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***Helicobacter pylori* DNA decreases pro-inflammatory cytokine production by dendritic cells and attenuates DSS-induced colitis**

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Abstract

Background & Aims—Recently there has been emerging epidemiological data to suggest *Helicobacter pylori* (*H. pylori*) may protect against certain chronic inflammatory diseases such as inflammatory bowel disease (IBD). However, the mechanism for the observed inverse association between *H. pylori* and IBD has not been described.

Methods—The frequency of immunoregulatory (IRS) to immunostimulatory (ISS) sequences within the genome of various bacteria was calculated using MacVector software. The induction of type I IFN and IL-12 responses by DNA-pulsed murine bone marrow-derived dendritic cells (BMDC) and human plasmacytoid dendritic cells (pDC) was analyzed by cytokine production. The effect of *H. pylori* DNA on *E. coli* DNA production of type I IFN and IL-12 was assessed. The in vivo significance of *H. pylori* DNA suppression was assessed in a DSS-model of colitis. The systemic levels of type I IFN were assessed in *H. pylori*-colonized and non-colonized patients.

Results—We showed that *H. pylori* DNA has a significantly elevated IRS:ISS ratio. In vitro experiments revealed the inability of *H. pylori* DNA to stimulate type I IFN or IL-12 production from mouse BMDCs or human pDCs. Additionally, *H. pylori* DNA was able to suppress *E. coli* DNA production of type I IFN and IL-12. Administration of *H. pylori* DNA prior to the induction of DSS colitis significantly ameliorated the severity of colitis as compared to *E. coli* DNA or vehicle control in both an acute and chronic model. Finally, the systemic levels of type I IFN were found to be lower in *H. pylori*-colonized patients versus non-colonized controls.

Conclusions—Overall, our study indicates that *H. pylori* DNA has the ability to down-regulate pro-inflammatory responses from DCs and this may in part explain the inverse association between *H. pylori* and IBD.

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Keywords

H. pylori; inflammatory bowel disease; mucosal immunology; dendritic cells

Introduction

Helicobacter pylori (*H. pylori*) is a highly prevalent infection. It colonizes the gastric mucosa of more than one-half of the world's population. Moreover, genetic studies support the notion that *H. pylori* is a commensal organism as it has co-existed with the human race for over 50,000 years (1, 2). The bacteria is clearly linked in the pathogenesis of peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (3–5). However, only a minority of infected patients will develop these complications. Epidemiological data indicate less than 15% of patients develop peptic ulcer disease and less than 1% develop gastric cancer (6). Additionally, in a subset of patients *H. pylori* may in fact offer benefit. For example, emerging data suggest a protective effect of *H. pylori* against gastroesophageal reflux disease and certain chronic inflammatory diseases such as asthma (7, 8).

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the intestinal tract. It has increasingly become a world-wide health burden, with a significant rise in incidence over the past two decades. Of note, this rise in incidence coincides with the development of effective anti-*H. pylori* therapy (9). Furthermore, we found a significant inverse association between *H. pylori* colonization and IBD (10). The results from our meta-analysis were further strengthened by the publication of two recent studies illustrating this relationship (11, 12). Despite these epidemiological data, there has been no mechanistic support for this inverse association.

Microbes, such as *H. pylori*, possess the unique characteristic of containing immunomodulatory sequences within their genome. Through direct interaction with immune cells, bacterial DNA can either stimulate or dampen host immune responses. This interaction is dependent upon the presence of toll-like receptors (TLR), which are proteins found on immune cells that act as sensors for antigens. More specifically, TLR-9 functions as an immune sensor for invading pathogenic bacterial DNA. Dendritic cells (DCs) are professional antigen-presenting immune cells that highly express TLR-9 and are therefore critical to the interaction with bacterial DNA. In humans there exists a certain subset of DCs termed plasmacytoid dendritic cells (pDCs) (type I-interferon (IFN) producing cells). pDCs, when stimulated by microbial DNA, are the main producers of type I IFN. Type I IFN is an important pro-inflammatory cytokine composed primarily of IFN- β and multiple subsets of IFN- α . Its pro-inflammatory mechanism is multi-focal and includes induction of a Th1 response (13–15). In fact, type I IFN has been implicated in the pathogenesis of certain Th1-driven diseases such as celiac disease, in that blocking type I IFN inhibited IFN- γ , a pro-inflammatory type II IFN, expression from ex vivo tissue cultures from celiac patients challenged with gluten (16). However, its influence on the pathogenesis of IBD has not been clearly defined.

