Cryptosporidium parvum Infection of Human Intestinal Epithelial Cells Induces the Polarized Secretion of C-X-C Chemokines

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Cryptosporidium parvum infects intestinal epithelial cells and does not invade deeper layers of the intestinal mucosa. Nonetheless, an inflammatory cell infiltrate that consists of neutrophils and mononuclear cells is often present in the lamina propria, which underlies the epithelium. This study investigated the host epithelial cell response to *C. parvum* by assessing in vitro and in vivo the expression and production of proinflammatory cytokines by intestinal epithelial cells after infection. The human colon epithelial cell lines HCT-8 and Caco-2 and human intestinal xenografts in SCID mice were infected with *C. parvum*. The expression and secretion of the C-X-C chemokines interleukin-8 (IL-8) and GROα were determined by reverse transcription-PCR analysis and enzyme-linked immunosorbent assay. Our results demonstrate that upregulated expression and secretion of IL-8 and GROα after *C. parvum* infection of intestinal epithelial cells first occurred 16 to 24 h after infection and increased over the ensuing 1 to 2 days. The kinetics of C-X-C chemokine production by *C. parvum*-infected epithelial cells contrast markedly with the rapid but transient expression of C-X-C chemokines by epithelial cells infected with invasive enteric bacteria. C-X-C chemokine secretion in *C. parvum*-infected epithelial cells occurred predominantly from the basolateral surface in polarized monolayers of Caco-2 cells grown in Transwell cultures, whereas cell lysis occurred at the apical surface. The basolateral secretion of IL-8 and GROα from *C. parvum*-infected epithelial cells suggests that C-X-C chemokines produced by those cells contribute to the mucosal inflammatory cell infiltrate in the underlying intestinal mucosa.

Cryptosporidium parvum is a common cause of diarrhea in humans and animals (5). Infection most commonly involves the small intestine and is usually self-limited, and the diarrhea which accompanies infection is transient in immunocompetent hosts (6). However, in immunocompromised individuals (e.g., patients with AIDS), diarrhea persists and frequently the infection and pathological changes are more widespread, possibly involving the biliary tract, pancreas, stomach, esophagus, and respiratory tract as well as the small intestine (3, 12, 19, 35).

In the intestine, *C. parvum* resides in epithelial cells (31). The life cycle of *C. parvum* in the intestinal mucosa includes several stages (14). When oocysts are ingested, sporozoites excyst and rapidly infect host epithelial cells. *C. parvum* establishes itself intracellularly but extracytoplasmically in a parasitophorous vacuole beneath the apical membrane of the epithelial cell. After asexual multiplications in epithelial cells over the ensuing 16 to 48 h, type I and II merozoites are released and infect new epithelial cells. Type II merozoites differentiate into microgamonts and macrogamonts and initiate sexual reproduction between 3 and 5 days postinfection (p.i.). After macrogametes are fertilized by microgametes, they develop into oocysts, which sporulate in situ. By approximately 4 to 7 days p.i., oocysts either are shed in the feces or release sporo-

zoites, which can infect other host epithelial cells and initiate a new cycle of parasite development (5).

C. parvum infection results in a spectrum of pathologic changes in the intestine. The severity of mucosal injury correlates with the numbers of infecting organisms and is characterized by minimal-to-marked villus atrophy (22, 31). Moderate-to-severe infections can be accompanied by a patchy or prominent neutrophil and mononuclear infiltrate in the lamina propria and numerous intraepithelial neutrophils (11, 22). Nonetheless, the mechanism by which this epithelial infection can cause mucosal inflammation and injury is not known.

Chemokines are low-molecular-weight proteins with pleiotropic effects on the recruitment and activation of leukocytes at sites of inflammation (2). The C-X-C chemokines, such as interleukin-8 (IL-8) and GROα, play an important role in the chemoattraction of neutrophils to sites of inflammation and in the activation of those cells (2). We and others have previously reported rapid upregulated expression of members of the C-X-C chemokine family in human intestinal epithelial cells after invasion of those cells with gram-negative or gram-positive bacteria (9, 16, 20, 30). Those studies have suggested that epithelial cells which line the human intestinal mucosa can act as sensors for bacterial invasion and provide early signals for initiation of the mucosal inflammatory response (17, 20, 30). In contrast to enteroinvasive bacteria, *C. parvum* resides within intestinal epithelial cells and does not invade deeper layers of the intestinal mucosa (5). Nonetheless, infection with *C. parvum* is associated with the recruitment of leukocytes to the lamina propria of the mucosa and with marked diarrhea.

To investigate the host epithelial cell response to *C. parvum*

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infection, we assessed the expression and production of proinflammatory cytokines in intestinal epithelial cells after infection of human epithelial cell lines and human intestinal xenografts in SCID mice. The data reported here demonstrate that *C. parvum* infection of human intestinal epithelial cells results in upregulated expression and basolateral secretion of the C-X-C chemokines IL-8 and GROα.

