Characterization of *Entamoeba histolytica*-induced dephosphorylation in Jurkat cells

J E TEIXEIRA* and B J MANN*[,†,§]

Departments of Internal Medicine and Microbiology*[,†], University of Virginia, Charlottesville, VA 22908, USA*

[§]*Corresponding author (Fax, 434-924-0075; Email, bjm2r@virginia.edu)*

Entamoeba histolytica killing of host cells is contact dependent and mediated by a Gal/GalNAc lectin. Upon contact with amoeba a rapid and extensive dephosphorylation of tyrosine phosphorylated host cell proteins is observed. This effect is mediated by the Gal/GalNAc lectin. However, it requires intact cells, as purified lectin failed to induce dephosphorylation in Jurkat cells. The nonpathogenic, but morphologically identical amoeba, *Entamoeba moshkovskii* also did not induce dephosphorylation in target cells. Treatment of Jurkat cells with phosphotyrosine phosphatase inhibitors has shown that a host phosphatase is responsible for dephosphorylation. However, it was found that the CD45 phosphotase was not necessary for dephosphorylation of host cell proteins.

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1. Introduction

Entamoeba histolytica-induced killing of target cell is contact-dependent, extracellular and mediated by a galactose/N-acetyl D-galactosamine (Gal/GalNAc) inhibitable lectin (McCoy *et al* 1994). The mechanisms involved in target cell death are not completely understood. Many of the characteristics of target cell death are consistent with apoptosis. Cells killed by *E. histolytica* undergo nuclear chromatin condensation, membrane blebbing, internucleosomal DNA fragmentation, and caspase 3 activation (Ragland *et al* 1994; Huston *et al* 2000). *E. histolytica*-mediated cell death occurs by a mechanism that involves activation of target cell phosphotyrosine phosphatases (PTPases). Protein dephosphorylation is observed in target cells upon interaction with *E. histolytica* (Teixeira and Mann 2000). Dephosphorylation of host cell proteins is dependent upon cell contact through the Gal/GalNAc lectin. Pretreatment of target cells with phosphatase inhibitors prevents dephosphorylation of host cell proteins indicating that activation of a host cell phosphatase is responsible for the dephosphorylation. Inhibition of target cell PTPases with phenylarsine oxide also blocks target cell apoptosis induced by *E. histolytica*. One PTPase that is activated upon contact with amoeba is PTP1B.

Using Jurkat cells as a model system, the mechanisms and characteristics of *E. histolytica* induced dephosphorylation of host cell proteins were further explored. Dephosphorylation was found to require intact cells and did not occur when the nonpathogenic species *Entamoeba moshkovskii* was incubated with target cells. The surface expressed phosphotyrosine phosphatase CD45 was not required for dephosphorylation.

2. Materials and methods

2.1 *Polyclonal and monoclonal antibodies*

FITC-conjugated mouse IgG1, ***k*** (MOPC-21) and FITC-conjugated mouse anti-phosphotyrosine (FITC-pTyr-mAb), clone PT-66 were purchased from Sigma.

Keywords. Apoptosis; CD45; *Entamoeba moshkovskii*; Jurkat cells

Abbreviations used: CHO, Chinese hamster ovary; Gal/GalNAc, galactose/N-acetyl D-galactosamine; PTPases, phosphotyrosine phosphatases.

2.2 *Cell lines and culture*

E. histolytica (HM1 : IMSS) trophozoites were grown axenically in TYI-S-33 (trypticase yeast extract, iron and serum) medium supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin sulphate at 37°C as previously described (Diamond *et al* 1978). Trophozoites were harvested after 48–72 h during the logarithmic phase, by chilling the culture tubes on ice for 10 min. After centrifugation at 200 *g* at 4°C for 5 min, the trophozoites were resuspended in medium 199 (Gibco BRL, Grand Island, NY) supplemented with 5·7 mM cysteine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), and 0·5% bovine serum albumin (BSA) at pH 6·8 (M199s).

