

Effects of Cyclooxygenase-1 and -2 Gene Disruption on *Helicobacter pylori*-Induced Gastric Inflammation

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Background. Cyclooxygenases (COXs) play important roles in inflammation and carcinogenesis. The present study aimed to determine the effects of COX-1 and COX-2 gene disruption on *Helicobacter pylori*-induced gastric inflammation.

Methods. Wild-type (WT), COX-1 and COX-2 heterozygous (COX-1^{+/−} and COX-2^{+/−}), and homozygous COX-deficient (COX-1^{−/−} and COX-2^{−/−}) mice were inoculated with *H. pylori* strain TN2 and killed after 24 weeks of infection. Uninfected WT and COX-deficient mice were used as controls. Levels of gastric mucosal inflammation, epithelial cell proliferation and apoptosis, and cytokine expression were determined.

Results. COX deficiency facilitated *H. pylori*-induced gastritis. In the presence of *H. pylori* infection, apoptosis was increased in both WT and COX-deficient mice, whereas cell proliferation was increased in WT and COX-1-deficient, but not in COX-2-deficient, mice. Tumor necrosis factor (TNF)- α and interleukin-10 mRNA expression was elevated in *H. pylori*-infected mice, but only TNF- α mRNA expression was further increased by COX deficiency. Prostaglandin E₂ levels were increased in infected WT and COX-2-deficient mice but were at very low levels in infected COX-1-deficient mice. Leukotriene (LT) B₄ and LTC₄ levels were increased to a similar extent in infected WT and COX-deficient mice.

Conclusions. COX deficiency enhances *H. pylori*-induced gastritis, probably via TNF- α expression. COX-2, but not COX-1, deficiency suppresses *H. pylori*-induced cell proliferation.

Cyclooxygenases (COXs) are the rate-limiting enzymes in the production of prostanoids and are also the pharmacological target of nonsteroidal anti-inflammatory drugs (NSAIDs) [1]. There are 2 isoforms of COX:

COX-1 is considered to be the constitutive form and is believed to regulate gastrointestinal homeostasis via the generation of prostaglandins (PGs), and COX-2 is considered to be the inducible form and is involved in the proinflammatory actions of PGs during injury [2]. *Helicobacter pylori* infection is a major cause of chronic gastritis, peptic ulceration, and gastric carcinoma [3]. Studies have shown that COX-2 is induced in gastric mucosa during *H. pylori* infection [4–6] and that NSAIDs enhance *H. pylori*-induced gastric inflammation and ulceration [7–12]. However, the role played by COX-2 in *H. pylori*-induced gastric inflammation is not entirely understood.

A dynamic balance between epithelial cell proliferation and apoptosis is essential for maintaining normal gastric mucosal integrity. *H. pylori* infection induces apoptosis and proliferation of gastric epithelial cells and may lead to alterations in the balance of epithelial cell

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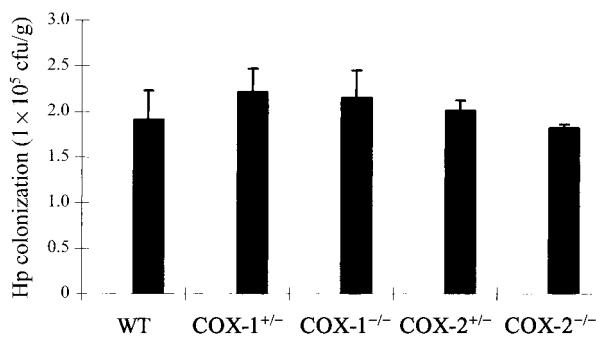


Figure 1. Levels of *Helicobacter pylori* (Hp) colonization in infected mice ($n = 12$ per group), as assessed by counting the no. of colony-forming units per gram of stomach tissue after bacterial culture. Data are means \pm SEs. COX, cyclooxygenase.

proliferation and apoptosis [13–17], which is believed to contribute to gastric ulcerogenesis or even carcinogenesis [18, 19]. However, whether COX-2 plays a role in the regulation of *H. pylori*-induced apoptosis and cell proliferation is unknown.

Previous studies have demonstrated that the *H. pylori*-induced immune response is skewed toward a Th1 phenotype, as is indicated by the predominance of cytokines—such as tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and interleukin (IL)-12—that contribute to the persistence of inflammation [20–23]. TNF- α , on the one hand, is a key proinflammatory cytokine in *H. pylori*-associated inflammation [24]; it is up-regulated early during *H. pylori* colonization and induces the production of other proinflammatory cytokines and chemokines, amplifying the *H. pylori*-induced inflammation [25]. IL-10, on the other hand, is an anti-inflammatory cytokine; it down-regulates the production of proinflammatory cytokines, such as TNF- α , IFN- γ , and IL-12 [26, 27]. In addition, it has been shown that PGE₂ derived from either COX-1 or COX-2 is involved in the regulation of gastric mucosal inflammation and also contributes to the maintenance of mucosal integrity during *H. pylori* infection [28]. However, whether COX-1 or COX-2 affects *H. pylori*-induced gastric inflammation through the regulation of cytokines and PGs needs to be investigated further.

