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Helicobacter pylori Outer Membrane Protein 18 (*Hp1125*) Induces Dendritic Cell Maturation and Function

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ABSTRACT

Background. Dendritic cells (DCs) are potent antigen-presenting cells that initiate T-cell responses. A robust adaptive Th1 immune response is crucial to an adaptive (Th2) immune response necessary for vaccine-induced protective immunity against *Helicobacter pylori.* It has been shown that several outer membrane proteins (Omps) induce a robust antibody response. However, it is also known that the antibodies generated are not protective. Moreover there is great variation in the recognition of high molecular weight *H. pylori* proteins by sera from infected patients. In contrast to the high molecular weight proteins, serologic responses to small molecular weight proteins provide assessment of current infection with *H. pylori* and also of its eradication.
Aim. The goal of the study was to analyze the activation of the immune response by a specific low molecular weight Omp that is universally expressed by all *H. pylori* strains. Therefore, we studied interaction of *H. pylori* Omp18 with DCs.
Methods. Activation of murine bone marrow-derived DCs and production of cytokines by Omp18 was assessed by fluorescence-activated cell sorter (FACS) for costimulatory markers and ELISA, respectively. The ability of Omp18 stimulated DCs to induce lymphocyte proliferation was measured in a mixed leukocyte reaction.
Results. Omp18 induced higher expression of the B7 (CD80 and CD86) costimulatory molecule after 18 hours indicating processing and presentation of the antigen on the surface by bone marrow-derived DCs. The maturing DCs also secreted significant levels of IL-12, but was 4-fold less than that stimulated by whole bacteria. Omp18-primed DCs induced proliferation and release of IFNγ by syngeneic splenocytes.
Conclusion. We concluded that Omp18 is capable of activating DCs initiating a Th1 immune response.
Keywords. Omps, Th1, CD80, CD86, IL-12.

Helicobacter pylori colonizes the human gastric epithelium causing chronic gastritis, which may progress to gastric adenocarcinoma in a subset of patients. Emerging antibiotic resistance, the differences in clinical outcome in infected individuals, and the chronic nature of the disease make treatment methods ineffective in preventing *H. pylori*-mediated gastric cancer [1–3]. Lifelong infection with *H. pylori* elicits a marked host response, including a robust gastric IgA response [4]. However, natural immunity fails to provide protection and is postulated to contribute to the development of complications related to the chronic infection. Initiation of the cellular inflammatory response to infection is typically through antigen-presenting cells (APCs). Dense infiltration of the gastric mucosa with immune cells (gastritis) seen in *H. pylori* infection suggest complex interactions with APCs and other immune cells, which may play an important role in the development of gastric cell injury. These immune responses are attributed to a subset of T helper lymphocytes (Th1 cells) that enhance cell-mediated immune responses and induce damage to the gastric epithelium [5,6]. Recent evidence indicates that antigen presentation by dendritic cells (DCs) is required to initiate a primary Th1 lymphocyte response [7]. Also, an influx of myeloid DCs in the lamina propria of the gastric mucosa is observed in *H. pylori* infection in mice [8,9].

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Microorganisms have conserved molecular features that in addition to having a variety of physiologic functions also act as antigens that can be recognized by receptors on cells of the innate immune system [10]. Among such molecules, bacterial outer membrane proteins (OMPs) are important to the organism due to their participation in ion transport, bacterial adherence, and osmotic and structural stabilities. They are also important due to their ability to generate an immune response. For example, in *Klebsiella pneumoniae* two cell wall components, outer membrane protein A (OmpA) and lipopolysaccharide (LPS), activate myeloid DCs through TLR2 and TLR4, respectively [11,12]. Since *H. pylori* LPS, unlike enterobacterial LPS, does not activate DCs [13], OMPs might be more important in initiating the immune response.