The human T leukemia-cell line Jurkat-E6-1 (American Type Culture Collection) or CD45-deficient J45.01 (gift of G A Koretzky) (Koretsky *et al* 1991) were grown in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin sulphate 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% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin sulphate and maintained at 37°C in a humidified 5% CO_2 atmosphere. CHO cells were harvested by trypsinization (0·25% for 3 min incubation) and suspended in M199s.

2.3 *Flow cytometry (FACS) analysis*

Quantification of intracellular phosphotyrosine was performed as previously described (Far *et al* 1994). Briefly, Jurkat cells (4×10^5) and *E. histolytica* trophozoites (4×10^4) 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 or trophozoites were preincubated for 15 min at 37°C with inhibitors, followed by two washes with M199s before they were suspended with amoebae. After incubation, cells were fixed with PBS-1% formaldehyde, pH 7·2 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-pTyr-mAb PT-66 (10 μg/ml) for 30 min in 100 μl of PBS-1% BSA. As 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. Amoebae and Jurkat cells were identified by size and density.

3. Results

3.1 E. histolytica-*induced dephosphorylation requires intact amoeba*

It has been previously shown that *E. histolytica* induces a rapid and extensive dephosphorylation in both CHO and Jurkat cells upon contact, while no changes in phosphorylated proteins are detected in the trophozoites (Teixeira and Mann 2002). NEWS It was further demonstrated that this effect was mediated by the Gal/GalNAc lectin since the addition of galactose blocked dephosphorylation. The ability of purified lectin to induce dephosphorylation in Jurkat cells was examined by FACS. Jurkat cells were incubated with amoebae or lectin as described in the methods. The cells were fixed, permeabilized and then stained with an antiphosphotyrosine antibody. The scan was gated on the Jurkat cells so that only the fluorescence intensity of the Jurkat cells was measured. Incubation of Jurkat cells with intact amoeba resulted in an 87·4% decrease in phosphorylation in the Jurkat cells (figure 1). When Jurkat cells were incubated with 40–210 μg/ml of purified lectin, no significant dephosphorylation in target cells was detected. In some experiments, a slight decrease in phosphorylation was observed, but this was not reproducible with independent experiments. Amoebic lysates also had no effect.

3.2 *Nonpathogenic* E. moshkovskii *does not induce dephosphorylation in target cells*

E. moshkovskii is a nonpathogenic amoeba that also colo-

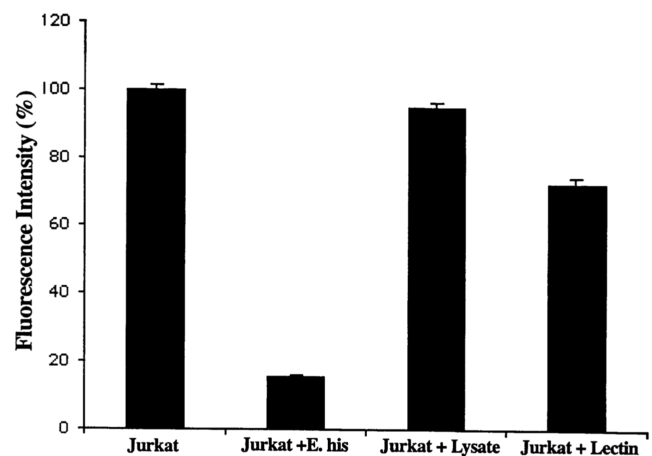

Figure 1. Purified Gal/GalNAc lectin or amoebic lysates fail to induce dephosphorylation in target cells. Cells were incubated with *E. histolytica* (1 : 10 ratio), 40 μg/l lectin or amoebic lysates for 15 min at 37°C, formaldehyde fixed, permeabilized and stained with FITC-ptyr Mab PT66. Phosphotyrosine protein levels in Jurkat cells only by FACS. Data are expressed as the percentage of fluorescence intensity of each sample compared to the basal level of Jurkat cells alone.