Because *H. pylori* does not colonize the intestine, we hypothesize the shedding of *H. pylori* DNA from the stomach into the distal intestine influences mucosal immunity. The presence of *H. pylori* DNA distal to the stomach has been shown, as multiple reports document *H. pylori* DNA both in the colon (17) and stool of infected patients (18, 19). We focus our study on bacterial DNA because of its immunomodulatory properties and the fact that bacterial DNA has been previously shown to influence susceptibility to experimentally-induced colitis (20).

In this work we highlight the unique immunoregulatory characteristics of the *H. pylori* genome. We then show certain bacterial DNA such as *E. coli* DNA can induce a rather significant pro-inflammatory response by DCs in vitro, and that *H. pylori* DNA lacks this ability. Further, we document the ability of *H. pylori* DNA to down-regulate production of pro-inflammatory cytokines by mouse and human DCs in vitro, and correlate this with an in vivo model in which administration of *H. pylori* DNA prior to induction of colitis significantly reduced the severity of colitis. Last, we show that patients infected with *H. pylori* have lower systemic levels of type I IFN compared to non-infected patients. Our results suggest *H. pylori* DNA may in part explain the epidemiologically observed inverse association between *H. pylori* and IBD.

Methods

Determination of IRS and ISS frequencies

Complete genomes for the following bacteria were obtained from the National Center for Biotechnology Information (NCBI) website: *H. pylori* Shi470, *H. pylori* 26695, *H. pylori* J99, *H. pylori* P12, *H. pylori* G27, *H. pylori* HPAG1, *Lactobacillus reuteri* JCM1112, *Bifidobacterium longum* DJO10A, *Lactobacillus acidophilus*, *Clostridium difficile* CD196, *Enterococcus faecalis* V583, *Mycobacterium avium* subspecies paratuberculosis K-10, *Bifidobacterium longum* subsp. infantis ATCC 15697, *E. coli* K27, and *Helicobacter hepaticus* ATCC 51449. Immunoregulatory (IRS) and immunostimulatory (ISS) sequences were obtained from previously published data (Table 1) (21). The number of IRS and ISS occurrences within each genome was calculated using MacVector® software. The number of IRS and ISS occurrences was then divided by the genome size of the bacteria to give a frequency value. Specifically:

$$\frac{\text{\# of IRS (or ISS) occurrences}}{\text{genome size}} = \text{frequency of IRS (or ISS)}$$

In order to normalize this value to the frequency of any random octomer sequence occurring in a genome, we divided our observed IRS and ISS frequency by $(1/4^8)$. Specifically:

$$\frac{\text{IRS (or ISS) frequency}}{(1/4^8)} = \text{normalized IRS (or ISS) frequency}$$

Mice

Specific-pathogen-free female C57/BL6 mice aged 8–10 weeks were obtained from the Charles River Laboratory and housed in the animal maintenance facility at the University of Michigan Health System. All animal experiments were reviewed and approved by the University Committee on Use and Care of Animals at the University of Michigan.

Bacterial DNA Preparations

Genomic DNA was isolated from *H. pylori* SS1, J99, and 26695 and *Escherichia coli* (*E. coli*) K27 using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's instructions. The purity of DNA was confirmed by measuring the UV 260/280 absorbance ratio, with an acceptable ratio being > 1.7 . To ensure that the DNA was free of bacterial LPS, an endotoxin removal step was performed using MiraCLEAN® Endotoxin Removal Kit (Mirus, Madison, WI) according to the manufacturer's protocol. All DNA was suspended in endotoxin-free TE buffer (QIAGEN).

