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Human Intestinal Epithelial Cells Produce Proinflammatory Cytokines in Response to Infection in a SCID Mouse-Human Intestinal Xenograft Model of Amebiasis

KARL B. SEYDEL,[1] ELLEN LI,[2,3] PAUL E. SWANSON,[4] AND SAMUEL L. STANLEY, JR.[1,2]*

Departments of Molecular Microbiology,[1] Medicine,[2] Biochemistry and Molecular Biophysics,[3] and Pathology,[4] Washington University School of Medicine, St. Louis, Missouri 63110

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The protozoan parasite *Entamoeba histolytica* causes amebic dysentery and amebic liver abscess, diseases associated with significant morbidity and mortality worldwide. *E. histolytica* infection appears to involve the initial attachment of amebic trophozoites to intestinal epithelial cells, followed by lysis of these cells and subsequent invasion into the submucosa. A recent in vitro study (L. Eckmann, S. L. Reed, J. R. Smith, and M. F. Kagnoff, J. Clin. Invest. 96:1269–1279, 1995) demonstrated that incubation of *E. histolytica* trophozoites with epithelial cell lines results in epithelial cell production of inflammatory cytokines, including interleukin-1 (IL-1) and IL-8, suggesting that intestinal epithelial cell production of cytokines might play a role in the inflammatory response and tissue damage seen in intestinal amebiasis. To determine whether intestinal epithelial cell production of IL-1 and IL-8 occurs in response to *E. histolytica* infection in vivo and as an approach to studying the specific interactions between amebic trophozoites and human intestine, we used a SCID mouse-human intestinal xenograft (SCID-HU-INT) model of disease, where human intestinal xenografts were infected with virulent *E. histolytica* trophozoites. Infection of xenografts with *E. histolytica* trophozoites resulted in extensive tissue damage, which was associated with the development of an early inflammatory response composed primarily of neutrophils. Using oligonucleotide primers that specifically amplify human IL-1β and IL-8, we could demonstrate by reverse transcription PCR that mRNA for both IL-1β and IL-8 is produced by human intestinal xenografts in response to amebic infection. The increase in human intestinal IL-1β and IL-8 in response to invasive amebiasis was confirmed by enzyme-linked immunosorbent assays specific for human IL-1β and IL-8. Using immunohistochemistry, we confirmed that human intestinal epithelial cells were the source of IL-8 in infected xenografts and established that IL-8 production can occur at sites distal to areas of intestinal mucosal damage. These results demonstrate that human intestinal epithelial cells can produce inflammatory cytokines in response to infection in vivo and establish the SCID-HU-INT model as a system for studying the interactions between *E. histolytica* and human intestine.

To establish disease, the protozoan parasite *Entamoeba histolytica* must adhere to and invade through the intestinal epithelial barrier. In intestinal amebiasis, invasion of the intestinal mucosa by amebic trophozoites may be accompanied by an inflammatory infiltrate composed primarily of neutrophils (11, 30). The early host responses that lead to this inflammatory reaction and the role of inflammation in tissue damage and diarrhea in amebiasis have not been well characterized. However, recent in vitro studies have provided new insights into the interactions between amebic trophozoites and epithelial cells that point to the possibility of an active role for epithelial cells in the inflammatory response to amebic infection (10, 18).

Epithelial cells, poised at the interface between an organism and its environment, have long been thought to function only as an absorptive and protective barrier, playing a passive role in interactions with the immune system. Recent studies have demonstrated that intestinal epithelial cells may play an active role in the host immune and inflammatory response, via the secretion of a variety of cytokines (4, 9, 10, 15, 17, 18, 21). The invasion of cultured epithelial cell lines by intracellular bacteria has been shown to result in the release of inflammatory cytokines, including interleukin-8 (IL-8), monocyte chemoattractant protein 1 (MCP-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor alpha (15). The interaction of *E. histolytica* with epithelial cell lines has been analyzed by Eckmann et al., who showed that coculture of *E. histolytica* with different cell lines, including intestinally derived cells, induced the production of mRNA transcripts for proinflammatory cytokines, including IL-8, GROα, GM-CSF, IL-1α, and IL-6, by the epithelial cells (10). The ability of *E. histolytica* to lyse epithelial cells and thus to release preformed IL-1α was implicated in inducing the production of other cytokines, such as IL-8, by these cells. These studies suggest that the production of cytokines such as IL-1 and IL-8 by intestinal epithelial cells could play a role in the pathogenesis of amebic infection by attracting neutrophils and increasing inflammation and tissue damage.

While the in vitro studies demonstrate that epithelial cell lines can produce inflammatory cytokines, the results of in vivo studies are conflicting. Inflammatory cytokines can be detected in biopsy specimens of intestinal mucosa from individuals with inflammatory bowel disease, celiac disease, or infectious colitis and in animal models of colitis (12, 15, 25–27, 33), but there are potential problems in distinguishing whether mucosally produced cytokines are derived from epithelial cells or mononuclear cells in the lamina propria. In the case of the two best-studied inflammatory cytokines, IL-1 and IL-8, some studies have localized mucosal IL-1 or IL-8 production to cells within the lamina propria (12, 33), while others have found evidence for IL-1 or IL-8 production by enterocytes (15, 26).

* Corresponding author. Mailing address: Washington University School of Medicine, 660 S. Euclid Ave., Box 8051, St. Louis, MO 63110. E-mail: stanley@im.wustl.edu.

