Infection and Immunity

Effects of Nonsteroidal Anti-Inflammatory Drugs on *Helicobacter pylori*-Infected Gastric Mucosae of Mice: Apoptosis, Cell Proliferation, and Inflammatory Activity

Tae Il Kim, Yong Chan Lee, Kwang Hyoung Lee, Jae Ho Han, Chae Yoon Chon, Young Myoung Moon, Jin Kyung Kang and In Suh Park

Infect. Immun. **2001, 69(8):5056. DOI: 10.1128/IAI.69.8.5056-5063.2001.**

INFECTION AND IMMUNITY, Aug. 2001, p. 5056–5063 Vol. 69, No. 8
0019-9567/01/$04.00+0 DOI: 10.1128/IAI.69.8.5056–5063.2001

Effects of Nonsteroidal Anti-Inflammatory Drugs on *Helicobacter pylori*-Infected Gastric Mucosae of Mice: Apoptosis, Cell Proliferation, and Inflammatory Activity

TAE IL KIM,[1]* YONG CHAN LEE,[1] KWANG HYOUNG LEE,[1] JAE HO HAN,[2] CHAE YOON CHON,[1] YOUNG MYOUNG MOON,[1] JIN KYUNG KANG,[1] AND IN SUH PARK[1]

Department of Internal Medicine and Institute of Gastroenterology[1] and Department of Pathology,[2] Yonsei University College of Medicine, Seoul, Korea

Received 29 January 2001/Returned for modification 14 March 2001/Accepted 29 April 2001

Helicobacter pylori **and nonsteroidal anti-inflammatory drugs (NSAIDs) are two well-known important causative factors of gastric damage. While *H. pylori* increases apoptosis and the proliferation of gastric epithelial cells and is an important factor in peptic ulcer and gastric cancer, NSAIDs induce cell apoptosis and have antineoplastic effects. We investigated the effects of NSAIDs (a nonselective cyclooxygenase [COX] inhibitor [indomethacin] and a selective COX-2 inhibitor [NS-398]) on the apoptosis and proliferation of gastric epithelial cells and gastric inflammation in *H. pylori*-infected mice. C57BL/6 mice were sacrificed 8 weeks after *H. pylori* SS1 inoculation. Indomethacin (2 mg/kg) or NS-398 (10 mg/kg) was administered subcutaneously once daily for 10 days before sacrifice. The following were assessed: gastric inflammatory activity, gastric COX protein expression by Western blotting; gastric prostaglandin E_2 levels by enzyme immunoassay, apoptosis by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling, and cell proliferation by Ki67 immunostaining. Compared to the controls, *H. pylori* infection and/or NSAID treatment increased COX-1 and COX-2 protein expression. Gastric prostaglandin E_2 levels, apoptotic index, cell proliferation index, neutrophil activity, and the degree of chronic inflammation were all increased by *H. pylori* infection, and these effects were significantly decreased by indomethacin treatment. However, NS-398 treatment after *H. pylori* infection did not induce a significant reduction, although it did result in a tendency to decrease. These results show that NSAIDs can reverse the increased apoptosis and proliferation of epithelial cells and inflammatory activity in the stomachs of *H. pylori*-infected mice and that, like COX-2 activation, COX-1 induction contributes to the change of gastric mucosal cell turnover and inflammation induced by *H. pylori* infection.**

Helicobacter pylori and nonsteroidal anti-inflammatory drugs (NSAIDs) are two well-known important causative factors of gastric injury such as gastritis and peptic ulcer. However, the interaction between these two factors in terms of their effects on gastric mucosa remains controversial. Some clinical studies have reported no interaction between *H. pylori* and NSAIDs or of a protective role of *H. pylori* in patients given NSAIDs (19, 24, 27). Other studies have shown harmful effects of *H. pylori* in patients given NSAIDs (1, 4, 25). There are many reasons for these controversies, including differences in the characteristics of subjects, differences in methods of approach, relatively small sample sizes, etc. Furthermore, the complicated mechanisms, including cyclooxygenase 2 (COX-2) and/or COX-1 activity, may be involved in the interaction between *H. pylori* and NSAIDs (nonselective and COX-2 selective) on the gastric mucosa. Therefore, an understanding of processes at the cellular level and large studies comparing COX-2 inhibitors with nonselective COX inhibitors will provide better information.

When one considers cell turnover and the pathogenic effects on the gastric mucosa, it becomes apparent that the dynamic balance between epithelial cell proliferation and apoptosis is essential for maintaining normal mucosal integrity and that an alteration of the balance in either direction could result in ulcerogenesis or carcinogenesis of the stomach (38, 44). *H. pylori* is associated in vivo with both increased apoptosis and the proliferation of gastric epithelial cells; accordingly, an alteration in the balance of these events may lead to increased injury or neoplastic transformation (2, 7, 34). On the other hand, NSAIDs induce cell apoptosis and have antineoplastic effects, and they reduce the incidence of and mortality from colon adenocarcinoma and gastric cancer (17, 57). However, in human clinical studies on the interaction of these two factors during cell turnover, some controversial aspects remain unresolved. Zhu et al. showed that NSAIDs could abrogate the apoptosis and proliferation enhanced by *H. pylori* (66). However, Leung et al. reported that the eradication of *H. pylori* prior to NSAID therapy significantly reduced the level of apoptosis in the gastric mucosa (29).

