Helicobacter pylori-secreted factors inhibit dendritic cell IL-12 secretion: a mechanism of ineffective host defense

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Kao, John Y., Sivapraaksh Rathinavelu, Kathryn A. Eaton, Longchuan Bai, Yana Zavras, Mimi Takami, Anna Pierzchala, and Juanita L. Merchant. Helicobacter pylori-secreted factors inhibit dendritic cell IL-12 secretion: a mechanism of ineffective host defense. Am J Physiol Gastrointest Liver Physiol 291: G73–G81, 2006. First published February 9, 2006; doi:10.1152/ajpgi.00139.2005.—Helicobacter pylori evades host immune defenses and causes chronic gastritis. Immunity against intestinal pathogens is largely mediated by dendritic cells, yet the role of dendritic cells in acute H. pylori infection is largely unknown. We observed the recruitment of dendritic cells to the gastric mucosa of H. pylori-infected mice. Bone marrow-derived dendritic cells from mice responded to live H. pylori by upregulating the expression of proinflammatory cytokine mRNA (i.e., IL-1α, IL-1β, and IL-6). The supernatant from dendritic cells stimulated with H. pylori for 18 h contained twofold higher levels of IL-12p70 than IL-10 and induced the proliferation of syngeneic splenocytes and type 1 T helper cell cytokine release (IFN-γ and TNF-α). These responses were significantly lower compared with those induced by Acinetobacter lwoffi, another gastritis-causing pathogen more susceptible to host defenses. Analysis of whole H. pylori sonicate revealed the presence of a heat-stable factor secreted from H. pylori that specifically inhibited IL-12 but not IL-10 release from dendritic cells activated by A. lwoffi. Our findings suggest that dendritic cells participate in the host immune response against H. pylori and that their suppression by H. pylori may explain why infected hosts fail to prevent bacterial colonization.

antigen-presenting cells; Acinetobacter lwoffi; inflammation; interferon-γ; interleukin-10

Helicobacter pylori causes chronic gastritis in virtually all infected individuals (7) and is associated with gastroduodenal ulcers and gastric cancer. This inflammatory reaction, which initially consists of the recruitment of neutrophils, followed by T cells, plasma cells, and antigen-presenting cells (APCs), is accompanied by epithelial cell damage (14). Because H. pylori seldom invades the gastric mucosa, the host response is believed to be triggered primarily by the attachment of bacteria to epithelial cells by means of binding to surface class II major histocompatibility complex (MHC) molecules leading to the secretion of inflammatory chemokines (11, 35). Infected individuals generate a vigorous systemic and mucosal humoral response (26) but fail to eradicate the offending organism and thus are susceptible to mucosal damage (31). Persistent H. pylori infection leads to the development of chronic gastritis characterized by a type 1 T helper cell response (Th1). Current understanding of the host response to H. pylori focuses on macrophages, because the APCs in the stomach regulate both early innate immunity as well as chronic adaptive cellular responses against this pathogen (31). However, growing evidence suggests that dendritic cells (DCs) may also participate in bacterial-specific pattern recognition and play a role in informing the adaptive arm of the immune system (4, 18).

Several lines of evidence have emerged implicating DC involvement. DCs are highly specialized APCs that, after in vivo and in vitro exposure to LPS or other bacterial products, undergo activation and maturation (28). DCs have also been shown to be capable of traversing the gut epithelium to sample luminal bacteria (29). Furthermore, Nishi et al. (24) demonstrated recruitment of myeloid DCs in response to H. pylori infection in neonatally thymectomized mice. In addition, human monocyte-derived DCs pulsed with H. pylori membrane proteins are capable of stimulating T cell proliferation (22). A DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN) was shown to have carbohydrate-specific affinity for natural surface glycans of human pathogenic H. pylori (2).