The serologic response to *H. pylori* low molecular weight OMPs decreases after successful eradication of the pathogen suggesting that these proteins are markers of an active infection. A serologic response implies recognition and processing of these proteins by APCs. In addition, Omp18 is an *H. pylori* protein routinely recognized by the immune system [14]. This protein is located on the outer membrane surface of the bacteria, and is expressed by all known *H. pylori* strains and was found to react specifically with sera from all *H. pylori* infected patients [15]. Omp18 is a peptidoglycan-associated lipoprotein precursor, which is structurally related but with low sequence similarity to *Campylobacter jejuni* Omp18, an immunodominant antigen that is used to vaccinate chickens [16]. Interferon gamma (IFNγ) producing Th1-polarized lymphocytes and activated natural killer cells (NK) during *H. pylori* infection play an important role in the development of pathology and in the elimination of colonized bacteria [30]. Understanding the role of different bacterial components in generating the immune response is crucial to the development of more effective therapies. In the current study, we demonstrate that *H. pylori* Omp18 can induce bone marrow-derived DC maturation, and prime Th1 lymphocytes to secrete IFNγ.

Materials and Methods

Bacterial Strains and Culture Conditions

Helicobacter pylori (26695 and SS1) strains were cultured on *Campylobacter*-selective agar supplemented with 5% sterile horse blood in (BD Diagnostics, Bedford, MA), trimethoprim (5 μg/ml), vancomycin (10 μg/ml), and nystatin (10 μg/ml) at 37 °C, in a humidified microaerophilic chamber (BBL Gas System, with CampyPak Plus packs, BD Microbiology, Sparks, MD). *HP1125* was polymerase chain reaction (PCR)-amplified and cloned using the DNA from *H. pylori* strain 26,695 as the template. The SS1 strain was used to stimulate DCs.

Generation of Recombinant Omp18

H. pylori (26695) genomic DNA was isolated using the DNeasy Tissue extraction kit. The full-length *Omp18* gene locus was amplified using the forward primer 5′-ATGAAGAGATCTTCTG-TATTTAGTTTCTTG-3′ and 5′-TTACT-TCATTAATTTG ACATCCACTCTTCT-3′ as the reverse primer. Amplification was performed in a total of 50 μl with high fidelity polymerase (Roche, Penzberg, Germany), 1 μl of template DNA (26 pg), and 5 μl of primers (10 μmol/l) in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA) for 30 cycles. Thermocycling conditions included an initial denaturation at 94 °C for 2 minutes, followed by two cycles at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds then extension at 72 °C for 90 seconds. The final amplification cycle included 30 cycles at 94 °C for 30 seconds, 50 °C for 30 seconds, and 72 °C for 90 seconds. The PCR products were resolved on a 1% agarose gel with ethidium bromide. The gel-purified product was cloned into pTrc-His TOPO TA vector (Invitrogen, Carlsbad, CA) and transformed into the BL21 (DE3) *Escherichia coli* strain. *HP1125* could not be successfully expressed in *E. coli*. So the gene was subcloned into a mammalian expression vector and expressed transiently in the AGS, human gastric cell line.

Orientation and sequence of *HP1125* in the pTrc-His vector were verified before subcloning the construct into the pTriEx2-Neo expression vector (Novagen, San Diego, CA). The pTriEx2-Neo vector, which added an amino terminal His tag to the full-length *HP1125*, was transiently transfected into AGS cells using the Fugene reagent (1μgDNA/well in a 6-well tissue culture plate). Transfected cells were grown for 48 hours, then washed once with PBS (pH 7.4) before collecting in ice cold PBS. Omp18 protein from the transiently transfected AGS cells was

purified with the His-Bind purification kit (Novogen) under denaturing conditions according to the manufacturer's instructions. The purified protein was dialyzed against PBS containing sequentially lower concentrations of urea for 48 hours at 4 °C to allow the protein to refold.

