INFECTION AND IMMUNITY, July 2003, p. 4163–4166 Vol. 71, No. 7
0019-9567/03/$08.00+0 DOI: 10.1128/IAI.71.7.4163–4166.2003

Helicobacter pylori Preferentially Induces Interleukin 12 (IL-12) Rather than IL-6 or IL-10 in Human Dendritic Cells

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Received 21 March 2003/Accepted 4 April 2003

Dendritic cells are potent antigen-presenting cells that are present in the gastrointestinal tract and are required for the induction of a Th1 T-cell acquired immune response. Since infection with the gastric pathogen *Helicobacter pylori* elicits a Th1 cell response, the interaction of these organisms with dendritic cells should reflect the Th1 bias. We incubated *H. pylori* with cultured human dendritic cells and measured the cytokine induction profile, comparing the response to that induced by *Salmonella enterica* serovar Typhimurium. We found that *H. pylori* induced little interleukin 6 (IL-6) and essentially no IL-10 in contrast to *S. enterica*. However, *H. pylori* induced levels of IL-12 that were 30% of those induced by *S. enterica*, indicating a Th1 response. An isogenic *cagE* mutant of *H. pylori* lost about 50% of its IL-12-inducing ability, suggesting a role for the *cag* type IV secretion system in the stimulation of dendritic cells.

Helicobacter pylori produces a chronic gastric infection that is widespread in the human population and is associated with severe disease sequelae including gastritis, peptic ulcers, mucoa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma (reviewed in reference 10). Even in people without symptoms, *H. pylori* produces histologic changes of gastritis, indicating a continuous inflammatory reaction of the gastric mucosa. Although *H. pylori* elicits both innate and acquired immune responses, the host is unable to eliminate the organism from the gastric mucosal surface, and indefinite infection is the usual outcome. In fact, this ineffective immune response is postulated to contribute substantially to the development of long-term disease syndromes (reviewed in reference 18). The innate immune response is driven, at least in part, by bacterial virulence factors such as the *cag* pathogenicity island (PAI) that stimulate production of proinflammatory mediators from the gastric epithelium (reviewed in reference 16). The acquired immune response includes both antibody- and cell-mediated components. A strong Th1 T-cell component has been described and has been implicated in perpetuating the inflammatory changes that lead to disease (18). Recent evidence indicates that antigen presentation by dendritic cells is required to initiate a primary Th1 T-cell immune response (5, 12). Furthermore, the Th1-biased response is facilitated by IL-12 elaboration in the context of the antigen presentation process. IL-12 is upregulated in the gastric mucosa of dyspeptic patients and is associated with presence of the *cag* PAI (9). These immunologic principles strongly suggest that *H. pylori* must interact with dendritic cells and elicit IL-12 secretion during infection of the gastric mucosa in order to induce the Th1 acquired immune response.

Dendritic cells are a group of antigen-presenting cells that are widely distributed in tissues, including the gastrointestinal mucosa (5, 12, 20). Immature dendritic cells in the mucosa-associated lymphoid tissue lymphoma, are phagocytic and specialize in antigen capture. Dendritic cells are capable of migrating through epithelial tight junctions to gain access to the gastrointestinal lumen (14, 15). Upon exposure to whole bacteria or bacterial components, immature dendritic cells differentiate into mature, antigen-presenting cells with upregulated expression of major histocompatibility complex class I and II, costimulatory, and adhesion molecules (5, 12).

Dendritic cells are derived from myeloid precursors and can be cultured in vitro by differentiation of human peripheral blood monocytes treated with IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (5, 17). The resulting immature dendritic cells are phagocytic and express Toll-like receptors capable of interaction with various bacterial components. The resulting signal transduction events stimulate cytokine release and differentiation into mature dendritic cells. For example, activation of Toll-like receptor 2 results in preferential secretion of IL-12 rather than IL-10 by human dendritic cells exposed to mycobacterial lipoprotein (17). We postulated that *H. pylori* would interact with immature dendritic cells to produce a characteristic cytokine pattern that also includes selective production of IL-12.

Human dendritic cells were derived and cultured in vitro from peripheral blood monocytes according to standard procedures (7, 17). Briefly, monocytes were isolated from the peripheral blood mononuclear cell fraction of healthy donors by adherence to plasma-coated plastic flasks. Monocytes were seeded into six-well plates and cultured in RPMI medium containing 10% fetal calf serum with IL-4 (1,000 U/ml) and GM-CSF (1,000 U/ml). After 5 days, the nonadherent cells were harvested by washing, resuspended in fresh RPMI medium containing 10% fetal calf serum with GM-CSF, and seeded into a 24-well plate at a concentration of 2×10^5 cells per well.

