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Expression of Amoebapores Is Required for Full Expression of *Entamoeba histolytica* Virulence in Amebic Liver Abscess but Is Not Necessary for the Induction of Inflammation or Tissue Damage in Amebic Colitis

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Entamoeba histolytica **trophozoites produce amoebapores, a family of small amphipathic peptides capable of insertion into bacterial or eukaryotic membranes and causing cellular lysis. Recently, *E. histolytica* trophozoites that are totally deficient in the production of amoebapore-A were created through a gene silencing mechanism (R. Bracha, Y. Nuchamowitz, and D. Mirelman, Eukaryot. Cell 2:295-305, 2003). Here we tested the virulence of amoebapore A(−) trophozoites in models of the two major forms of amebic disease: amebic liver abscess and amebic colitis. We demonstrate that amoebapore expression is required for full virulence in the SCID mouse model of amebic liver abscess, but *E. histolytica* trophozoites that do not express amoebapore-A can still cause inflammation and tissue damage in infected human colonic xenografts. These data are consistent with the concept that tissue damage may proceed by different mechanisms in amebic liver abscess compared to amebic colitis.**

One of the remarkable capacities of *Entamoeba histolytica* trophozoites is their ability to lyse eukaryotic cells on contact. This ability derives from amoebapores, a family of three pore-forming peptides (amoebapore A [AP-A], AP-B, and AP-C) (10, 12). Amoebapores insert into the membranes of bacteria or eukaryotic cells and form pores that result in lysis of the target cells. The addition of purified amoebapores to eukaryotic cells results in cell necrosis and possibly apoptosis (2, 4, 11). *E. histolytica* trophozoites expressing decreased levels of AP-A (by antisense inhibition) showed defects in cytolytic and bactericidal functions (5). Trophozoites with decreased levels of amoebapore were also less virulent in the hamster model of amebic liver abscess, a finding consistent with a role for amoebapores in liver abscess formation (5). Recently, it was found that transfection of *E. histolytica* trophozoites with plasmid psAP-1, a construct encoding the AP-A gene, flanked by its regulatory components or plasmid psAP-2, which contains only 470 bp of the 5′ upstream flanking region of the AP-A gene, results in transcriptional silencing of the AP-A gene, with resulting complete loss of AP-A expression (6). This complete gene silencing contrasts with the residual amoebapore activity seen when antisense inhibition was used, and the creation of totally AP-A-deficient *E. histolytica* trophozoites provides an unprecedented opportunity to assess the role of AP-A in amebic colitis and amebic liver abscess formation. Here, we examine the virulence of *E. histolytica* strain HM-1:IMSS trophozoites lacking AP-A expression in two well-established models of amebiasis: the severe combined immunodeficient mouse-human intestinal xenograft (SCID-HU-INT) model of amebic colitis and the SCID mouse model of amebic liver abscess.

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MATERIALS AND METHODS

Strain and culture conditions. *E. histolytica* HM1:IMSS trophozoites were grown at 37°C in TYI-S-33 media as previously described (14).

Plasmids and transfections. The pEhActNeo plasmid (1), which contains the Neo gene conferring resistance to G418, the 5′ and 3′ untranslated regions from the ameba actin 1 gene, and the *E. histolytica* autonomous replication sequence served as the initial control vector for these experiments. Plasmid psAP-2 was derived from pEhActNeo by inserting a fragment containing 470 bp of the upstream 5′ flanking region of the *ap-A* gene (6). Trophozoites transfected with the psAP-2 plasmid underwent selection with G418 to a maximum concentration of 60 μg/ml as previously described (6). After 2 months of selection, G418 was withdrawn and, 60 days later, AP-A expression was assessed by real-time PCR and immunoblotting. *E. histolytica* trophozoites used for liver challenges or human colonic xenograft challenges had been grown without G418 for at least 90 days.