Reverse Transcriptase-PCR

Total RNA from AGS cells transfected with the *HP1125* expression vector was extracted using the Trizol reagent (Invitrogen) and stored at –20 °C until further use. One microgram of RNA was reverse transcribed using random primers according to the manufacturer's instructions in a 20-µl reaction (reverse transcription system, Promega, Madison, WI). Five microliters of the cDNA from the above reaction was used to PCR amplify *HP1125* cDNA.

Fluorescent Antibody Staining

AGS stable transformants expressing Omp18 protein were selected using G418 at 1 mg/ml. The selected clones were cultured on sterile cover slips for 24–48 hours until they reached 60–70% confluency. The coverslips were then collected and fixed for 30 minutes in 100% methanol followed by an acetone rinse for 10 minutes at –20 °C. The dehydrated cells were then rehydrated with two rinses in PBS for 5 minutes. Nonspecific antigens were blocked with 5% fetal bovine serum (FBS) in tris-buffered saline (TBS), and Fc blocker (PharMingen, San Diego, CA) for 30 minutes. The supernatant was washed three times with TBS containing 0.5% Tween 20 (TBST) and overlaid with Omp18 rabbit antibody at a 1 : 10,000 dilution for 1 hour at 37 °C in a humidified chamber. AGS cells stably transfected with the empty vector served as a control. Unbound primary antibody was washed away with TBST; counterstained with antirabbit FITC antibody, mounted using Gel Mount (Biomedia, CA), and viewed under the Olympus BX60 fluorescent microscope.

Western Blot Analysis

Purified Omp18 protein was resolved on 15% Tricine-SDS mini gels. The resolved proteins were subsequently electrotransferred onto nitrocellulose membranes (Hybond-C Extra, Amersham Biosciences, Piscataway, NJ). Nonspecific binding sites were blocked using 5% nonfat milk in TBST overnight at 4 °C followed by incubation with anti-Omp18 antibodies for 1 hour at 37 °C. Following three 10-minute washes with TBST, the membrane was incubated with antibody to rabbit IgG conjugated to horseradish peroxidase (HRP, 1: 5000), in 5% nonfat milk in TBST for 1 hour at 37 °C. After washing with TBST, the signal was detected by chemiluminescence (SuperSignal WestPico Chemiluminescent Substrate, Pierce, Rockford, IL).

Harvesting of Bone Marrow Dendritic Cells

Female C57BL/6 or BALB/c mice (6–8 week) were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed in the specific pathogen-free Animal Maintenance Facility at the University of Michigan. Erythrocyte-depleted bone marrow cells were harvested and cultured at a density of 1×10^6 cells/ml with 10 ng/ml of recombinant mouse GM-CSF (granulocyte-macrophage colony-stimulating factor) and IL-4 10 ng/ml (R & D Systems, Minneapolis, MN). On day 6, non-adherent DCs were harvested and enriched by density gradient centrifugation using the Optiprep density solution (Sigma, St Louis, MO). The interface containing the DCs was washed twice with complete RPMI-1640 media and cultured with GM-CSF before use. The University of Michigan Animal Care and Use Committee approved all animal experiments.

FACS Analysis of Dendritic Cells

Approximately 1×10^6 cells in 1 ml of RPMI media were coincubated with PBS, 100 ng/ml of Omp18, 100 ng/ml of *E. coli* LPS (Sigma), and *H. pylori* (1×10^8/ml) for 18–24 hours. Following the incubation, DCs were washed and resuspended in Hank's balanced salt solution with 1% bovine serum albumin (HBSB), before blocking with the Fc blocker for 30 minutes (1 µg/ml, PharMingen). After blocking, the DCs were stained with phycoerythrin (PE) or fluorescein isothiocynate (FITC) conjugated CD40, CD80, and CD86 primary antibodies along with their isotype controls (PharMingen) for 20 minutes. Unconjugated antibodies were removed by washing in HBSB. Labeled DCs were resuspended in 500 µl of HBSB and analyzed by fluorescence-activated cell sorter (FACS, Coulter's XL Flow Cytometer, Hialeah, FL).

