine whether protein tyrosine dephosphorylation played a role in signaling pathways affecting Entamoeba histolytica-mediated host cell killing, we investigated the involvement of PTPases during the attachment of E. histolytica to target cells. We observed a rapid decrease in cellular protein tyrosine levels in Jurkat cells, as measured with an antiphosphotyrosine monoclonal antibody, following adherence to E. histolytica. Ameba-induced protein dephosphorylation was contact dependent and required intact parasite, since blocking amebic adherence with galactose inhibited tyrosine dephosphorylation and amebic lysates had no effect on phosphorylative levels. Moreover, disruption of amebic adherence with galactose promoted recovery of phosphorylation in Jurkat cells, indicating that dephosphorylation precedes target cell death. The evidence suggests that ameba-induced dephosphorylation is mediated by host cell phosphatases. Prior treatment of Jurkat cells with phenylarsine oxide, a PTPase inhibitor, blocked ameba-induced dephosphorylation. We also found proteolytic cleavage of the PTPase 1B (PTP1B) in Jurkat cells after contact with ameba. The calcium-dependent protease calpain is responsible for PTP1B cleavage and enzymatic activation. Pretreatment of Jurkat cells with calpeptin, a calpain inhibitor, blocked PTP1B cleavage and inhibited ameba-induced dephosphorylation. In addition, inhibition of Jurkat cell PTPases with phenylarsine oxide blocked Jurkat cell apoptosis induced by E. histolytica. These results suggest that E. histolytica-mediated host cell death occurs by a mechanism that involves PTPase activation.

The protozoan Entamoeba histolytica is the causative agent of amebiasis, a common parasitic disease characterized by dysentery and, occasionally, liver abscesses. Colonization and invasion of the colonic mucosa involve adherence of trophozoites to glycoprotein mucins and host epithelial cells. In the cecum, the parasite is able to evade host immune defenses in part by secreting cysteine proteases that degrade immunoglobulins and proteins of the complement system and by killing host defense cells such as lymphocytes and neutrophils. E. histolytica-induced cell killing is contact dependent and is mediated by a galactose- and N-acetyl-D-galactosamine-specific (Gal/GalNAc) amebic lectin (21, 31). Upon contact, the pore-forming protein, amoebapore, is secreted by the parasite resulting in the formation of ion channel pores spanning the membrane of the target cell (17). E. histolytica induces a rapid and irreversible rise in target cell calcium concentration, [Ca^2+]_i, that is associated with membrane blebbing and precedes cell death (29). Changes in ion flux in both the ameba and the target cell are involved in amebic killing. Studies with the calcium chelators EDTA and EGTA demonstrate an absolute requirement for extracellular Ca^{2+} in amebic cytolysis of target cells (28). Moreover, treatment of target cells with verapamil, a slow calcium channel blocker, protects cells from lysis by the ameba (30). Thus, [Ca^{2+}]_i may act as a second messenger, triggering host cell signaling transduction pathways leading to cell death.

Interference in host cell signaling machinery is a strategy used by a number of microorganisms, such as Yersinia, Leishmania, and Mycobacterium spp., as a manner of either evading or inhibiting cellular mechanisms of host defense (13, 22). What these pathogens have in common is their ability to modulate tyrosine phosphorylation events in host cells by secreting or activating protein tyrosine phosphatases (PTPases). Studies have shown that PTPase activation and protein dephosphorylation are associated with cell deactivation and cellular death (5, 22, 25, 26). Leishmania donovani and Mycobacterium tuberculosis are able to impair macrophage functional responses by inhibiting mitogen-activated protein kinase signaling events via activation of the PTPase SHP-1 (5, 22). Pathogenic yersiniae resist phagocytosis by eukaryotic cells by inducing protein dephosphorylation, which is mediated by the Yersinia virulence protein YopH (26). YopH shares functional homology with PTPase 1B (PTP1B), a ubiquitous PTPase that has been implicated in the control and modulation of several tyrosine phosphorylation-controlled signaling pathways (2, 3, 19, 23, 25). PTP1B can be activated by calpain, a calcium-dependent cysteine proteinase. In response to elevations in intracellular [Ca^{2+}]_i, calpain cleaves PTP1B resulting in a two- to threefold increase in its enzymatic activity (11, 32). Cleavage of PTP1B is associated with extensive cellular protein dephosphorylation and is correlated with the appearance of a 42-kDa enzymatically active form of the phosphatase (11, 24). Specific proteolysis of PTP1B is observed in some models of programmed cell death as demonstrated by cytoplasm accumulation of the 42-kDa form in apoptotic-sensitive cell lines (ME-180, MCF-7, BT-20, and MDA-361) (25).

