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Stromal Regulation of Human Gastric Dendritic Cells Restricts the Th1 Response to *H. pylori*

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Abstract

Background and Aims—Mucosal dendritic cells (DCs) play a key role in initiating the Th1 response to *H. pylori*. To further elucidate the mucosal response to *H. pylori*, we examined whether gastric stromal factors condition DCs to support tolerance to *H. pylori*, analogous to intestinal stromal factor-driven macrophage tolerance to commensal bacteria.

Methods—To model mucosal DC development, we isolated and cultured cell-depleted human stroma/extracellular matrix from fresh gastric and intestinal mucosa to generate stroma-conditioned media (S-CM). We then analyzed the capacity of S-CM-treated monocyte-derived DCs and primary human gastric and intestinal DCs pulsed *in vitro* with *H. pylori* to induce T cell proliferation and IFN- γ secretion.

Results—Stromal factors in gastric mucosa suppressed *H. pylori*-stimulated DC activation and the ability of DCs to drive a Th1 proliferative and cytokine response to *H. pylori*. The ability of gastric stromal factors to down-regulate DC function was similar to that of intestinal stromal factors and was independent of transforming growth factor- β , prostaglandin E₂, interleukin (IL)-10 and thymic stromal lymphopoietin. S-CM-induced reduction in DC-stimulated Th1 responses was associated with reduced DC release of IL-12.

Conclusion—Gastric stromal factors down-regulate DC responsiveness to *H. pylori*, resulting in a dampened gastric Th1 response. We speculate that stroma-induced down-regulation of DC function contributes to the permissiveness of both gastric and intestinal mucosa to colonization by persistent residential microbes.

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Keywords

Dendritic cells; mucosa; *H. pylori*; stomach; gastritis

Introduction

Helicobacter pylori gastritis is the most common antecedent lesion associated with peptic ulcer and gastric cancer worldwide^{1, 2}. To elucidate the early events in the pathogenesis of *H. pylori* gastritis, we recently identified gastric dendritic cells (DCs) as important cellular elements involved in the induction of the T cell response to *H. pylori*³. Specifically, DCs isolated from normal human gastric mucosa and pulsed *in vitro* with *H. pylori* induced a prominent Th1 cell response, analogous to the gastric Th1 response in both human and murine *H. pylori* infection *in vivo*^{4, 5}.

Despite the Th1 response that may progress to severe mucosal damage during *H. pylori*-associated inflammation, the infection is characterized by prolonged bacterial colonization⁶. Local host factors that contribute to the permissive mucosal environment in *H. pylori* gastritis are not well understood; in contrast, bacterial factors have received intense investigative attention⁷. Newly appreciated mucosal factors include soluble mediators derived from mucosal epithelial cells, immune cells and stromal cells that bind to proteoglycans in the extracellular matrix (stroma) of the mucosal lamina propria (stromal factors)^{8, 9}. In the intestine, epithelial cell-derived factors condition DCs to suppress pro-inflammatory responses, thereby contributing to immune homeostasis in the mucosa^{10–13}. Among the stromal factors in human intestinal mucosa, transforming growth factor β (TGF- β) regulates the differentiation of pro-inflammatory blood monocytes into non-inflammatory intestinal macrophages, as we have reported^{14–16}. Whether stromal factors in human gastric mucosa condition resident antigen-presenting cells, thereby regulating the response to *H. pylori*, is not known. Therefore, we isolated cell-depleted human gastric stroma/extracellular matrix and generated stroma-conditioned media (S-CM) to determine whether soluble gastric stromal factors regulate DC phenotype and function. In addition, since *H. pylori* infection may involve the proximal duodenum¹⁷, and intestinal DCs reportedly take up *Helicobacter* antigens¹⁸, we also generated conditioned media from isolated intestinal stroma. We show that factors released from human gastric and intestinal stroma down-regulate DC responses to *H. pylori*, thereby promoting a reduced T cell response that in turn may contribute to bacterial persistence and prolonged *H. pylori* gastritis.

Materials and Methods

Human subjects

Gastric and small intestinal tissue specimens were obtained with institutional review board approval from healthy human subjects undergoing elective gastric bypass surgery for obesity or from non-obese transplant donors. Heparinized blood samples were obtained from the same subjects or other healthy adult volunteers. All subjects were free of *H. pylori* infection as determined by CLO-Test and/or serology.