In vitro stimulation of murine bone marrow-derived DCs

Erythrocyte-depleted murine bone marrow cells were cultured in RPMI medium containing 5% fetal bovine serum, penicillin, streptomycin, and glutamine. Additionally, the medium was supplemented with 10 ng/mL GM-CSF and 5 ng/mL IL-4 at 10^6 cells/mL. On day 6, nonadherent bone marrow derived (BM-DCs) were harvested by gradient centrifugation using OptiPrep® density solution (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. The low-density interface containing DCs was collected by gentle aspiration. The BM-DCs (10^6 per reaction well) were cultured with bacterial DNA in the presence of DOTAP liposomal transfection reagent (Roche, Germany) for 8 hours after which the supernatant was collected. Protein levels of IFN- α and IFN- β from mice were measured using ELISA (PBL Interferon Source, Piscataway, NJ). ELISA (BD Biosciences Pharmingen, San Diego, CA) was also used to measure concentration of IL-12.

In vitro stimulation of human plasmacytoid dendritic cells (pDCs)

Peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coat of blood donated by two healthy volunteers according to the manufacturer's instructions (Ficoll-Paque, Amersham Biosciences, Sweden). Using the protocol described by the Diamond plasmacytoid dendritic cell isolation kit (Miltenyi Biotec, Germany), pDCs were isolated by negative selection. Once isolated, pDCs (10^4 per reaction) were co-cultured with bacterial DNA in the presence of DOTAP liposomal transfection reagent (Roche, Germany) overnight after which the supernatant was collected. Protein level of IFN- α was measured using ELISA (PBL Interferon Source, Piscataway, NJ).

In vivo induction of colitis

To induce acute colitis, 3% dextran sodium sulfate (DSS) (MP Biomedicals, Solon, OH) was given 2 hours after oral administration of respective endotoxin-free DNA (50 μ g) on Day 0. Intra-gastric intubation was performed for oral administration of DNA. DSS was given in the drinking water for 7 days and changed every 2 days to ensure purity. On Day 7, DSS water was switched to regular drinking water, and a recovery period of 7 days was observed. The mice were sacrificed on Day 14. A chronic DSS colitis model was also utilized. This involved oral administration of 20 μ g of endotoxin-free DNA 2 hours prior to induction of colitis with 2% DSS. DSS was given in the drinking water for 5 days, followed by substitution of regular water for DSS for the next 6 days (days 6–11). This cycle was repeated two more times, at the end of which the mice were sacrificed.

Clinical assessment

Through out the study multiple clinical parameters were measured. Specifically, % initial weight, stool consistency, and bleeding were recorded daily and graded for severity on a scale from 0 (less severe) to 4 (most severe). In order to calculate a disease activity score, each parameter was then rescored on a scale from 0 to 3. The scoring for each parameter were as follows: % initial weight (0=>99%, 1=92–99%, 2=85–92%, 3=<85%); stool consistency (0=normal, 1=slightly soft, 2=loose, 3=liquid); bleeding (0=negative, 1=faint blue on Hemocult, 2=blue on Hemocult, 3=gross red). The average of these parameters represented the disease activity score.

Gross and Histological Analysis

Inflammation-induced reduction in colon length has been previously used as a marker for severity of DSS-induced inflammation to the colon (22). Therefore, following sacrifice of the animals on day 14, the entire colon was removed and measured.

Swiss roll of the large colon was fixed in formalin for histological analysis. Histological sections were scored for severity of colitis based on the following parameters (ranging from 0–3) multiplied by the extent of involvement (times one for less than 10%, times two for 10–25%, and times three for greater than 25%): crypt damage, leukocyte infiltration, submucosal edema, and hemorrhage. A value of four was added to the histological score if there was evidence for transmural involvement (23). The scoring was performed in a blinded fashion by two independent investigators (JL and JYK).

Determination of type I IFN levels in patients

Male patients aged 50–79 years undergoing routine screening colonoscopy at the University of Michigan Health Systems and Ann Arbor Veterans Affairs Medical Center were recruited for participation. Serum was collected from consenting individuals. No patients included in this analysis carried a diagnosis of IBD or were on interferon therapy. Each serum sample was tested for the presence of *H. pylori* IgG antibodies using an *H. pylori* IgG enzyme immunoassay kit (Diamedix®, Miami, FL). IFN- α levels were measured using ELISA (PBL Interferon Source, Piscataway, NJ).

