INFECTION AND IMMUNITY, May 2006, p. 2697–2705 Vol. 74, No. 5
0019-9567/06/$08.00+0 doi:10.1128/IAI.74.5.2697–2705.2006

Campylobacter jejuni Induces Maturation and Cytokine Production in Human Dendritic Cells

Lan Hu, Mechelle D. Bray, Manuel Osorio, and Dennis J. Kopecko*

Laboratory of Enteric and Sexually Transmitted Diseases, Center for Biologics Evaluation and Research, Food and Drug Administration, 29 Lincoln Drive, NIH Campus, Building 29/420, HFM440, Bethesda, Maryland 20892

Received 28 October 2005/Returned for modification 27 November 2005/Accepted 1 February 2006

***Campylobacter jejuni* is a leading bacterial cause of human diarrheal disease in both developed and developing nations. Colonic mucosal invasion and the resulting host inflammatory responses are thought to be the key contributing factors to the dysenteric form of this disease. Dendritic cells (DCs) play an important role in both the innate and adaptive immune responses to microbial infection. In this study, the interaction between human monocyte-derived dendritic cells and *C. jejuni* was studied. We found that *C. jejuni* was readily internalized by DCs over a 2-h period. However, after a prolonged infection period (24 or 48 h) with *C. jejuni*, only a few viable bacteria remained intracellularly. Minimal cytotoxicity of *C. jejuni* to dendritic cells was observed. *C. jejuni* induced the maturation of dendritic cells over 24 h, as indicated by up-regulation of cell surface marker proteins CD40, CD80, and CD86. In addition, *Campylobacter*-infected DCs triggered activation of NF-κB and significantly stimulated production of interleukin-1β (IL-1β), IL-6, IL-8, IL-10, IL-12, gamma interferon, and tumor necrosis factor alpha (TNF-α) compared to uninfected DCs. Active bacterial invasion of DCs was not necessary for the induction of these cytokines, as heat-killed *C. jejuni* stimulated similar levels of cytokine production as live bacteria. Purified lipooligosaccharide of *C. jejuni* appears to be the major stimulant for the increased production of cytokines by DCs. Taken together, these data indicate that during infection, *Campylobacter* triggers an innate inflammatory response through increased production of IL-1β, IL-6, IL-8, and TNF-α and initiates a Th1-polarized adaptive immune response as predicted from the high level of production of IL-12.**

Campylobacter jejuni is a spiral, gram-negative, microaerophilic bacterium. This pathogen is a leading bacterial cause of human diarrheal disease throughout the world. In the United States, about 2 million cases of diarrhea per year are caused by *C. jejuni*. Illness caused by *C. jejuni* is characterized by fever, headache, abdominal cramping, diarrhea, and the presence of erythrocytes and leukocytes in the stool (4, 5). Two to 3 weeks postinfection, *C. jejuni* is sometimes associated with the extraintestinal manifestations of Guillain-Barré syndrome or reactive arthritis (43). The pathogenic mechanisms of *C. jejuni* are not yet well understood. In developing countries, *C. jejuni* triggers primarily a noninflammatory, watery diarrhea. However, in developed countries infection via bacterial invasion of the colonic mucosa is thought to lead to intestinal inflammation and microulceration of the colonic mucosa. As is the case with shigellosis, the acute host inflammatory responses are thought to contribute heavily to disease pathogenesis (reviewed in reference 22).

Dendritic cells (DCs) play important roles in both the innate and adaptive immune responses to microbial pathogens. They are the major antigen-presenting cells (APCs) and are widely distributed in tissues, including the intestinal mucosa (reviewed in references 26, 32, 53). Dendritic cells regulate the type of T-cell-mediated immune response to an offending agent and are also a source of proinflammatory cytokines. They are the only cells that are able to initiate proliferation in naïve T cells, thereby inducing the primary immune response and permitting the establishment of immunological memory.

Immature DCs can capture antigens by phagocytosis, macropinocytosis, and endocytosis (17, 37, 38). Exposure of DCs to inflammatory stimuli converts these cells from antigen-capturing immature DCs to antigen-presenting mature DCs. This process is accompanied by up-regulation of major histocompatibility complex (MHC) class I and II molecules, costimulatory receptors, and adhesion molecules (2, 7, 11). Once a DC has captured an antigen, its ability to capture additional antigens rapidly declines. The process of differentiation from an immature DC into a mature professional APC can be induced by whole bacteria, their components, or cytokines as well as other inflammatory stimuli and infectious agents. Upon activation, DCs migrate from the inflammatory site into the lymphoid tissues, such as the lymph nodes and spleen, down-regulate their phagocytic ability, and up-regulate their antigen-presenting capacity (2).

Numerous studies have shown that DCs play an important role in the host-pathogen interaction during infection with enteric pathogenic bacteria. For example, DCs that phagocytose *Salmonella enterica* serovar Typhimurium can process and present bacterial antigens and produce cytokines that are critical to the immune response (25, 56). *Helicobacter pylori* also induces maturation and cytokine release from human DCs (24). *Shigella flexneri* infection of DCs leads to up-regulation of interleukin-1β (IL-1β) and IL-18 and rapid DC death (8). In contrast, *Yersinia enterocolitica* is able to invade DCs and does not induce necrosis or apoptosis but impairs DC function (40).

* Corresponding author. Mailing address: Laboratory of Enteric and Sexually Transmitted Diseases, Center for Biologics Evaluation and Research, Food and Drug Administration, 29 Lincoln Drive, NIH Campus, Bldg. 29/420, HFM440, Bethesda, MD 20892. Phone: (301) 496-1893. Fax: (301) 402-8701. E-mail: kopecko@cber.fda.gov.

During infection, *Campylobacter* can invade and traverse the epithelial barrier and comes into contact with numerous leukocytes (49, 50). In addition, DCs are capable of traversing the tight junctions of the intestinal mucosa, which enables them to interact directly with bacteria on the mucosal surface (35, 39). Although the infection of macrophages by *C. jejuni* has been studied previously (14, 19, 20, 42, 44, 51), *Campylobacter*'s interaction with DCs has not been reported to date. Understanding the interaction between DCs and *C. jejuni* will provide insight into the role of DCs in stimulating *Campylobacter*-induced inflammatory pathology and in controlling the infection. The goal of this work was to study the interaction between human DCs and *C. jejuni* with respect to DC activation and induction of a number of cytokines known to be involved in both the innate and adaptive immune responses.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *Campylobacter jejuni* strain 81-176, obtained following a disease outbreak in Minnesota, has been shown to cause colitis in two human challenge studies (4, 23; D. Tribble et al., unpublished data). *C. jejuni* 81-176 and its mutant RY213 (a *cheY*$^+$ diploid) that is noninvasive (55), as well as the genome-sequenced *C. jejuni* NCTC 11168 (33), were grown in Mueller-Hinton (M-H) biphasic broth or on M-H agar (Difco) at 37°C under a *Campylobacter* gas atmosphere of 10% carbon dioxide, 5% oxygen, and 85% nitrogen (16).

