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Campylobacter jejuni-Induced Cytokine Responses in Avian Cells

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***Campylobacter jejuni* is a major cause of human inflammatory enteritis. During the course of human disease numerous proinflammatory cytokines are produced. Little is known, however, about the cytokine responses produced during the interaction of this bacterium with the avian host. *Campylobacter* has been considered a commensal of the avian host. Any differences in innate responses to this pathogen between the human and avian hosts should lead to a greater understanding of the disease process in humans. We have demonstrated expression of proinflammatory cytokines and chemokines in response to *Campylobacter* infection in avian primary chick kidney cells and the avian macrophage cell line HD11. The data indicate that *Campylobacter* can stimulate the avian host in a proinflammatory manner. The data strongly suggest that the lack of pathology in vivo is not due to an inability of *Campylobacter* to stimulate a proinflammatory response from avian cells.**

Campylobacter jejuni causes severe gastroenteritis in humans. The pathology includes severe inflammation of the intestinal mucosa with an influx of professional phagocytes (25, 41–43). Histological analysis of biopsy samples from patients with *C. jejuni* colitis has shown that the bacteria invade the colonic mucosa. In contrast *Campylobacter* infection of the chicken leads to high-level colonization of the intestinal tract in an apparently commensal association, with little or no pathology (6). *Campylobacter* can invade human epithelial cell monolayers (11, 12), causing disruption to the epithelium and gaining access to its basal side (41, 45). Interleukin-1β (IL-1β), IL-8, and nitric oxide (NO) are produced during human *Campylobacter* infections (10), and in vitro experiments with human-derived epithelial cell lines have shown that *C. jejuni* can induce the secretion of a range of cytokines and chemokines (2, 3, 16, 17, 19).

During human infection *Campylobacter* can invade and traverse the epithelial barrier (41, 42). Given the range of chemokines produced in vitro and the observed attraction of a range of leukocytes in vivo, it is probable that *Campylobacter* interacts with leukocytes. Human monocytes produce a range of cytokines and chemokines, including IL-1β, IL-6, tumor necrosis factor alpha, and IL-8 when infected with *Campylobacter*, and their stimulation could contribute to disease pathology (20, 39).

Two *C. jejuni*-derived factors that can induce IL-8 production from epithelial cells have been defined: the cytolethal-distending toxin (CDT) and the adhesion factor Jlp (17, 19). CDT has an effect in disease models, but it is not required for induction of cytokines by live *C. jejuni* during infection of either epithelial or monocytic cells (14, 17, 20).

While the production of chemokines by human cells in response to *Campylobacter* infection has only just been described (3), their role in infection may be crucial to the development of inflammatory disease. The chicken has a reduced chemokine repertoire compared to mammals (18) and possesses two homologues of IL-8, 9E3/CEF4 (also known as IL-8/CAF and referred to as IL-8 in this paper) and K60, both of which are found on chromosome 4 (38).

Little is known about the stimulation of avian cells by *Campylobacter*, but studies have been carried out with *Salmonella* infections. Kaiser et al. (23) used chick kidney cells (CKCs) as a model for bacterial interaction with avian epithelial cells. HD11 cells (7) can be used as a model for interactions with avian macrophages. Colonization of the chicken by *Campylobacter* generates an antibody response as indicated by the production of both secretory and serum antibodies (8, 32). Therefore, it could be expected that this is driven by an innate reaction. In this paper, we aimed to investigate whether *Campylobacter* could induce proinflammatory cytokines in HD11 cells and CKCs following in vitro invasion.

MATERIALS AND METHODS

Bacteria. *C. jejuni* 11168H and NCTC11168 *cdtB* have been described previously (20, 24, 34, 35). NCTC11168 *cdtB* has an insertion mutation in the *cdtB* gene and lacks CDT-dependent cytotoxicity (35). *C. jejuni* G1 was isolated from a patient who went on to develop Guillain-Barré syndrome (30). All strains were cultured for 2 days in Mueller-Hinton broth, from which they were diluted 1/50 into fresh prewarmed Mueller-Hinton medium and grown for 12 h prior to experimentation. The optical density was measured at 600 nm, and then the bacteria were centrifuged and resuspended in prewarmed phosphate-buffered saline (PBS) to the desired cell density for inoculation of eukaryotic cell cultures at a multiplicity of infection (MOI) of 100 bacteria per cell. All cultures were grown at 37°C in a modified gas atmosphere of 10% CO_2, 5% O_2, and 85% N_2. Heat-killed bacteria were prepared in the same way, but after suspension in PBS they were heated to 70°C for 20 min. All heat-killed cultures were assessed for nonviability by plating on sheep blood agar plates.