COX expression and prostaglandin (PG) production may have some mechanistic influence on the interaction between *H. pylori* and NSAIDs in the gastric mucosa. It is well known that COX-2 is the inducible form, involved in the inflammatory reaction and tumorigenesis, and that COX-1 is the constitutive form, related to physiologic cytoprotection. In *H. pylori*-infected gastric tissue, upregulated COX-2 expression and increased PGE_2 production may be important for cell proliferation (13, 46). In the case of NSAID-induced gastric injury, the

* Corresponding author. Mailing address: Department of Internal Medicine, Yonsei University College of Medicine, 134 Shinchon-dong Seodaemun-ku, Seoul, 120-752, Korea. Phone: (822) 361-5410. Fax: (822) 393-6884. E-mail: taeilkim@yumc.yonsei.ac.kr.

most important mucosal injury factor appears to be the decrease in mucosal PG production by COX inhibition and apoptosis induction (16, 30). It has also been shown that selective COX-2 inhibitors have antineoplastic effects, as do nonselective COX inhibitors, and that they induce less mucosal damage than nonselective COX inhibitors (27, 49, 54). However, it has not been determined whether there are any differences between the effects of nonselective and selective COX-2 inhibitors on gastric epithelial cell kinetics in the *H. pylori*-infected stomach. Therefore, in this study, we evaluated the interaction between *H. pylori* and NSAIDs in terms of gastric epithelial cell apoptosis, proliferation, and inflammation in a mouse model. In particular, we investigated the differences between nonselective and selective COX-2 inhibitors on these interactions.

MATERIALS AND METHODS

Animals and *H. pylori* challenge. Rat and mice models have been used to investigate the NSAID-induced gastric damage and the effects of NSAIDs in ulcer healing in many studies (37, 52, 61). *H. pylori* SS1 infection of C57BL/6 mice has been used as standardized mouse model of *H. pylori* infection, inducing chronic gastritis similar to *H. pylori*-induced gastritis in humans (28). Therefore, in the present study, we used the C57BL/6 mouse as the animal model.

Specific-pathogen-free, inbred C57BL/6 female mice 7 weeks of age were purchased from Jackson Laboratory (Bar Harbor, Maine). All animals were housed in the specific-pathogen-free barrier area of Department of Laboratory Animal Medicine, Yonsei University College of Medicine, for the duration of the experiments. All experiments and procedures involving the animals were reviewed by the Yonsei University Committee for the Care and Use of Laboratory Animals according to university's the guidelines and regulations for use and care of animals.

The mice were divided into six groups A to F, and treated according to the following schedule: A, control ($n = 7$); B, *H. pylori* infection ($n = 7$); C, *H. pylori* infection followed by indomethacin treatment ($n = 7$); D, *H. pylori* infection followed by NS-398 treatment ($n = 5$); E, indomethacin treatment ($n = 7$); and F, NS-398 treatment ($n = 5$). *H. pylori* SS1 ($vacA^+$ $cagA^+$), obtained from Daewoong Research and Development, Daewoong Pharmaceutical Co., Ltd., was grown on blood agar plates containing sterile sheep blood (5%, vol/vol) in blood agar base (Becton Dickinson and Co., Cockeysville, Md.). The *H. pylori* colonies were suspended in phosphate-buffered saline and administered intragastrically to the mice. The animals were dosed three times in a 3-day period with 0.1 ml of bacterial suspension (approximately 10^9 organisms/ml). The animals that were not inoculated with *H. pylori* were inoculated with identical volumes of sterile phosphate-buffered saline alone.

Administration of NSAIDs. Indomethacin and NS-398 were purchased from Sigma Chemical Co. (St. Louis, Mo.) and Calbiochem (La Jolla, Calif.), respectively. In other experiments, NS-398 was used as a selective COX-2 inhibitor in doses of less than 10 mg/kg (14, 37, 52); in our experiment, we found that a 13-mg/kg dose of NS-398 reduced the levels of PGE_2 in mouse stomachs ($n = 5$) by approximately 35%, while a 10-mg/kg dose of NS-398 did not reduce the gastric PGE_2 level. Therefore, we decided to set the dose of selective COX-2 inhibitor at 10 mg of NS-398 per ml. Indomethacin (2 mg/kg), a nonselective COX inhibitor, and NS-398 (10 mg/kg), a selective COX-2 inhibitor, suspended in 1% carboxymethyl cellulose solution were administered subcutaneously in a volume of 0.25 ml/100 g of body weight once daily for 10 days before sacrifice. The animals that were not treated with indomethacin or NS-398 received identical volumes of vehicle (carboxymethyl cellulose) alone. Each animal was killed 8 weeks after *H. pylori* inoculation, and the stomach was longitudinally divided into three pieces. One piece was fixed in 10% neutral buffered formalin for histological examination and immunohistochemistry, and the other two pieces were immediately stored in liquid nitrogen until assayed for the measurement of gastric PGE_2 levels and Western blotting.