Cytokines Measurements

The media from coincubation experiments was collected, spun at 4 °C to remove the cells, and frozen at –20 °C until assayed for cytokines by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (PharMingen). Briefly, the supernatant was collected after 18–24 hours of coculture. Polystyrene microtiter plates (Costar, Fernwald, Germany) were coated with the first antibody diluted in coating buffer overnight at 4 °C. Various concentrations of recombinant cytokines were added as external standards. Standards and supernatants were incubated for 2 hours in dilutions of 1: 5. Bound cytokine was detected with biotinylated secondary antibody, streptavidin–peroxidase conjugate, and a chromogenic marker. The reaction was stopped with H_2SO_4 (0.5 mol/l) and the optical densities were measured using an ELISA plate reader. Cytokine concentrations were calculated using standard curves that were performed for each ELISA plate in order to minimize interassay variability. The results from three experiments were expressed as the mean concentrations of duplicate aliquots.

Mixed Lymphocyte Reaction (MLR)

Splenocytes isolated from the spleen (2×10^5) of a syngeneic mouse were coincubated with antigen, PBS, or *H. pylori* treated and irradiated (5000 rads) 10^3–10^5 DCs. After 72 hours, 100 µl of the culture medium was removed and the wells were pulsed with 0.5 µCi of ^{3}H-thymidine/well (Amersham) for 8 hours. The contents of the plates were frozen at –20 °C until assayed. After thawing, the cells were collected on nylon filters, washed, and counted (Beckman Scintillation Counter, Fullerton, CA). The replicate supernatants collected were pooled and stored at –70 °C until they were analyzed for cytokine content by ELISA.

PMA/ionomycin (10 ng/ml and 1 µmol/l, respectively) were used as positive controls in each experiment and cell proliferation was measured as above at 24 hours.

Proliferation was expressed as counts per minute (c.p.m.) ± SD of incorporated ^{3}H-thymidine.

Statistical Analysis

Data were analyzed by Student's *t*-test and one-way ANOVA and expressed as the mean ± SD. *p*-values < .05 were considered to be significant.

Results

Generation of Recombinant Omp18 Protein

To study the contribution of Omp18 towards recognition and induction of an adaptive immune response against *H. pylori* infection, we cloned the *HP1125* gene from *H. pylori* 26695 and generated Omp18 fusion protein.

Reverse transcriptase-polymerase chain reaction (RT-PCR) using gene specific primers confirmed *HP1125* expression in transiently transfected AGS cells. A 537 bp PCR product was detected (Fig. 1A). cDNA from the cells transfected with the empty vector was used as a negative control. The gene product was of the expected size that was observed with *H. pylori* DNA. Further confirmation of the mammalian