Production of recombinant chicken IFN-γ by COS-7 cells. Recombinant chicken gamma interferon (IFN-γ) was produced in COS-7 cells as described previously (28). Briefly, 5×10^5 COS-7 cells/ml were transfected with 37.5 μg of DNA (pCI-neo-chIFN-γ) per ml by using a DEAE-dextran-based method. COS-7 supernatant containing recombinant chicken IFN-γ was harvested at 72 h after transfection, and bioactivity was confirmed by titration in a macrophage activation assay, using HD11 cells (28). Recombinant chicken IFN-γ (ex-COS) was used at a dilution (1/200) that induced a half-maximal NO response in HD11 cells.

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TABLE 1. Primers and probes

Target	Probe or primer[a]	Sequence[b]	GenBank accession no.
28S	Probe	5′-(FAM)-AGGACCGCTACGGACCTCCACCA-(TAMRA)-3′	X59733
	F	5′-GGCGAAGCCAGAGGAAACT-3′	
	R	5′-GACGACCGATTTGCACGTC-3′	
IL-1β	Probe	5′-(FAM)-CCACACTGCAGCTGGAGGAAGCC-(TAMRA)-3′	AJ245728
	F	5′-GCTCTACATGTCGTGTGTGATGAG-3′	
	R	5′-TGTCGATGTCCCGCATGA-3′	
IL-6	Probe	5′-(FAM)-AGGAGAAATGCCTGACGAAGCTCTCCA-(TAMRA)-3′	AJ250838
	F	5′-GCTCGCCGGCTTCGA-3′	
	R	5′-GGTAGGCTGAAAGGCGAACAG-3′	
IL-8	Probe	5′-(FAM)-GCCCTCCTCCTGGTTTCAG-(TAMRA)-3′	AJ009800
	F	5′-TGGCACCGCAGCTCATT-3′	
	R	5′-TCTTTACCAGCGTCCTACCTTGCGACA-3′	
K60	Probe	5′-(FAM)-TGGCTCTTCTCCTGATCTCAATG-(TAMRA)-3′	AF277660
	F	5′-GCACTGGCATCGGAGTTCA-3′	
	R	5′-TCGCTGAACGTGCTTGAGCCATACCTT-3′	
IL-10	Probe	5′-(FAM)-CGACGATGCGGCGCTGTCA-(TAMRA)-3′	AJ621614
	F	5′-CATGCTGCTGGGCCTGAA-3′	
	R	5′-CGTCTCCTTGATCTGCTTGATG-3′	
iNOS	Probe	5′-(FAM)-TCCACAGACATACAGATGCCCTTCCTCTTT-(TAMRA)-3′	U46504
	F	5′-TTGGAAACCAAAGTGTGTAATATCTTG-3′	
	R	5′-CCCTGGCCATGCGTACAT-3′	

[a] F, forward primer; R, reverse primer.
[b] FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

Cells and culture conditions. HD11 cells (7) were cultured in RPMI 1640 medium containing 20 mM L-glutamine (Life Technologies), 2.5% newborn calf serum, 2.5% chicken serum, and 10% tryptose phosphate broth. Cells were seeded at 4×10^6 cells/ml (1 ml per well) in 24-well tissue culture plates, and cells were incubated at 42°C for 2 days prior to use. Cells were washed three times in PBS at 37°C, and fresh antibiotic-free medium was added. All infections were carried out by inoculating bacteria as suspensions in PBS at an MOI of 100:1, unless stated otherwise. Controls consisted of mock infections using PBS alone or positive controls of *Escherichia coli* O55:B5 lipopolysaccharide (LPS) (Sigma, Poole, United Kingdom) at a final concentration of 5 μg/ml unless stated otherwise. Primary CKCs were prepared from the kidneys of 1- to 2-week-old Rhode Island Red chicks as previously described (5). CKCs were seeded in 24-well plates at 1.2×10^6 cells/ml (1 ml per well) in Dulbecco's modified Eagle's medium supplemented with 12.5% newborn calf serum, 10% tryptose phosphate broth, and 1% HEPES and incubated at 37°C with 5% CO_2 prior to use. Human epithelial INT407 cells were cultured in RPMI 1640 medium containing 20% newborn calf serum and 10% tryptose phosphate broth. Cells were seeded at 1.2×10^6 cells/ml and cultured at 37°C for 2 days prior to use.

