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Cutting Edge: Cyclooxygenase-2 Activation Suppresses \( \text{Th1} \) Polarization in Response to \textit{Helicobacter pylori} \(^1\)

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Helicobacter pylori infection causes a \( \text{Th1} \)-driven mucosal immune response. Cyclooxygenase (COX)-2 is up-regulated in lamina propria mononuclear cells in H. pylori gastritis. Because COX-2 can modulate \( \text{Th1}/\text{Th2} \) balance, we determined whether H. pylori activates COX-2 in human PBMCs, and the effect on cytokine and proliferative responses. There was significant up-regulation of COX-2 mRNA and PGE\(_2\) release in response to \( \text{H. pylori} \) preparations. Addition of COX-2 inhibitors or an anti-PGE\(_2\) Ab resulted in a marked increase in \( \text{H. pylori} \)-stimulated IL-12 and IFN-\( \gamma \) production, and a decrease in IL-10 levels. Addition of PGE\(_2\) or cAMP, the second messenger activated by PGE\(_2\), had the opposite effect. Similarly, stimulated cell proliferation was increased by COX-2 inhibitors or anti-PGE\(_2\), Ab, and was decreased by PGE\(_2\). Our findings indicate that COX-2 has an immunosuppressive role in \( \text{H. pylori} \) gastritis, which may protect the mucosa from severe injury, but may also contribute to the persistence of the infection. The Journal of Immunology, 2003, 171: 3913–3917.

\( \text{H. pylori} \) is a Gram-negative, microaerophilic bacterium, which selectively colonizes the mammalian stomach, and causes gastritis, peptic ulcers, and gastric cancer. The human host mounts a vigorous innate and adaptive immune response, yet this results only in lifelong gastritis without eradication of the organism. \( \text{H. pylori} \) has evolved several strategies to enhance its own survival in the face of this immune response. For example, we have reported that while the host produces NO derived from inducible NO synthase in response to soluble products of \( \text{H. pylori} \), an arginase enzyme expressed by the bacterium competitively inhibits host NO production and prevents NO-mediated killing (3). In addition, \( \text{H. pylori} \) induces macrophage apoptosis via activation of polyamine synthesis (4), and \( \text{Th} \) cell apoptosis mediated by Fas (5), both of which are likely to also diminish the effectiveness of the immune response.

There is consistent evidence that the \( \text{H. pylori} \)-induced immune response is skewed toward a \( \text{Th1} \) phenotype indicated by a predominance of IFN-\( \gamma \) (6–8). This polarization has been suggested to contribute to the persistence of inflammation and to the inhibition of a possibly beneficial \( \text{Th2} \) response. However, data has now emerged from mouse model studies using cytokine-deficient mice (9) and adoptive transfer of selected splenocytes into SCID mice (10) that an inadequate \( \text{Th1} \) response may actually contribute to the pathogenesis of the infection. Although not studied with \( \text{H. pylori} \) infection, lymphocyte immune responses have been reported to be down-regulated by PGE\(_2\), with inhibition of both \( \text{T} \) cell proliferation and production of the \( \text{Th1} \) cytokines IL-12 and IFN-\( \gamma \) (11) and stimulation of \( \text{Th2} \) cytokine production (12). This effect has been attributed to elevation of the intracellular second messenger cAMP (13).

We and others have demonstrated that the inducible form of cyclooxygenase, (COX)\(^1\)–\(^2\), is up-regulated in human \( \text{H. pylori} \) gastritis tissues and localizes to lamina propria mononuclear cells (14, 15). Increased levels of PGE\(_2\), have also been demonstrated in the infected gastric mucosa (16). Accordingly, the aim of this study was to determine whether COX-2 activation is involved in \( \text{H. pylori} \) pathogenesis via inhibition of the \( \text{Th1} \)-predominant response to the infection. Although often considered a noninvasive pathogen, \( \text{H. pylori} \) itself and CagA have both been shown to invade gastric epithelial cells (17), and \( \text{H. pylori} \) proteins, including urease, have been demonstrated in the lamina propria of infected patients (18). Therefore, we used \( \text{H. pylori} \) preparations added to human PBMCs, and found that COX-2 mRNA expression and PGE\(_2\) production were induced in these cells. Inhibition of COX-2 or neutralization of PGE\(_2\) potentiated a \( \text{Th1} \) cytokine response and lymphocyte proliferation and reduced \( \text{Th2} \) response, while addition of exogenous PGE\(_2\) or cAMP had the opposite effect. We suggest that induction of COX-2 may be a host defense strategy that limits mucosal inflammation, but ultimately contributes to bacterial infection.