Statistical Analysis

Statistical significance was determined by unpaired Student *t* test and one-way ANOVA testing using commercially available software (PRISM, GraphPad, San Diego, CA). Values of $P < 0.05$ were considered significant. All data are presented as mean $\pm$ SEM.

Results

H. pylori DNA has a high immunoregulatory (IRS) to immunostimulatory (ISS) ratio

To address the mechanism by which *H. pylori* may influence intestinal mucosal immunity, we turned our attention to DNA for multiple reasons. First, *H. pylori* DNA has been documented in both the stool and colon of infected patients (17–19). Second, microbial DNA possess certain sequences within their genome that can be either immunoregulatory (IRS) or immunostimulatory (ISS). These sequences are imperative in determining the host's response to an organism, as immune cells contain toll-like receptors which allow them to interact with microbial DNA.

We evaluated the IRS:ISS ratios of 6 known strains of *H. pylori* (G27, Shi470, HPAG1, P12, 22695, J99) and compared these values to other bacteria. We found that the values for the 6 *H. pylori* strains range from 19.5 to 37.01 (mean: 29.4) (Figure 1). In contrast, the IRS:ISS ratios of *E. coli* K27 and *E. faecalis*, two common intestinal flora with the ability to cause acute infection, are 0.36 and 0.27, respectively. *Mycobacterium* paratuberculosis, a bacteria previously implicated in the pathogenesis of IBD (24), has an IRS:ISS ratio of 0.70. *Clostridium difficile* has a ratio of 1.16, while *Helicobacter hepaticus* has a ratio of 3.08. Probiotic bacteria, such as *Bifidobacterium. infantis* and *longum* and *Lactobacillus reuteri* and *acidiphillus*, all have ratios less than 1 (mean: 0.47). The fact that probiotic DNA have ratios of less than 1 suggests their immunoregulatory properties are mediated through a different mechanism, perhaps a mechanism independent of type I IFN production. This data clearly illustrate *H. pylori* is unique among bacteria in that its genome has a high ratio of IRS to ISS.

H. pylori DNA does not induce a pro-inflammatory response by DCs and can suppress *E. coli*-induced pro-inflammatory responses

It has been previously shown that ISS can stimulate production of type I IFN by DCs through a toll-like receptor 9-dependent mechanism (25). Based on our previous data which revealed IRS and ISS frequencies in various bacteria, we hypothesized that bacteria with

high ISS, such as *E. coli*, would stimulate pro-inflammatory cytokines, whereas bacteria with low ISS, such as *H. pylori*, would be unable to stimulate DCs. In fact we observed significant production of type I IFN and IL-12 by mDCs stimulated with *E. coli* DNA to levels similar to that of lipopolysaccharide (LPS) stimulation. *H. pylori* DNA, however, stimulated marginal production of these pro-inflammatory cytokines (Figure 2). Additionally, Duramad *et al.* showed that IRS has the ability to block ISS-stimulated DC production of type I IFN (26). Of note, pDCs, as compared to conventional DCs, are the primary source of type I IFN in humans (13). Therefore, we conducted our experiments both in DCs from mice (mDC) and from humans (pDC). Because *H. pylori* DNA has a higher IRS:ISS than *E. coli* DNA, we hypothesized *H. pylori* DNA would have the capability to suppress *E. coli*-induced production.

As seen in Figure 2b, *H. pylori*-DNA displayed the ability to significantly suppress *E. coli*-induced type I IFN production in a dose-dependent manner (mDC: 2.6 fold reduction, $p < 0.05$, pDC: 4.7 fold reduction, $p < 0.001$). To exclude DOTAP toxicity as the primary cause for our observed reduction, increasing doses of calf-thymus DNA were cultured with *E. coli*-DNA-treated DCs. In contrast to *H. pylori* DNA, calf-thymus DNA was unable to suppress type I IFN production even at high doses (data not shown). The suppressive effects of *H. pylori* DNA on *E. coli* DNA were also observed with IL-12.