Cell isolation and enrichment

Gastric and intestinal lamina propria cells were isolated from surgical specimens using our previously described protocol³. Briefly, mucosa was dissected from the underlying muscularis propria and submucosa, washed twice for 20 min in HBSS (Mediatech Inc., Manassas, VA) supplemented with 0.2 mg/mL dithiothreitol (DTT, Sigma, St. Louis, MO), and epithelial cells were removed by incubation in Hank's buffered salt solution containing

1.25 mM EDTA plus 0.2 mg/mL DTT (3×30min). The tissue then was minced and transferred to a solution containing 0.5 FALGPA U/mL collagenase L (Sigma), 0.2 mg/mL DNase (Sigma), 20 mM HEPES (Mediatech Inc.) plus antibiotics in RPMI (Mediatech). After each of three 45 min collagenase treatments, lamina propria mononuclear cells (lymphocytes, macrophages and DCs) were collected by centrifugation. To enrich human leukocyte antigen (HLA) DR^{high} DCs, positive MACS® selection was used with a low concentration of anti-HLA-DR beads (0.2 µL per 10⁶ cells; Miltenyi Biotec, Auburn, CA). For T cell stimulation assays, mucosal DCs were further purified to >95% by FACS sorting (FACSVantage Diva, Becton Dickinson, San Jose, CA) using fluorescent-labeled anti-HLA-DR antibodies with gates set to include HLA-DR^{high} cells and to exclude lymphocytes, debris and propidium iodide-positive dead cells.

Stroma-conditioned media and tissue-conditioned media

Stroma- and tissue-conditioned media were prepared as described previously^{14, 16}. After tissue digestion (see previous paragraph), the remaining stroma consisted of cell-depleted extracellular matrix (collagen and proteoglycans), which contained only few residual epithelial cells, fibroblasts, endothelial cells and leukocytes, as determined by H&E staining. The stroma was cultured in serum-free RPMI (containing 20 mM HEPES and antibiotics) at 37°C for 24 h (1 g tissue wet weight/mL). The resultant supernatants were harvested, sterile-filtered (0.2 µm syringe filter, Corning Inc., NY), and frozen at -70°C. T-CM was derived from fresh intact gastric or intestinal mucosa that had been minced into 0.25 cm² pieces and cultured in RPMI at 37°C for 24 h at 0.5 g tissue/mL. Endotoxin and protein concentrations were determined by the Limulus Amebocyte Lysate assay (Cambrex, Walkersville, MD) and the BCA™ Protein Assay Kit (Thermo Scientific, Rockford, IL), respectively. Since some S-CM samples contained LPS (up to 15 endotoxin units/mL), polymyxin B (5 µg/mL, Sigma) was added to all cell cultures to block residual LPS effects on DCs.

Monocyte-derived DCs (MoDCs)

MoDCs were differentiated from MACS-isolated CD14⁺ blood monocytes by culturing 2×10⁶ monocytes per well in 24-well plates in complete medium (RPMI1640, 10% heat-inactivated human AB serum and antibiotics) supplemented with recombinant human granulocyte-monocyte colony stimulating factor (GM-CSF, 25 ng/mL) and interleukin 4 (IL-4, 17 ng/mL), both from R&D Systems, Minneapolis, MN. To recapitulate DC differentiation in the gastric or intestinal mucosal microenvironment, MoDC cultures were maintained in the presence or absence of gastric or intestinal S-CM or gastric T-CM at the previously determined optimal concentration of 500 µg protein/mL. Non-adherent cells were harvested as MoDCs by vigorous pipetting after four days of culture.

DC treatment with *H. pylori*

To elucidate the DC response to *H. pylori*, 3 – 5×10⁵ MoDCs were transferred into fresh medium (RPMI with 10% human Ab serum and IL-4/GM-CSF) with or without S-CM for 1 h, after which *H. pylori* 60190 (CagA⁺, VacA *s1m1*) was added at a multiplicity of infection (MOI) of 10 for 48 h, as previously described³. Surface marker expression was determined by flow cytometry.

