JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1989, p. 2772–2777
0095-1137/89/122772-06$02.00/0

Vol. 27, No. 12

Thiol Proteinase Expression and Pathogenicity of *Entamoeba histolytica*

SHARON L. REED,[1] WILLIAM E. KEENE,[2,3] AND JAMES H. McKERROW[3]*

Departments of Medicine and Pathology, Division of Infectious Diseases, University of California, San Diego, California 92037[1]; Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, California 94720[2]; and Department of Pathology, University of California, San Francisco, California 94143[3]

Received 19 May 1989/Accepted 21 August 1989

Expression of the 56-kilodalton (kDa) neutral thiol proteinase has been shown to correlate with the potential of clinical isolates of *Entamoeba histolytica* to produce invasive disease. A 56-kDa band was identified by gelatin substrate gel electrophoresis in 10 of 10 isolates from patients with colitis or amebic liver abscesses, but in only 1 of 10 isolates from asymptomatic patients. Pathogenic isolates appear capable of releasing significantly larger quantities of the proteinase, as measured by cleavage of a synthetic peptide substrate, ZRR-AMC (benzyloxycarbonyl-arginine-arginine-4-amino-7-methylcoumarin). We have also shown that the proteinase is released during the course of clinical invasive amebic disease, as demonstrated by the presence of circulating antibodies detectable by enzyme-linked immunosorbent assay. These studies support the importance of the 56-kDa thiol proteinase in the pathogenesis of invasive amebiasis.

Amebiasis, defined as infection by the protozoan parasite *Entamoeba histolytica*, affects over 500 million people worldwide. While most infections are silent, approximately 10% of amebiasis patients develop symptomatic disease. In severe infections, trophozoites invade the bowel wall and cause either mucosal ulceration and colitis or extraintestinal disease, especially liver abscesses.

At present, there is no consensus about the properties that promote the virulence of *E. histolytica*. The old idea that *E. histolytica* can be subdivided into pathogenic and nonpathogenic strains (4) did not achieve wide consideration until Sargeaunt and others identified distinctive isoenzyme patterns (zymodemes) that correlated with the presence of symptomatic disease (25, 26). Only 10 of the 23 well-defined zymodeme groups have been associated with symptomatic, invasive infections; these are the pathogenic zymodemes (24). The isoenzyme profiles of many thousands of clinical isolates from around the world have now been characterized, and without exception, all amebiasis patients with colitis or liver abscesses have been infected with one of these 10 pathogenic zymodemes. Additional evidence that there may be important strain differences is provided by the finding that isolates from patients with invasive disease are resistant to complement-mediated lysis, in contrast to strains from asymptomatic carriers (22). Furthermore, a recent report suggests that surface antigen and genomic differences may exist between pathogenic and nonpathogenic amebae (27).

It remains unclear, however, whether an apparently noninvasive phenotype represents a stable characteristic of the parasite strain or a transient state influenced by the host or other environmental factors (18–20, 24). The implications of zymodeme typing for treatment remain controversial (1, 8, 13). It would therefore be of great value to devise another assay of pathogenicity, in particular one directly related to the pathogenesis of the disease.

We have been studying a neutral thiol (cysteine) proteinase secreted by virulent laboratory strains of *E. histolytica* (12). This protein (M_r, ~56,000) probably plays a key role in tissue invasion, since it degrades collagen, fibronectin, and laminin. Furthermore, it causes the cytopathic effect of amebae and amebic extracts on cell culture monolayers, one of the in vitro hallmarks of virulence (7, 12). Assays of two widely used laboratory strains, HM-1 and HK-9, showed that the more virulent HM-1 releases more of this proteinase (12). This observation confirmed earlier reports correlating virulence with the amount of crude proteolytic activity in both amebic secretions and lysates (14, 15, 17). Furthermore, thiol proteinases were shown to be involved with the production of tissue necrosis in rat models of acute amebiasis (3).

We now report that the clinical histories of 20 patients correlate with both the zymodeme pattern and the capacity of their isolates to secrete the neutral thiol proteinase. Moreover, we also show that the proteinase is secreted during the course of symptomatic clinical amebiasis by demonstrating the production of antibodies to this proteinase in patients with clinical histories of invasive amebiasis. In contrast, asymptomatic patients infected with amebae of nonpathogenic zymodemes remained seronegative.