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adherence to *E. histolytica*. We also found proteolytic cleavage of PTP1B (the 42-kDa form). Calpeptin, a calpain inhibitor, blocked the PTP1B cleavage and inhibited ameba-induced dephosphorylation. Thus, we hypothesize that *E. histolytica* causes host cell death by a mechanism of intracellular [Ca\(^{2+}\)]\_ influx and PTPase activation.

**MATERIALS AND METHODS**

**Chemical and reagents.** Phenylarsine oxide (PAAO), protein-phosphotyrosine (6-4), orthovanadate, NaF, and a protease inhibitor cocktail ([2-aminoethyl])benzenesulfonyl fluoride (AEBSF), bestatin, leupeptin, aprotinin, E-64, and sodium EDTA were all purchased from Sigma (St. Louis, Mo.). Calpeptin was purchased from Calbiochem (La Jolla, Calif.).

**Polyclonal and monoclonal antibodies.** Horseradish peroxidase-conjugated rabbit anti-immunoglobulin G (IgG), fluorescein isothiocyanate (FITC)-conjugated mouse IgG1 (MOPC-21) and FITC-conjugated mouse antiphosphotyrosine (FITC-Pyr-MAB), clone PT-66, were purchased from Sigma. Horseradish peroxidase-conjugated recombinant antiphosphotyrosine (HRP-Pyr-Ab), clone RC20, was purchased from Transduction Laboratories (Lexington, Ky.). Monoclonal anti-PTP1B, clone FG6-1G, which is specific for the catalytic domain (N terminal), was purchased from Calbiochem.

**Cell lines and culture.** Trophozoites of *E. histolytica* (HM1:IMSS) were grown axenically in TYI-S-33 (Trypsin yeast extract, iron, and serum) medium supplemented with 100 μM of penicillin and 100 μg of streptomycin sulphate/ml at 37°C as previously described (9). Trophozoites were harvested after 48 to 72 h during the logarithmic phase by chilling the culture tubes on ice for 10 min. After centrifugation, the trophozoites were resuspended in medium 199 (Gibco-BRL, Grand Island, N.Y.) supplemented with 5.7 mM cytochrome, 25 mM HEPES, and 0.5% bovine serum albumin (BSA) at pH 6.8 (M199s).

The human leukemia T-cell line Jurkat-E6-1 (American Type Culture Collection) was grown in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum and 1% penicillin and 100 μg of streptomycin sulphate/ml at 37°C in a humidified 5% CO\(_2\) atmosphere. Chinese hamster ovary (CHO) cells were grown in 25-cm\^2\_ flasks in 7 ml of MEM-a medium (Gibco) supplemented with 10% fetal bovine serum, 100 μU of penicillin/ml, and 100 μg of streptomycin sulphate/ml and maintained at 37°C in a humidified 5% CO\(_2\) atmosphere. CHO cells were harvested by trypsinization (0.25% for a 3-min incubation) and suspended in M199s.

**Flow cytometry (FACS) analysis.** Intracellular phosphotyrosine quantification was performed as previously described (10). Jurkat cells were chosen for this assay because the level of phosphorylated proteins was high enough to be detected by fluorescence-activated cell sorting (FACS) and because they represent a physiologically relevant target cell. Briefly, Jurkat cells (4 × 10\(^5\)) and *E. histolytica* trophozoites (4 × 10\(^5\)) were suspended in 0.5 ml of M199s, centrifuged at 200 × g for 3 min, and incubated for 15 min at 37°C. When indicated, Jurkat cells were preincubated for 15 min at 37°C with inhibitors, followed by two washes with M199s before they were suspended with amebae. After incubation, cells were fixed with paraformaldehyde (1% for 30 min at 4°C and centrifuged at 200 × g for 3 min. The supernatant was discarded, and the cells were permeabilized with PBS–0.05% saponin for 10 min at room temperature. Nonspecific binding was blocked with PBS–1% BSA for 30 min. The cells were then centrifuged, followed by incubation with FITC-Pyr-MAB (10 μg/ml) for 30 min in 100 μl of PBS–1% BSA. As a negative control, the cells were incubated with irrelevant FITC-conjugated MAB MOPC-21 (10 μg/ml). The cells were washed twice and resuspended in PBS for flow cytometry analysis. Amebac and Jurkat cells were identified by size and density.

