Entamoeba histolytica Trophozoites Induce an Inflammatory Cytokine Response by Cultured Human Cells through the Paracrine Action of Cytolytically Released Interleukin-1α

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Abstract

Infection with the protozoan parasite *Entamoeba histolytica* results in a high mortality worldwide. To initiate infection, *E. histolytica* trophozoites in the bowel lumen penetrate the epithelium, and cause extensive lysis of host cells. The acute amebic lesions in animal models are characterized by infiltration with inflammatory cells, particularly neutrophils. The acute host response is likely important for determining whether the infection will spread systemically, but little is known regarding the signals which initiate an acute inflammatory response to *E. histolytica*. In the studies reported herein, we used an in vitro model system to define the proinflammatory signals produced by epithelial and other host cells in response to infection with *E. histolytica* trophozoites. Coculture of human epithelial and stromal cells and cell lines with trophozoites is shown to increase expression and secretion of an array of chemoattractant and proinflammatory cytokines, including IL-8, GROα, GM-CSF, IL-1α, and IL-6. Moreover, high-level secretion of those cytokines is regulated by the paracrine action of cytolytically released IL-1α. A second mechanism for trophozoite-induced IL-8 production involves trophozoite-target cell contact via a galactose-inhibitable amebic adherence protein, and appears to be mediated through increased intracellular calcium levels. These studies define novel mechanisms through which acute inflammation can be initiated in the host in response to a cytolytic pathogen, such as *E. histolytica*. (*J. Clin. Invest.* 1995. 96:1269–1279.) Key words: amebiasis • neutrophils • interleukin-8 • inflammation • epithelial cells

Introduction

Entamoeba histolytica infects ~ 500 million people worldwide and results in a mortality, among parasitic infections, that is exceeded only by malaria and schistosomiasis (1). The majority of individuals remain asymptomatic and spontaneously clear the infection (2). Nonetheless, in ~ 10% of individuals, the trophozoites invade the bowel or enter the circulation and cause dysentery or hepatic abscesses. The course and final outcome of infection depends on a number of parasite factors, including the virulence of the infecting strain (3–12), and host factors which can alter the risk and severity of invasive disease (13–18).

To initiate symptomatic infection, *E. histolytica* trophozoites in the bowel lumen penetrate the mucus layer and adhere to the underlying mucosa. Trophozoite attachment to host epithelial and inflammatory cells and to colonic mucins and bacteria is mediated by a lectin specific for galactose/*N*-acetyl-galactosamine (19–21). In rodents, changes are seen in epithelial cells, including microvillus shortening and damage to the apical portion of cells, before direct trophozoite contact (22). Initial steps in tissue invasion may be aided by the release from trophozoites of neutral cysteine proteases which are capable of degrading components of the extracellular matrix (23). Trophozoites then lyse colonic epithelial cells and penetrate into the underlying mucosa, where they are in close contact with stromal cells such as fibroblasts and smooth muscle cells, and inflammatory cells. Trophozoite penetration into the mucosa is associated with a local infiltration with PMNs in experimental models (22, 24, 25), but data on the earliest lesions in humans are limited. Lysis of host cells may be mediated by pore-forming peptides (26), phospholipases (27), or hemolysins (28). Similar events in the liver indicate that the host inflammatory response in that site is initiated also by the interaction between trophozoites and host cells (29–31). In contrast to the acute *E. histolytica* infection, chronic amebic abscesses in the liver contain few acute inflammatory cells (32).

Little is known regarding the host signals that initiate an acute inflammatory response to *E. histolytica*. The studies reported herein have used an in vitro model system to define the proinflammatory signals produced by host cells in response to infection with *E. histolytica* trophozoites. We show that coculture of human epithelial cells with *E. histolytica* trophozoites results in secretion of an array of potent chemoattractant and proinflammatory cytokines, including IL-8, GROα, GM-CSF, IL-1α, and IL-6. Moreover, we demonstrate that increased IL-8 and GM-CSF secretion after *E. histolytica* infection is regulated predominantly by the cytolytic release of preformed IL-1α. A second mechanism for trophozoite induced IL-8 production involves trophozoite-target cell contact via a galactose-inhibitable amebic adherence protein. These studies define novel mechanisms through which acute inflammation can be initiated in the host in response to a cytolytic pathogen such as *E. histolytica*.

Methods

Reagents. The following cytokines and antibodies were used in these studies: recombinant human (rh)[1] TNFα (Genentech, Inc., South San Francisco, CA); rh IL-1α and IL-1β (Immunex Corp., Seattle, WA);

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1. *Abbreviation used in this paper:* rh, recombinant human; IL-1Ra, IL-1 receptor antagonist.

rh IL-8 (R&D Systems, Inc., Minneapolis, MN); rh IL-1 receptor antagonist (IL-1Ra) (R&D Systems, Inc.), polyclonal goat antibodies against human IL-1α, IL-1β, and TNFα (R&D Systems, Inc.); monoclonal mouse antibodies against human IL-1α (Genzyme Corp., Cambridge, MA) and normal goat IgG (Sigma Chemical Co., St. Louis, MO). D-galactose and β-D-fructose were obtained from Calbiochem Corp. (La Jolla, CA). D-(+)-glucose and *N*-acetyl-D-galactosamine were purchased from Sigma Chemical Co.

E. histolytica trophozoites. The axenic *E. histolytica* strain HM-1:IMSS was obtained from the American Type Culture Collection (ATCC, Rockville, MD), and trophozoites were grown in TYI-S-33 medium at 37°C. Before infection of cell monolayers, trophozoites were washed twice in ice-cold PBS and resuspended in DME with 10% FCS. Trophozoites were viable under these conditions for at least 6–8 h. Conditioned medium was prepared by incubating trophozoites (10^7/ml) in PBS for 3 h and collecting the supernatant after centrifugation at 3000 *g* for 5 min (23, 33).

Cells. The following human cell lines were obtained from the ATCC: HT29 colon adenocarcinoma cells (HTB 38), SW620 colon adenocarcinoma cells (CCL 227), I407 embryonic intestinal cells (CCL 6), CCD-18Co normal colon fibroblasts (CRL 1459), HISM normal jejunal smooth muscle cells (CRL 1692) (34), HeLa cervix epithelioid carcinoma cells (CCL 2), WI38 normal diploid lung fibroblasts (CCL 75), and HepG2 hepatocellular carcinoma cells (HB 8065). Cells were grown in DME medium supplemented with 10% FCS, 20 mM Hepes, and 2 mM glutamine in 10% CO_2, 90% air at 37°C. T84 human colon carcinoma cells were obtained initially from K. Dharmsathaphorn and were grown in 50% DME, 50% Ham's F12 medium supplemented with 5% newborn calf serum and 2 mM glutamine. PMNs were prepared from freshly drawn venous blood as described by others (35).

Infection protocol. Cells were seeded into 6- or 24-well plates and grown to confluency. Monolayers were washed once, and washed trophozoites in growth medium were added. Cultures were incubated for 8 h and supernatants were removed, filtered through a 0.22-μm filter and cytokine concentrations were measured. To determine the number of viable cells in the monolayers, the cultures were washed twice with ice-cold PBS to remove dead and adherent trophozoites, and the remaining cells were removed from the plates using trypsin/EDTA. Cells were centrifuged, resuspended in 0.4% trypan blue in PBS, and viable cells were counted. Control experiments had shown that the number of viable cells determined by this method correlated closely with the total protein content of the monolayers.