TGF-β neutralization and replacement

For antibody neutralization experiments, S-CM (500 µg protein/mL) was incubated with anti-human TGF-β1/2/3 antibody (R&D Systems, 100 µg/mL) for 30 min at 37°C before adding the S-CM to the MoDC cultures. To recapitulate the potential effects of TGF-β on MoDCs, active rhTGF-β1 (R&D Systems) was added to MoDC cultures at 6.25 – 100 pg/

mL, a range that includes the TGF- β 1 concentrations routinely measured in gastric and intestinal S-CM at 500 μ g protein/mL.

PGE₂ neutralization and replacement

To determine the effect of exogenous PGE₂ on DC differentiation, PGE₂ (Sigma) was added to MoDC cultures at 0.06 – 1,000 ng/mL, a range that includes the PGE₂ concentrations in gastric and intestinal S-CM at 500 μ g protein/mL. For antibody neutralization, S-CM was incubated with anti-PGE₂ (45 μ g/mL, clone 2B5¹⁹, Cayman Chemicals, Ann Arbor, MI) or an irrelevant isotype-matched control (BD Pharmingen, San Diego, CA) for 60 min at 37°C before adding the S-CM to the MoDC cultures.

T cell stimulation assays

For DC-T cell stimulation assays, primary, FACS-purified gastric and intestinal HLA-DR^{high} DCs or day 4 MoDCs were pulsed with *H. pylori* (MOI=10) for 2 h at 37°C, washed twice and plated at 2×10^4 /well on 96-well plates. Autologous T cells were isolated by MACS using anti-CD4 microbeads or the Naive CD4⁺ T Cell Isolation Kit II (Milteny Biotec) and were added to the DCs at a DC:T cell ratio of 1:10, unless indicated otherwise. In some experiments, recombinant human IL-12p70 (1.25 – 20 ng/mL, R&D Systems) was added to the co-cultures. After 4 days, cell proliferation was determined by bromodeoxyuridine (BrdU) ELISA (Roche Diagnostics, Mannheim, Germany), and cell-free supernatants were collected for cytokine analysis.

Cell staining and flow cytometry

The following mAbs labeled with fluorescein isothiocyanate, phycoerythrin, peridinin-chlorophyll-protein, allophycocyanin or allophycocyanin-CyTM7, were used: CD11c (B-ly6), CD45 (2D1), CD80 (BB1), CD83 (HB15e), CD86 (FUN-1), HLA-DR (L243), DC-SIGN (DCN46), and appropriate isotype control antibodies, all from Becton Dickinson (San Jose, CA). For flow cytometric analysis, $0.3 - 1 \times 10^6$ cells were blocked with 10% human AB serum for 20 min, then treated with the mAbs for 20 min at 4°C, washed, fixed in Cytofix, and analyzed on an LSRII flow cytometer (Becton Dickinson). Data from 10,000 cells per sample were analyzed with FlowJo 7.5.5 software (TreeStar, Ashland, OR). To compare results from different experiments, relative geometric mean was calculated as $f(x) = (\text{geomean fluorescence [stained sample]} / \text{geomean fluorescence [isotype control of sample]}) / (\text{geomean fluorescence [stained control cells]} / \text{geomean fluorescence [isotype control of control cells]})$.

Cytokine levels

The amounts of IL-10, IL-12p70, interferon- γ (IFN- γ), PGE₂, TGF- β 1 and thymic stromal lymphopoietin (TSLP) in the S-CMs or culture supernatants were determined by ELISA (R&D Systems); the TGF- β 1 was assayed after acid activation of the S-CM.

Statistical analysis

Data were analysed using Microsoft® Excel 2003 and Analyse-it® for Excel, version 1.73. Results are presented as mean $\pm$ SEM. Differences between values were analysed for statistical significance by 1-way analysis of variance (ANOVA) with pairwise comparisons and Tukey's post hoc test, the two-tailed Student *t* test, or the Mann-Whitney U test, as indicated. Differences were considered significant at $P < 0.05$.