MATERIALS AND METHODS

Cell lines and cell culture. The human ileocecal adenocarcinoma cell line HCT-8 (ATCC CCL244) was obtained from the American Type Culture Collection, Rockville, Md., and the human ileocecal adenocarcinoma cell line Caco-2 was kindly provided by S. Tzipori, Tufts University. Cells were maintained in growth medium (RPMI 1640 with heat-inactivated fetal bovine serum [FBS] [10% FBS for HCT-8 cells and 20% FBS for Caco-2 cells], 2 mM L-glutamine, 50 U of penicillin G per ml, and 50 μg of streptomycin per ml).

Polarized monolayers of Caco-2 cells were prepared by seeding 5×10^5 Caco-2 cells on the tops of collagen-coated microporous supports (0.4-μm pore size; 4.7-cm^2 growth area) in Transwell chambers (Costar, Cambridge, Mass.). The medium was replaced every 2 days, and cells were allowed to differentiate for 15 days before use. The medium volumes in the apical and basal compartments were 1.5 and 2.6 ml, respectively. The differentiation of polarized Caco-2 monolayers was verified by transmission electron microscopy, demonstrating the formation of microvilli and tight junctions, and by the increased expression over time of alkaline phosphatase, as determined by enzymatic assay (15) (data not shown). In addition, the formation of tight junctions was functionally assessed by measurements of electrical resistance across monolayers by using a Millicell electrical resistance system (Millipore, Bedford, Mass.). The electrical resistance of uninfected monolayers in the experiments reported here ranged from 430 to 495 $\Omega \times$ cm^2 after subtraction of resistance across a cell-free filter.

Purification and excystation of *C. parvum* oocysts. *C. parvum*, initially isolated from an infected child (1), was maintained in calves at the INRA, Nouzilly, France. *C. parvum* oocysts, isolated from feces by filtration and diethyl ether sedimentation, were treated with 1.25% sodium hypochlorite, washed, and then stored at 4°C in phosphate-buffered saline (PBS; pH 7.4) containing 50 U of penicillin G per ml, 50 μg of streptomycin per ml, and 0.25 μg of amphotericin B per ml. With this treatment, subsequent excystation rates in growth medium were 40 to 60%.

Oocysts in medium equilibrated with 5% CO_2–95% air were gently agitated for 2 h at 37°C to enhance excystation. Various numbers of oocysts that had been excysted in growth medium were used to infect Caco-2 cells, whereas various numbers of oocysts that had been excysted in supplemented growth medium (34) (the supplement consisted of 50 mM glucose, 35 μg of ascorbic acid per ml, 1.0 μg of folic acid per ml, 4 μg of 4-aminobenzoic acid per ml, 2.0 μg of calcium pantothenate per ml, 0.1 U of insulin per ml, and 0.25 μg of amphotericin B per ml) were used to infect HCT-8 cells. For heat inactivation, oocysts were incubated at 57°C in growth medium for 2 h prior to use.

Infection protocols for cell lines. For the infection of HCT-8 cells, 2×10^6 cells were seeded into six-well Costar tissue culture plates and cultured for 24 h to form confluent monolayers. Oocysts, prepared in supplemented growth medium as described above, were added to cell monolayers and allowed to infect cells for 5 h, after which cells were washed three times before fresh supplemented growth medium was added. For the infection of Caco-2 cells, 5×10^5 cells were seeded either into six-well Costar tissue culture plates or onto microporous supports of Costar Transwell chambers and allowed to differentiate for 15 days, during which the medium was changed every 2 days. For the infection of polarized monolayers, *C. parvum* oocysts were added to the upper compartment for 5 h, after which cells were washed and fresh medium was added. The ratios of oocysts to epithelial cells used for infection were calculated as the ratio of excysted oocysts to epithelial cells. The fraction of excysted oocysts in oocyst preparations was determined by light microscopy.

The infectivity of oocyst preparations for the epithelial cells used in this study was confirmed by immunofluorescence. Cells were fixed for 5 min in methanol and then incubated with either a monoclonal mouse anti-*C. parvum* oocyst antibody (immunoglobulin M [IgM] isotype) that also detects the intracellular stages of *C. parvum* (Chemicon International, Temecula, Calif.) or a polyclonal rat antiserum against *C. parvum* sporozoites produced in our laboratory by previously described methods (36). Phycoerythrin-labeled goat anti-mouse IgM or fluorescein isothiocyanate-labeled goat anti-rat IgG was used as the secondary antibody. On average, at 48 h p.i. ~10 to 30% of epithelial cells were infected, depending on the *C. parvum* inoculum.

Cytokine ELISAs and lactate dehydrogenase (LDH) assays. IL-8 in culture supernatants was assayed by enzyme-linked immunosorbent assay (ELISA) as described before (8). The IL-8 ELISA was sensitive to 20 pg/ml. The GROα and ENA-78 ELISAs used goat anti-human GROα and goat anti-human ENA-78 (R&D Systems, Minneapolis, Minn.), respectively, as capturing antibodies, monoclonal mouse anti-human GROα and monoclonal mouse anti-human ENA-78 (R&D Systems), respectively, as detecting antibodies, and horseradish peroxidase-labeled goat anti-mouse IgG (Sigma Chemical Co., St. Louis, Mo.) as

the second-step antibody. Bound horseradish peroxidase was visualized with the substrate 3,3′5,5′-tetramethylbenzidine (Calbiochem, La Jolla, Calif.). The GROα ELISA was sensitive to 30 pg/ml, and the ENA-78 ELISA was sensitive to 100 pg/ml.

