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cDNA sequence analysis of a 29-kDa cysteine-rich surface antigen of pathogenic *Entamoeba histolytica*

(fusion protein/monoclonal antibodies)

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ABSTRACT **A λgt11 cDNA library was constructed from poly(U)-Sepharose-selected *Entamoeba histolytica* trophozoite RNA in order to clone and identify surface antigens. The library was screened with rabbit polyclonal anti-*E. histolytica* serum. A 700-base-pair cDNA insert was isolated and the nucleotide sequence was determined. The deduced amino acid sequence of the cDNA revealed a cysteine-rich protein. DNA hybridizations showed that the gene was specific to *E. histolytica* since the cDNA probe reacted with DNA from four axenic strains of *E. histolytica* but did not react with DNA from *Entamoeba invadens*, *Acanthamoeba castellanii*, or *Trichomonas vaginalis*. The insert was subcloned into the expression vector pGEX-1 and the protein was expressed as a fusion with the C terminus of glutathione *S*-transferase. Purified fusion protein was used to generate 22 monoclonal antibodies (mAbs) and a mouse polyclonal antiserum specific for the *E. histolytica* portion of the fusion protein. A 29-kDa protein was identified as a surface antigen when mAbs were used to immunoprecipitate the antigen from metabolically ^{35}S-labeled live trophozoites. The surface location of the antigen was corroborated by mAb immunoprecipitation of a 29-kDa protein from surface-^{125}I-labeled whole trophozoites as well as by the reaction of mAbs with live trophozoites in an indirect immunofluorescence assay performed at 4°C. Immunoblotting with mAbs demonstrated that the antigen was present on four axenic isolates tested. mAbs recognized epitopes on the 29-kDa native antigen on some but not all clinical isolates tested.**

Entamoeba histolytica causes extensive mortality and morbidity worldwide through diarrheal disease and organ abscess formation. The understanding of membrane constituents is important to the study of potential mechanisms of parasite–host dynamics, and for selection of parasite proteins with potential for immunomodulation. In addition, analysis of the structure and function of parasite surface antigens is integral to the understanding of the parasite biochemistry. In previous studies, we identified, purified, and characterized the cellular distribution of the 96-kDa surface antigen of *E. histolytica* (1, 2). Utilizing surface antigens for detailed genetic and biochemical characterization is also a logical step in understanding the biochemical nature of the parasite. In this report, we describe the molecular cloning and nucleotide sequence of a cDNA that encodes a 29-kDa *E. histolytica* trophozoite antigen.** The antigen was located on the surface of the trophozoites and was cysteine-rich, suggesting a structural or functional role for a select cysteine-rich domain.

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MATERIALS AND METHODS

***E. histolytica* Strains and Culture Conditions.** Strains used included HM-1:IMSS (ATCC 30459), H-303:NIH (ATCC 30885), H-302:NIH (ATCC 30887), and HM-3:IMSS (ATCC 30890). Amoebae were cultivated axenically in TYI-S-33 medium (3) and harvested as described (1). Zymodeme-typed lysates of polyxenically cultivated clinical isolates were kindly provided by Sharon L. Reed (University of California, San Diego): SAW 1519, FAT 1014, FAT 967 (pathogenic zymodemes XIV, II, II, respectively), and FAT 1010, SD 107, SD 130 (nonpathogenic zymodeme I). Polyxenic cultures were prepared and 99.9% of bacteria adherent on the surface of the trophozoites were removed by washing (2).

cDNA Library. Total RNA was isolated from midlogarithmic-phase *E. histolytica* trophozoites (strain H-302:NIH) by the guanidinium isothiocyanate method (4). Poly(A)$^+$ RNA was selected by column chromatography using poly(U)-Sepharose 4B in 4 M guanidinium isothiocyanate (5). The first- and second-strand cDNA was synthesized from *E. histolytica* poly(A)$^+$ RNA as described (6), except that RNase inhibitor was present at 1 unit/μl for the first-strand reaction (7). Double-stranded cDNA was inserted into λgt11 and packaged (8), and an amplified library was prepared by plating on the bacterial host Y1088 (9). The quality of the library was assessed using a bovine actin cDNA probe (7, 10).