The bacterial strains used for infection were *H. pylori* SD4 (a clinical isolate described previously and shown to induce IL-8 secretion in epithelial cells [4]) and *Salmonella enterica* serovar Typhimurium 14028s (7). Cultures were maintained on Columbia agar plates containing 5% laked sheep or horse blood and

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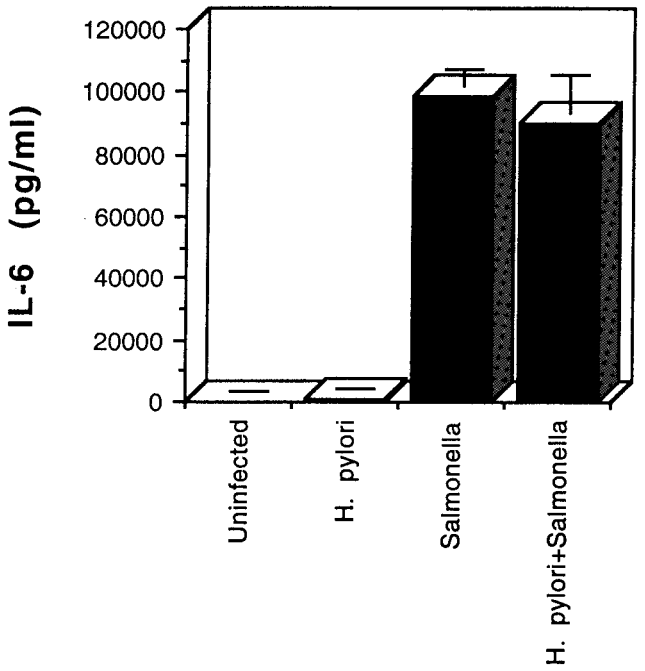

FIG. 1. IL-6 secretion by human dendritic cells infected with *H. pylori* or *Salmonella enterica*. IL-6 levels in the supernatants collected after 48 h from triplicate culture wells were measured by ELISA for each of the infection conditions. Error bars represent standard deviations.

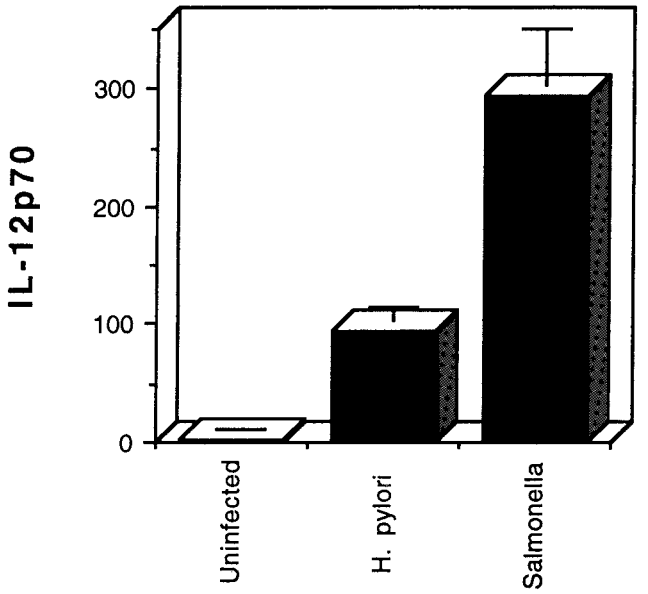

FIG. 2. IL-12 p70 (picograms per milliliter) produced by human dendritic cells after infection with *H. pylori* or *Salmonella enterica* by using the same samples as shown in Fig. 1. Error bars represent standard deviations.

1% fungizone (amphotericin B; Omega Scientific), as described previously (4). Bacteria were maintained in an atmosphere of 10% CO_2, 5% O_2, and 85% N_2. For dendritic cell experiments, bacteria from 1- or 2-day-old plates were resuspended in RPMI medium and counted with a Petroff-Hausser chamber.