Although the issue of whether IFN-γ-deficient mice can be vaccinated or not remains equivocal, IL-12-deficient mice failed to prevent H. pylori infection following vaccination (1, 13). Thus it is tempting to speculate that infected hosts fail to clear H. pylori due to the inability of the adaptive immune mechanism to induce a robust Th1 response. However, no ideal gastric pathogen exists to compare the adequacy of a Th1 response in achieving bacterial clearance in the stomach, because the commonly used bacterial controls (e.g., Escherichia coli and Campylobacter jejuni) do not cause gastritis. Our laboratory recently demonstrated the ability of A. lwoffi to induce H. pylori-like gastritis (36). Although A. lwoffi was not recovered from the infected mouse stomach, Acinetobacter 16S rRNA was found in the stomach tissue from inoculated animals, indicating the presence of low levels of replicating bacteria. In contrast, H. pylori was readily recovered from the infected mouse stomach. Evidently, both H. pylori and A. lwoffi induce gastric inflammation but differ in their ability to achieve chronic colonization of the mouse stomach. In fact, whereas H. pylori causes chronic gastritis in most infected persons, A. lwoffi normally is nonpathogenic in humans but is capable of causing opportunistic infections, such as septicemia, pneumonia, endocarditis, meningitis, wound sepsis, and urinary tract infections (27). We hypothesized that H. pylori induces a less-robust DC-mediated host response than that induced by A. lwoffi, which results in a failure to clear H. pylori from the...
overnight in Luria-Bertani (LB) medium at 37°C. For in vivo study, Diagnostics, Bedford, MA) supplemented with 5% sterile horse blood, and H. pylori/H9262 murine bone marrow cells were cultured in complete medium with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. The following recombinant cytokines (R&D Systems, Minneapolis, MN) were diluted in complete medium: mouse granulocyte/macrophage colony-stimulating factor (GM-CSF; 10 ng/ml) and mouse IL-4 (10 ng/ml).

Bacterial strains and culture conditions. Helicobacter species (H. pylori and H. felis) were grown on Campslobacter-selective agar (BD Diagnostics, Bedford, MA) supplemented with 5% sterile horse blood, trimethoprim (5 µg/ml), vancomycin (10 µg/ml), and nystatin (10 µg/ml) (37) for 2 days at 37°C in a humidified microaerophilic chamber (BBL Gas System, CampyPak Plus packs, BD Microbiology, Sparks, MD). Green fluorescent protein (GFP)-expressing H. pylori (designated as GFP-HP) was a kind gift of Andrew Wright of Tufts University and was constructed using the H. pylori M6 (type I) strain, which expresses GFP under the control of the hepsin promoter. The M6 strain, originally isolated from a human patient, can colonize mice and is easily transformed in vitro (10). In vitro experiments were performed using H. pylori SS1, H. pylori 399, and H. felis, which were all grown as described above. E. coli and A. lwoffi were cultured overnight in Luria-Bertani (LB) medium at 37°C. For in vivo study, GFP-HP [10^8 organisms in 200 µl of brain heart infusion (BHI) medium] was harvested and used to inoculate mouse stomachs by oral intubations.

H. pylori LPS isolation. H. pylori LPS was isolated from the SS1 strain using the Westphal method (33). Briefly, H. pylori culture was lyophilized and resuspended in distilled water at 68°C. Water-saturated phenol (Sigma-Aldrich, St. Louis, MO) was then added and then centrifuged to separate the phases. The water phase was further treated with protease K, RNase, and DNase (Sigma-Aldrich). The final sample was reextracted with water-saturated phenol to remove any remaining traces of protein, dialyzed against water, aliquoted, and lyophilized. Aliquots were concentrated 10 times and evaluated for remaining traces of protein, dialyzed against water, aliquoted, and reextracted with water-saturated phenol to remove any protein contamination and the condition of the LPS by Coomasie blue- and silver-stained SDS gels. Protein contamination (100 µg/ml) was determined by the absorbance spectrum from 300 to 260 nm. The endotoxin activity was 30 endotoxin units (EU/ml) as determined by a semiquantitative assay using E-Toxate kits (Sigma-Aldrich).

Generation of bone marrow-derived DCs. Erythrocye-depleted murine bone marrow cells were cultured in complete medium with 10 ng/ml GM-CSF and 10 ng/ml IL-4 at 1 x 10^6 cells/ml (17). On day 6, nonadherent DCs were harvested by vigorous pipetting and enriched by gradient centrifugation using the OptiPrep density solution (Sigma) according to manufacturer’s instruction. The low-density interface containing the DCs was collected by gentle aspiration. The recovered DCs were washed twice with RPMI-1640 and cultured in complete medium with GM-CSF (10 ng/ml).