Preparation of human monocyte-derived dendritic cells. Mononuclear cells were obtained by apheresis of normal volunteer donors as performed by the Blood Services Section of the Department of Transfusion Medicine at the National Institutes of Health Warren G. Magnuson Clinical Center (Bethesda, MD). The mononuclear cells were further enriched for monocytes by centrifugal elutriation as performed by the Cell Processing Section of the Department of Transfusion Medicine. Similar to the work of Pickering et al. (34), the elutriated monocytes were then cultured (1×10^6/well) in six-well tissue culture plates with 3 ml/well of RPMI 1640 medium (Mediatech, Inc., Herndon, VA) containing 5% human AB serum (Nabi, Miami, FL), 800 U/ml granulocyte-macrophage colony-stimulating factor (GM-CSF; Peprotech, Rocky Hill, NJ), and 500 U/ml IL-4 (Peprotech). The nonadherent and loosely adherent cells were harvested after incubating the plates for 6 days at 37°C in 5% CO_2. The cells were centrifuged for 10 min at 1,200 rpm in a Beckman GS-6KR centrifuge and then plated in 96- (50,000 cells/well) or 6-well plates (10^6 cells/well) containing fresh RPMI (Mediatech) with 5% human AB serum (Nabi) and 800 U/ml GM-CSF (Peprotech). This resulted in the generation of immature monocyte-derived dendritic cells that were positive for CD11c but negative for CD14 (data not shown).

Infection of DCs. *C. jejuni* was added to the cultured DCs at different multiplicities of infection (MOIs). Bacterium-DC interactions were initiated by centrifugation at 1,000 rpm in a Beckman GS-6KR centrifuge for 5 min, followed by incubation at 37°C in 5% CO_2. For assessment of the number of intracellular bacteria, 2×10^5 infected DCs/well in 24-well plates were cultured for 2, 4, 24, and 48 h. After the 4-h time point, gentamicin (20 μg/ml) was added to inhibit the growth of extracellular bacteria (19). At the specified time points (2, 4, 24, and 48 h), cells were washed three times with RPMI 1640, followed by incubation of DCs for 2 h in fresh medium including 100 μg/ml gentamicin to kill any remaining extracellular bacteria. The infected monolayers were then washed three times to remove the gentamicin. The DCs were lysed with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 15 min. Following serial dilutions in PBS, the viable internalized bacteria were enumerated by plate count on M-H agar. For cytokine measurements, the infected culture supernatants in 96-well plates were harvested, centrifuged, and frozen at −80°C prior to analysis (12, 34).

In control studies, 100 μg/ml gentamicin was found to kill all extracellular bacteria within 2 h. The MIC of gentamicin at which 50% of the *C. jejuni* 81-176 organisms were inhibited was ~6.25 μg/ml for *C. jejuni* 81-176. At 20 μg/ml of gentamicin, we found that extracellular *C. jejuni* could not multiply in cell culture medium. We used 20 μg/ml gentamicin to block any extracellular growth of released *C. jejuni* over longer periods (i.e., 24 to 48 h), because higher antibiotic concentrations over long periods can leak into the host cells and cause death of some intracellular bacteria.

Cell cytotoxicity assay. The CytoTox 96 assay (Promega, Madison, WI) was used to determine host cell cytotoxicity over time induced by bacterial preparations that were added to the DCs. This assay qualitatively measures supernatant lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis. The DC number was evaluated via the assay instructions and determined to be optimal at 50,000 cells/well. The ratios of effector cells to bacteria used to assess host cell cytotoxicity were 1:1, 1:10, 1:20, and 1:100. Supernatants were collected following centrifugation at 4, 24, and 48 h postinfection with *C. jejuni* 81-176. Maximum LDH release was determined by measuring the amount of LDH release from uninfected DCs that were treated with lysis buffer. The percentage of cytotoxicity was calculated according to the following formula (OD is an abbreviation for optical density): $[(OD_{sample}) - (OD_{medium}/OD_{max\ LDH\ release}) - OD_{adjusted\ medium}] \times 100$.

Cytokine measurement of DC supernatants by Luminex assay. Immature DCs were incubated in 96-well plates (5×10^4 DCs/well) for 4, 24, and 48 h with live *C. jejuni* 81-176, heat-killed (i.e., 70°C for 30 min) *C. jejuni* 81-176, lipooligosaccharide (LOS) of *C. jejuni* 81-176 (extracted by using the hot phenol-water technique [52]), or hot phenol-water-extracted lipopolysaccharide (LPS) of *Escherichia coli* (Sigma, St. Louis, MO). PBS (Invitrogen Corporation)-incubated DCs served as unstimulated controls. Cytokine assays were performed by using human cytokine 10-plex and IL-12p70 antibody bead kits (Biosource International, Camarillo, CA) and a Luminex 100 analyzer (Luminex Corporation, Austin, TX) for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and GM-CSF. Cytokine data analysis was performed using the MasterPlex QT quantitation software (MiraiBio, Alameda, CA).

Flow cytometry. Flow cytometric analysis was performed in order to examine the cell surface maturation markers CD40, CD80, and CD86 in *Campylobacter*-infected DCs. Dendritic cells were infected as described above. Maturation of DCs was determined at 24 and 48 h after infection with *C. jejuni* (MOI, 10) by checking the expression of the surface maturation markers by flow cytometry. Cell samples (5×10^5 cells) were incubated (20 min, 4°C) with 4% human AB serum (Nabi) in PBS (Invitrogen Corporation) to block nonspecific binding sites and Fc receptors. The cells were washed via centrifugation (1,200 rpm; 10 min) with fluorescence-activated cell sorter buffer containing 0.2% bovine serum albumin (Sigma) and 0.1% sodium azide (Sigma) in PBS (Invitrogen Corporation) and then incubated (45 min, 4°C) with anti-CD40 (5C3), anti-CD 80 (L307.4), or anti-CD86 (2331 [FUN-1]) fluorescein isothiocyanate-labeled antibody (1 μl; Becton Dickinson, San Jose, CA) or the isotype-matched (1 μl of immunoglobulin G1; X40) control antibody (Becton Dickinson). After washing again, the cells were resuspended in 0.5% paraformaldehyde (Sigma) in cold PBS (Invitrogen) and 0.1% sodium azide. The cell-associated immunofluorescence was measured with a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) and analyzed using CellQuest software (B-D Biosciences).

NF-κB transcription factor assay. The TransAM NF-κB kit p65 (Active Motif, Carlsbad, CA) was employed to detect the activation of NF-κB. At 4 h postinfection, the nuclear extracts were prepared as per the manufacturer's instructions. Protein concentrations were determined using a 2′-benzoyloxycinnamaldehyde protein assay reagent kit (Pierce, IL). Nuclear extracts (10 μg/ml) were added into each well of a 96-well plate and incubated with anti-p65, and NF-κB concentrations were determined spectrophotometrically per the kit instructions.

Statistical analysis. Results are presented as the mean ± standard error of the mean from three independently conducted assays. *P* values were calculated with Student's *t* test.