**Immunoblotting.** Jurkat or CHO cells (5 × 10\(^5\)) and amebae (5 × 10\(^5\)) were suspended in 0.5 ml of M199s and centrifuged at 200 × g for 3 min. After incubation for the indicated times at 37°C, cells were pelleted, resuspended in 30 μl of 2× Laemmli sample buffer with protease and phosphatase inhibitors (100 μM orthovanadate, 100 μM NaF, 800 μM AEBSF, 50 μM bestatin, 0.4 μM leupeptin, 0.15 μM aprotinin, 0.6 μM E-64, and 400 μM EDTA), and immediately boiled for 5 min. The lysates were microcentrifuged for 1 min and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10 to 12% polyacrylamide gel. After electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) filters (Millipore Corp., Bedford, Mass.) at 400 mA for 2.5 h at 4°C. The blots were blocked overnight at 4°C in Tris-buffered saline–1% Tween 20 (pH 7.4) containing 5% BSA and probed with HRP-Pyr-Ab RC20 or anti-PTB-1B MAB FG6-1G for 4 h at 4°C. Filters were washed five times in Tris-buffered saline–1% Tween 20. Peroxidase-conjugated secondary antibody was applied for 2 h at room temperature when indicated. The blots were developed by using enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, N.J.).

**RESULTS**

*E. histolytica*-induced tyrosine dephosphorylation in target cells. Activation of host PTPases is a common mechanism used by pathogens to avoid host immune responses (5, 13, 22, 26). To investigate whether activation of host cell PTPases might be involved in the pathogenicity of *E. histolytica*, we measured the level of phosphotyrosine proteins in target cells and amebae. We observed a rapid and extensive dephosphorylation in target cells after adherence to *E. histolytica*. Western blot analyses revealed tyrosine dephosphorylated bands from 190 to 35 kDa in both CHO and Jurkat cells lysates following contact with *E. histolytica*, while no detectable changes in the amebic tyrosine phosphorylation profile were observed (Fig. 1). Analysis of cellular phosphorylation was also carried out by flow cytometry.
Fig. 2A). After incubation of Jurkat cells with amebae, the cells were fixed in formaldehyde, and the phosphotyrosine protein levels were determined in Jurkat cells with a fluorescent antiphosphotyrosine monoclonal antibody. *E. histolytica* caused a reduction in target cell phosphotyrosine levels by 30.5% to 96.9% depending on the cell ratio (100:1 to 1:1 Jurkat-*E. histolytica*) utilized in the FACS analysis (Fig. 2B). A time course experiment revealed dephosphorylation in Jurkat cells as quickly as 2.5 min after contact with amebae. After a 30-min incubation, the cellular protein tyrosine level was reduced by 82.4% ± 0.72% (Fig. 3). Ameba-induced protein dephosphorylation was shown to be cell contact dependent, since blocking amebic adherence with galactose (25 mg/ml) inhibited tyrosine dephosphorylation by 66.3% ± 10.4%, while amebic lysates had no effect (100% ± 3.7%) (Fig. 4).

Dephosphorylation in adherent Jurkat cells is reversible. To rule out the possibility that the dephosphorylation observed in target cells was simply a side effect of cell death, the level of tyrosine phosphorylation was measured after blocking amebic adherence to cells with galactose (25 mg/ml).

Fig. 2. FACS analysis of *E. histolytica*-induced protein dephosphorylation in Jurkat cells. (A) Jurkat cells were incubated with *E. histolytica* trophozoites at a 1:10 Jurkat cell-*E. histolytica* ratio for 15 min at 37°C, formaldehyde fixed, permeabilized, and stained with FITC-pTyr-MAb PT-66. The data express the fluorescence intensities from Jurkat cells only. (A) FACS profile of the Jurkat cell population in medium alone (dotted line) or after incubation with amebae (continuous line, shaded area). (B) Phosphotyrosine protein levels in Jurkat cell after incubation with *E. histolytica* at different cell ratios. Cells were incubated for 15 min at 37°C prior to pTyr-FACS analysis. Cells were formaldehyde fixed, permeabilized, and stained with FITC-pTyr-MAb PT-66 for FACS analysis. Data are expressed as the percentage of fluorescence intensity of each sample compared to the basal phosphotyrosine level from Jurkat cells alone (100%). (P < 0.03 at a 100:1 cell ratio and P < 0.001 at 50:1, 10:1, 2:1, and 1:1 cell ratios compared to Jurkat cells alone).