Cytokine assays. Cytokine concentrations were determined by ELISA. IL-8 ELISAs were performed as described previously (36) and were sensitive to 20 pg/ml. Bioactivity of secreted IL-8 was assessed by measuring secretion of myeloperoxidase from purified human PMNs (35). IL-1α ELISAs used optimal concentrations of monoclonal mouse anti-human IL-1α antibody (Genzyme Corp.) as capturing antibody, and polyclonal rabbit anti-human IL-1α antibody (Endogen, Inc., Boston, MA) as detecting antibody. Alkaline phosphatase-labeled monoclonal mouse anti-rabbit IgG (Sigma Chemical Co.) was used as a second-step antibody. Bound alkaline phosphatase was visualized with the substrate *p*-nitrophenylphosphate (Sigma Chemical Co.). The IL-1α ELISA was sensitive to 5 pg/ml recombinant IL-1α. IL-6 ELISAs used monoclonal mouse anti-human IL-6 (R&D Systems Inc.) as capturing antibody, polyclonal rabbit anti-human IL-6 (Endogen Inc.,) as detecting antibody and the same second-step reagents as described for IL-1α. The IL-6 ELISA was sensitive to 50 pg/ml. The GM-CSF and GROα ELISAs (Quantikine; R&D Systems Inc.,) were sensitive to 5 pg/ml and 20 pg/ml, respectively.

RNA extraction and reverse transcription-PCR analysis. Total cellular RNA was extracted from cells using an acid guanidinium thiocyanate-phenol-chloroform method (37). Quantitative reverse transcription-PCR analysis of mRNA levels for IL-8, IL-1α, GM-CSF, IL-6, and β-actin was performed as described previously using internal RNA standards (38). This method was sensitive to $< 10^3$ mRNA molecules per microgram total RNA.

Reporter gene constructs and transfection experiments. To construct the IL-8 reporter vector pIL8/luc, we amplified a 1.4-kb fragment of the IL-8 promoter (39) from human DNA by PCR using the oligonucleotides 5′-ATCTGAAGCTTGAGCTCGAATTCAGTAACCCAGGCATTATTTTATC-3′ and 5′-TTGTCCTAGAAGCTTGTGTGCTCTGCTGTC-3′. The PCR product was inserted into the unique HindIII site of p19LUC, a plasmid containing the gene for firefly luciferase (40). The identity of the insert was confirmed by dideoxy sequencing using Sequenase (United States Biochemical Corp. Cleveland, OH). To construct the β-actin reporter vector pβact/luc, the luciferase gene from the vector pGL2Basic (Promega Corp., Madison, WI) was amplified using the oligonucleotides 5′-GTCAGTCGACCGGTACTGTTGGTAAAATGG-3′ and 5′-CTGCAAGCTTCGCTGAATACAGTTACATTTTAC-3′. The amplified fragment was inserted between the unique SalI and HindIII sites of the plasmid pHβAPr-1-neo which contains a 4.3-kb fragment of the human β-actin promoter (41). To correct for differences in transfection efficiencies and cell numbers, the expression vector pRSV/lacZ (Promega Corp.) was used in which the expression of the gene for bacterial β-galactosidase is controlled by the long terminal repeat of Rous sarcoma virus.

Cells were transfected with reporter gene constructs using a calcium precipitation method as described previously (42). Briefly, subconfluent HeLa cell cultures in 10-cm dishes were transfected with 10 μg each of pIL8/luc and pRSV/lacZ, or pβact/luc and pRSV/lacZ. After 24 h, cells were seeded into 24-well plates and incubated for an additional 24 h, after which they were used for infections. Cell lysates were prepared using 200 μl/well of 0.5% Triton X-100, 0.1 M potassium phosphate (pH 7.8), 1 mM DTT. Luciferase activity was assayed by addition of 100 μl extract to 100 μl of 1 mM D-luciferin and 100 μl of 20 mM ATP (pH 7.4) in a buffer of 35 mM glycylglycine (pH 7.8), 20 mM $MgCl_2$, and light release was quantitated for 10 s using a luminometer (Monolight 2010; Analytical Luminescence Laboratory, San Diego, CA). β-galactosidase activity was determined using the chemiluminescent substrate AMPGD (3-(4-methoxyspiro[1,2-dioxetane-3,2′-tricyclo[3.3.1.1^{3,7}]decan]-4-yl)phenyl-β-D-galactopyranoside; Tropix Inc., Bedford, MA) as described before (43). Briefly, 50 μl extract was added to 500 μl of 16 μg/ml AMPGD, 0.1 M sodium phosphate (pH 8.4), 1 mM $MgCl_2$ and incubated for 60 min at room temperature. Light release was induced by addition of 100 μl 0.2 N NaOH containing 10% Emerald enhancer (Tropix Inc.), and quantitated for 10 s in a luminometer. Increased transcription from the IL-8 and β-actin promoters in response to *E. histolytica* infection were calculated by comparing ratios of luciferase to β-galactosidase activities in cells cotransfected with pIL8/luc and pRSV/LacZ, or pβact/luc and pRSV/lacZ, respectively, with the ratios in uninfected controls. Nontransfected cells were used as a background control.

Western blot analysis. Cell lysates were prepared by sonication of confluent monolayers in 6-well plates in 2 ml DME supplemented with 10% FCS. Immunoprecipitations were carried out using optimal concentrations of monoclonal mouse anti-human IL-1α (R&D Systems Inc.,) as primary antibody and affinity-purified anti-mouse IgG agarose (Sigma Chemical Co.) as secondary antibody. Samples were denatured by boiling in 2% SDS, size-fractionated on a 12.5% SDS-polyacrylamide gel, and electrophoretically transferred to a nitrocellulose membrane (0.1-μm pore size). Immunodetection was performed using optimal concentrations of polyclonal rabbit anti-human IL-1α antibody (Endogen Inc., Boston, MA) as primary antibody, and a peroxidase-labeled anti-rabbit IgG antibody (Amersham Corp., Arlington Heights, IL) as secondary antibody. Specifically bound peroxidase was detected by enhanced chemiluminescence (ECL System; Amersham Corp) and exposure to x-ray film for 15–30 s. Band intensities were quantitated with a densitometer.

Results

Human cell lines cocultured with E. histolytica trophozoites secrete an array of chemoattractant and proinflammatory cytokines, including IL-8, GROα, GM-CSF, and IL-6. Neutrophils accumulate rapidly at the site of acute *E. histolytica* infection (24, 25, 29–31), yet the responsible host signals are not known.

*Table I. E. histolytica Trophozoites Induce IL-8 Secretion by Cultured Human Cells**

Cells	Addition to culture: None — IL-8 secreted	*E. histolytica* — IL-8 secreted	*E. histolytica* — Fold increase over control	TNFα — IL-8 secreted	TNFα — Fold increase over control
	pg/ml	*pg/ml*		*pg/ml*	
T84 colon epithelial cells	68±21‡	752±121‡	11	11,251±4,593‡	165
HT29 colon epithelial cells	96±20	740±108	7.7	95,810±22,264	998
SW620 colon epithelial cells	92±37	478±112	5.2	4,334±780	47
I407 embryonic intestinal cells	212±65	18,401±933	87	4,500±524	21
CCD-18Co colon fibroblasts	118±25	1,919±576	16	36,328±10,891	308
HISM intestinal smooth muscle cells	416±26	3,566±96	8.6	38,325±6,847	92
HeLa cervix epithelial cells	179±87	27,969±4,808	156	5,418±1,517	30
WI38 lung fibroblasts	1,403±111	92,088±7,717	66	225,835±7,151	161
HepG2 liver cells	54±9	668±140	12	1,281±77	24

* Confluent monolayers of human cell lines in 6-well plates were infected with 2–5 × 10^5 *E. histolytica* trophozoites/well in a 2-ml vol and incubated for 8 h. Parallel cultures were incubated for 8 h in the presence of 200 ng/ml TNFα or no added stimuli. After incubation, supernatants were removed and the concentration of IL-8 was determined by ELISA, as described in Methods. ‡ Numbers represent mean±SEM of the results of at least three independent experiments.