MATERIALS AND METHODS

Isolation of amebae and maintenance of cultures. Clinical isolates were cultured directly from stools or liver abscesses into the medium of Robinson (23) and subsequently transferred and maintained in TYSGM (Trypticase [BBL Microbiology Systems, Cockeysville, Md.] soy-serum-gastric mucin) media containing *Escherichia coli* 0111 (6). Amebae were subcultured into fresh TYSGM every 2 to 3 days. Six pathogenic and five nonpathogenic strains were a gift of Peter Sargeaunt of the London School of Hygiene and Tropical Medicine (denoted by SAW [see Table 1]). The other nine strains were isolated by the Microbiology Laboratory at the University of California, San Diego, Medical Center from fecal samples or liver abscesses.

Axenic *E. histolytica* HM-1:IMSS were originally obtained from the American Type Culture Collection, Rockville, Md., and cultured in TYI-S-33 medium as previously descibed (5).

Zymodeme analysis. Lysates and starch gel electrophore-

* Corresponding author.

sis were performed as described by Sargeaunt et al. (26), and strains were assigned to zymodemes according to the patterns of malic enzyme, phosphoglucomutase, glucose phosphate isomerase, and hexokinase (25, 26).

Preparation of secretions and lysates. Amebic cultures of clinical isolates were purified over a discontinuous Percoll gradient followed by three low-speed washes to remove starch and bacteria as previously described (22). Purified trophozoites were suspended at a concentration of 10^7/ml in phosphate-buffered saline (PBS), pH 7.0, containing 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 20 mM cysteine and incubated at 37°C for 3 h on a rocking platform in Microfuge tubes (Beckman Instruments, Inc., Fullerton, Calif.). The pellets and supernatants were separated by centrifugation at 10,000 × *g* for 10 min. The pellets were lysed by five freeze-thaw cycles in liquid N_2.

Bacterial controls were prepared by direct subcultures of culture supernatants from amebic cultures into Trypticase soy broth. The bacteria from an overnight culture were pelleted, washed twice in PBS, and suspended in 1 ml of PBS at concentrations 2 to 3 orders of magnitude higher than those in secretions or lysates of the amebic samples.

Secretions from axenic strains were collected as previously described (12). Briefly, trophozoites were harvested by chilling culture flasks on ice, concentrated by low-speed centrifugation, washed in PBS, and incubated for 3 to 4 h in PBS at 37°C, and the amebae and secretions were separated by high-speed centrifugation. The viability of organisms during collection of secretions was assessed by microscopic examinations of morphology and motility as well as trypan blue exclusion (12) and was consistently >95%.

Expression of the neutral thiol proteinase activity. Proteinase activity was assessed by gelatin substrate gel electrophoresis as previously described (12). Briefly, 10 μl of a 1:50 dilution of the secretions from clinical amebic isolates or bacterial controls suspended in 10 μl of sample buffer without dithiothreitol were electrophoresed in 10% sodium dodecyl sulfate-polyacrylamide gels copolymerized with 0.1% gelatin. After removal of the sodium dodecyl sulfate with 0.1% Triton X-100 and incubation of the gels in Tris-dithiothreitol buffer (100 mM Tris, 2 mM dithiothreitol, 5 mM EDTA, pH 7.4) for 18 h at 37°C, proteinase activity was detected by gelatin degradation, indicated by a clear band on Coomassie blue-stained gels. Inhibition of proteolytic activity by E-64 [L-*trans*-epoxysuccinyl-leucylamido-(4-guanidino)-butane] (Sigma Chemical Co., St. Louis, Mo.), a specific inhibitor of thiol proteinases (2), was assessed by preincubating parallel samples for 20 min at room temperature in sample buffer containing a final concentration of 100 μM E-64.

The absolute level of enzyme activity was assayed by measuring the activity of amebic secretions and lysates on a specific peptide substrate, ZRR-AMC (benzyloxycarbonyl-arginine-arginine-4-amino-7-methylcoumarin) (Enzyme System Products, Livermore, Calif.) as previously described (12). The initial velocity of the reaction (i.e., cleavage of the AMC group) was determined on a continuously recording spectrofluorometer. Activity was measured as the amount of substrate cleaved per unit time by a 10-μl sample (equivalent to 10^5 trophozoites) of secretions or lysate from each strain.

Specific proteinase activity was detected by electrophoresing 1:10 dilutions of secretions in nondenaturing gels and incubating these gels for 18 h in contact with a cellulose acetate overlay impregnated with a similar peptide substrate, ZRR-7-amino-4-trifluoromethyl coumarin (ZRR-AFC; Enzyme System Products). Substrate cleavage was identified by UV fluorescence of the AFC group.