Induction of proliferative splenocyte responses in vitro. Irradiated (5,000 rad) DCs were cocultured with syngeneic splenocytes (2 x 10^5 cells) from naïve mice for 72 h in complete medium. The proliferation of splenocytes was quantified using the ViaLight Plus Cell Proliferation and Cytotoxicity BioAssay Kit (Cambrex Bio Science Rockland, Rockland, ME) according to the manufacturer’s instruction.

Animal studies. C57BL/6 mice were inoculated with PBS or GFP-HP and killed 6, 48, and 120 h after inoculation. The mouse stomachs were removed and cut along the greater curvature. Strips (2 mm) composed of the fundus and antrum were embedded in Tissue-Tek optimum cutting temperature compound (Sakura Finetek, Torrance, CA) and immersed in liquid nitrogen; 4-µm sections were cut and stored at -80°C until immunofluorescent microscopy was performed. Paraffin sections were also prepared for hematoxylin and eosin (H&E) staining.

Immunohistochemistry. Frozen sections (5 µm thick) were fixed with cold acetone for 5 min and then washed with PBS. After a 30-min incubation with 10% goat serum and Fc Block (1 µg/100 µl, BD Biosciences Pharmingen, San Diego, CA), the sections were stained with either H&E, Alexa-488-conjugated anti-mouse CD11b antibodies, or PE-conjugated anti-mouse CD11c antibodies with their respective isotype controls (BD Biosciences Pharmingen). The sections were mounted with 6-diamidino-2-phenylindole (DAPI)-containing aqueous mounting solution to label the cell nuclei, and images were obtained with a fluorescent microscope (Olympus BX60 with a Diagnostics Instruments SPOT camera). In separate experiments, frozen sections of GFP-HP-infected stomachs were stained similarly with PE-conjugated CD11c and primary rabbit myeloperoxidase (MPO) Ab-1 (Lab Vision, Fremont, CA) with secondary Alexa-488-conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA).

Confocal microscopy. Bone marrow-derived DCs were cocultured with GFP-HP (1:100) for 3 h and then washed three times in PBS. DCs were labeled with PE-CD11c antibodies (1:100) for 1 h, and images were captured with an Olympus FV 300 confocal microscope. Composite images were generated using Adobe Photoshop.

RNase protection assay. Total RNA extracts from bone marrow-derived DCs were prepared using TRIzol reagent (Invitrogen) and analyzed by RNase protection assay (with a RiboQuant mCK-2b set, BD Biosciences Pharmingen). mRNA levels for IL-12p35, IL-12p40, IL-1ß, IL-1ß, IL-10, IL-6, IFN-γ, and migration inhibitory factor (MIF) were measured. GAPDH mRNA was quantified to normalize the results. The biotin-labeled riboprobes were transcribed using biotin-14-CTP (Invitrogen) and a MAXiScript T7/T3 In Vitro Transcription Kit (Ambion, Austin, TX). The RNase protection assay was performed using the SuperSignal RNA III chemiluminescent detection kit (Pierce, Rockford, IL) according to the manufacturer’s instructions. Results were quantified using ImageQuant software (Amersham Biosciences, Piscataway, NJ) and expressed as the fold change normalized by the value for the GAPDH band.

Fluorescence-activated cell sorting analysis. Bone marrow-derived DCs were washed twice with ice-cold PBS containing 0.5% BSA and sodium azide. After a 30-min incubation with Fc Block (1 µg/100 µl, BD Biosciences Pharmingen), the cells were incubated with FITC- and/or PE-conjugated antibodies or isotype control antibodies (1:100 dilution). These cells were washed, resuspended in ice-cold 2% paraformaldehyde, and analyzed using a Coulter XL Flow Cytometer (Hialeah, FL). For intracellular cytokine staining, cells were permeabilized with Perm/Fix Solution (BD Biosciences Pharmingen) before staining. Dot plots and histograms were obtained using WinMDI version 2.8.

Cytometric bead analysis. A mouse Th1/Th2 cytokine cytometric bead analysis kit was purchased from BD Biosciences Pharmingen and used according to the manufacturer’s instructions. Briefly, cell culture supernatants and standards were incubated with capture beads and PE detection reagent and analyzed with BD FACSCalibur (BD Biosciences, San Jose, CA) using software supplied by the manufacturer.

ELISA. Supernatants of DCs and T cell proliferation assays were collected and stored immediately at -20°C. ELISA kits for mouse IL-12p70, IL-10, and TNF-α (R&D Systems, Minneapolis, MN) were used according to the manufacturer’s instructions.