Northern Analysis. Northern analysis was performed by standard methods (8). In brief, 1 μg of *E. histolytica* mRNA was electrophoresed in a 1% agarose/2.2 M formaldehyde gel in Mops buffer and transferred to a nylon membrane (Hybond-N; Amersham) by capillary action (11). RNA was probed using ^{32}P-labeled bovine actin cDNA (2.5×10^6 cpm/ml; specific activity, 2.5×10^6 cpm/μg of DNA) or ^{32}P-labeled *E. histolytica* cDNA (10^6 cpm/ml; specific activity, 10^7 cpm/μg).

Isolation of DNA and Hybridizations. Cell lysates (5×10^6 cells) of four axenic *E. histolytica* strains, *Entamoeba invadens*, *Acanthamoeba castellanii*, and *Trichomonas vaginalis* were prepared for DNA hybridization (12). The lysates were applied to a nylon membrane (Hybond-N) using the Hybri-Slot manifold apparatus (Bethesda Research Laboratories) and hybridized to the 700-base-pair (bp) cDNA ^{32}P-labeled probe (10^6 cpm/ml; specific activity, 10^7 cpm/μg) (8). For

Abbreviations: GST, glutathione *S*-transferase; FP, fusion protein; mAb, monoclonal antibody.

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. M35635).

isolation of genomic DNA, *E. histolytica* HM-1:IMSS trophozoites were digested with proteinase K (200 μg/ml) in 0.02 M Tris Cl, pH 8/0.01 M EDTA/0.1 M NaCl/1% SDS at 37°C overnight. DNA was extracted with phenol/chloroform and precipitated with ethanol. Purified genomic DNA (0.5 μg) was used as a positive control.

Screening for Recombinants. The λgt11 library was screened with whole rabbit antiserum to *E. histolytica* trophozoites (1) (1:100 dilution) followed by peroxidase-conjugated protein A (Zymed Laboratories), then developed with 4-chloro-1-naphthol and H_2O_2. A second screening was performed using serum that had been adsorbed to and eluted from formalin-fixed trophozoites to enhance the chance of identifying clones expressing surface antigens. Five clones were obtained and subcloned into the plasmid vector pGEM-4 (Promega) for sequencing. One clone designated p47, which contained a 700-bp insert, was selected for further characterization (since it reacted with surface-adsorbed antibody) and was subcloned into a pGEX expression vector (13).

pGEX-1 transformants were screened with protein A-purified rabbit anti-*E. histolytica* serum (1:100 dilution) essentially as described (14), but positive reactions were detected with peroxidase-conjugated goat antiserum to rabbit immunoglobulins (1:200; Zymed Laboratories), and 4-chloro-1-naphthol and H_2O_2. Colonies showing positive reactions were selected for isolation of the fusion protein (FP).

Affinity Purification of the p47 FP. The FP was purified by a modification of the procedure of Smith and Johnson (13). An overnight culture of the transformant was diluted 1:10 in 250 ml of Luria–Bertani broth (8) and incubated for 1 hr at 37°C with shaking. Isopropyl β-D-thiogalactopyranoside (IPTG) was added to 0.1 mM and the culture was incubated for 4 hr. The cells were pelleted at 10,000 × *g* for 15 min and 20 ml of 25 mM Tris Cl, pH 8/50 mM glucose/10 mM EDTA containing lysozyme at 2 mg/ml was added to the pellet. The suspension was kept on ice for 30 min and then frozen at −70°C. The thawed lysate was sonicated on ice for 2 min and centrifuged at 10,000 × *g* for 5 min at 4°C. Three milliliters of a 50% suspension of glutathione-agarose beads (Sigma) was added to the supernatant and the mixture was incubated with rocking for 5 min. The agarose beads were washed three times in phosphate-buffered saline (PBS: 0.01 M phosphate/0.15 M NaCl, pH 7.6). The FP was eluted with 2 ml of 3 M sodium thiocyanate and dialyzed against PBS. The purified FP was used in ELISAs and immunoblotting procedures and for the production of mouse polyclonal antiserum and monoclonal antibodies (mAbs).