Human sera. Patient sera were obtained from a variety of sources. At the University of California, San Diego, blood samples were allowed to clot for 30 min. at room temperature and the serum was separated by centrifugation at 250 × *g* for 15 min. All samples were stored at −70°C until use. Other patient sera were kindly provided by Conrad Casavant at the University of California, San Francisco, George Healy and Henry Mathews at the Centers for Disease Control, Atlanta, Ga., and S. Zakaria and A. Mohammed, Department of Endemic Medicine, Cairo University, Cairo, Egypt. These serum samples were obtained from patients with a spectrum of amebic diseases, including asymptomatic cyst passing, colitis, and hepatic abscess. Amebae were not available from all patients for isolation and zymodeme determination. Uninfected controls included serum samples from patients with ulcerative colitis, rheumatoid arthritis, and hepatocellular carcinoma, as well as from healthy volunteers with no history of amebiasis.

Amebic serologies were determined by agar gel diffusion (LMD Laboratories, San Diego, Calif.).

ELISA. The 56-kilodalton (kDa) proteinase was purified from axenic strain HM-1 as previously described (12). Plastic microdilution plates were incubated with antigen diluted in enzyme-linked immunosorbent assay (ELISA) buffer (1% bovine serum albumin–0.5% Tween 20 in Tris-buffered saline) overnight at 4°C. The plate was washed with buffer and incubated at room temperature for 2 h in a blocking solution (100 μl per well) of 4% bovine serum albumin in ELISA buffer. The plate was washed three times with buffer and incubated for 1 h with 50 μl of a 1:100 dilution of patient sera. After three more washes, the plate was incubated with secondary antibody (horseradish peroxidase-conjugated goat anti-human immunoglobulins G, A, and M; Zymed Laboratories, Inc., South San Francisco, Calif.) diluted 1:500 with buffer for 1 h at room temperature. The plate was again washed and incubated with 50 μl of substrate [1 mM 2,2′-azino-bis(3-ethylbenzthiazolinesulfonic acid); Sigma] per well in 100 mM sodium citrate buffer, pH 4.5–HCl with 0.1% (vol/vol) H_2O_2 (30%; Sigma). A_{414} was read after 20 min of development on an ELISA reader (Titertek). All samples were tested in duplicate. Samples were considered positive if the A_{414} was more than three standard deviations above the mean of the negative controls.

RESULTS

All 10 *E. histolytica* cultures isolated from patients with colitis or extraintestinal disease were of pathogenic zymodeme groups, and all released the 56-kDa thiol proteinase as assessed by gelatin substrate gel electrophoresis (Fig. 1; Table 1). While the 56-kDa band was always the most prominent, a minor band at approximately 30 kDa was seen frequently. Both the major and minor proteolytic species were inhibited by E-64, a specific, irreversible thiol proteinase inhibitor. In contrast, 9 of 10 samples from asymptomatic patients (all 10 of which were nonpathogenic by zymodeme analysis) did not degrade gelatin.

The 56-kDa proteinase was shown to possess enzymatic activity against both gelatin and synthetic peptide substrates by overlaying the gel with cellulose acetate impregnated with a synthetic peptide in which the ZRR group is coupled to 7-amino-4-trifluoromethyl coumarin. Cleavage of the AFC group is demonstrated with UV light (Fig. 1). Since detection by membrane overlays is less sensitive, a fivefold-higher

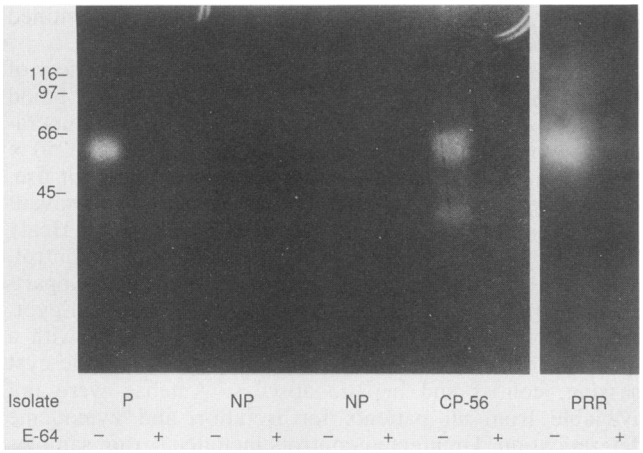

FIG. 1. Secretions of typical pathogenic and nonpathogenic isolates compared by gelatin substrate gel electrophoresis. Clearing at ~56 kDa is seen with the pathogenic (P) but not nonpathogenic (NP) isolates (numbers at left indicate kilodaltons). CP-56, Partially purified enzyme from secretions; PRR, pathogenic isolate overlaid with ZRR-AFC cellulose acetate paper as seen under UV light. Proteolytic activity was inhibitable by E-64.

concentration (1:10 dilution) of the secretions was required. No enzymatic activity could be detected in nonpathogenic strains (1749, 102, 95, and 103) under identical conditions.

