Helicobacter pylori-Pulsed Dendritic Cells Induce *H. pylori*-Specific Immunity in Mice

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Vaccine, dendritic cells, Th1/Th2, regulatory T cells, CD25 depletion.

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Abstract

Background: The growing concern over the emergence of antibiotic-resistant *Helicobacter pylori* infection is propelling the development of an efficacious vaccine to control this highly adaptive organism.
Aim: We studied the use of a dendritic cell (DC)-based vaccine against *H. pylori* infection in mice.
Methods: The cellular immune responses to murine bone marrow-derived DCs pulsed with phosphate-buffered saline (PBS-DC) or live *H. pylori* SS1 (HP-DC) were assessed in vitro and in vivo. The protective immunity against *H. pylori* SS1 oral challenge was compared between HP-DC or PBS-DC immunized mice. The effect of regulatory T-cell (Treg) depletion by anti-CD25 antibody on HP-DC vaccine efficacy was also evaluated.
Results: HP-DC induced a Th1-dominant response in vitro. In vivo, HP-DC immunized mice were characterized by a mixed Th1/Th2 peripheral immune response. However, in the stomach, HP-DC immunized mice expressed a higher level of IFN-γ compared to PBS-DC immunized mice; no difference was found for interleukin-5 expressions in the stomach. A lower bacterial colonization post-*H. pylori* challenge was observed in HP-DC immunized mice compared to PBS-DC immunized mice with no significant difference in gastritis severity. *H. pylori*-specific Th1 response and protective immunity were further enhanced in vivo by depletion of Treg with anti-CD25 antibody.
Conclusion: DC-based anti-*H. pylori* vaccine induced *H. pylori*-specific helper T-cell responses capable of limiting bacterial colonization. Our data support the critical role of effector cellular immune response in the development of *H. pylori* vaccine.

Helicobacter pylori causes chronic gastritis in most infected individuals and it is associated with gastroduodenal ulcers and malignancies. Although *H. pylori* can be eradicated by a combination of antibiotics and proton-pump inhibitors, the emergence of antibiotic-resistant *H. pylori* strains [1–3] remains a significant threat to the half of the world's population infected with this pathogen. An improved understanding of the interplay between *H. pylori* and the host immune response will contribute to the development of an effective immunotherapy targeting *H. pylori*.

Studies in mice have revealed that interleukin (IL)-12, a type 1 helper T cell (Th1)-promoting cytokine, plays a critical role in vaccine-induced protective immunity [4,5]. We have previously shown that dendritic cells (DCs) pulsed with *H. pylori* (HP-DC) secrete lower levels of IL-12 than *Escherichia coli* or *Acinetobacter lwoffi*, possibly leading to an ineffective host response and failure to eradicate *H. pylori* [6]. Since immunization with *ex vivo* antigen-pulsed DCs leads to the induction of in vivo antigen-specific protective immunity against tumors [7], we speculate that an HP-DC vaccine may prime effective protective immunity against *H. pylori.* Although several prototype vaccines for *H. pylori* have been assessed with some degree of success [8,9], the advantage of DC vaccine is the priming of potent adaptive immunity which, in tumor models, were shown to be more efficacious than non-DC-based vaccines [10]. The goal of the current study was to examine the efficacy of an HP-DC vaccine against *H. pylori* infection in vivo.

Our results showed that HP-DC stimulate the in vitro proliferation of Th1 cells. In vivo, HP-DC stimulated

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H. pylori-specific Th responses, including Th1 and Th2, and a lower bacterial colonization was observed in HP-DC mice compared to DCs pulsed with phosphate-buffered saline (PBS-DC). Depletion of Tregs with anti-CD25 antibodies in mice immunized with HP-DC augments *H. pylori*-specific immunity. These data indicate that the goal of *H. pylori* vaccine should include both augmentation of Th1 effector response and controlled Treg response.

Materials and Methods

Mice

Specific pathogen-free female C57BL/6 mice aged 8–10 weeks were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and housed in the Animal Maintenance Facility at the University of Michigan Health System. Experiments were conducted on mice aged 10–14 weeks. All animal experiments were approved by the University of Michigan Animal Care and Use Committee.

Media, Cytokines, and Neutralizing Antibodies

Complete medium (CM) consisted of RPMI-1640 with 10% heat-inactivated fetal calf serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Two recombinant cytokines (R&D Systems, Minneapolis, MN, USA) were diluted in CM: mouse granulocyte/macrophage colony-stimulating factor (GM-CSF, 10 ng/mL) and mouse IL-4 (10 ng/mL). The antibodies used to deplete CD25+ cells were purified using protein G columns (Amersham Biosciences, Piscataway, NJ, USA) from the supernatant of the PC61 hybridoma cell line (American Type Culture Collection, Manassas, VA, USA).