Production of FP-Specific Polyclonal Antiserum and mAbs. Polyclonal antiserum to the FP was produced in BALB/c mice. Six mice were immunized i.p. with 100 μg of purified FP (1 mg/ml in PBS) diluted 1:1 in adjuvant (Ribi Immunochem), which was given in three doses over the course of 5 weeks. Mice were bled by cardiac puncture, and serum was pooled and stored at −70°C. A serum pool from unimmunized mice served as a negative control.

mAbs specific for the *E. histolytica* portion of the FP were produced by the method of Köhler and Milstein (15). Mice were immunized i.p. with 100 μg of purified FP (1 mg/ml) diluted 1:1 in adjuvant (Ribi Immunochem). The mice were rested 21 days, given an i.p. booster injection of 100 μg of FP, rested for 20 days, and then given a final i.v. injection of 100 μg of FP. The cell fusion and cloning by limiting dilution were carried out as described (16). Screening was performed by ELISA using purified FP at 10 μg per well. Purified glutathione *S*-transferase (GST) was used as a negative control (at 100 μg per well). Ascites was generated by a published protocol (17) and protein A purification of antibody from ascites was carried out by standard methods. mAbs were isotyped with a kit (HyClone).

SDS/PAGE and Immunoblotting. Affinity-purified FP (1 μg per lane) or 5 × 10^5 solubilized whole *E. histolytica* trophozoites were electrophoresed in SDS/10% polyacrylamide gels (18) under reducing conditions and stained with Coomassie blue or transferred to nitrocellulose (19). Immunoblotting (20) used mouse polyclonal antiserum to the FP at a 1:750 dilution or mAb at a 1:500 or 1:1000 dilution. Bound antibody was detected with protein A peroxidase conjugate (1:1000; Zymed Laboratories) and stained with 4-chloro-1-naphthol and H_2O_2. Prestained molecular weight standards (Diversified Biotech, Newton Centre, MA) were included in each gel. Controls included isotype-matched mAb specific for *Pneumocystis carinii*, nonimmune mouse serum, or peroxidase conjugate alone.

Radioimmunoprecipitation. For the first immunoprecipitation procedure, live trophozoites labeled with [^{35}S]methionine/cysteine (Tran^{35}S-label; ICN) were incubated with mAb at 4°C and immunoprecipitation was carried out as described (2). The reaction mixture was maintained at 4°C to prevent capping and shedding or internalization of antibody (21). Alternatively, trophozoites metabolically labeled with ^{35}S or surface labeled with ^{125}I (1, 21) were solubilized in Nonidet P-40 prior to immunoprecipitation (1). Controls included isotype-matched mAb specific for *P. carinii* with protein A-Sepharose CL-4B or protein A-Sepharose CL-4B alone.

Immunofluorescence. Formalin-fixed amoebae (5 × 10^3) were fixed to glass slides with acetone, incubated with mouse polyclonal antiserum diluted in PBS containing 1% bovine serum albumin for 1 hr at 37°C, and then incubated with fluorescein-conjugated rabbit antiserum to mouse immunoglobulins (Zymed Laboratories). Alternatively, the indirect immunofluorescence procedure was performed at 4°C, using live organisms as described (1), except that the amoeba–mAb complexes were fixed in 1% formalin for 1 hr after incubation with the primary antibody (excess antibody was washed off prior to fixation) to preserve cellular morphology.

DNA Sequence Analysis. The nucleotide sequence of both strands of the 700-bp cDNA was determined by the dideoxy method (22, 23) using Sequenase version 2.0 (United States Biochemical) and SP6 and T7 promoter primers according to the manufacturer's instructions except that template–primer annealing was at 37°C for 10 min. Discrepancies between strands were resolved by using custom oligonucleotide primers (Louisiana State University Biotechnology Center). The sequence was analyzed using the computer program PC/GENE (IntelliGenetics/Genofit).