Several cytokines are known to be potent chemoattractants and activators of neutrophils, including members of the C-X-C family of chemokines such as IL-8 and GROα, and hemopoietic growth factors such as GM-CSF. Thus, we asked whether human cells cocultured with *E. histolytica* are induced to secrete these cytokines. We first investigated IL-8 as a prototype member of the C-X-C family of chemokines. As shown in Table I, coculture of several human epithelial cell lines, nontransformed human fibroblasts and intestinal smooth muscle cells and human liver cells with *E. histolytica* trophozoites resulted in a 5- to 150-fold increase in IL-8 secretion. The lower IL-8 response of some cells to *E. histolytica* infection (e.g., T84, HT29) was not related to the inability to secrete high levels of IL-8, since stimulation with TNFα increased IL-8 secretion by those cells to levels comparable to that of the higher responding cells (Table I).

In addition to IL-8, secretion of GROα and GM-CSF, cytokines that influence the localization, activation, and survival of neutrophils, was increased in response to *E. histolytica* infection, as shown in Table II for HeLa and WI38 cells. We also analyzed secretion of IL-6 since *E. histolytica* infection can induce an acute phase response (44). IL-6 secretion increased after coculture of HeLa and WI38 cells with *E. histolytica* trophozoites (Table II). Taken together, these findings indicate that cells respond to *E. histolytica* infection with increased secretion of an array of cytokines, which includes IL-8, GROα, GM-CSF, and IL-6.

Since secretion of all the proinflammatory cytokines tested increased in response to *E. histolytica* infection, our subsequent studies focused on the mechanisms that underlie increased secretion of the prototypic proinflammatory cytokine IL-8. As shown in Fig. 1, maximal IL-8 secretion was confined to a relatively narrow range of trophozoite inoculum. Little IL-8 secretion was seen below 5 × 10^4 trophozoites/well using 6-well plates (9.3 cm^2 growth area), and maximal levels of IL-8 secretion were observed at 2.5–5 × 10^5 trophozoites/well, which equals a cell:trophozoite ratio of ~ 15:1 to 2:1 (Fig. 1). However, further increases in the ratio of trophozoites to cells resulted in decreased IL-8 secretion, a finding which likely related to the decreased number of viable cells at the end of the incubation period in monolayers that were infected with these larger trophozoite inocula (Fig. 1). Comparison of the dose–response curves for IL-8 secretion and cell lysis indicated that maximal IL-8 secretion was obtained when ~ 50% of the monolayer was lysed.

Secreted IL-8 was bioactive, as tested in HeLa cells, since supernatants of *E. histolytica*–infected cell cultures stimulated the release of myeloperoxidase by freshly isolated human PMNs (35), and this activity was completely blocked by addition of anti–IL-8 antibodies (data not shown). No IL-8 activity was released by trophozoites cultured alone. Moreover, incubation of rhIL-8 with *E. histolytica* trophozoites in the culture medium used in these studies for 8 h did not alter IL-8 bioactivity.

Time course of increased IL-8 secretion and IL-8 mRNA

*Table II. Increased Secretion of GROα, GM-CSF, and IL-6 in HeLa and WI38 Cells after E. histolytica Infection**

Cytokine	Cells	Cytokine secretion: Control (pg/ml)	+ *E. histolytica* (pg/ml)	Ratio infected/control
GROα	HeLa	< 20	2,967±1,129‡	> 148
	WI38	194±45	10,689±635	55
GM-CSF	HeLa	< 5	187±34	> 37
	WI38	14±3	357±12	26
IL-6	HeLa	54±24	6,458±2,182	120
	WI38	168±40	16,184±6,852	96

* Confluent monolayers of HeLa and WI38 cells in 6-well plates were infected with 2–5 × 10^5 *E. histolytica* trophozoites and incubated for 8–12 h, after which cytokine concentrations in the supernatants were determined by ELISA. In parallel, uninfected cultures were used as controls. ‡ Data are means±SEM of the results from three independent experiments.

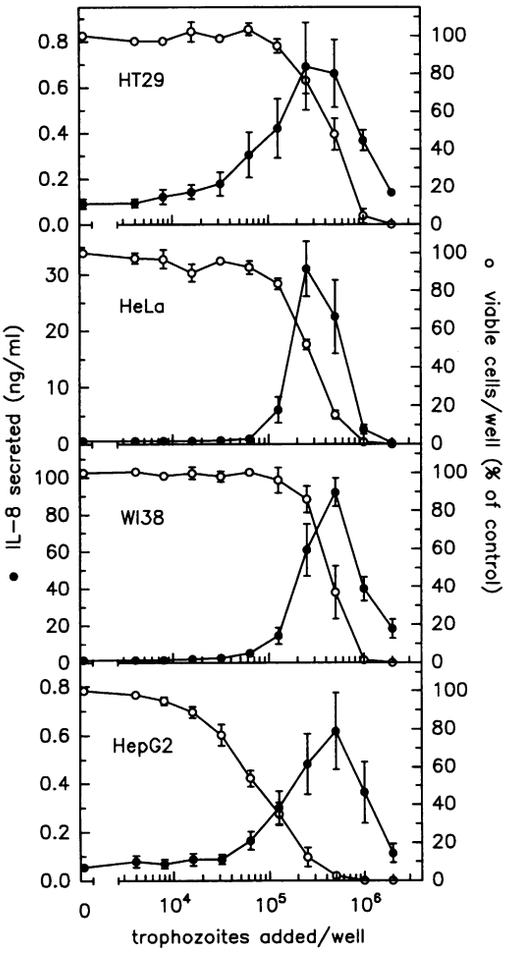

Figure 1. Relationship between *E. histolytica* trophozoite inoculum and IL-8 secretion. Confluent monolayers of HT29, HeLa, WI38, and HepG2 cells in 6-well plates were cocultured for 8 h with varying numbers of *E. histolytica* trophozoites in a 2-ml vol, after which IL-8 concentrations in the supernatants were measured by ELISA (•). In parallel, the number of viable cells remaining in the monolayers at the end of the culture period was determined by trypan blue dye exclusion, and is expressed as a percentage of the number of viable cells present in uninfected control cultures (○). Values are mean ±SEM of the results from at least three independent experiments. Control cultures contained a mean of 3.7×10^6 (*HT29*), 7.1×10^5 (*HeLa*), 1.7×10^6 (*WI38*), and 3.6×10^6 (*HepG2*) cells/well.