Bacterial Strains and Culture Conditions

H. pylori (SS1 strain) organisms were grown on *Campylobacter*-selective agar (BD Diagnostics, Bedford, MA, USA) supplemented with 5% sterile horse blood, trimethoprim (5 μg/mL), vancomycin (10 μg/mL), and nystatin (10 μg/mL) [11] for 2 days at 37 °C in a humidified microaerophilic chamber (BBL Gas System, with CampyPak Plus packs, BD Microbiology, Sparks, MD, USA).

Generation of Bone Marrow-Derived DCs

Erythrocyte-depleted murine bone marrow cells were cultured in CM with GM-CSF (10 ng/mL) and IL-4 (10 ng/mL) at 1×10^6 cells/mL [12]. On day 6, nonadherent bone marrow-derived DCs were harvested by vigorous pipetting and enriched by gradient centrifugation using the Optiprep density solution (Sigma, St. Louis, MO, USA). The low-density interface containing the DCs was collected by gentle aspiration. The recovered bone marrow-derived DCs were washed twice with RPMI-1640 and cultured in CM with GM-CSF (10 ng/mL).

Induction of Proliferative T-cell Responses In Vitro

After overnight stimulation with *H. pylori* SS1 (1×10^8 CFU/mL) followed by washing to remove bacteria, irradiated (5000 rads) bone marrow-derived DCs (1×10^4 cells/mL) were cocultured with naïve syngeneic C57BL/6 splenocytes (1×10^5 cells/mL) for 48 hours. Tritiated deoxythymidine (Amersham Biosciences) was added to each microtiter well (1 μCi/well), and the plates were incubated for 24 hours [7]. The plates were harvested at completion and the radioactivity was measured using a scintillation counter. Responses were reported as the mean count per minute ± standard error of the mean (SEM) from duplicate samples in three separate experiments.

Animal Studies

Naïve C57BL/6 mice (n = 15 per group) were given an intraperitoneal (IP) injection of *H. pylori* SS1 (1×10^8 CFU/mL)-stimulated DCs (10^6 cells per injection) on day 0 and day 14. Phosphate-buffered saline-treated unstimulated DCs served as controls. Beginning on day 21, all mice were infected with *H. pylori* SS1 – an oral gavage of 10^8 colony-forming units (CFU) per milliliter of live organisms was given to each mouse, three times over 1 week. Mice were sacrificed 120 days after the first infection on day 0, and spleens and stomachs were removed for analyses. The stomachs were cut along the greater curvature into 2-mm strips that included fundic and antral tissue. Paraffin sections were prepared for hematoxylin and eosin (H&E). The spleens were flushed with RPMI-1640 and CD4+ T cells were isolated using Magnetic-Activated Cell Sorting (MACS) microbeads (Miltenyi Biotech, Auburn, CA, USA). Histologic scores were determined as mean ± SEM according to the Eaton scoring method [13]. The pathologist was blinded to the experimental manipulation of each mouse to eliminate observer bias.

Delayed-Type Hypersensitivity Measurement

To measure delayed-type hypersensitivity, mice were given 10 μg of *H. pylori* sonicate by injection into the right hind footpad 1 day before sacrifice. The left hind footpad was given an equal volume of sterile saline. Footpad thickness was measured with a dial thickness gauge 24 hours later, immediately before sacrifice, and the difference in thickness between the control and the sonicate-treated footpads was recorded.

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Cytometric Bead Analysis

Mouse Th1/Th2 Cytokine Cytometric Bead Array Kit (BD Biosciences PharMingen, San Diego, CA, USA) was 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 the BD FACSCalibur™ system (BD Biosciences, San Jose, CA, USA) using software supplied by the manufacturer.

Preparation of Conditioned Medium and Whole Cell Sonicate

Bacteria were grown overnight at a concentration of 1×10^9 CFU/mL. *H. pylori* was grown in brain–heart infusion medium. The cultures were spun down for 15 minutes at 14,500 ***g***. The culture supernatants were designated as conditioned media. The pellets were sonicated on ice as previously described [14] and then spun down for 10 minutes at 280 ***g***. The supernatants were stored and designated as bacterial sonicate.

Quantification of *Helicobacter* Colonization and Gastric Tissue mRNA for IFN-γ and IL-5 by Real-Time Polymerase Chain Reaction