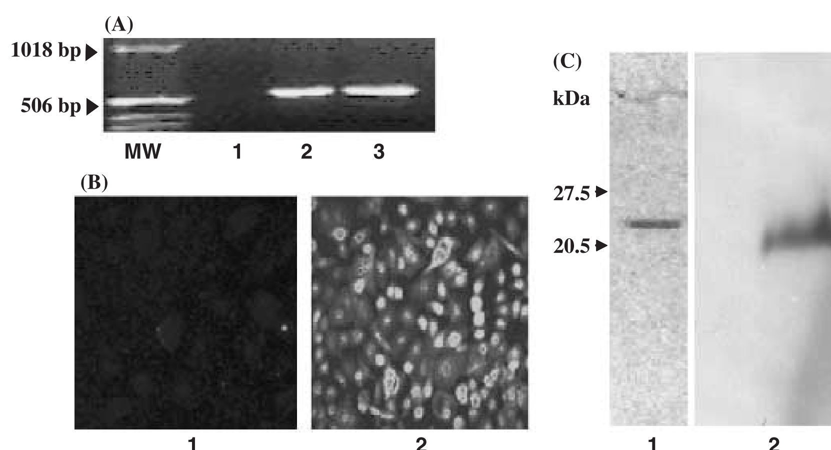

Figure 1 Generation of recombinant Omp18 in AGS cells. (*A*) RT-PCR on AGS cells transfected with the recombinant plasmid showing *HP1125* gene product. Lane 1-AGS cells transfected with empty vector, lane 2-AGS cells transfected with *HP1125* construct, lane 3-PCR-positive control (*H. pylori* 26695 DNA). (*B*) immunofluorescent staining of stably transfected AGS cells – 1, cells transfected with empty vector; 2, cells transfected with the *HP1125* construct showing Omp18 expression. (*C*) Coomassie Brilliant Blue staining 1, and Western blot; 2, probed with Omp18 antibody of the purified protein from AGS cells.

expression was achieved by performing immunofluorescence on stable transformants expressing Omp18 (Fig. 1B). Polyclonal Omp18 antibody detected Omp18 protein expressed in AGS stable transformants, but did not cross-react with control cells transfected with empty vector. Recombinant Omp18 protein from the transfected cells was purified by affinity chromatography using nickel-agarose columns and resolved on a 15% SDS gel before transferring to a nitrocellulose membrane. The membrane was probed with Omp18 antibodies. As illustrated in Fig. 1(B), a single protein band was observed at about 25 kDa. Cells transfected with the empty vector did not show the corresponding protein band (Fig. 1C).

Omp18 Stimulates DC Maturation and IL-12 Secretion

Omp18 protein was added to cultures of bone marrow-derived DCs to test the effect of this *H. pylori* OMP on DC differentiation. FACS analysis was used to quantify the number of cells expressing mature DC surface markers. More than 80% of the freshly isolated DCs were CD11c and/or MHC II, CD80 and CD86 positive (data not shown). The cells had low levels of CD40 expression at baseline indicating the immature state of the isolated DCs. When DCs were cocultured with *E. coli* LPS, Omp18, or *H. pylori* for 24 hours, expression of CD80 and CD86 molecules increased in comparison to basal conditions (medium alone or with PBS, Fig. 2). However, no significant stimulation of CD40 expression was observed (Fig. 2).

Initially to address whether the DCs isolated from C57BL/6 mice with a Th1 immune response influenced induction of inflammatory cytokines, we compared the response of C57BL/6 bone marrow-derived DCs to DCs from BALB/c mice that tend to generate a Th2 skewed immune response. We found no significant difference in IL-12p70 release (C57BL/6 = 418 ± 52 pg/ml versus Balb/C = 353 ± 50 pg/mL, $p > .05$) and splenocyte proliferation index (C57BL/6 = 3.4 ± 0.7 versus Balb/C = 3.9 ± 0.8, $p > .05$) using live *H. pylori* bacteria. Thus there was no evidence to suggest that the mouse strain altered the DC response.

ELISA was used to determine if Omp18-activated DCs secreted IL-12 and IL-10 cytokines into the media (Fig. 3). The results showed significant induction of IL-12 release although not to the same extent as observed with live *H. pylori*. Nevertheless, induction of IL-12 secretion by Omp18 was significant compared to the controls, but lower than that induced by *E. coli* LPS (Fig. 3A). By comparison, there was 50% less IL-10 (355 ± 39.06 pg/ml) than IL-12 (755 ± 105.74 pg/ml) released by *H. pylori* and no IL-10 induced by Omp18 (Fig. 3B).