Histologic gastritis and *H. pylori* infection. A single experienced pathologist examined hematoxylin-eosin (H&E)- and Giemsa-stained paraffin sections of mouse stomachs without knowledge of the experimental schedule. As semiquantitative estimates, the two parameters in inflammation, active inflammatory activity (neutrophil score) and chronic inflammatory activity (mononuclear cell score), were graded from 0 to 3, based on the updated Sydney system (9), and the presence of spiral organisms was also examined in Giemsa-stained paraffin sections.

Western blot analysis for the measurement of gastric COX protein expression. For analysis of gastric COX protein expression, frozen gastric tissues of mice were homogenized and lysed in buffer containing 40 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.5% NP-40, 1 mM phenylmethylsulfonyl fluoride, and 10 μg of leupeptin per ml. The lysate were incubated on ice for 60 min and centrifuged at 12,000 rpm for 20 min. After denaturation 20-μg aliquots of extracted proteins were electrophoresed on sodium dodecyl sulfate–10% polyacrylamide gels and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, Mass.). The PVDF membrane was blocked with 5% nonfat milk in TBST (10 mM Tris [pH 7.4], 100 mM NaCl, 0.5% Tween 20) and then probed for 1 h with 1:1,000-diluted goat anti-COX-1 or anti-COX-2 polyclonal immunoglobulin Ig G (IgG; Santa Cruz Biotechnology, Santa Cruz, Calif.). Bound antibodies were detected with horseradish peroxidase-conjugated anti-goat IgG (Santa Cruz Biotechnology), using the enhanced chemiluminescence detection system.

Enzyme immunoassay for the measurement of gastric PGE_2 levels. The frozen specimens were weighed and put in a tube containing 100% ethanol plus 10 μg of indomethacin per ml. The samples were then minced with scissors, homogenized, and centrifuged for 10 min at 12,000 rpm at 4°C. The supernatant of each sample was used for determination of PGE_2, using a Bicyclo PGE_2 enzyme immunoassay kit (Cayman Chemical Company. Ann Arbor, Mich.). as described by the manufacturer.

Determination of apoptosis and proliferation. A single experienced pathologist blinded to treatment schedule quantitated gastric epithelial cell apoptosis and proliferation by using immunohistochemical stains. Epithelial cell apoptosis was determined in situ from paraffin-embedded tissue sections by the terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL) technique (apoTACS-Basic kit; R&D Systems, Minneapolis, Minn.) as described by the manufacturer. All samples were processed under the same conditions with positive and negative controls. Positive control sections were pretreated with DNase I and negative controls were processed without terminal deoxynucleotidyl-transferase. For each mouse, 50 well-oriented, representative gastric glands were scored in a tissue section, and the results of apoptosis are expressed as apoptotic index, defined as the number of positive cells per 50 glands.

Epithelial cell proliferation was determined by immunohistochemical staining for Ki67. Briefly, sections were deparaffinzed, placed in citrate buffer (pH 6.0) (10 mmol/liter), and heated in a 700-W microwave oven for 10 min. Endogenous peroxidase activity was quenched with hydrogen peroxide, and after washing in immunoassay buffer, the slides were incubated with a monoclonal mouse IgG raised against Ki67 (PharMingen, San Diego, Calif.) in a humidified chamber. Slides then were incubated with biotinylated anti-mouse IgG and streptavidin-horseradish peroxidase (LSAB2 kit; DAKO Corporation, Carpinteria, Calif.), developed in aminoethylcarbazole (AEC+; DAKO), and counterstained with hematoxylin. The results of cell proliferation are expressed as proliferation index, defined as the number of positive cells per 50 glands.

Statistics. Two-tailed statistical tests were used to evaluate the data. The Mann-Whitney *U* test for nonparametric data was used to compare scores between animal groups. The correlation among apoptosis, proliferation, and inflammatory activity was evaluated using Spearman's rho test for nonparametric data. The data are presented as medians in figures and median (range) in Table 1. Significance was defined as a P value of <0.05.

RESULTS

***H. pylori* infection and chronic gastritis.** *H. pylori* infection in the stomach was confirmed by Giemsa staining in all *H. pylori*-inoculated mice (Fig. 1A), and organisms were found throughout the glandular stomach, colonizing both the antrum and the body, as found in a previous study (28). Increased levels of polymorphonuclear neutrophils and mononuclear cells were found in both the antrum and the body of the stomachs of *H. pylori*-infected mice (Fig. 1B), whereas atrophy, intestinal metaplasia, and ulceration were not observed.

PGE_2 levels and COX expression. To assess the effects of *H. pylori* infection and treatment with the NSAIDs indomethacin and NS-398 on PGE_2 production in gastric tissues, PGE_2 levels in the gastric tissues of mice were examined by enzyme immunoassay.