Results

Gastric and intestinal stromal factors inhibit DC activation

DCs differentiate from monocytes or DC precursors under the influence of tissue-specific stromal factors^{20, 21}. Using our established *in vitro* model of the mucosal microenvironment^{14–16}, MoDCs were generated in GM-CSF plus IL-4 in the presence or absence of conditioned media prepared from cell-depleted mucosal stroma/extracellular matrix (S-CM). DCs then were exposed to *H. pylori* bacteria in the continuous presence of S-CM. As shown in Figure 1A, *H. pylori* stimulation caused the up-regulation of CD80, CD83 and CD86 expression on MoDCs cultured in medium, consistent with previous findings^{3, 22–24}. Importantly, both gastric and intestinal S-CM blocked inducible CD80, CD83 and CD86, as well as differentiation (GM-CSF plus IL-4)-induced CD86 expression. Gastric and intestinal S-CM were nearly equivalent in their capacity to suppress MoDC differentiation and *H. pylori*-induced activation. Both gastric and intestinal S-CM also suppressed DC activation by *Salmonella enterica* Typhimurium (MOI = 50, data not shown), indicating that inducible activation was not specific for *H. pylori*.

To dissect whether S-CM inhibition of DCs occurred during differentiation or activation, MoDCs were generated in the presence or absence of S-CM (days 1–4) and then stimulated with *H. pylori*, also in the presence or absence of S-CM (days 4–6) (Figure 1B,C). The presence of either gastric or intestinal S-CM exclusively during MoDC differentiation (days 1–4) suppressed CD80, CD83 and CD86 expression (Figure 1B) to the same level as the continuous presence of S-CM during both MoDC generation and *H. pylori* stimulation (days 1–6) (Figure 1A). In contrast, the presence of S-CM exclusively during stimulation with *H. pylori* (days 4–6) reduced DC activation marker expression by only a small amount (Figure 1C). Supernatants generated from intact gastric mucosa (T-CM) had the same suppressive effects as gastric S-CM, indicating that inhibition of DC activation by S-CM was not caused by collagen fragments or other products resulting from the collagenase treatment (Figure 2). These findings suggest that stromal conditioning of DCs occurs primarily during DC differentiation, rather than during activation, and that such conditioning has lasting inhibitory effects on the differentiation of mucosal DCs, consistent with the notion that mucosal DCs retain their phenotype after they leave the mucosa and migrate to draining lymph nodes.

Stromal inhibition of DCs is independent of TGF- β , IL-10, TSLP and PGE₂

To explore the mechanism by which gastric and intestinal stromal factors suppress DCs, we determined whether TGF- β , IL-10, TSLP or PGE₂, soluble mediators that regulate immune cell functions at mucosal sites^{11, 14, 15, 25–27}, contribute to stromal regulation of DCs. Both gastric S-CM (3,043 $\pm$ 327 μ g protein/mL; n=12) and intestinal S-CM (3,115 $\pm$ 390 μ g protein/mL; n=12) contained the anti-inflammatory cytokine TGF- β 1, although the concentration of TGF- β 1 was significantly lower in gastric S-CM (39 $\pm$ 15 pg/mL) than in intestinal S-CM (146 $\pm$ 42 pg/mL; $P < 0.01$) (Figure 3A). In contrast, gastric S-CM contained significantly higher levels of PGE₂ (17,020 $\pm$ 3,528 pg/mL) than intestinal S-CM (4,030 $\pm$ 1,422 pg/mL; $P < 0.01$). IL-10 and TSLP were undetectable in both gastric and intestinal S-CM. We first examined whether the inhibitory effect of S-CM on DC activation marker expression was due to stromal TGF- β . Neutralization of TGF- β with anti-TGF- β 1/2/3 antibody (100 μ g/mL), shown previously to inhibit TGF- β activity in intestinal S-CM^{14, 15}, did not prevent gastric or intestinal S-CM inhibition of *H. pylori*-induced MoDC expression of CD83 (Figure 3B) or CD80 and CD86 (data not shown). Furthermore, the addition of 6.25 – 100 pg/mL rhTGF- β 1 to MoDC cultures, which was equivalent to the amount of TGF- β 1 present in gastric and intestinal S-CM at a concentration of 500 μ g protein/mL, did not inhibit *H. pylori*-induced up-regulation of CD80, CD83 and CD86 (data not shown).