A standard curve was generated by extracting total RNA, using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), from *H. pylori SS1* bacterial cultures with densities ranging from 10^3 to 10^9 total bacteria. Total RNA also was isolated from stomach tissue using TRIzol Reagent. Primer pairs C97 and C98 [15] were used to amplify the 16S rRNA species that is specific for *Helicobacter*, generating an amplicon of about 400 base pairs [16]. Additional primer sequences used were: IFN-γ (sense: GGCTGTTTCTGGCT GTTACTGCCACG, antisense: GACAATCTCTTCCCCACCCC GAATCAG) and IL-5 (sense: GCAATGGAAGGCTGAGGCTG, antisense: GGGTATGTGATCCTCCTGCGT C). Polymerase chain reaction (PCR) amplifications were performed in a total volume of 25 μL, containing 10 × PCR buffer with $MgCl_2$, 10 nmol/L dNTPs, 200 nmol/L primers, 5 μL cDNA, 100 nmol/L Taq polymerase GOLD, and 2.5 μL Sybr Green (Molecular Probes, Carlsbad, CA, USA). Each PCR amplification was performed in duplicate wells in a Bio-Rad I-Cycler (I-Cycler IQ Real-Time PCR Detection System, Bio-Rad Laboratories, Hercules, CA, USA) at 94 °C for 10 minutes, followed by 35 two-temperature cycles at 94 °C for 1 minute and 55 °C for 1 minute.

Fluorescence-Activated Cell Sorting (FACS)

DCs were washed twice with ice-cold PBS containing 0.5% bovine serum albumin and sodium azide. After a 30-minute incubation with Fc Block (1 μg/100 μL, BD Biosciences PharMingen), the cells were incubated with either FITC and/or PE-conjugated antibodies or with isotype control antibodies (1 : 100 dilution). The cells were washed, resuspended in ice-cold 2% paraformaldehyde, and analyzed using a Coulter XL Flow Cytometer (Beckman Coulter, Miami, FL, USA). For intracellular cytokine staining, cells were permeabilized with Perm/Fix Solution (BD Biosciences PharMingen) before staining. Both dot plots and histograms were obtained using WinMDI version 2.8. The percentage of CD4+CD25+ Treg determination was measured using Mouse Regulatory T Cell Staining Kit (eBioscience, San Diego, CA, USA).

H. pylori-Specific In Vitro Stimulation

Erythrocyte-depleted splenic CD4+ T cells (1×10^5 cells/mL) were isolated using MACS (Miltenyi Biotech) and then stimulated for 7 days with syngeneic bone marrow-derived DCs (1×10^6 cells/mL) and *H. pylori* SS1 sonicate (5 μg/mL). CD4+ T cells stimulated with DCs alone or *H. pylori* sonicate alone served as controls.

Enzyme-Linked Immunosorbent Spot (ELISpot)

In vitro stimulated *H. pylori*-specific CD4+ T cells were cultured with bone marrow-derived DCs (1 : 10) and *H. pylori* SS1 sonicate (10 μg/mL) for 24 hours (to measure IFN-γ secretion) or for 48 hours (to measure IL-5 secretion). ELISpot (BD Biosciences Pharmingen) assays were performed.

In Vivo Depletion of Regulatory T Cells (Tregs)

C57BL/6 mice (n = 9 or 10 per group) were treated IP with either PBS or a single 1-mg dose of anti-CD25 mAb (PC61). The success of Treg depletion was determined after 30 days by FACS analysis of isolated splenocytes.

Statistical Analysis

Statistical significance was determined by nonparametric Student *t*-test using commercially available software (PRISM, GraphPad, San Diego, CA, USA). $p < .05$ was considered significant.

Results

H. pylori-Pulsed DCs Stimulated Th1 Response In Vitro

The activation of antigen-presenting cells by bacterial antigens can be measured by the surface expression of

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costimulatory molecules (e.g. CD80 and CD86) and by the level of intracellular cytokine expression. To determine whether *H. pylori* SS1 activates DCs, bone marrow-derived DCs were cocultured with live *H. pylori* SS1 or PBS for 18 hours and cell surface expressions of CD40, CD80, and CD86 and intracellular cytokine expression were determined by FACS analysis. As shown in Fig. 1A, *H. pylori* stimulated a significant upregulation of IL-12, which suggests that bone marrow-derived DCs recognize *H. pylori* in vitro and may prime a Th1 response. To determine the type of Th response induced by HP-DC, we measured the ability of bone marrow-derived DCs to stimulate T-cell proliferation of splenocytes and the Th1/Th2 cytokine profiles of the proliferating cells. HP-DC induced significantly higher levels of cellular proliferation than PBS-DC in a dose-dependent fashion (Fig. 1B,C). The cytokine profiles of the proliferating cells as determined by cytometric bead assay showed a Th1-dominant response with increased levels of tumor necrosis factor (TNF)-α and interferon (IFN)-γ and no measurable levels of IL-4 or IL-5 (Fig. 1D). These data suggest that HP-DC induce a Th1-dominant response in vitro, a finding that is consistent with reports using human monocyte-derived DCs [17,18].