The LDH activity in culture supernatants was assayed by a colorimetric method based on the reduction of pyruvate to lactate in the presence of LDH and NADH. The remaining pyruvic acid was colorimetrically detected after a reaction with 2,4-dinitrophenylhydrazine to form a colored hydrazone (LDH-LD, Sigma Chemical Co.). The absorbance was determined at 450 nm.

RNA extraction and RT-PCR analysis. Total cellular RNA was extracted from cells with Trizol reagent (Gibco BRL, Gaithersburg, Md.). Epithelial monolayers were directly lysed in Trizol reagent, whereas mucosal scrapings from intestinal xenografts were first snap frozen in liquid nitrogen and then homogenized in Trizol reagent with a Potter-Elvehjem tissue grinder. mRNA levels for IL-8, GROα, and β-actin were determined by quantitative reverse transcription (RT)-PCR analysis with internal RNA standards as previously described (16). The RT-PCR was specific for the respective human mRNAs, since amplification of mouse spleen RNA or DNA did not yield any PCR products.

Human fetal intestinal xenografts. The human fetal intestinal xenograft model used in the present work has been described in detail previously (29). Briefly, human fetal small intestine (gestational age, 10 to 14 weeks) was transplanted subcutaneously into C.B-17 severe combined immunodeficient (SCID) mice. Xenografts were allowed to develop for 10 weeks prior to use, at which time the epithelium and underlying mucosa are fully differentiated (29). Xenografts were infected with 5×10^6 or 5×10^7 *C. parvum* oocysts resuspended in 100 μl of PBS. Xenograft tissue was removed 5 days after infection. For histology, portions were fixed in 10% formalin in PBS and embedded in paraffin, and sections were prepared and stained with hematoxylin and eosin. For RNA extractions, mucosal scrapings were prepared and immediately snap frozen in liquid nitrogen. Xenograft studies were performed with full approval from the Cambridge Local Ethics Committee and in accordance with the Home Office guidelines specified in the Polkinghorne Report (29).

Transmission electron microscopy. Tissue pieces were immersion fixed in 4% glutaraldehyde–2% sucrose–0.1 M sodium phosphate (pH 7.2) for 1 to 2 h at room temperature. Postfixation was performed for 1 h in 1% osmium tetroxide–0.1 M sodium cacodylate (pH 7.2), followed by 1 h of staining in 2% aqueous uranyl acetate. Samples were dehydrated in ethanol and propylene oxide and embedded in Araldite epoxy resin (Sigma Chemical Co.). All processing was carried out at room temperature. Sections were stained with uranyl acetate and lead citrate.

RESULTS

Increased IL-8 and GROα release in response to *C. parvum* infection of HCT-8 and Caco-2 cells. IL-8 and GROα are potent chemoattractants and activators of neutrophils. We first assessed IL-8 and GROα production in response to *C. parvum* infection of HCT-8 cells, a human intestinal epithelial cell line known to support the development of *C. parvum* in vitro (33). The levels of IL-8 and GROα release were determined for up to 96 h after infection by ELISA. As shown in Fig. 1, HCT-8 cells constitutively secreted low levels of IL-8 and GROα and the release of these chemokines increased two- to threefold after *C. parvum* infection. Increases in IL-8 and GROα release were first noted at 16 to 24 h p.i. and continued for up to 72 to 96 h p.i.

Increased chemokine release required infection with viable *C. parvum* organisms, as heat-killed *C. parvum* organisms did not induce IL-8 or GROα release (data not shown). To relate increased chemokine release to cell lysis, which is a consequence of *C. parvum* infection, lysis of HCT-8 cells after infection was assessed by LDH release. As shown in Fig. 1, cell lysis increased markedly between 16 and 48 h p.i. and the LDH levels in supernatants were maximal at between 48 and 72 h p.i., indicating that the time course of cell lysis after infection overlapped that of the increases in IL-8 and GROα release. The maximum levels of LDH release from monolayers infected with *C. parvum* were ~50% of the levels obtained by lysing parallel monolayers by sonication.