nizes humans and is morphologically indistinguishable from *E. histolytica* in both the cyst and trophozoite stages. Jurkat cells were incubated with either *E. histolytica* or *E. moshkovskii* in a 1 : 10 ratio. The cells were incubated for 15 min at 37°C, then prepared for FACS analysis. In this set of experiments, interaction with *E. histolytica* induced a 70·2% drop in phosphotyrosine levels, whereas incubation with *E. moshkovskii* produced an insignificant drop of 9·4% (figure 2).

3.3 *CD45 tyrosine phosphatase is not involved in* E. histolytica-*induced dephosphorylation*

The tyrosine phosphatase CD45 is a key regulator of antigen receptor signalling in T and B cells. Phosphotyrosine kinase activation by the T cell receptor requires the surface expression of CD45. To test whether CD45 played a role in *E. histolytica*-induced dephosphorylation, trophozoites were incubated with a Jurkat cell line, J45.01, that lacks CD45 (Koretzky *et al* 1991). Phosphorylation levels in the Jurkat cells were measured by FACS. *E. histolytica* produced a 81·5% drop in tyrosine phosphorylation in wild-type Jurkat cells, and a 82·3% decrease in CD45-Jurkat cells (figure 3). These results suggest that CD45 does not play a significant role in the amoeba-induced dephosphorylation of target cell proteins.

4. Discussion

Upon contact with target cells *E. histolytica* induces a rapid dephosphorylation by activating host PTPases. This may be an early step in the eventual apoptotic death of target cells. Interaction of target cells with the amoebic Gal/GalNAc lectin is necessary for dephosphorylation to occur. Target cell death is also mediated by the Gal/GalNAc lectin (Saffer and Petri 1991). However, both of these activities require intact cells. Jurkat cells incubated with purified Gal/GalNAc lectin or amoebic lysates failed to induce any measurable dephosphorylation. This suggests that even the early cytolytic events are complex processes.

The failure of *E. moshkovskii* to induce dephosphorylation in target cells supports the connection between dephosphorylation and pathogenicity. *E. moshkovskii* is a human commensal organism that was originally identified as a free-living amoeba in sewage from a Moscow sewer. Later the *E. histolytica*-like amoeba known as the Laredo strain was determined to be *E. moshkovskii* (Clark and Diamond 1991). The original Laredo strain was isolated from a Laredo, Texas resident who was experiencing diarrhea, weight loss and epigastric pain. However, these symptoms were later found to be due to a benign tumour of the colon. Although *E. histolytica* and *E. moshkovskii* are morphologically very similar, a comparison of rRNA genes suggest that they are not closely related (Clark and Diamond 1991). Sequencing of more genes from *E. moshkovskii* should help to further define this relationship. Unpublished observations suggest that *E. moshkovskii* may lack a homologue of the Gal/GalNAc lectin.

Modulation of host cell PTPases is used by disparate pathogens as a means of evading host defenses and/or establishing a niche within the host environment. *Leishmania donovani* and *Mycobacterium tuberculosis* are able