Substrate gels provide a qualitative, but not a quantitative, measure of proteolytic activity. Therefore, we measured the total cysteine proteinase activity by determining the rate of cleavage of a synthetic peptide substrate, ZRR-AMC, in lysates and secretions of pathogenic and nonpathogenic trophozoites. We found that there was significant day-to-day variation in the proteinase activity of any one strain of up to 100-fold, depending on time after passage of the culture. We took two approaches to compensate for this variability. First, we measured the ability of each strain to cleave ZRR-AMC two or more times (36 determinations on 8 pathogenic strains and 27 determinations on 10 nonpathogenic strains) and found that pathogenic strains were more active (Fig. 2A; $P < 0.05$ by Student's t test). We next compared the highest value obtained from each strain and found that the difference between pathogenic and nonpathogenic strains was even more pronounced (Fig. 2B; $P < 0.02$). Although the total cysteine proteinase activity of a single pathogenic or nonpathogenic strain may overlap on a given day, pathogenic isolates clearly release larger quantities of the proteinase. Proteolytic activity could not be ascribed to the cocultured bacteria. After isolation of amebic trophozoites from clinical cultures, 10^5 to 10^7 bacteria per ml were present. No measurable protease activity was detected when concentrations of bacteria 2 to 3 orders of magnitude higher than in the amebic samples were assayed against ZRR-AMC. Moreover, there were no protease bands detectable by gelatin gel electrophoresis of bacterial culture supernatants alone.

TABLE 1. Amebic isolates characterized by zymodeme group, clinical syndrome of the patients, and presence of 56-kDa proteinase activity on substrate gel electrophoresis

Isolate[a]	Zymodeme	Clinical syndrome (location[b])	56-kDa proteinase
Pathogenic			
SAW 1528	XIV	Colitis (India)	+
53	II	Colitis and liver abscess (U.S.)	+
SAW 1721	XIX	Colitis (U.K.)	+
SAW 1760	XIX	Colitis (U.K.)	+
SAW 1516	XIV	Colitis (India)	+
SAW 1519	XIV	Colitis (India)	+
SAW 1706	XIX	Colitis (U.K.)	+
104	II	Colitis (U.S.)	+
92	II	Colitis (U.S.)	+
73	II	Liver abscess (U.S.)	+
Nonpathogenic			
98	I	Asymptomatic (U.S.)[c]	–
10	III	Asymptomatic (U.S.)[d]	+
SAW 1719	I	Asymptomatic (Australia)	–
95	I	AIDS[e], *Mycobacterium avium* infection, and diarrhea (U.S.)	–
SAW 1718	I	Asymptomatic (U.K.)	–
SAW 1776	III	Asymptomatic (U.K.)	–
SAW 1749	III	Asymptomatic (U.K.)	–
100	I	One day diarrhea, then asymptomatic (U.S.)[c]	–
102	II	Pneumonia (U.S.)[c]	–
103	III	Asymptomatic (U.S.)[c]	–

[a] SAW indicates isolate obtained from Peter Sargeaunt.
[b] U.S., United States; U.K., United Kingdom.
[c] Gay male.
[d] Mental patient.
[e] AIDS, Acquired immunodeficiency syndrome.

Serum samples from 42 patients and 6 negative controls were tested by ELISA with the purified neutral thiol proteinase as the antigen. More than 80% of patients with histories of invasive amebiasis were seropositive, including 16 of 20 with liver abscesses, 7 of 7 with colitis, and 1 of 2 with ameboma (Fig. 3). Of 13 people with asymptomatic infections, 11 were seronegative; two were weakly positive. All controls, including three patients with ulcerative colitis, rheumatoid arthritis, and hepatocellular carcinoma, were negative. The five patients with invasive disease (four with liver abscesses and one with ameboma) without antibody to the proteinase were also seronegative by the conventional agar gel diffusion. Since at least 10% of patients may not have detectable antibody by agar gel diffusion in the early, acute stages of infection (11), it is possible that development of an immunoglobulin G response to the amebic proteinase may follow a similar time course.