RNA preparation and real-time PCR. A total of 10^6 *E. histolytica* trophozoites in log phase were harvested, washed twice by suspending them in cold phosphate-buffered saline (PBS), and centrifuged at 430 × *g* for 5 min; the pellet was suspended in TRIzol, and RNA was isolated according to the manufacturer's protocol (Invitrogen Life Technologies, Carslbad, Calif.). Reverse transcription was performed with 2 μg of total RNA, oligo(dT) as primers and avian myeloblastosis virus reverse transcriptase (Fisher Research, Pittsburg, Pa.). Primers for PCR were designed by using Primer Express Software (Applied Biosystems, Foster City, Calif.) and are shown in Table 1. Real-time PCR was carried out in a total volume of 10 μl with 200 nM primer pairs, 5 μl of 2× SYBR Green PCR Mastermix (Applied Biosystems), and various concentrations of cDNA template under conditions of 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, followed by 60°C for 1 min by using a ABI 7700 (Applied Biosystems). Each reaction was run in triplicate.

Immunoblotting. Soluble extracts from *E. histolytica* trophozoites that had been previously transfected with psAP-2 or control *E. histolytica* trophozoites were prepared as previously described (18), separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions, transferred to nitrocellulose membranes, and reacted with rabbit polyclonal antibodies against AP-A (a gift of Matthias Leippe, University of Wurzburg, Wurzburg, Germany).

SCID mouse model of amebic liver abscess. BALB/c SCID mice, male, 6 to 8 weeks of age, were used for all experiments. For studies of amebic liver abscess, 10^6 AP-A(−) *E. histolytica* trophozoites or 10^6 trophozoites of the parent HM1:IMSS *E. histolytica* strain were directly inoculated into the liver of SCID mice as previously described (7). After 48 h, animals were sacrificed, and the livers were removed and weighed. The abscessed region of the liver was cut out and weighed, and the percent liver abscessed was calculated.

TABLE 1. Primers used for real-time PCR

Gene	Forward primer sequence	Reverse primer sequence
AP-A	5′-CACTAAGGGAGCTGATAAAGTAAAAGATTA-3′	5′-TCCAAAATCAAGAACTTTAGTGCAA-3′
AP-B	5′-TGGTGCTCAAGCTGTTAGACAATAT-3′	5′-AAGGATTTTTTCACAAAGAGTTCCA-3′
AP-C	5′-TCCTGTTTGTACATCACTTGTTGGA-3′	5′-GCACAGAGTGTTTCAAGATAATCAGTTAC-3′
Actin	5′-TGTAGATAATGGATCAGGAATGTGTAAA-3′	5′-CAATGGATGGGAATACAGCTCTT-3′
18S RNA	5′-TGGTGCATGGCCGTTCTTA-3′	5′-AATTAATAGGTTTCAGTCTCGTTCGTT-3′

SCID-HU-INT mouse model of amebic colitis. Human colonic xenografts were transplanted into the subscapular region of SCID mice (age, 6 to 8 weeks) and allowed to mature for 8 weeks to generate SCID-HU-INT mice as previously described (14). Log-phase cultures of *E. histolytica* HM1:IMSS AP-A(−) or the parental HM1:IMSS strain trophozoites were chilled on ice for 10 min, pelleted by centrifugation at 500 × *g* for 5 min, and resuspended in TYI-S-33 medium at 10^6 trophozoites/100 μl. To establish amebic colitis, 100 μl of the amebic suspension was injected directly into the lumen of the grafts via a 26-gauge needle. A small square of gelfoam was placed over the injection site upon removal of the needle in order to prevent leakage. The incision was closed with 5-mm Michel clips (14). At 24 h after inoculation, SCID-HU-INT mice were sacrificed, and segments of the intestinal xenografts were assayed for cytokine and myeloperoxidase (MPO) production as previously described (15). Fifteen SCID-HU-INT mice were used in each challenge group; 5 SCID-HU-INT mice served as the uninfected controls.