RESULTS

Viable intracellular bacteria in dendritic cells after *C. jejuni* infection. *C. jejuni* was added to DCs at MOIs of 1, 10, 20, and 100. After a 2-, 4-, 24-, or 48-h invasion period, followed by a 2-h gentamicin (100 μg/ml) treatment to kill all extracellular bacteria, the numbers of viable, intracellular *Campylobacter* were quantified by plate count after host cell lysis. At 2 and 4 h postinfection, the uptake was generally dose dependent, and higher doses of bacteria led to greater levels of bacterial internalization by DCs (Fig. 1). The average number of viable intracellular bacteria per DC was ~1.5 bacteria/DC on average at 2 or 4 h at an MOI of 10 and increased about fourfold at an MOI of 100. By 24 or 48 h postinfection, however, the numbers

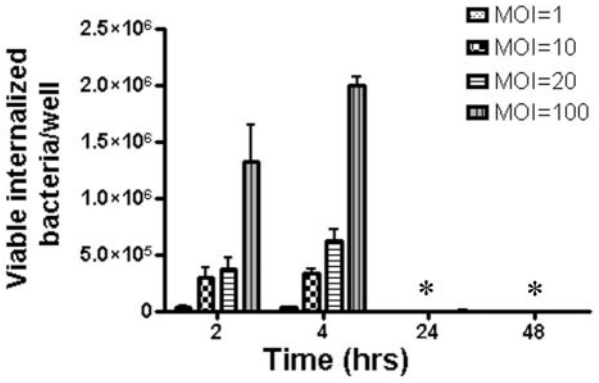

FIG. 1. Measurement of viable intracellular bacteria in dendritic cells after *C. jejuni* infection. *C. jejuni* was added to DCs at MOIs of 1, 10, 20, and 100. After various invasion periods, the cells were washed three times, followed by an incubation of the DCs for 2 h in fresh medium containing 100 μg/ml gentamicin to kill any remaining extracellular bacteria. The data represent the mean ± standard deviation of duplicate wells from three independent assays. *, the number of viable intracellular bacteria had markedly decreased at 24 to 48 h and does not appear on this graph.

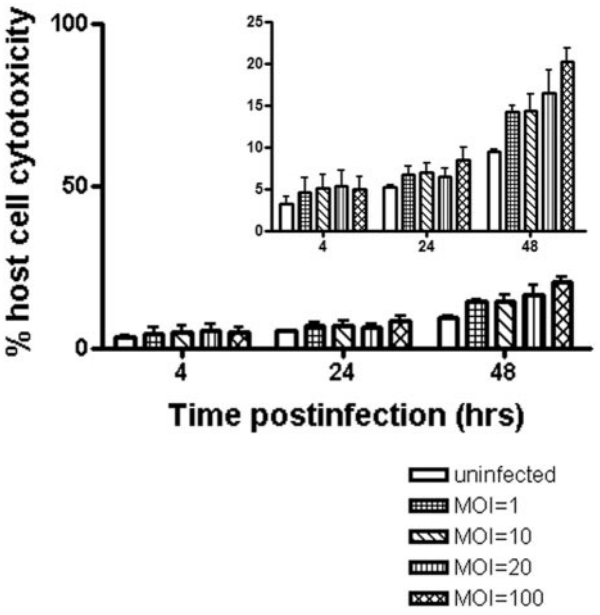

FIG. 2. DC viability after infection with *C. jejuni* 81-176. LDH in the supernatants of infected and uninfected DCs was sampled and measured at 4, 24, and 48 h after infection at various MOIs. The data are presented as means ± standard errors of the means from three separate experiments. The values are expressed as percent host cell cytotoxicity, relative to the uninfected cell control obtained by lysing uninfected DCs. The insert shows the data on an expanded scale for detailed comparison.

of viable intracellular bacteria were dramatically reduced. For example, at an MOI of 100, only ~1% of DCs contained one viable bacterium, with even less observed at 48 h. These data suggest that *C. jejuni* is internalized at early time points, but most intracellular bacteria are killed by 24 and 48 h postinfection. This assumption is predicated on little or no cytotoxicity to DCs during bacterial infection, which was studied next.

DC viability after infection with *C. jejuni*. To assess the viability over time of DCs following infection with *C. jejuni*, LDH release assays were performed using the supernatants of both uninfected and infected cells. No significant differences in DC viability were detected at 4 and 24 h after infection at any bacterial MOI, in comparison to the uninfected controls (Fig. 2). At 48 h postinfection, all infected DCs showed a minimal increase in cytotoxicity over the uninfected control. However, the 9.5% dead cells in the uninfected control at 48 h was only doubled to ~20% death in the infected DCs at the highest MOI. These data suggest that limited host cell cytotoxicity was associated with both long infection times and higher MOIs. However, infection-induced DC death over 48 h was very limited even at the highest MOI.

***C. jejuni* effects on DC maturation.** To study the effects on DC maturation of *Campylobacter* infection, the expression of cell surface costimulatory molecules CD40, CD80, and CD86 was measured with flow cytometry. Compared to uninfected cultures, *C. jejuni*-infected DCs showed increased expression of all three surface molecules at 24 h postinfection, as indicated by increased fluorescence intensity of DCs (Fig. 3). The averaged mean fluorescence intensity measurements for CD40, CD80, and CD86 at 24 h in all three sets of control DCs were 12.52 ± 0.99, 7.01 ± 0.38, and 45.28 ± 10.83, respectively. Infection with *C. jejuni* 81-176 significantly increased these maturation marker averaged mean fluorescence intensity measurements to 21.66 ± 3.84 ($P < 0.05$), 25.99 ± 10.83 ($P < 0.01$), and 96.16 ± 7.35 ($P < 0.005$), respectively. As a positive control, purified *E. coli* LPS induced DC maturation similarly to that observed with live *C. jejuni* (data not shown). These data show that *C. jejuni* infection stimulates an increase in the expression of DC surface costimulatory molecules, which are indicators of DC maturation.

***C. jejuni* infection induces the production of cytokines in human DCs.** The kinetics and profile of cytokine secretion from human DCs during *Campylobacter* infection were analyzed. Cell culture supernatants were collected at 4, 24, and 48 h from uninfected DCs or after infection with strain 81-176 at different MOIs, and cytokine levels were measured in multiplex bead assays. *Campylobacter* infection of DCs induced enhanced production of IL-1β, IL-6, IL-8, IL-10, IL-12, IFN-γ, and TNF-α (Fig. 4) but not IL-2, IL-4, or IL-5 (data not shown).

At 4 h postinfection with *C. jejuni* at an MOI of 10, the titers of IL-6, IL-8, and TNF-α were increased ~29-fold (837.80 ± 83.00 pg/ml), 34-fold (1,073.70 ± 282.90 pg/ml), and 1,959-fold (46,596.30 ± 11,977.78 pg/ml), respectively, compared to the uninfected controls (Fig. 4B, C, and G). The production of IL-6 and IL-8 as well as IL-12 and IFN-γ (Fig. 4E and F) was significantly increased at 24 and 48 h postinfection in comparison to the 4-h time point. TNF-α was detected at high levels at all time points from infected DCs. *C. jejuni* 81-176 induced comparatively lower quantities of IL-1β or IFN-γ than the other cytokines; however, IL-1β and IFN-γ levels were significantly increased at 24 and 48 h postinfection relative to the 4-h time point (Fig. 4A and F). Finally, substantial increases in IL-10 were only observed at the 48-h time point (Fig. 4D). These data show that increases in the *Campylobacter* MOI from 10 to 100 had little impact on overall cytokine production. Cytokine production increased over time, with peak amounts at 24 or 48 h.

