Use of recombinant Entamoeba histolytica cysteine proteinase 1 to identify a potent inhibitor of amebic invasion in a human colonic model

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Cysteine proteinases are key virulence factors of the protozoan parasite *Entamoeba histolytica*. We have shown that cysteine proteinases play a central role in tissue invasion and disruption of host defenses by digesting components of the extracellular matrix, immunoglobulins, complement, and cytokines. Analysis of the *E. histolytica* genome project has revealed more than 40 genes encoding cysteine proteinases. We have focused on *E. histolytica* cysteine proteinase 1 (EhCP1) because it is one of two cysteine proteinases unique to invasive *E. histolytica* and is highly expressed and released. Recombinant EhCP1 was expressed in *Escherichia coli* and refolded to an active enzyme with a pH optimum of 6.0. We used positional-scanning synthetic tetrapeptide combinatorial libraries to map the specificity of the P1 to P4 subsites of the active site cleft. Arginine was strongly preferred at P2, an unusual specificity among clan CA proteinases. A new vinyl sulfone inhibitor, WRR483, was synthesized based on this specificity to target EhCP1. Recombinant EhCP1 cleaved key components of the host immune system, C3, immunoglobulin G, and pro-interleukin-18, in a time- and dose-dependent manner. EhCP1 localized to large cytoplasmic vesicles, distinct from the sites of other proteinases. To gain insight into the role of secreted cysteine proteinases in amebic invasion, we tested the effect of the vinyl sulfone cysteine proteinase inhibitors K11777 and WRR483 on invasion of human colonic xenografts. The resultant dramatic inhibition of invasion by both inhibitors in this human colonic model of amebiasis strongly suggests a significant role of secreted amebic proteinases, such as EhCP1, in the pathogenesis of amebiasis.

The intestinal protozoan parasite *Entamoeba histolytica* is the etiologic agent of amebic colitis and liver abscess, which cause high rates of morbidity and mortality worldwide (49). The mechanism by which *Entamoeba histolytica* is able to invade and damage the host’s target tissues has been the subject of intense research. Several virulence factors have been identified, including secreted cysteine proteinases (39, 42). These amebic enzymes have been implicated in the in vitro cytopathology of cell monolayers (20, 23), which correlates with the observed separation of colonic epithelial cells before invasion (51). Other correlates with invasion include the ability of cysteine proteinases to degrade extracellular matrix components (19) and colonic mucin (31, 32). Furthermore, cysteine proteinases enable *E. histolytica* to evade the host’s immune defenses by activating and locally depleting complement (43), and by degrading anaphylotoxins C3a and C5a (41), human immunoglobulin G (IgG) (53), human IgA (21), and interleukin-18 (IL-18) (37).

The recent completion of the *Entamoeba histolytica* genome project has revealed the presence of at least 40 genes encoding cysteine proteinases (25). Of all the cysteine proteinase genes, only *ehcp1* and *ehcp5* are unique to *E. histolytica*, as their orthologs are either absent (*ehcp1*) or nonfunctional (*ehcp5*) in *Entamoeba dispar*, a morphologically identical but noninvasive *Entamoeba* species (5, 6, 54). Surprisingly, only a small subset of these genes are expressed in cultured *Entamoeba histolytica* trophozoites (6), and only three, *ehcp1*, *ehcp2*, and *ehcp5*, account for more than 90% of the cysteine proteinase-specific transcripts in culture (5, 6). Quantitative studies of the expression of the major *Entamoeba histolytica* cysteine proteinases have shown that *E. histolytica* cysteine proteinase 1 (EhCP1) is one of the most highly expressed and released cysteine proteinases in cultured trophozoites (6, 8, 17). In a recent study of gene expression in a mouse model of amebic colitis, EhCP1 expression was increased almost twofold following invasion, while expression of EhCP5, the other *E. histolytica*-specific proteinase, was not (11). To further characterize this impor-
tant amebic cytochrome proteins, we have cloned, expressed, and refolded recombinant EhCP1 (rEhCP1) to obtain active proteinase. We now show that rEhCP1 can cleave physiologic substrates, such as the third component of complement, pro-IL-18, and IgG, components of the host immune response which must be circumvented for the amebae to invade. We designed a new inhibitor based on the marked preference of EhCP1 for arginine in the P2 position. We now show for the first time that specific inhibitors of cytochrome proteinases block invasion in the human colon.