TABLE 1. Primers used in this study and their sequences

Primer	Sequence at:	
	5′ end	3′ end
IL-1β	ATGGCAGAAGTACCTGAGCTC	GCACATAAGCCTCGTTATCCCATG
IL-6	ATGAACTCCTTCTCCACAAGC	CTACATTTGCCGAAGAGCCCT
IL-8	ATGACTTCCAAGCTGGCCGTGGC	CTCTTCAAAAACTTCTCCACAACCCTC
Actin	GACTTCGAGCAGGAGATGGCCAC	CTCCTGCTTGCTGATCCACATC

The goal of the present studies was to determine whether cytokine production by human intestinal epithelial cells occurs in response to amebic infection in vivo. To approach this problem and as a means of studying the interactions between *E. histolytica* trophozoites and the human intestine, we generated a SCID mouse-human intestinal xenograft (SCID-HU-INT) model by engrafting human fetal intestinal tissue into the subcutaneous space of mice with severe combined immunodeficiency (SCID mice). Because the intestinal xenograft tissue in this system is of human origin, while inflammatory cells are of host (murine) origin, we could use human-specific oligonucleotides and enzyme-linked immunosorbent assays (ELISAs) to determine whether inflammatory cytokines are derived from the human intestinal xenografts. A similar approach was used recently to demonstrate intracellular cell adhesion molecule type 1 (ICAM-1) expression by *Salmonella*-infected intestinal epithelial cells (13). Here we show that intestinal epithelial cells from human intestinal xenografts infected with *E. histolytica* trophozoites produce the inflammatory cytokines IL-1β and IL-8 and that synthesis of IL-8 is induced at sites distal to regions of amebic invasion.

MATERIALS AND METHODS

Amebas. *E. histolytica* HM1:IMSS (originally provided by V. Tsutsumi, Center for Research and Advanced Studies, National Polytechnic Institute, Mexico City, Mexico) was grown in axenic B1-S-33 media. The ameba were passaged bimonthly through gerbil livers to maintain virulence. *E. moshkovskii* (Laredo strain) was obtained from the American Type Culture Collection (Rockville, Md.) and grown as previously described (6).

Animals. SCID mice (used at 6 to 8 weeks of age) of the C.B.-17 background were bred and maintained in a barrier facility at Washington University School of Medicine. The mice were screened for the leaky phenotype by an ELISA for total immunoglobulin G (IgG) in serum, and only animals with <2 μg/ml were used.

Human tissue. Human intestines from 100- to 130-day (gestational age) fetal tissues destined for discard following prostaglandin-saline-induced therapeutic abortions were obtained from the Center for Embryology, University of Washington Health Sciences Center. Intestinal tissue was placed in ice-cold Dulbecco's modified Eagle's medium for transport to the laboratory. On receipt, the tissue was washed in a change of medium, cleaned of all mesentery, and cut into approximately 5-cm sections.

Engraftment of tissue. Tissue implanted essentially as previously described (31). Briefly, the SCID mice were anesthetized, and a small incision was made in both rear flanks and both suprascapular regions. A subcutaneous tunnel was then produced between the two ipsilateral incisions by blunt dissection. A thin forcep was inserted through the tunnel, and the tissue segment was threaded through the tunnel. The tissue was trimmed as necessary, and the incision was closed with a 7-mm Michel clip. The grafts were allowed to develop for 8 weeks before manipulation.

Infection of tissue. Log-phase (72-h) cultures of virulent *E. histolytica* HM1:IMSS trophozoites were chilled on ice for 5 min, pelleted by a 10-min centrifugation at $500 \times g$, counted, and resuspended at 10^6 ameba/100 μl of B1-S-33 medium without calf serum. After anesthetization, a midline incision along the spine was made, allowing for visualization of the grafts on both sides. Then 10^6 ameba in 100 μl of medium were injected directly into the lumen of the graft through a 26-gauge needle. Upon removal of the needle, the injection site was covered with Gelfoam to prevent seepage. The contralateral control grafts received 100 μl of medium alone. The incisions were closed with 7-mm Michel clips. After 4, 24, or 48 h, the animals were sacrificed and the engrafted tissue was removed. The specimens were examined grossly, and then sections were obtained for histologic testing (fixed in formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin [H&E]), ELISA, and reverse transcription-PCR (RT-PCR).

RT-PCR. Total cellular RNA was extracted from homogenized tissue samples with Tri-reagent as previously described (3). Briefly, 100 mg of tissue was suspended in 1 ml of TRIZOL reagent (Gibco BRL, Gaithersburg, Md.). The tissue was homogenized for 15 s with a Polytron. The samples were then subjected to phase separation with chloroform. The aqueous layer was removed, and the RNA was precipitated with isopropyl alcohol. After a 15-min $12{,}000 \times g$ centrifugation, the RNA pellet was washed in 70% ethanol, dried, resuspended in diethylpyrocarbonate-treated water, and quantitated by measuring the absorbance at 260 nm. cDNA was synthesized from 2 μg of total RNA by priming with 0.5 μg of oligo(dT) primer–50 mM dithiothreitol–10 μM each deoxynucleoside triphosphate–200 U of RNase H^- Moloney murine leukemia virus reverse transcriptase (Gibco BRL) in a final volume of 20 μl at 37°C for 1 h. cDNA equivalent to 0.2 μg of starting RNA was used for each PCR. PCR was performed in a 100 μl volume containing 0.5 μM each specific sense and antisense oligonucleotide primer, 200 mM each deoxynucleoside triphosphate, 5% dimethyl sulfoxide, 1 U of *Taq* polymerase (Boehringer Mannheim, Indianapolis, Ind.), and the supplied 10× buffer. PCR was performed in a Hybaid thermal reactor (National Labnet, Woodbridge, N.J.) with a program of 35 cycles of denaturing at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min. A 20-μl volume of the PCR product was subjected to electrophoresis in a 1.5% agarose gel and stained with ethidium bromide for visualization. The primers used are listed in Table 1. The expected product lengths are 339 bp for IL-1β, 638 bp for IL-6, 281 bp for IL-8, and 419 bp for actin.