DISCUSSION

These results suggest a strong relationship between release of the major neutral thiol proteinase of *E. histolytica* and the clinical course of infection. Sodium dodecyl sulfate-gelatin-polyacrylamide gel electrophoresis analysis allowed us to distinguish two broad groups, but it is possible that these groups overlap. This could explain the one exception we saw in which protease activity at 56 kDa was detectable in an asymptomatic patient. This patient was a native of Mexico, and the amebae isolated had a nonpathogenic zymodene. While follow-up data are not available on the subsequent clinical course of his infection, we cannot exclude the possibility that despite an early asymptomatic course, the expression of a tissue-destructive proteinase heralded either a shift to a potentially more pathogenic infection or an undetectable (by zymodeme testing) coinfection or preinfection with a pathogenic strain. We plan to carry out larger prospective studies in the future to more directly address this issue.

Comparisons between individual isolates using the ZRR-AMC assay, however, were more difficult than with the

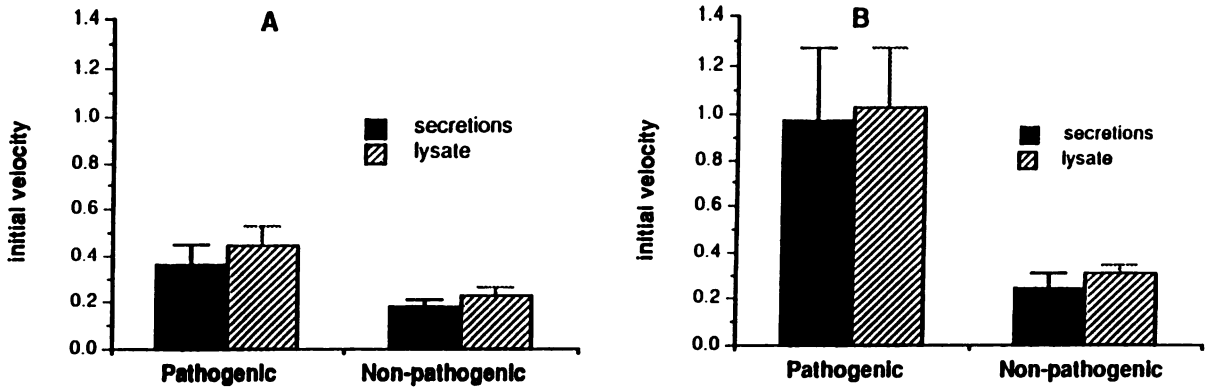

FIG. 2. (A) Total thiol proteinase activity of eight pathogenic (strains SAW 1528, 53, SAW 1721, SAW 1519, SAW 1706, 104, 92, and 73) (36 determinations) and 10 nonpathogenic (27 determinations) isolates measured by the initial velocity of cleavage of the synthetic peptide substrate ZRR-AMC in lysates and secretions. Values are means ± standard errors of the mean ($P < 0.05$ by Student's t test). (B) Maximal thiol proteinase activity of 8 pathogenic and 11 nonpathogenic strains in lysates and secretions. Values are means ± standard errors of the mean ($P < 0.02$ by Student's t test).

gelatin substrate gel assay, because the level of proteinase activity of each strain varied considerably with time in culture. Moreover, the enzyme activity is very unstable in storage, even at −20°C. Over a 1-month period in culture, the level of proteinase activity of one pathogenic strain showed variation of up to 100-fold. Although the overlap in total cysteine proteinase activity between pathogenic and nonpathogenic strains was such that a single quantitative measure of enzyme activity was not sufficient to distinguish between pathogenic strains, these pathogenic isolates appear capable of releasing larger quantities of the proteinase. Considerable variability has also been observed in cultures of clinical isolates for phosphoglucomutase, one of the enzymes used in the determination of zymodemes (P. Sargeaunt, personal communication), as well as for the secretion of a metallocollagenase by axenic trophozoites (21); this may reflect variation in the metabolic activity of the trophozoites during different stages of growth in vitro.