**MATERIALS AND METHODS**

*Entamoeba* cultures and purification of genomic DNA. *E. histolytica* strain HM1:IMSS was grown axenically in TYI-S-33 medium (9) and subcultured every 48 to 72 h. *E. histolytica* genomic DNA was purified from trophozoite nuclei with the DNeasy Kit (QIAGEN, Valencia, CA).

Isolation and purification of released amebic cysteine proteinases. Released proteinases in conditioned medium (CM) from *Entamoeba histolytica* trophozoites were prepared as previously described in phosphate-buffered saline supplemented with 20% CM which was preincubated for 15 min at room temperature in buffer alone or containing 80 μM E-64 (an irreversible inhibitor of all cysteine proteinases) and the cleavage products were analyzed by SDS-PAGE (24) (30). The pH optimum of rEhCP1 was determined by preincubating rEhCP1 (50 nmol) in standard assay buffer (17) (for pH 7.0 to 9.0 or with 25 mM sodium acetate for pH 4.0 to 6.5) and the remaining enzymatic activity was determined as described above.

Cloning of biologic substrates by purified, active rEhCP1. Human C3 (1 μg; Quidel, San Diego, CA) was incubated with increasing amounts of active purified rEhCP1 or CM which was preincubated for 15 min at room temperature in buffer alone or containing 80 μM E-64 (an irreversible inhibitor of all cysteine proteinases) for 60 min at 37°C, and the cleavage products were analyzed by SDS-PAGE as previously described (43). Cleavage of native human IgG (2 μg; Sigma, St. Louis, MO) was performed under similar conditions except for 16-h incubation. The resulting immunoblots were probed with goat anti-human IgG–horseradish peroxidase conjugate (1:2000; Zymed, San Francisco, CA) and developed with SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL). Human pro-IL-18 was expressed in *E. coli* as previously detailed (22, 37) and incubated with increasing amounts of purified active rEhCP1 (active CM) in 1% (v/v) 3-[(3-cholamidopropyl)dimethylammonio] propane sulfonate (CHAPS) (37, 50) or 0.1% Triton X-100, and for immunoblots, rEhCP1 was electrophoresed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. For immunoblot assays, rEhCP1 was electrophoresed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis using the MiniPROTEAN III apparatus (Bio-Rad Laboratories, Hercules, CA) in 50 mM Trıton X-100 (v/v) buffer under reducing conditions. The proteinase activity was determined by measuring the release of the fluorescent leaving group, 4-amino-7-methylcoumarin (AMC group) from AMC substrates using a Genios pro Microplate Spectrophotometer (Tecan, Mannedorf, Switzerland) for elapsed time (40). The pH optimum of rEhCP1 was determined by preincubating rEhCP1 (20 to 300 nM) to 10-fold dilutions of inhibitor (500 nM to 100 μM) with 10 μM Z-Arg-Arg-AMC (Km = 2 μM) on a Flex Station with robotics (Molecular Devices) and an N-terminal read time. The value of ka observation, the rate constant for loss of enzymatic activity, was determined from an equation for pseudo-first-order dynamics using Prism 4 (GraphPad). When ka observation varied linearly with inhibitor concentration, Km, was determined by linear regression analysis (41). If the variation was hyperbolic, indicating saturation inhibition kinetics, ka observation and Km were determined from an equation describing a two-step irreversible inhibitor mechanism (ka observation = ka observation [I] + Km + |Km + (I + [S])[Km/H1]| and nonlinear regression analysis using Prism (41).

**Amebic infection of a human intestinal xenograft model.** Human intestinal xenografts were transplanted subcutaneously into the backs of SCID mice and infected with *E. histolytica* trophozoites as previously detailed (28, 46, 55). Xenografts were injected with medium alone, *E. histolytica* trophozoites (1 × 106) in medium, or trophozoites preincubated with K11777 (20 μM) with 19 amino acids; and the remaining two positions were randomized. The pH optimum of rEhCP1 was determined by preincubating rEhCP1 (30 nmol) in standard assay buffer (17) (for pH 7.0 to 9.0 or with 25 mM sodium acetate for pH 4.0 to 6.5) and the remaining enzymatic activity was determined as described above.