ELISAs. A quantitative ELISA kit for detecting human IL-8 was obtained from R&D Systems (Minneapolis, Minn.). A similar kit detecting human IL-1β was obtained from Endogen (Cambridge, Mass.). Protein samples were prepared by homogenizing tissue at 50 mg/ml in a solution of phosphate-buffered saline (PBS) with 1 μg of aprotinin per ml, 1 μg of leupeptin per ml, and 1 μg of pepstatin A per ml. The samples were then centrifuged for 5 min at $500 \times g$ to remove particulate matter. Supernatants from this centrifugation were processed as specified by the manufacturer. The sensitivity of the assays are 20 pg/ml for IL-8 and 1 pg/ml for IL-1β.

Immunofluorescence. Sections from control or infected grafts were flash-frozen in Tissue-Tek OCT compound (Miles, Naperville, Ill.) in methyl butane chilled with dry ice. Sections (4 μm) were cut and fixed for 5 min in 4°C ethanol. They were stained with DM/C7, a murine monoclonal antibody specific for human IL-8 (generously provided by M. Bolanowski, Monsanto Corp.), for 12 h at 4°C. DM/C7 binding was detected with donkey anti-mouse IgG antibody conjugated to the fluorophore Cy3 (Jackson Immunochemicals, West Grove, Pa.).

RESULTS

Establishment of SCID-HU-INT mice. We engrafted human fetal intestines (either small intestine or colon) into bilateral subcutaneous tunnels on the backs of SCID mice. After 10 weeks, the intestinal xenografts remained viable, grew in circumference, became vascularized, and secreted mucus. By microscopic examination of histologic sections, the engrafted tissue appeared to have developed normally, with the appropriate small intestinal (elongated villi, Paneth cells) (Fig. 1A) or colonic (blunted villi, large number of goblet cells) morphology present (Fig. 1B).

Infection of SCID-HU-INT mice with *E. histolytica*. To determine whether human intestinal xenografts were susceptible to amebic infection, we inoculated 10^6 axenically cultured *E. histolytica* trophozoites directly into the lumen of one of the grafts of each animal; the contralateral control graft was inoculated with medium alone. As soon as 4 h after infection, significant damage was visible microscopically in intestinal grafts inoculated with amebae, while no damage was detected

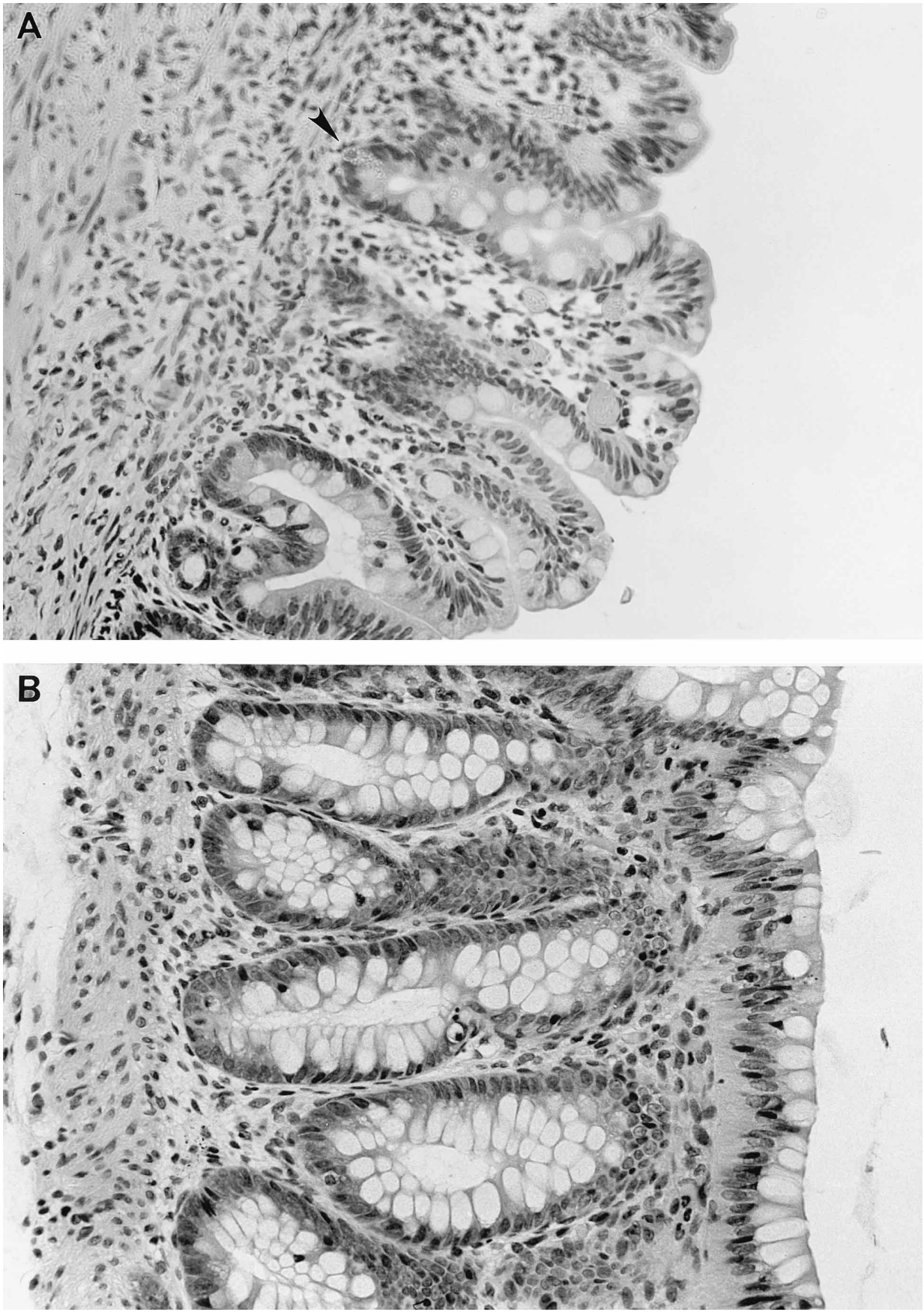

FIG. 1. (A) Photomicrograph of an H&E-stained section of a human small intestinal xenograft 8 weeks after engraftment. Note the elongated villi and the presence of a Paneth cell (arrowhead). (B) Photomicrograph of an H&E-stained section of a human colonic xenograft 8 weeks after engraftment. Note the absent or blunted villi and the preponderance of goblet cells. Magnification, ×250.