To determine whether PGE₂ contributes to S-CM suppression of DC activation, we next showed that exogenous PGE₂ added during DC differentiation blocked *H. pylori*-induced DC expression of CD83 (Figure 3C), CD80 and CD86 (not shown) in a dose-dependent manner, with maximum suppression seen at ≥ 10 ng/mL PGE₂. However, gastric and intestinal S-CM diluted to 500 μ g protein/mL routinely contained less PGE₂ than the amount of exogenous PGE₂ needed to achieve the same level of suppression. Consequently, antibody neutralization of PGE₂ in gastric and intestinal S-CM failed to restore the ability of the MoDCs to upregulate CD83 (Figure 3D), CD80 and CD86 (not shown), although antibody neutralization of 2.7 ng/mL of exogenous PGE₂, a dose calculated to match the PGE₂ concentration in the gastric S-CM used in the experiment, abolished the suppressive effects of the PGE₂. Exogenous PGE₂ at 0.06 ng/mL, the concentration present in the intestinal S-CM used in the experiment, did not suppress *H. pylori*-induced DC activation. These findings indicate that stromal factors other than TGF- β 1, IL-10, TSLP and PGE₂ suppress DCs in gastric and small intestinal mucosa.

Intestinal and gastric stromal factors reduce the ability of DCs to drive a Th1 response to *H. pylori*

We next determined whether S-CM inhibition of *H. pylori*-induced DC activation was associated with inhibition of inducible responses by CD4⁺ T cells, the predominant cell type in inflammatory infiltrates in *H. pylori* gastritis⁴. MoDCs differentiated in the presence or absence of gastric or intestinal S-CM were loaded with *H. pylori*, co-cultured with autologous CD4⁺ T cells, and the proliferative and cytokine responses of the T cells were determined 4 days later. MoDCs loaded with *H. pylori* drove a strong proliferative response, whereas MoDCs generated in the presence of either gastric or intestinal S-CM induced significantly less *H. pylori*-specific T cell proliferation (Figure 4A).

The Th1 response driven by *H. pylori* is characterized by T cell secretion of high levels of IFN- γ ³⁻⁵. Therefore, we examined whether S-CM modulates the ability of *H. pylori*-pulsed MoDCs to induce T cell IFN- γ secretion. Control MoDCs loaded with *H. pylori* elicited a strong T cell IFN- γ response, but MoDCs generated in the presence of either gastric or intestinal S-CM were significantly impaired in their ability to induce T cell IFN- γ secretion in response to *H. pylori* (Figure 4B). In contrast, neither gastric nor intestinal S-CM altered the ability of *H. pylori*-pulsed MoDCs to stimulate IL-10 secretion by T cells (Figure 4C). T cell proliferation and cytokine responses did not differ between total CD4⁺ T cells and naïve CD4⁺ T cells (data not shown).

Because DCs induce Th1 responses through IL-12²⁸, and because *H. pylori* stimulates DC IL-12 production^{22, 23, 29}, we next evaluated the effect of S-CM on *H. pylori*-stimulated MoDC secretion of IL-12p70. Importantly, both gastric and intestinal S-CM potently down-regulated *H. pylori*-induced MoDC secretion of IL-12 (Figure 4D). Addition of rhIL-12p70 to co-cultures of S-CM-treated, *H. pylori*-pulsed DCs and CD4⁺ T cells partially restored T cell IFN- γ secretion (Figure 4E). Thus, both gastric and intestinal S-CM significantly reduced the ability of *H. pylori*-pulsed MoDCs to induce an IFN- γ -mediated Th1 response, likely through impaired DC IL-12 release.

Reduced capacity of primary gastric and intestinal DCs to induce T cell expansion in response to *H. pylori*

Dendritic cells that reside in normal gastric and intestinal mucosa are exposed to mucosal stromal factors in their tissue environment. Therefore, we next determined whether primary human mucosal DCs are impaired in their response to *H. pylori*, as suggested by the preceding *in vitro* findings. Primary gastric and intestinal HLA-DR^{high}/CD13^{low} DCs isolated by our previously described protocols^{3, 30} constituted $5.3 \pm 1.2\%$ and $5.3 \pm 1.8\%$ of