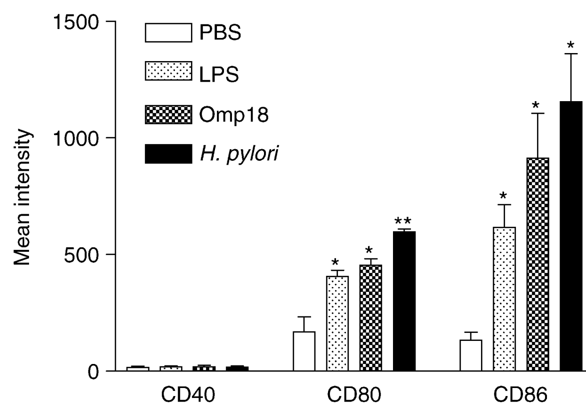

Figure 2 Induction of DC maturation by Omp18 analyzed by flow cytometry. Bone marrow-derived DCs were cocultured with PBS, Omp18, *E. coli* LPS, and *H. pylori* for 18–24 hours and co-stimulatory markers were assessed by FACS. The graph shows the difference in the mean intensity for CD40, CD80, and CD86 for DCs stimulated with *E. coli* LPS, Omp18, or *H. pylori* in comparison to PBS control. Mean values from three experiments with SD are shown. *$p < .004$, **$p < .003$.

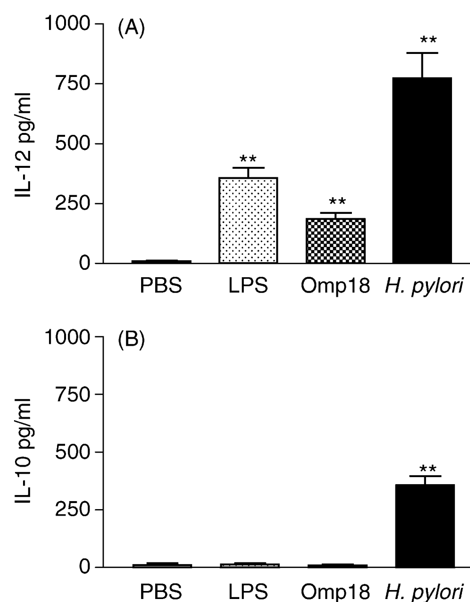

Figure 3 Omp18 stimulated IL-12 and IL-10 secretion from DCs. DCs were cocultured with PBS, *E. coli* LPS, *H. pylori* Omp18 at 100 ng/ml or with *H. pylori* bacteria (10^8). Eighteen to 24 hours after the addition, cytokines were measured in the culture media by ELISA. (*A*) IL-12 secretion by DCs. (*B*) Measurement of IL-10 released by DCs. Shown is the mean ± SD for three experiments. **$p < .004$, ***$p < .0001$.

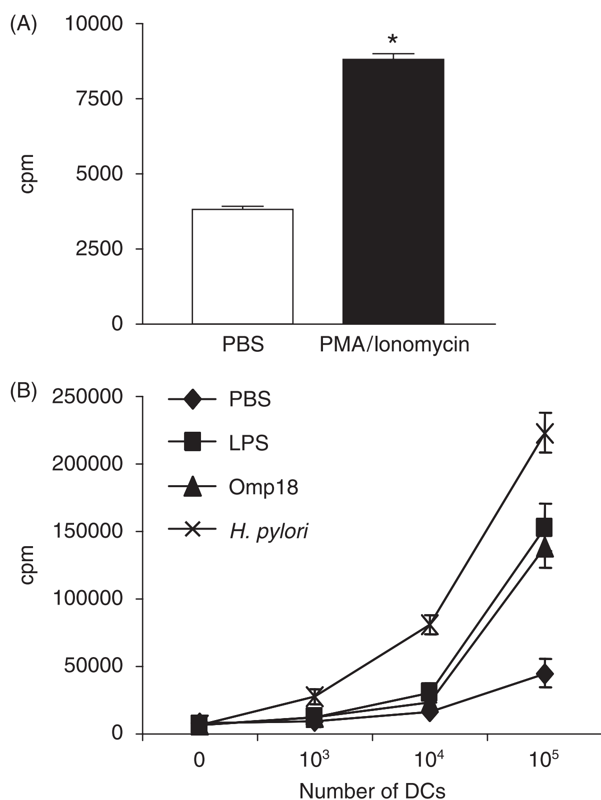

Figure 4 Induction of splenocyte proliferation by Omp18. (*A*) Graph depicting the significant proliferation of splenocytes treated with PMA/Ionomycin compared to the control at 24 hours. (*B*) DCs primed by Omp18 stimulate autologous splenocyte proliferation in MLR assay at 72 hours. Antigen- (*E. coli* LPS, Omp18) or *H. pylori*- (10^8) primed irradiated DCs at varying densities, when cocultured with constant congeneic splenocytes (2×10^5) stimulated significant autologous splenocyte proliferation over the PBS-primed DCs. Graph represents mean ± SD from three experiments $^{**}p < .004$.