Fig. 3. Time course analysis of *E. histolytica*-induced protein dephosphorylation in Jurkat cells. *E. histolytica* trophozoites incubated with Jurkat cells at 37°C were evaluated at different time points (0, 2.5, 10, 20, and 30 min) and then fixed with formaldehyde. Cells were permeabilized, stained with FITC-pTyr-MAb PT-66, and analyzed by FACS. Symbols: ○, Jurkat cells alone; □, Jurkat cells incubated with *E. histolytica* (P < 0.03 at 10 min and P < 0.005 at 20 and 30 min compared to Jurkat cells alone). The mean fluorescence intensity for the entire Jurkat cell population is expressed in arbitrary units (a.u.).

Fig. 4. Effect of galactose on *E. histolytica*-induced protein dephosphorylation in Jurkat cells. *E. histolytica* trophozoites were incubated with Jurkat cells (at a 10:1 Jurkat cell-*E. histolytica* ratio) for 15 min at 37°C in control medium (open bar) or in the presence of either galactose (25 mg/ml) (solid bar) or amebic lysates (gray bar). Cells were formaldehyde fixed, permeabilized, and stained with FITC-pTyr-MAb PT-66 for FACS analysis. Data are expressed as the percentage of fluorescence intensity of each sample compared to the basal phosphotyrosine level from Jurkat cells alone (100%). * P < 0.03 compared to the control medium.
Jurkat cells were incubated for 5 min, and then galactose was added in order to disrupt amebic adherence to cells. The cell suspensions were further incubated for 15, 30, and 60 min at 37°C (solid bars). Error bars represent the percentage of Jurkat cell phosphorylation by 28.9% ± 2.5%, whereas prior exposure to amebae had no effect (Fig. 6B).

**Calpeptin inhibits E. histolytica-induced tyrosine dephosphorylation.** Calpain has been shown to induce proteolytic modification and activation of proteins associated with multiple signaling events, such as protein tyrosine kinases (pp60<sup>src</sup> and focal adhesion kinase [FAK]) and PTPases (PTP1B and SHP-1) (1, 7, 24). In this study we observed that calpeptin, a cell-permeable calpain inhibitor, inhibited ameba-induced dephosphorylation in Jurkat cells (Fig. 7). This effect was dependent on the cell ratio. No inhibition in dephosphorylation was observed at a 10:1 Jurkat cell-E. histolytica cell ratio. However, Jurkat cell dephosphorylation was inhibited by 11.7% ± 4.99% and 50.2% ± 5.7%, respectively, when the cells were incubated at 50:1 and 100:1 cell ratios (Fig. 7). We concluded that the inhibition of host cell dephosphorylation with calpeptin could be overcome by decreasing the number of amebae interacting with the host cells.

**E. histolytica-induced cleavage of PTP1B.** PTP1B may be implicated in ameba-induced protein dephosphorylation since it is activated by calpain-mediated proteolytic cleavage. In order to confirm this hypothesis, Western blot analyses were carried out to determine if cleavage of PTP1B occurred after exposure of Jurkat cells to E. histolytica. Jurkat cells (10<sup>6</sup> cells) were incubated with E. histolytica (10<sup>7</sup> cells) for 15 min at 37°C and immediately lysed with boiling sample buffer. The cell lysates were subjected to SDS-PAGE, followed by immunoblot analysis with monoclonal antibody against the catalytic domain (N-terminal portion) of PTP1B. In control samples (Jurkat cells alone), PTP1B migrated as a 50-kDa band, corresponding to the full-length form of the phosphatase (Fig. 8). The antibody also detected two additional PTP1B-like proteins at 36 and 40 kDa. This PTP1B immunoblot profile was similar to that previously described for ME-180 cell lysates (25). Exposure of Jurkat cells to E. histolytica induced the appearance of a PTP1B cleaved form at 42 kDa. Incubation of the cells in the presence of galactose (25 mg/ml) or EGTA (5 mM) inhibited PTP1B cleavage. Moreover, the 42-kDa cleaved form of PTP1B was not detected when Jurkat cells were pretreated with calpeptin (1 mM). These results indicate that, upon contact, E. histolytica induces PTP1B cleavage in Jurkat cells and that Ca<sup>2+</sup> and calpain may play a role in this event.