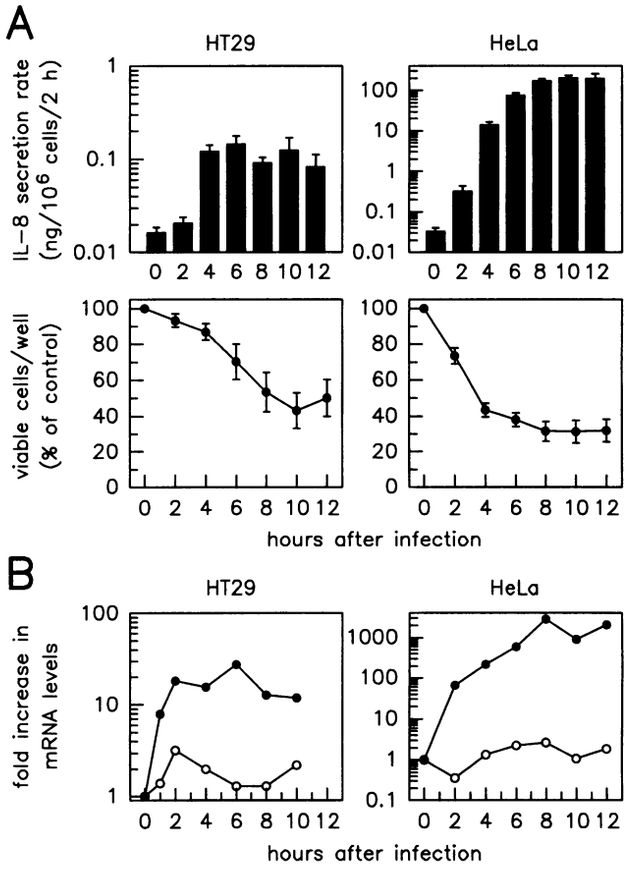

Figure 2. IL-8 secretion rates and IL-8 mRNA levels after addition of *E. histolytica* trophozoites. Confluent monolayers of HT29 and HeLa epithelial cells in 6-well plates were infected with $2-5 \times 10^5$ *E. histolytica* trophozoites/well in a 2-ml vol. (*A*) Supernatants were removed at different times after infection, and the IL-8 concentration was assayed by ELISA. In parallel, the number of viable cells in the monolayers was determined by trypan blue exclusion. Values are mean±SEM of the results of three independent experiments. (*A, top*) IL-8 secretion rates. IL-8 secretion for consecutive 2-h periods was calculated by subtracting the amount of IL-8 in the supernatants at the beginning of each 2-h period from the amount at the end of that period. To obtain the IL-8 secretion rate, the amount of IL-8 secreted during each 2-h period was divided by the number of viable cells in the monolayers at the end of the respective 2-h period. *Hours after infection* refers to the end of each 2-h period after infection. The "0 h" value refers to the IL-8 secretion rate in uninfected cultures during a 2-h period. (*A, bottom*) Number of viable cells. The number of viable cells remaining at different times after infection was determined, and is expressed as a percentage of the number of viable cells present in uninfected control cultures. Control cultures contained a mean of 4.3×10^6 (*HT29*) and 1.0×10^6 (*HeLa*) cells/well. (*B*) Total cellular RNA was extracted at different times after addition of trophozoites to the monolayers. mRNA levels for IL-8 (•) and β-actin (○) were determined by quantitative reverse transcription-PCR analysis using internal standard RNAs, as described in Methods, and are expressed as fold increase over the levels in unstimulated control cultures. Control cultures contained 1.1×10^4 (*HT29*) and 1.1×10^5 (*HeLa*) IL-8 mRNA molecules μg total cellular RNA, and 5.0×10^6 (*HT29*) and 1.8×10^8 (*HeLa*) β-actin mRNA molecules/μg RNA.

levels after addition of E. histolytica trophozoites. A time course analysis of the IL-8 response revealed that IL-8 secretion by cells was increased within 2 to 4 h after addition of *E. histolytica* trophozoites, and reached a maximum at 4–6 h in HT29 cells, and 8 h in HeLa cells (Fig. 2 *A, top*). Cell lysis was observed within 2 h and cell numbers continued to decrease for 6–10 h after the addition of trophozoites (Fig. 2 A, bottom). After 8–10 h in culture, most trophozoites were dead and, concomitantly, no further reduction in the number of cells in the monolayers was seen beyond this point.

Levels of IL-8 mRNA after infection of HT29 and HeLa monolayers with *E. histolytica* were assayed at different times after infection by quantitative reverse transcription-PCR (38). As shown in Fig. 2 *B*, levels of IL-8 mRNA increased within 1 to 2 h after addition of the trophozoites to the monolayers and reached a maximum between 2 (HT29) and 8 h (HeLa) after infection. IL-8 mRNA levels in HT29 cells increased by 10- to 20-fold, while levels in HeLa cells increased ~1,000-fold. No significant differences in levels of β-actin mRNA were

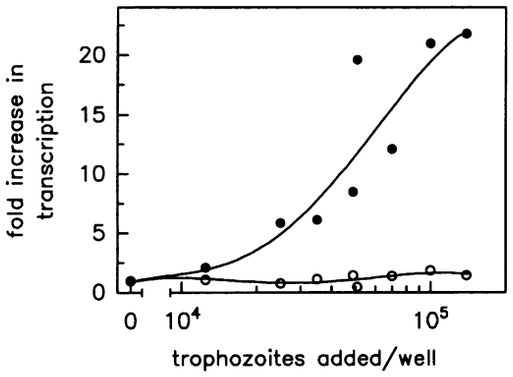

Figure 3. Transcription from the IL-8 promoter is increased in HeLa cells after *E. histolytica* infection. HeLa cells were cotransfected with the reporter constructs pIL8/luc and pRSV/lacZ, or pβact/luc and pRSV/lacZ, as described in Methods. 48 h after transfection, the monolayers were infected with different numbers of *E. histolytica* trophozoites as indicated and 10 h after infection, cell extracts were prepared and assayed for luciferase and β-galactosidase activities. Changes in transcription from the IL-8 (•) and β-actin (○) promoters in response to *E. histolytica* infection were assessed by comparing the ratios of luciferase to β-galactosidase activity in cells cotransfected with pIL-8/luc and pRSV/lacZ, or pβact/luc and pRSV/lacZ. Increased transcription is expressed as the fold increase in transcription in *E. histolytica*–infected compared to uninfected control cells.

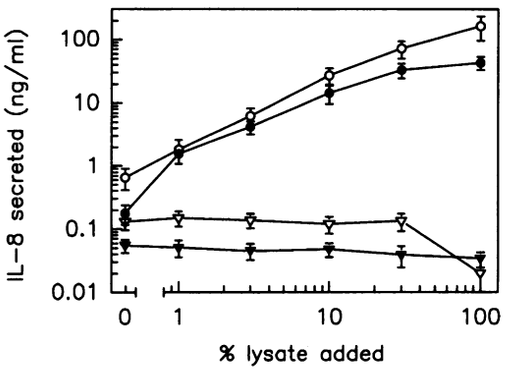

Figure 4. Induction of IL-8 secretion by cell lysates. HeLa (•), WI38 (○), HepG2 (▼) and HT29 (▽) cells were seeded into 6-well plates and grown to confluency. To prepare cell lysates, monolayers from some of the wells were harvested in 0.2 ml growth media by scraping and then sonicated. Different amounts of these lysates were then added to the remaining viable monolayers of the same cell line that were cultured in parallel, i.e., HeLa lysates were added to HeLa monolayers. The final volume in each well was 2 ml. Lysates were used undiluted for the highest lysate concentration (100%). Cultures were incubated for 8–10 h after which IL-8 concentrations in the supernatants were determined. The amount of IL-8 present in lysates alone before culture ranged from 20 to 350 pg/ml IL-8 (equivalent to $4–50 \times 10^{-18}$ grams/cell), and was subtracted as background. Data points are means±SEM of the results of three independent experiments.

detected in infected and control cultures (Fig. 2 *B*). mRNA levels for GM-CSF and IL-6 were similarly increased 1,000- and 250-fold, respectively, in HeLa cells after coculture with *E. histolytica* trophozoites. Thus, unstimulated HeLa cells contained 1×10^3 GM-CSF and 4×10^4 IL-6 mRNA molecules/μg total cellular RNA, respectively. In contrast, after 8 h coculture with *E. histolytica,* HeLa cells contained 1×10^6 GM-CSF and 1×10^7 IL-6 mRNA molecules/μg total RNA, respectively.

Increased transcription from the IL-8 promoter in cells cocultured with E. histolytica. To assess whether increased IL-8 mRNA levels in response to *E. histolytica* infection were due to increased IL-8 gene transcription, we constructed a vector containing a chimeric reporter gene in which a 1.4-kb fragment of the human IL-8 promoter was placed in front of the gene for firefly luciferase (pIL8/luc). An expression vector containing the bacterial β-galactosidase gene under the control of the long terminal repeat of Rous Sarcoma Virus (pRSV/lacZ) and a luciferase vector driven by the human β-actin promoter (pβact/luc) were used as controls. pIL8/luc and pRSV/lacZ, or pβact/luc and pRSV/lacZ were cotransfected into HeLa cells, and *E. histolytica* trophozoites were added to the cultures 48 h later. At different times after infection, cell extracts were assayed for luciferase and β-galactosidase activities. Increased transcription from the IL-8 promoter was observed in HeLa cells within 2 h after infection. By 10 h after infection, transcription was increased by > 20-fold, whereas transcription of the β-actin gene was unchanged (Fig. 3).