The magnitude of the chemokine response was dependent on the inoculum of infecting oocysts per epithelial cell. Thus, infection of HCT-8 cells with increasing ratios of *C. parvum* oocysts per HCT-8 cell was paralleled by increased IL-8 release. At ratios of 0.3, 1, and 3 oocysts/HCT-8 cell, IL-8 release
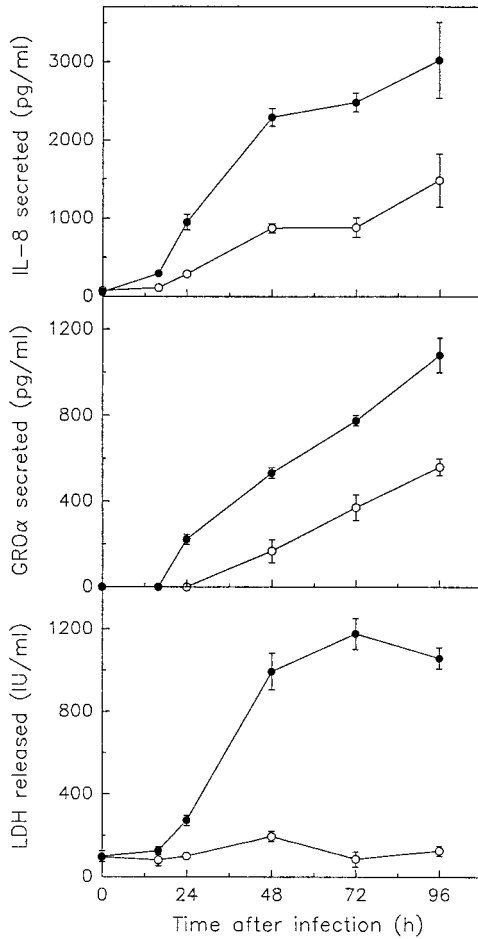
FIG. 1. Kinetics of IL-8 secretion by HCT-8 cells infected with *C. parvum*. Confluent monolayers of HCT-8 cells were infected for 5 h at a ratio of 2 oocysts per cell, as described in Materials and Methods, after which cells were washed and incubated for an additional 96 h without changing the medium. Control uninfected cells were cultured in parallel. Supernatants were collected at the indicated times p.i. IL-8 (top) and GROα (middle) levels were determined by ELISA, and LDH activity (bottom) was determined by enzymatic assay. Data points from infected (●) and control (○) cultures are means ± standard deviations of triplicate cultures from a single representative experiment. Similar data were obtained in at least eight additional experiments.
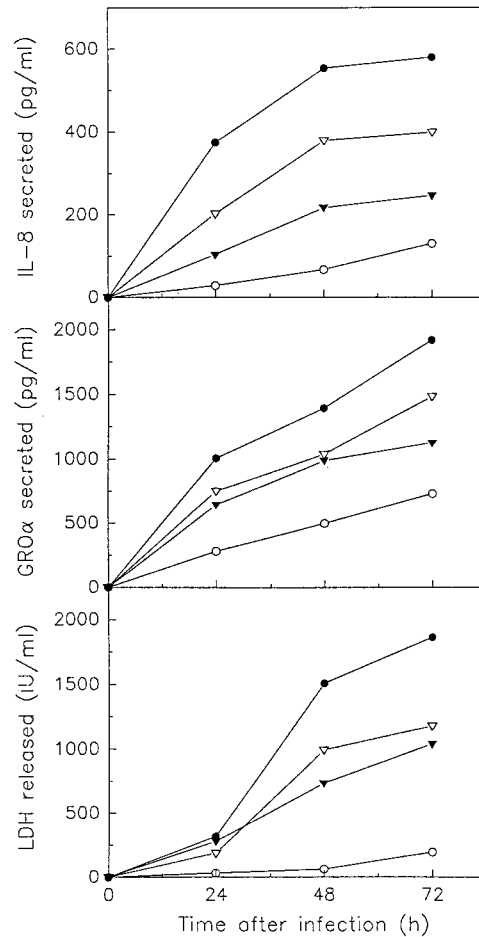

FIG. 2. Kinetics of IL-8 secretion by Caco-2 cells infected with *C. parvum*. Confluent differentiated monolayers of Caco-2 cells on six-well plates were infected with oocysts at oocyst/cell ratios of 0.3 (▼), 1 (▽), and 3 (●) for 5 h, after which cells were washed and incubated for an additional 72 h without changing the medium. Control uninfected cells were cultured in parallel (○). IL-8 (top) and GROα (middle) levels in supernatants were determined by ELISA, and LDH activity (bottom) was determined by enzymatic assay. Data points are from single wells in a representative experiment. Similar results were obtained in two repeated experiments.

increased (1.3 ± 0.3)-, (3.4 ± 0.4)-, and (5.1 ± 0.7)-fold, respectively, relative to that of uninfected controls (data are means ± standard errors of the means of six separate experiments).

Caco-2 is another human intestinal epithelial cell line known to support *C. parvum* infection (4). In addition, Caco-2 cells differentiate in culture and can be grown as polarized monolayers on microporous supports. As shown in Fig. 2, *C. parvum* infection of Caco-2 cells increased IL-8 and GROα release in a dose- and time-dependent manner, with kinetics similar to those in the HCT-8 line. Whereas HCT-8 cells infected with *C. parvum* released higher levels of IL-8 than of GROα, the opposite was true for Caco-2 cells. Neither cell line produced detectable levels of the C-X-C chemokine ENA-78 before or after *C. parvum* infection (<100 pg/ml). Lysis of Caco-2 cells, like that of HCT-8 cells, increased markedly between 24 and 48 h p.i. and the LDH levels in supernatants continued to increase over the 72-h culture period (Fig. 2).