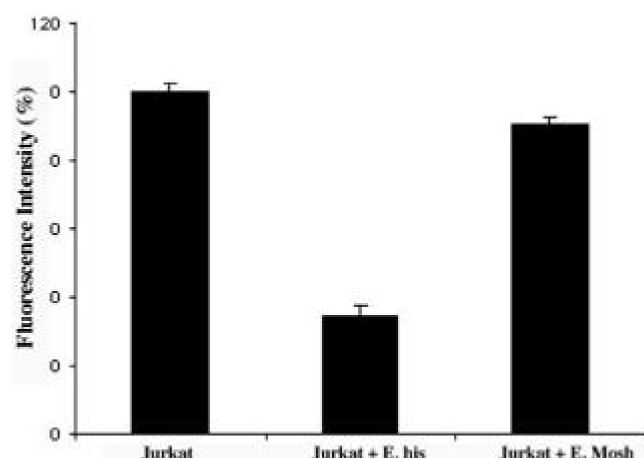

Figure 2. *E. moshkovskii* fails to induce dephosphorylation in Jurkat cells. Jurkat cells were incubated with *E. histolytica* or *E. moshkovskii* at a 1 : 10 ratio for 15 min at 37°C prior to analysis. Phosphotyrosine protein levels in Jurkat cells only by FACS. Data are expressed as the percentage of fluorescence intensity of each sample compared to the basal level of Jurkat cells alone.

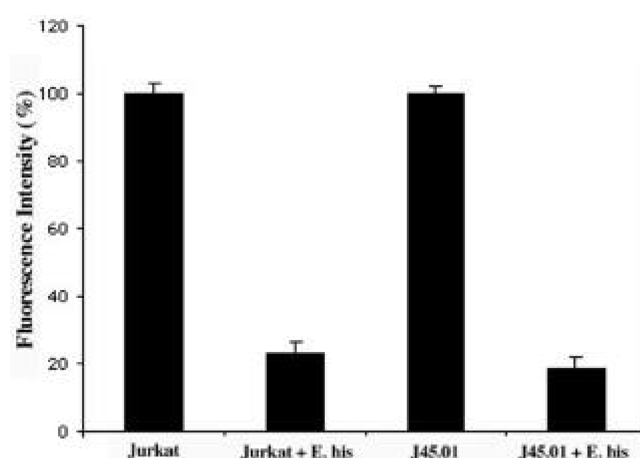

Figure 3. *E. histolytica* induces dephosphorylation in Jurkat cells lacking CD45 phosphatase. Jurkat cells or Jurkat cell line J45.01 were incubated with *E. histolytica* at a 1 : 10 ratio for 15 min at 37°C prior to analysis. Phosphotyrosine protein levels in Jurkat cells only by FACS. Data are expressed as the percentage of fluorescence intensity of each sample compared to the basal level of Jurkat cells alone.

to inhibit macrophage responses by activating SHP-1, a phosphatase that regulates mitogen-activated protein kinase signalling cascades (Blanchette *et al* 1999; Nandan *et al* 2000) *Yersinia* sp. avoid phagocytosis by introducing a bacterial PTPase, YopH into host cells (Persson *et al* 1997). YopH has some similarity of PTP1B, a ubiquitous PTPase that controls a number of signalling pathways. Activation of PTP1B occurs in some cell lines undergoing apoptosis, suggesting an involvement of PTP1B in program cell death (Perez *et al* 1999). PTP1B activation occurs upon contact with *E. histolytica* as evidenced by the appearance of the active form of the enzyme (Teixeira and Mann 2002). PTP1B is cleaved and activated by the calcium-dependent protease calpain (Frangioni *et al* 1993). Treatment of target cells with calpeptin, a calpain inhibitor, results in blocked PTP1B activation and an inhibition of dephosphorylation. Calpain, a calcium-dependent proteinase, has been shown to be an upstream promoter of apoptosis (Squier and Cohen 1997).

To begin to examine whether other phosphatases were involved or required for *E. histolytica*-induced dephosphorylation, *E. histolytica* was incubated with a Jurkat cell line that lacks the CD45 phosphatase. CD45 is a phosphatase that is found on the surface of B and T cells (Koretzky *et al* 1991). CD45 is required for activation of T cell receptor-associated tyrosine kinase as well as the phosphatidylinositol pathway. *E. histolytica*-induced dephosphorylation in Jurkat cells lacking CD45 was comparable to wild-type Jurkat cell. These results demonstrate that the *E. histolytica*-induced dephosphorylation of target cells is independent of CD45 regulated pathways.