IL-1α in cell lysates activates IL-8 secretion. Since maximal IL-8 secretion occurred under conditions which resulted in cell lysis, we reasoned that cell components released during lysis may stimulate the remaining viable cells in the monolayers to secrete IL-8. To test this possibility, cell lysates were prepared from intact monolayers by sonication, and their effect on IL-8 secretion by viable cell monolayers was determined. As shown in Fig. 4 for HeLa and WI38 cells, cell lysates induced IL-8 secretion by viable monolayers of those cells. Moreover, this response was comparable in magnitude to that seen after *E. histolytica* infection of HeLa and WI38 cells (Table I). Although the cell lysates added to the monolayers themselves contained small amounts of IL-8 ($4–50 \times 10^{-18}$ grams/cell), this accounted for < 0.5% of the total IL-8 in the lysate-stimulated culture supernatants. Similar findings were obtained with lysates from I407, T84, and CCD-18Co cells. In contrast, lysates from HT29 and HepG2 cells did not stimulate IL-8 secretion by those cells (Fig. 4).

Several cytokines, including TNFα, IL-1α, and IL-1β, are known to induce increased IL-8 secretion by HeLa and WI38 cells (36). Moreover, epithelial cells and fibroblasts can express TNFα, IL-1α, and IL-1β (38). Therefore, we tested the possibility that one or more of these cytokines might be present in the cell lysates and responsible for increased IL-8 secretion after addition of cell lysates to the monolayers. For these experiments, anticytokine antibodies were added to lysates from HeLa, T84, I407, and WI38 cells, after which IL-8 secretion by viable test cells (HeLa) was assessed. As shown in Table III, anti–IL-1α antibodies or IL-1Ra blocked > 90% of the lysate-induced IL-8 response, whereas antibodies against IL-1β or TNFα had no effect on this response. Anti–IL-1α antibodies or IL-1Ra, but not anti–IL-1β or anti-TNFα antibodies, also were shown to block > 50% of the IL-8 inducing activity in lysates from CCD-18Co colon fibroblasts and human intestinal smooth muscle (HISM) cells. In all cell lysates tested, some of the IL-8 stimulating activity remained and could not be neutralized, even with high concentrations of inhibitors of IL-1α activity.

A possible explanation for the inability of lysates of HT29 and HepG2 cells to induce IL-8 secretion by these cells was a lack of IL-1α responsiveness of those cells. However, this was not the case since IL-8 secretion of HT29 and HepG2 cells

*Table III. Cell Lysates Contain Bioactive IL-1α**

Experiment	Stimulators added	IL-8 secreted (pg/ml)‡ No inhibitor added	+ Anti-IL-1α	+ Anti-IL-1β	+ Anti-TNFα	+ Normal goat IgG	+ IL-1 receptor antagonist
1	HeLa Lysate	29,540±1,287	648±36	32,193±3,818	29,855±1,377	30,369±1,738	791±94
	IL-1α (50 pg/ml)	65,110±4,545	255±21	56,574±781	49,825±10,222	60,644±3,482	321±40
	IL-1β (50 pg/ml)	58,076±1,595	79,218±5,189	265±28	67,090±1,683	70,945±4,304	404±8
	TNFα (500 pg/ml)	3,000±157	2,469±324	2,513±337	235±36	2,936±23	2,338±119
	None	365±61	251±32	295±48	319±75	297±48	337±81
2	T84 Lysate	1,035±75	62±3	927±29	970±23	876±26	86±1
	I407 Lysate	60,812±2,517	120±8	55,458±2,896	59,962±4,030	59,346±1,041	397±5
	TNFα (200 ng/ml)	4,313±74	N.D.§	N.D.	N.D.	N.D.	N.D.
	None	43±3	N.D.	N.D.	N.D.	N.D.	N.D.
3	WI38 Lysate	749±51	76±3	711±65	782±38	772±8	76±2
	TNFα (200 ng/ml)	2,727±69	N.D.	N.D.	N.D.	N.D.	N.D.
	None	40±3	N.D.	N.D.	N.D.	N.D.	N.D.

* Confluent monolayers of HeLa cells in 24-well plates were incubated with combinations of stimulators and inhibitors for 8 h, after which IL-8 concentrations in the supernatants were determined by ELISA. The following stimulators and inhibitors were used: lysates from HeLa cells (10% final concentration), T84 cells (50% final concentration), I407 cells (2% final concentration), WI38 cells (10% final concentration), rhIL-1α, rhIL-1β, rhTNFα, purified goat anti-human IL-1α, IL-1β, or TNFα antibodies (10 μg/ml each), normal goat IgG (10 μg/ml), and rhIL-1Ra (400 ng/ml). Cell lysates were prepared from confluent monolayers in 10-cm dishes in a 2-ml vol using DME medium, supplemented with 10% fetal calf serum. ‡ Numbers are mean±SEM of the results of triplicate cultures from a representative experiment. Similar results were obtained in at least one additional experiment for each tested cell lysate. § N.D., not done.

increased after IL-1α stimulation (10 ng/ml IL-1α stimulated a 240-fold and 100-fold increase in IL-8 secretion by HT29 and HepG2 cells, respectively). Despite a requirement for a greater IL-1α concentration to stimulate IL-8 secretion by HT29 cells (ED_{50} = 1,500 pg IL-1α/ml) and HepG2 cells (ED_{50} = 140 pg IL-1α/ml) compared with HeLa cells (ED_{50} = 50 pg IL-1α/ml) or WI38 cells (ED_{50} = 55 pg IL-1α/ml), addition of lysates from HeLa or WI38 cells to HT29 or HepG2 cells induced IL-8 secretion. In contrast, lysates from HT29 and HepG2 cells did not increase IL-8 secretion by HeLa or WI38 cells. Furthermore, HT29 and HepG2 cell lysates did not appear to contain inhibitors of IL-1α activity since no inhibition of IL-1α bioactivity was seen when titrated doses of rhIL-1α were added to HT29 or HepG2 cell lysates. Thus, the failure of HT29 and HepG2 cell lysates to stimulate increased IL-8 secretion appears to reflect a lack of IL-1α activity in the lysates of these cells. This conclusion is supported by the previous finding that HT29 cells had $< 10^3$ IL-1α mRNA molecules/μg cellular RNA (38).