Cytokine assays. Protein samples for enzyme-linked immunosorbent assay (ELISA) were prepared by homogenizing tissue at 50 mg/ml in a solution of PBS containing 1 μg each of aprotinin, leupeptin, and pepstatin A (Sigma, St. Louis, Mo.)/ml. The homogenized samples were centrifuged at 12,000 × *g* for 15 min. Supernatants were processed as specified by the suppliers of the ELISA kits. ELISAs for interleukin-1β (IL-1β) and IL-8 were obtained from Endogen (Woburn, Mass.). The sensitivities were 1 pg/ml for IL-1β and 2 pg/ml for IL-8.

MPO assay. Tissue samples were homogenized for 30 s at a concentration of 50 mg/ml in a solution of PBS with 1 μg each of aprotinin, leupeptin, and pepstatin A/ml. Samples were spun at 12,000 × *g* for 15 min, and the pellet was resuspended in the same volume of 80 mM sodium phosphate–1% hexadecyltrimethylammonium bromide (Sigma)–5 mM EDTA (pH = 5.4). Samples were subjected three times to a freeze-thaw cycle and spun at 2,000 × *g* for 15 min, and the supernatants were used in the subsequent assay. Samples (25 μl) were combined with 125 μl of 80 mM sodium phosphate (pH 5.4) and 25 μl of 1.28 mM 3,3′,5,5′-tetramethylbenzidine dihydrochloride (Sigma) in dimethyl sulfoxide. A total of 25 μl of H_2O_2 in 80 mM sodium phosphate was added immediately prior to analysis to yield a final concentration of 0.24 mM and a final reaction volume of 200 μl. Conversion of the substrate was read at 650 nm. Dilutions of purified MPO (Sigma) were used as standards.

Measurement of intestinal permeability. Dextrans of approximate mass of 4,000 Da labeled with fluorescein isothiocyanate (FITC) were purchased from Sigma and resuspended in endotoxin-free PBS at a concentration of 5 mg/ml. At 4 h prior to sacrifice, the renal pedicle was tied off to prevent excretion of the fluorophore, and 50 μl of the solution was injected directly into the lumen of the graft. At the time of sacrifice, animals were bled, and 20 μl of blood was diluted into 400 μl of 150 mM NaCl–50 mM Tris (pH 10.3) and spun at 2,000 × *g* for 15 min. The supernatants from this spin were analyzed on a Cytofluor 23000 fluorescent plate reader (Millipore, Bedford, Mass.)

RESULTS

Reduction of AP-A expression in psAP-2-transfected *E. histolytica*. We first confirmed that *E. histolytica* trophozoites that had been transfected earlier with the psAP-2 plasmid had undergone silencing of the *ap-A* gene (6). Real-time PCR was performed on three different RNA samples obtained from psAP-2-transfected *E. histolytica* trophozoites 3 months after removal of G418 selection and from three different RNA samples obtained from the parental *E. histolytica* HM1:IMSS strain. Primers specific for AP-A, AP-B, and AP-C were used, along with primers for actin and *E. histolytica* 18S rRNA as controls. The psAP-2-transfected trophozoites demonstrated a >5,000-fold decrease in AP-A expression compared to the parental control trophozoites (Table 2). AP-B expression was also significantly decreased (>400-fold), but we found no evidence for a decrease of expression of AP-C. Transcripts for actin and 18S RNA were present in roughly equivalent numbers in all samples. The results from the real-time PCR analysis were confirmed by immunoblotting with polyclonal antibodies against purified AP-A. As shown in Fig. 1, lysates obtained from AP-A(−) trophozoites showed little detectable AP-A compared to lysates obtained from the parental *E. histolytica* HM1:IMSS strain.