RESULTS

Isolation of Clone p47, Northern Analysis, and DNA Hybridizations. A cDNA library was generated from *E. histolytica* poly(A)$^+$ RNA and screened with rabbit polyclonal antiserum to *E. histolytica*. Clone p47, which contained a 700-bp insert, was subcloned into plasmid pGEM-4 for DNA sequencing and for further characterization, as it was suspected to be a surface antigen since the secondary λgt11 phage plaques reacted with surface-adsorbed and eluted antiserum. Northern blot analysis of poly(U)-selected *E. histolytica* mRNA with the 700-bp fragment as a probe identified a single 1050-nucleotide mRNA band (Fig. 1). By DNA·DNA hybridization, the 700-bp probe reacted with purified genomic DNA isolated from *E. histolytica* HM-1-IMSS as well as with DNA extracted from cell lysates of four axenic strains of *E. histolytica* but not with DNA extracted from *E. invadens*, *A. castellanii*, or *T. vaginalis* (Fig. 2 and data not shown for *T. vaginalis*).

Analysis of the FP Expressed in pGEX. The p47 insert was subcloned into pGEX expression vectors, which have frame-shifted cloning sites that direct the synthesis of the foreign polypeptide as a fusion with the C terminus of GST under the

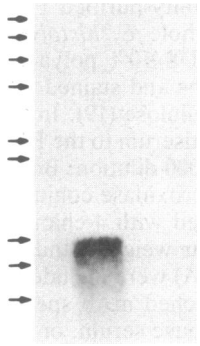

FIG. 1. Northern analysis of *E. histolytica* mRNA probed with the 700-bp cDNA. Arrows at left correspond to DNA molecular size standards of 23, 9.4, 6.6, 4.36, 2.3, 2.0, 1.35, 1.078, and 0.87 kilobases, in descending order.

control of the inducible *tac* promoter (13). The 700-bp insert was expressed only in pGEX-1, in contrast to vectors pGEX-2T and pGEX-3X. The FP was purified under nondenaturing conditions by affinity chromatography using glutathione-agarose beads and was analyzed by SDS/PAGE. The recombinant DNA expressed as a FP with the 27.5-kDa GST carrier migrated at 52.5 kDa (Fig. 3*A*, lane 1), suggesting that the *E. histolytica* portion of the protein encoded by p47 had an approximate size of 25 kDa. A 29-kDa protein of *Escherichia coli* copurified with the FP during affinity chromatography (Fig. 3*A*, lane 1). Bacterial proteins can associate with the FP when sonication is too severe and thus appear as contaminants after affinity purification (24). Fig. 3*A*, lane 2, shows the protein from the purified GST carrier alone.

mAbs. Twenty-two independent mAb-secreting clones were produced to the FP and when screened by ELISA, all of the mAb-bearing culture supernatants reacted with the FP but not with the purified GST carrier. Fourteen of the 22 mAb-bearing ascitic fluids later produced and tested as ascites or protein A-purified antibody were positive by immunoblot with the FP. Eight mAb-bearing ascitic fluids were negative by immunoblot but immunoprecipitated a native *E. histolytica* protein.

Antibody Specificity. Immunoblotting was used to analyze the antigenic specificity of mouse polyclonal antiserum and murine mAbs. Intense staining of the 52.5-kDa FP was detected with mouse polyclonal antiserum, whereas no reactivity to the GST carrier was detected, demonstrating that the antibodies were specific for the *E. histolytica* portion of the FP (Fig. 3*B*, lane 1). Nonimmune mouse serum tested at a 1:25 dilution with conjugate or conjugate alone did not react with the FP or with the purified GST (Fig. 3*B*). When immunoblotting was performed using early logarithmic-phase solubilized whole axenic *E. histolytica* trophozoites, the mouse polyclonal antiserum and mAb (1:1000) detected a single band, which comigrated with the 29-kDa standard (Fig. 3*C*, lane 1; Fig. 3*D*). This is 4 kDa larger than the cDNA-

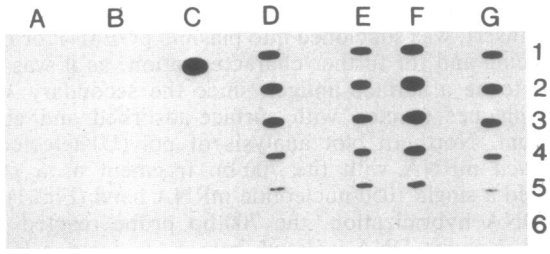

FIG. 2. DNA hybridizations of DNA extracted from *E. invadens*, *A. castellanii*, and four strains of *E. histolytica*. Column A, *E. invadens*; column B, *A. castellanii*; column C, genomic DNA isolated from *E. histolytica* HM-1:IMSS (0.5 μg); columns D–G, *E. histolytica* HM-3:IMSS, H-303:NIH, H-302:NIH, and HM-1:IMSS, respectively. Row 1, DNA extracted from 2.5×10^6 cells; rows 2–6, successive dilutions by a factor of two of the cell lysates used. For column C (genomic DNA), only one dilution was tested.