Because NSAIDs exert pharmacological effects via COX-dependent and -independent mechanisms (the inhibition of COX activity is only one of them), the observed effects produced by NSAIDs may not necessarily reflect the physiological roles played by the COX isoforms [29–33]. To overcome this, COX-1- and COX-2-deficient mice can be used in a novel approach to help clarify the role that COX plays in *H. pylori*-induced gastric inflammation [34, 35]. The aim of the present study was to determine the effects of the COX-1 and COX-2 isoforms on *H. pylori*-induced gastric inflammation, apoptosis, cell proliferation, and cytokine expression by studying long-term *H. pylori*-infected wild-type (WT) and COX-deficient mice. Additionally, because *H. pylori* infection is associated with

increased gastric mucosal expression of leukotrienes (LTs), such as LTB₄ and LTC₄ (LTs are a class of lipids that are derived from 5-lipoxygenase [LOX] [36–38]), a possible change of LTs and a potential role for the LOX pathway after *H. pylori* infection in COX-deficient mice were also investigated.

MATERIALS AND METHODS

Animals. COX-1-deficient (COX-1^{+/-} [heterozygous] and COX-1^{-/-} [homozygous]) and COX-2-deficient (COX-2^{+/-} [heterozygous] and COX-2^{-/-} [homozygous]) mice derived from strain C57BL/6-129/Ola and their WT littermates (which have been described elsewhere [34, 35]) were used in the present study. Mice were maintained on a 12-h-light/12-h-dark cycle at 22°C in Plexiglas cages with autoclaved water and were fed autoclaved standard laboratory chow ad libitum. All procedures were performed with the approval of the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong.

Genotyping. The genotype of each mouse was determined 3–4 weeks after birth by a polymerase chain reaction (PCR)-based method that has been described elsewhere [39]. Briefly, DNA from the tail was extracted using the DNeasy Tissue Kit (Qiagen), in accordance with the manufacturer's protocol. The sample from the tail was lysed using proteinase K, and the lysate was loaded into a minicolumn. After the column was washed, DNA was eluted in a buffer ready for PCR. Three primers were used in each PCR. For COX-1, the primers were 5'-AGGAGATGGCTGCTGAGTTGG-3' (COX-1 5', for the WT allele), 5'-GCAGCCTCTGTTCCACATACAC-3' (COX-1 Neo, for the mutant allele), and 5'-AATCTGACTTCTGAGTTGCC-3' (the 3' primer; COX-1 3'), which yielded fragments of 560 and 650 bp for the COX-1 WT and mutant alleles, respectively. For COX-2, the primers were 5'-ACACACTCTATCACTGGC-ACC-3' (COX-2 Δ 9, for the WT allele), 5'-ACGCGTCACCTT-AATATGCG-3' (NeoPro, for the mutant allele), and 5'-TCCC-TTCACTAAATGCCCTC-3' (the 3' primer; TGC2-3), which yielded fragments of 760 and 900 bp for the COX-2 WT and mutant alleles, respectively.

Experimental design. A total of 120 six-week-old mice weighing 17–22 g were used in the experiments. They included 24 WT, 24 COX-1^{+/-}, 24 COX-1^{-/-}, 24 COX-2^{+/-}, and 24 COX-2^{-/-} mice. The *H. pylori* strain used was TN2, which produces a vacuolating cytotoxin and possesses the *cag* pathogenicity island (and which was used in our previous study [40]). This strain shares an ancestral strain with TN2GF4, which has been reported to induce gastric cancer in Mongolian gerbils [41].

After overnight fasting, mice (12 WT, 12 COX-1^{+/-}, 12 COX-1^{-/-}, 12 COX-2^{+/-}, and 12 COX-2^{-/-}) were inoculated 3 times by gavage with 300 μ L of *H. pylori* organisms (1×10^8 cfu/mL), with a 1-day interval between inoculations. Age-matched control mice were inoculated with 300 μ L of medium (Brucella broth).