Whole cell sonicate and conditioned medium preparation. Bacteria were grown in LB or BHI medium overnight (~1 x 10^9 colony-forming units/ml). The cultures were spun down for 15 min at 36,000 rpm. The culture supernatants were designated as conditioned media. The endotoxin activity of the conditioned media used in DC coculture
experiments was 12 EU/ml as determined by a semiquantitative assay using the E-toxase kits (Sigma-Aldrich). In separate experiments, H. pylori-conditioned media were pretreated with polymyxin B (10 U/ml) for 6 h to neutralize the effect of LPS. The pellets were sonicated on ice as previously described (9) and then spun down for 10 min at 5,000 rpm. The supernatants were stored and designated as bacterial sonicate.

**RT-PCR.** Total RNA from the normal or H. pylori SS1-infected mouse stomach was extracted using TRIzol reagent and stored at −20°C. RNA (1 μg) was reverse transcribed using random primers in a 20-μl reaction according to the manufacturer’s instructions (reverse transcription system, Promega, Madison, WI). cDNA (5 μl) from the above reaction was used to amplify cDNA using PCR. Primer pairs C97 and C98 were used to amplify the 16S rRNA species specific for Helicobacter and to generate an amplicon of ~400 bp (12). The primer pairs used for amplifying IL-12p40 and IL-10 mRNA were designed according to Zhu et al. (38): IL-12 p40 (5'-GAA GTA TTC AGT GTC CTG CC-3' forward and 5'-TGT CTT CTC TAC GAG GAA CGC-3' reverse, 343 bp) and IL-10 (5'-CCC AGA AAT CAA GAA GAA TTT G-3' forward and 5'-CAT GTA TGC TTC TAT GCA GGT G-3' reverse, 211 bp). The GAPDH genes served as the loading control. In separate experiments, total RNA from bone marrow-derived DCs was extracted using TRIzol reagent. An additional primer pair was used: IL-12p35 (5'-GAG GAC TTG AAG ATG TAC CAG-3' forward and 5'-TTC TAT CTG TGT GAG GGC-3' reverse, 325 bp).

**Statistical analysis.** Statistical significance was determined by an unpaired Student’s t-test using commercially available software (PRISM, GraphPad, San Diego, CA). P < 0.05 was considered significant.

**RESULTS**

Recruitment of DCs to mouse stomach during acute H. pylori infection. DCs are not found in healthy gastric mucosa. Therefore, to determine whether DCs are recruited to the stomach after H. pylori colonization, we examined GFP-HP-infected stomachs at early time points of acute H. pylori infection. Naïve mice infected with actively growing GFP-HP recruited gastric CD11c+ DCs as early as 6 h postinfection (Fig. 1A) and continued to do so 48 h after infection. These CD11c+ cells coexpress MHC class II molecules and are thus less likely to be polymorphonuclear neutrophils, which also express low levels of CD11c (Fig. 1B). Further characterization of these CD11c+ cells for the presence of MPO, which is expressed by neutrophils, showed that CD11c+ cells in the infected mouse stomach do not express MPO (Fig. S3; supplemental data may be found at http://ajpgi.physiology.org/cgi/content/full/00139.2005/DC1). Visualization of GFP-HP on the epithelial surface of the gastric antrum (Fig. 1C) and the detection of Helicobacter-specific 16S RNA by RT-PCR (Fig. 1D) confirmed that these mice were colonized with GFP-HP. Of note, DCs were rarely seen 120 h after infection (Fig. 1A). Thus we demonstrated that during acute H. pylori infection, DCs are recruited fairly rapidly to the stomach and then leave the stomach. We speculate that the recruited DCs may migrate to the nearby draining lymph nodes to encounter resting lymphocytes. The rapid recruitment and disappearance of DCs in response to antigen challenge has also been shown in the lungs (25). We also detected a slight increase in tissue IL-12 mRNA expression 48 h postinfection, indicating possible activation of DCs by H. pylori in vivo.