HP-DC Induced a Delayed-Type Hypersensitivity Reaction Against *H. pylori* Sonicate In Vivo

Research suggests that the induction of an *H. pylori*-specific Th1 response is a crucial component of protective immunity against *H. pylori* [4,5]. Since HP-DC prime a Th1 response in vitro, we examined this response in vivo. Using a general protocol for inducing protective antitumor immunity, mice were injected IP with HP-DC or PBS- PBS-DC. A booster injection was given 2 weeks later. The mice were then challenged with live *H. pylori* to assess the induction of anti-*H. pylori* immunity. After footpad injection with *H. pylori* SS1 sonicate to measure *H. pylori*-specific delayed-type hypersensitivity reaction, a significant increase in footpad thickness was measured in the HP-DC injected mice compared to the PBS-DC injected mice (Fig. 2). These data indicate that HP-DC induce *H. pylori*-specific cellular immune responses.

Induction of *H. pylori*-Specific Th1 and Th2 Responses In Vivo

To determine the ability of HP-DC to induce *H. pylori*-specific adaptive immune responses, CD4+ T cells isolated from splenocytes of PBS-DC and HP-DC mice were stimulated *ex vivo* with *H. pylori* sonicate. Naïve BM-DCs were included as stimulators. ELISpot assays showed significantly higher numbers of *H. pylori*-specific Th1 (i.e. IFN-γ-producing CD4+) and Th2 (i.e. IL-5-producing CD4+) cells in HP-DC mice (Fig. 3A,B). Thus, HP-DC are capable of inducing Th1 and Th2 responses in vivo.

Induction of a Th1-dominant Gastric Response in HP-DC Mice

To characterize further the effective of HP-DC on host response to *H. pylori* infection, total gastric RNA of mice was assessed for IFN-γ and IL-5 expressions in order to better define the Th1/Th2 responses at the gastric tissue level. We found increased gastric IFN-γ mRNA in HP-DC mice compared to PBS-DC mice and no measurable difference for the IL-5 mRNA expression (Fig. 4A). Despite detecting a mixed Th1/Th2 response in the peripheral (spleen) immune compartment, HP-DC induced a Th1-dominant response in the gastric tissue. This finding indicates HP-DC primes a Th1-dominant response in the gastric tissue despite a mixed Th1/Th2 in the periphery. Therefore, it is critical to evaluate the gastric Th response separately from peripheral Th response when determining in vivo *H. pylori*-specific immunity.

A Reduced *H. pylori* Colonization was Observed in HP-DC Mice Compared to PBS-DC Mice

To determine whether the *H. pylori*-specific Th1-dominant response induced by HP-DC could limit *H. pylori* colonization, we performed quantitative reverse transcriptase (RT)-PCR, which is a sensitive method of determining the number of bacterial CFU in the stomach. There was a trend toward lower bacterial colonization in HP-DC mice compared to PBS-DC mice ($p = .1$) (Fig. 4B). To evaluate whether this trend was due to more severe gastritis in HP-DC mice, the degree of gastritis also was assessed using the Eaton gastritis score for *H. pylori*-induced gastritis [14]. Quantification of *H. pylori* by the culture method showed a similar trend (data not shown). No significant differences in gastritis or polymorphonuclear neutrophil infiltration scores were measured (Fig. 4C,D). Thus, HP-DC induced a *H. pylori*-specific Th response and reduced *H. pylori* colonization.

Depletion of Tregs by CD25 Antibody Further Enhanced the Ability of HP-DC Mice to Prime *H. pylori*-Specific Immunity

H. pylori-infected individuals have been shown to express higher levels of Foxp3, a regulatory T-cell (Treg) marker. We speculate that a depletion of Tregs by the anti-CD25 antibody PC61 may further enhance the ability of HP-DC to limit *H. pylori* colonization. To examine this, PC61-treated or nontreated mice vaccinated with HP-DC were challenged with *H. pylori* and then analyzed 7 days