The *cagE* mutation in *H. pylori* SD4 was constructed by insertion of a chloramphenicol resistance gene (*cat,* originally isolated from *Campylobacter coli,* obtained from D. Taylor [19]) in the *Eco*RI site, 756 bp from the 5′ start site of the published *cagE* gene sequence from strain NCTC 11638 (3). The 1.2-kb *cagE* gene was obtained by PCR from *H. pylori* strain SD14 (4). The resulting construct, on plasmid PCR-Script (Stratagene, La Jolla, Calif.), was transformed into *H. pylori* SD4 by standard procedures (8). Briefly, bacteria were inoculated as a patch on Columbia blood agar plates, and 5 μg of plasmid DNA prepared from *E. coli* DH5α was spread over the patch. After 24 h of incubation at 37°C in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2, bacteria were streaked for single-colony isolation to plates containing 20 μg of chloramphenicol per ml and incubated further. Single colonies were isolated, expanded, and checked for proper insertion of the *cat* gene by PCR, performed by using one primer located in the *cagE* gene and one the *cat* gene. Mutants were confirmed by identification of an appropriate-sized band by Southern hybridization.

All bacterial strains were inoculated into tissue culture wells at a ratio of 10 organisms per dendritic cell. This inoculum was chosen on the basis of preliminary dose-response experiments indicating that 10:1 was the optimal ratio for cytokine secretion. For the dual infections, *H. pylori* SD4 was added 1 h before the *S. enterica*. Infected cultures were centrifuged at 200 × *g* for 5 min and incubated for 1 h at 37°C in 5% CO_2. Subsequently, gentamicin was added at 20 μg/ml and the incubation was continued for 48 h. At this time, the supernatant was aspirated, centrifuged, and frozen in aliquots at −70°C until used for the cytokine assays. *H. pylori* infection of AGS gastric epithelial cells was done as described previously (4). Cytokine assays were performed by using commercial enzyme-linked immunosorbent assay (ELISA) kits for IL-6, IL-8, IL-10, and IL-12 p70.

Initially, we examined the production of IL-6 by dendritic cells infected with equal numbers of *H. pylori* or *S. enterica* serovar Typhimurium cells. Figure 1 shows that serovar Typhimurium was a potent stimulator of IL-6 secretion, while by comparison, *H. pylori* induced little IL-6 release. To determine whether *H. pylori* was actively inhibiting IL-6 secretion by dendritic cells, we infected the cells first with *H. pylori* and then with *Salmonella*. As seen in Fig. 1, preinfection with *H. pylori* had no effect on the IL-6 levels induced by serovar Typhimurium, indicating the lack of an inhibitory activity in *H. pylori*.

Next, we tested the ability of *H. pylori* and *S. enterica* to stimulate IL-12 p70 secretion by dendritic cells, as shown in Fig. 2. In contrast to IL-6, *H. pylori* infection produced levels of IL-12 that were approximately 30% of those induced by *S. enterica*. These results suggest that *H. pylori* stimulates differential cytokine production by dendritic cells.

To determine the role of the *cag* PAI type IV protein secretion system in the interaction of *H. pylori* with dendritic cells, we constructed a *cagE* knockout mutation in strain SD4. We verified that strain SD4 stimulated IL-8 secretion in gastric epithelial cells by a *cag* type IV secretion-system-dependent mechanism. As shown in Fig. 3, AGS cells were infected with either wild-type *H. pylori* SD4 or its isogenic *cagE*-negative mutant, and IL-8 secretion was determined 24 h after infec-

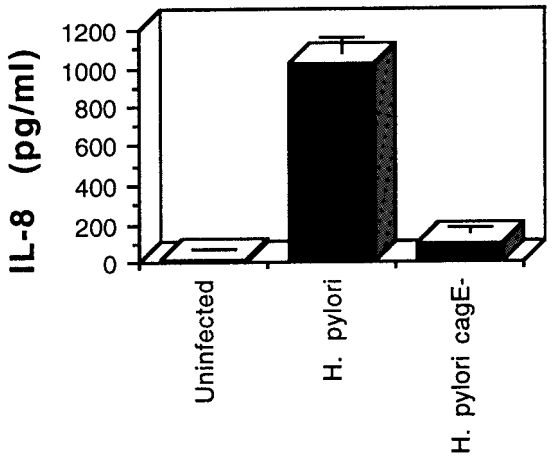

FIG. 3. IL-8 secretion by human gastric epithelial cells infected with *H. pylori* SD4 or an isogenic *cagE* mutant defective in type IV protein secretion function. IL-8 levels in the culture supernatants from triplicate wells collected 24 h after infection were measured by ELISA. Error bars represent standard deviations.

tion. The results indicate that SD4 stimulation of IL-8 secretion from gastric epithelial cells is largely dependent on *cagE*. We then infected dendritic cells with wild-type *H. pylori* or the *cagE* mutant. As shown in Fig. 4, the *cagE* mutant was about 50% less active at inducing IL-12 than the wild type. Similar results were obtained with another *cagE* mutant isolate of *H. pylori* SD4. This study indicates that *H. pylori* stimulates IL-12 secretion by both *cagE*-dependent and *cagE*-independent mechanisms.