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\(^4\) Abbreviations used in this paper: COX, cyclooxygenase; cag, cytotoxin-associated gene; FP, French press lysate.
Materials and Methods

Reagents

All the reagents for cell culture and RNA analysis were obtained from Life Technologies (Gaithersburg, MD). Recombinant H. pylori urease was obtained and used as described (2, 19). Monoclonal anti-PGE$_2$ Ab 2B5 and MOPC21 isotype-matched (IgG) control Ab were provided by S. J. Minch and J. P. Pantanov (G. D. Searle, St. Louis, MO); 1.5 μg/ml 2B5 effectively neutralizes 1 ng/ml PGE$_2$ (20). The COX-2 inhibitors, NS-398 and 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl) phenyl-2(5H)-furanone (DFU), were obtained from Cayman Chemical (Ann Arbor, MI) and C. C. Chan (Merck Frost Centre for Therapeutic Research, Kirkland, Quebec, Canada), respectively. The cAMP analogues, dibutyryl-cAMP and 8-bromo-cAMP, were obtained from Sigma-Aldrich (St. Louis, MO) and Calbiochem-Novabiochem (La Jolla, CA), respectively. PGE$_2$ was purchased from Sigma-Aldrich.

Cells and culture conditions

PBMCs were isolated from venous blood obtained from H. pylori-negative donors using density gradient centrifugation with Histopaque-1077 (Sigma-Aldrich) and cultured in complete RPMI 1640 medium as described (19). Cells were plated at 2 × 10$^5$ cells/ml. For the RNA experiments, six-well plates were used (2 ml/well); for all other studies, round-bottom 96-well plates (200 μl/well) were used. Cultures were maintained in a humidified atmosphere (37°C) enriched with CO$_2$ (5%) in the presence or absence of various H. pylori preparations.

Bacteria

H. pylori strain UMB 41 (cytotoxin-associated gene (cag)A-positive) was used and grown on Brucella agar plates containing 10% sheep blood under anaerobic conditions as described (1, 19). For experiments, H. pylori was harvested into sterile PBS and concentration was determined by OD (1, 19). Lysates were prepared with a French press (1). Intact bacteria, French press lysates (FP), and recombinant urease were used at a protein concentration of 50 μg/ml, equal to 2.28 × 10$^9$ bacteria/ml (1), because this concentration of bacterial preparations elicits a maximal cytokine response in PBMCs (19).

mRNA analysis

PBMCs were cocultured with H. pylori preparations and after incubations, cells were washed twice with PBS, and total RNA was isolated using TRIzol reagent. The cDNA was synthesized using random primers and subjected to PCR analysis.

Cytokine and PGE$_2$ assays

Culture supernatants were immediately triplicated for cytokine concentrations using commercially available ELISA Kits (Quantikine; R&D Systems, Minneapolis, MN) for IL-10, IL-12p40, and IFN-γ (19). PGE$_2$ was measured by enzyme immunoassay (Cayman Chemical).

Proliferation studies

Tritium incorporation was used as an estimate for cell growth and DNA synthesis as described (19). After 24 h of cell culture, 1 μCi [methyl-3H]thymidine (Amersham, Arlington Heights, IL) was added to triplicate PBMC cultures for 12 h. Incorporated radioactivity was measured in cpm by liquid scintillation counting.