Gastric PGE_2 levels showed a tendency to increase upon *H. pylori* infection, although this was not significant, and further

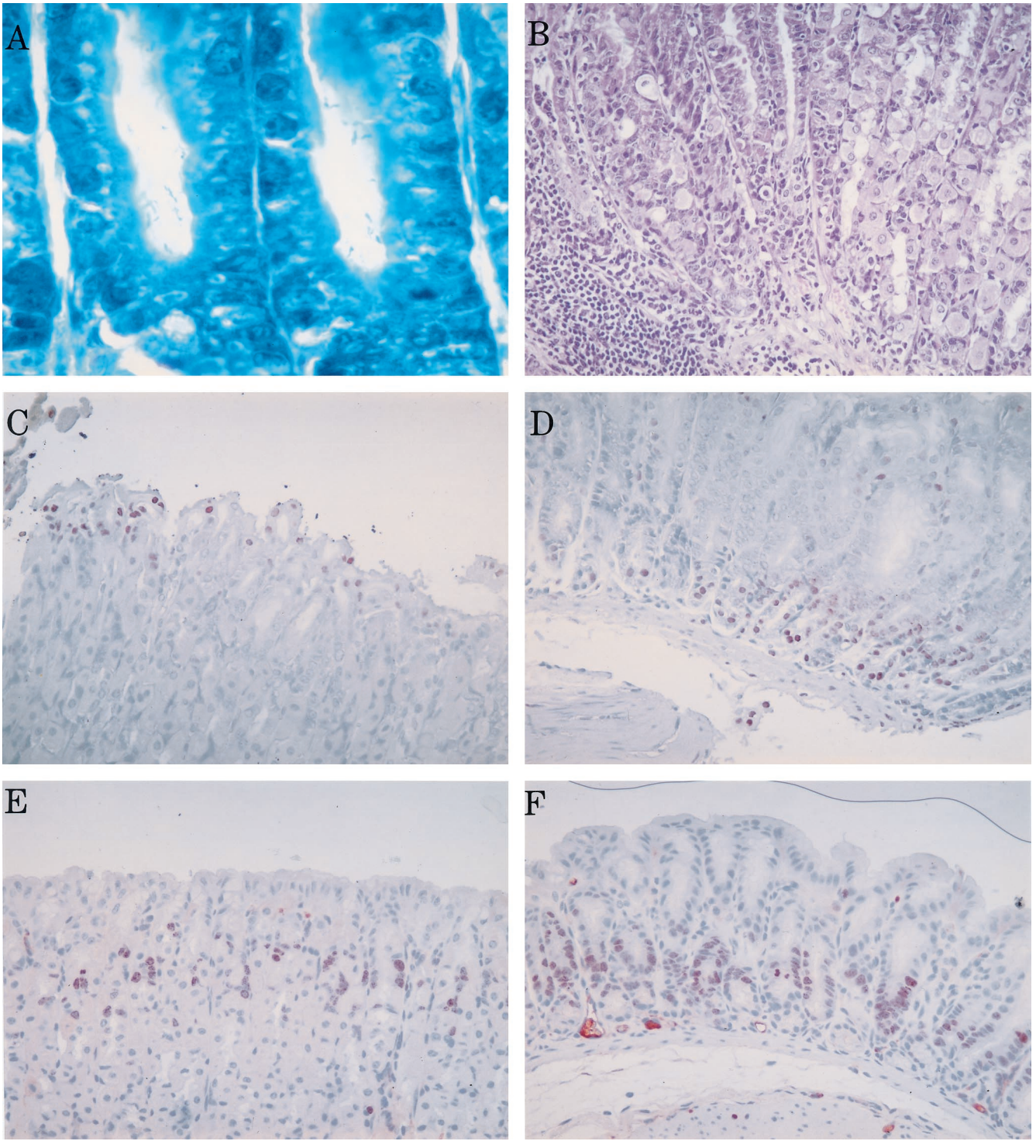


FIG. 1. *H. pylori* infection, chronic gastritis, apoptosis, and proliferation in stomachs of *H. pylori*-infected mice. (A) Light microscopic photograph of gastric tissue from a C57BL/6 mouse colonized with *H. pylori* SS1 showing the S shape of the bacteria. *H. pylori* infections were confirmed by Giemsa staining in all cases of *H. pylori*-inoculated mice (magnification ×730). (B) Findings of chronic gastritis with many inflammatory cells in the stomach of an *H. pylori*-infected mouse (H&E, ×292). (C) Increased positive apoptotic epithelial cells in the superficial portion of the stomach of an *H. pylori*-infected mouse (TUNEL stain, ×292). (D) Increased numbers of positive apoptotic epithelial cells in the proliferation compartment and superficial portion of the antral mucosa of the stomach of an *H. pylori*-infected mouse (TUNEL stain, ×292). (E and F) Increased number of Ki67-positive cells in the proliferation compartment of the body (E) and antrum (F) of the stomach of an *H. pylori*-infected mouse (Ki67 immunostain, ×292).