IL-1α is released during E. histolytica infection and its effect on IL-8 secretion is blocked by IL-1α specific inhibitors. To confirm that IL-1α is released during *E. histolytica* infection, cell monolayers were cocultured with increasing numbers of trophozoites, and IL-1α concentrations in the culture supernatants were determined by ELISA. In parallel, monolayers were lysed by sonication and the concentration of released IL-1α was measured. As shown in Fig. 5 for I407 cells, *E. histolytica* trophozoites were effective at stimulating IL-1α release over a range of inocula. The highest *E. histolytica* inoculum caused rapid and complete cell lysis of I407 cells and, in these cultures, the IL-1α concentration in supernatants was similar to that measured in cell monolayers that were lysed by sonication. This confirmed that *E. histolytica*–induced cell lysis and cell lysis after sonication were equally effective in causing the release of preformed IL-1α. Since less cell lysis was observed at lower *E. histolytica* inocula, if IL-1α was derived solely from the release of preformed cytokine, lower concentrations of IL-1α would be expected in the supernatants. However, substantially greater quantities of IL-1α than predicted were present in these supernatants (Fig. 5), indicating that, in addition to release of preformed IL-1α, IL-1α synthesis was increased during infection. Similarly, increased IL-1α concentrations were detected in HeLa and WI38 monolayers after *E. histolytica* infection

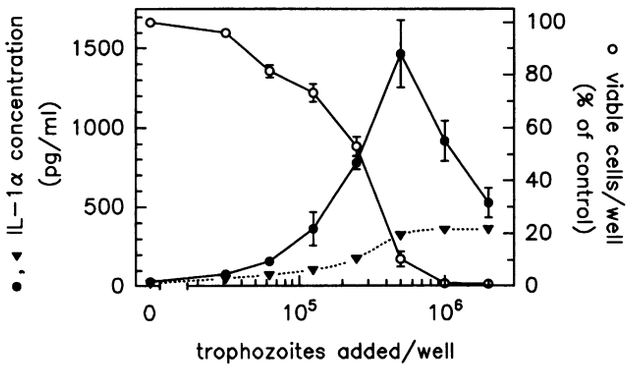

Figure 5. Release of IL-1α in *E. histolytica* infected cell cultures. Confluent monolayers of I407 cells in 6-well plates were infected with different numbers of *E. histolytica* trophozoites in a 2-ml vol. After 8 h in culture, supernatants were removed and IL-1α concentrations were determined by ELISA (•). In parallel, the number of viable cells in the monolayers was determined, and is expressed as a percentage of the number of viable cells in uninfected control cultures (○). Control cultures contained a mean of 3.9×10^6 cells per well. To estimate the contribution of preformed intracellular IL-1α to the total IL-1α in the supernatants for each *E. histolytica* inoculum, uninfected monolayers were lysed by sonication in a 2-ml volume and the IL-1α concentration in the sonicate was multiplied by the fraction of cells that were lysed in response to each *E. histolytica* inoculum (▾, dotted line). Data points are means±SEM of the results of three independent experiments.

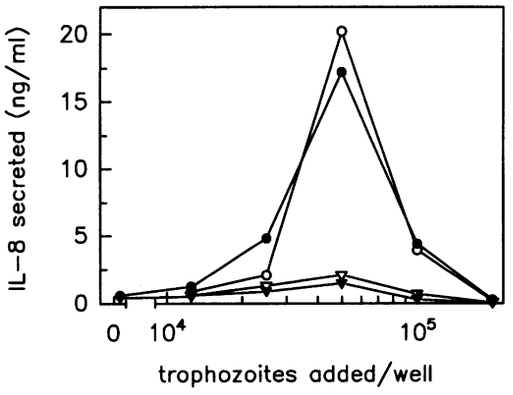

Figure 6. Inhibitors of IL-1α activity block IL-8 secretion by HeLa cells in response to *E. histolytica.* Confluent HeLa cell monolayers in 24-well plates were infected with varying numbers of *E. histolytica* trophozoites in a 0.4-ml vol in the presence of 20 μg/ml goat anti-IL-1α antibodies (▼), 300 ng/ml rhIL-1Ra (▽), 20 μg/ml normal goat IgG (○), or without added inhibitors (●). After 8 h, IL-8 concentrations in the supernatants were assayed. Data represent the mean of triplicate cultures from a representative experiment. SEM, omitted from the figure for clarity, were < 20% for all data points. Similar results were obtained in two additional experiments using either polyclonal goat anti-IL-1α or monoclonal mouse anti-IL-1α antibodies (10 μg/ml).

(HeLa cells: controls < 5 pg/ml, infected 94 pg/ml; WI38 cells: controls < 5 pg/ml, infected 59 pg/ml). In further studies, increased IL-1α mRNA levels were found after *E. histolytica* infection. Thus, unstimulated HeLa and WI38 cells contained 5×10^4 and 1×10^3 IL-1α mRNA molecules/μg total cellular RNA, respectively. In contrast, after 8 h coculture with *E. histolytica,* HeLa and WI38 cells contained 4×10^6 and 3×10^5 IL-1α mRNA molecules/μg total RNA, respectively.

Cell lysates contained soluble IL-1α, as determined for HeLa cells, since ultracentrifugation (100,000 *g,* 30 min) did not decrease the ability of HeLa cell lysates to induce IL-8 secretion by these cells. Moreover, as tested in I407 cell lysates, > 95% of the IL-1α had a molecular mass of 33 kD, as assessed by Western blot analysis, indicating that most IL-1α in the cell lysates was present as the unprocessed precursor. In contrast, supernatants from I407 cells cocultured with *E. histolytica* contained only 50% of the IL-1α as the 33-kD precursor and the remainder as the 17-kD mature form. A similar 1:1 distribution of IL-1α precursor and mature form was observed after incubation of I407 cell lysates with trophozoites for 8 h at 37°C, whereas the distribution of precursor and mature form was not altered after incubation of the I407 cell lysates under identical conditions without trophozoites (i.e., an ~ 20:1 ratio of precursor: mature form). This indicates that *E. histolytica* trophozoites or their secreted products can convert IL-1α precursor into the mature form of IL-1α.

We next determined if IL-1α mediated the increase in IL-8 secretion that was observed after infection of the cell monolayers with *E. histolytica.* As shown in Fig. 6, addition of anti-IL-1α antibodies or IL-1Ra to HeLa cells cocultured with *E. histolytica* reduced IL-8 secretion > 90%, whereas control antibodies had no effect on increased IL-8 secretion. Similarly, increased GM-CSF secretion was not seen when anti-IL-1α antibodies were added to *E. histolytica*-infected HeLa cell cultures, indicating that IL-1α also mediated the induction of that cytokine. The extent of *E. histolytica*-induced HeLa cell lysis was not affected by antibody addition. In further experiments, increased IL-8 secretion by I407 cells in response to *E. histolytica* infection was inhibited > 80% if anti-IL-1α antibodies or IL-1Ra was added to culture. In contrast, increased IL-8 secretion by HepG2 and HT29 cells after infection of the monolayers with *E. histolytica* was not affected by the addition of anti-IL-1α antibodies or IL-1Ra, further documenting that increased IL-8 secretion by those cells was not mediated by IL-1α release.

Induction of IL-8 secretion requires viable E. histolytica trophozoites. These studies indicate that release of IL-1α is a crucial host cell mechanism to induce high-level IL-8 secretion after *E. histolytica* infection. To address the parasite factors which are important for this response, we first assessed whether *E. histolytica* membrane molecules or secreted *E. histolytica* products affect IL-8 secretion. Nonviable trophozoites (boiled for 3 min, or fixed with 1% formaldehyde for 30 min at 4°C) did not induce IL-8 secretion by HeLa cells (21 pg IL-8/ml in the supernatants of uninfected confluent control monolayers in 24-well plates, 12,046 pg/ml in cultures infected with 5×10^4 viable trophozoites, 20 pg/ml in cultures infected with 5×10^4 boiled trophozoites, and 27 pg/ml in cultures infected with 5×10^4 fixed trophozoites).

Similarly, freshly prepared lysates of *E. histolytica,* obtained by sonication on ice and tested at a range of concentrations (50–650 μg/ml total amebic protein), had little effect on IL-8 secretion by HeLa cells (57 pg IL-8/ml in the supernatants of untreated confluent control monolayers in 24-well plates, 43 pg/ml in the presence of 50 μg/ml amebic lysate, and 52 pg/ml with 650 μg/ml amebic lysate), and HT29 cells (174 pg IL-8/ml in the supernatants of untreated confluent control monolayers in 24-well plates, 204 pg/ml in the presence of 50 μg/ml amebic lysate, and 312 pg/ml with 650 μg/ml amebic lysate), and no effect on IL-8 secretion by T84 cells (< 30 pg IL-8/ml in control cultures and in the presence of 650 μg/ml amebic lysate).