Characterization of bone marrow-derived DCs. Isolation of adequate samples of DCs from H. pylori-infected stomachs to characterize DC function and response further was not possible. Therefore, we decided to assess the responsiveness of bone marrow-derived DCs to H. pylori. The identity and purity of DCs were verified by morphological and surface markers. The typical appearance of bone marrow-derived DCs with long surface projections was observed in culture (Fig. S1A). The purity of DCs, which was determined by the presence of DC surface markers (e.g., CD11c and MHC class II molecules), ranged from 70% to 80% (Fig. S1B). Surface costimulatory molecules characteristic of DCs were also expressed (i.e., CD54, CD80, and CD86). The intracellular cytokine contents of these DCs showed TNF-α but not IFN-γ, IL-12, or IL-10 expression (Fig. S1C). Overall, the surface marker and cytokine profiles observed were characteristic of immature DCs.

**DC-phagocytosed H. pylori.** DCs are capable of taking up bacterial products by means of phagocytosis, an ability that is enhanced in immature DCs. After antigen uptake, DCs become activated and process phagocytosed antigens for presentation to T cells (4). To demonstrate that DCs take up H. pylori, we used GFP-HP to trace the intracellular localization of H. pylori. GFP-HP cocultured with DCs for 3 h and the cellular localization of GFP-HP within DCs was determined both by fluorescent and confocal microscopy. Figure 2 shows a micrograph of GFP-HP within DCs and confirmation of intracellular GFP-HP by a Z-series confocal microscopic image. In addition, we observed the colocalization of GFP-HP with a CD11c+ cytosolic vesicle and speculated this may represent a phagosome containing GFP-HP (Fig. 2B). These results clearly demonstrate the ability of DCs to take up H. pylori.

H. pylori stimulates the expression of proinflammatory cytokines. To determine the cytokine profile of DCs after an encounter with H. pylori SS1, cytokine mRNA profiles of PBS-treated and H. pylori-stimulated DCs were compared using an RNase protection assay. H. pylori stimulation for 18 h induced increased levels of IL-1α, IL-1β, and IL-6 mRNA (fold change: 6.05 ± 0.12, 12.69 ± 0.29, and 3.59 ± 0.53, respectively, n = 3, P < 0.05). No significant increase in IL-12p35, IL-10, IL-18, IL-1Ra, and IFN-γ was detected (Fig. 3B). The level of MIF was lower after H. pylori stimulation (~0.57 ± 0.09-fold). These findings suggest that DCs may contribute to the maintenance of gastritis by significantly increasing the production of proinflammatory cytokines such as IL-1α, IL-β, and IL-6. Interestingly, mRNA levels for the immunomodulatory cytokine IL-12p40 were only slightly elevated, supporting our hypothesis that DCs may not induce a robust Th1 response against H. pylori. Similar results were found using RT-PCR (Fig. 3C). The lack of IL-10 expression and the modest IL-12 expression are consistent with the pattern seen in vivo (Fig. 1E).

H. pylori induces lower IL-12 release by DCs compared with A. lwoffi. It has been suggested that H. pylori may not mount a sufficiently vigorous Th1 immune response to eradicate the bacteria (8). IL-12 is an important Th1-type cytokine secreted by APCs necessary for Th1 priming. Therefore, we compared DC IL-12 release stimulated by H. pylori vs. another gastritis-causing bacteria, A. lwoffi. We found that H. pylori stimulation increased the production of IL-12 by DCs, and this response was significantly lower than that evoked with A. lwoffi stimulation (Fig. 4A). To determine whether H. pylori-stimulated DCs favor Th1 or Th2 priming, we also measured IL-10 release by DCs and found a lower level of IL-10
compared with IL-12 in both *H. pylori* and *A. lwoffi* groups (Fig. 4B). Similar results occurred when *H. pylori* was compared with *E. coli*, another immunostimulatory bacteria. These findings suggest that both organisms favor Th1 priming and that *H. pylori* induced a weaker response than *A. lwoffi*.

**Comparison of the Th1 response by *H. pylori* vs. *A. lwoffi***

To determine whether *H. pylori*-stimulated DCs can prime a Th1 helper cell response, mixed leukocytes derived from naïve mouse spleens were cocultured with stimulated DCs for 72 h. The Th1/Th2 cytokine release was quantified, and cell proliferation was measured. As Fig. 5A shows, *H. pylori*-stimulated DCs were capable of inducing the release of IFN-γ and TNF-α (Th1 cytokines) from splenocytes, but the response was significantly less than that seen with *A. lwoffi*. After coculture for 72 h, IL-2 was undetectable in all groups (data not shown). Cell proliferation measurement after a 72-h stimulation revealed a DC-dependent proliferative response in the *H. pylori* and *A. lwoffi* groups (Fig. 5B); and, again, the *H. pylori* group exhibited a lower proliferative response compared with that of *A. lwoffi*.