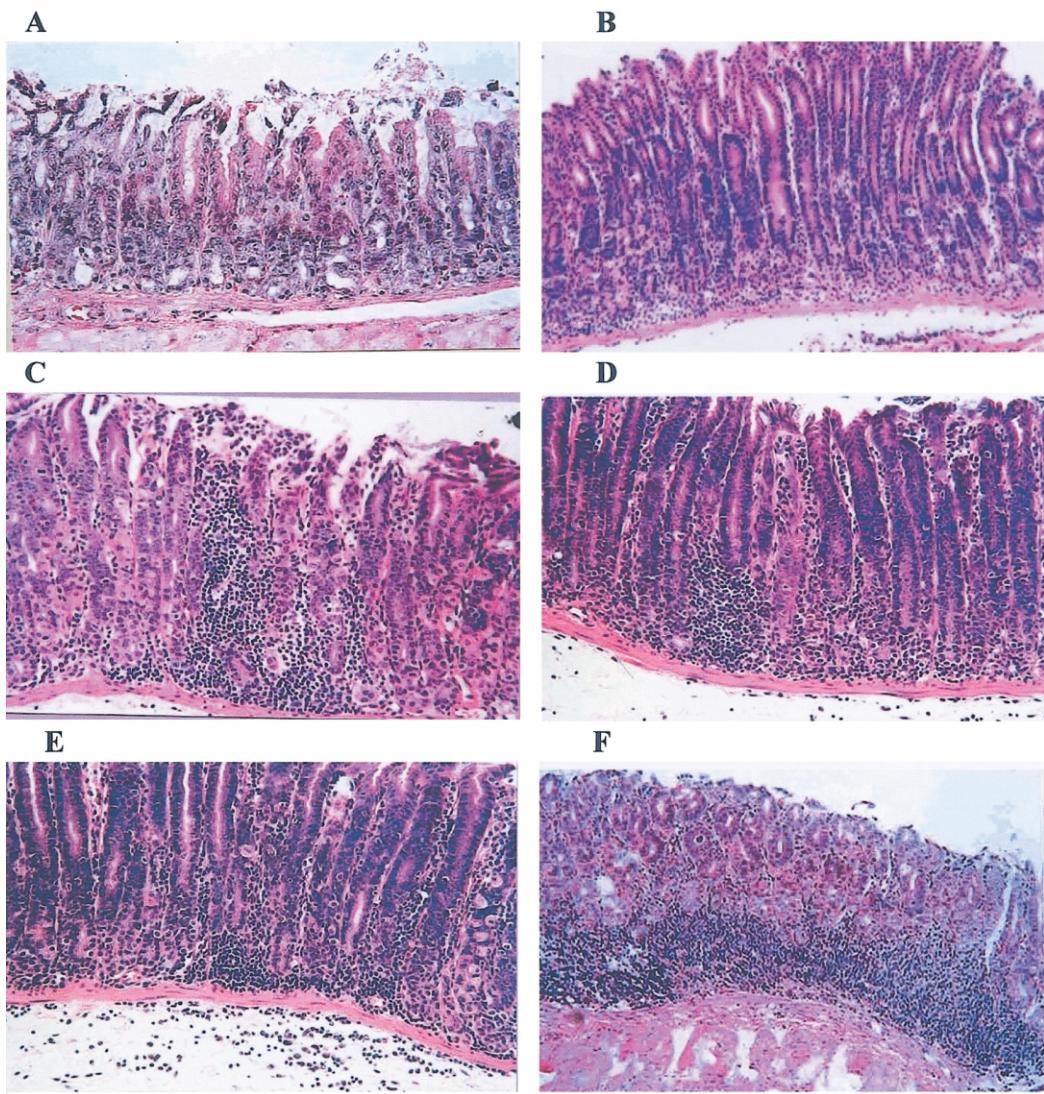


Figure 2. Assessment of changes in *Helicobacter pylori*-induced gastric mucosal inflammation in wild-type (WT) and cyclooxygenase (COX)-deficient mice 24 weeks after inoculation. Representative hematoxylin-eosin-stained stomach sections from uninfected WT mice (*A*) and from infected WT (*B*), COX-1^{+/−} (*C*), COX-1^{−/−} (*D*), COX-2^{+/−} (*E*), and COX-2^{−/−} (*F*) mice (original magnification, $\times 200$).

Mice were killed by cervical dislocation 24 weeks after inoculation, and their stomachs were removed, weighed, opened along the great curvature, and longitudinally divided into 6 pieces. Three pieces were immediately frozen in liquid nitrogen and stored at -80°C for measurement of levels of cytokines (TNF- α and IL-10), PGE₂, LTB₄, and LTC₄; 2 pieces were fixed in 10% neutral buffered formalin for histological examination and immunohistochemistry; and 1 piece was used for quantitative culture. Control groups consisted of 12 WT, 12 COX-1^{+/−}, 12 COX-1^{−/−}, 12 COX-2^{+/−}, and 12 COX-2^{−/−} mice.

Quantitative culture of *H. pylori*. For quantitative assessment of *H. pylori* colonization, 1 piece of weighed stomach tissue was homogenized in 1 mL of Brucella broth by use of a hand pestle (Kontes), and the homogenate was diluted 10- and

100-fold in Brucella broth. One hundred microliters of each dilution was plated on selective medium containing 10% horse blood (Hong Kong Jockey Club), 100 $\mu\text{g}/\text{mL}$ vancomycin, 50 $\mu\text{g}/\text{mL}$ cefsulodin, 50 $\mu\text{g}/\text{mL}$ trimethoprim lactate, and 50 $\mu\text{g}/\text{mL}$ amphotericin B (Oxoid). Plates were incubated under microaerophilic conditions produced by a gas-generating system (CampyGen; Oxoid) for 5–7 days. *H. pylori* was identified by Gram staining and by positive urease, oxidase, and catalase tests. *H. pylori* colonies were then counted, to determine the number of colony-forming units per gram of stomach tissue.

Histological examinations. Gastric specimens were embedded in paraffin and cut into 4- μm -thick sections. The sections were stained with hematoxylin-eosin for semiquantitative examination of the activity (neutrophil infiltration score) and

Table 1. Neutrophil infiltration and mononuclear cell infiltration into the stomachs of wild-type (WT) and cyclooxygenase (COX)-deficient mice infected or not infected (control) with *Helicobacter pylori*.

Infiltration	WT		COX-1 ^{+/−}		COX-1 ^{−/−}		COX-2 ^{+/−}		COX-2 ^{−/−}	
	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>
Neutrophil	0.2 ± 0.2	1.0 ± 0.1 ^a	0.2 ± 0.1	1.4 ± 0.2 ^a	0.4 ± 0.3	1.6 ± 0.2 ^{b,c}	0.3 ± 0.1	1.5 ± 0.1 ^{a,c}	0.4 ± 0.2	1.7 ± 0.3 ^{b,c}
Mononuclear cell	0.2 ± 0.2	1.1 ± 0.1 ^a	0.1 ± 0.1	1.5 ± 0.2 ^a	0.2 ± 0.2	1.9 ± 0.3 ^{a,c}	0.2 ± 0.1	1.7 ± 0.1 ^{a,c}	0.2 ± 0.2	1.9 ± 0.3 ^{a,c}

NOTE. Data are mean ± SE infiltration scores 24 weeks after inoculation (explained in detail in Materials and Methods).