Cloning of biologic substrates by purified, active rEhCP1. Human C3 (1 μg; Quidel, San Diego, CA) was incubated with increasing amounts of active purified rEhCP1 or CM which was preincubated for 15 min at room temperature in buffer alone or containing 80 μM E-64 (an irreversible inhibitor of all cysteine proteinases) for 60 min at 37°C, and the cleavage products were analyzed by SDS-PAGE as previously described (43). Cleavage of native human IgG (2 μg; Sigma, St. Louis, MO) was performed under similar conditions except for 16-h incubation. The resulting immunoblots were probed with goat anti-human IgG–horseradish peroxidase conjugate (1:2000; Zymed, San Francisco, CA) and developed with SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL). Human pro-IL-18 was expressed in *E. coli* as previously detailed (22, 37) and incubated with increasing amounts of purified active rEhCP1 (active CM) in 1% (v/v) 3-[(3-cholamidopropyl)dimethylammonio] propane sulfonate (CHAPS) (37, 50) or 0.1% Triton X-100, and for immunoblots, rEhCP1 was electrophoresed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis using the MiniPROTEAN III apparatus (Bio-Rad Laboratories, Hercules, CA) in 50 mM Trıton X-100 (v/v) buffer under reducing conditions. The proteinase activity was determined by measuring the release of the fluorescent leaving group, 4-amino-7-methylcoumarin (AMC group) from AMC substrates using a Genios pro Microplate Spectrophotometer (Tecan, Mannedorf, Switzerland) for elapsed time (40). The pH optimum of rEhCP1 was determined by preincubating rEhCP1 (20 to 300 nM) to 10-fold dilutions of inhibitor (500 nM to 100 μM) with 10 μM Z-Arg-Arg-AMC (Km = 2 μM) on a Flex Station with robotics (Molecular Devices) and an N-terminal read time. The value of ka observation, the rate constant for loss of enzymatic activity, was determined from an equation for pseudo-first-order dynamics using Prism 4 (GraphPad). When ka observation varied linearly with inhibitor concentration, Km, was determined by linear regression analysis (41). If the variation was hyperbolic, indicating saturation inhibition kinetics, ka observation and Km were determined from an equation describing a two-step irreversible inhibitor mechanism (ka observation = ka observation [I] + Km + |Km + (I + [S])[Km/H1]| and nonlinear regression analysis using Prism (41).
xenografts infected with *E. histolytica*. A standard curve to measure the number of *E. histolytica* trophozoites invading human intestinal xenografts was generated by adding trophozoites (10^2 to 10^6) to 25 to 50 mg of human intestinal xenograft tissue and extracting the DNA with the PUREGENE DNA purification kit (Gentra Systems, Minneapolis, MN). Real-time PCRs were carried out using the SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA) with 100 ng of the extracted DNA (from xenografts and standards) and 100 nM (final concentration) of both the forward primer (5'-H11032- AAATCAATTGTGAAGTTATGGAGTGA-3'/H11032) and the reverse primer (5'-H11032- TCCTACTCCTCTTTACTTTTATCTGCT-3'/H11032), to amplify a 92-bp region of the *E. histolytica* peroxiredoxin gene (7). Real-time PCR was performed under conditions previously described (40) with a standard curve and negative controls consisting of reaction mixtures without template or containing noninfected xenograft DNA (100 ng).

The potential presence of any PCR inhibitor in each of the human intestinal xenograft DNA samples was tested by the quantitative measure of amplifiable human genomic DNA with primers specific for the detection of human *Alu* sequences (56). Xenograft DNA (0.1 ng) was amplified with 100 nM of sense (5'-ACG CCT GTA ATC CCA GCA CTT 3') and antisense (5'-TCG CCC AGG CTG GAG TGC A 3') human *Alu* primers, under the same PCR conditions as described above. Negative controls, positive controls, and the experimental samples were run in duplicate. The threshold cycle values from each sample were averaged, and the standard deviation was calculated.

**RESULTS**

Expression, purification, and refolding of thio-pro-EhCP1. Expression of the thio-pro-EhCP1 recombinant protein in *E. coli* resulted in an overexpressed protein of ~50 kDa, corresponding to the predicted molecular mass for thio-pro-EhCP1 of 50.3 kDa (36.3 kDa for the zymogen form of EhCP1, 13 kDa for the thioredoxin peptide, and 0.9 kDa for the six-His tag) (Fig. 1). rEhCP1 was purified by nickel affinity chromatography and reacted with rabbit polyclonal anti-EhCP1 Ab and monoclonal antithioredoxin Ab (Fig. 1).