***C. parvum* infection upregulates IL-8 mRNA levels in HCT-8 cells.** To assess whether increased chemokine secretion was paralleled by increased mRNA levels, the IL-8 mRNA levels in control and *C. parvum*-infected HCT-8 cells were assessed. As shown in Table 1, the levels of IL-8 mRNA transcripts increased by 5-fold at 24 h p.i., peaked at 12-fold by 48 h p.i., and

TABLE 1. Time course of increased IL-8 mRNA levels after
C. parvum infection of HCT-8 cells[a]

Time p.i. (h)	IL-8 mRNA levels		Infected/control ratio	β-Actin mRNA levels		Infected/control ratio
	Control	+ *C. parvum*		Control	+ *C. parvum*	
24	3.8×10^5	1.9×10^6	5.0	1.3×10^7	2.0×10^7	1.5
48	5.0×10^5	6.0×10^6	12.0	2.0×10^7	1.9×10^7	1.0
72	8.3×10^5	2.0×10^6	2.6	2.4×10^7	2.0×10^7	0.8
96	1.1×10^6	1.0×10^6	0.9	1.5×10^7	2.1×10^7	1.4

[a] Confluent monolayers of HCT-8 cells in six-well plates were infected (+ *C. parvum*) at an oocyst/cell ratio of 3. Total cellular RNA was extracted at the indicated times p.i., and mRNA levels for IL-8 and the housekeeping gene β-actin were determined by quantitative RT-PCR with internal standards. Data are the numbers of mRNA transcripts per microgram of cellular RNA.

decreased to baseline thereafter. These data suggest that the increased IL-8 secretion in response to *C. parvum* infection was due in large part to pretranslational events. In contrast, the β-actin mRNA levels in infected cells remained relatively constant throughout the same period (Table 1).

Mediators released from lysed cells are not responsible for increased IL-8 production. We asked whether increased IL-8 production after *C. parvum* infection of HCT-8 cells was due to the release of a stable IL-8-stimulating activity from infected cells. For these experiments, cell lysates and supernatants were combined from HCT-8 cells that had been infected with *C. parvum* for 48 h, the earliest time point after infection at which the majority of cells had lysed, and were added to fresh monolayers of HCT-8 cells. The lysates and supernatants did not induce significant IL-8 production in fresh HCT-8 monolayers (data not shown). This result suggests that the IL-8 produced in infected cultures derived directly from *C. parvum*-infected cells, not from neighboring uninfected cells that had been activated by mediators released from infected or lysed cells.

Polarized basolateral secretion of IL-8 and GROα after *C. parvum* infection. Intestinal epithelial cells are functionally polarized. If IL-8 and GROα produced by *C. parvum*-infected epithelial cells play a physiologic role in the influx of neutrophils into the mucosa, they predictably would be released from the basolateral surfaces of infected epithelial cells. To determine the polarity of chemokine secretion by *C. parvum*-infected epithelial cells, Caco-2 cells were cultured as polarized monolayers in Transwell chambers. The impermeability of monolayers to IL-8 was established by the addition of a relatively high dose of IL-8 (10 ng/ml) to the basolateral compartment of uninfected monolayers. This resulted in the appearance of only 3% of the added IL-8 in the apical compartment 24 h later. After apical infection with *C. parvum*, the medium in the top and bottom compartments of Transwell chambers was changed daily and the IL-8 and GROα levels, as well as LDH release, in the apical and basolateral compartments were determined. As shown in Fig. 3, 80% or more of IL-8 and GROα was found in the basolateral compartment. This was not related to a reduced barrier function of infected monolayers, since LDH was released predominantly into the apical compartment (Fig. 3) and the electrical resistance across monolayers decreased only slightly after infection (430 to 495 $\Omega \times cm^2$ in controls and 380 and 270 $\Omega \times cm^2$ at 24 and 48 h, respectively, after infection at an oocyst/epithelial cell ratio of 3). Thus, the secretion of IL-8 and GROα in *C. parvum*-infected epithelial cells occurs predominantly from the basolateral surface. In controls, polarized Caco-2 monolayers stimulated with IL-1α for 24 h secreted 110-fold more total IL-8 than did controls and 84% of IL-8 was found in the basolateral compartment.