In further experiments, *E. histolytica* conditioned medium (see Methods) was tested for its ability to stimulate IL-8 secretion by HeLa, HT29, and T84 cells under serum-free conditions, since serum components can inhibit the activity of some of the enzymes secreted by *E. histolytica* (23, 33). The adherent cell monolayers detached from the plates after addition of increasing concentrations of conditioned media, a cytopathic effect which has been associated with cysteine proteases released by *E. histolytica* (11). Nonetheless, conditioned media did not increase IL-8 secretion at all concentrations tested (2–50% final concentration) (data not shown). Taken together, these results suggest that viable trophozoites, and not simply membrane molecules or secreted products of *E. histolytica,* are required for increased IL-8 secretion.

Role of the galactose-inhibitable amebic adherence protein in the epithelial cell IL-8 response to E. histolytica. Adherence of viable *E. histolytica* to target cells, and subsequent cell lysis, is mediated by a galactose-inhibitable adherence protein (20, 21, 45). To investigate the role of this protein in the IL-8 response to *E. histolytica,* HeLa and HT29 monolayers were cocultured with trophozoites in the presence of galactose or *N*-acetyl-galactosamine, carbohydrates which are reported to specifically block the adherence lectin (20, 45), or glucose and fructose as osmotic controls. As shown in Fig. 7, *A* and *B,* galactose shifted the dose-response curve for both IL-8 secretion and cell lysis by HeLa cells to higher trophozoite inocula, but did not affect maximal IL-8 secretion compared with the osmotic controls. In the presence of galactose, a twofold higher

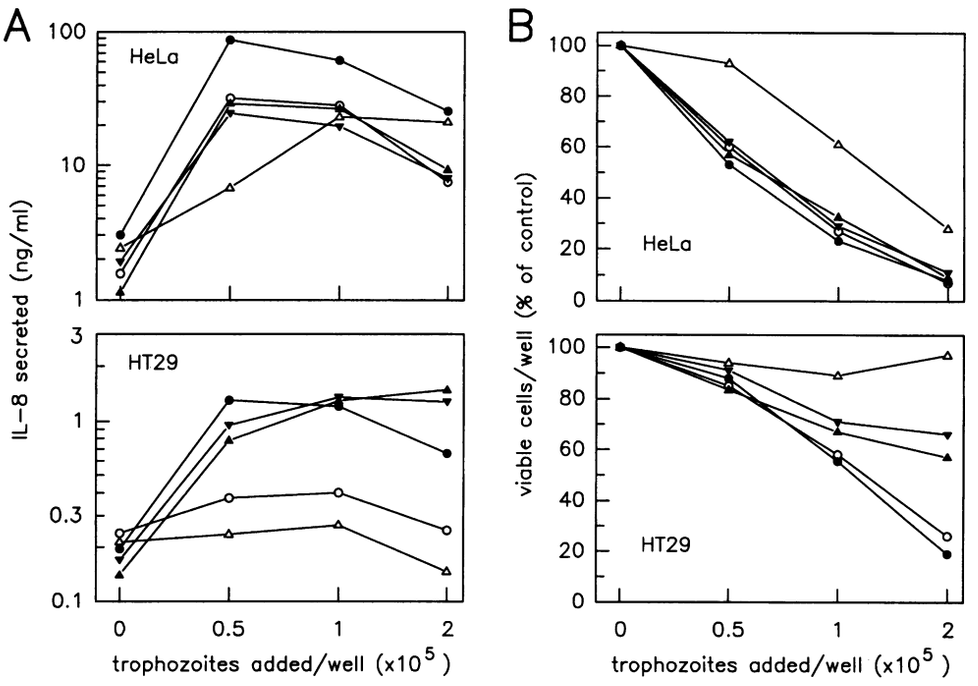

Figure 7. Carbohydrate inhibition of *E. histolytica*–induced IL-8 secretion. Confluent monolayers of HeLa and HT29 cells in 24-well plates were infected with 0.5–2 × 10^5 *E. histolytica* trophozoites in the presence of 100 mM each of galactose (▽), *N*-acetyl-galactosamine (○), glucose (▼), fructose (▲), or without added carbohydrates (●), and incubated for 6 h. Trophozoite viability was not affected by the added carbohydrates during the 6-h culture period. Values are means of the results from three independent experiments. (*A*) Supernatants were removed, and IL-8 concentrations were determined by ELISA. The two- to threefold inhibition of maximal IL-8 secretion in response to *E. histolytica* infection of HeLa cells in the presence of added carbohydrates likely is a nonspecific effect and was also noted after IL-1α or TNFα stimulation (data not shown). (*B*) The number of viable cells remaining in the monolayers at the end of the culture period was determined by trypan blue dye exclusion, and is expressed as a percentage of the number of viable cells present in uninfected control cultures. Control cultures contained a mean of 3.9 × 10^5 (*HeLa*) and 1.1 × 10^6 (*HT29*) cells/well.

number of trophozoites was required to induce maximal IL-8 secretion compared with *E. histolytica*–infected control cultures containing glucose or fructose. The finding that IL-8 secretion and target cell lysis are closely correlated in HeLa cells after *E. histolytica* infection supports the conclusion that target cell lysis and the release of IL-1α are important for the induction of IL-8 secretion in response to *E. histolytica* infection.

In HT29 cells, IL-8 secretion was not closely correlated with target cell lysis, indicating that the IL-8 response of these cells was not dependent on cell lysis. IL-8 secretion by HT29 cells in response to *E. histolytica* was almost completely blocked by both galactose and *N*-acetyl-galactosamine (Fig. 7 *A*) irrespective of whether or not cell lysis was blocked (Fig. 7 *B*) (i.e., galactose blocked cell lysis whereas *N*-acetyl-galactosamine did not). Further, glucose and fructose did not inhibit the increase in IL-8 secretion, although they also significantly decreased target cell lysis after *E. histolytica* infection. These data indicate that the contact through the galactose-inhibitable amebic adherence protein, rather than cell lysis, is most important for the IL-8 response of HT29 cells to *E. histolytica* infection. Moreover, the data with HT29 cells define a further mechanism in addition to the cytolytic release of cellular IL-1α for the induction of IL-8 after *E. histolytica* infection. This mechanism appears to be most relevant for cells that lack preformed IL-1α.

Increases in intracellular calcium are paralleled by moderate increases in IL-8 secretion. Increased levels of intracellular calcium in target cells after contact with trophozoites are thought to be required as an intermediate event for subsequent cell lysis (46). Moreover, the increased intracellular calcium levels have been reported to be mediated by the galactose-inhibitable amebic adherence protein (46). Since the above studies showed that the galactose-inhibitable amebic adherence protein was important for the IL-8 response to *E. histolytica,* we tested the possibility that increases in intracellular calcium would increase IL-8 secretion. As shown in Table IV, stimulation of HT29 cells (which lack preformed IL-1α) or T84 cells (which do not respond to IL-1α) with the calcium elevating agents, thapsigargin (47) and ionomycin, resulted in an increased IL-8 secretion to levels approximating those seen after *E. histolytica* infection. The same agents also elevated IL-8 secretion moderately in HeLa cells, although the increases were considerably lower than those seen after *E. histolytica* infection (Table I). These data are consistent with the possibility that the amebic adherence protein could mediate increases in IL-8 secretion, at least in part, by an increase in intracellular calcium levels after *E. histolytica* infection.

*Table IV. Calcium-elevating Agents Induce IL-8 Secretion by Epithelial Cells**

Cells	IL-8 secreted (pg/ml)		
	Control	+ Thapsigargin	+ Ionomycin
HT29	400	3,394	699
T84	73	1,249	514
HeLa	631	3,217	1,482

* Confluent monolayers of epithelial cell lines in 24-well plates were cultured for 6 h in the presence of 1 μM thapsigargin or 1 μM ionomycin. Since stocks of these agents were prepared in DMSO, control cultures were incubated with the same final concentration of DMSO (0.02%). Supernatants were removed, and IL-8 concentrations were determined by ELISA. Numbers are means of the results of two or three independent experiments.