Administration of *H. pylori* DNA attenuates the severity of acute DSS-induced colitis

We next examined the effects of *H. pylori* DNA in vivo. Specifically, we assessed the effect of *H. pylori* DNA on the severity of DSS-induced colitis. Mice were pre-treated with either *E. coli* DNA, *H. pylori* DNA, or vehicle control (TE) before administration of 3% DSS in the drinking water for 7 days. A recovery period, during which regular water was substituted for DSS-containing drinking water, was allowed for the next 7 days. Because *H. pylori* DNA can act directly on dendritic cells, and dendritic cells have been shown to be important mediators for DSS-induced colitis (22), we speculated that animals receiving pre-treatment with *H. pylori* would experience less severe colitis.

Accordingly, *H. pylori* DNA-treated animals experienced less bleeding, weight loss, and loss of stool consistency compared to *E. coli* DNA- or vehicle-treated mice (Figure 3a). The differences between each group became more evident following the removal of DSS from the drinking water (day 7), emphasizing the importance of *H. pylori* DNA during the recovery phase. These clinical markers suggest less DSS-induced inflammatory changes in the *H. pylori* DNA mice. Consequently, the disease activity score was significantly lower in the *H. pylori* DNA mice compared to the *E. coli* and vehicle-control groups (0.75 vs. 1.21 and 1.22, respectively, $P < 0.01$) (Figure 3a).

In accordance with the clinical data, certain gross and histological parameters also favored the *H. pylori* DNA-treated mice suggesting less severe disease. For example, the average length of colons in the *H. pylori* DNA-treated mice (7.60cm) was longer than the *E. coli* DNA group (6.78cm, $p < 0.05$) and the vehicle controls (6.94cm), suggesting less inflammation (Figure 3b). Additionally, the average histological score from cecal biopsies in the *H. pylori* group (15.88) was lower than the *E. coli* group (22.25, $P < 0.05$) and the vehicle control group (21.33, $P < 0.05$) indicating less inflammation (Figure 3c).

Administration of *H. pylori* DNA attenuates the severity of chronic DSS-induced colitis

Inflammatory bowel disease is a chronic inflammatory disorder. Therefore, we sought to examine the effect of *H. pylori* DNA on the severity of colitis in a chronic inflammatory model. Therefore, we examined the effect of administration of *H. pylori* DNA orally delivered to mice prior to each of three cycles consisting of 5 days of 2% DSS water

followed by 6 days of regular water. As compared to mice treated with *E. coli* DNA or TE, *H. pylori*-DNA treated mice experience less bleeding and less diarrhea (lower stool consistency score) at the end of three cycles of DSS, suggesting less inflammatory changes (Figure 4a). There was no difference in weight change between the groups, as all mice maintained their baseline weight throughout the experiment. In correlation with the clinical data, *H. pylori*-treated mice had lower cecal histological scores (HP 10.5, EC 16.5, TE 13.9) (Figure 4b). There was no statically significant difference in the cecal level of type I mRNA expression, however there is a trend towards lower levels in the *H. pylori* DNA mice as compared to *E. coli* DNA-treated mice (Figure 4c). There was, however, significantly less systemic type I IFN in the *H. pylori*-DNA treated mice, suggesting less systemic inflammation (Figure 4d).

The systemic level of type I IFN is lower in *H. pylori*-infected patients as compared to controls

H. pylori has the unique ability to evade host immune response through down-regulation of pro-inflammatory responses. Based on our previous observations, we speculated that *H. pylori*'s down-regulation of the pro-inflammatory type I IFN in vitro would correlate to the human model and may explain another mechanism by which *H. pylori* promotes its own survival. We hypothesized that the systemic levels of type I IFN would be lower in *H. pylori*-infected patients versus controls. To test this, patients presenting for routine colonoscopy were tested for *H. pylori* chronic infection (serum IgG status) followed by assessment of systemic levels of type I IFN.