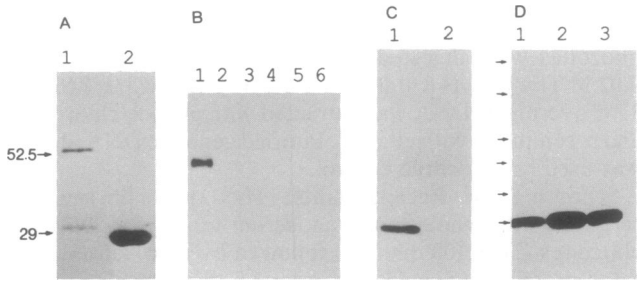

FIG. 3. Immunoblot analysis of mouse polyclonal antiserum and mAbs to purified FP or whole axenic *E. histolytica* trophozoite antigen. (*A*) Coomassie-stained gel. Lane 1, purified FP, 52.5 kDa; lane 2, purified GST, 27.5 kDa. (*B*) Immunoblot of FP and GST with mouse polyclonal antiserum (lanes 1 and 2), nonimmune mouse serum (lanes 3 and 4), and conjugate control (lanes 5 and 6). Lanes 1, 3, and 5, FP; lanes 2, 4 and 6, GST. (*C*) Immunoblot of whole trophozoite (HM-1:IMSS) antigen with mouse polyclonal antiserum (lane 1) and nonimmune mouse serum (lane 2). (*D*) Immunoblot of different strains of whole *E. histolytica* trophozoites with mAb FP-14. Lane 1, HM-1:IMSS; lane 2, H-303:NIH; lane 3, H-302:NIH. Arrows correspond to molecular size standards of 200, 95.5, 55, 43, 36, and 29 kDa, in descending order.

expressed protein, suggesting that the latter is missing ≈40 amino acids from the N terminus. mAb-bearing ascitic fluid FP-14 (1:1000) also reacted with the 29-kDa antigen on all four axenic strains tested (Fig. 3*D*, data shown for three strains). Six clinical isolates were tested with three of the mAb-bearing ascitic fluids (FP-14, FP-33, and FP-36; 1:500 dilution). All three mAbs reacted with a 29-kDa antigen on isolate FAT 967, and two of the three antibodies (FP-14 and FP-36) reacted with this antigen on isolate FAT 1014 (Fig. 4). mAbs FP-14 and FP-36 showed weak reactivity to the 29-kDa antigen on two nonpathogenic isolates, SD 130 and FAT 1010, respectively (data not shown). Nonimmune mouse serum, anti-*Pneumocystis* mAb, or conjugate alone did not react with *E. histolytica* antigens (data not shown).

Immunoprecipitation of antigen from live, metabolically ^{35}S-labeled trophozoites resulted in visualization of a single band at 29 kDa (Fig. 5*A*). Membrane integrity of trophozoites was demonstrated to be 100% by trypan blue exclusion after excess antibody was removed from the live organism–antibody mixture by four 10-ml washes in PBS, pH 7.6, at 4°C. When solubilized ^{35}S-labeled trophozoites were used for immunoprecipitation, a single 29-kDa band was also detected (data not shown). Immunoprecipitation of trophozoites sur-

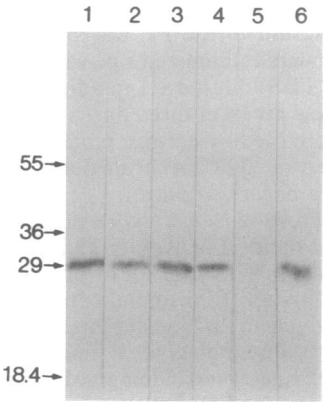

FIG. 4. mAb immunoblotting of polyxenically cultivated *E. histolytica* from two patients with liver abscess infection. Lanes 1–3, amoebae (5×10^5) from FAT 967; lanes 4–6, amoebae (5×10^5) from FAT 1014. Lanes 1 and 4 were immunoblotted with mAb FP-36; lanes 2 and 5, mAb FP-33; lanes 3 and 6, mAb FP-14. Molecular size standards are at left (kDa).