***E. histolytica* trophozoites lacking AP-A expression show reduced virulence in the SCID mouse model of amebic liver abscess.** When *E. histolytica* trophozoites are directly inoculated into the livers of SCID mice, they cause liver abscesses, which reach maximum size approximately 48 h after inoculation (7, 19). We challenged groups of 13 SCID mice with intrahepatic inoculations of 10^6 E. histolytica AP-A(−) trophozoites or 10^6 trophozoites of the control, parental HM1:IMSS strain and assessed liver abscess size at 48 h. As shown in Fig. 2, *E. histolytica* trophozoites lacking AP-A caused significantly smaller liver abscesses than control HM1:IMSS trophozoites ($P < 0.05$ in a two-tailed t test comparing the mean percentage of the liver occupied by abscess in each group) after direct inoculation into the livers of SCID mice. Histologic examination of microscopic sections of the amebic liver ab-

TABLE 2. Real-time PCR analysis of amoebapore expression in AP-A(−) *E. histolytica* trophozoites and the parental *E. histolytica* HM1:IMSS strain[a]

Gene	Sample	Mean Ct	ΔCt	ΔΔCt	Fold difference
AP-A	AP(−)	27.5	12.4		
	Control	15.2	0	−12.4	5,404.704
AP-B	AP(−)	23.6	8.5		
	Control	14.9	−0.3	−8.8	445.7219
AP-C	AP(−)	15	−0.1		
	Control	14.8	−0.4	−0.3	1.231
18S rRNA	AP(−)	15.1			
	Control	15.2			

Values shown for mean threshold cycle (Ct) were obtained from three different samples of RNA obtained from cultures of AP-A(−) *E. histolytica* trophozoites [AP(−)] or the parental *E. histolytica* HM1:IMSS strain (control). The ΔCt represents the difference in cycle time for the indicated gene between AP(−) and control *E. histolytica* trophozoites; the ΔΔCt values were obtained by using the values for 18S RNA as the internal control. Similar results were seen when actin was used as the standard (data not shown). Relative quantification of amoebapore gene expression was performed using the comparative Ct method (Applied Biosystems) by using the expression $2^{-\Delta\Delta Ct}$ to calculate the fold difference in transcript level.

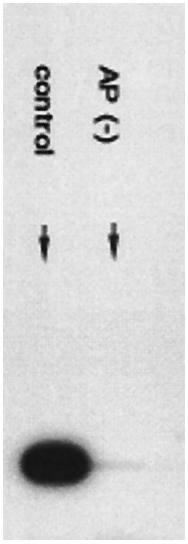

FIG. 1. AP-A(−) *E. histolytica* trophozoites produce little detectable AP-A by immunoblotting. Lysates from AP-A(−) *E. histolytica* trophozoites [AP(−)] or the parental *E. histolytica* HM1:IMSS strain (control) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and reacted with a polyclonal antiserum to AP-A. A prominent band at ~5 kDa (the region where amoebapores migrate) is visible in the control lane but is barely detectable in the lane loaded with lysates from AP-A(−) trophozoites.

scesses did not reveal obvious differences between abscesses induced by AP-A(−) trophozoites and those induced by the parental *E. histolytica* HM1:IMSS strain (data not shown).

***E. histolytica* trophozoites lacking AP-A show a mixed phenotype in the SCID-HU-INT model of amebic colitis.** We next examined whether amebic trophozoites lacking AP-A showed altered virulence in the SCID-HU-INT model of amebic colitis. The lumens of human colonic xenografts in SCID-HU-INT mice were inoculated with 10^6 trophozoites of the parent HM1:IMSS strain or 10^6 AP-A (−) *E. histolytica* trophozoites. *E. histolytica* trophozoites induce the production of inflammatory mediators, including interleukin-1β (IL-1β) and IL-8, in human intestinal xenografts (14, 15, 20). The mean levels of IL-1β and IL-8 from 15 human intestinal xenografts infected with AP-A(−) *E. histolytica* trophozoites were significantly higher than the mean levels of IL-8 and IL-1β from five uninfected human intestinal xenografts ($P < 0.01$ [two-tailed Student *t* test]), but were significantly lower ($P < 0.05$ [two-tailed Student *t* test]) than the mean levels obtained from 15 human colonic xenografts infected with the parental HM1:IMSS strain (Fig. 3A and B).