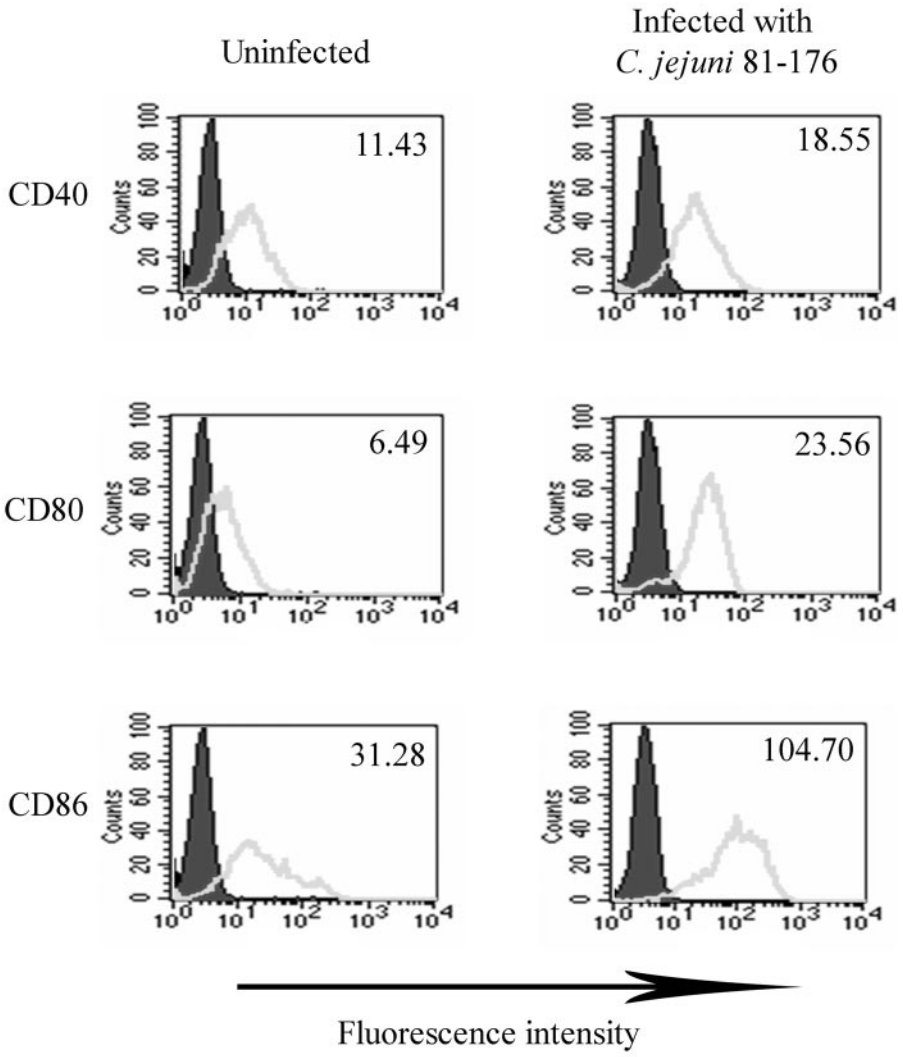

FIG. 3. Surface molecules of DCs as determined by flow cytometry 24 h after exposure to *C. jejuni* 81-176. The DCs were stained with fluorescence-conjugated monoclonal antibodies to human CD40, CD80, and CD86. Histograms depict the level of surface expression, and the value indicated on each histogram is the mean fluorescence intensity of the marker-specific antibody from one representative experiment of three different donors. Black histograms indicate the levels of fluorescence using isotype control antibodies, and the open histograms represent the cell surface molecule expression in infected or uninfected DCs.

Is active bacterial invasion or bacterial viability necessary to induce cytokine production in DCs? Wild-type invasive *C. jejuni* 81-176, the noninvasive RY213 mutant strain of 81-176 (55), NCTC 11168, a minimally invasive *C. jejuni* strain (1), and heat-killed 81-176 were compared for their ability to trigger DC secretion of selected cytokines. *C. jejuni* 81-176 was heat treated at 70°C for 30 min and subsequently shown by plate count to be nonviable. The production levels of IL-1β, IL-6, IL-8, IFN-γ, and TNF-α (Fig. 5A, B, C, F, and G) at 24 h postinfection were similar for all strains. The mutant RY213 and NCTC 11168 showed slightly lower IL-10 and IL-12 induction levels compared to live 81-176 (Fig. 5D and E). Heat-killed 81-176 cells generally induced slightly lower cytokine levels than live 81-176 cells, but this difference was, in most cases, not significant. Using an MOI of 100, the heat-killed bacteria did not induce any higher cytokine levels than when at an MOI of 20 (data not shown). These data suggest that neither active bacterial invasion of DCs nor bacterial viability is necessary for *C. jejuni* to induce cytokine production, but live wild-type 81-176 cells generally result in slightly better induction than other stimulants.

Comparing polysaccharides of *C. jejuni* and *E. coli* for ability to induce cytokines in DCs. LOS was prepared from *C. jejuni* 81-176 by the hot phenol-water extraction method and identified as LOS on silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (data not shown). LOS (100 ng/ml) was added to DCs for 24 h. An equal amount of purified LPS from *E. coli*, known to induce cytokine production in DCs, was used as a control. Both LOS and LPS induced cytokine levels similar to that seen with live *C. jejuni* (Fig. 6), with the exception of a lower IL-10 induction by *C. jejuni* LOS. These data suggest that LOS of *C. jejuni* is a key contributor to the induction of cytokine production by DCs.

NF-κB activation after infection of DCs with *C. jejuni*. The host transcription factor NF-κB promotes transcription of various im-