Intracellular bacterial counts. The number of intracellular bacteria per eukaryotic cell culture was assessed by using a gentamicin protection assay. At 1 h postinfection, the culture supernatant was supplemented with medium containing gentamicin to give a final concentration of 100 μg/ml and incubated at 37°C and 5% CO_2. The concentration of 100 μg/ml was previously found to be sufficient to kill 100% of noninternalized *Campylobacter* organisms by 1 h under equivalent conditions (data not shown). At set time points the cell medium was removed, and the cells were washed three times in warm PBS. The cell monolayer was lysed in cold 0.5% (vol/vol) Triton X-100. The viable bacterial counts were determined by plating serial dilutions of the lysate on sheep blood agar plates and are expressed as CFU per milliliter, where the volume of a well was 1 ml.

Quantitative RT-PCR. RNA expression was determined by quantitative reverse transcription-PCR (RT-PCR) with the ABI PRISM 7700 sequence detection system (Perkin-Elmer Applied Biosystems, Boston, Mass.) as described previously (15, 23, 27, 44). Primers and probes for 28S rRNA, IL-1β, IL-6, IL-8, K60, and IL-10 have been described previously (Table 1) (23, 36, 44). The sequences for the inducible nitric oxide synthase (iNOS) primer and probe set were kindly provided by Bas Baaten (personal communication). RT-PCR was performed with the RT qRT-PCR Mastermix (Eurogentec, Seraing, Belgium). Amplification and detection of specific products were performed with the ABI PRISM 7700 with the following cycle profile: one cycle of 50°C for 2 min, 96°C for 5 min, 60°C for 30 min, and 95°C for 5 min and 40 cycles of 94°C for 20 s and 59°C for 1 min. Each RT-PCR experiment contained three no-template controls, test samples, and a standard $\log_{10}$ dilution series. Each experiment was performed in triplicate with replicates performed on different days. Regression analysis of the mean values from six replicate RT-PCRs for the $\log_{10}$-diluted RNA was used to generate standard curves.

Data were calculated as fold changes compared to the mock-infected samples. All of the data shown are from three independent experiments and represent the averages and standard deviations (SD) of the fold changes between experiments. Statistical analysis was carried by analysis of variance between experiments.

Measurement of NO by the Griess assay. Nitric oxide production was measured by assaying cell culture supernatant fluid for the presence of nitrite, using the standard Griess assay (9, 40). The absorbance was read at 550 nm, using an Anthos Lab Systems microtiter plate reader (Labtech, Ringmer, United Kingdom). Serial dilutions of sodium nitrite (Sigma) were used to determine a standard curve.

RESULTS

Intracellular survival of *C. jejuni* in cells. *C. jejuni* strains 11168H, NCTC11168 *cdtB*, and G1 were taken up by (invaded) both HD11 and CKC cells to the same extent (Table 2). Both human INT407 and CKC cells were invaded to the same extent by *C. jejuni* 11168H, and the bacteria did not persist (Fig. 1), being undetectable after a period of 24 h (data not shown). To determine whether priming of macrophages would affect invasion and survival, *C. jejuni* 11168H infection was also assessed with HD11 cells treated with or without IFN-γ (Fig. 2) ($P < 0.002$). We monitored intracellular survival of *C. jejuni* 11168H

TABLE 2. Invasion counts for bacteria

C. jejuni strain	Intracellular bacterial count[a] ($\log_{10}$ CFU/ml) in:	
	CKCs	HD11 cells
11168H	4.46 ± 0.02	5.66 ± 0.08
11168 *cdtB*	4.51 ± 0.01	5.27 ± 0.07
G1	4.63 ± 0.06	5.89 ± 0.06

[a] Intracellular bacterial counts at 2 h postinfection. Data represent the averages from three independent experiments ± SD.