The optimal refolding conditions to produce active rEhCP1 were determined by using a 16-condition screen as previously reported (3, 38). The most effective conditions were identified by cleavage of the synthetic peptide substrate Z-Arg-Arg-AMC. Refolded rEhCP1 was unique in several ways. (i) Refolded rEhCP1 did not require preincubation at low pH, which is usually required for the self-activation from the proform to the mature form of eukaryotic cysteine proteinases. (ii) Refolded rEhCP1 could be activated in a Tris-EDTA, phosphate-EDTA, or citrate-EDTA buffer at neutral or slightly alkaline pH (7.0 to 7.5) with 2 to 5 mM dithiothreitol (DTT). (iii) Refolded rEhCP1 could be activated with 5 to 10 mM cysteine alone. rEhCP1 activation by cysteine is of importance as it would not affect the in vitro cleavage of several of its putative biological substrates, particularly C3, which is readily denatured in the presence of other thiol-reducing agents such as DTT (43) or 2-mercaptoethanol, each of which is widely used for the in vitro activation of cysteine proteinases.

![FIG. 1.](image1.png)

**FIG. 1.** Purification of recombinant thio-pro-EhCP1 and refolded, active rEhCP1. Recombinant thio-pro-EhCP1 was purified by Ni affinity chromatography, analyzed by 12% SDS-PAGE, and visualized by Coomassie blue staining (lane 1), probing of an immunoblot with polyclonal rabbit anti-EhCP1 Ab (lane 2), and probing of an immunoblot with mouse monoclonal antithioredoxin Ab (lane 3). Hi-Trap-Q-purified, refolded, and active EhCP1 was analyzed by SDS-PAGE (12% gels, silver stained) (lane 4), gelatin zymography (12% SDS-PAGE, 0.1% gelatin, run under nonreducing conditions) (lane 5), and immunoblotting (rabbit polyclonal anti-EhCP1 Ab) (lane 6). Numbers at left are molecular masses in kilodaltons.

![FIG. 2.](image2.png)

**FIG. 2.** Substrate specificity determined by synthetic combinatorial libraries. A P1 complete diverse library and P2, P3, and P4 sublibraries of the P1-lysine fixed library were used to determine the substrate specificities of EhCP1. Activities are displayed as percentages of the maximum at each position. Amino acids are represented by the single-letter code (“n” is norleucine). Error bars represent the standard deviations from the results of duplicate experiments.

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Arg-Arg-AMC, typical cathepsin B substrates, were readily cleaved, whereas Z-Phe-Arg-AMC, a typical cathepsin L substrate, was not (data not shown). To further define the substrate requirements of rEhCP1, we mapped the specificity of the rEhCP1 active site using two synthetic tetrapeptide combinatorial libraries (Fig. 2). An almost absolute requirement for arginine at the P2 position was found.

**pH optimum of rEhCP1.** rEhCP1 was found to have a pH optimum of 6.0, retaining most of its catalytic activity in the pH range of 5.5 to 7.5 (data not shown). A 50% or higher reduction in the catalytic activity is seen at pH values of ≤5.0 or ≥8.0.

**Biological substrate specificity of rEhCP1.** To determine if rEhCP1 had the same biological substrate specificity as the secreted native proteinases, human C3, IgG, and pro-IL-18 were incubated with equal amounts of active purified rEhCP1 or native Eh-secreted proteinases (CM). rEhCP1 cleaves C3 in a fashion identical to that of the secreted native proteinases (43), generating the α′ subunit (Fig. 3A). rEhCP1 also cleaves the human IgG heavy chain in a dose- (Fig. 3B) and time-dependent manner (53) (data not shown), similar to native proteinases. Human pro-IL-18 is also cleaved by rEhCP1, similar to the degradation seen by released amebic proteinases (CM) and rEhCP5 (Fig. 3C) (37).

**Intracellular localization of EhCP1.** In order to determine the intracellular localization of EhCP1, Entamoeba histolytica trophozoites were labeled with rabbit polyclonal anti-EhCP1 Ab and imaged by fluorescence, confocal, and electron microscopy. EhCP1 localizes within large cytoplasmic vesicles, which are distinct from those containing EhCP3 (Fig. 4A). These results were confirmed by immunoelectron microscopy (Fig. 4B).