the total gastric and intestinal lamina propria cells, respectively (n=4). Gastric and intestinal HLA-DR^{high} DCs also expressed similar levels of CD11c, DC-SIGN and CD206 (Figure 5). Both DC populations displayed equally low baseline expression of CD80, CD83 and CD86, consistent with an immature phenotype and low level activation. Having previously shown that *H. pylori* infection enhances primary mucosal DC maturation and activation³, we assessed the ability of primary gastric and intestinal DCs isolated from the same tissue donor and pulsed with *H. pylori* to induce T cell proliferation and cytokine secretion. *H. pylori*-pulsed primary gastric and intestinal HLA-DR^{high} DCs triggered only marginal T cell expansion (Figure 6A), consistent with the ability of S-CM to down-regulate MoDC-induced proliferative responses (Figure 4A). However, both mucosal DC populations induced T cell secretion of IFN- γ , indicative of a Th1 response (Figure 6B and C). In all experiments, *H. pylori*-pulsed gastric DCs and intestinal DCs induced nearly identical T cell responses. Thus, recapitulating our *in vitro* results with S-CM-treated MoDCs, primary DCs isolated from gastric and intestinal mucosa are phenotypically similar and induce similar levels of T cell IFN- γ secretion in the absence of strong T cell proliferation in response to *H. pylori*.

Discussion

We recently showed that human gastric DCs are key initiators of the Th1 response to *H. pylori*³. To elucidate the role of the mucosal microenvironment on gastric DC phenotype and function, we investigated the ability of gastric stromal factors to modulate DC differentiation/activation markers and the ability of DCs to present *H. pylori* to T cells. Using S-CM derived from cell-depleted gastric lamina propria stroma/extracellular matrix as a surrogate for the gastric lamina propria, we show that exposure to gastric stromal factors prevented *H. pylori*-induced CD80, CD83 and CD86 expression. MoDCs exposed to stromal factors during differentiation from monocytes, but not during activation by *H. pylori*, retained their down-regulated phenotype, supporting the concept that mucosal DCs retain their tissue-specific characteristics after leaving the mucosae^{31–33}. Importantly, T-CM derived from intact gastric mucosa had similar effects as S-CM, indicating that the suppressive effects of the S-CM were not a result of the collagenase treatment. Stromal factors also down-regulated MoDC and primary gastric DC induction of T cell proliferation and IFN- γ secretion in response to *H. pylori*, likely through inhibition of IL-12p70 secretion. Thus, stromal down-regulation of gastric DCs, and macrophages as reported elsewhere^{14, 15}, indicate a potential role for the stroma in the innate suppression of pro-inflammatory responses to colonizing bacteria, thereby contributing to the maintenance of mucosal homeostasis^{14, 34–38}.

In the gastrointestinal mucosa, epithelial cells, resident immune cells and lamina propria stromal cells secrete a complex array of cytokines, chemokines and other soluble factors that bind to the extracellular matrix, creating a reservoir of immunoactive “stromal factors”^{8, 9, 39, 40}. In this connection, previous studies from our laboratory have shown that stromal TGF- β 1 contributes to inflammation anergy in intestinal lamina propria macrophages^{14, 15}. TGF- β 1 was present in both gastric and intestinal S-CM, but antibody neutralization of TGF- β 1, 2 and 3 did not alter the effect of either S-CM on DC activation, suggesting that stromal regulation varies among mucosal compartments and target cell populations. TGF- β 1 has been reported to inhibit DC differentiation *in vitro*, albeit at a higher concentration (5 ng/mL) than the levels of TGF- β 1 in gastric and intestinal S-CM (6.5 and 24 pg/mL, respectively for 500 μ g total protein/mL)⁴¹.

PGE₂, an important stroma-derived mediator, also has the capacity to down-modulate DCs^{10, 27, 42}. Indeed, PGE₂ added to MoDC cultures during differentiation had an inhibitory effect on *H. pylori*-induced DC activation similar to that of gastric and intestinal S-CM.

Importantly, PGE₂ was present in both gastric and intestinal S-CM, although at a low concentration. However, antibody neutralization of PGE₂ in gastric and intestinal S-CM failed to restore the ability of MoDCs to respond to *H. pylori*-stimulation. Our intriguing results that gastric and intestinal S-CM downregulation of DCs was independent of TGF- β , PGE₂, IL-10 and TSLP point to a major role for other DC-regulatory stromal factors, which are a subject of ongoing investigation in our laboratory.