*H. pylori*-secreted factors inhibit activated DC IL-12 release. To address whether the induction of a weaker Th1 response by *H. pylori*-stimulated DCs is a result of poor bacterial immunogenicity or inhibited activation of DCs, we investigated whether *H. pylori* bacterial products inhibited the release of IL-12 by *A. lwoffi*-stimulated DCs. We found that *H. pylori* SS1 whole cell sonicate as well as *H. pylori* SS1-conditioned medium both significantly inhibited IL-12 release by *A. lwoffi*-stimulated DCs (Fig. 6A), indicating that *H. pylori* can inhibit the DC adaptive immune function by suppressing the release of IL-12. Interestingly, IL-10 release was not significantly affected (Fig. 6B). Because DC viability may be different between experimental conditions and may affect our interpretation of the data, we performed a trypan blue exclusion assay to determine the viability of DCs after they had been cocultured with *A. lwoffi* in the presence of *H. pylori*-conditioned medium or BHI (control medium). We found no difference in DC viability between DCs cocultured with *A. lwoffi* or DC with *A. lwoffi* and *H. pylori*-conditioned medium (viable-to-nonviable ratio: 0.80 vs. 0.83, respectively). This is also supported by the finding that both had similar levels of IL-10 (Fig. 6B) and TNF-α (821.8 ± 1.5 vs. 816.2 ± 3.8 pg/ml, respectively, *n* = 3, *P* > 0.05). The *H. pylori* factors appeared to be heat stable, because incubation at 65 or 100°C for 10 min did not abolish the inhibition of IL-12 release (Fig. 6A). This inhibitory property, which was observed in other *Helicobacter* species (e.g., *H. pylori* J99 and *H. felis*) but not in *E. coli*, is independent of *H. pylori*-derived LPS (Fig. 6C). Further support for an LPS-independent mechanism was shown by the use of polymyxin B to block the effect of LPS. Polymyxin B is known to deorganize the cell wall of gram-negative bacteria and, therefore, can be used to prevent LPS activity potentially present in the *H. pylori*-conditioned media. Polymyxin B did not reverse the inhibitory activity of *H. pylori* J99-conditioned medium on DC IL-12 release (Fig. S4).

**DISCUSSION**

Our current understanding of how *H. pylori* induces chronic gastritis is based on a paradigm that has yet to incorporate recent knowledge regarding the importance of DCs in mucosal immunity (31). Whereas many studies have implicated the vital role of DC-mediated adaptive immunity in their response to a variety of intestinal bacterial pathogens (20, 21, 30), the role of DCs in *H. pylori*-induced gastritis is not well understood. Because vaccine-induced protection against *H. pylori* may require a Th1-adaptive immune response (1), the prospect of DCs playing a central role in determining whether an infected

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**Fig. 2.** Phagocytosis of GFP-HP by DCs. Actively growing GFP-HP was cocultured with bone marrow-derived DCs for 3 h. **A**: fluorescent microscopic image shows an arrow pointing to GFP-HP (green) within a cultured DC (×1,000). **B**: bone marrow-derived DCs were cocultured with GFP-HP for 3 h and then stained with PE-conjugated (red) anti-CD11c antibodies to identify the DC surface membrane. Z-series confocal microscopic images were taken and showed GFP-HP within a DC (solid arrow) and the overlap of GFP-HP with a CD11c-expressing vacuole (arrowhead).