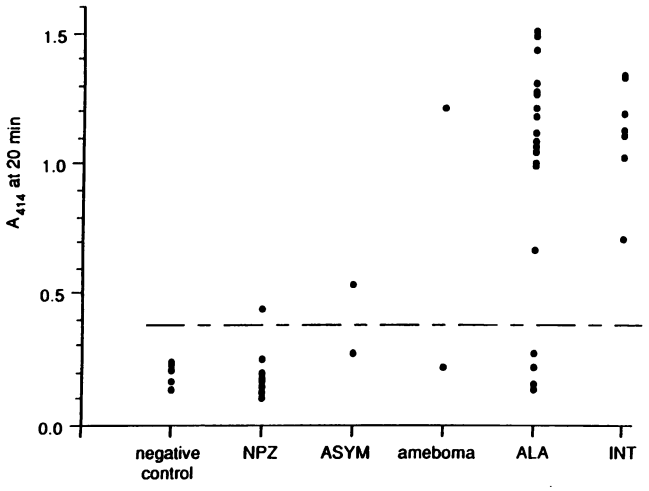

FIG. 3. ELISA of purified proteinase and sera from 20 patients with amebic liver abscess (ALA), 7 patients with colitis (INT), 2 patients with ameboma, 2 asymptomatic patients who were passing cysts of *E. histolytica* of unknown zymodeme groups (ASYM), 13 asymptomatic patients harboring amebae of nonpathogenic zymodemes (NPZ), and 6 controls. Values are averages of duplicate samples. — - — -, Mean + 3 standard deviations of the negative controls. Values above the line were considered positive.

The ZRR-AMC data suggest that nonpathogenic isolates also secrete this proteinase, albeit in lower quantities. The apparent discrepancy between lack of gelatinolytic activity of nonpathogenic strains despite cleavage of synthetic peptide substrates in solution is not solely a function of the lower enzyme activity of nonpathogenic strains. The use of a cellulose acetate overlay impregnated with synthetic peptide substrate demonstrated that the 56-kDa enzyme could cleave both small and large substrates. No activity was detectable in nonpathogenic strains, but with a less sensitive assay, we cannot rule out the possibility that the enzyme in nonpathogenic strains has retained its ability to cleave small substrates but not larger ones such as gelatin. Purified enzyme is active against both substrates, and specific inhibitors of the enzyme block degradation of both substrates (12).

A brief discussion of the relationship of the 56-kDa proteinase assayed in this study to other cysteine proteinase species identified in *E. histolytica* (3, 7, 12, 14, 15, 17) is appropriate at this juncture. Using our protocols and assays (as described in this study and reference 12), we identified three proteinase species released by virulent strains of *E. histolytica*. While we consistently found that the major species is of 56 kDa, we often saw species of approximately 40 and 30 kDa in gelatin substrate gel electrophoresis. We are currently carrying out structural studies of each of these proteinase species. The 30-kDa species (which can occasionally be seen in gelatin gel electrophoresis of the recent clinical isolates described in this study) has been purified, and an amino-terminal sequence analysis indicated that it is identical to the 27-kDa species described by Luaces and Barrett (14). Other studies using immunologic and molecular probes suggest that there is a single cysteine protease gene in *E. histolytica* and that the various cysteine proteinase forms we and others have observed may represent posttranslational modifications of a single gene product (J. Bouvier and A. Sikes, unpublished data). However, at this time we are not yet sure whether the 56-kDa species represents an active proenzyme or a non-disulfide-linked dimer of the 27- to 30-kDa species.

If the neutral thiol proteinase is also secreted by pathogenic amebae in vivo and is immunogenic, it should induce an antibody response in patients with colitis or extraintestinal infections. On the other hand, patients infected only with nonpathogenic lumen-dwelling strains might not pro-

duce antibodies, even if these strains do produce small amounts of proteinase. Our results, shown in Fig. 3, support this hypothesis. Crude trophozoite lysate is used as the antigen in the current serologic tests for amebiasis. The specificities of these tests are quite good. Sensitivity has been more of an issue, particularly for intestinal infections. Seropositivity may also persist long after treatment and apparent cure (9). Our serologic results suggest that a larger prospective trial would be of interest to assess the antibody response to the proteinase after treatment. The results obtained by ELISA with the thiol protease as the antigen are similar to those of Mathews and co-workers with a specific soluble fraction of *E. histolytica* trophozoites (16). Joyce and Ravdin (10) identified a 59-kDa mannose-containing glycoprotein in amebic extracts that was recognized by immune human sera from 11 patients cured of amebic liver abscesses. It will be interesting to see whether the secreted neutral thiol protease, which is estimated to have a molecular weight of about 56,000, is present in either of these antigenic fractions. The use of a purified antigen for the detection of amebic antibody offers the potential for improved standardization of serodiagnostic reagents, especially with the eventual production of recombinant antigen.