**Inhibition of EhCP1 by vinyl sulfone inhibitors.** We first tested the inhibition of purified rEhCP1 by K11777, a vinyl sulfone inhibitor which has been shown to cure Trypanosoma cruzi infection in animals (10), has undergone extensive toxicity testing (1), and is approaching clinical trials for Chagas’ disease. The $K_{\text{ass}}$ ($k_{\text{inact}}/k_{\text{app}}$) of K11777 against EhCP1 was 350 (1/M·1/s). We next synthesized a peptidomimetic vinyl sulfone with an arginine substituted for phenylalanine in the P2 position based on the distinct specificity for arginine in P2 detected by active site mapping (Fig. 2). The resulting $K_{\text{ass}}$ for WRR483 was 849,000 (1/M·1/s), an increase of ~2,500-fold.
Effect of the cysteine proteinase inhibitors K11777 and WRR483 on amebic invasion in the intestinal xenograft model.

We next tested the efficacy of the inhibitors in an in vivo model of invasion of human intestine. The pathology of human colon xenografts infected for 24 h with virulent *Entamoeba histolytica* trophozoites closely mimics human disease, with undermined ulcers containing trophozoites interspersed with normal mucosa (Fig. 5, left). When colon xenografts were infected with *E. histolytica* trophozoites preincubated with the cysteine proteinase inhibitor K11777 (1) (data not shown) or WRR483 (Fig. 5, right), no invasion was seen. Amebic invasion in human colon xenografts was quantified by a real-time PCR assay that could detect as few as 10 trophozoites (data not shown). The amplification of human *Alu* sequences in all of the xenografts was equivalent (15.93 ± 1.3) (56). Xenografts infected with *E. histolytica* trophozoites preincubated with the specific irreversible vinyl sulfone cysteine proteinase inhibitor K11777 had a >80% reduction in the number of detectable *E. histolytica* trophozoites (Fig. 6A). Inhibition of amebic invasion with the vinyl sulfone cysteine proteinase inhibitor WRR483 was even more dramatic, with a >95% reduction in the number of detectable trophozoites in the xenograft tissue (Fig. 6B).

**DISCUSSION**

Released cysteine proteinases play a fundamental role in invasion of target tissues by *E. histolytica* trophozoites. Analysis of the *E. histolytica* genome reveals that 40 genes encode cysteine proteinases (25). Studies of the expression of the cysteine proteinase genes have shown that only three, *ehcp1*, *ehcp2*, and *ehcp5*, account for more than 90% of the cysteine proteinase-specific transcripts (5, 6) and more than 95% of secreted amebic proteinases in vitro (6, 8, 17). We focused on EhCP1 because it is unique to *E. histolytica*, there being no homologous gene in the closely related but noninvasive *E. dispar* (5). It is also one of the most highly expressed and released cysteine proteinases (16, 17). Because of the difficulty in separating the large number of similar native cysteine proteinases, we focused on the expression of active, recombinant enzyme. We have previously expressed active EhCP2 and EhCP3 in baculovirus (36) and EhCP5 in *Pichia* (37). We expressed the proenzyme of EhCP1 in *E. coli* as a thioredoxin fusion with a 3’ histidine tag which autocatalytically processed itself to the mature, active enzyme during refolding (Fig. 1). Surprisingly, purified, refolded rEhCP1 did not require activation by preincubation at low pH or with thiol reagents, such as DTT, 2-mercaptoethanol, or cysteine, which distinguishes rEhCP1 from previously purified recombinant parasite cysteine proteinases (16, 33, 44, 45, 47, 48). Purified refolded rEhCP1 has a pH optimum of 6.0, although 50% or more of its catalytic activity is retained across a pH range of 5.0 to 8.5. This broad pH range is consistent with the release of native EhCP1 in the neutral environment of the large bowel (29).