^a $P < .01$, compared with the corresponding uninfected mice.

^b $P < .05$, compared with the corresponding uninfected mice.

^c $P < .05$, compared with the infected WT mice.

severity (mononuclear cell infiltration score) of chronic inflammation, which were graded (in accordance with the updated Sydney system [42]) as follows: 0, minimal; 1, mild; 2, moderate; and 3, marked. The presence of spiral organisms was examined in Giemsa-stained sections.

Determination of apoptosis and cell proliferation. Epithelial cell apoptosis was determined in situ from paraffin-embedded tissue sections by use of the TUNEL technique (Apop Tag In Situ Apoptosis Detection Kit; Intergen Company), in accordance with the manufacturer's protocol. Epithelial cell proliferation was determined by immunohistochemical staining for Ki-67. Briefly, sections were deparaffinized, placed in citrate buffer (10 mmol/L; pH 6.0), and heated in a 700-W microwave oven for 20 min. Endogenous peroxidase activity was quenched by use of hydrogen peroxide. After being washed in immunoassay buffer, the slides were incubated with a mouse monoclonal IgG against Ki-67 (DakoCytomation) in a humidified chamber. Slides were incubated with biotinylated rabbit anti-rat IgG and peroxidase-conjugated streptavidin (DakoCytomation), developed using the DAB+ substrate-chromogen system (DakoCytomation), and counterstained with hematoxylin (Sigma).

An experienced pathologist who was blinded to treatment schedule read the stained slides. For determination of the level of apoptosis or cell proliferation, cells were counted in 25 well-orientated gastric glands in the gastric corpus and antrum. The apoptosis index (AI) and the cell proliferation index (PI) were defined as the percentage of positively stained cells per the total number of cells counted (~1000 cells).

Reverse-transcription (RT)-PCR analysis of gastric TNF- α and IL-10 mRNA expression. Total RNA was isolated using TRIzol reagents (GIBCO BRL) and was reverse transcribed using the ThermoScript RT-PCR System (Invitrogen) in a total reaction volume of 20 μ L that contained 4 μ g of RNA. The RT product (cDNA; 2 μ L) was amplified by PCR with 1.5 U of HotStar-Taq DNA polymerase (Gene Company) and 10 pmol of forward and reverse TNF- α or IL-10 primers (Genset Singapore Biotech), and the same amounts of forward and reverse β -actin primers were included in the multiplex PCR, as an internal control for the efficiency of the RT and the amount of RNA. Each PCR cycle

consisted of a denaturation step (45 s at 94°C), an annealing step (45 s at 52°C), and an elongation step (45 s at 72°C). There was a total of 35 cycles for TNF- α and IL-10 and 30 cycles for β -actin, which were followed by an additional extension step (7 min at 72°C). The primer sequences and the sizes of the PCR products were as follows: for TNF- α , 5'-CATGGATCTCAAAGA-CAACCAA-3' (forward) and 5'-GCTGGTAGAGAATGGATG-AAC-3' (reverse), with a product size of 385 bp; for IL-10, 5'-ACCTCTGATACCTCAGTTCCA-3' (forward) and 5'-CAA-TTGAAAGGACACCAGTAGCA-3' (reverse), with a product size of 268 bp; and for β -actin, 5'-ACCCAGATCATGTTGAGA-CCT-3' (forward) and 5'-CTGCTCGAAGTCTAGAGCAACA-3' (reverse), with a product size of 318 bp. PCR products were electrophoresed on 1.5% agarose gel with 0.5 μ g/mL ethidium bromide. Stained bands were visualized under UV light, photographed, and digitized using the Bio Imagine Detection System (GS-700; Bio-Rad), and band intensity was quantitated using a Macintosh computer and imaging and analysis software (GeneTools; version 3.06; Syngene).

ELISAs for gastric PGE₂, LTB₄, and LTC₄ levels. Gastric specimens were weighed and homogenized at 4°C in lysis buffer (50 mmol/L Tris-HCl [pH 7.4], 100 mmol/L NaCl, 1 mmol/L CaCl₂, 1 mg/mL glucose, and 28 mmol/L indomethacin). Homogenates were vortexed and centrifuged at 12,000 rpm for 30 min at 4°C. PGE₂, LTB₄, and LTC₄ levels in the supernatants were measured by use of commercially available ELISA kits (Caymen), in accordance with the manufacturer's protocol. Plates were read at 410 nm by use of a microtiter plate reader.

Statistical analyses. All numerical data are presented as means ± SEs. The Mann-Whitney *U* test was used for assessment of the differences between groups. Statistical analyses were performed using SPSS (version 12.0 for Windows; SPSS). Significance was defined as $P < .05$ (2-tailed).

RESULTS

Quantitative culture of *H. pylori*. *H. pylori* colonized in all mice inoculated with the pathogen, as determined by culture. There were no significant differences in the numbers of *H. pylori* colony-forming units in gastric mucosa between the COX-de-