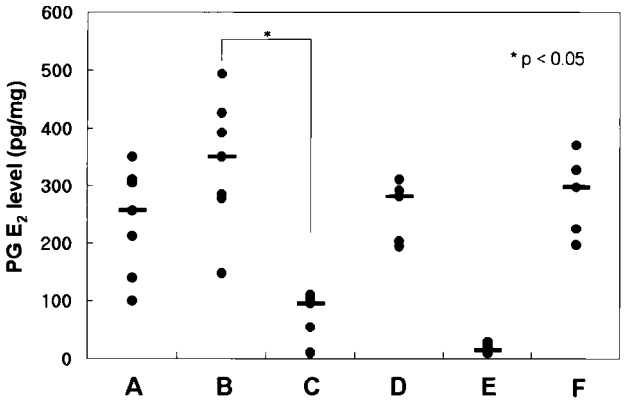
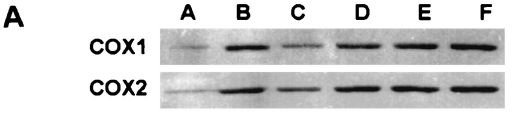


FIG. 2. PGE_2 levels in stomachs of *H. pylori*-infected mice with or without NSAID treatment (A, control; B, *H. pylori* infection; C, *H. pylori* infection followed by indomethacin treatment; D, *H. pylori* infection followed by NS-398 treatment; E, indomethacin treatment; F, NS-398 treatment). Symbols show the PGE_2 levels in the gastric tissues of mice, measured by enzyme immunoassay. Horizontal lines show the median for each group. *, significant difference ($P < 0.05$) as calculated by the Mann-Whitney *U* test.



B

	A	B	C	D	E	F
COX1	282 (122, 415)	2492* (1688, 3386)	1890* (1056, 2814)	1958* (1584, 2739)	1723* (1705, 2095)	2080* (1704, 2153)
COX2	301 (277, 401)	1613* (826, 2533)	1579* (1522, 2968)	2148* (1177, 3019)	1505* (1022, 1559)	1603* (848, 1738)

FIG. 3. COX expression as detected by Western blotting in stomachs of *H. pylori*-infected mice with or without NSAID treatment (A, control; B, *H. pylori* infection; C, *H. pylori* infection followed by indomethacin treatment; D, *H. pylori* infection followed by NS-398 treatment; E, indomethacin treatment; F, NS-398 treatment). (A) Western blot analysis of COX-1 and COX-2 proteins in the gastric tissues of mice. Extracted proteins from the gastric tissues were electrophoresed, transferred to a PVDF membrane, and probed with anti-COX-1 and anti-COX-2 antibodies. (B) The bands were measured densitometrically, and the data were combined. Values are medians (ranges) of optical density units in each mice group. *, significant difference ($P < 0.05$) compared to the control group as calculated by the Mann-Whitney *U* test.

administration of indomethacin significantly decreased this heightened PGE_2 level ($P = 0.002$) (Fig. 2). However, the effect of NS-398 treatment after *H. pylori* infection on PGE_2 production was not significant, although it tended to decrease to the level of the controls (Fig. 2). In the absence of *H. pylori* infection, indomethacin treatment markedly decreased the PGE_2 level ($P = 0.002$), but NS-398 treatment resulted in no difference compared with the control group (Fig. 2).

We determined COX-1 and COX-2 expression levels by Western blotting to evaluate the effect of *H. pylori* and/or NSAID treatment. Levels in mouse stomachs were found to be increased by *H. pylori* infection and/or NSAID treatment (Fig. 3A), and this result was confirmed by densitometric band measurements (Fig. 3B).

Apoptosis of gastric epithelial cells. Because *H. pylori* and gastric mucosal inflammation contribute to cell turnover, and NSAIDs have anti-inflammatory and apoptotic effects, we investigated whether epithelial cell apoptosis is altered by *H. pylori* infection and how these apoptotic effects are affected by NSAIDs.

Apoptotic cells were detected by the TUNEL method, and positive staining was found to be confined to the nucleus. In the *H. pylori*-negative control group, TUNEL-positive cells were few in number and usually situated at the superficial part of the glands. However, in *H. pylori*-infected mice, apoptotic cells in the superficial portion of the body and antrum were increased in number and positive cells were often detected in the proliferating basal zone of the antral mucosa, which is an important site for epithelial replication and the regeneration of mucosal glands and surface epithelium (Fig. 1C and D).

To investigate the effect of NSAIDs on apoptosis in *H. pylori*-infected gastric mucosa, we measured the apoptotic index in the gastric mucosa of *H. pylori*-infected mouse with or without NSAID treatment. The apoptotic index was significantly increased upon *H. pylori* infection ($P = 0.003$), and this effect was significantly decreased after further treatment with indomethacin ($P = 0.025$) (Fig. 4). The apoptotic index was also lower in *H. pylori*-infected mice after NS-398 treatment than in nontreated *H. pylori*-infected mice, but this difference was not statistically significant (Fig. 4). When noninfected mice were treated with indomethacin or NS-398, no significant change in the apoptotic indices was observed compared to the control group (Fig. 4).

Proliferation of gastric epithelial cells. To determine whether *H. pylori* infection increases proliferation in mice mucosa and how NSAIDs affect the gastric epithelial cell hyperproliferation induced by *H. pylori*, epithelial cell proliferation was quantified after challenge with *H. pylori* and/or NSAIDs.