We recruited 46 patients for our analysis. As seen in Figure 4a, the average level of type I IFN was significantly lower in *H. pylori*-colonized patients (10.4 pg/mL) (n=22) versus controls (41.1 pg/mL) (n=24) ($P < 0.05$). Furthermore, the percentage of patients with detectable levels of type I IFN was lower in the *H. pylori*-infected group (Figure 4B). This data suggest that the systemic level of type I IFN is lower in chronically infected *H. pylori* patients.

Discussion

In this study we highlight the high ratio of IRS to ISS within the genome of *H. pylori* and correlate this finding with in vitro data illustrating decreased production of pro-inflammatory cytokines such as type I IFN and IL-12 by DCs. We further show the ability of *H. pylori* DNA to suppress *E. coli* DNA-induced pro-inflammatory responses by DCs in vitro. This in vitro data correlated with an in vivo model of colitis in which administration of *H. pylori* DNA prior to DSS ameliorated the severity of colitis. The decreased production of type I IFN by DCs correlated with a reduced systemic level of the cytokine in patients infected with *H. pylori*.

H. pylori are bacteria that strictly colonize the upper gastrointestinal tract. Therefore, it is interesting to consider that this infection may impact immune homeostasis distally in the small bowel and colon. Our group has previously demonstrated a protective effect of *H. pylori* colonization in mice against experimental colitis (27). Specifically, when mice were colonized with *H. pylori* SS1 6 weeks prior to the induction of experimental colitis, the severity of inflammation was markedly reduced as compared to mice not colonized with *H. pylori*. The mechanism for this observation, however, remained elusive. In this paper, we suggest that the physical connection between its effect in the upper GI tract and more distally is the DNA which is shed into the stool and colon of infected patients. Therefore, the interaction between shed DNA and immune cells such as DCs may alter immunity. Specifically, if the mucosal barrier is damaged as in our DSS model of colitis and as seen in patients with IBD, luminal *H. pylori* DNA may down-regulate and interfere with the ability

of other bacteria, such as *E. coli*, to interact with DCs and promote significant inflammatory responses.

The role of ISS and type I IFN in the pathogenesis of colitis has been previously studied. Consistent with our results, Obermeier and colleagues demonstrated significant weight loss, reduction in colon length, and higher histological scores in mice treated with CpG motifs (ISS) compared to controls using acute and chronic DSS-colitis models (23). They further showed that the administration of sequences from adenoviral DNA with immunoregulatory properties was able to attenuate colitis in CpG-treated mice in a chronic DSS model (22). In contrast, Rachmilewitz *et al.* found ISS administration prior to the induction of DSS attenuated the severity of colitis (20). This same group showed that the protective effect of ISS was mediated by conventional dendritic cells. Specifically, they showed wild-type B6 mice recovered to their baseline weight quicker than IFN- α/β R^{-/-} mice after 7 days of DSS-induced colitis (14 versus 21 days following induction) (28, 29). It was speculated that this was a result of differential recruitment of monocytes and neutrophils to sites of inflammation. Therefore, the role of ISS and IRS as well as type I IFN in the pathogenesis of colitis remains unclear. However, there are abundant previously described pro-inflammatory mechanisms for type I IFN, of which includes its imperative role both in the ability of tumor necrosis factor to cause inflammation and in maintaining a Th1 response in celiac disease (15, 16).

Clinically, the role of type I IFN also remains controversial. There have been multiple case reports associating the development of IBD with the initiation of type I IFN therapy (30–33). For example, a recent report from Morimoto and colleagues describes the development of endoscopically-proven pouchitis in a patient who had been previously doing well following total colectomy for medication-resistant ulcerative colitis (34). However, the patient's symptoms acutely worsened following initiation of IFN- α therapy for chronic hepatitis C. In contrast, certain studies have examined the potential therapeutic role of type I IFN for IBD (35–38). A meta-analysis of three studies comparing IFN treatment versus placebo for ulcerative colitis in a randomized fashion found no difference in induction of remission (39). Just as is the case with murine models of colitis, the role of type I IFN in the pathogenesis of human colitis remains controversial.