Increased IL-8 and GROα expression by intestinal epithelial cells in vivo in a human intestinal xenograft model infected with *C. parvum*. We next asked whether *C. parvum* infection induces IL-8 and GROα expression in intestinal epithelial cells in vivo. To infect human intestinal epithelial cells with *C. parvum*, we used a human fetal intestinal xenograft model in which human fetal intestine (gestational age, 10 to 14 weeks) is transplanted subcutaneously into SCID mice. Such xenografts develop a fully differentiated epithelial layer of entirely human origin over a 10- to 20-week period (29). To establish that human intestinal xenografts could be infected with *C. parvum*, xenografts were injected intraluminally with *C. parvum* oocysts and tissues were removed 5 days later and analyzed on hematoxylin- and eosin-stained paraffin sections and by transmission electron microscopy. Infection showed a patchy distribution, with *C. parvum* localized to the luminal surface of intestinal
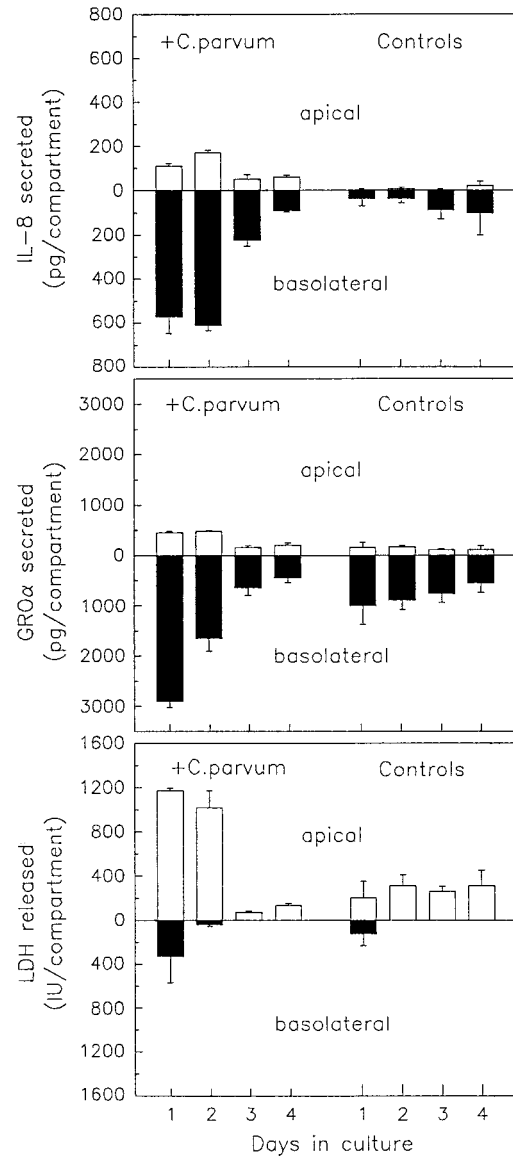

FIG. 3. Basolateral IL-8 and GROα secretion and apical LDH release by polarized Caco-2 cells. Polarized monolayers of Caco-2 cells were infected (+ *C. parvum*) at an oocyst/epithelial cell ratio of 3. Cultures were incubated for 4 days, during which supernatants were removed every 24 h from the apical and basolateral compartments and replaced with fresh medium. IL-8 secretion (top) and GROα secretion (middle) in 24-h supernatants were determined by ELISA, and LDH activity (bottom) was determined by enzymatic assay.

epithelial cells (Fig. 4A). The characteristic developmental stages of *C. parvum* were observed by transmission electron microscopy (Fig. 4C), indicating that *C. parvum* infection proceeded normally in xenografts. Furthermore, infection elicited an inflammatory host response, since small cell infiltrates with mostly neutrophils were observed beneath the epithelium (Fig. 4B). Based on the nuclear morphology, infiltrating neutrophils appeared to be of mouse origin.