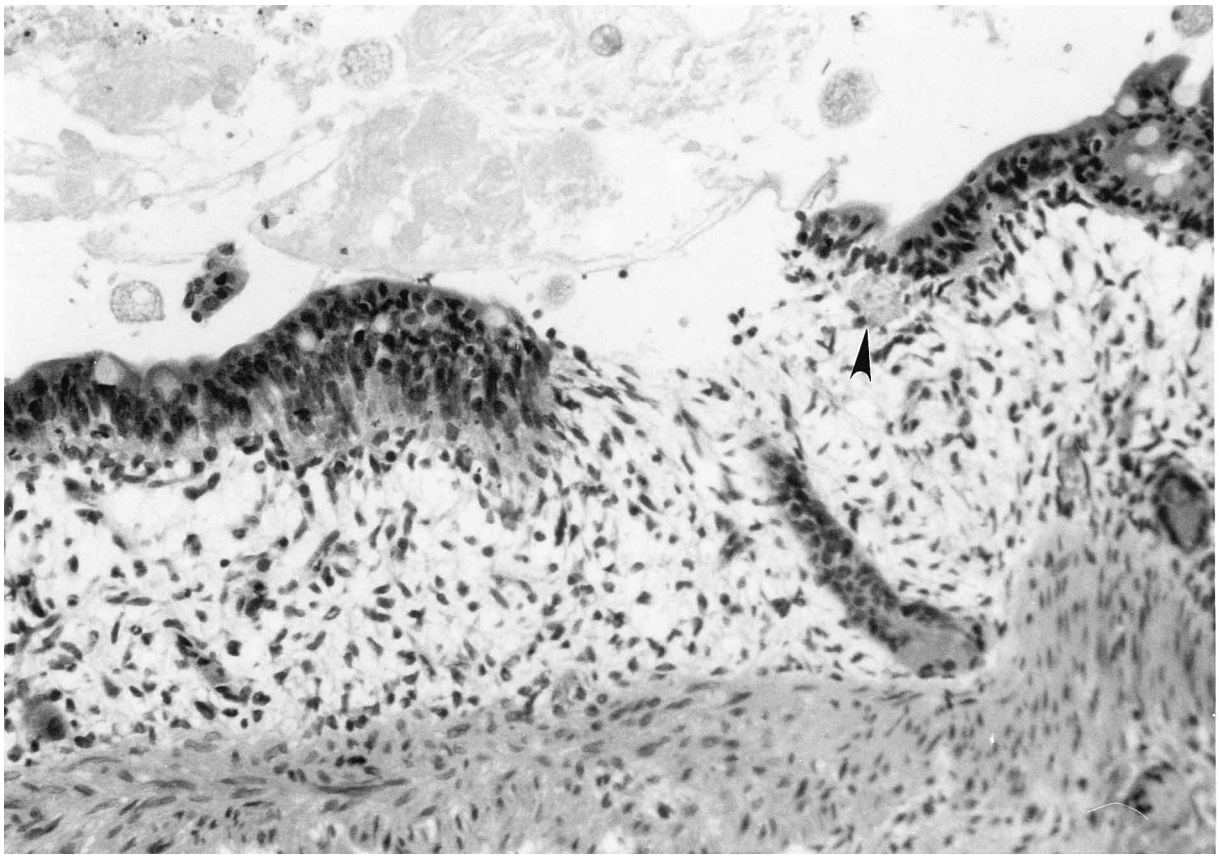

FIG. 2. Photomicrograph of an H&E-stained section of a human intestinal xenograft obtained 4 h after infection with 10^6 *E. histolytica* trophozoites. A fibrin clot with numerous trophozoites can be seen covering an ulceration in the intestinal mucosa. An amebic trophozoite (arrowhead) can be seen under the epithelial layer in the lamina propria. Magnification, ×250.

in grafts receiving medium alone. Disease was detected in intestinal grafts derived from both fetal small intestine and fetal colon. As shown in Fig. 2, classic amebic ulcers, with invasion through the mucosa, an overlying fibrin cap, hemorrhage, and amebae present in the lamina propria, were detected in sections obtained at the 4-h time point. Disease was spotty, with regions of normal epithelium abutting areas of ulceration and invasion. Few or no inflammatory cells were detectable in the lesions at the 4-h time point. After 24 h of infection, some sections revealed nearly complete destruction of the intestinal mucosa, with abundant trophozoites in the submucosal areas. In contrast to the findings at 4 h, inflammatory cells (predominantly neutrophils) were present at many of the lesions, and a striking finding in many sections was the presence of microabscesses with amebic trophozoites surrounded by polymorphonuclear cells (Fig. 3). These were detected in the muscularis mucosa or, in some cases, appeared to extend the full thickness through the bowel wall into underlying tissues. At time points longer than 48 h, there was evidence for continued progression of disease, with much of the intestinal xenograft showing complete loss of mucosal tissue and an intense inflammatory response in the submucosal tissues.