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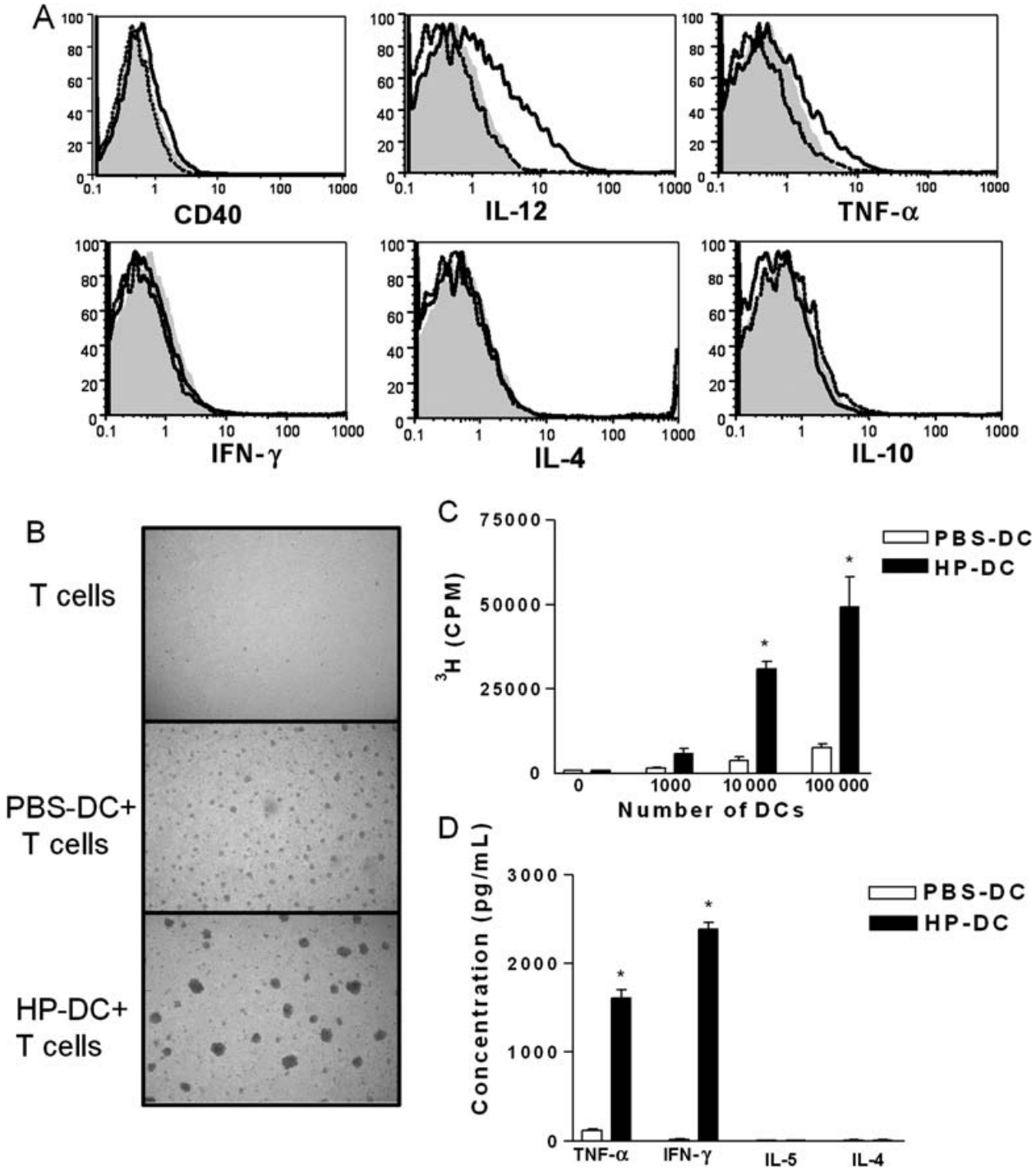

Figure 1 *H. pylori*-pulsed dendritic cells (HP-DC) induce Th1 helper response in vitro. (*A*) *H. pylori* induces increased expression of DC costimulatory molecules and intracellular cytokines. DCs were cocultured with PBS or *H. pylori* SS1 (MOI 1 : 100) for 18 hours. The expressions of surface costimulatory molecules CD40, CD80, and CD86 and intracellular cytokines interleukin (IL)-4, IL-10, IL-12, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ were assessed by fluorescence-activated cell sorting (FACS). Representative histograms from at least three independent experiments are shown (isotype control: shaded area, phosphate-buffered saline (PBS) control: dotted line, and *H. pylori*-pulsed DCs: solid line) (*B*, *C*). HP-DC stimulate splenocyte proliferation. Irradiated (5000 rads) HP-DC were cocultured with naïve syngeneic splenocytes for 72 hours. Micrographs show intense splenocyte clustering in the HP-DC culture (×100). Splenocyte proliferation was quantified in a dose–response manner using a tritiated thymidine (^{3}H) incorporation assay. (*D*) HP-DC stimulate a Th1 response. The production of Th1/Th2 cytokines was measured using cytometric bead assays. HP-DC induced a type 1 helper T cell (Th1)-dominant response with increased levels of TNF-α and IFN-γ. Data shown represent results from three independent experiments.

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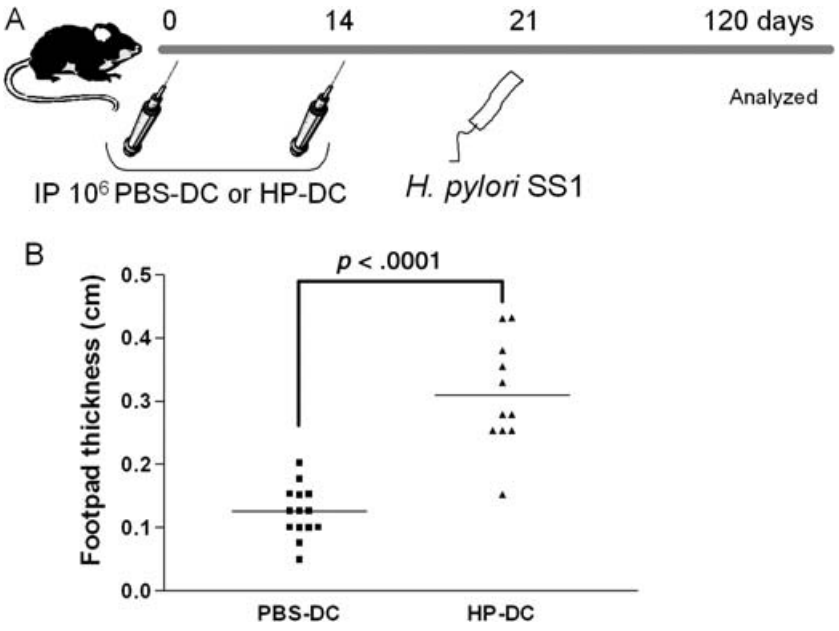