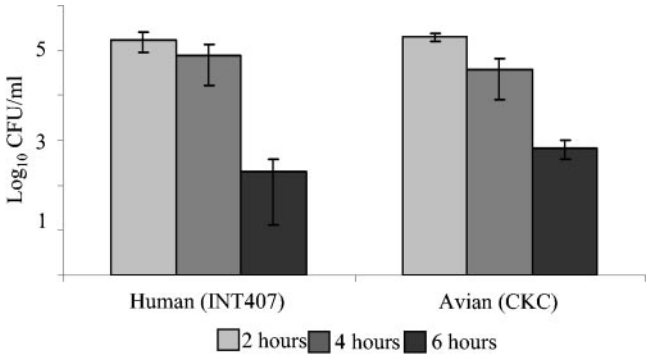

FIG. 1. Invasion of INT407 cells and CKCs by *Campylobacter*. Cells seeded at equivalent levels (1.2×10^6 cells/ml) were infected at an MOI of 100:1 with *C. jejuni* 11168H. Internalized bacteria were assessed by gentamicin protection at 2, 4, and 6 h postinfection. Data are representative of those from three independent experiments. Values shown are averages and SD from three independent samples.

in HD11 cells, and killing was more rapid in the presence of IFN-γ (Fig. 2) ($P < 0.002$). Intracellular *Campylobacter* was undetectable after 24 h postinfection of HD11 cells with and without IFN-γ (data not shown).

Induction of iNOS and production of NO by CKCs and HD11 cells. The fold change in iNOS transcripts from CKCs and HD11 cells was measured at 4 h after *Campylobacter* infection (MOI of 100:1). iNOS was induced by both CKCs and HD11 cells (Fig. 3). The production of NO from the infected HD11 cells and CKCs was determined by using the Griess assay. Nitric oxide was produced by both infected CKCs (Fig. 4) and HD11 cells (Fig. 5). The production of NO from infected HD11 cells pretreated with or without chicken IFN-γ

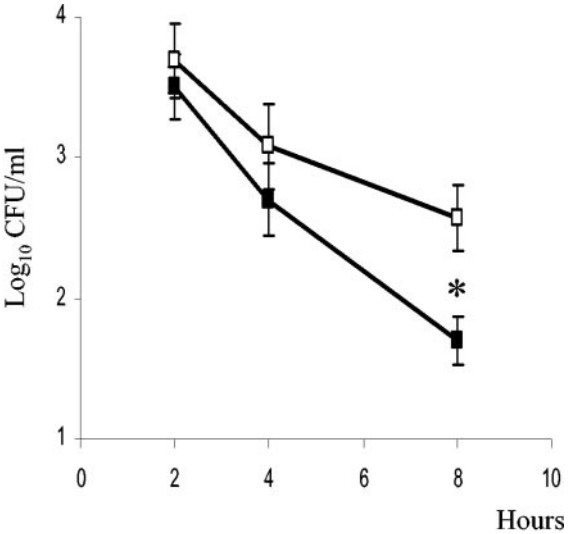

FIG. 2. Survival of *C. jejuni* 11168H within HD11 cells. At 1 h prior to infection, cells were either treated with IFN-γ (closed symbols) or not treated (open symbols). Cells were infected at an MOI of 100:1. Internalized bacteria were assessed by gentamicin protection assay, and intracellular bacteria were counted at 2, 4, and 8 h postinfection. Data shown are the averages and SD from three replicate samples. *, significant difference ($P < 0.002$) between IFN-γ-treated and nontreated cells.