Infected human intestinal xenografts produce IL-1β, IL-8, and IL-6. Because one of the most prominent features of disease at the 24-h time point was the presence of a marked neutrophilic inflammatory response, and based on in vitro studies demonstrating the production of inflammatory cytokines by epithelial cells coincubated with *E. histolytica* trophozoites (3), we set out to determine whether the human intestinal xenografts were producing inflammatory cytokines in response to amebic invasion. To assess this, we used primers specific for the human cytokines IL-1β, IL-6, IL-8, and IL-12p40, and we used the RT-PCR technique to assess human intestinal production of mRNA for these cytokines in response to amebic infection. These primers were designed to specifically amplify human IL-1β, IL-6, IL-8, and IL-12p40, and testing of RNA obtained from activated murine peripheral blood leukocytes revealed that the primers used did not amplify any transcripts from a murine source (data not shown). When we examined samples from human colonic or small intestinal xenografts that had been challenged 24 h earlier with amebae, we detected mRNA transcripts for IL-1β and IL-8 in infected human xenografts which were not amplified in the contralateral medium-challenged graft (Fig. 4). A transcript for IL-6 mRNA could be detected in both control and *E. histolytica*-infected xenografts but appeared to increase in quantity in the *E. histolytica*-infected xenografts (Fig. 4). Transcripts for human actin mRNA were not upregulated in the ameba-infected graft compared to the control graft, which received an injection of medium alone (Fig. 4), and no transcript was amplified for IL-12p40 in human intestinal xenografts challenged with medium or amebae (data not shown). To establish whether invasion of intestinal mucosa was required for induction of cytokines, we also infected xenografts with the noninvasive, nonpathogenic ameba *E. moshkovskii*. Gross and histologic examination of *E. moshkovskii*-infected xenografts revealed no

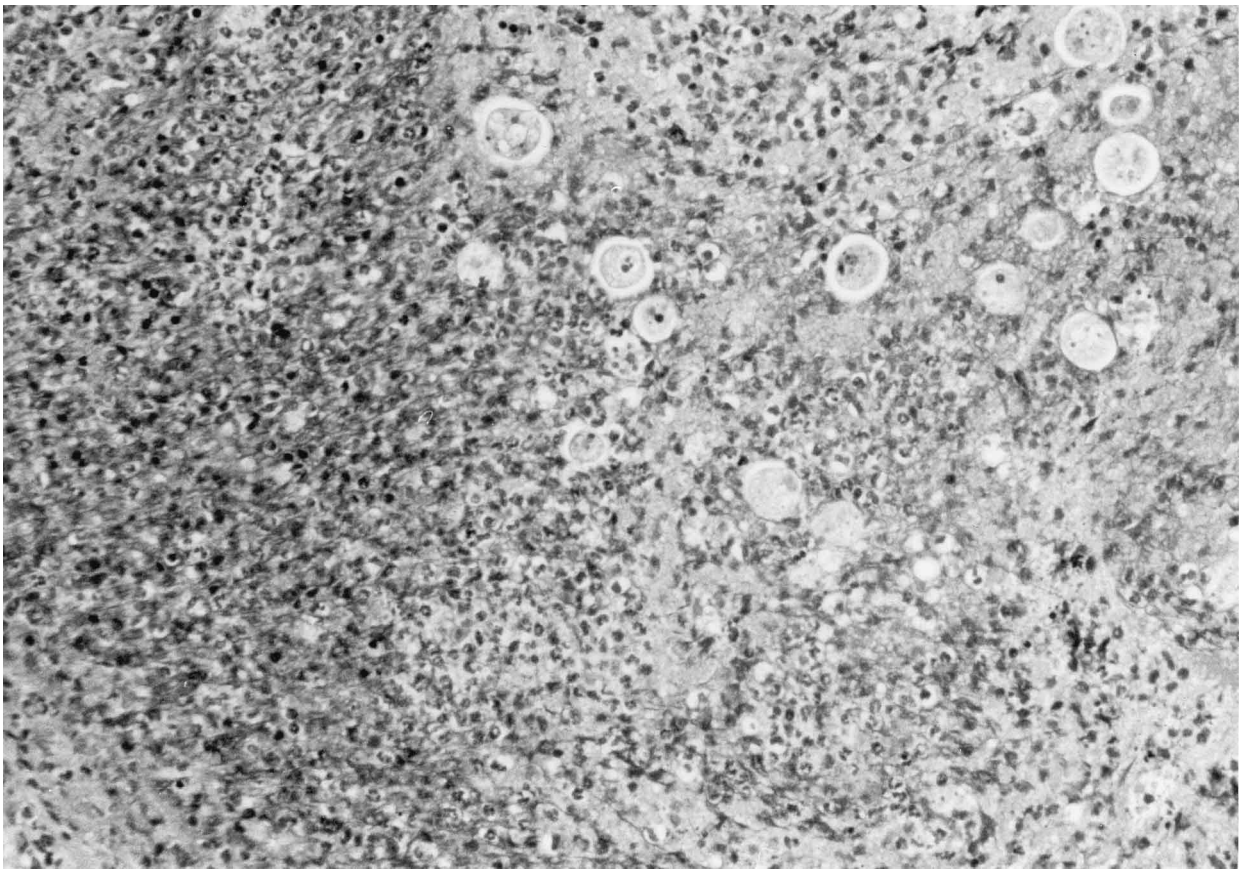

FIG. 3. Photomicrograph of an H&E-stained section of a human intestinal xenograft 24 h after infection with 10^6 *E. histolytica* trophozoites. Amebic trophozoites are surrounded by neutrophils in the submucosa, forming a microabscess. Magnification, ×250.

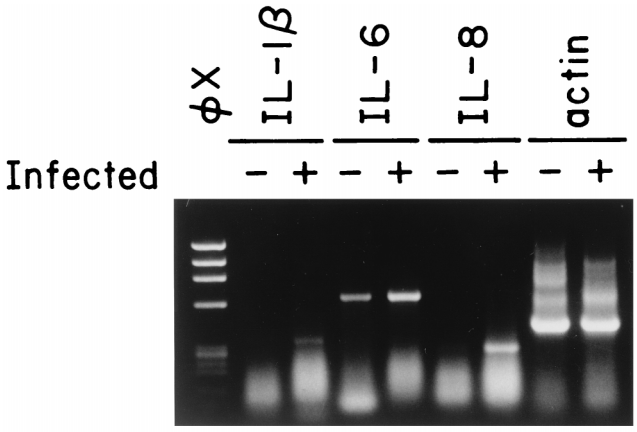

FIG. 4. mRNA transcripts for human IL-1β and IL-8 are induced by intestinal xenograft infection with *E. histolytica*. The results of an RT-PCR assay for mRNA transcripts of IL-1β, IL-8, IL-6, and actin from tissue samples from an intestinal xenograft 24 h after infection with *E. histolytica* (lanes +) and a control xenograft (lanes −) 24 h after inoculation with medium are shown. Transcripts (of the predicted size) for IL-1β and IL-8 are amplified in *E. histolytica*-infected xenografts but are not detectable in the control xenografts. A transcript for IL-6 is amplified in both control and infected xenografts, while the equivalent intensity of the actin control suggests that equivalent quantities of cDNA were present in the samples. Representative samples of PCR on 12 separate grafts are shown. Similar results were seen in tissue infected for 4 h. The ϕX lane is ϕX174RF/*Hae*III DNA size standards.