Several important conclusions can be drawn from the results of this study. The zymodeme patterns of clinical isolates of *E. histolytica* have now been correlated with a specific putative virulence factor, namely, the neutral thiol proteinase. The in vitro activity of this enzyme against extracellular matrix macromolecules and its ability to digest the anchoring proteins connecting epithelial cells to the basement membrane and to each other (12, 28) can be more plausibly related to virulence per se than can the metabolic enzymes which are the basis of zymodeme analysis. Nevertheless, the two tests correlate very well.

The ability of only pathogenic *E. histolytica* isolates to degrade gelatin in substrate gels and their greater activity against synthetic substrates suggest a role for the neutral thiol proteinase in the production of disease. Secretion during the course of clinical invasive disease, but not during asymptomatic infection, as demonstrated by the presence of circulating antibodies, strongly supports this hypothesis. This study is unique because it was performed with fresh clinical isolates that have been correlated with the zymodeme profile and the clinical syndrome of the patient. Other studies of putative virulence factors have primarily used axenized strains. Since to date only pathogenic zymodemes have been axenized, this is the first study to compare expression of a virulence factor in pathogenic and nonpathogenic zymodemes. Our results confirm the importance of this approach.

These results do not directly address the issue of whether the pathogenic phenotype, associated with certain zymodemes and now with expression of the thiol proteinase, represents a stable, intrinsic characteristic of certain strains of *E. histolytica* or whether it is subject to change over time. If differences in pathogenicity reflect the presence or absence of genes coding for certain virulence factors, for example, specific molecular or immunological probes may allow us to address this question. If these differences reflect differential expression of genes present in both pathogenic and nonpathogenic strains, the issue will be both more interesting and more difficult to resolve.

Our results indicate that assay of the neutral thiol proteinase by sodium dodecyl sulfate-gelatin-polyacrylamide gel electrophoresis can be done reliably with amebae recently isolated from patients as well as with axenic laboratory strains. We found a strong association between zymodeme class and secretion of the enzyme when assayed by substrate gel. Patients with histories of invasive amebiasis generally have elevated levels of antibodies against this protein that are detectable by ELISA. These findings substantiate the importance of this secreted proteinase in the pathogenesis of invasive amebiasis. Of equal importance, we have correlated the presence of a putative virulence factor identified in axenic laboratory strains with recent clinical amebic isolates.

ACKNOWLEDGMENTS

Sharon L. Reed is a Lucille P. Markey Scholar, and this work was supported in part by grants from the Lucille P. Markey Charitable Trust, A120067 and the University of California Task Force on AIDS.

We appreciate the kind gift of strains from Peter Sargeaunt.