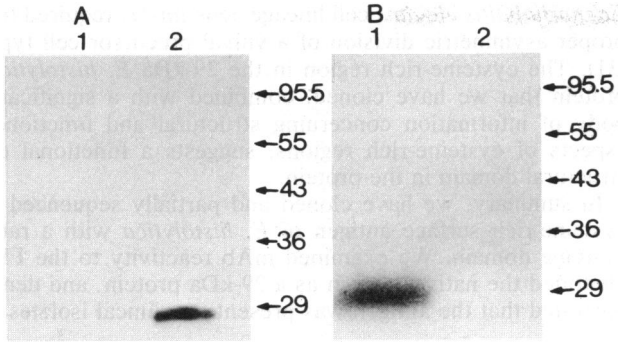

FIG. 5. Immunoprecipitations of metabolically ^{35}S-labeled protein from live axenic *E. histolytica* trophozoites and solubilized surface-^{125}I-labeled trophozoites. Molecular size standards are at right (kDa). (*A*) ^{35}S-labeled live trophozoites. Lane 1, immunoprecipitation using *P. carinii* mAb; lane 2, mAb FP-21. (*B*) Surface-^{125}I-labeled trophozoites. Lane 1, immunoprecipitation using mAb FP-21; lane 2, *P. carinii* mAb.

face-labeled with ^{125}I also demonstrated a 29-kDa protein (Fig. 5*B*). Whole trophozoites were obtained after iodination by using a Percoll gradient (21), and trypan blue exclusion demonstrated that membranes of iodinated trophozoites were intact. Whole metabolically ^{35}S-labeled and surface-^{125}I-labeled *E. histolytica* profiles were identical to those previously described (ref. 1; data not shown). mAb specific for *P. carinii* failed to immunoprecipitate antigens from ^{35}S-labeled live trophozoites or solubilized ^{35}S- or ^{125}I-labeled trophozoites.

Immunofluorescence. Indirect immunofluorescence of formalin-fixed *E. histolytica* trophozoites showed uniform staining over the entire organism, suggesting surface staining (Fig. 6*A*). Staining of live trophozoites also demonstrated surface specificity, showing even fluorescence over the entire surface of the organism (Fig. 6*B*). Immunofluorescence was not detected when live and formalin-fixed organisms were tested with nonimmune mouse serum (1:12.5 dilution), mAb to *P. carinii*, or fluorescein conjugate only (data not shown).

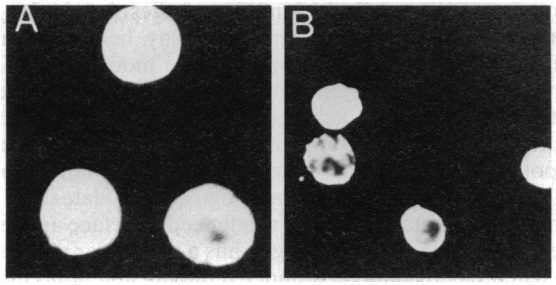

FIG. 6. Indirect immunofluorescence profiles of formalin-fixed and live axenic *E. histolytica* trophozoites showing uniform staining of the organisms. (*A*) Formalin-fixed *E. histolytica* stained with mouse polyclonal antiserum to the FP. (×630.) (*B*) Live *E. histolytica* stained with mAb FP-28. (×400.)

Nucleotide Sequence. The nucleotide sequence was determined for p47 cDNA. A single long open reading frame of 226 amino acids was defined, encoding a polypeptide of 25.3 kDa (Fig. 7). The deduced amino acid sequence revealed a cysteine-rich protein, with one putative immunodominant epitope identified in a hydrophilicity plot (ref. 25; data not shown) by using the program PC/GENE.

