Cutting Edge: Cyclooxygenase-2 Activation Suppresses Th1 Polarization in Response to *Helicobacter pylori*


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Helicobacter pylori infection causes a 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 Th1/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 PGE₂ release in response to H. pylori preparations. Addition of COX-2 inhibitors or an anti-PGE₂ Ab resulted in a marked increase in H. pylori-stimulated IL-12 and IFN-γ production, and a decrease in IL-10 levels. Addition of PGE₂ or cAMP, the second messenger activated by PGE₂, had the opposite effect. Similarly, stimulated cell proliferation was increased by COX-2 inhibitors or anti-PGE₂ Ab, and was decreased by PGE₂. Our findings indicate that COX-2 has an immunosuppressive role in 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.

**Cutting Edge: Cyclooxygenase-2 Activation Suppresses Th1 Polarization in Response to Helicobacter pylori**

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Helicobacter 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. 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 H. pylori (1, 2), an arginase enzyme expressed by the bacterium competitively inhibits host NO production and prevents NO-mediated killing (3). In addition, H. pylori induces macrophage apoptosis via activation of polyamine synthesis (4), and T 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 H. pylori-induced immune response is skewed toward a Th1 phenotype indicated by a predominance of IFN-γ (6–8). This polarization has been suggested to contribute to the persistence of inflammation and to the inhibition of a possibly beneficial 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 Th1 response may actually contribute to the pathogenesis of the infection. Although not studied with H. pylori infection, lymphocyte immune responses have been reported to be down-regulated by PGE₂, with inhibition of both T cell proliferation and production of the Th1 cytokines IL-2 and IFN-γ (11) and stimulation of 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 H. pylori gastritis tissues and localizes to lamina propria mononuclear cells (14, 15). Increased levels of PGE₂ 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 H. pylori pathogenesis via inhibition of the Th1-predominant response to the infection. Although often considered a noninvasive pathogen, H. pylori itself and CagA have both been shown to invade gastric epithelial cells (17), and H. pylori proteins, including urease, have been demonstrated in the lamina propria of infected patients (18). Therefore, we used H. pylori preparations added to human PBMCs, and found that COX-2 mRNA expression and PGE₂ production were induced in these cells. Inhibition of COX-2 or neutralization of PGE₂ potentiated a Th1 cytokine response and lymphocyte proliferation and reduced Th2 response, while addition of exogenous PGE₂ 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...
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. Mnich and J. P. Ponzano (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 x 10$^6$ 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 UMB41 (cytotoxin-associated gene [cag]A-positive) was used and grown on Brucella agar plates containing 10% sheep blood under microaerobic 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 pressure cell (1). Intact bacteria, French press lysates (FP), and recombinant urease were used at a protein concentration of 50 µg/ml, equal to 2.28 x 10$^8$ 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. Reverse transcription, PCR cycle conditions, primer sequences, and digital capillaries were as described (9). The COX-2 inhibitors, NS-398 or DFU. Data with control values. 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), but only a minimal decrease in IL-10 levels was observed (Fig. 3, bottom panel).
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 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 mouse macrophage cell lines, peritoneal macrophages, and splenocytes (27). However, inducible PGE₂ production by lymphocytes has also been reported (28). Our data indicate that urease is a potent inducer of COX-2 expression; consistent with this we have found that concentrated supernatants of H. pylori response to urease A, B, and C in triplicate. A, **p < 0.01 vs control without urease, §§ p < 0.01 vs urease alone. B, **p < 0.01 vs urease alone.

**FIGURE 4.** COX-2 activity regulates H. pylori urease-stimulated cell proliferation in PBMCs, as determined by uptake of [³H]thymidine. A, Addition of PGE₂ inhibits proliferation. B, Neutralization of endogenous PGE₂ or inhibition of COX-2 increases proliferation; anti-PGE₂ Ab 2B5, control Ab MOPC21, and NS-398 were added as in Fig. 2. A, Values are mean cpm ± SEM; B, data are standardized to the cpm attributable to urease stimulation, to allow for variation in baseline and stimulated proliferation between experiments using the different inhibitors. For A and B, n = 3, in triplicate. A, **p < 0.01 vs control without urease; §§ p < 0.01 vs urease alone. B, **p < 0.01 vs urease alone.

References