Dendritic cells are highly tissue specific with special subsets present in different organs and tissue compartments^{43, 44}. Surprisingly, we show that the gastric and the intestinal stromal microenvironment have remarkably similar down-regulatory effects on DC phenotype and function. Intestinal stromal factors suppressed DC activation, IL-12 secretion and their ability to drive T cell proliferation and IFN- γ secretion, likely contributing to intestinal tolerance. Teleologically, the requirement for “tolerance” is less evident in the gastric mucosa. First, commensal bacteria have not yet been identified in the human stomach, except possibly *H. pylori*, which has features of both a commensal and pathogenic microorganism^{7, 45, 46}. Second, the gastric mucosal barrier is considerably tighter than that of the intestine, due to a thick pre-epithelial mucusbicarbonate-phospholipid layer, which prevents luminal antigens from accessing the gastric lamina propria⁴⁷. Consequently, a default tolerogenic response may not be necessary in the gastric mucosa, and indeed, bacterial colonization of the gastric mucosa with *H. pylori* consistently results in chronic inflammation³⁴. Nevertheless, as shown here, gastric stromal factors did indeed suppress DC responses. Thus, gastric and small intestinal mucosa may have co-evolved as a common immunoregulatory compartment in which local DC function is conditioned by stromal factors for tolerogenic responses to colonizing and/or commensal bacteria.

In summary, we present new evidence that gastric stromal factors down-regulate *H. pylori*-induced DC activation and T cell stimulatory capacity, resulting in a dampened Th1 response to the bacteria. Thus, gastric mucosal tolerance may contribute to a permissive environment that facilitates prolonged *H. pylori* colonization, similar to the intestinal mucosal tolerance that permits colonization by commensal bacteria. In this regard, systemic Th1-promoting vaccines conferred a greater protection of mice from *H. pylori* challenge than mucosal vaccines^{48, 49}. Our results offer a potential mechanism whereby a systemic vaccine to *H. pylori* that bypasses down-regulated gastric DCs may be more effective than a mucosal vaccine, suggesting that preventive strategies for *H. pylori*-infection in humans should target non-mucosal DC populations.

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Abbreviations

ANOVA	analysis of variance
BrdU	Bromodeoxyuridine
DC	dendritic cell
DTT	dithiothreitol

ELISA	enzyme-linked immunosorbent assay
GM-CSF	granulocyte monocyte colony stimulating factor
HLA	human leukocyte antigen
IFN-γ	interferon gamma
IL	interleukin
MoDC	Monocyte-derived dendritic cell
MOI	multiplicity of infection
PGE₂	prostaglandin E ₂
rh	recombinant human
S-CM	stroma-conditioned media
TGF-β	transforming growth factor beta
Th1	T helper 1
TSLP	thymic stromal lymphopoeitin

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Figure 1.

Conditioned media from gastric and intestinal stroma (S-CM) down-modulate *H. pylori*-induced MoDC activation. MoDCs were differentiated from blood monocytes, stimulated with *H. pylori* and then analyzed for activation marker expression by FACS. DCs were exposed to gastric or intestinal S-CM (500 µg protein/mL) during (A) both DC differentiation (days 1–4) and *H. pylori*-induced activation (days 4–6) (n=8), (B) DC differentiation only (n=4) or (C) activation only (n=3). Bars represent geometric mean fluorescence normalized to medium control samples (where control=1) and corrected for isotype control antibody. Values are shown as mean ± SEM; statistical significance **P*≤.05 was determined by 1-way ANOVA with Tukey's post hoc test.

Figure 2.

T-CM derived from gastric mucosal tissue down-modulate *H. pylori*-induced MoDC activation. MoDCs were exposed to medium alone, gastric S-CM or gastric T-CM (500 μ g protein/mL) during DC differentiation, stimulated with *H. pylori* and then analyzed for activation marker expression by FACS (n=3). Bars represent geometric mean fluorescence normalized to medium control samples (where control=1) and corrected for isotype control antibody. Values are represented as mean $\pm$ SEM; statistical significance $*P \leq 0.05$ was determined by 1-way ANOVA with Tukey's post hoc test.

Figure 3.