PTPase inhibitor (PAO) inhibits E. histolytica-induced Jur-
kat cell apoptosis. *E. histolytica* has been shown to induce apoptosis in several cell types (16, 27). To determine whether host cell protein tyrosine dephosphorylation might play a role in ameba-induced apoptosis, we investigated the effect of PAO on Jurkat cell DNA fragmentation by *E. histolytica*. Prior exposure of Jurkat cells to PAO (1.0 mM) blocked DNA fragmentation by amebae (Fig. 9A), suggesting that host cell PTPases may be involved in this amebic killing mechanism. We also evaluated the effect of calpeptin on Jurkat cell DNA fragmentation. Pretreatment of Jurkat cells with calpeptin had no effect on blocking DNA fragmentation by amebae. These experiments were carried out at 10:1 and 50:1 cell ratios (Jurkat cells to *E. histolytica*). We were not able to test the effect of calpeptin at a 100:1 cell ratio because of the low sensitivity of the method, i.e., no DNA fragmentation was detected in the controls at this condition (Fig. 9B). The fact that calpeptin was not able to block Jurkat cell DNA fragmentation was expected, since calpeptin only partially inhibited ameba-induced dephosphorylation.

**FIG. 7.** Effect of calpeptin on *E. histolytica*-induced protein dephosphorylation in Jurkat cells. Pretreatment of Jurkat cells with calpeptin had no effect on blocking DNA fragmentation by amebae. The y axis represents the percentage of inhibition of ameba-induced dephosphorylation.

**FIG. 8.** Detection of PTP1B cleavage by Western blot analysis. Jurkat cells were incubated with *E. histolytica* (at a 10:1 Jurkat cell-*E. histolytica* ratio) for 15 min at 37°C in medium alone or in the presence of galactose (25 mg/ml), EGTA (5 mM), or calpeptin (1 mM) and lysed with boiling sample buffer. Proteins from lysates were separated by SDS–10% PAGE, transferred to PVDF membrane, and incubated with anti-PTP1B MAb, clone FG6-1G. The relative migration of 50-, 42-, 40-, and 36-kDa PTP1B proteins are depicted with arrows and are based upon estimates of molecular standards.
DISCUSSION

Modulation of tyrosine phosphorylation-based signaling pathways represents an important mechanism whereby pathogens may interfere with host cell biology. In this study we demonstrated that contact with *E. histolytica* induced a rapid dephosphorylation in CHO and Jurkat target cells. Ameba-induced dephosphorylation required Gal/GalNAc lectin-mediated contact between amebae and target cells, since blocking amebic adherence with galactose inhibited tyrosine dephosphorylation. Dephosphorylation occurred before cell death as demonstrated by recovery of phosphorylation in Jurkat cells after disruption of ameba-adherent cells with galactose. Prior exposure of Jurkat cells to PAO, but not to *E. histolytica*, protected the cells from ameba-induced dephosphorylation, suggesting that PTPases are activated in the target cells. Intracellular Ca$^{2+}$ influx after contact with amebae may trigger dephosphorylation in target cells. Studies have shown that calpain, a calcium-activated cysteine proteinase, is able to cleave and thus activate PTPases such as PTP1B and SHP-1 (11, 24, 32). Specific proteolysis of PTP1B stimulates its enzymatic activity, possibly through disruption of its association with the endoplasmic reticulum membrane facilitating its access to cellular substrates (11, 12). PTP1B cleavage and activation has been demonstrated in many cell types and is associated with extensive cellular protein dephosphorylation (24, 25). In the present study we observed proteolytic cleavage of PTP1B (42-kDa fragment) in Jurkat cells after adhesion to *E. histolytica*. Calpain seems to participate in this process since its inhibition by calpeptin blocked PTP1B cleavage and also protected Jurkat cells from ameba-induced dephosphorylation.