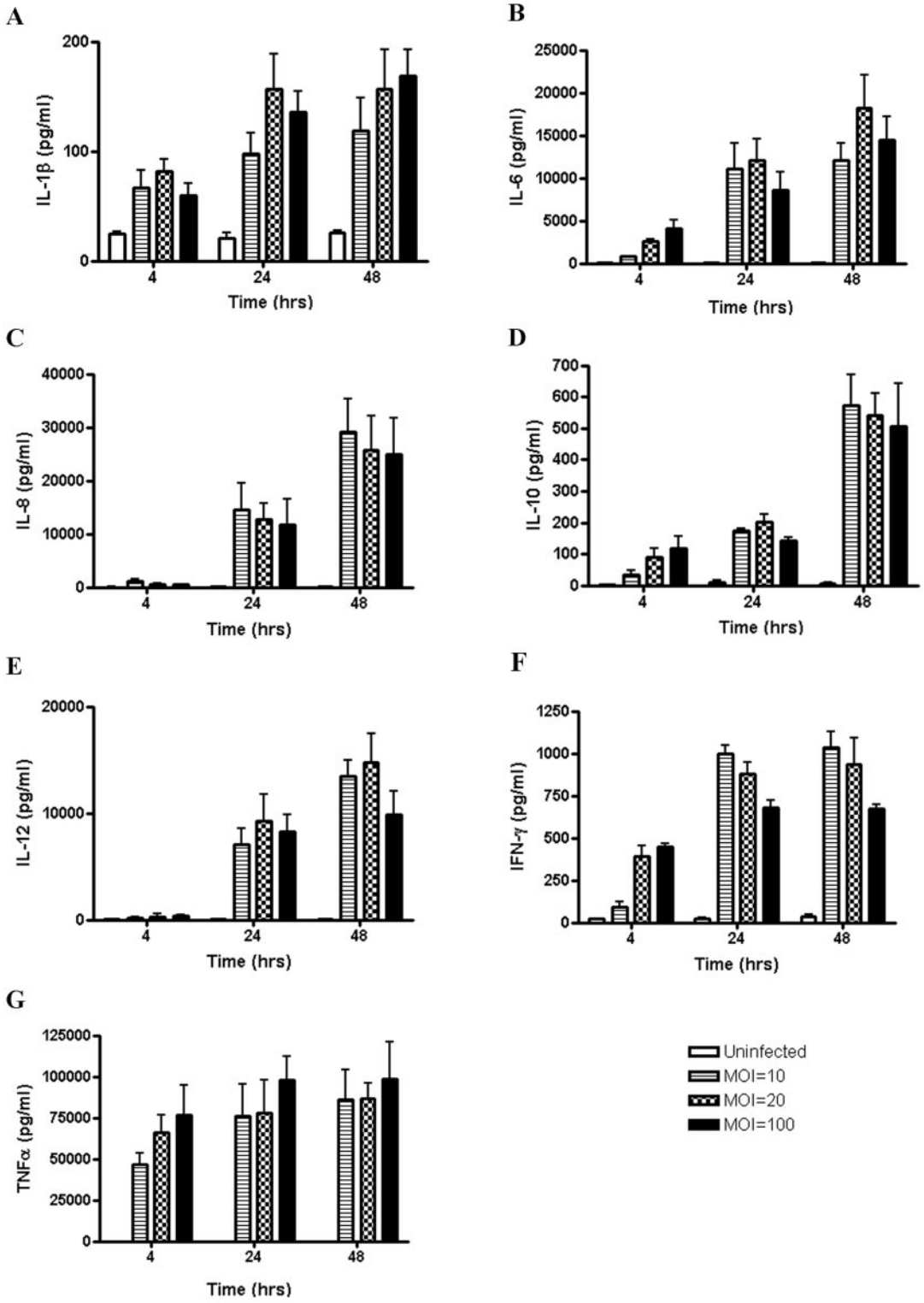

FIG. 4. Cytokine induction in human DCs by *C. jejuni* 81-176 at different MOIs. Culture supernatants were tested at 4, 24, and 48 h postinfection with *C. jejuni* 81-176 at an MOI of 10, 20, or 100 and compared to that of uninfected DCs. The release of IL-1β, IL-6, IL-8, IL-10, IL-12, IFN-γ, and TNF-α is shown (A, B, C, D, E, F, and G, respectively). Data are presented as the mean ± standard deviation of duplicate wells from three independent assays.

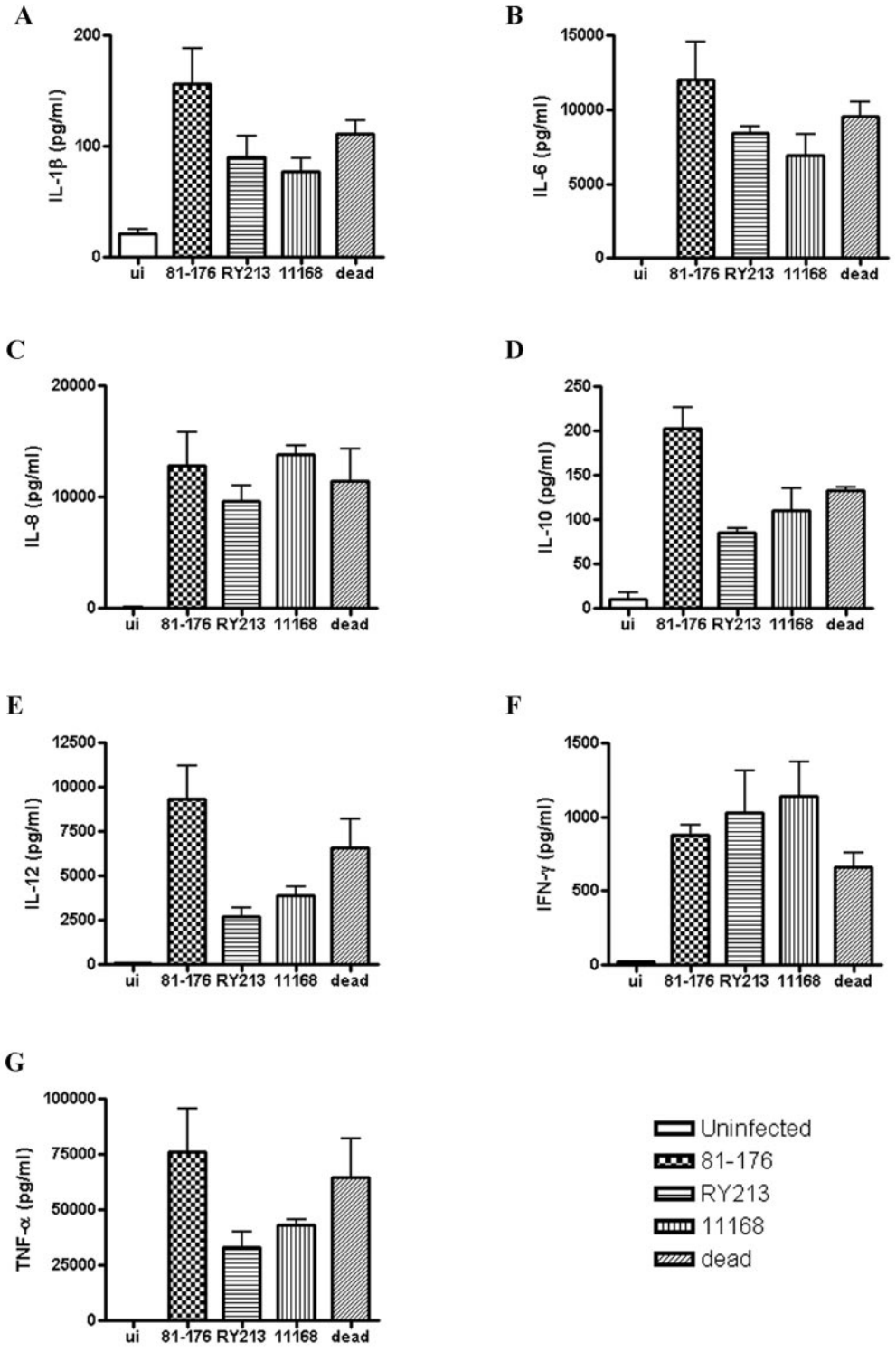

FIG. 5. Comparison of cytokine levels induced in DCs infected by live invasive, noninvasive, or heat-killed strain 81-176. Culture supernatants were tested 24 h postinfection with *C. jejuni* at an MOI of 20. The release of IL-1β, IL-6, IL-8, IL-10, IL-12, IFN-γ, and TNF-α is shown (A, B, C, D, E, F, and G, respectively). Data are presented as the mean ± standard deviation of duplicate wells from three independent assays.

mune response genes, including cytokines (10). The nuclear extracts exhibited low NF-κB activation in the uninfected control DCs. In contrast, NF-κB activation increased threefold ($P < 0.05$) in DCs infected with *C. jejuni* 81-176 for 4 h (Fig. 7).