Figure 2 *H. pylori*-pulsed dendritic cells (HP-DC) induce a delayed-type hypersensitivity reaction to *H. pylori* sonicate. (*A*) Schematic representation of experimental treatment of mice (DCs pulsed with phosphate-buffered saline (PBS-DC) mice vs. HP-DC mice). C57BL6 mice were injected with PBS-DC or HP-DC on day 0 and day 14. Oral challenge with *H. pylori* SS1 (10^8 live organisms) was administered three times in 1 week beginning on day 21. Mice were sacrificed on day 120. (*B*) HP-DC vaccination resulted in an *H. pylori*-specific delayed-type hypersensitivity reaction. The right hind footpads of the mice from each group were injected with 10 μg in 30 μL of *H. pylori* SS1 sonicate and the left hind footpads with 30 μL of PBS. Footpad thickness was measured 24 hours later and expressed as change of thickness from PBS control. Results are from three independent experiments.

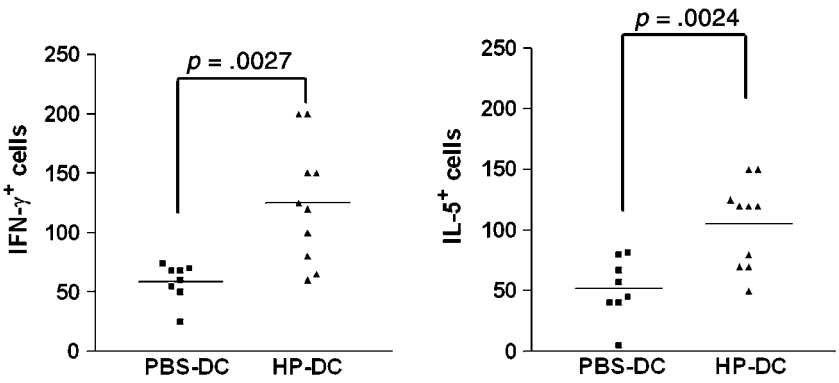

Figure 3 Induction of in vivo *H. pylori*-specific Th1 and Th2 responses by *H. pylori*-pulsed dendritic cells (HP-DC). CD4+ T cells were isolated using MACS microbeads from DCs pulsed with phosphate-buffered saline (PBS-DC) (n = 15) or HP-DC (n = 16) mice. CD4+ T cells were stimulated in vitro for 7 days with *H. pylori* SS1 sonicate and naïve bone marrow-derived DCs. *H. pylori*-specific interferon (IFN)-γ-producing (Th1) and interleukin (IL)-5-producing (Th2) CD4+ T cells were quantified using ELISpot (*A*, *B*). Data are from three independent experiments.

postinfection. We found that PC61-treated mice had a lower percentage of splenic CD4+CD25+ T cells (Fig. 5A,B) and an increased *H. pylori*-specific Th1 response (Fig. 5C) with a significant reduction of *H. pylori* colonization in the stomach ($p = .0028$) compared to non-PC61-treated HP-DC immunized mice (Fig. 5D). No significant gastritis was noted at this early time point in either group (data no shown). These data indicate further enhancement of *H. pylori*-specific Th1 response by removal of Tregs.

Discussion

Research has shown that human DCs pulsed with *H. pylori* stimulate a Th1-dominant response [17,18]. Although the use of DC vaccine as a routine immunization strategy for *H. pylori* infection may appear impractical and too technically complex, the role of DC-based vaccine may benefit patients infected with antibiotic-resistant *H. pylori*, especially those with mucosa-associated lymphoid tissue (MALT) lymphoma in which eradication of *H. pylori* may lead to disease regression [19]. We explored the possibility of inducing bacteria-specific immunity with a DC-based vaccine in a mouse model of *H. pylori* infection. Similar to human DCs, mouse bone marrow-derived DCs pulsed with live *H. pylori* stimulated a Th1-dominant response. HP-DC mice challenged with *H. pylori* exhibit an *H. pylori*-specific delayed-type hypersensitivity reaction, whereas PBS-DC mice challenged with *H. pylori* do not. Analysis of HP-DC-stimulated helper T-cell responses showed that HP-DC induce a mixed peripheral Th1/Th2 response but a Th1-dominant gastric response that lowered *H. pylori* colonization. We also showed that enhancing the *H. pylori*-specific Th1 response by Treg depletion further augmented *H. pylori* immunity.