Discussion

E. histolytica trophozoites stimulated increased secretion of an array of chemoattractant and proinflammatory cytokines, including IL-8, GROα, GM-CSF, IL-6, and IL-1α from human epithelial and stromal cell lines of varying origins. Moreover, as shown for IL-8, increased cytokine secretion was paralleled by increased gene transcription and steady state mRNA levels. These cytokines, particularly IL-8, GROα, and GM-CSF, are known to govern PMN localization and function. Thus, IL-8 and GROα are potent PMN chemoattractants in vitro and in vivo (48). In addition, IL-8 increases the secretion of myeloperoxidase and other antimicrobial proteins by PMNs (35), and GM-CSF increases PMN survival and the capacity of PMNs to phagocytose microbial pathogens (49). The local release of these cytokines, and possibly additional cytokines induced through mechanisms similar to those described herein, along with a direct effect of *E. histolytica* products on PMN migration (50), can explain the histopathologic finding of PMN infiltration in acute experimental *E. histolytica* infection (22, 24, 25).

The cytolytic release of IL-1α was important for initiating the secretion of inflammatory cytokines in response to *E. histolytica* infection. IL-1α appears particularly well suited for this function since it differs in several aspects from most other proinflammatory cytokines. The unprocessed 31–33 kD IL-1α precursor is bioactive with a specific activity that is only twofold lower than that of the mature 17-kD form (51), whereas the precursor form of IL-1β, for example, requires proteolytic processing by an IL-1β converting enzyme for bioactivity (52). Moreover, IL-1α induces physiologic responses at low concentrations (1–10 pg/ml), whereas other proinflammatory cytokines, particularly the chemokines, require substantially higher concentrations (> 10 ng/ml) to mediate their biological activity (48). Unlike other cytokines, IL-1α lacks an amino terminal signal peptide required for efficient secretion (53), which suggests that IL-1α may exert some of its activities in the absence of secretion (54). Despite the absence of a signal peptide, it is known that IL-1α can be secreted by cells in the mature form in response to various stimuli (53). For example, murine macrophages secrete IL-1α after LPS stimulation (53), or after infection with *Shigella flexneri* (55). In contrast, keratinocytes do not secrete IL-1α, although they have high levels of intracellular IL-1α whose biological functions are unknown (53).

These studies suggest a physiologic role of intracellular IL-1α in the host response to lytic pathogens. In this role, membrane damage causes the release of constitutively expressed, intracellular IL-1α precursor, which can activate a cascade of proinflammatory events in the immediate vicinity of the lysed cells. Membrane damage and subsequent cell lysis may be secondary to contact with a cytolytic pathogen such as *E. histolytica,* but this principle may also apply to other pathogens which cause cell lysis such as *Chlamydia trachomatis* or Rotavirus. Further, we showed that IL-1α released by damaged cells can increase IL-1α production by neighboring cells. Such a mechanism could serve to amplify the inflammatory response by increasing the capacity of neighboring cells to release additional IL-1α when they are damaged.

Whereas most epithelial cell lines contained preformed, intracellular IL-1α, some epithelial cell lines, like HT29, lacked this cytokine. Consistent with this finding, only a fraction of intestinal epithelial may express a particular cytokine as shown, for example, for IL-6 (56). This suggests that a minimum number of intestinal epithelial cells (i.e., a lower threshold number) must be lysed before sufficient IL-1α is released to initiate secretion of IL-8 and other proinflammatory cytokines. In contrast to some of the epithelial cells, the stromal cells tested (i.e., fibroblasts and smooth muscle cells) expressed preformed IL-1α, suggesting that once *E. histolytica* has penetrated through the intestinal epithelium deeper into the intestinal mucosa, lysis of a comparatively smaller number of mucosal host cells is sufficient to initiate an inflammatory response.

Increased IL-8 secretion was not mediated by released IL-1α in HT29 and HepG2 cells cocultured with trophozoites, and these cells showed a lower IL-8 response to *E. histolytica* infection. Other cells, like T84, express IL-1α but do not respond to that cytokine since they lack type I IL-1 receptors (Kagnoff, M. F., unpublished data). Nonetheless, the dose–response curves for IL-8 secretion and for target cell lysis were comparable in all these cells, indicating that those cells were not resistant to the effects of *E. histolytica.* Our data indicate that IL-8 secretion by HT29 and HepG2 cells may be induced by signalling pathways that result in increased intracellular calcium levels. This was shown directly by demonstrating that pharmacologic agents that increased intracellular calcium levels induced IL-8 secretion, and indirectly by showing that blocking a galactose-inhibitable amebic adherence protein known to mediate an increase in intracellular calcium levels (46) also blocked IL-8 secretion in HT29 cells. The lower IL-8 response of HT29 to *E. histolytica*, compared to other cells, may also be explained by the fact that direct trophozoite target cell contact, which is required for increased levels of intracellular calcium (46) ultimately leads to cell lysis (45).

IL-8 and GROα, produced by host epithelial and stromal cells in response to *E. histolytica,* are potent chemoattractants and activators of PMNs. In addition, *E. histolytica* products can attract human PMNs (50, 57). The relative contribution of host cell-derived and ameba-derived activities to the chemoattraction of PMNs is not known. However, the host cytokine response demonstrated herein also can activate PMN functions, such as the release of microbicidal enzymes (35), and initiate mechanisms that result in tissue inflammation. For example, IL-1 upregulates adhesion molecules for inflammatory cells on the endothelium (53), and induces prostaglandin production which may contribute to the diarrhea often associated with *E. histolytica* infection (58). Infiltration of host tissues with PMNs is seen early in the response to *E. histolytica* infection in animal models (22, 24, 25). However, the role of PMNs in the host's defense to acute *E. histolytica* infections has not been clearly defined. Thus, nonactivated PMNs were lysed by virulent *E. histolytica* trophozoites in vitro (57). In contrast, cytokine-activated PMNs killed the free living amebas, *Acanthamoeba* and *Naegleria* (59, 60), as well as virulent *E. histolytica* trophozoites (61). These data suggest that PMNs may play a role in the first line of defense against acute *E. histolytica* infection, for example, by reducing the initial trophozoite load.

These studies used cell lines which may have lost or altered differentiated cell functions during the transformation process. However, the data suggest that the observed cellular responses to *E. histolytica* are likely representative of the physiologic host response in vivo. Thus, essentially identical cytokine responses to *E. histolytica* were observed using cells of different origin, cell lineage and differentiation stage, and in several nontransformed cells including CCD-18Co colon fibroblasts and HISM jejunal smooth muscle cells (34). Cell lines have the advantage over freshly isolated cells that they permit the study of cell types in isolation. This is crucial, for example, for demonstrating

the importance of low levels of intracellular IL-1α in the cellular response to *E. histolytica,* since even small numbers of highly IL-1α expressing cells in freshly isolated cell populations, such as macrophages (53), would have confounded the results.

Finally, we further note that cytokines secreted in response to *E. histolytica,* in addition to attracting and activating PMNs, may contribute to other aspects of the clinical picture in acute *E. histolytica* infections. In this respect, IL-1 is a potent pyrogen (53), and fever is commonly seen in acute systemic infection with *E. histolytica* (58). Moreover, IL-6, locally produced at the infection site, can induce the production of acute phase proteins, which are detected in patients with amebic liver abscesses (44, 62). Finally, increased serum IL-8 levels, as we have noted in some patients with amebic liver abscesses (Eckmann, L., and S. L. Reed, unpublished observations), may contribute to the leukocytosis found in patients with invasive amebiasis (63).

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