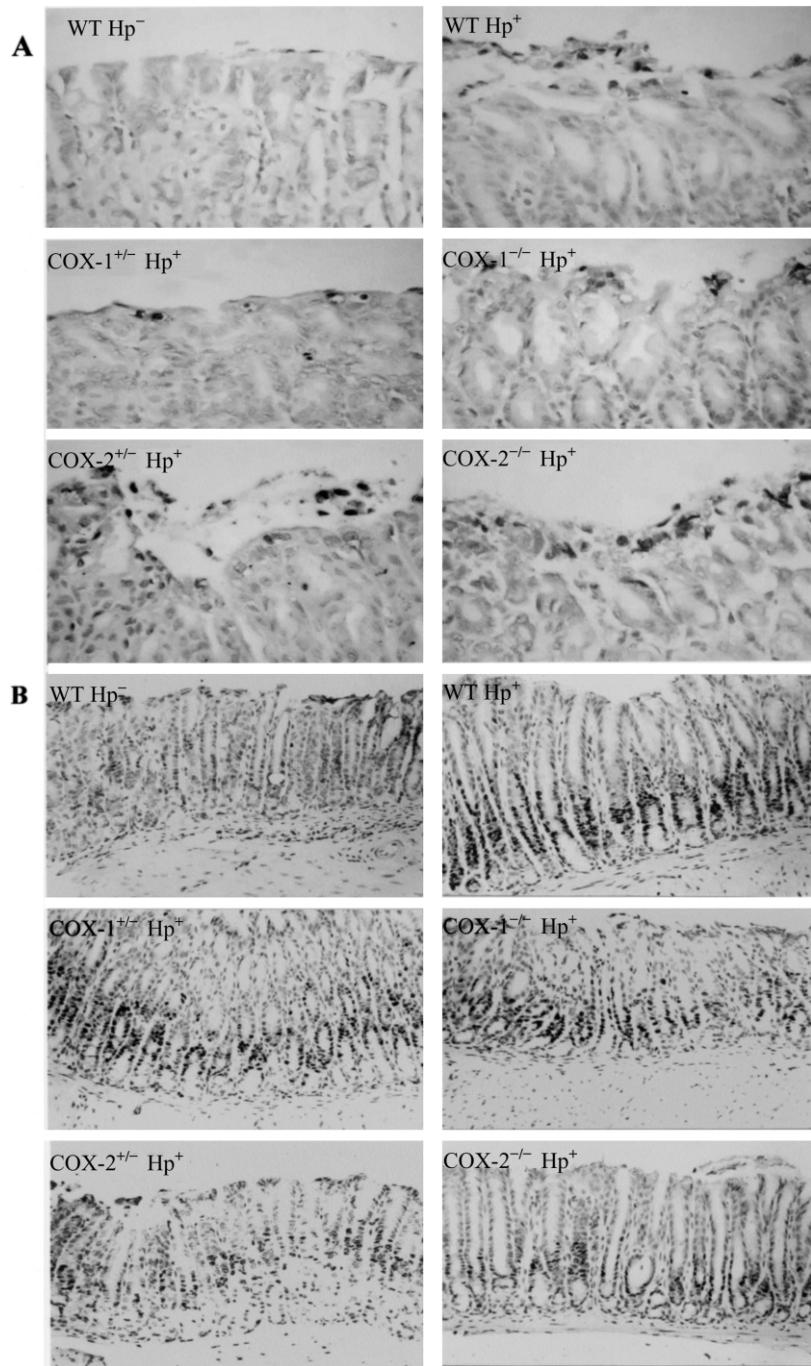


Figure 3. Representative TUNEL-stained sections of the gastric corpus (A; original magnification, $\times 400$) and Ki-67-stained sections of the gastric antrum (B; original magnification, $\times 100$) from uninfected wild-type (WT) mice and from *Helicobacter pylori* (Hp)-infected WT and cyclooxygenase (COX)-deficient mice 24 weeks after inoculation.

ficient mice and the WT mice when assayed 24 weeks after inoculation (figure 1). *H. pylori* was not detected in the uninfected mice.

Inflammation of gastric mucosa. No gastric mucosal inflammation was present in the uninfected WT and COX-deficient mice (figure 2A). However, increased levels of polymorphonuclear neutrophils and mononuclear cells were found

in the stomachs of the infected WT and COX-deficient mice (figure 2B-F); nonetheless, gastric atrophy, intestinal metaplasia, and ulceration were not observed in the infected mice.

Neutrophil infiltration was significantly increased in all infected mice, compared with that in the uninfected mice. In the infected COX-1^{-/-}, COX-2^{+/-}, and COX-2^{-/-} mice, neutrophil infiltration was significantly increased, compared with that in

Table 2. Apoptotic index (AI) and proliferation index (PI) for gastric epithelial cells in wild-type (WT) and cyclooxygenase (COX)-deficient mice infected or not infected (control) with *Helicobacter pylori*.

Index	WT		COX-1 ^{+/−}		COX-1 ^{−/−}		COX-2 ^{+/−}		COX-2 ^{−/−}	
	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>	Control	<i>H. pylori</i>
AI	0.03 ± 0.01	0.15 ± 0.01 ^a	0.03 ± 0.00	0.14 ± 0.01 ^a	0.03 ± 0.00	0.14 ± 0.01 ^a	0.05 ± 0.01	0.16 ± 0.02 ^a	0.05 ± 0.01	0.17 ± 0.01 ^a
PI	0.17 ± 0.03	0.39 ± 0.01 ^a	0.15 ± 0.02	0.39 ± 0.01 ^a	0.17 ± 0.01	0.40 ± 0.01 ^a	0.15 ± 0.03	0.22 ± 0.02 ^b	0.18 ± 0.04	0.19 ± 0.03 ^b

NOTE. Data are mean ± SE index values 24 weeks after inoculation (explained in detail in Materials and Methods).

^a $P < .05$, compared with the corresponding uninfected mice.