We have shown previously by homology modeling that the structures of EhCP2, EhCP3, and EhCP5 are characteristic of cathepsin L proteins with the ERFNIN motif, but their ability to accept positively charged amino acids at P2 resembles cathepsin B proteins (36). To further investigate this peptide substrate preference, we mapped the specificity of the active site cleft of rEhCP1 using two positional-scanning synthetic tetrapeptide combinatorial libraries (15, 27). We found an almost absolute preference for arginine at the P2 position (Fig. 2), an unusual specificity among clan CA proteinases. We took advantage of this unique substrate specificity to design a vinyl sulfone inhibitor, WRR483, from the scaffold compound K11777.
rEhCP1 cleaves a number of key physiologic substrates equivalently to the native enzymes. For example, active rEhCP1 readily cleaves the α chain of human complement component C3 (Fig. 3A), which we have shown mimics the cleavage of C3 convertases, producing an active C3b molecule (43). The cleavage site is actually one amino acid residue distal to native convertases, placing an arginine in the P2 position of EhCP1 (43). The released C3a anaphylatoxin fragment is further degraded by the amebic cysteine proteinases (41), limiting the host inflammatory response. Human IgG is also cleaved by refolded active rEhCP1 (Fig. 3B) as previously shown with purified amebic proteinases (53). This observation is consistent with recent clinical findings that systemic antiamebic IgG responses do not protect people from reinfection with E. histolytica (12, 13, 14). Previously, we have demonstrated that rEhCP5, the only other cysteine proteinase unique to E. histolytica, degraded pro-IL-18, a finding that implicates amebic cysteine proteinases in the observed lack of neutrophils in amebic lesions (37). Now we have found that rEhCP1 produces a similar dose- (Fig. 3C) and time-dependent degradation of pro-IL-18.

The intracellular localization is known for only a few amebic cysteine proteinases. EhCP5 (18), EhCP2 (35, 36), and EhCP112 (34) are membrane associated, while EhCP3 is intracellular (17, 36). By using confocal and immunoelectron microscopy, we found that EhCP1 localizes to large cytoplasmic vesicles, sites distinct from the vesicles containing EhCP3 (Fig. 4). These are likely the larger, prephagocytic, nonacidic vesicles, sites distinct from the vesicles containing EhCP3 (Fig. 4). These are likely the larger, prephagocytic, nonacidic vesicles, sites distinct from the vesicles containing EhCP3 (Fig. 4).

Animal models of amebiasis have been problematic as only humans and higher primates are naturally susceptible to infection. Cysteine proteinase inhibitors such as E-64 and an EhCP5 antisense construct, which blocked expression of multiple cysteine proteinases, inhibited amebic liver abscess formation in SCID mice (50) and hamsters (2). The trophozoites had to be injected directly into the liver, however, which bypasses the normal route of infection through the colon. To avoid this limitation, we closely mimicked human infection by using the human xenograft model (55). This localization is consistent with the higher pH optimum (6.0) of EhCP1 as well. We had previously shown that EhCP1, EhCP2, and EhCP5 are passively released during phagocytosis (17). This release is likely to be part of the newly described EhRab11B-associated secretory pathway, as overexpression of EhRab11B led to increased release of all three proteinases (30).

We next tested the new vinyl sulfone inhibitor WRR483, which was synthesized with arginine at the P2 position instead of phenylalanine to specifically target EhCP1. This specific inhibitor was almost 2,500-fold more potent than K11777 against rEhCP1 (849,000 1/M · 1/s). WRR483 is even more effective than K11777 at blocking amebic invasion in the human intestinal xenograft model (Fig. 5, right), as it reduced invasion by more than 95% (Fig. 6B).

Cysteine proteinases are an attractive target for agents designed to disrupt invasion by E. histolytica. The unique requirement of EhCP1 for arginine in the P2 position and the demonstrated ability of a specific cysteine proteinase inhibitor with these particular structural features (WRR483) to block amebic invasion of the bowel support the feasibility of this approach. We have now shown that the expression of active recombinant enzymes will enable us to dissect the role of individual cysteine proteinases in the virulence of this important protozoan parasite. Also, these recombinant enzymes will be a fundamental tool as we address the question of whether E. histolytica releases a higher level of cysteine proteinases facilitating invasion, in contrast to noninvasive E. dispar, or whether the key determinants of its invasiveness are the unique proteinases, EhCP1 and EhCP5. We expect that our continuing efforts in specific inhibition of these unique proteinases will shed yet more light on their roles in the pathogenesis of amebiasis. In the meantime, our current studies have proven that inhibition of amebic cysteine proteinases blocks invasion of the human bowel and have identified a promising, orally available scaffold inhibitor.

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