Activated DCs Induce Splenocyte Proliferation

Having observed a significant increase in IL-12 secretion by Omp18-primed DCs, we next investigated whether the activated DCs were capable of priming naïve syngeneic splenocytes by performing a mixed leukocyte reaction. Naive splenocytes isolated from a syngeneic mouse spleen were cocultured with irradiated DCs treated with Omp18 or *H. pylori*. Splenocyte proliferation was higher when cocultured with Omp18-primed DCs than when cocultured with PBS-primed DCs (Fig. 4). *H. pylori*-primed DCs induced splenocyte proliferation 2-fold higher compared to Omp18 consistent with the higher level IL-12 from *H. pylori*. In addition, proliferation with *H. pylori*-stimulated DCs could be detected with as few as 10^3 DCs; whereas, the isolated proteins required 10^5 DCs. *E. coli* LPS showed a similar response as Omp18. PMA/ionomycin used as positive controls induced significant proliferation of lymphocytes compared to PBS controls (Fig. 4A).

Table 1 Secretion of IFN-γ by proliferating splenocytes

	pg/ml ± SD (*n* = 3)
DCs alone	Undetectable
LPS	69.60 ± 15.12
rOmp18	133.40 ± 57.17
H. pylori	402.02 ± 131.46

Omp18 Primes a Th1 Response from Proliferating Splenocytes

Next, we quantified the cytokine released from the proliferating splenocytes to determine whether there was induction of the Th1/Th2 immune response. IL-4, IL-5 (Th2), IL-2, IFNγ, and TNFα (Th1) cytokines in the MLR reaction supernatant were assessed using ELISA as described above. Incubation of splenocytes with Omp18 and *H. pylori*-pulsed DCs significantly enhanced IFNγ secretion after 72 hours of coculture with no detectable levels in the PBS-primed controls (Table 1). The other Th1 or Th2 cytokines tested were not detectable in the supernatant, confirming that the response was skewed towards Th1. Interestingly, the induction of IFNγ was about twice what was observed with *E. coli* LPS.

Discussion

Both innate and acquired immune responses are elicited by *H. pylori* infection; however, these immune responses are not sufficient to eliminate the infection [17]. Microorganisms have conserved molecular features that the receptors on immune cells recognize. These pattern-recognition receptors when triggered activate the innate immune response [18]. Prominent among these immune cells are the DCs, which have the receptors for sampling bacterial LPS, OMPs and secreted factors. *H. pylori* LPS, unlike enterobacterial LPS, has lower biologic activity and hence does not activate DCs, indicating the importance of bacterial OMPs in inducing an immune response [13].

Recent studies have demonstrated the importance of *H. pylori* small molecular weight proteins in diagnosis, therapy, and vaccine development [14]. The immune response to

some of these proteins wanes as the infection is eradicated. Among those proteins that generate an adaptive immune response, we tested the Omp18 (*HP1125*) antigen, an OMP that is expressed in all known *H. pylori* strains [15]. Our results clearly demonstrate recognition of the *H. pylori* Omp18 antigen by immature bone marrow-derived DCs, which mature and present the antigen to splenocytes subsequently priming Th1 lymphocytes.