^b $P < .01$, compared with the infected COX-1-deficient mice and the infected WT mice.

the infected WT mice ($P < .05$, for all) (table 1). Mononuclear cell infiltration was also significantly increased in all infected mice, compared with that in the uninfected mice. In the infected COX-1^{−/−}, COX-2^{+/−}, and COX-2^{−/−} mice, mononuclear cell infiltration was significantly increased, compared with that in the infected WT mice ($P < .05$, for all) (table 1).

Apoptosis and proliferation of gastric epithelial cells.

Apoptosis, assessed on the basis of TUNEL-positive cells, was observed in the surface epithelium of the gastric corpus mucosa of the infected WT and COX-deficient mice (figure 3A). AIs were significantly increased in the infected WT and COX-deficient mice, compared with those in the uninfected mice ($P < .01$) (table 2). However, there was no difference between the AIs in the infected COX-deficient mice and those in the infected WT mice.

Immunostaining for Ki-67, which reflects cell proliferation, was localized in the nuclei of epithelial cells located within the proliferating compartment in the basal zone of the corpus and antrum (figure 3B). Furthermore, *H. pylori* infection caused a significant increase in the PIs in the WT and COX-1-deficient mice ($P < .01$) but not in the COX-2-deficient mice ($P = .10$, for the COX-2^{+/−} mice; $P = .73$, for the COX-2^{−/−} mice). Compared with those in the infected WT and COX-1-deficient mice, PIs were significantly lower in the infected COX-2-deficient mice ($P < .01$) (table 2).

TNF- α and IL-10 mRNA expression in gastric mucosa.

TNF- α mRNA was detected by RT-PCR in all infected mice, but expression was absent or low in the uninfected mice (figure 4A). Expression of TNF- α mRNA was significantly increased in the infected COX-1^{−/−}, COX-2^{+/−}, and COX-2^{−/−} mice, compared with that in the infected WT mice ($P < .05$), although there was no significant difference between expression in the infected COX-1^{+/−} mice and that in the infected WT mice (figure 4B). On the other hand, there was no difference in IL-10 mRNA expression between the infected mice and the uninfected mice and between the infected WT mice and the infected COX-deficient mice (figure 4C).

Gastric PGE₂, LTB₄ and LTC₄ levels. *H. pylori* infection significantly increased gastric PGE₂ production in the WT (1.6-fold), COX-2^{+/−} (1.3-fold), and COX-2^{−/−} (1.4-fold) mice, compared with that in the corresponding uninfected mice ($P < .05$,

for all). However, PGE₂ was barely detectable in the gastric specimens from the COX-1-deficient mice, both those that were infected and those that were uninfected ($P < .001$, compared with the WT mice) (figure 5A). Both LTB₄ and LTC₄ levels were increased in the infected mice, compared with those in the uninfected mice ($P < .05$); this result was independent of COX deficiency (figure 5B and 5C).

DISCUSSION

In the present study, we have shown that there is no apparent difference in the bacterial density in gastric mucosa between WT and COX-deficient mice, suggesting that *H. pylori* colonization is not affected by the absence of COX-1 or COX-2 expression. Several studies have demonstrated that NSAIDs enhance *H. pylori*-induced gastric mucosal inflammation and injury [7–10, 28, 43, 44]—for example, Takahashi et al. reported that NS-398, a COX-2-specific inhibitor, and indomethacin, a dual COX inhibitor, promoted *H. pylori*-induced neutrophil infiltration and lymphoid follicle formation [44]; Tanigawa et al. showed that inhibition of COX-1 (by use of SC-560) or COX-2 (by use of NS-398) enhanced neutrophil infiltration into gastric mucosa in *H. pylori*-infected mice [28]; and Yoshida et al. reported that *H. pylori* infection potentiated aspirin-induced gastric mucosal injury in Mongolian gerbils [10]. However, other studies have shown that NSAIDs have no effect or even protective effects on *H. pylori*-induced gastritis—Kim et al. reported that indomethacin and NS-398 decreased gastric inflammation induced by *H. pylori* infection in mice [11], and, in a clinical study conducted by Scheiman et al., rofecoxib, a COX-2 inhibitor, did not significantly affect gastritis scores [12]. Various factors, such as the duration of *H. pylori* infection and/or duration of NSAID treatment, may contribute to the discrepant findings of these studies. Thus, a regulatory role for COX in *H. pylori*-induced gastritis cannot be directly derived from studies that use NSAIDs. Furthermore, these drugs have many pharmacological activities in addition to the inhibition of COX activity [29–33]. Therefore, the *H. pylori*-infected COX-deficient mouse model is useful for clarifying the physiological roles played by the COX-1 and COX-2 isoforms in *H. pylori*-induced gastritis. The present study demonstrated