Immunostaining of Ki67 was localized in the nuclei, and

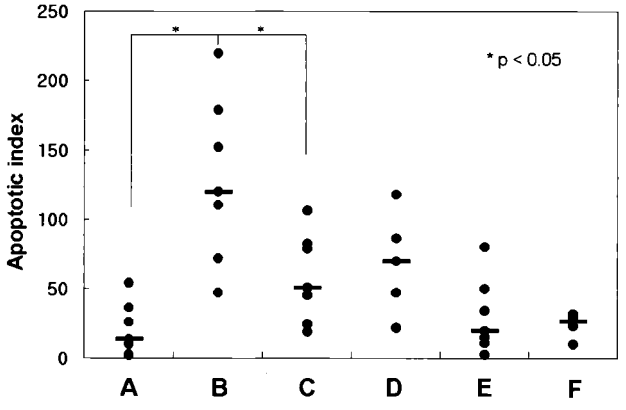


FIG. 4. Apoptotic indices in stomachs of *H. pylori*-infected mice with or without NSAID treatment (A, control; B, *H. pylori* infection; C, *H. pylori* infection followed by indomethacin treatment; D, *H. pylori* infection followed by NS-398 treatment; E, indomethacin treatment; F, NS-398 treatment). Symbols show the apoptotic index (number of positive cell per 50 glands) determined by TUNEL staining. Horizontal lines show the median for each group. *, significant difference ($P < 0.05$) as calculated by the Mann-Whitney *U* test.

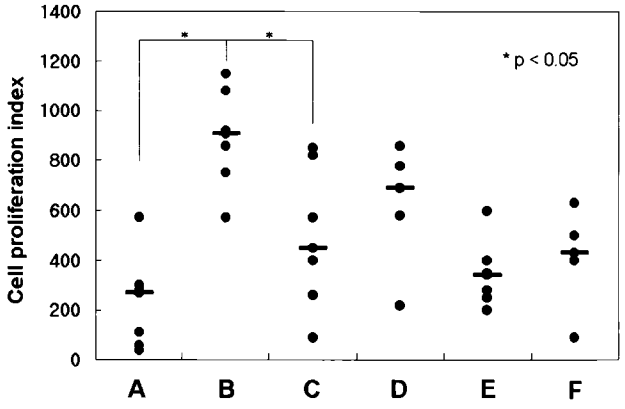


FIG. 5. Cell proliferation indices in stomachs of *H. pylori*-infected mice with or without NSAID treatment (A, control; B, *H. pylori* infection; C, *H. pylori* infection followed by indomethacin treatment; D, *H. pylori* infection followed by NS-398 treatment; E, indomethacin treatment; F, NS-398 treatment). Symbols show the cell proliferation index (number of positive cell per 50 glands) determined by Ki67 immunostaining. Horizontal lines show the median for each group. *, significant difference ($P < 0.05$) as calculated by the Mann-Whitney *U* test.

virtually all epithelial cells that stained positively for Ki67 were located within the proliferating compartment, which is located in the upper one third, the glandular neck of the body, and the basal zone of the antrum (Fig. 1E and F). The cell proliferation index was significantly increased after *H. pylori* infection ($P = 0.002$), and this effect was significantly decreased by indomethacin ($P = 0.011$) (Fig. 5). On the other hand, NS-398 treatment after *H. pylori* infection led to lower cell proliferation indices compared to the *H. pylori*-infected mice, but this difference was not significant (Fig. 5). When indomethacin or NS-398 was administered in the absence of *H. pylori* infection, no significant changes in cell proliferation were observed compared to the controls (Fig. 5).

Inflammation of gastric mucosa. To determine how NSAIDs affect *H. pylori* infection-induced inflammatory activity in the stomach, we quantified neutrophils as indicators of active inflammatory activity and mononuclear cells as indicators of chronic inflammatory activity, based on the updated Sydney system (9).

The neutrophil score was significantly increased after *H. pylori* infection ($P = 0.004$), and this effect was decreased significantly by indomethacin ($P = 0.012$) (Table 1). The mononuclear cell score showed a similar pattern, increasing after *H. pylori* infection ($P = 0.001$) and decreasing after further treatment with indomethacin ($P = 0.007$) (Table 1). NS-398 treatment after *H. pylori* infection did not induce significant reductions in the neutrophil and mononuclear cell scores compared to the *H. pylori*-infected mice (Table 1).

When indomethacin or NS-398 treatment was used in the absence of *H. pylori* infection, no significant change was observed in the neutrophil and mononuclear cell scores, except in the case of the indomethacin-treated mice, which showed a significant increase in the mononuclear cell score ($P = 0.018$), compared to the controls (Table 1).

Correlations between cell apoptosis, proliferation, and inflammatory activity. To determine whether increased apoptosis and proliferation may be related in a compensatory manner and whether the effect of *H. pylori* on mucosal cell cycle events in vivo may be associated with mucosal inflammation, we examined the relationships between apoptosis, proliferation indices, and inflammatory activity. In no group of mice was a correlation found between increased apoptosis and proliferation indices of epithelial cells or between increased apoptosis or proliferation index and inflammatory activity.

TABLE 1. Neutrophil activity and degree of chronic inflammation in stomachs of *H. pylori*-infected mice with or without NSAID treatment

Group[a]	Median score (range)[b]	
	Neutrophil	Mononuclear cell
A	0 (0–0.5)	0 (0–0.5)
B	1.5 (0.5–2.0)[c]	1.0 (0.5–1.5)[c]
C	0.5 (0–1.0)[d]	0 (0–1.0)[d]
D	0.5 (0.5–1.5)	0.5 (0–1.5)
E	0.5 (0–1.5)	0.5 (0–1.0)[c]
F	0 (0–1.0)	0 (0–1.0)

[a] A, control; B, *H. pylori* infection; C, *H. pylori* infection followed by indomethacin (2 mg/kg) treatment; D, *H. pylori* infection followed by NS-398 (10 mg/kg) treatment; E, indomethacin treatment; F, NS-398 treatment.
[b] Graded from 0 to 3 by a single pathologist.
[c] Significantly different ($P < 0.05$) from controls.
[d] Significantly different ($P < 0.05$) from group B as calculated by the Mann-Whitney *U* test.