evidence of mucosal damage (data not shown), but 4 of 18 *E. moshkovskii*-infected xenografts examined showed induction of IL-1β and IL-8 mRNA transcripts by RT-PCR (data not shown).

We used ELISAs specific for human IL-1β and IL-8 to measure the production of each of these cytokines in human intestinal xenografts 24 h after *E. histolytica* or *E. moshkovskii* infection. As shown in Fig. 5, human xenografts infected with *E. histolytica* showed a roughly 50-fold increase in IL-1β production compared with medium-inoculated grafts and an approximately 5-fold increase compared with *E. moshkovskii*-infected grafts. Analysis of these data indicated that the difference in IL-1β production between *E. histolytica*-infected and *E. moshkovskii*-infected intestinal grafts was statistically significant ($P < 0.05$). Similar results were seen when IL-8 was measured (Fig. 6). *E. histolytica*-infected human xenografts showed a fivefold increase in IL-8 production over grafts inoculated with medium alone and a threefold increase over human xenografts infected with *E. moshkovskii*. The difference between IL-8 production in *E. histolytica*-infected human intestinal xenografts and *E. moshkovskii*-infected xenografts was statistically significant ($P < 0.05$).

Localization of IL-8 production in infected human xenografts. Infection of the human colon with *E. histolytica* usually results in localized areas of disease (ulcers) surrounded by regions of relatively normal-appearing colon. This finding was maintained in the *E. histolytica*-infected human xenografts, where areas of normal-appearing xenograft adjoined regions of amebic invasion and ulceration. We were interested in deter-

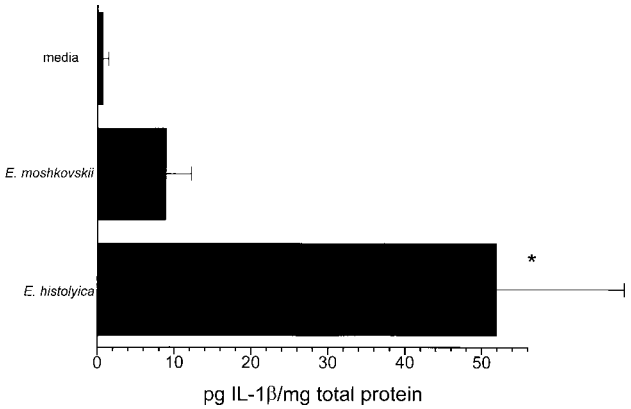

FIG. 5. Human IL-1β is produced by human intestinal xenografts in response to infection with *E. histolytica*. The mean quantity of IL-1β detected in human intestinal xenografts infected 24 h previously with *E. histolytica* ($n = 23$), *E. moshkovskii* ($n = 18$), or medium controls ($n = 4$) is shown. The asterisk indicates that the difference between *E. histolytica* and *E. moshkovskii* is statistically significant at $P < 0.05$.

mining whether the induction of proinflammatory cytokines in intestinal tissue occurs only at the sites of tissue damage or whether it can occur at morphologically normal xenograft distal to the areas of amebic invasion. To study this, we stained sections of human intestinal xenografts obtained from sites of tissue damage and sites distal to damage (as visualized by H&E staining of the sections) with a monoclonal antibody which specifically recognizes IL-8. IL-8 staining was detected by the appearance of immunofluorescence in regions of tissue damage in *E. histolytica*-infected xenografts (data not shown), but, more strikingly (Fig. 7A), IL-8 staining was readily detectable in morphologically normal regions of intestinal xenograft distal to areas of damage. In contrast, uninfected grafts that received only a medium injection showed no detectable IL-8 staining (Fig. 7B).

DISCUSSION

Our studies involving a SCID-HU-INT model of amebiasis indicate that infection of human intestinal xenografts with *E. histolytica* induces the production of the inflammatory cytokines IL-1β and IL-8 from intestinal epithelial cells. These findings are consistent with recent observations suggesting that intestinal epithelial cells may play an active role in host inflammatory and immune responses. In situ hybridization studies have shown that IL-1β mRNA is expressed by enterocytes in the basal portions of the crypts in acetic acid-induced colitis in rats (26). Intestinally derived cell lines produce mRNA for IL-1α, IL-1β, and IL-8 (as well as other cytokines) constitutively (8) and can produce increased levels of IL-1 and IL-8 mRNA in response to bacterial invasion (15). When the intestinally derived HT-29 cell line was stimulated with IL-1β and then cocultured with endothelial cell monolayers, an increased transepithelial migration of activated neutrophils was detected. This neutrophil migration could be blocked by anti-IL-8 (17). Recently, it was shown that coculture of a number of different epithelial cell lines with *E. histolytica* trophozoites resulted in increased expression of the proinflammatory cytokines IL-1α, IL-6, IL-8, GROα, and GM-CSF from the mammalian cells (10). Increased levels of IL-8 have been found in intestinal biopsy specimens from patients with inflammatory bowel disease, and freshly isolated human colonic cells produce IL-8 (5, 12, 14, 15, 20, 28). However, there have been questions about whether IL-8 detected in intestinal biopsy specimens from patients with inflammatory bowel disease is derived from intestinal epithelial cells or monocytes and macrophages within the lamina propria (12). In addition, in the case of cytokine production by epithelial cell lines, there are concerns that transformed cell lines could have altered the expression or regulation of genes due to the transformation process and that it is difficult for a single cell line to represent the large number of cell types at various stages of development in the human intestine. In this regard, there can be significant differences in the cytokine response of individual epithelial cell lines to the same stimuli (8).