To determine chemokine expression in *C. parvum*-infected xenografts, RNA was extracted from mucosal scrapings and analyzed by quantitative RT-PCR. As shown in Table 2, the levels of human IL-8 and GROα mRNAs increased by >20-fold after *C. parvum* infection of intestinal xenografts, whereas β-actin mRNA levels were only slightly increased after infec-
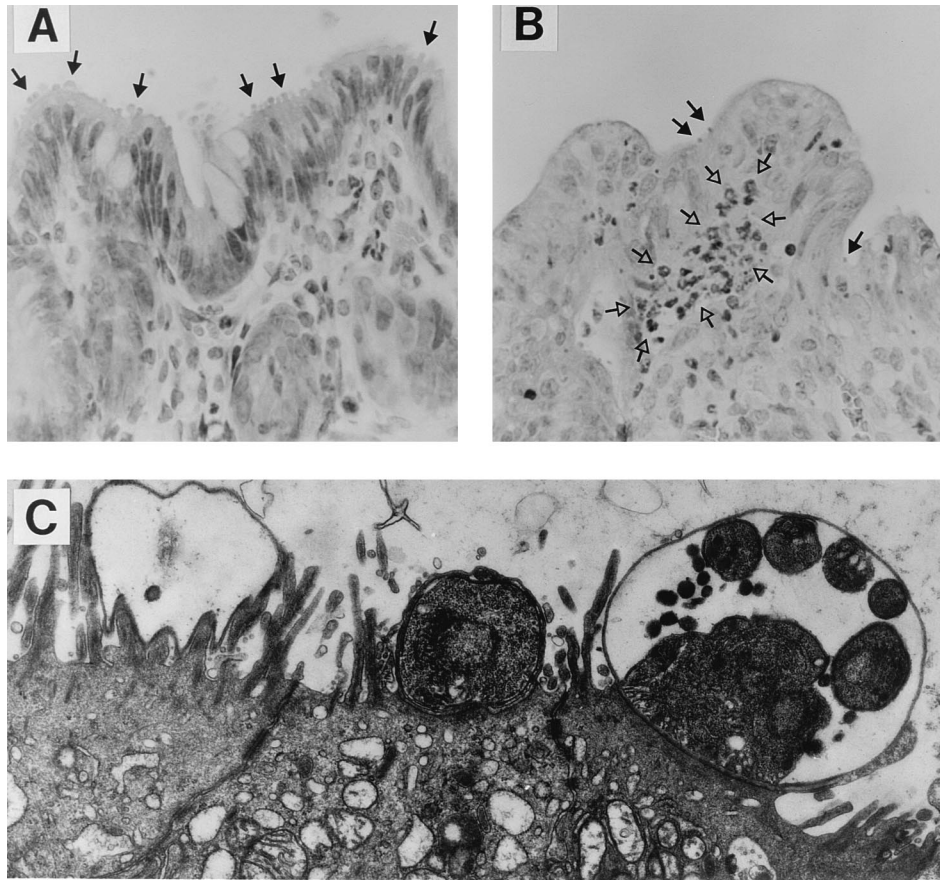
FIG. 4. *C. parvum* infection of human small intestinal xenografts. Human intestinal xenografts in SCID mice were infected with 5×10^6 *C. parvum* oocysts by transcutaneous injection. Xenografts were collected 5 days after infection, and paraffin sections were prepared and stained with hematoxylin and eosin (A and B). In parallel, sections were prepared for transmission electron microscopy (C). (A) Two atrophied villi with multiple *C. parvum* parasite stages (arrows) at the apical surface of the intestinal epithelium; original magnification, ×400. (B) Damaged villi with several *C. parvum* parasite stages (arrows with solid arrowheads) and a small cell infiltrate containing mostly neutrophils (arrows with open arrowheads); original magnification, ×400. (C) Three parasite stages of *C. parvum* at the apical surfaces of three intestinal epithelial cells; original magnification, ×5,000. On the right side, a schizont containing several merozoites can be seen. In the middle, a trophozoite is visible, and on the left either the remains of a schizont which released its merozoites or a schizont whose merozoites were not sectioned is shown. Epithelial microvilli and tight junctions between epithelial cells are also shown.

tion (4.2- and 1.5-fold in two separate experiments). Since intestinal epithelial cells are the most abundant human cell type in the mucosa of the intestinal xenograft model and only those cells were infected with *C. parvum*, these data suggest that epithelial cells were most likely responsible for the increases in IL-8 and GROα expression after infection.

DISCUSSION

C. parvum infects and undergoes its life cycle within epithelial cells that line the human intestinal mucosa but does not invade deeper layers of the human gastrointestinal mucosa (5, 31). Nonetheless, moderate-to-severe infection of the gastrointestinal tract with *C. parvum* is often associated with prominent infiltration of neutrophils and mononuclear phagocytes in the lamina propria, which underlies the epithelium, and intraepithelial neutrophils (11, 22). The mechanisms by which an infection with a pathogen, such as *C. parvum*, that is confined to epithelial cells results in subepithelial inflammation are not well understood. The present studies used in vitro and in vivo models to demonstrate that intestinal epithelial cells infected with *C. parvum* upregulate the expression and polarized basolateral secretion of the C-X-C chemokines IL-8 and

GROα, which are known chemoattractants and activators of neutrophils (2). This epithelial response to *C. parvum* appears to be well suited for contributing to the accumulation of neutrophils at the site of infection by establishing a chemotactic gradient within the underlying mucosa and to the activation of these cells.

The secretion of IL-8 and GROα after *C. parvum* infection occurred mostly from the basolateral surface in polarized monolayers of intestinal epithelial cells. These data suggest that increased basolateral IL-8 secretion did not simply result from cell lysis, since LDH (as a marker of cell lysis) was found predominantly in the apical compartment after *C. parvum* infection, a finding that is consistent with previous observations (13). Similar to *C. parvum*-infected epithelial cells, polarized Caco-2 cells stimulated with the proinflammatory agonist IL-1α and polarized T84 human colon epithelial cells stimulated with the agonist tumor necrosis factor alpha (8) predominantly secreted IL-8 at the basolateral surface. In general, secreted proteins that are not specifically targeted to the apical surfaces of polarized epithelial cells appear to be predominantly secreted at the basolateral surfaces of those cells (27). Although this has not been specifically documented for IL-8 or other chemokines, a similar default mechanism is likely re-

TABLE 2. Increased chemokine mRNA levels after *C. parvum* infection of human small intestinal xenografts[a]