To measure inflammation in *E. histolytica*-infected human intestinal xenografts, we measured the levels of MPO, a neutrophil/early monocyte-specific marker. MPO levels increase in *E. histolytica*-infected xenografts, correlating with the degree of neutrophil influx, but are undetectable in uninfected xenografts (15). We found that the mean MPO levels were significantly higher in the human intestinal xenografts infected with the parental *E. histolytica* HM1:IMSS strain or the *E. histolytica* AP-A(−) trophozoites compared to uninfected xenografts ($P < 0.01$ for each comparison by using a two-tailed Student *t* test) (Fig. 3C). Although the levels of MPO were lower in human colonic xenografts infected with AP-A(−) *E. histolytica* trophozoites compared to the parental HM1:IMSS strain, the difference did not reach statistical significance ($P = 0.46$ [two-tailed Student *t* test]).

We also looked for differences in intestinal permeability between human colonic xenografts infected with AP-A-deficient *E. histolytica* trophozoites or the parental HM1:IMSS strain by introducing fluoresceinated dextran (FITC-dex) into the lumen of the human colonic xenografts and measuring its levels in the serum of SCID-HU-INT mice 24 h later (15). Wild-type *E. histolytica* trophozoites damage the intestinal permeability barrier, allowing the flux of fluoresceinated dextran from the lumen of colonic xenograft into the circulation of the SCID-HU-INT mice. Uninfected human intestinal xenografts retain their permeability barrier, preventing the flow of fluoresceinated dextran into the circulatory system of the SCID-HU-INT mouse. Human intestinal xenografts infected with *E. histolytica* AP-A(−) trophozoites or the parental *E. histolytica* HM1:IMSS strain both showed damage to the intestinal permeability barrier, with detection of fluoresceinated dextran in the serum of the SCID-HU-INT mice (Fig. 3D). Levels of fluoresceinated dextran were significantly higher in human intestinal xenografts infected with the parental HM1:IMSS strain or the AP-A(−) *E. histolytica* trophozoites compared to uninfected human colonic xenografts ($P < 0.01$ for each comparison [two-tailed *t* test]). Although serum levels of FITC-dex were lower in SCID-HU-INT mice with colonic xenografts infected with AP-A(−) *E. histolytica* trophozoites compared to serum obtained from SCID-HU-INT mice with human colonic xenografts infected with the parental HM1:IMSS strain, this

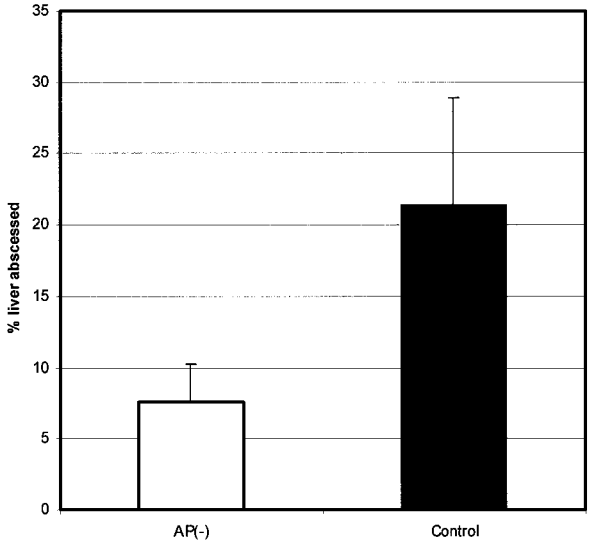

FIG. 2. AP-A(−) *E. histolytica* trophozoites cause significantly smaller amebic liver abscesses compared to the parental HM1:IMSS strain in SCID mice. The mean percentage of liver abscessed from SCID mice ($n = 13$) infected with AP-A(−) *E. histolytica* trophozoites [AP(−)] or SCID mice ($n = 13$) infected with the parental *E. histolytica* HM1:IMSS strain (control) is shown. The standard deviation of each mean value is shown in the error bars. The difference in the mean percentage of liver abscessed between livers infected with AP-A(−) trophozoites compared to the parental HM1:IMSS strain was significant at $P < 0.05$ as determined by a two-tailed Student *t* test.