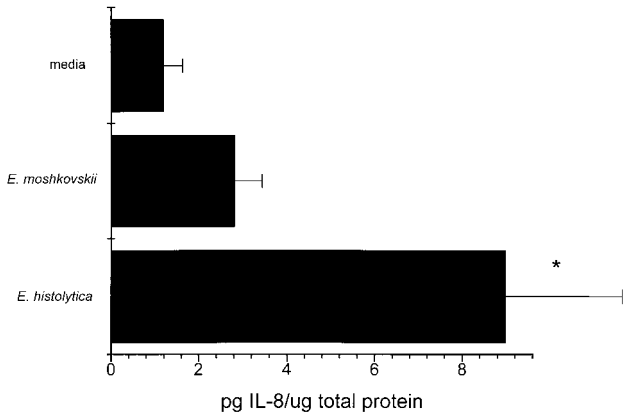

FIG. 6. IL-8 is produced by human intestinal xenografts in response to infection with *E. histolytica*. The mean quantity of IL-8 detected in human intestinal xenografts infected 24 h previously with *E. histolytica* ($n = 23$), *E. moshkovskii* ($n = 18$), or medium controls ($n = 4$) is shown. The asterisk indicates that the difference between *E. histolytica* and *E. moshkovskii* is statistically significant at $P < 0.05$.

The use of the SCID-HU-INT model allowed us to deal with each of these potential concerns. The intestinal epithelial cells in the xenografts are of human origin, while the inflammatory cells (neutrophils, monocytes, and macrophages) are primarily of murine origin. Thus, our detection of human IL-1β and IL-8 in *E. histolytica*-infected human xenografts establishes that intestinal epithelial cells produce proinflammatory cytokines in response to infection in vivo. These findings were confirmed by immunofluorescence, which enabled us to localize IL-8 production to intestinal epithelial cells in *E. histolytica*-infected human xenografts. While the SCID-HU-INT system overcomes the potential confounding factors with transformed cells and allows differentiation of intestinal cell production of cytokines from those of inflammatory cells, we cannot exclude the possibility that cytokine production by the human fetal tissue used in this system differs from the response of the mature intestine.

One of the striking findings in our infected human xenografts was the intense neutrophilic inflammatory response, including the formation of microabscesses, seen 24 h after amebic infection. Neutrophilic infiltration has been described in early lesions of animal models of intestinal amebiasis and amebic liver abscess (reviewed in reference 30) and in biopsy specimens from patients with amebic dysentery (11), and it has been proposed that neutrophils, by releasing their lysosomal enzymes, may play a significant role in the tissue damage seen in amebiasis (2, 29). One important question is what serves as the stimulus to attract neutrophils to the site of amebic invasion. Our findings support the model proposed by Eckmann et al., where an intestinally produced cytokine cascade of IL-1 and IL-8 could play an important role in the recruitment of