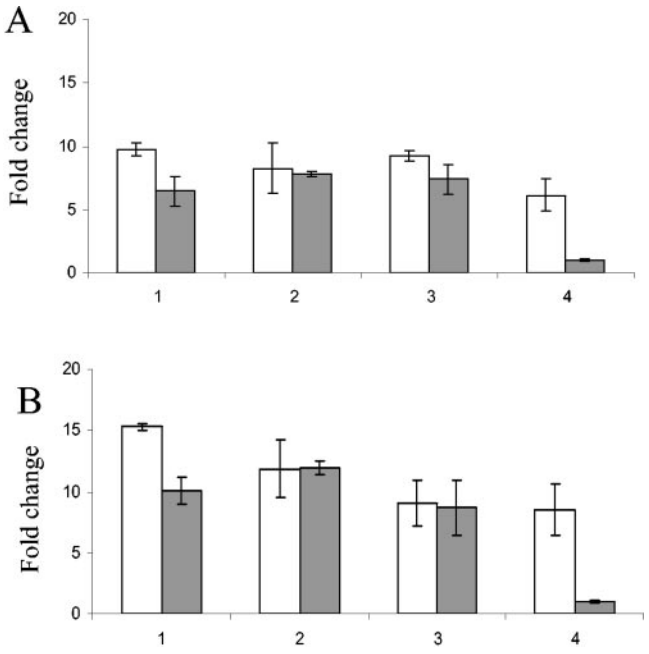

FIG. 3. Quantification of iNOS transcripts from CKCs (A) and HD11 cells (B) at 4 h postinfection. Open bars, live bacteria; closed bars, heat-killed bacteria. Bars: 1, *C. jejuni* 11168H; 2, *C. jejuni* G1; 3, *C. jejuni* 11168 *cdtB*; 4, LPS control *E. coli* O55:B55 (5 μg/ml) (open bar) and mock infection (closed bar). RNA was isolated at 4 h postinfection. Data are representative of those from three independent experiments. Mean values and SD from three replicate samples are shown.

was also assessed. As expected, priming of the HD11 cells with IFN-γ increased their response to *Campylobacter* infection. There was a significant increase in NO production during infection in the presence of IFN-γ compared to infection without IFN-γ (Fig. 5). At 24 h, maximal NO production had been reached in both IFN-γ-treated and untreated cells (data not shown). To determine whether stimulation of the cells required active invasion by the bacteria, the production of NO

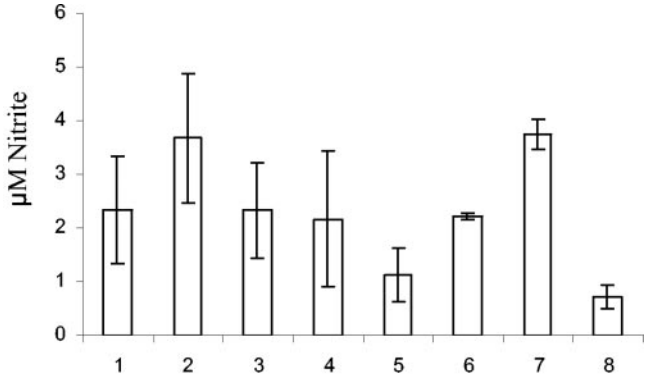

FIG. 4. Production of NO by CKCs at 4 h postinfection. Bars: 1, live *C. jejuni* 11168H; 2, live *C. jejuni* G1; 3, live *C. jejuni cdtB*; 4, heat-killed *C. jejuni* 11168H; 5, heat-killed *C. jejuni* G1; 6, heat-killed *C. jejuni cdtB*; 7, LPS control *E. coli* O55:B55 (5 μg/ml); 8, mock infection. Bacterial infections were carried out at an MOI of 100:1. Data shown are averages and SD from three independent experiments.

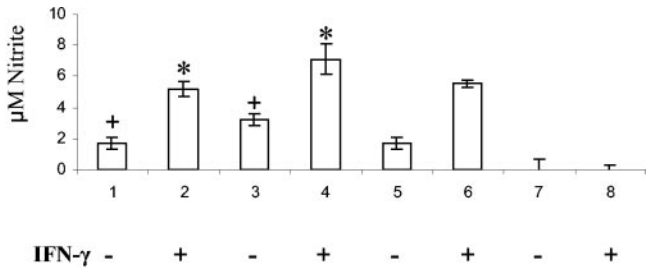

FIG. 5. Production of NO by HD11 cells at 6 h postinfection with and without IFN-γ. Bars: 1 and 2, live *C. jejuni* 11168H; 3 and 4, heat-killed *C. jejuni* 11168H; 5 and 6, *E. coli* O55:B55 LPS at 1 μg/ml (positive control); 7 and 8, mock-infected controls with no IFN-γ and IFN-γ alone, respectively. Cells for bars 2, 4, 6, and 8 were pretreated with IFN-γ for 1 h prior to infection. Bacterial infections were carried out at a MOI of 100:1. The data shown are representative of those from two independent experiments and are the averages and SD from four replicate samples. *, significant difference ($P < 0.002$) between IFN-γ-treated and nontreated cells; +, no significant difference ($P > 0.05$) between infection with heat-killed and live cells.

was also assessed after infection with heat-killed bacteria at an MOI equivalent to 100:1. No significant difference in NO production was observed between cells infected with either heat-killed or live bacteria (Fig. 4 and 5) ($P > 0.05$).