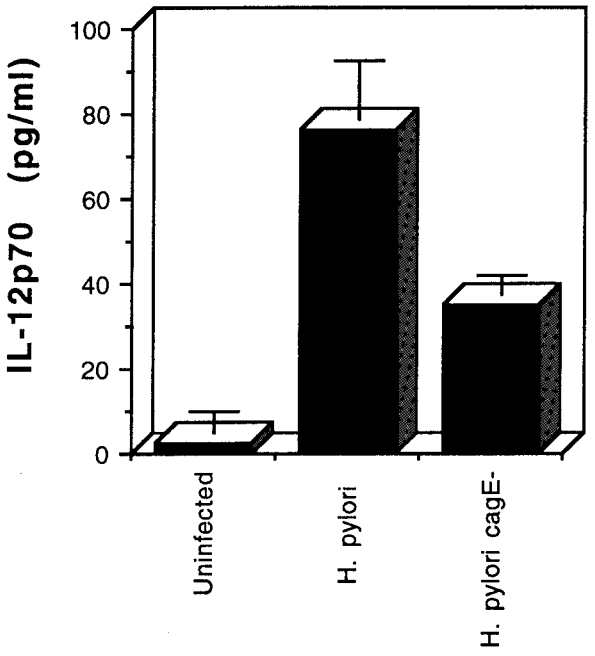

FIG. 4. Effect of a *cagE* mutation on IL-12p70 secretion by human dendritic cells infected with *H. pylori*. Supernatants were collected from triplicate wells 48 h after infection and assayed by ELISA. Error bars represent standard deviations. The difference in IL-12 production by cells infected with the wild-type strain compared to the *cagE* mutant is significant at $P < 0.01$ (t test)

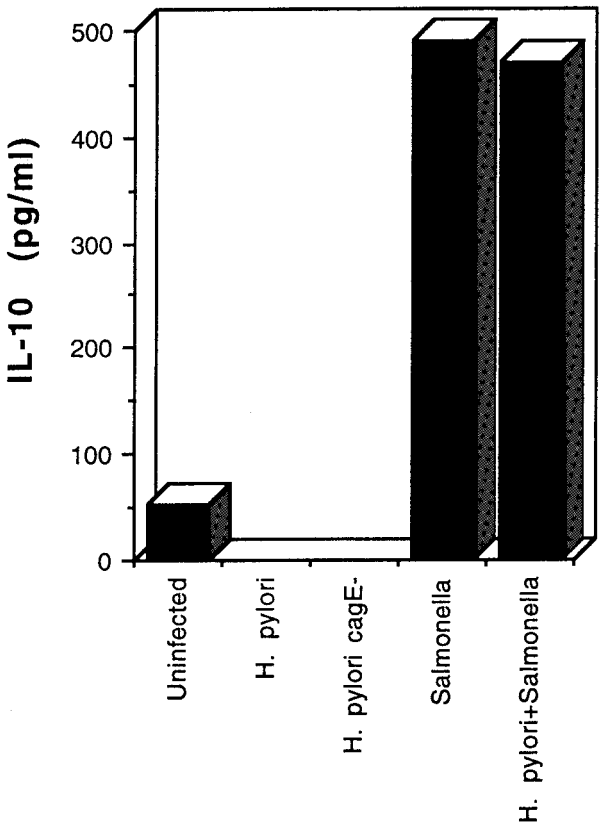

FIG. 5. IL-10 secretion by human dendritic cells infected with *H. pylori* or *Salmonella enterica.* Values represent the means of culture supernatants collected from duplicate wells and assayed by ELISA. The experiment was repeated independently with similar results.

Results reported in studies of the inhibition of growth and intracellular survival of *H. pylori* in human monocyte-derived macrophages have varied, depending on the presence or absence of the *cag* PAI (1, 11, 13). We examined whether there was a difference in survival of the wildtype and *cagE* mutant in our assay system. Therefore, we incubated SD4 and SD4*cagE* with dendritic cells as above (multiplicity of infection, 10), treated the cultures with gentamicin (20 μg/ml) for 1 h, added saponin (0.1%), sonicated the cells for 30 s in a water bath, and plated the resulting lysed cells for intracellular bacteria. We were unable to recover viable bacteria from the gentamicin-treated cultures, while in the absence of gentamicin we recovered approximately equal numbers of *H. pylori* SD4 and SD4*cagE,* representing 1 to 2% of the original inocula. The results indicate that *H. pylori* survives poorly under these assay conditions, but no significant difference was found between wild-type and *cagE* mutants.