The mechanism of cell death in Jurkat cells, a T cell line, occurs by a mechanism that involves dephosphorylation. The mechanism by which PTP1B and perhaps other PTPases becomes activated by the amoeba is still not understood. Identification of receptor for the Gal/GalNAc lectin and other parasite and host molecules should help to delineate the process of *E. histolytica*-mediated cell death.

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References

Blanchette J, Racette N, Faure R, Siminovitch K A and Olivier M 1999 *Leishmania*-induced increases in activation of macrophage SHP-1 tyrosine phosphatase are associated with impaired IFN-*g*-triggered JAK2 activation; *Eur. J. Immunol.* **29** 3737–3744

Clark C G and Diamond L S 1991 The Laredo strain and other *Entamoeba histolytica*-like amoebae are *Entamoeba moshkovskii*; *Mol. Biochem. Parasitol.* **46** 11–18

Diamond L S, Harlow D R and Cunnick C C 1978 A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*; *Trans. R. Soc. Trop. Med. Hyg.* **72** 431–432

Far D F, Peyron J F, Imbert V and Rossi B 1994 Immunofluorescent quantification of tyrosine phosphorylation of cellular proteins in whole cells by flow cytometry; *Cytometry* **15** 327–334

Frangioni J V, Oda A, Smith M, Salzman E W and Nell B G 1993 Calpain-catalyzed cleavage and subcellular relocation of protein phosphotyrosine phosphatase 1B (PTP 1B) in human platelets; *EMBO J.* **12** 4843–4856

Huston C D, Houpt E R, Mann B J, Hahn C S and Petri W A Jr 2000 Caspase 3-dependent killing of host cells by the parasite *Entamoeba histolytica*; *Cell. Microbiol.* **2** 617–625

Koretzky G A, Picus J, Schultz T and Weiss A 1991 Tyrosine phosphatase CD45 is required for T-cell antigen receptor and CD2-mediated activation of a protein tyrosine kinase and interleukin 2 production; *Proc. Natl. Acad. Sci. USA* **88** 2037–2041

McCoy J J, Mann B J and Petri W A Jr 1994 Adherence and cytotoxicity of *Entamoeba histolytica* or how lectins let parasites stick around; *Infect. Immun.* **62** 3045–3050

Nandan D, Knutson K L, Lo R and Reiner N E 2000 Exploitation of host cell signaling machinery: activation of macrophage phosphotyrosine phosphatses as a novel mechanism of molecular microbial pathogenesis; *J. Leul. Biol.* **67** 464–470

Perez M, Haschke B and Donato N J 1999 Differential expression and translocation of protein tyrosine phosphatase 1B-related proteins in ME-180 tumour cells expressing apoptotic sensitivity and resistance to tumour necrosis factor; potential interaction with epidermal growth factor receptor; *Oncogene* **18** 967–978

Persson C, Carballeira N, Wolf-Watz H and Fullman M 1997 The PTPase YopH inhibits uptake of *Yersina*, tyrosine phosphorylation of $p130^{cas}$ and FAK, and the associated accumulation of these proteins in peripheral focal adhersions; *EMBO J.* **16** 2307–2318

Ragland B D, Ashley L S, Vaux D L and Petri W A Jr 1994 *Entamoeba histolytica*: target cells killed by trophozoites undergo DNA fragmentation which is not blocked by Bcl-2; *Exp. Parasitol.* **79** 460–467

Saffer L D and Petri W A Jr 1991 Role of the galactose lectin of *Entamoeba histolytica* in adherence-dependent killing of mammalian cells; *Infect. Immun.* **59** 4681–4683

Squier M K T and Cohen J J 1997 Calpain, an upstream regulator of thymocyte apoptosis; *J. Immunol.* **158** 3690–3697

Teixeira J E and Mann B J 2002 *Entamoeba histolytica*-induced dephosphorylation in host cells; *Infect. Immun.* **70** 1816–1823