DISCUSSION

While *E. histolytica* continues to be a serious worldwide health concern and a leading cause of morbidity and mortality among diarrheal diseases, little information is available concerning structure and function of surface antigens that may play an integral role in the modulation of host–parasite

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                                                        10
      GGG TGT TGT AAA AAA GAA TGT CAA GAG AAA GAA TGT TGT AAA GAA TGT TGT
NH2... Gly Cys Cys Lys Lys Glu Cys Gln Glu Lys Glu Cys Cys Lys Glu Cys Cys>
                  20                                    30
      TGT CCA AGA ATA AAA GCA TTT AAG AAA TTT ATA AAC ACA TTT GAA AAA GCA
      Cys Pro Arg Ile Lys Ala Phe Lys Lys Phe Ile Asn Thr Phe Glu Lys Ala>
                        40                                          50
      CAA ATT GGA AAA GAA GCC CCA GAA TTT AAA GCA CCA GCA TAT TGT CCA TGT
      Gln Ile Gly Lys Glu Ala Pro Glu Phe Lys Ala Pro Ala Tyr Cys Pro Cys>
                                 60
      GGT TCA ATC AAA GAG ATT GAT ATT AAT GAA TAT AAA GGA AAA TAT GTT GTA
      Gly Ser Ile Lys Glu Ile Asp Ile Asn Glu Tyr Lys Gly Lys Tyr Val Val>
           70                                    80
      TTG TTG TTT TAT CCA TTG GAT TGG ACA TTT GTT TGT CCA ACA GAA ATG ATT
      Leu Leu Phe Tyr Pro Leu Asp Trp Thr Phe Val Cys Pro Thr Glu Met Ile>
                        90                                          100
      GGA TAT AGT GAA CTT GCA GGA CAA TTG AAA GAA ATC AAT TGT GAA GTT ATT
      Gly Tyr Ser Glu Leu Ala Gly Gln Leu Lys Glu Ile Asn Cys Glu Val Ile>
                              110
      GGA GTG AGT GTA GAT TCA GTT TAT TGT CAT CAA GCA TGG TGT GAA GCA GAT
      Gly Val Ser Val Asp Ser Val Tyr Cys His Gln Ala Trp Cys Glu Ala Asp>
      120                                   130
      AAA AGT AAA GGA GGA GTA GGA AAG TTG ACA TTC CCA TTA GTA TCA GAT ATT
      Lys Ser Lys Gly Gly Val Gly Lys Leu Thr Phe Pro Leu Val Ser Asp Ile>
                  140                                         150
      AAG AGA TGC ATT TCT ATC AAA TAT GGA ATG TTA AAT GTC GAA GCA GGA ATT
      Lys Arg Cys Ile Ser Ile Lys Tyr Gly Met Leu Asn Val Glu Ala Gly Ile>
                              160                                         170
      GCA AGA AGA GGA TAT GTC ATC ATT GAT GAT AAA GGA AAA GTA AGA TAC ATT
      Ala Arg Arg Gly Tyr Val Ile Ile Asp Asp Lys Gly Lys Val Arg Tyr Ile>
                                    180
      CAA ATG AAT GAT GAT GGA ATT GGA AGA TCA ACG GAA GAA ACA ATC AGA ATA
      Gln Met Asn Asp Asp Gly Ile Gly Arg Ser Thr Glu Glu Thr Ile Arg Ile>
            190                                   200
      GTT AAA GCA ATT CAA TTC AGT GAT GAA CAT GGA GCA GTT TGT CCA CTC AAT
      Val Lys Ala Ile Gln Phe Ser Asp Glu His Gly Ala Val Cys Pro Leu Asn>
                        210                                         220
      TGG AAA CCA GGC AAA GAC ACC ATT GAA CCA ACA CCA GAT GGA ATT AAG AAA
      Trp Lys Pro Gly Lys Asp Thr Ile Glu Pro Thr Pro Asp Gly Ile Lys Lys>

      TAT TTA ACA GCA CAT [TAA] AAC AAA CAA GAT AAT TTC C
      Tyr Leu Thr Ala His End
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FIG. 7. Nucleotide sequence of the 700-bp cDNA and the deduced amino acid sequence.

interactions. Especially lacking are studies on cloning and sequencing of surface antigens that may be important as potential vaccinogens or as biochemical modulators of cellular functions of this pathogenic protozoan. Since evidence exists implicating cell-mediated immunity to *E. histolytica* as the primary mechanism in protection from invasive disease, the potential for vaccine development should not be overlooked, and surface antigens are logical candidates.