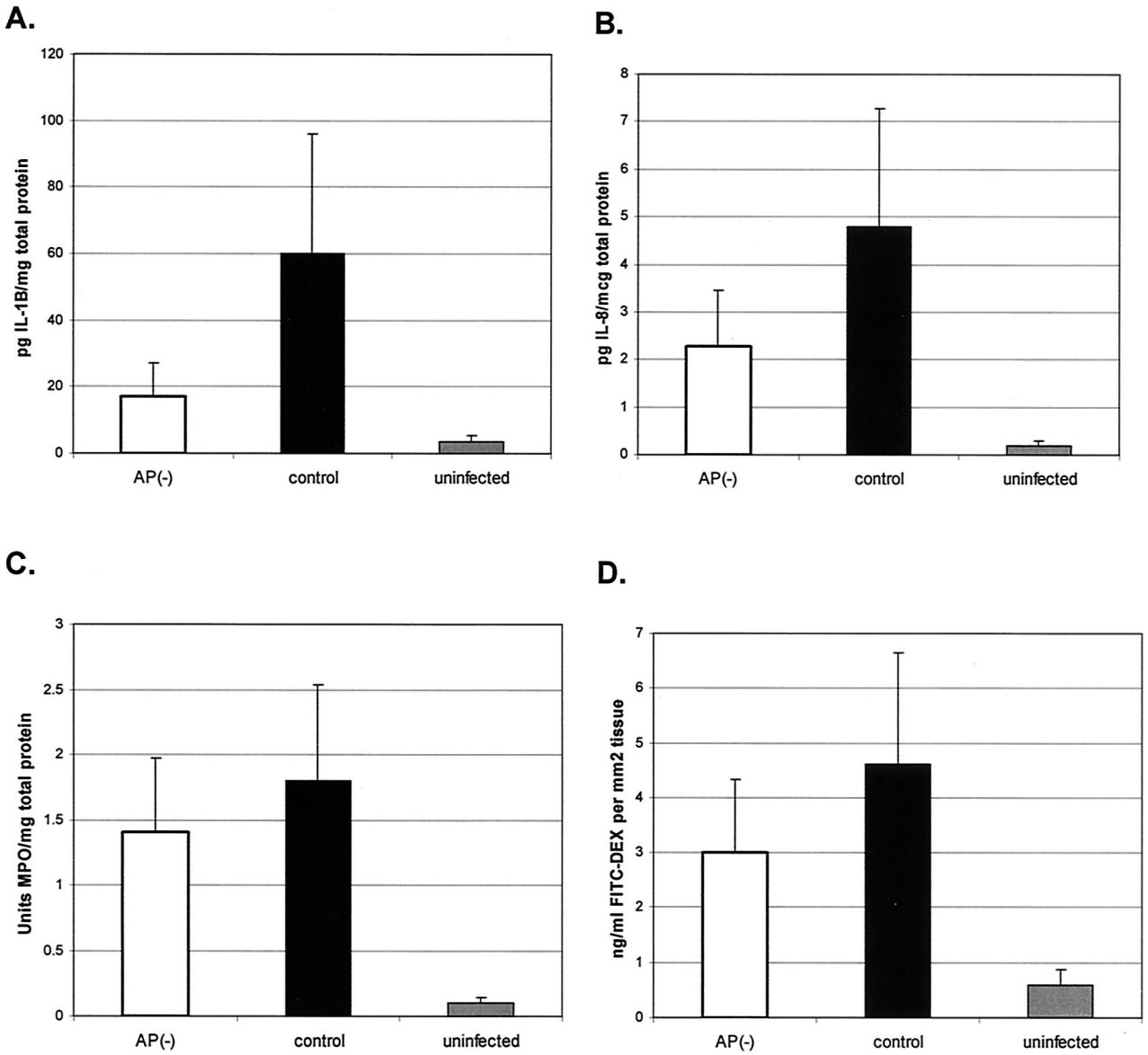

FIG. 3. Comparison of amebic colitis in human colonic xenografts obtained from SCID-HU-INT mice after xenograft infection with either AP-A(−) *E. histolytica* trophozoites or the parental *E. histolytica* HM1:IMSS strain. In all panels, values are the mean ± the standard deviation for 15 human colonic xenografts infected with AP-A(−) *E. histolytica* trophozoites [AP-A(−)], or 15 human colonic xenografts infected with the parental *E. histolytica* HM1:IMSS strain (control) or five uninfected human colonic xenografts (uninfected). Shown are values for IL-1β, expressed as picograms of IL-1β per milligram of total protein (A); IL-8, expressed as picograms of IL-8 per microgram of total protein (B); MPO, expressed as MPO per milligram of total protein (C); or the levels of FITC-dex in the serum of the host SCID-HU-INT mice, expressed as nanograms of FITC-dex/ml/mm^2 (D).

difference was not statistically significant ($P = 0.24$ [two-tailed Student *t* test]). Histologic examination of microscopic sections did not reveal differences between sections obtained from human intestinal xenografts infected with AP-A(−) *E. histolytica* trophozoites and those infected with the parental HM1:IMSS strain (data not shown).

DISCUSSION

The use of genetic approaches (dominant-negative mutants, antisense expression) has facilitated the effort to define virulence factors of *E. histolytica* (16). Key roles for the amebic Gal/GalNAc specific lectin (21), amebic cysteine proteinases (3), and amoebapores (5) in the formation of amebic liver abscesses in gerbil or hamster models have been established through these approaches. One limitation of the current genetic approaches using antisense to reduce the expression of a targeted *E. histolytica* gene is the inability to completely block gene expression. In the case of amebic cysteine