Furthermore, our work highlights *H. pylori*'s unique interaction with the host's immune system.. It has been previously shown that *H. pylori* induces a regulatory T-cell response and also down-regulates the pro-inflammatory Th17 pathway (40–43). Our data provide another mechanism which *H. pylori* can down-regulate pro-inflammatory responses, specifically through a reduction in type I IFN production. Therefore, it is feasible that *H. pylori*, by attempting to promote its own survival, offers protective benefit against other chronic inflammatory conditions such as IBD and asthma through these mechanisms.

In summary, we show the ability of *H. pylori* DNA, which has the unique characteristic of containing a significantly high IRS:ISS ratio, to attenuate the severity of chemically-induced colitis. We correlate this amelioration with decreased production of type I IFN by dendritic cells. Our results suggest that the clinically observed negative association between *H. pylori* and IBD is in part explained by *H. pylori* DNA-induced decrease in type I IFN production. However, further studies are needed to elucidate the role of type I IFN in pathogenesis of colitis. Additionally, further investigation into other potential protective mechanisms by *H. pylori*, such as up-regulation of IL-10 and TGF-beta and down-regulation of IL-17 in the cecum and mesenteric lymph nodes of infected individuals, are warranted.

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Summary Box

What is already known about this subject?

- Over the past two decades, the worldwide incidence of inflammatory bowel disease has increased substantially
- *H. pylori*, whose incidence has decreased over the past two decades, can induce a T-regulatory cell response thereby suppressing pro-inflammatory responses
- There is an epidemiological inverse association between *H. pylori* and certain chronic inflammatory conditions, such as asthma and IBD

What are the new findings?

- *H. pylori* DNA, which is shed into the distal intestinal system in colonized patients, has unique immunoregulatory properties
- *H. pylori* DNA can attenuate an experimental model of colitis
- *H. pylori* colonized patients have lower systemic levels of type I interferon

How might it impact on clinical practice in the foreseeable future?

- *H. pylori*, through its immunoregulatory properties, may offer benefit to colonized patients
- Because of its potential benefits, asymptomatic *H. pylori* colonization may be of value, precluding a need for global vaccination

Figure 1. An elevated IRS:ISS ratio within the genome of *H. pylori*

Using previously published data describing IRS (immunoregulatory sequences) and ISS (immunostimulatory sequences), the ratio of the frequencies of IRS to ISS were calculated for various bacteria. The highest IRS:ISS can be found in all six strains of *H. pylori*. *H. hepaticus* has a IRS:ISS ratio of greater than one which corresponds with its lack of colonic pathogenic ability in the gut of immunocompetent C57/BL6 mice (44). Although *C. difficile* has an IRS:ISS ratio of greater than one, its ability to cause infection is predicated on toxin formation. Interestingly, probiotic bacteria have low IRS:ISS ratios suggesting their ability to modulate the immune response is mediated through a different mechanism.

(a)

(b)

(c)

Figure 2. *H. pylori* DNA does not induce a pro-inflammatory response by DCs and suppresses *E. coli*-induced pro-inflammation

Mouse mDC were cultured with 2.5ug/mL of *E. coli* DNA or *H. pylori* DNA. Additionally, mDCs were cultured with 2.5ug/mL of *E. coli* DNA and 1ug/mL, 3ug/mL, or 5ug/mL of *H. pylori* DNA for 8 hours. The same experiment was performed using human pDCs, although cultures were performed overnight.. Averaged data from three experiments from mouse data are shown. The human data were obtained from two separate donors. (a) *E. coli* DNA stimulates significant production of type I IFN from mouse myeloid dendritic cells (mDCs) as compared to *H. pylori* DNA (37.21 pg/mL vs 1.86 pg/mL, ** = $p < 0.01$). Further, *H. pylori*-DNA displayed the ability to significantly suppress *E. coli*-induced type I IFN production in a dose-dependent manner from mDCs (37.2 pg/mL to 11.37 pg/mL, * = $p < 0.05$). (b) *H. pylori*-DNA displayed the ability to significantly suppress *E. coli*-induced type I IFN production in a dose-dependent manner from human plasmacytoid dendritic cells (pDCs) (263.42 pg/mL to 30.63 pg/mL, ** = $p < 0.01$). (c) Last, *E. coli* DNA stimulated significantly more IL-12 production from mDCs as compared to *H. pylori* DNA (588.83 pg/mL versus 23.46 pg/mL, *** = $p < 0.001$). Further, *H. pylori*-DNA displayed the ability to significantly suppress *E. coli*-induced type I IFN production in a dose-dependent manner from pDCs (588.83 pg/mL to 303.84 pg/mL, *** = $p < 0.001$).