DISCUSSION

DCs play important roles in both the innate and adaptive immune responses to microbial pathogens (26). The intestinal

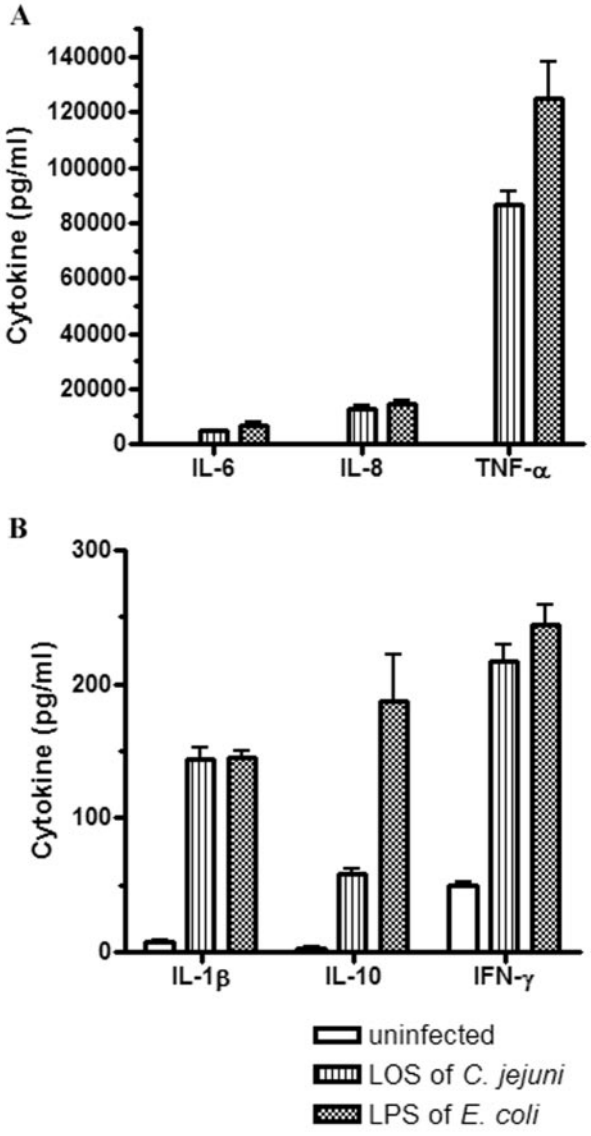

FIG. 6. Comparison of the potential of *C. jejuni* LOS and *E. coli* LPS to induce cytokine production in human DCs. LOS (100 ng/ml) from *C. jejuni* and LPS (100 ng/ml) from *E. coli* were added to DCs for 24 h. The release of IL-6, IL-8, and TNF-α (A) or IL-1β, IL-10, and IFN-γ (B) are compared to values for uninfected DCs (due to the scale, uninfected DC data are too low to be visible in panel A). Data are presented as the mean ± standard deviation of duplicate wells from three independent assays.

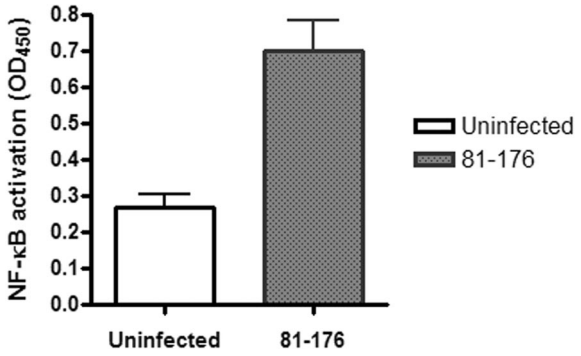

FIG. 7. *C. jejuni*-induced activation of NF-κB in DCs. DCs were incubated with *C. jejuni* 81-176 for 4 h. Nuclear extracts were prepared and incubated with anti-NF-κB p65 per the instructions of the manufacturer. The NF-κB concentrations were determined spectrophotometrically. The data represent the mean ± standard deviation from three independent assays.

immune system DCs lie beneath the mucosal surface in Peyer's patches and form an early line of defense against invading pathogens. Intestinal DCs can internalize bacterial pathogens following their uptake through M cells or more directly through paracellular dendrite extensions into the intestinal lumen (35). Here, we analyzed the interaction between *C. jejuni* and human DCs to investigate DC activation, potential bacterial or DC toxicity, and the induction of cytokines associated with disease inflammation and immune responses to infection. Our data show that DCs internalize *C. jejuni* at early time points (i.e., 2 h postinfection), taking up one to six bacteria per DC on average at MOIs of 10 to 100. Ninety-nine percent of internalized *C. jejuni* were killed within 24 h, and there was virtually no cytotoxicity to DCs induced over 24 h, with only minimal increased DC death (~10%) after 48 h. Wassenaar et al. (51) found that activated human monocytes/macrophages also efficiently killed *C. jejuni*, causing a 5-log reduction in 24 h. Our results differ from those of Siegesmund et al. (42), who reported that *C. jejuni* F38011 induced apoptosis in 63% of THP-1 (human monocyte line) cells 48 h after inoculation.

In striking contrast, many studies report that *Salmonella* is internalized by and survives within both human and murine DCs (6, 9, 18, 29, 45), although *Salmonella* has been reported to rapidly kill infected DCs and macrophages (21, 25, 48). *Shigella* infection of human DCs results in rapid IpaB-dependent DC death (8), which probably dampens the adaptive immune response to this enteric pathogen. Similar to our findings with *Campylobacter*, *Y. enterocolitica* enters DCs and does not induce necrosis or apoptosis (40). However, *Yersinia* reverses the activation of DCs, reduces DCs' abilities to stimulate an adaptive response, and prolongs infection. For *Campylobacter* and some enteric pathogens, it is still largely unclear as to what primary factor(s) determines intracellular bacterial survival or triggers host cell necrosis/apoptosis.

In this study, *C. jejuni* stimulation of immature DCs resulted in their maturation to APCs, as evidenced by enhanced expression of cell surface costimulatory molecules CD40, CD80, and CD86. Similarly, other enteric pathogens, such as *S. enterica* serovar Typhimurium (30) or *Helicobacter pylori* (24), have been shown to up-regulate DC surface expression of MHC class II, CD40, CD80, and CD86 molecules. Coordinated up-regulation of the costimulatory molecules and translocation of MHC molecules to the cell surface are essential molecular events for subsequent antigen presentation and ultimate activation of both $CD4^+$ and $CD8^+$ T cells (36).

Monocytes/macrophages are considered early host responders to infection and are also an important source of proinflammatory cytokines. *C. jejuni* has recently been reported to induce IL-1, IL-6, IL-8, and TNF-α in human monocytes (14, 19). Dendritic cells also produce various cytokines and chemokines upon interaction with pathogenic bacteria (8, 24, 25, 56). Our data show that *C. jejuni* activates the transcriptional factor

NF-κB and induces the production of significant amounts of IL-1β, IL-6, IL-8, IL-10, IL-12, IFN-γ, and TNF-α in DCs. These cytokines play crucial roles in the induction of inflammation and in adaptive immune responses (28, 31, 47).

Proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α were induced rapidly in DCs infected with *C. jejuni* and were secreted and maintained at high levels over 48 h. The cytokine expression pattern was not markedly altered by increasing the MOI from 10 to 100. These findings add to the growing body of information which shows that *C. jejuni* triggers the innate immune system to produce proinflammatory cytokines, which likely serve to initiate and modulate local inflammation in the Peyer's patches, leading to disease symptoms. Previous studies of *C. jejuni*-infected human cell lines have shown that this pathogen also induces epithelial cell secretion of IL-8 and some chemokines (13, 15, 27), attracting phagocytic cells that may be important in both containing and in exacerbating the infection (54). Consistent with these results, our studies show that many proinflammatory cytokines, including IL-8, were stimulated in a significant and rapid fashion in DCs.