Expt no.	mRNA species	No. of mRNA transcripts/μg of total RNA		Infected/control ratio
		Control	+ *C. parvum*	
1	IL-8	$<5 \times 10^2$	1.1×10^4	>22
	GROα	$<5 \times 10^2$	1.0×10^4	>20
	β-Actin	1.8×10^6	7.6×10^6	4.2
2	IL-8	1.0×10^4	6.0×10^5	60
	β-Actin	8.0×10^6	1.2×10^7	1.5

[a] Human intestinal xenografts were infected with 5×10^6 and 5×10^7 *C. parvum* oocysts in experiments 1 and 2, respectively. Paired xenograft samples derived from the same donors were left uninfected as controls. Total cellular RNA was extracted from mucosal scrapings of individual xenografts obtained 5 days after infection, and equal amounts of RNA were pooled from five to eight xenografts. mRNA levels for IL-8, GROα, and β-actin were determined by quantitative RT-PCR with internal standards.

sponsible for the predominance of basolateral IL-8 secretion after *C. parvum* infection or agonist stimulation of intestinal epithelial cells.

Upregulated IL-8 and GROα secretion after *C. parvum* infection of cell lines declined to control levels by 48 to 72 h p.i. This can be explained by the limited ability of *C. parvum* to undergo repeated rounds of its life cycle in epithelial cell lines (4, 23) and by our finding that increased IL-8 secretion appeared to be limited to *C. parvum*-infected cells. Since the majority of infected cells have lysed after 2 to 3 days in culture and few additional cells become infected thereafter, increased IL-8 secretion decreases. This likely contrasts with *C. parvum* infection in vivo, as shown with infected intestinal xenografts at 5 days p.i., since the parasite can undergo repeated cycles of reinfection (14) and conceivably could induce a more prolonged epithelial chemokine response.

The kinetics of the epithelial cell C-X-C chemokine response to *C. parvum* infection differed markedly from those seen after infection of epithelial cells with invasive bacteria or the rapidly cytolytic pathogen *Entamoeba histolytica* (9, 10, 20, 37). The upregulation of IL-8 and GROα mRNA levels and secretion after *C. parvum* infection of epithelial cells was delayed for 16 to 24 h after infection, and chemokine secretion continued at increased levels for at least 48 h. In contrast, epithelial cell infection with invasive pathogens (e.g., *Salmonella*, *Yersinia*, or enteroinvasive *Escherichia coli* strains) resulted in rapid (2 to 3 h) but brief (4 to 10 h) upregulated IL-8 mRNA expression and IL-8 secretion by epithelial cells (9, 20, 30). Similarly, maximal IL-8 and GROα mRNA expression and production occurred within 4 to 8 h after infection of epithelial cell cultures with *E. histolytica* trophozoites (10, 37). However, the kinetics of epithelial cell IL-8 and GROα production after *C. parvum* infection are similar to those we recently noted after infection of intestinal epithelial cells with *Chlamydia trachomatis* (26), a bacterial pathogen which, like *C. parvum*, resides and replicates in epithelial cells (24).

In prior reports, the cumulative levels of IL-8 and GROα produced by epithelial cells in response to infection with *E. histolytica*, a rapidly cytolytic protozoan parasite, or *Chlamydia trachomatis*, a slowly cytolytic intracellular bacterium, were often greater than those stimulated by *C. parvum* infection in the present studies (10, 26). In the former cases, the IL-1α released from epithelial cells lysed by infection amplified the IL-8 or GROα response in culture by stimulating increased production of those chemokines by neighboring intact cells. In contrast, the IL-8 response after *C. parvum* infection of HCT-8

and Caco-2 cells appeared to be due to the production of IL-8 and GROα by infected cells themselves and was not amplified by mediators released from *C. parvum*-infected cells.

Our data suggest that epithelial cells play an active role in the host response to *C. parvum* infection. After infection, increased epithelial chemokine production may attract inflammatory cells into the subepithelial region, and those cells may subsequently migrate into and across the epithelium (21, 22, 25). In this regard, neutrophils in mucosal secretions have previously been shown to retain the ability to phagocytose and kill pathogens (7). In addition, neutrophils may function to regulate other aspects of the inflammatory response at the site of *C. parvum* infection through the secretion of cytokines (18).

Human small intestinal xenografts were shown to be a good model for studying important aspects of the host response to *C. parvum* infection. This model has several advantages over clinical studies, since it is possible to characterize the response of normal human intestinal epithelial cells to the infection in a controlled and easily accessible environment (32). In contrast, clinical studies of immunocompetent patients are difficult to conduct since cryptosporidiosis in these patients is generally transient (6); for this reason, such patients are rarely biopsied. Moreover, immunodeficient patients with *C. parvum* infection often have multiple other opportunistic pathogens, which complicates the selection of appropriate controls and the interpretation of findings. The xenograft model consists of a chimeric mixture of human and mouse cells (29). Nonetheless, our finding that mouse neutrophils accumulated under the human epithelium after *C. parvum* infection suggests that epithelial cell-derived chemotactic signals were active on mouse cells. Consistent with this notion, human IL-8 has previously been shown to activate and chemoattract mouse neutrophils (28).

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