Modulation of host cell PTPase is a strategy evolved by various microorganisms to evade cellular host defense (5, 13, 22, 26). Thus, enhanced intracellular PTPase activity might play a role in *E. histolytica* pathogenicity. YopH, a virulence *Yersinia* PTPase, has been shown to cause dephosphorylation of target cell proteins, interrupting early phosphotyrosine signaling events associated with integrin-mediated bacterial uptake (8). Upon contact with the eukaryotic target cell, the bacteria secrete YopH, which translocates through the plasma membrane into the interior of the cell. YopH acts by interacting and dephosphorylating cytoskeleton-associated proteins such as p130Cas and FAK, resulting in disruption of F-actin stress fibers and focal adhesion complexes (4, 26). Similar to YopH, PTP1B also negatively regulates integrin-mediated signaling pathways (6, 20). Both the full-length and the proteolytic cleaved forms (42 kDa) of PTP1B are able to bind and dephosphorylate p130Cas (15, 18, 32). Overexpression of PTP1B in rat 3Y1 fibroblasts was shown to interfere with cell spreading, cytoskeletal architecture, and the formation of focal adhesion complexes (2, 20). It is interesting that, upon contact with *E. histolytica*, target cells rapidly become round, a phenomenon that also occurs when target cells are exposed to YopH and is indicative of disruption of cytoskeleton-associated proteins. It is therefore possible that *E. histolytica* has evolved a strategy similar to *Yersinia* to evade or inhibit cellular mechanisms of host defense. Thus, *E. histolytica* might resist phagocytosis by inducing extensive protein dephosphorylation in host cells. Calpain-mediated cleavage and activation of PTP1B may play a general role in this process by promoting dephosphorylation of focal adhesion-associated proteins.

*E. histolytica*-induced protein dephosphorylation might also be part of an endogenous pathway associated with programmed cell death. *E. histolytica* can kill host cells by apoptosis (27). Several studies have shown that cells killed by *E. histolytica* present characteristics of apoptosis, such as membrane blebbing, chromatin condensation, and internucleosomal DNA fragmentation (16, 27, 33). The molecular pathways involved in *E. histolytica*-induced apoptosis are not fully understood. *E. histolytica* has been shown to induce apoptosis in murine hepatocytes by a non-Fas-dependent and non-tumor...
necrosis alpha-dependent pathway (33). In a more recent study, it was demonstrated that E. histolytica-induced apoptosis required activation of host cell caspase-3 and was independent of caspase-8 and caspase-9 (16).

In this study we demonstrated that E. histolytica-induced Jurkat cell apoptosis was blocked by PAO, suggesting that host cell PTPases may play a role in this killing mechanism. Although calpeptin inhibited amebic-induced dephosphorylation in Jurkat cells at a 100:1 cell ratio, we were not able to demonstrate its effect on ameba-induced apoptosis under this condition. The fact that calpeptin only partially inhibited host cell dephosphorylation suggests that calpain and PTP1B activation might participate in E. histolytica-induced apoptosis. However, PTPases other than PTP1B may also be involved in this mechanism. Protein dephosphorylation has been reported to be involved in apoptosis (34, 35). Exposure of renal epithelial cells to nephrotoxic drugs causes dephosphorylation of FAK, resulting in the loss of focal adhesion and actin stress fibers, which precedes the onset of apoptosis. These events are independent of caspase activation and occur before caspase-3 activity (35). Focal adhesion organization seems to be important in the maintenance of cell survival signaling. It has been proposed that apoptotic suppressive signaling pathways downstream from FAK could be lost as a consequence of the dephosphorylation of focal-adhesion-associated proteins (34). PTP1B might be involved in this apoptotic mechanism, since focal-adhesion-associated proteins are dephosphorylated by this phosphatase (14, 15). Moreover, cleavage of PTP1B must be a key step in this mechanism. In support of this is the fact that treatment of apoptosis-sensitive cell lines with tumor necrosis factor results in the accumulation of PTP1B-related proteins, including the 42-kDa form, in the cytoplasm, whereas the treatment of resistant cells has no effect (25). Thus, we suggest that E. histolytica may induce apoptosis in host cells by a mechanism whereby protein dephosphorylation may play an important role. We propose a model where Ca\(^{2+}\) acts as a second messenger initiating ameba-induced apoptosis by activating host cell proteases. Host cell PTPases are then activated by these proteases, resulting in cellular protein tyrosine dephosphorylation. For example, PTP1B may be cleaved and activated by calpain, facilitating its access to cellular substrates. Focal-adhesion-associated proteins, such as p130Cas, FAK, and paxillin, are then dephosphorylated by PTP1B, resulting in the disruption of focal adhesions and actin stress fibers. As a result, signaling survival pathways are inactivated and the apoptotic machinery is activated (caspase-3 activation).

In this study we investigated the mechanism of host cell death in Jurkat cells, a T-lymphocyte cell line. It is possible that E. histolytica may induce cell death by a different mechanism in other cell types. Future studies will determine which host cell PTPases are being activated in this process. This will allow us to investigate whether a similar process is occurring in other cell types.

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