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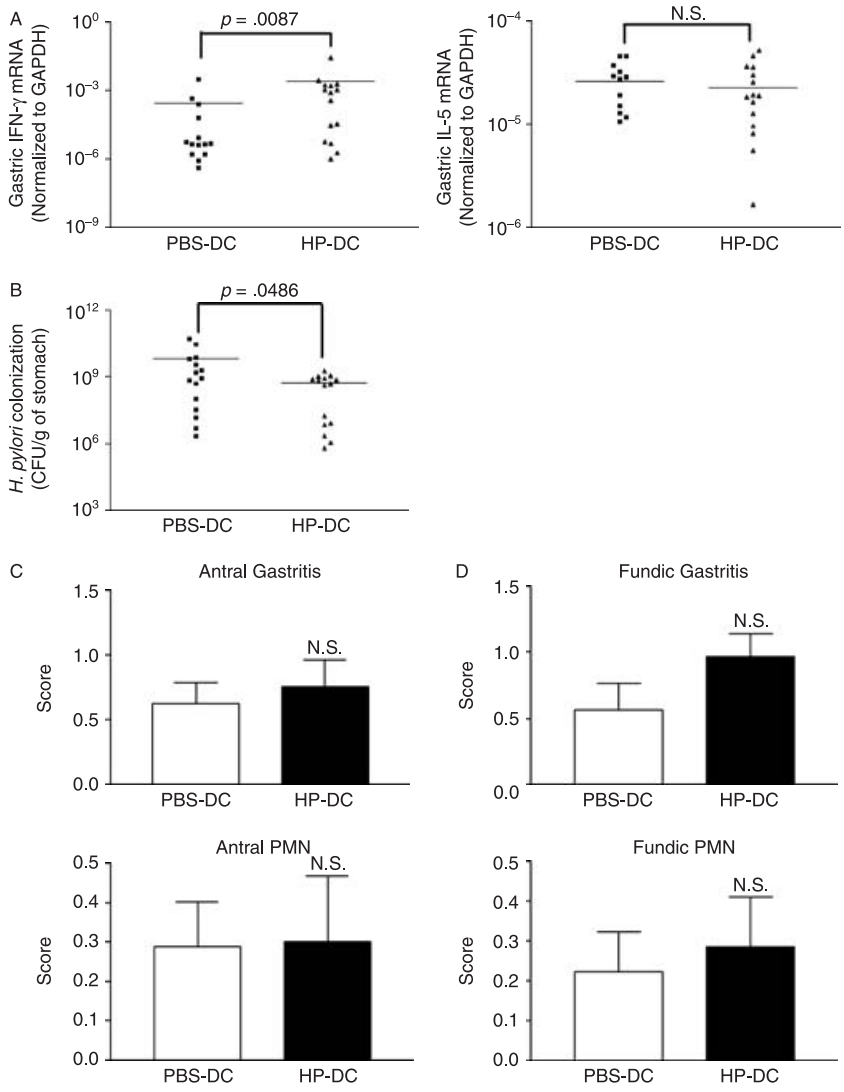

Figure 4 Induction of a Th1-dominant gastric immune response that limited *H. pylori* colonization. Total stomach RNA was isolated from DCs pulsed with phosphate-buffered saline (PBS-DC) or *H. pylori*-pulsed dendritic cells (HP-DC) mice. Quantitative reverse transcriptase PCR was performed to measure mRNA expressions of interferon-γ and interleukin-5 (*A*), and to quantify *H. pylori* colonization (*B*). HP-DC vaccine reduced *H. pylori* colonization. (*C*, *D*) Histologic and polymorphic neutrophil scores of stomach tissue from HP-DC were similar to the scores from PBS-DC mice. Results are from three independent experiments.

Otsu et al. reported that the transfer of *H. pylori*-pulsed immortalized murine DC cell line (JAWS II) effectively induced therapeutic immunity against *H. pylori* infection (reported as a 2-log reduction in bacterial colonization) [20]. In our study, HP-DC induced a 1.25-log reduction in bacterial colonization compared to PBS-DC. The difference in these results may be explained by their use of immortalized DC cell lines versus bone marrow-derived primary DCs used in this study. Immortalized cells may have higher efficacy in vivo due to prolonged antigen priming. Another difference between the two studies is the methods used to quantify *H. pylori* colonization (i.e. culture by Otsu et al. vs. real-time PCR in this study). In our laboratory, we find that real-time PCR quantification of *H. pylori* colonization is more sensitive than culturing methods (data not shown).