Production of cytokines by CKCs. The levels of IL-1β, IL-6, K60, and IL-8 transcripts in CKCs were measured at 4 h postinfection with *C. jejuni* strains 11168H, G1, and NCTC11168 *cdtB* and heat-killed bacteria of the same strains (Fig. 6). Mock-infected cells and those infected with *E. coli* LPS at 5 μg/ml were used as controls throughout. As can be seen, all three bacterial strains induced the production of IL-1β, IL-6, K60, and IL-8 (Fig. 6). Heat-killed bacteria produced equivalent stimulation of the proinflammatory signals (Fig. 6). IFN-γ production was measured and, as expected, was not induced (data not shown). There was no significant difference in the level of IL-10 between infected and uninfected cells ($P > 0.05$) (data not shown).

Production of cytokines by HD11 cells. The levels of IL-1β, IL-6, K60, and IL-8 transcripts in HD11 cells were measured at 4 h postinfection with *C. jejuni* strains 11168H, G1, and NCTC11168 *cdtB* and heat-killed bacteria of the same strains (Fig. 7). Mock-infected cells and cells infected with *E. coli* LPS at 5 μg/ml were used as controls throughout. All three bacterial strains, whether live or heat killed, induced the production of IL-1β, IL-6, K60, and IL-8 in HD11 cells (Fig. 7). IFN-γ was measured and, as expected, was not produced (data not shown). There was no significant difference in the level of IL-10 between infected and uninfected cells ($P > 0.05$) (data not shown).

DISCUSSION

Previous studies of cytokine stimulation by *C. jejuni* have concentrated on the interaction of the bacterium with human cell lines (2, 16, 17, 19, 20, 31). In this study we describe the interaction of *C. jejuni* with avian cells. We used CKCs as a model for epithelial cells and an avian macrophage cell line, HD11, to study the potential effects on tissue macrophages if *C. jejuni* penetrated the epithelial layer. The results clearly show that *C. jejuni* can invade CKCs to a level equivalent to that seen with human enterocytes and that the rate of killing is similar.

To determine whether there was an inflammatory response to *Campylobacter*, we measured the induction of iNOS and the production of NO from both CKCs and HD11 cells after infection with *Campylobacter.* Both CKC and HD11 cells showed