Statistics

Results are expressed as mean ± SEM. For comparisons between multiple groups, the Student Newman-Keuls test was used, and for single comparisons between two groups, the Student t test was used as appropriate.

Results

H. pylori preparations induce COX-2 expression and activity in PBMCs

To determine whether H. pylori could up-regulate COX-2 in human mononuclear cells in vitro, we exposed freshly isolated ex vivo human PBMCs to different bacterial preparations. As shown in Fig. 1A, intact H. pylori bacteria, lysates, or recombinant urease each markedly increased COX-2 mRNA expression from undetectable basal levels. PGE$_2$ levels were measured in response to these preparations as an indicator of COX-2 activity (Fig. 1B), with significant, 25- to 90-fold, increases detected. These increases were completely abolished by the COX-2 inhibitors NS-398 or DFU. Data with H. pylori lysate are shown; similar inhibition of PGE$_2$ release with COX-2 inhibitors was observed with stimulation by intact H. pylori or recombinant urease.

PGE$_2$ inhibits IL-12 and IFN-γ production and increases IL-10 response to H. pylori

Because we found that H. pylori induced PGE$_2$ release, we sought to directly assess the effect of PGE$_2$ on H. pylori-stimulated cells. As shown in Fig. 2A, addition of exogenous PGE$_2$ resulted in a concentration-dependent inhibition of IL-12 and IFN-γ production, by 57–92% and 68–97%, respectively, and an up-regulation of IL-10 production, by 170–360% of control values.

cAMP reproduces the PGE$_2$ effect

Because PGE$_2$ is known to activate the intracellular second messenger cAMP, we also determined whether addition of cAMP analogues could reproduce the effect of PGE$_2$ (Fig. 2B). Dibutyryl-cAMP significantly inhibited the H. pylori-stimulated IL-12 and IFN-γ production and simultaneously increased IL-10 levels, in the same pattern as observed with addition of PGE$_2$. Similar results occurred with another cAMP analog, 8-bromo-cAMP (data not shown).

COX-2 expression and PGE$_2$ production down-regulate Th1 and enhance Th2 response to H. pylori

Because we found that H. pylori induced COX-2, we sought to determine whether the functional activity of COX-2 resulted in modulation of the Th1-driven immune response to H. pylori. Inhibition of COX-2 with NS-398 resulted in a significant further increase in both IL-12 and IFN-γ levels above that due to stimulation with H. pylori alone (Fig. 3, top and middle panels).
indicative of a potentiation of the Th1-like response. This occurred in a similar fashion with all three preparations of *H. pylori*. Similar results were observed with COX-2 inhibition by DFU (data not shown). Additionally, neutralizing Ab to PGE2, the main COX-2 product in mononuclear cells, effectively increased the IL-12 and IFN-γ/H9253 production as well (Fig. 3). Importantly, both NS-398 and anti-PGE2 Ab decreased the IL-10 response to the three *H. pylori* preparations (Fig. 3, bottom panel). Taken together, these data suggest that COX-2 activity, and generation of PGE2, specifically, normally act to limit Th1 response and enhance Th2 response.

**COX-2 inhibits lymphocyte proliferation in response to *H. pylori* products via PGE2 synthesis**

Because lymphocyte proliferation is an important part of the amplification of the mucosal immune response, we determined whether COX-2 activity could also regulate this process. We studied modulation of proliferation induced by recombinant urease at 50 μg/ml, because we previously reported that urease was a more potent inducer of proliferation than intact or lysed *H. pylori* and that this was the concentration at which peak stimulation occurred (19). Addition of PGE2 caused a concentration-dependent 44–82% inhibition of proliferation (Fig. 4A). Consistent with this, addition of NS-398 or neutralization of PGE2 resulted in a significant 3- to 4-fold increase in cell proliferation (Fig. 4B). Similar results occurred with COX-2 inhibition by DFU (data not shown).