DISCUSSION

According to epidemiological studies (40, 56), *H. pylori*-infected subjects have a significantly higher risk of developing peptic ulcer or gastric malignancy. It has been hypothesized that the alteration of the balance between apoptosis and the proliferation of gastric epithelial cells, induced by *H. pylori* infection, contributes to either gastric injury such as gastritis and peptic ulcer or carcinogenesis of the stomach (2, 7, 34). Although *H. pylori* decreases epithelial cell proliferation and induces apoptosis in vitro (5, 45, 47, 59), *H. pylori* infection in vivo is associated with both increased apoptosis and proliferation (12, 41, 42). These differences between the in vivo and in vitro results suggest that in vivo, factors including inflammatory cells, extracellular matrix proteins, cytokines, and adhesion molecules may also contribute to epithelial cell turnover. The present study also confirmed that *H. pylori* infection increases the rates of gastric epithelial cell apoptosis and proliferation, as reported previously (12, 23, 34, 39, 41, 42).

Epidemiological, clinical, and animal studies have shown that NSAIDs have an antineoplastic effect in colon cancer, and NSAIDs also appear to reduce the incidence of and mortality from gastric cancer (17, 57). The mechanisms responsible for these remarkable antineoplastic effects are not understood, but emerging data indicate that NSAIDs are likely to target several steps along the carcinogenic pathway. These include NSAID-induced inhibition of cell turnover, such as the induction of apoptosis and inhibition of cell proliferation, stimulation of immune surveillance, and antiangiogenic effects (22, 51, 55). In the case of NSAID-induced gastric damage, it has been also shown that NSAIDs induce the apoptosis of epithelial cells and delay ulcer healing (52), and in combination with decreased

gastric PG production, this apoptosis may serve as an important prerequisite for the gastric damage caused by NSAIDs (34, 44, 53). In the present study, NSAID administration without *H. pylori* infection induced no significant changes in the proliferative and apoptotic activities of gastric epithelial cells. In other studies, the gastric mucosal cell proliferation rates in subjects given NSAIDs have been reported to be enhanced (31) or to be unaffected (48), and the increased turnover of gastric mucosa has been hypothesized to represent an adaptive response, which is a process that gradually resolves early gastric mucosal damage, despite continued exposure to NSAIDs (15, 31). Therefore, we believe that our results regarding effects of NSAIDs on the epithelial cell turnover rate of gastric mucosa may be explained in part by the adaptive response of gastric mucosa.

Considering the combined effects of *H. pylori* and NSAIDs on cell turnover in the stomach, interestingly, indomethacin suppressed the apoptotic activities enhanced by *H. pylori* infection and reversed *H. pylori*-mediated cell proliferation in our study. Similar findings were reported in a clinical human study (66). From these results, although the mechanism underlying this effect is unclear and complicated, it appears that NSAID administration can suppress uncontrolled apoptosis and proliferation in *H. pylori*-infected subjects and that this effect might be extended to subsequent processes involving neoplastic changes. We also found that both of the inflammatory activities increased by *H. pylori* infection were also significantly reduced by indomethacin, suggesting the anti-inflammatory activity of NSAIDs in the *H. pylori*-infected stomach. These interesting results on cell turnover and inflammation lead to speculation on the complicated mechanisms involving inflammatory cells, cytokines, direct *H. pylori* stimuli, etc., in the interaction between *H. pylori* and NSAIDs in the gastric mucosa.

Our results also suggest that apoptosis and proliferation may be compensatory mechanisms, counteracting excessive cell proliferation and apoptosis, respectively (35). However, we found no correlation between cell proliferation and apoptosis in *H. pylori*-infected and/or NSAID-treated mice, in agreement with the results of other studies (41, 66). This observation supports the notion that the induction of apoptosis and proliferation in vivo are independent events regulated by different factors, including the direct effects of *H. pylori* stimuli and host inflammatory and immune responses, involving cytokines, nitric oxide, gastrin, etc. (8, 20, 36, 50).

In addition, because chronic inflammation may be a recognized risk factor for epithelial carcinogenesis (63), we can speculate about the association between cell turnover and inflammatory activity. However, in many other reports, inflammatory cytokines, oxygen free radicals, direct *H. pylori* stimuli, and other factors following *H. pylori* infection are believed to contribute to the increased gastric epithelial cell turnover (11, 32, 33) rather than the inflammation itself. Although the number of subjects involved was limited, our data also show that there is no correlation between the degree of inflammation and apoptosis or proliferation, as reported previously (33, 39, 66).