(A) Gastric and intestinal S-CM were analyzed for the presence of anti-inflammatory mediators TGF-β1 (n=13), IL-10 (n=5), TSLP (n=5) and PGE₂ (n=8) by ELISA. Values represent mean ± SEM; Mann-Whitney U test: **P≤.01. Detection limits for IL-10 and TSLP were 3.9 pg/mL and 3.5 pg/mL, respectively. (B) MoDCs were differentiated in the presence of medium, gastric or intestinal S-CM (500 μg protein/mL), or gastric or intestinal S-CM (500 μg protein/mL) pre-incubated with anti-TGF-β1/2/3 (100 μg/mL), stimulated with *H. pylori* and then analyzed for CD83 expression. Black histogram and legend correspond to cells treated with *H. pylori*, grey histogram and legend to cells treated with medium, and filled grey histogram to cells plus isotype control antibody; n=2. (C) MoDCs were differentiated from blood monocytes in the presence of medium, gastric or intestinal S-CM (500 μg/mL) or PGE₂ (0.1 – 1,000 ng/mL), stimulated with *H. pylori* and then analyzed for CD83 expression. Mean ± SEM of two independent experiments. (D) MoDCs were generated in the presence of medium, PGE₂ (2.7 or 0.06 ng/mL), gastric S-CM (2.7 ng/mL PGE₂, 500 μg protein/mL) or intestinal S-CM (0.06 ng/mL PGE₂, 500 μg protein/mL), all either pre-incubated with anti-PGE₂ (45 μg/mL) or an irrelevant isotype control for 1 h at 37°C. DCs then were stimulated with *H. pylori* and analyzed for CD83 expression. Results from a representative experiment (n=2).

Figure 4.

Gastric and intestinal stromal factors inhibit the ability of DCs to prime a Th1 response through suppression of DC IL-12p70 secretion. (A) MoDCs were differentiated in medium alone or in the presence of gastric or intestinal S-CM (500 μ g protein/mL), harvested, and pulsed with *H. pylori* bacteria for 2 h. Proliferation of autologous CD4⁺ T cells in response to untreated or *H. pylori*-pulsed DCs was determined after 4 days by BrdU ELISA. Proliferation is expressed as T cell proliferation index, which was calculated as absorption of DC-T cell co-culture relative to absorption of CD4⁺ T cells alone. (B) IFN- γ and (C) IL-10 secretion by autologous CD4⁺ T cells in response to untreated DCs or *H. pylori*-pulsed DCs was determined for the 4-day culture supernatants by ELISA. (D) Gastric and

intestinal S-CM block *H. pylori*-induced IL-12p70 secretion by MoDCs. MoDCs differentiated in the presence or absence of S-CM were stimulated with *H. pylori* for 48 h, and culture supernatants were analyzed for IL-12p70 by ELISA (detection limit 5 pg/mL). (A – D) Diamonds represent cumulative data from three experiments with one or two S-CM each; lines represent mean values. * $P \leq .05$, 1-way ANOVA with Tukey's post hoc test. (E) Co-cultures of naïve CD4⁺ T cells and S-CM-treated or untreated DCs pulsed with *H. pylori* were established as described above, with rhIL-12p40 added at 5 ng/mL. T cell IFN- γ secretion was determined by ELISA analysis of 4-day culture supernatants. Results from a representative experiment (n=4).

Figure 5.

Phenotype profile of primary human gastric and intestinal DCs. DCs were isolated from healthy human stomach and jejunum by collagenase digestion followed by HLA-DR MACS purification as described in the Materials and Methods. Surface marker expression was determined on DCs gated as HLA-DR^{high} cells. **(A)** Representative dotplots and **(B)** mean ± SEM proportion of cells that expressed the indicated surface markers (n=3–5).

Figure 6.

Primary gastric and intestinal DCs are weak inducers of T cell proliferation but trigger T cell IFN- γ secretion. Freshly isolated gastric and intestinal HLA-DR^{high} DCs derived from healthy subjects were pulsed with *H. pylori* bacteria for 2 h and then co-cultured with autologous T cells. (A) Proliferation of autologous blood T cells in response to DCs treated with medium alone or with *H. pylori* was determined after 4 days by BrdU ELISA. Proliferation is expressed as T cell proliferation index and was calculated as absorption of DC-T cell co-cultures relative to absorption of T cells alone. Data are representative of 3 experiments with cells from separate subjects. (B,C) IFN- γ secretion by autologous T cells in response to *H. pylori*-pulsed or medium-treated DCs for 4-day culture supernatants was

determined by ELISA. **(B)** Cumulative data from 5 experiments at a DC-T cell ratio of 1:10 (dots) with mean values shown as bars, and **(C)** the DC dilution curve for values from the subject shown in **(A)**. Values correspond to mean $\pm$ SEM.