Studies examining the interaction of the whole bacteria with DCs have clearly demonstrated rapid DC maturation and activation [19]. Increased expression of MHC class II molecules and up-regulation of costimulatory molecules is associated with activation of CD4+, NK cells and differentiation of splenocytes into Th1 or Th2 helper cells [20,21]. In our study coincubation with *H. pylori*, Omp18 induced significant up-regulation of CD86 and to a lesser extent CD80, confirming maturation of immature DCs. Relative expression levels of CD80 and CD86 on DCs influence the immune outcome by influencing the level of T-regulatory cell activation [22]. With Omp18, there was high expression of CD86 and lower expression of CD80, which indicate response to foreign antigen signals such as bacterial LPS and potent membrane protein antigens that enhance the splenocyte response [23]. Also increased expression of B7 molecules (CD80 and CD86) is important in amplifying the signaling processes at the immunologic synapse established between DCs and other cells of the immune system, thereby determining the type of immune response [24]. The results reported here confirm that *H. pylori* Omp18 protein that is not injected into cells is processed and presented on the surface of DCs. This suggests that surface *H. pylori* proteins are valid antigens capable of activating the immune system.

In recent studies, interaction between DCs and other immune cells during infection with *H. pylori* have shown remarkable stimulation of IL-12 secretion in DC preparations with very low IL-10 secretion [19,25]. Data from our study indeed confirm this observation. However, in our study Omp18 induced significant levels of IL-12 with no detectable levels of IL-10 from the bone marrow-derived DCs. This contradicts the observation made by Voland et al., who found higher levels of both IL-10 and IL-12 secreted in response to Omp18 stimulation in vitro [15]. The difference between the two studies may be due to the human plasmacytoid type of DCs used in the Voland et al. study compared to the myeloid DCs used in our study, each of which respond differently. Myeloid DCs respond to bacteria while plasmacytoid DCs are more responsive to viruses inducing production of IFNα rather than of IL-12 [26,27]. In addition the difference in the two studies may also be due to the higher amounts of protein used to stimulate the DCs in their study, 1 µg/ml in contrast to 100 ng/ml used in this study which is closer to physiologic levels. Furthermore, our findings demonstrate no difference between DCs from C57BL/6 mice (Th1 bias) used in this study and DCs from BALB/c mice (Th2 bias) in inflammatory cytokine production and induction of splenocyte proliferation. Also, we acknowledge that there are differences in mouse and human physiology; however, further studies using human myeloid DCs will determine the significance of our findings in patients with *H. pylori* infection. That said, results from our study are consistent with data from humans showing that *H. pylori* infection is a Th1 disease.

Induction of IL-12 secretion suggests internalization of the protein and subsequent activation of DCs, initiating a Th1 response and enhancing cell-mediated immunity. IL-12 release from DCs plays a key role in the polarization of naïve splenocytes into Th1 cells mediating an adaptive immune response. Vaccination studies in knockout mice also demonstrate the importance of Th1-polarized splenocytes in providing protection from infection with *H. pylori* [28,29]. Interestingly, compared to the whole bacteria, *H. pylori* membrane preparations are more potent in priming a Th1 effector response [30]. In our study, coculture of Omp18-pulsed DCs with naïve splenocytes induced significant proliferation of splenocytes. Proliferating splenocytes released significant levels of IFNγ. Our observations were in accordance with reports indicating that gastric splenocytes from *H. pylori*-infected individuals and animals produce predominantly IFNγ and not IL-4, IL-5 as typical for a Th1 polarized splenocyte [31–33].

In summary, our study concurs with Voland et al. who demonstrated recognition and processing of Omp18 by DCs. However, we also demonstrate that Omp18 may act as one of several antigens that stimulate a Th1 immune response by interacting with the DCs. Results from our study also demonstrate the existence of a difference between OMPs and whole bacteria

in priming an immune response to *H. pylori* infection. Moreover, the ability of Omp18 to activate APCs demonstrates the ability of a *H. pylori* protein to interact with the DCs inducing a Th1 effector response, which maybe important in eliminating the infection. Understanding the role of OMPs in triggering an immune response may prove useful in future vaccination regimens.

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