proteinases, residual proteinase activity of 10 to 20% of wild-type levels was found in amebic trophozoites expressing the antisense construct, (22). Similarly, amebic trophozoites expressing an antisense construct to the *E. histolytica* alcohol dehydrogenase 2 (EhADH2) gene had residual alcohol dehydrogenase that was

ca. 15% of wild-type levels (9). The recent discovery that expression of AP-A gene could be silenced by transfection with a plasmid expressing the gene and its regulatory elements, provides a new tool for analyzing the role of AP-A in disease (6). However, while essentially free of AP-A, these trophozoites may not be completely free of amoebapore activity, since we found that they still express transcripts for one of the isoforms, AP-C. This isoform only accounts for ca. 3% of the total amoebapore content (13) but is reported to have the highest ion pore-forming activity (11).

Previous studies with AP-A(−) trophozoites showed that AP-A expression was required for amebic liver abscess formation in hamsters, since no hamsters inoculated with AP-A(−) trophozoites developed amebic liver abscesses (6). In the present study we examined whether a similar effect could be seen in the SCID mouse model of amebic liver abscess. Our results confirmed the decreased virulence of AP-A (−) trophozoites, since liver abscesses were significantly smaller in SCID mice challenged with AP-A(−) trophozoites. However, unlike experiments in hamsters, the difference was not absolute, since most SCID mice challenged with AP-A(−) trophozoites still developed amebic liver abscesses. This may reflect an increased susceptibility of SCID mice to amebic liver abscesses, the different timing of liver abscess assessment in each model (48 h in the SCID mouse model versus 7 days in hamsters), or possible differences in the role of the amoebapore in each animal model.