**Discussion**

COX-2 has gained extensive notoriety as a proneoplastic factor, which has been attributed to factors such as inhibition of epithelial apoptosis (22), stimulation of proliferation (23), and angiogenesis (24). We and others have shown that *H. pylori* increases COX-2 expression in human gastric mucosa (14, 15) and in gastric epithelial cell lines (21, 25). *H. pylori* is strongly linked to gastric cancer (26), and the chronic inflammation associated with the longstanding infection is presumed to be the main cause. It has been suggested that chronic overexpression of COX-2 may be a contributing factor in this process via effects on epithelial cells (21, 25). We now present new evidence that an important role of COX-2 in *H. pylori* pathogenesis is also dysregulation of the immune response. Using human PBMCs exposed to various *H. pylori* preparations, we show that COX-2...
is potently induced in these cells and the resulting PGE₂ production causes attenuation of IL-12 and IFN-γ production and lymphocyte proliferation, and enhancement of IL-10 response.

It is expected that the primary source of COX-2 and PGE₂ production in the PBMCs is monocytes; consistent with this, we have observed significant COX-2 expression and activity in macrophage cell lines, peritoneal macrophages, and splenocytes (27). However, inducible PGE₂ production by lymphocytes has also been reported (28). Our data indicate that this is a potent inducer of COX-2 expression; consistent with this we have found that concentrated supernatants of ureA-deficient isogenic mutant strains have decreased COX-2 inducing activity in mouse macrophages (27) and we have reported that this is a major inducer of iNOS expression in macrophages (2). Additionally, our data are expected to have direct relevance to the events in the gastric mucosa, because we have observed that COX-2⁻/⁻ mice infected with *H. pylori* have both increased gastritis and up-regulated IFN-γ and IL-12 expression compared with wild-type mice (29). It is also likely that the presence of both monocytes and lymphocytes in the PBMCs provides a synergistic effect in the *H. pylori* response. For example, IL-12 is primarily derived from monocytes and acts to induce IFN-γ synthesis by lymphocytes, as evidenced by our previous report that neutralization of IL-12 can inhibit *H. pylori*-stimulated IFN-γ production (19). Although the mucosal response to *H. pylori* is Th1-predominant, Th2 cytokine generation by T cell clones derived from *H. pylori*-infected hosts has been demonstrated in a substantial number of cases (7, 30), consistent with our findings of *H. pylori* induced IL-10 response in this study and in our prior report (19). Therefore, the ability of COX-2 to modulate both the Th1 and Th2 cytokine response, as we have shown in this report, is likely to be significant.

It is probable that *H. pylori* may often behave as a commensal organism that more frequently exerts a pathological role in the case of strains possessing the catg pathogenicity island or in unusually susceptible hosts (31, 32). A common feature of the infection is chronic persistent gastritis, and inability of the host to eradicate the organism despite the mucosal immune response (31, 32). We suggest that our data may explain, at least in part, the persistence of the bacterium. Mouse studies have directly shown that Th1 responses are associated with increased gastritis, because IFN-γ⁻/⁻ mice had decreased gastritis (9) and SCID mice infected with *H. pylori* required reconstitution with CD4⁺ T cells for gastritis, with inflammation most severe in mice receiving splenocytes from IL-10⁻/⁻ mice and least severe with cells received from IFN-γ⁻/⁻ mice (10). However, these studies have also elucidated the critical point that a decreased Th1 response is associated with increased bacterial colonization. IFN-γ⁻/⁻ and IL-12⁻/⁻ mice could not be immunized against *H. pylori* infection, in contrast to wild-type or Th2-deficient IL-4⁻/⁻ mice (9). Additionally, SCID mice adaptively transferred with varying splenocyte preparations exhibited an inverse correlation between severity of gastritis associated with Th1 response and bacterial colonization (10). We conclude that the chronic expression of COX-2 and production of PGE₂ in mononuclear and other cells of the gastric mucosa results in inhibition of the effectiveness of the mucosal immune response by enhancing a state of tolerance (33) that may prevent eradication of the organism and contribute to the risk for complications from *H. pylori* infection, including gastric cancer.

**References**