(a)

(b)

(c)

TE

EC

HP

Figure 3. *H. pylori* DNA attenuated the severity of acute DSS-induced colitis

Mice were pre-treated with oral gavage of 50ug of *H. pylori* DNA, *E. coli* DNA, or vehicle control prior to DSS administration for 7 days. This was followed by a 7 day recovery period. Mice receiving *H. pylori* DNA displayed (a) less weight loss, less bleeding measured with Hemocult, and greater stool consistency compared to the *E. coli* or vehicle control groups. The differences in each parameter widened during the 7 day recovery phase. Consequently, *H. pylori* DNA treated mice had lower disease activity scores, most pronounced at day 14 (*H. pylori* DNA: 0.75, *E. coli* DNA: 1.22, TE: 1.21, ** = $p < 0.01$). Data were taken from all 8 mice per group. Furthermore, (b) *H. pylori* DNA-treated mice had significantly longer colons than *E. coli* DNA mice (* = $p < 0.05$), and (c) less severe histological scores compared to *E. coli*- or vehicle-treated mice (* = $p < 0.05$). Notice the loss of glandular architecture and significant lymphocyte infiltration in the histological sections from the TE and *E. coli*-DNA treated mice. This is less severe in the *H. pylori* DNA-treated mouse section. All 8 mice per group were scored. One section, representative of that group,

is displayed. The black arrows highlight the epithelium in each histological section. As illustrated, there is significant more lymphocytic infiltration and loss of glandular architecture in the *E. coli* and TE groups as compared to the *H. pylori* DNA group.

(a)

Figure 4. *H. pylori* DNA attenuated the severity of chronic DSS-induced colitis

Oral administration of 20 μ g of endotoxin-free DNA 2 hours prior to induction of colitis with 2% DSS was performed. DSS was given in the drinking water for 5 days, followed by substitution of regular water for the next 6 days (days 6–11). This cycle was repeated two more times, at the end of which the mice were sacrificed. Data are shown as an average from all 5 mice per group. As seen in (a), mice treated with *H. pylori* DNA experienced less bleeding and diarrhea as compared to mice treated with *E. coli* DNA or TE. Further, there was less severe inflammation histologically in mice treated with *H. pylori* DNA (b). The black arrows highlight the epithelium in which there is more lymphocytic infiltration and loss of glandular architecture in the *E. coli* DNA and TE treated groups. As seen in (c), there

was no statistical difference in the level of cecal mRNA expression of type I IFN between groups, however the level of systemic type I IFN in the *H. pylori* DNA-treated group was significantly less as compared to the *E. coli* DNA or TE-treated groups (d).

Figure 5. *H. pylori*-colonized patients have lower systemic levels of type I IFN

Serum samples from volunteers presenting for routine screening colonoscopy were collected. The serum was then tested for *H. pylori* IgG antibody as well as for levels of type I IFN. As seen in (a), patients colonized with *H. pylori* had significantly lower levels of type I IFN in their serum compared to non-colonized controls (* = $p < 0.05$). Additionally, the number of patients with detectable type I IFN levels was higher in non-colonized patients versus *H. pylori*-colonized patients (b).

Table 1

Nucleotide sequences composing IRS and ISS (21)

IRS	ISS
TGTAGGGG	TGACGTTC
TTTAGGGG	TAACGTTG
TTGAGGGG	TGACGTTG
TGGAGGGG	AGACGTTA
TGGCGGGG	TGACGTTT
AGTGGGGG	CAACGTTG
ATTGGGGG	TAACGTTC
	TGTCGTTC