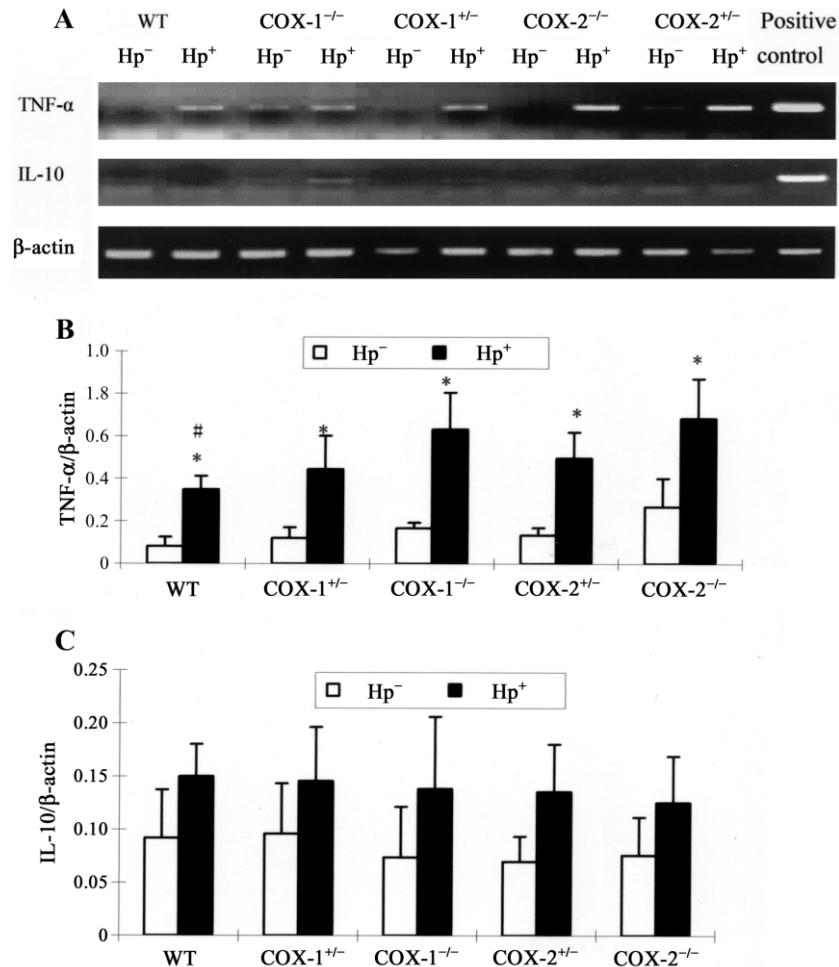


Figure 4. Gastric tumor necrosis factor (TNF)- α and interleukin (IL)-10 mRNA levels in uninfected and *Helicobacter pylori* (Hp)-infected wild-type (WT), cyclooxygenase (COX)-1-deficient, and COX-2-deficient mice. TNF- α , IL-10, and β -actin mRNA levels in representative gastric specimens from infected and uninfected WT, COX-1-deficient, and COX-2-deficient mice, as well as a mouse splenic specimen (as a positive control), were determined by semiquantitative reverse-transcription polymerase chain reaction (A). TNF- α , IL-10, and β -actin mRNA levels in uninfected and infected mice ($n = 12$ per group) were then determined by densitometry, and TNF- α and IL-10 levels were standardized against β -actin levels (B and C). Experiments were performed in triplicate. Data are means \pm SEs. * $P < .05$, compared with the corresponding uninfected mice; # $P < .05$, compared with the infected COX-1^{-/-}, COX-2^{+/-}, and COX-2^{-/-} mice.

that genetic deficiency of the COX-1 or COX-2 isoform exacerbated the severity of *H. pylori*-induced gastritis and that heterozygous COX-deficient mice, which have a partial deficiency of COX expression, displayed an intermediate phenotype. Our data, along with those from other animal studies [43, 44], suggest that COX-1 and COX-2 independently contribute to the down-regulation of gastric mucosal inflammation induced by *H. pylori* infection.

It has been consistently demonstrated that chronic *H. pylori* infection increases apoptosis and proliferation of gastric epithelial cells and may eventually result in an imbalance between apoptosis and proliferation, which may contribute to either gastric ulceration due to excessive apoptosis or even carcinogenesis due to hyperproliferation [18, 19, 45-49]. Our animal experiments showed that *H. pylori* infection increased both

apoptosis and proliferation of gastric epithelial cells in WT mice, a finding that is consistent with those of previous studies. Moreover, we observed, for the first time, the simultaneous increase in apoptosis and lack of an increase in cell proliferation in *H. pylori*-infected COX-2-deficient mice, suggesting that COX-2 disruption is protective against *H. pylori*-induced gastric carcinoma. We propose that, in the presence of *H. pylori* infection, COX-2 is involved in the up-regulation of cell proliferation but not of apoptosis and, thus, that COX-2 disruption blocks the up-regulation of cell proliferation but has little effect on apoptosis. In other words, COX-2 is involved in *H. pylori*-induced gastric cell proliferation, and the inhibition or disruption of COX-2 production may be associated with a decreased risk of *H. pylori*-induced gastric carcinoma because COX-2 blocks the up-regulation of cell proliferation without affecting