Of greater interest was whether AP-A(−) trophozoites would show decreased virulence in the SCID-HU-INT model of amebic colitis. This model, which allows one to study the interaction of *E. histolytica* trophozoites and human colon in vivo, has proven useful for analyzing both host responses to infection and the role of specific *E. histolytica* molecules in the pathophysiology of amebic colitis (14, 15, 20, 22). Previous studies have established that infection of human colonic xenografts with *E. histolytica* trophozoites results in the activation of epithelial cell NF-κB, with production of inflammatory mediators, including IL-1, IL-8, tumor necrosis factor alpha, and COX-2, and the resultant influx of neutrophils and other inflammatory cells into the mucosa and lumen of the human colonic xenograft (15, 20, 23). The combination of mucosal invasion by *E. histolytica* trophozoites and the resultant inflammatory response leads to intestinal mucosal damage with ulceration and loss of the intestinal permeability barrier (15, 23).

The AP-A(−) phenotype in the SCID-HU-INT model of amebic colitis proved complex. All parameters associated with infection (cytokine production, neutrophil influx, and damage to the permeability barrier) were significantly higher in human colonic xenografts infected with AP-A(−) trophozoites than in uninfected human colonic xenografts, indicating that the failure to express AP-A does not render *E. histolytica* trophozoites avirulent. However, compared to the parental HM1:IMSS strain, we found that AP-A(−) trophozoites induced significantly lower levels of human IL-1β and IL-8 from human colonic xenografts. Thus, in one component of infection (induction of human cytokine production from intestinal epithelial cells) AP-A(−) trophozoites are clearly less efficient than the parental HM1:IMSS strain. We and others have previously hypothesized that *E. histolytica* lysis of intestinal cells might result in the release of preformed mediators (e.g., IL-1α) (8) or cytokine precursors (pIL-1β), which could initiate the inflammatory response (15). The reduced levels of these cytokines in colonic xenografts infected with *E. histolytica* AP-A(−) trophozoites is consistent with some role for amoebapore-mediated intestinal cell lysis in the higher cytokine levels seen with *E. histolytica* colonic infection.

Since both IL-1β and IL-8 are potent inflammatory mediators, one might predict that the decreased levels of each of these cytokines seen in human colonic xenografts infected with AP-A(−) trophozoites would be associated with lower tissue levels of MPO, a marker for neutrophil influx into the infected xenograft. Levels of MPO were lower in human colonic xenografts infected with AP-A(−) trophozoites than in colonic xenografts infected with the parental HM1:IMSS strain, but this difference was not statistically significant. This may reflect the fact that the levels of IL-1β and IL-8, although reduced, were still above the threshold needed to induce inflammatory responses. Alternatively, the levels of crucial mediators and cytokines other than IL-1β and IL-8 may not be reduced in human colonic xenografts infected with AP-A(−) trophozoites. There was also a trend toward less damage to the intestinal permeability barrier in human colonic xenografts infected with AP-A(−) trophozoites compared to the parental HM1:IMSS strain, but again this difference did not reach statistical significance. These results, along with the histologic findings, which were essentially indistinguishable between human colonic xenografts infected with AP-A(−) trophozoites compared to those infected with the parental strain, indicate that *E. histolytica* trophozoites that do not express AP-A(−), show relatively little attenuation of virulence in the SCID-HU-INT model of colitis. These results contrast with our findings on the role of *E. histolytica* cysteine proteinases in amebic colitis, where proteinase-deficient amebas showed defects in their ability to invade into the intestinal mucosa and induced significantly less inflammation and tissue damage than the control parental strain (22).

In summary, amoebapores produced by *E. histolytica* trophozoites play a critical role in the formation of amebic liver abscess in the SCID mouse model of disease Our results link amoebapores to *E. histolytica* induction of intestinal epithelial cell cytokine production but show that trophozoites lacking AP-A expression can still cause inflammation and tissue damage in the SCID-HU-INT mode of amebic colitis. In addition to demonstrating that amoebapores may not be necessary in the pathogenesis of amebic colitis, these data are also consistent with the concept that tissue damage in amebic liver abscess may develop through pathways different than those observed in amebic colitis (17).

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