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**Fig. 1.** Dendritic cells (DCs) are recruited to the stomach during acute *Helicobacter pylori* (HP) infection. C57BL/6 mice were orally inoculated with green fluorescent protein-expressing *H. pylori* (GFP-HP) and analyzed for inflammation and DC recruitment at 6, 48, and 120 h after infection. Uninfected mice served as controls. **A**: time course of acute *H. pylori* gastritis and DC recruitment. Paraffin sections of stomachs were stained with hematoxylin and eosin (H&E; top, magnification ×400), and frozen sections of stomachs were stained with PE-conjugated anti-mouse CD11c antibodies for DCs (red) and counterstained with 6-diamidino-2-phenylindole (DAPI; blue) to outline the background (bottom, magnification ×400). Arrows indicate increased inflammatory infiltrate at 48 h. **B**: CD11c+ cells also express major histocompatibility complex (MHC) class II molecules. Frozen sections of mouse spleens (positive control) and stomachs from mice infected with GFP-HP for 48 h were dual labeled with PE-conjugated CD11c and FITC-conjugated MHC class II antibodies. Left, merged image of dual-stained spleen section (×400) right, merged image of a stomach section 48 h after infection. Arrows indicate a single DC within the lamina propria of the stomach. **C**: GFP-HP colonization of infected mouse stomach 48 h after oral inoculation. Arrows indicate the presence of GFP-HP on the surface epithelium of the stomach (×1,000). **D**: RT-PCR using uninfected and *H. pylori*-infected mouse stomach RNA to amplify 16S rRNA species specific for *Helicobacter* confirmed *H. pylori* colonization 48 h after infection. **E**: expression of IL-12 mRNA but not IL-10 was upregulated 48 h after infection. No increase in IL-10 expression was measured. Data shown represent 1 of 3 independent experiments.
host clears the bacteria or goes on to develop chronic inflammation seems plausible. In fact, such a model was shown in a study of Listeria monocytogenes infection of mice, in which susceptibility to microbes was shown to be associated with DC responsiveness (21). We speculate that DCs may play a role in mediating H. pylori gastritis.

A central argument against the involvement of DCs in H. pylori infection is the lack of evidence that DCs exist in the stomach (6, 18, 30). This assertion was recently challenged by Nishi et al. (24), who showed gastric recruitment of myeloid DCs 12 wk after H. pylori infection in a neonatally thymectomized mouse model. DC involvement in the first 7 days of acute H. pylori infection, however, has not been studied. Because the host immune response during an acute infection is likely to determine the final outcome, that is, eradication or colonization, it was critical to ascertain whether DCs are recruited during acute H. pylori infection. We showed in C57BL/6 mice that acute infection with H. pylori resulted in an influx of CD11c+ DCs to the lamina propria of the antral stomach at 6 h and disappeared by 5 days. The possibility that the observed CD11c+ cells were neutrophils was addressed by the coexpression of MHC class II molecules, an APC marker normally absent on neutrophils (Fig. 1B), and the lack of neutrophil marker, MPO (Fig. S3). These data suggest a biphasic mucosal immune response with the development of acute inflammation during the first 3 days, which then resolves 1 wk after H. pylori infection. A second or chronic phase begins at around 12 wk after infection. It is conceivable that a failure to eradicate H. pylori during the acute phase may result in an aberrant immune response leading to chronic gastritis.

The presence of DCs during acute Helicobacter infection may signify the recognition of H. pylori by DCs. Several

Fig. 3. H. pylori-stimulated DCs express high levels of IL-1α, IL-1β, and IL-6 but a low level of IL-12. Bone marrow-derived DCs were cocultured with PBS or H. pylori SS1 for 18 h, after which, cytokine mRNA levels were measured by RNase protection assay. A: cytokine mRNA bands detected by cytokine-specific RNA probes. B: fold change in cytokine mRNA levels normalized to GAPDH. Data are shown as means ± SE from 3 independent experiments.

Fig. 4. H. pylori-stimulated DCs secrete less IL-12 and IL-10 than A. lwoffi-stimulated DCs. Bone marrow-derived DCs were cocultured with PBS, H. pylori SS1, A. lwoffi, or E. coli (EC) for 18 h. The amount of IL-12p70 (A) and IL-10 (B) released by DCs was determined by ELISA. Data are shown as means ± SE from 3 independent experiments. *P < 0.05 compared with the H. pylori group.
reports have shown that H. pylori stimulates the maturation and cytokine production of human monocyte-derived DCs (15, 16, 19). The observation of GFP-HP within a cytosolic vesicle shows that DCs can take up H. pylori (Fig. 2B). Although the specific mechanism for the uptake is unknown, the DC surface receptor DC-SIGN has been described to have high affinity for H. pylori surface glycans (2) and DC-SIGN is believed to play an important role in allowing DCs to engulf pathogens (4). It is not clear whether a noninvasive pathogen such as H. pylori comes in contact with APCs in the lamina propria; however, DCs have been shown to traverse the tight junction of the intestinal epithelium to sample luminal bacteria (29) and may have the ability to sample mucosal H. pylori in the stomach as well.