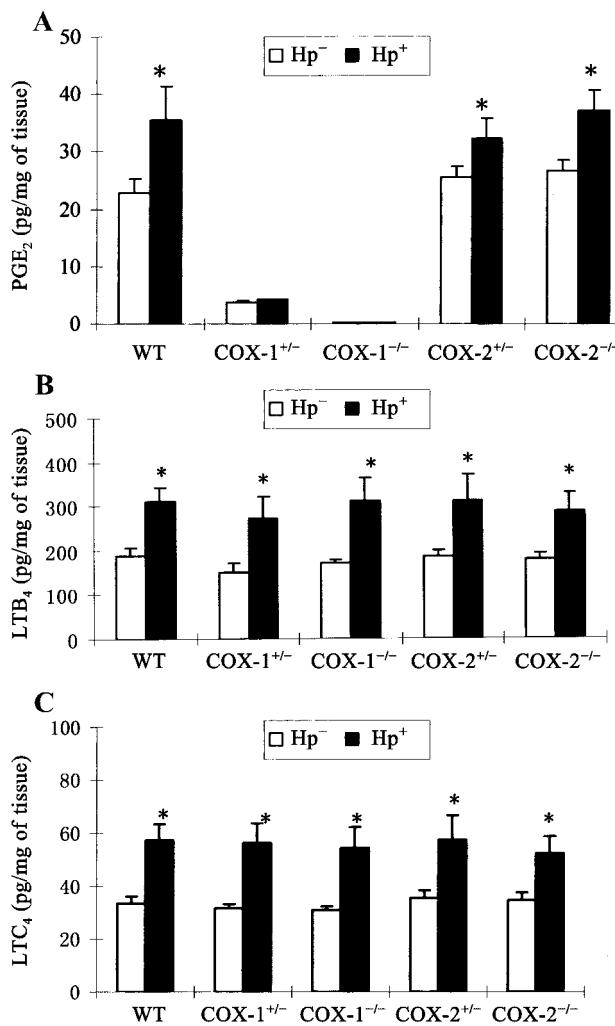


Figure 5. Expression of gastric prostaglandin E (PGE₂) (A), leukotriene (LT) B₄ (B), and LTC₄ (C) in uninfected and *Helicobacter pylori* (Hp)-infected wild-type (WT), cyclooxygenase (COX)-1-deficient, and COX-2-deficient mice ($n = 12$ per group). Experiments were performed in triplicate. Data are means \pm SEs. * $P < .05$, compared with the corresponding uninfected mice.

apoptosis. Indeed, previous studies have shown that NSAIDs have antineoplastic effects for gastric carcinoma [50, 51], and several possible mechanisms that might underlie the effects—including the induction of apoptosis, the inhibition of cell proliferation, antiangiogenic activities, and immune surveillance—have been proposed [52–56].

The observations that deficiency of the COX-1 or COX-2 gene enhanced the severity of *H. pylori*-induced gastritis and that COX-2 deficiency increased apoptosis without inducing a corresponding increase in cell proliferation raise the concern that the risk of gastric erosion, or even of peptic ulcer, may increase. However, no gastric ulceration was observed in the present study. In COX-1-deficient mice, the balance between apoptosis and cell proliferation—and, thus, the integrity of gastric mucosa—may

be maintained, as we found both to be increased at similar levels. In COX-2-deficient mice, we found the production of PGE₂ to be unchanged, which may be responsible for the maintenance of gastric mucosal integrity. Moreover, the 2 COX isoforms might have compensatory functions for one another in the regulation of gastrointestinal homeostasis and inflammation during injury, even though COX-1 is constitutive and COX-2 is inducible [57]. Kirtikara et al. reported compensatory PGE₂ biosynthesis in COX-1- or COX-2-deficient lung fibroblasts [57]. However, Langenbach et al. did not find any compensatory COX-2-mediated PGE₂ production in glandular gastric specimens from COX-1-deficient mice [34], a finding that is consistent with our observation here.

In the present study, *H. pylori* infection elevated the gastric mucosal expression of TNF- α mRNA and IL-10 mRNA in WT mice, a finding that is in agreement with those of other studies [24, 25, 58, 59]. Moreover, we observed that TNF- α mRNA expression, but not IL-10 mRNA expression, was further increased in COX-deficient mice, indicating that the 2 COX isoforms play an anti-inflammatory role by suppressing the expression of TNF- α . Our finding is supported by a previous study conducted in mice that showed that *H. pylori* infection elevated TNF- α mRNA expression in the stomach and that the expression of TNF- α mRNA was further increased by the inhibition of both COX-1 and COX-2, indicating that both isoforms are involved in *H. pylori*-induced gastric inflammation via inhibition of the expression of TNF- α [28].

We observed that basal PGE₂ levels were almost undetectable in the gastric mucosa of COX-1^{-/-} mice. Basal PGE₂ levels were reduced by $\sim 85\%$ in COX-1^{+/-} mice, compared with those in their WT counterparts. Moreover, *H. pylori* infection did not increase PGE₂ levels, although the severity of gastric mucosal inflammation was enhanced in COX-1-deficient mice. In contrast, basal PGE₂ levels in COX-2-deficient mice were similar to those in WT mice, and the production of PGE₂ was significantly increased by *H. pylori* infection. These observations indicate that, in both infected and uninfected mice, PG synthesis is dependent on COX-1 only.

Previous studies have shown that *H. pylori*-induced gastritis is associated with increased levels of gastric mucosal LTs, such as LTB₄ and LTC₄, and that these products may amplify the damaging effects that the bacterium has on gastric mucosa [36–38]. To our knowledge, however, there has been no study of whether COX-1 and COX-2 down-regulate *H. pylori*-induced gastritis via inhibition of LTs or whether the LOX pathway is involved in the increased *H. pylori*-induced gastritis observed in COX-deficient mice. In the present study, the gastric levels of both LTB₄ and LTC₄ were increased in *H. pylori*-infected WT and COX-deficient mice, compared with those in the corresponding uninfected mice. However, there was no further compensatory LTB₄ and LTC₄ production in COX-deficient mice,

compared with that in WT mice. These findings suggest that LTB₄ and LTC₄ levels increased during *H. pylori* infection independently of COX and that the LOX pathway was not involved in the increased *H. pylori*-induced gastritis in COX-deficient mice.

In conclusion, in the present 24-week *H. pylori* colonization model, COX-1 and COX-2 deficiency enhances *H. pylori*-induced gastritis, probably via TNF- α expression. COX-2 deficiency, but not COX-1 deficiency, suppresses the cell proliferation induced by *H. pylori* infection.

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