Differential secretion of IL-12 compared to IL-10 has been reported for dendritic cells treated with bacterial lipoprotein (13). Accordingly, we examined the ability of *H. pylori* and *S. enterica* to induce IL-10 secretion in dendritic cells. The results are shown in Fig. 5. Neither strain SD4 nor its *cagE* mutant induced detectable levels of IL-10 in the dendritic cell cultures. In contrast, IL-10 was readily detected in cultures infected with *S. enterica*. Similar to the IL-6 result, preinfection with *H. pylori* did not inhibit the activity of *S. enterica* in this system, indicat-

ing that the lack of IL-10 induction by *H. pylori* is not due to an inhibitory effect.

These results demonstrate that *H. pylori* induces IL-12 secretion from monocyte-derived human dendritic cells but does not elicit IL-10 production. Secretion of IL-6 in response to *H. pylori* was detectable but occurred only at low levels. In contrast, an equal number of *S. enterica* bacteria induced secretion of all three cytokines from dendritic cells. Compared to *S. enterica*, *H. pylori* was much more active in eliciting an IL-12 response than in eliciting an IL-6 response (see Fig. 2 and 3). We chose *S. enterica* serovar Typhimurium as a positive control in these studies since it has been used extensively in cytokine induction experiments, including those performed with dendritic cells (7, 20). For a comparison of this organism to *H. pylori*, we infected cells with equal numbers of bacteria. Although this clearly means that the amount of any bacterial component, such as protein or lipopolysaccharide (LPS), will be different in the inocula, we reasoned that the organism load is a relevant biological parameter in an infection. We recognize that for any given cytokine, comparisons between different organisms are difficult to interpret. However, the data clearly indicate that *H. pylori* preferentially stimulates IL-12 secretion over that of IL-6 and IL-10 from human dendritic cells.

The results suggest that *H. pylori* components interact with dendritic cells to selectively induce the IL-12 secretion pathway, while *S. enterica* lacks this selectivity. The difference between *H. pylori* and *Salmonella* may be partly explained by the structures and relative biological effects of their LPS. *H. pylori* has an atypical lipid A that is markedly less immunostimulatory than *Salmonella* LPS (2). It is likely that much of the cytokine-inducing activity of *S. enterica* in these studies is due to its LPS, since purified *Salmonella* LPS has been shown to induce these responses in human dendritic cells. The relatively poor ability of *H. pylori* to induce IL-6 and IL-10 may be due to the low endotoxin activity of its LPS. This explanation implies that IL-12 secretion is due to a different bacterial stimulus than that causing secretion of IL-6 or IL-10 in the case of *H. pylori.* Our data suggest that a component of this stimulus is the *cag* PAI-encoded type IV secretion system. We found that a *cagE* knockout mutant has lost about 50% of its IL-12-inducing ability. The CagE protein is absolutely required for the activity of the type IV system, as evidenced by the marked loss of IL-8-inducing activity in epithelial cell infections (Fig. 4). The *cag* PAI is known to activate both the mitogen-activated protein kinase and NF-κB signal transduction pathways in host cells (16). However, the *cag* PAI is only partially responsible for IL-12 induction, since residual activity remains with the *cagE* mutant. The evidence suggests that IL-12 induction by *H. pylori* is multifactorial.

The selective IL-12 secretion from dendritic cells during *H. pylori* infection is consistent with the ability of *H. pylori* to elicit a Th1-type acquired cellular immune reaction. IL-12 and IL-18 appear to be key cytokines produced by antigen-presenting cells to preferentially stimulate a Th1 T-cell response (6, 17). In contrast, IL-10 has immunosuppressive effects and can downregulate the Th1 response (17). Therefore, the lack of IL-10 induction by *H. pylori* would also favor a Th1 response. The presence of bacterial factors that stimulate IL-12 production in an organism with low endotoxin activity may account in part for the propensity of *H. pylori* to modulate the acquired immune response toward a Th1 bias.

This work was supported by PHS grant R01 DK53649.

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Editor: F. C. Fang