Attempts to understand the pathogenic mechanisms that lead to H. pylori gastritis have been inconclusive. H. pylori infection induces a vigorous systemic and mucosal humoral response (26). This antibody production does not lead to eradication of the pathogen and chronic infection ensues. Evaluation of mucosal H. pylori-specific immune responses has shown that H. pylori-specific T cells generally present a Th1 phenotype (32). Moreover, stimulation of human monocyte-derived DCs by live H. pylori in vitro results in a Th1-biased response (15, 16, 19). Furthermore, the ability to promote a Th1 response is required for vaccine-induced protective immunity against H. pylori. Several immune escape mechanisms have been proposed that include the induction of Fas-mediated apoptosis of H. pylori-specific T cell clones by H. pylori (32) and the presence of H. pylori-specific regulatory T cells that actively suppress memory CD4+ T cell responses to H. pylori (22). We speculate that another contributing factor is the suppression of APCs by H. pylori.
secreted proteins, because VacA, an *H. pylori*-secreted protein, has been shown to interfere with B cell antigen presentation (23).

Our data revealed that the level of IL-12 released and the induction of the Th1 response by DCs was significantly lower in the *H. pylori*-stimulated group when measured against another gastritis-causing gram-negative bacteria, *A. lwoffi*. Differences in the kinetics of cytokine induction between *H. pylori* and *A. lwoffi* may explain why *H. pylori*-stimulated DCs release lower levels of IL-12. We performed time-course experiments using *H. pylori* SS1 and *A. lwoffi* and showed that for each time point, IL-12 release was higher in the *A. lwoffi*-stimulated group than for the *H. pylori*-stimulated group. The dose-response experiments also showed higher IL-12 release with *A. lwoffi* at three different multiplicity of infection (Fig. S2). Thus the low level of IL-12 released by *H. pylori*-stimulated DCs is not due to differences in bacterial stimulatory kinetics.

Two possible explanations for the low IL-12 release by *H. pylori*-stimulated DCs are 1) *H. pylori* is weakly immunogenic and 2) *H. pylori* inhibits DC IL-12 release. Our data support the latter in that the secreted factors were present in the *H. pylori*-conditioned medium (Fig. 6, A and C). The inhibitory activity was not significantly affected by the 10-min heat treatment, neither at 65°C nor at 100°C, which suggests that the secreted factors are nonproteinaceous or small peptides. However, Bergman et al. (3) reported that carbohydrate-rich Lewis antigens (Le*) on *H. pylori* LPS can bind to a DC receptor DC-SIGN by means of carbohydrate recognition domains, leading to suppression of Th1 development and DC IL-6 release. Our data indicate that other LPS-independent pathways are likely involved in the observed inhibition of DC IL-12 release because *H. pylori* LPS failed to suppress DC IL-12 release (Fig. 6A) and polymyxin B failed to block the inhibitory activity (Fig. S4). Further support comes from a study using human DCs, which reports that the addition of polymyxin B (1 µg/ml) has no effect on *H. pylori*-induced MHC class II and IL-12 upregulation (16). One would expect polymyxin B treatment to increase IL-12 release if DCs were inhibited by *H. pylori* LPS in that study.

The failure to induce a strong protective Th1 response against *H. pylori* would permit bacterial persistence and possibly lead to chronic gastritis, perhaps due to chronic antigenic stimulation of the APCs in the lamina propria. As our data indicate, DCs respond to *H. pylori* by upregulating IL-1α, IL-1β, and IL-6, cytokines that have been shown to be present in large amounts in the stomach tissue of patients with *H. pylori* gastritis (5, 34, 35). DCs may be a significant contributor in fueling the inflammatory reaction seen in *H. pylori* gastritis. Further in vivo studies are required to confirm the above findings.

In summary, the response of DCs recruited to the stomach during an acute *H. pylori* infection is inhibited by *H. pylori*-secreted factors. Our work describes a potentially defective host immune mechanism against *H. pylori*, which, after further research, may contribute to the development of effective vaccine strategies against *H. pylori*.

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**REFERENCES**

H. PYLORI INHIBITS DENDRITIC CELL IL-12 RELEASE