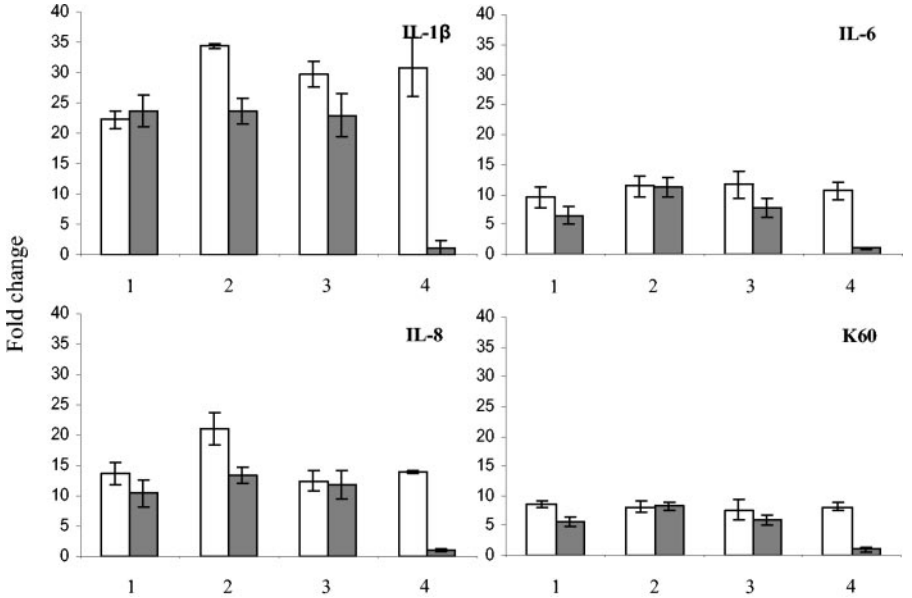

FIG. 6. Quantification of cytokine and chemokine transcripts from CKCs at 4 h postinfection. Open bars, live bacteria; closed bars, heat-killed bacteria. Bars: 1, *C. jejuni* 11168H; 2, *C. jejuni* G1; 3, *C. jejuni* 11168 *cdtB*; 4, LPS control *E. coli* O55:B55 (5 μg/ml) (open bar) and mock infection (closed bar). RNA was isolated at 4 h postinfection. Data are representative of those from three independent experiments. Mean values and SD from three replicate samples are shown.

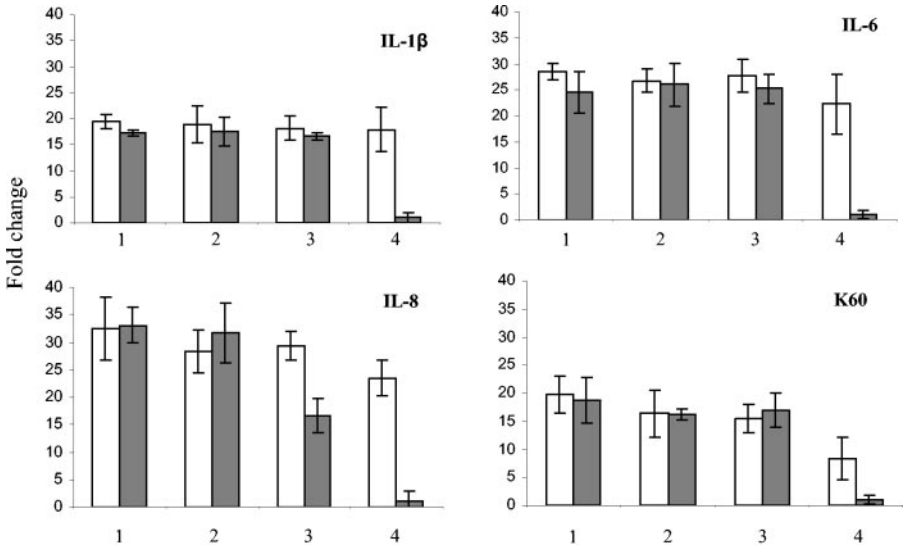

FIG. 7. Quantification of cytokine and chemokine transcripts from HD11 cells at 4 h postinfection. Open bars, live bacteria; closed bars, heat-killed bacteria. Bars: 1, *C. jejuni* 11168H; 2, *C. jejuni* G1; 3, *C. jejuni* 11168 *cdtB*; 4 LPS control *E. coli* O55:B55 (5 μg/ml) (open bar) and mock infection (closed bar). RNA was isolated at 4 h postinfection. Data are representative of those from three independent experiments. Mean values and SD from three replicate samples are shown.

a measurable increased in iNOS transcription at 4 h postinfection and the production of NO at 6 h postinfection. Analysis of primary transcripts from infected CKCs shows that *Campylobacter* is capable of inducing the expression of the proinflammatory cytokines IL-1β and IL-6 and the proinflammatory chemokines K60 and IL-8. Thus, it would appear that CKCs, a well established model for chicken epithelial cells, are capable of being stimulated by *Campylobacter* in a similar manner to that for human epithelial cells. Work with *Salmonella*, both in vitro invasion of CKCs and in vivo infections (23, 44), strongly suggests these data can be extrapolated to responses that may occur in the intestine.