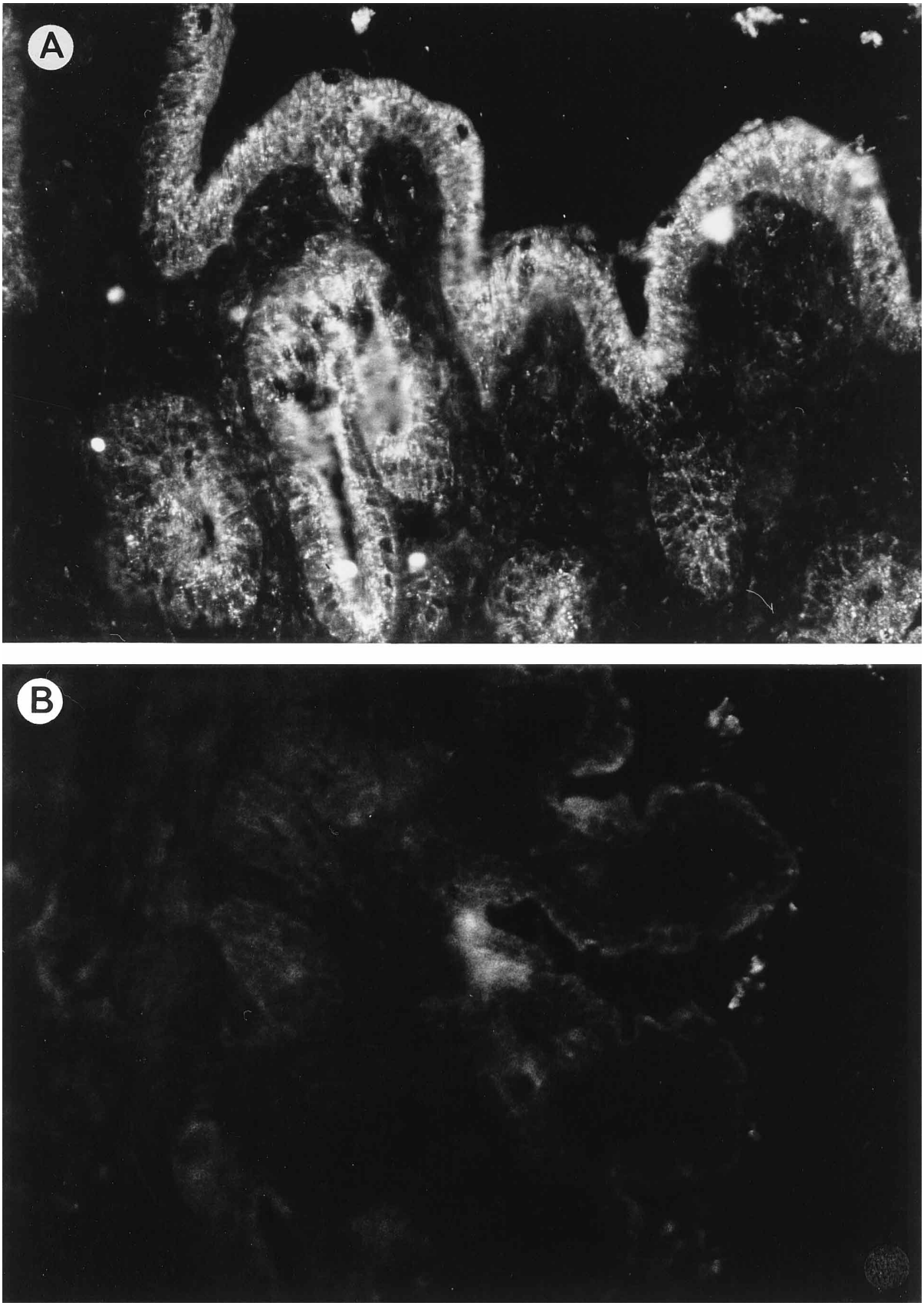

FIG. 7. Human intestinal epithelial cells in both crypts and villi produce IL-8 after xenograft infection with *E. histolytica*. Sections of an intestinal xenograft were obtained 24 h after infection with 10^6 *E. histolytica* trophozoites (A) or 24 h after medium inoculation (B) and stained for IL-8 with monoclonal anti-human IL-8 antibody DM/C7 as the primary antibody and donkey anti-mouse IgG conjugated to Cy3 as the secondary antibody. Immunofluorescent staining is found only in the *E. histolytica*-infected graft (A) and appears to be limited to intestinal epithelial cells in villi and crypts. No IL-8 staining is detected in the medium-inoculated human intestinal xenograft (B). Representative staining from one of two separate experiments is shown. Magnification, ×250.

neutrophils to the site of amebic invasion, with IL-1 stimulating the endothelial cells for neutrophil attachment and inducing the production of IL-8, a potent neutrophil chemoattractant (10). It is interesting that in this system, an inflammatory response by human tissue is capable of recruiting murine neutrophils to the site of damage. The exact nature of this cross-reactivity is unknown, although it should be noted that both murine IL-1 receptors and the tumor necrosis factor R1 are responsive to the human cytokine counterparts (7, 19). In this regard, it should also be noted that while no murine structural homolog of IL-8 has yet been identified, human IL-8 is a potent chemoattractant for and activator of murine neutrophils (28, 34). Studies with human IL-8 in SCID mice have confirmed that human IL-8 can attract murine neutrophils to sites of inflammation, and blockade of human IL-8 inhibits neutrophil migration (24, 32). Our studies were able to confirm that IL-8 is produced in response to *E. histolytica* infection in vivo and demonstrate that synthesis of IL-8 is induced in epithelial cells throughout the infected xenograft and is not confined to regions of amebic invasion. This is consistent with the concept that some factor released by damaged cells (putatively IL-1) can induce IL-8 production in distant regions of the graft. Interestingly, we also found that infection with the noninvasive ameba, *E. moshkovskii*, which causes no visible damage to the xenografts, induced transcripts of IL-1β, IL-6, and IL-8 in some grafts, suggesting that cytokine induction may occur in the absence of histologically detectable tissue damage. The physiologic relevance of the cytokine induction in *E. moshkovskii*-infected xenografts is unclear, since there were no signs of inflammation in these grafts and quantitatively the levels of IL-1β and IL-8 produced were significantly smaller than those seen in *E. histolytica* infected grafts.

E. histolytica infects only humans and nonhuman primates, and there has been limited success in establishing reproducible animal models of intestinal amebiasis. In this regard, it is important to emphasize that amebiasis in the SCID-HU-INT model closely mimicked the pathological findings reported in cases of human amebic colitis (reviewed in reference 1), with an early phase of mucosal damage with subsequent invasion of amebae into submucosal tissues and the formation of amebic ulcers. Interestingly, we were able to induce disease in xenografts derived from human small intestine or colon. Clinically, intestinal amebic disease is usually confined to the colon, with small intestinal involvement occurring in only approximately 5% of infected individuals (1, 16). Our findings suggest that there may be no inherent differences between small intestinal and colonic tissue in susceptibility to invasive amebiasis, but other factors occurring in the human host, such as the enhanced motility of the small bowel, differences in the bacterial flora of each compartment, or differences between fetal and mature intestine, may influence disease localization in natural infection.

A number of studies have suggested a role for the host bacterial flora in the pathogenesis of invasive amebiasis (reviewed in reference 22). In one study, axenically cultured amebae did not cause cecal disease in germ-free guinea pigs but could cause disease in animals with normal intestinal flora (23). Since our intestinal xenografts did not possess either human or murine intestinal flora, our findings demonstrate that the normal colonic flora or any associated bacteria are not required for successful invasion of the human intestine by axenically cultured amebae and, given the severity of disease seen in our xenografts, raise the possibility that components of the normal flora play a protective role in infection. However, the lack of motility and flow of intestinal contents in our xenografts may make them more susceptible to amebic invasion than intestinal tissue in the human host; hence, an exacerbative or protective role for the normal flora in natural infection should not be excluded on the basis of our results.

In summary, we used a SCID-HU-INT model of intestinal amebiasis and human-cytokine-specific reagents to establish that *E. histolytica* infection results in increased production of IL-1β and IL-8 in human intestinal tissue in vivo. These results suggest that signals produced by the intestinal epithelial cells may play an important role in inducing the early host inflammatory response to infection and raise the possibility that interventions that directly target intestinal epithelial cell production of inflammatory cytokines might alter the course of disease.

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