The current study shed light on the complexity of vaccine development against *H. pylori*. As the debate over the requirement of a Th1 response for vaccine-induced protective immunity continues [4,5], our finding supports the current paradigm that an effective Th1 response protects against *H. pylori* infection. Ineffective Th1 effector response, suggested by our previous study of a defective Th1 priming by dendritic cells [6], may result in the persistent colonization and the development of chronic gastritis. Using a potent inducer of antigen-specific cellular immunity with DC vaccine, we show that *H. pylori*-specific Th1 response can be induced in vivo. A Th2 response also was augmented by DC vaccine but its role is less well-defined as protective immunity could be achieved in B-cell-deficient mice [21,22]. Additional support for the importance of effector Th1 response was reported by Rad

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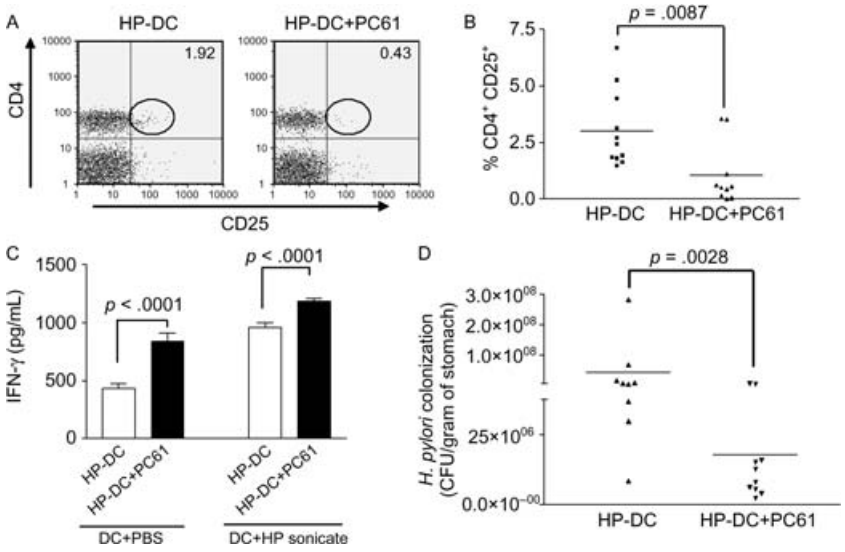

Figure 5 Depletion of Tregs by CD25 antibody enhanced the ability of *H. pylori*-pulsed dendritic cells (HP-DC) mice to prime *H. pylori*-specific immunity. C57BL/6 mice were injected intraperitoneally with phosphate-buffered saline (PBS) or PC61 mAb (1 mg per mouse) on day 0. Mice were then immunized with *H. pylori*-stimulated DCs (10^6 cells per injection) on day 0 and day 14. Mice were orally challenged with *H. pylori SS1* (10^8 organisms) three times in 1 week starting on day 21. Mice were sacrificed on day 28. Dot plots (*A*) and percentages (*B*) of CD4+CD25+ Tregs from splenocytes were determined by Fluorescence-Activated Cell Sorting. Treatment with PC61 reduced the percentage of Tregs in mouse spleen (n = 9 or 10). (*C*) PC61 treatment significantly increased *H. pylori*-specific interferon (IFN)-γ production by CD4+ T cells. CD4+ T cells were isolated from mouse spleens using Magnetic-Activated Cell Sorting microbeads and stimulated with PBS or *H. pylori* sonicate in the presence of unstimulated DCs (see Materials and Methods). IFN-γ concentration was measured by ELISA. (*D*) *H. pylori* colonization in PC61-treated and nontreated groups. *H. pylori* colonization in each mouse was determined by quantitative RT-PCR. 16s rRNA expression specific for *H. pylori* was determined in each mouse by quantitative reverse transcriptase PCR. The graph shows the level of *H. pylori* colonization in HP-DC– or HP-DC+PC61-treated mice.

et al., where depletion of CD4+CD25+ Tregs correlated with a reduction in *H. pylori* colonization [23]. In our study, a depletion of CD4+CD25+ Tregs further enhances DC vaccine primed *H. pylori*-specific Th1 response and reduced bacterial colonization. As an intact Th1 response is essential for vaccine-induced protection against *H. pylori*, a balanced, nonrestrictive Treg response may be the other determinant of whether *H. pylori* sterilization can be achieved. Understanding the interaction between *H. pylori* and host immune system with respect to induction of Th and Treg response will be critical for the success of a vaccination strategy. Furthermore, since Treg is also involved in regulation of gastric inflammation [23], manipulations to upregulate this response may prove to be beneficial in suppressing chronic gastritis.

In summary, our study shows that DC-based vaccine is capable of inducing protective *H. pylori*-specific immunity. Treg depletion with anti-CD25 antibodies enhanced the ability of DC vaccine to prime a *H. pylori*-specific immunity. Our study supports the critical role of a Th1-skewed response in anti-*H. pylori* immunity. These studies may shed light on novel strategies to modulate the host response to eradicate *H. pylori* infection.

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