Thus, we characterized and sequenced a surface antigen of *E. histolytica*. The native antigen has a molecular mass of 29 kDa when electrophoresed under reducing and nonreducing conditions. Northern analysis detected an mRNA of 1.05 kilobases, a size sufficient to code for the entire protein. Lack of identification of a multimer when the antigen was solubilized under nonreducing conditions and electrophoresed does not *a priori* indicate that the antigen cannot form multimers through other than disulfide-bond interaction. Location of the antigen on the surface of the trophozoites was determined by immunoprecipitation with mAbs and live, metabolically ^{35}S-labeled organisms; indirect immunofluorescence performed on live organisms; and iodination of whole organisms followed by immunoprecipitation. The protein was determined to be intrinsic to *E. histolytica* by metabolic labeling. Immunoblotting demonstrated that the protein was expressed in four axenic pathogenic *E. histolytica* strains tested. Further, immunoblotting demonstrated that three of the mAbs generated to the FP recognized epitopes on the 29-kDa native antigen on some but not all clinical isolates tested, consistent with our previous observations with mAb to the 96-kDa surface antigen, which demonstrated differential reactivity among polyxenically cultivated isolates (2). DNA hybridization experiments showed that the gene was present in four axenic *E. histolytica* strains tested but was not present in *E. invadens*, *T. vaginalis*, or *A. castellanii*.

Computer analysis of the amino acid sequence with the program PC/GENE revealed a hydrophilic region (amino acids 117–122, Glu-Ala-Asp-Lys-Ser-Lys) predicted to be an antigenic determinant for this protein (25). The cDNA sequence suggests a remarkably cysteine-rich protein (7%) with multiple cysteine doublets and a triplet near the 5′ end of the cDNA, although the projected missing 40 amino acids at the 5′ end of the gene may alter the total cysteine content. Analysis of homology of the entire cDNA sequenced and deduced protein sequence revealed no similarity to other proteins or genes in current data banks (GenBank Release 55 and EMBL Release 19).

The deduced sequence of amino acids 2–18 is Cys-Cys-Lys-Lys-Glu-Cys-Gln-Glu-Lys-Glu-Cys-Cys-Lys-Glu-Cys-Cys-Cys. In a search of protein data banks, this Cys_2 . . . Cys . . . Cys_2 . . . Cys_3 formation was found in only two other proteins, in inverse order, both possessing an extra single Cys. One of the sequences was located in the peplomer protein E2 of porcine coronavirus, which mediates binding to the host cell receptor and is involved in cellular membrane fusion (26). The other similar cysteine-rich sequence was found in the SCMK-2BA protein from the high-sulfur fraction of wool keratin (27). The function of the SCMK-2BA cysteine-rich regions was suggested to be involved in structural rigidity on the basis of a multiple 10-codon sequence repeated five times. Cysteine-rich regions of proteins are generally highly conserved and are implicated in functional or structural aspects of several proteins recently reported. Cysteine residues contribute to the function of the human immunodeficiency virus type 1 envelope (28). The insulin gene enhancer-binding protein Isl-1 contains both homeo- and Cys-His domains (29), and metal-binding regions of a number of proteins contain conserved cysteine residues generally separated by two amino acids (30). Lastly, a novel cysteine-rich homeodomain is present in the product of the nematode *Caenorhabditis elegans* cell lineage gene *lin-11*, required for proper asymmetric division of a vulval precursor cell type (31). The cysteine-rich region in the 29-kDa *E. histolytica* protein that we have cloned, combined with a significant body of information concerning structural and functional aspects of cysteine-rich regions, suggests a functional or structural domain in the protein.

In summary, we have cloned and partially sequenced a cysteine-rich surface antigen of *E. histolytica* with a rare cysteine domain. We examined mAb reactivity to the FP, identified the native antigen as a 29-kDa protein, and demonstrated that the antigen was present on clinical isolates.

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