In humans, bacterial infection of epithelial cells stimulates the production of chemokines which are involved in the attraction of leukocytes (3, 21). *Campylobacter* can traverse human epithelial cells and therefore interact with leukocytes to further stimulate the immune response. It has been suggested that the stimulation of leukocytes is significant and could contribute to the pathology of disease (20). To investigate whether *Campylobacter* can stimulate avian leukocytes in a similar manner, we measured the production of cytokines and chemokines in the HD11 macrophage cell line (7). The different *Campylobacter* strains are taken up to the same level by HD11 cells and are rapidly killed, with live bacteria being undetectable by gentamicin protection assay at 24 h postinfection. This result is equivalent to that seen previously with peritoneal macrophages (33). IFN-γ increases the sensitivity of HD11 cells to bacterial stimulation, and bacterial killing was enhanced by the addition of IFN-γ prior to infection. *Campylobacter* clearly stimulates the HD11 cells in a proinflammatory manner, inducing iNOS, the production of NO, the proinflammatory cytokines IL-1β and IL-6, and the proinflammatory chemokines K60 and IL-8.

The production of cytokines by enterocytes has been associated with the active invasion of bacteria (21). To determine whether the stimulation that we observe requires active invasion, we used heat-killed bacteria at an MOI equivalent to that of live infection. The up-regulation of cytokine mRNA levels and NO was not significantly different between live and heat-killed bacterial infection ($P > 0.05$). This stimulation by the heat-killed bacteria could be multifaceted. Although we have ruled out a requirement for CDT by using the *cdtB* knockout strain, *jlpA* (19) may still be active, and the heat-killed bacteria will contain many potential innate ligands, such as lipooligosaccharides and flagellin, any of which may be involved in stimulation.

This paper clearly shows that *Campylobacter* can stimulate inflammatory responses from avian cells. The induction of IL-8 in CKCs suggests that there should be an attraction of peripheral blood mononuclear cells (4). To our knowledge, no in vivo data to indicate any leukocyte migration in response to *Campylobacter* colonization in the chicken have been published. The biological roles of the avian IL-8 homologues measured in this study are undefined (22), although there is circumstantial evidence for a true functional homologue of IL-8 (26). Mammalian IL-8 is involved in the attraction of neutrophils, while the avian IL-8 homologue attracts heterophils and monocytes.

Reports of invasion and pathology by *Campylobacter* in the chicken are limited to day-of-hatch chicks (37). Day-of-hatch birds have no established gut flora and possess an immature mucosal immune system, and analysis of published infection data suggests that the chicken develops resistance to *Campylobacter* invasion with age. The only published studies on the avian immune response to *Campylobacter* describe adaptive immunity (8, 32). To induce the adaptive response, an innate response is first required.

Even though *Campylobacter* colonizes the intestinal tracts of chickens to a high level (10^9 CFU/g), very little invasion is observed and the bacteria do not elicit inflammatory disease, through either a lack of contact with the relevant receptors or induction of tolerance. If *Campylobacter* does not invade in

sufficient numbers to cause severe inflammation, it may still cause local inflammation, which could be sufficient to control the bacteria and also drive the adaptive immune response. This local response may be self-limiting and so not lead to severe pathology. This question will be addressed in further studies.

While iNOS, IL-1β, IL-6, and IL-8 are major markers of inflammatory disease, other factors, such as IL-10 and suppressors of cytokine signaling (1, 29), which are key in regulation of inflammation, may be modulated in different manners in humans and chickens and may play a crucial role in the development of disease. We measured the levels of IL-10 transcripts in infected and uninfected CKCs and HD11 cells and found no significant differences between samples. However, this does not rule out IL-10 production in vivo by other cell types. Recent evidence from *Salmonella enterica* serovar Infantis infections of gnotobitic pigs shows that polymorphonuclear leukocyte influx can be uncoupled from the disease process and preempt a virulent infection, thus protecting against challenge with virulent *Salmonella* (13). Obviously, therefore, factors other than the signals that attract polymorphonuclear leukocytes are required for inflammatory disease.

Evidence exists for a role of other chemokines in human infection (3), but nothing is known about their production in chickens during *Campylobacter* infection. Given the importance of chemokines in *Salmonella* infections (46), key differences in their production may dictate the outcome of the balance between colonization and disease.

Finally, we cannot rule out that there may be fundamental differences between host physiologies that preclude disease. There may be a crucial, as yet undefined, difference between human and avian hosts. The ability of the bacteria to induce inflammatory cytokines may be sufficient to prevent disease in poultry, and either the physical interaction of *Campylobacter* with the infected host or the regulation of those responses may be crucial to the outcome of infection.

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