As DCs mature, they synthesize cytokines essential for the development of T-cell interactions (i.e., adaptive responses). Compared to other types of APCs, DCs are 1,000-fold more efficient in activating resting T cells (3). Interleukin-12 appears to be a key cytokine produced by APCs to stimulate a Th1-directed response (7, 46). *C. jejuni* induced very high levels of IL-12 at 24 and 48 h after infection. IL-10 is an important Th2-type cytokine that up-regulates humoral and down-regulates cell-mediated immune responses (31, 41). The high IL-12 production (i.e., 10,000-fold induction) and relatively low IL-10 induction (i.e., 100- to 500-fold induction) by *C. jejuni* favors a Th1 response.

The induction of cytokine production in DCs was not significantly different between live and heat-killed bacteria, except for IL-10, which was slightly lower with the heat-killed bacterial stimulus. Similar findings were previously reported in *C. jejuni*-infected monocytes (19). The noninvasive RY213 strain and *C. jejuni* NCTC 11168, which have low invasion efficiencies (1), induced cytokines IL-10, IL-12, and TNF-α at levels reduced only by about one-half versus strain 81-176. These data argue that invasion is not necessary for the induction of these cytokines. In fact, purified LOS from strain 81-176 induced high levels of the selected cytokines studied, with the exception of IL-10. These data indicate that the predominant induction of cytokines in DCs may be due to interaction with LOS. Some alternate pathway is likely needed for full IL-10 induction. The signal transduction pathways modulated by the interaction of *C. jejuni* with DCs are currently being investigated in our lab. Taken together, these data suggest that DCs are an important component of the host-pathogen interaction with *Campylobacter*. Further, DCs play a role in triggering inflammatory cytokines likely involved in disease pathogenesis and in initiating a Th1-directed adaptive immune response during *C. jejuni* infection.

ACKNOWLEDGMENTS

We acknowledge T. T. Wai for help in preparing *Campylobacter* LOS and thank R. I. Walker, M. Akkoyunlu, and S. Dharwan for critical review of the manuscript.

REFERENCES

1. **Bacon, D. J., R. A. Alm, D. H. Burr, L. Hu, D. J. Kopecko, C. P. Ewing, T. J. Trust, and P. Guerry.** 2000. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81–176. Infect. Immun. **68:**4384–4390.
2. **Banchereau, J., and R. M. Steinman.** 1998. Dendritic cells and the control of immunity. Nature **392:**245–252.
3. **Bhardwaj, N., J. W. Young, A. J. Nisanian, J. Baggers, and R. M. Steinman.** 1993. Small amounts of superantigen, when presented on dendritic cells, are sufficient to initiate T cell responses. J. Exp. Med. **178:**633–642.
4. **Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. J. Blaser.** 1988. Experimental *Campylobacter jejuni* infection in humans. J. Infect. Dis. **157:**472–479.
5. **Blaser, M. J.** 1997. Epidemiologic and clinical features of *Campylobacter jejuni* infections. J. Infect. Dis. **176**(Suppl. 2)**:**S103–S105.
6. **Cano, D. A., M. Martinex-Moya, M. G. Pucciarelli, E. A. Groisman, J. Casadesus, and F. Garcia-Del Portillo.** 2001. *Salmonella enterica* serovar Typhimurium response involved in attenuation of pathogen intracellular proliferation. Infect. Immun. **69:**6463–6474.
7. **de Saint-Vis, B., I. Fugier-Vivier, C. Massacrier, C. Gaillard, B. Vanbervliet, S. Ait-Yahia, J. Banchereau, Y.-J. Liu, S. Lebecque, and C. Caux.** 1998. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J. Immunol. **160:**1666–1676.
8. **Edgeworth, J. D., J. Spencer, A. Phalipon, G. E. Griffin, and P. J. Sansonetti.** 2002. Cytotoxicity and interleukin-1β processing following *Shigella flexneri* infection of human monocyte-derived dendritic cells. Eur. J. Immunol. **32:**1464–1471.
9. **Garcia-Del Portillo, F., H. Jungnitz, M. Rohde, and C. A. Guzman.** 2000. Interaction of *Salmonella* enterica serotype Typhimurium with dendritic cells is defined by targeting to compartments lacking lysosomal membrane glycoproteins. Infect. Immun. **68:**2985–2991.
10. **Ghosh, S., M. J. May, and E. B. Kopp.** 1998. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu. Rev. Immunol. **16:**225–260.
11. **Guermonprez, P., J. Valladeau, L. Zitvogel, C. Thery, and S. Amigorena.** 2002. Antigen presentation and T cell stimulation by dendritic cells. Annu. Rev. Immunol. **20:**621–667.
12. **Guiney, D. G., P. Hasegawa, and S. P. Cole.** 2003. *Helicobacter pylori* preferentially induces interleukin 12 (IL-12) rather than IL-6 or IL-10 in human dendritic cells. Infect. Immun. **71:**4163–4166.
13. **Hickey, T. E., S. Baqar, L. Bourgeois, C. P. Wesing, and P. Guerry.** 1999. *Campylobacter jejuni*-stimulated secretion of interleukin-8 by INT407 cells. Infect. Immun. **67:**88–93.
14. **Hickey, T. E., G. Majam, and P. Guerry.** 2005. Intracellular survival of *Campylobacter jejuni* in human monocytic cells and induction of apoptotic death by cytolethal distending toxin. Infect. Immun. **73:**5194–5197.
15. **Hu, L., and T. E. Hickey.** 2005. *Campylobacter jejuni* induces secretion of proinflammatory chemokines from human intestinal epithelial cells. Infect. Immun. **73:**4437–4440.
16. **Hu, L., and D. J. Kopecko.** 1999. *Campylobacter jejuni* 81–176 associates with microtubules and dynein during invasion of human intestinal cells. Infect. Immun. **67:**4171–4182.
17. **Inaba, K., M. Inaba, M. Deguchi, K. Hagi, R. Yasumizu, S. Ikehara, S. Muramatsu, and R. M. Steinman.** 1993. Granulocytes, macrophages, and dendritic cells arise from a common major histocompatibility complex class II-negative progenitor in mouse bone marrow. Proc. Natl. Acad. Sci. USA **90:**3038–3042.
18. **Jantsch, J., C. Cheminay, D. Chakravortty, T. Lindig, J. Hein, and M. Hensel.** 2003. Intracellular activities of *Salmonella enterica* in murine dendritic cells. Cell. Microbiol. **5:**933–945.
19. **Jones, M. A., S. Totemeyer, D. J. Maskell, C. E. Bryant, and P. A. Barrow.** 2003. Induction of proinflammatory responses in the human monocytic cell line THP-1 by *Campylobacter jejuni*. Infect. Immun. **71:**2626–2633.
20. **Kielhbauch, J. A., R. A. Albach, L. L. Bauum, and K.-P. Chang.** 1985. Phagocytosis of *Campylobacter jejuni* and its intracellular survival in mononuclear phagocytes. Infect. Immun. **48:**446–451.
21. **Knodler, L. A., and B. B. Finlay.** 2001. *Salmonella* and apoptosis: to live or let die? Microbes Infect. **3:**1321–1326.
22. **Kopecko, D. J., L. Hu, and K. J. Zaal.** 2001. *Campylobacter jejuni*-microtubule-dependent invasion. Trends Microbiol. **9:**389–396.
23. **Korlath, J. A., M. T. Osterholm, L. A. Judy, J. C. Forfang, and R. A. Robinson.** 1985. A point-source outbreak of campylobacteriosis associated with consumption of raw milk. J. Infect. Dis. **152:**592–596.
24. **Kranzer, K., A. Eckhardt, M. Aigner, G. Knoll, L. Deml, C. Speth, N. Lehn, M. Rehli, and W. Schneider-Brachert.** 2004. Induction of maturation and cytokine release of human dendritic cells by *Helicobacter pylori*. Infect. Immun. **72:**4416–4423.
25. **Marriott, I., T. G. Hammond, E. K. Thomas, and K. L. Bost.** 1999. *Salmonella* efficiently enter and survive within cultured $CD11c^+$ dendritic cells initiating cytokine expression. Eur. J. Immunol. **29:**1107–1115.
26. **Medzhitov R., and C. A. Janeway, Jr.** 1997. Innate immunity: the virtues of a nonclonal system of recognition. Cell **91:**295–298.