LITERATURE CITED

1. **Allason-Jones, E., A. Mindel, P. Sargeaunt, and P. Williams.** 1986. *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. N. Engl. J. Med. **315**:353–356.
2. **Barrett, A. J., A. A. Kembhavi, M. A. Brown, H. Kirschke, C. Knight, M. Tamai, and K. Hanada.** 1982. L-*trans*-Epoxysuccinyl-leucylamido(4-guanidino)butane (E-64) and its analogues as inhibitors of cysteine proteinases including cathepsins B, H and L. Biochem. J. **201**:189–198.
3. **Becker, I., R. Pérez-Tamayo, I. Montfort, A. M. Alvizouri, and R. Perez-Montfort.** 1988. *Entamoeba histolytica*: role of amebic proteinases and polymorphonuclear leukocytes in acute experimental amebiasis in the rat. Exp. Parasitol. **67**:268–280.
4. **Brumpt, E.** 1925. Etude sommarie de l'*Entamoeba dispar* n. sp. amibe a kystes quadrinuclées, parasite de l'homme. Bull. Acad. Natl. Med. **94**:942–952.
5. **Diamond, L. S.** 1984. Lumen dwelling protozoa: *Entamoeba*, trichomonads, and *Giardia*, p. 65–109. *In* J. B. Jensen (ed.), In vitro cultivation of protozoan parasites. CRC Press, Boca Raton, Fla.
6. **Diamond, L. S., D. R. Harlow, and C. C. Cunnick.** 1978. A new medium for the axenic cultivation of *Entamoeba histolytica* and other Entamoeba. Trans. R. Soc. Trop. Med. Hyg. **72**:431–432.
7. **Gadasi, H., and D. Kobiler.** 1983. *Entamoeba histolytica*: correlation between virulence and content of proteolytic enzymes. Exp. Parasitol. **55**:105–110.
8. **Goldmeier, D., P. G. Sargeaunt, A. B. Price, P. E. Munday, P. Billington, I. Dixon, P. Borriello, J. M. Carder, S. A. J. Hilton, and D. J. Jeffries.** 1986. Is *Entamoeba histolytica* in homosexual men a pathogen? Lancet **i**:641–644.
9. **Healy, G.** 1986. Immunologic tools in the diagnosis of amebiasis: epidemiology in the United States. Rev. Infect. Dis. **8**:239–246.
10. **Joyce, M. P., and J. I. Ravdin.** 1988. Antigens of *Entamoeba histolytica* recognized by immune sera from liver abscess patients. Am. J. Trop. Med. Hyg. **38**:74–80.
11. **Katzenstein, D., V. Rickerson, and A. I. Braude.** 1982. New concepts of amebic liver abscess derived from hepatic imaging, serodiagnosis and hepatic enzymes in 67 consecutive cases in San Diego. Medicine **61**:237–246.
12. **Keene, W. E., M. G. Petitt, S. Allen, and J. H. McKerrow.** 1986. The major neutral proteinase of *Entamoeba histolytica*. J. Exp. Med. **163**:536–549.
13. **Krogstad, D. J.** 1986. Isoenzyme patterns and pathogenicity in amebic infection. N. Engl. J. Med. **315**:390–391.
14. **Luaces, A. L., and A. J. Barrett.** 1988. Affinity purification and biochemical characterization of histolysin, the major cysteine proteinase of *Entamoeba histolytica*. Biochem. J. **250**:903–909.
15. **Lushbaugh, W. B., A. F. Hofbauer, and F. E. Pittman.** 1984. Proteinase activities of *Entamoeba histolytica* cytotoxin. Gastroenterology. **87**:17–27.
16. **Mathews, H. M., D. M. Moss, and G. S. Visvesvara.** 1986. Analysis of antigens from membrane and soluble fractions of *Entamoeba histolytica*. J. Protozool. **33**:328–334.

17. **McGowan, K., C. F. Deneke, G. M. Thorne, and S. L. Gorbach.** 1982. *Entamoeba histolytica* cytotoxin: purification, characterization, strain virulence, and protease activity. J. Infect. Dis. **146**:616–625.
18. **Mirelman, D.** 1987. Amoeba-bacterium relationship in amebiasis. Microbiol. Rev. **51**:272–284.
19. **Mirelman, D.** 1987. Effect of culture conditions and bacterial associates on the zymodemes of *Entamoeba histolytica*. Parasitol. Today **3**:37–40.
20. **Mirelman, D., R. Gracha, A. Wexler, and A. Chayen.** 1986. Changes in isoenzyme patterns of a cloned culture of nonpathogenic *Entamoeba histolytica* during axenization. Infect. Immun. **54**:827–832.
21. **Muñoz, M., J. Calderón, and M. Rojkind.** 1982. The collagenase of *Entamoeba histolytica*. J. Exp. Med. **155**:42–51.
22. **Reed, S. L., J. G. Curd, I. Gigli, F. D. Gillin, and A. I. Braude.** 1986. Activation of complement by pathogenic and nonpathogenic *Entamoeba histolytica*. J. Immunol. **136**:2265–2270.
23. **Robinson, G.** 1968. The laboratory diagnosis of human parasitic amoebae. Trans. R. Soc. Trop. Med. Hyg. **62**:285–293.
24. **Sargeaunt, P. G.** 1987. The reliability of *Entamoeba histolytica* zymodemes in clinical diagnosis. Parasitol. Today **3**:40–43.
25. **Sargeaunt, P. G., and J. E. Williams.** 1979. Electrophoretic isoenzyme patterns of the pathogenic and non-pathogenic intestinal amoebae of man. Trans. R. Soc. Trop. Med. Hyg. **73**: 225–227.
26. **Sargeaunt, P. G., J. E. Williams, and J. D. Greene.** 1978. The differentiation of invasive and noninvasive *E. histolytica* by isoenzyme electrophoresis. Trans. R. Soc. Trop. Med. Hyg. **72**:519–521.
27. **Strachan, W. D., P. L. Chiodini, W. M. Spice, A. H. Moody, and J. P. Ackers.** 1988. Immunological differentiation of pathogenic and non-pathogenic isolates of *Entamoeba histolytica*. Lancet **i**:561–563.
28. **Wyler, D. J.** 1987. Fibronectin in parasitic diseases. Rev. Infect. Dis. **9**:s391–s400.