27. **Mellits, K. H., J. Mullen, M. Wand, G. Armbruster, A. Patel, P. L. Connerton, M. Skelly, and I. F. Connerton.** 2002. Activation of the transcription factor NF-κB by *Campylobacter jejuni*. Microbiology **148:**2753–2763.
28. **Nicod, L. P., and J. M. Dayer.** 1999. Cytokines in the functions of dendritic cells, monocytes, macrophages and fibroblasts. Oxford University Press, New York, N.Y.
29. **Niedergang, F., J. C. Sirard, C. T. Blanc, and J. P. Kraehenbuhl.** 2000. Entry and survival of *Salmonella typhimurium* in dendritic cells and presentation of recombinant antigens do not require macrophage-specific virulence factors. Proc. Natl. Acad. Sci. USA **97:**14650–14655.
30. **Norimatsu, M., V. Chance, G. Dougan, C. J. Howard, and B. Villarreal-Ramos.** 2004. Live *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) elicit dendritic cell responses that differ from those induced by killed *S. typhimurium*. Vet. Immunol. Immunopathol. **98:**193–201.
31. **Opal, S. M., J. C. Wherry, and P. Grint.** 1998. Interleukin-10: potential benefits and possible risks in clinical infectious diseases. Clin. Infect. Dis. **27:**1497–1507.
32. **Palucka, K., and J. Banchereau.** 2002. How dendritic cells and microbes interact to elicit or subvert protective immune responses. Curr. Opin. Immunol. **14:**420–431.
33. **Parkhill, J., B. W. Wren, K. Mungall, J. M. Ketley, C. Churcher, D. Basham, T. Chillingworth, R. M. Davies, T. Feltwell, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Moule, M. J. Pallen, C. W. Penn, M. A. Quail, M. A. Rajandream, K. M. Rutherford, A. H. van Vliet, S. Whitehead, and B. G. Barrell.** 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature **403:**665–668.
34. **Pickering, A. K., M. Osorio, G. M. Lee, V. K. Grippe, M. Bray, and T. J. Merkel.** 2004. Cytokine response to infection with *Bacillus anthracis* spores. Infect. Immun. **72:**6382–6389.
35. **Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli.** 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. **2:**361–367.
36. **Rescigno, M., M. Martino, C. L. Sutherland, M. R. Gold, and P. Ricciardi-Castagnoli.** 1998. Dendritic cell survival and maturation are regulated by different signaling pathways. J. Exp. Med. **188:**2175–2180.
37. **Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia.** 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J. Exp. Med. **182:**389–400.
38. **Sallusto, F., and A. Lanzavecchia.** 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J. Exp. Med. **179:**1109–1118.
39. **Scheinecker, C., R. McHugh, E. M. Shevach, and R. N. Germain.** 2002. Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. J. Exp. Med. **196:**1079–1090.
40. **Schoppet, M., A. Bubert, and H. I. Huppertz.** 2000. Dendritic cell function is perturbed by *Yersinia enterocolitica* infection in vitro. Clin. Exp. Immunol. **122:**316–323.
41. **Sharma, S., M. Stolina, Y. Lin, B. Gardner, P. W. Miller, M. Kronenberg, and S. M. Dubinett.** 1999. T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. J. Immunol. **163:**5020–5028.
42. **Siegesmund, A. M., M. E. Konkel, J. D. Klena, and P. F. Mixter.** 2004. *Campylobacter jejuni* infection of differentiated THP-1 macrophages results in interleukin 1 beta release and caspase-1-independent apoptosis. Microbiology **150:**561–569.
43. **Skirrow, M. B., and M. J. Blaser.** 2000. Clinical aspects of *Campylobacter* infection, p. 969–988. *In* I. Nashamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, D.C.
44. **Smith, C. K., P. Kaiser, L. Rothwell, T. Humphrey, P. A. Barrow, and M. A. Jones.** 2005. *Campylobacter jejuni*-induced cytokine responses in avian cells. Infect. Immun. **73:**2094–2100.
45. **Svensson, M., C. Johansson, and M. J. Wick.** 2000. *Salmonella enterica* serovar Typhimurium-induced maturation of bone marrow-derived dendritic cells. Infect. Immun. **68:**6311–6320.
46. **Thoma-Uszynski, S., S. M. Kiertscher, M. T. Ochoa, D. A. Bouis, M. V. Norgard, K. Miyake, P. J. Godowski, M. D. Roth, and R. L. Modlin.** 2000. Activation of toll-like receptor 2 on human dendritic cells triggers induction of IL-12, but not IL-10. J. Immunol. **165:**3804–3810.
47. **Trinchieri, G.** 1998. Immunobiology of interleukin-12. Immunol. Res. **17:** 269–278.
48. **van der Velden, A. W., M. Velasquez, and M. N. Starnbach.** 2003. *Salmonella* rapidly kill dendritic cells via a caspase-1-dependent mechanism. J. Immunol. **171:**6742–6749.
49. **Walker, R. I., M. B. Caldwell, E. C. Lee, P. Guerry, T. J. Trust, and G. M. Ruiz-Palacios.** 1986. Pathophysiology of *Campylobacter* enteritis. Microbiol. Rev. **50:**81–94.
50. **Wallis, M. R.** 1994. The pathogenesis of *Campylobacter jejuni*. Br. J. Biomed. Sci. **51:**57–64.
51. **Wassenaar, T. M., M. Engelskirchen, S. Park, and A. Lastovica.** 1997. Differential uptake and killing potential of *Campylobacter jejuni* by human peripheral monocytes/macrophages. Med. Microbiol. Immunol. (Berlin) **186:** 139–144.
52. **Westphal, O., and K. Jann.** 1965. Methods in carbohydrate chemistry, vol. 5, p. 83. Academic Press, New York, N.Y.
53. **Wick, M. J.** 2002. The role of dendritic cells during *Salmonella* infection. Curr. Opin. Immunol. **14:**437–443.
54. **Wuyts, A., P. Proost, J. P. Lenaerts, A. Ben-Baruch, J. Van Damme, and J. M. Wang.** 1998. Differential usage of the CXC chemokine receptors 1 and 2 by interleukin-8, granulocyte chemotactic protein-2 and epithelial-cell-derived neutrophil attractant-78. Eur. J. Biochem. **255:**67–73.
55. **Yao, R., D. H. Burr, and P. Guerry.** 1997. CheY-mediated modulation of *Campylobacter jejuni* virulence. Mol. Microbiol. **23:**1021–1031.
56. **Yrlid, U., M. Svensson, C. Johansson, and M. J. Wick.** 2000. *Salmonella* infection of bone marrow-derived macrophages and dendritic cells: influence on antigen presentation and initiating an immune response. FEMS Immunol. Med. Microbiol. **27:**313–320.

Editor: J. T. Barbieri