In a clinical report on the interaction between NSAIDs and *H. pylori*, Hawkey et al. showed that *H. pylori* eradication in long-term users of NSAIDs, with past or current peptic ulcer or troublesome dyspepsia, led to a impaired healing of the gastric ulcers (19), implying that under certain circumstances some patients with *H. pylori* are less prone to NSAID-induced ulceration than noninfected patients. This may be due to the opposing effects of *H. pylori* and NSAIDs on PG synthesis in the gastric mucosa (18). However, other studies have shown that the eradication of *H. pylori* prior to NSAID therapy reduces the occurrence of peptic ulcers (4) and the level of apoptosis in gastric mucosae (29), and that NSAID users infected with *H. pylori* carry a greater risk of peptic ulcer than noninfected NSAID users (1). It was also reported that *H. pylori* infection may reduce the adaptation threshold and that the eradication of *H. pylori* restored the ability of the gastric mucosa to adapt to aspirin (26). However, in our results showed no significant difference between the proliferative, apoptotic, and inflammatory activities of NSAID-treated mice with *H. pylori* infection and NSAID-treated mice without *H. pylori* infection, suggesting that *H. pylori* infection may not induce significant additive harmful effects in the stomachs of subjects administered NSAIDs, as reported previously (27, 64).

The aforementioned antineoplastic and anti-inflammatory effects of NSAIDs are commonly attributed to the inhibition of COX-2, the inducible form of COX (62). The levels of PGs and COX-2 gene expression are elevated in colon cancer tissue (10, 65), and the overexpression of COX-2 in epithelial cells inhibits apoptosis (54, 58). Although some NSAIDs were shown to induce apoptosis by a mechanism that may be unrelated to the ability of these drugs to inhibit PG synthesis (43), PG and COX-2 have an important role in cell proliferation and carcinogenesis and are elevated in *H. pylori*-infected gastric mucosa (13). In terms of COX expression as measured by Western blotting in our mouse model, both COX-2 and COX-1 expression levels were found to be elevated in the gastric tissue of *H. pylori*-infected mice compared with control mice. Wallace et al. showed that an inflammatory response could be elicited in COX-$2^{-/-}$ mice as well as wild-type mice and that this response could be reduced in both groups by indomethacin but not by a selective COX-2 inhibitor, which strongly suggests that COX-1 plays a role in the inflammatory response (60). Furthermore, other studies have suggested that like COX-2, COX-1 can be induced by *H. pylori* (3, 21) or by stressful stimuli such as radiation injury (6). In addition, in our present study, along with the increased expression of both COX-1 and COX-2 in the gastric tissue of *H. pylori*-infected mice, indomethacin treatment after *H. pylori* infection induced more significant decreases in the enhanced apoptotic, proliferative, and inflammatory activities of the *H. pylori*-infected stomach than NS-398 treatment after *H. pylori* infection. Therefore, it is possible that like COX-2 activation, some degree of COX-1 induction may also contribute to increased cell turnover and the inflammatory activity induced by *H. pylori* infection in gastric tissue. Although it is questionable that these effects translate to human disease, recently Jackson et al. showed the importance of COX-1 in *H. pylori*-infected humans (21). Therefore, from the viewpoint of both gastric injury and carcinogenesis, it will be interesting to compare the effects of nonselective and selective COX-2 inhibitors on gastric events including epithelial cell kinetics, gastric injury, and its molecular mechanism in *H. pylori*-infected humans.

In summary, NSAIDs can reverse the increased apoptosis

and proliferation of epithelial cells and the inflammatory activity induced by *H. pylori* infection in the mouse stomach, and *H. pylori* does not potentiate significantly the effects on cell turnover and inflammation induced by NSAID treatment. In addition, like COX-2 activation, some degree of COX-1 induction might also contribute to the change in gastric mucosal cell turnover and inflammation induced by *H. pylori* infection.

REFERENCES

1. **Aalykke, C., J. M. Lauritsen, J. Hallas, S. Reinholdt, K. Krogfelt, and K. Lauritsen.** 1999. *Helicobacter pylori* and risk of ulcer bleeding among users of non-steroidal anti-inflammatory drugs: a case-control study. Gastroenterology **116:**1305–1309.
2. **Anti, M., A. Armuzzi, A. Gasbarrini, and G. Gasbarrini.** 1998. Importance of changes in epithelial cell turnover during *Helicobacter pylori* infection in gastric carcinogenesis. Gut **43**(Suppl. 1)**:**S27–S32.
3. **Byrne, M. F., D. J. Fitzgerald, J. C. Atherton, and F. E. Murray.** 2000. *Helicobacter pylori* induces cyclooxygenase-1 as well as cyclooxygenase-2 in endothelial cells. Gastroenterology **118:**A738.
4. **Chan, F. K. L., J. J. Y. Sung, S. C. S. Chung, K. F. To, M. Y. Yung, V. K. S. Leung, Y. T. Lee, C. S. Y. Chan, E. K. M. Li, and J. Woo.** 1997. Randomised trial of eradication of *Helicobacter pylori* before non-steroidal anti-inflammatory drug therapy to prevent peptic ulcers. Lancet **350:**975–979.
5. **Chen, G., E. M. Sordillo, W. G. Ramey, J. Reidy, P. R. Holy, S. Krajewski, J. C. Reed, M. J. Blaser, and S. F. Moss.** 1997. Apoptosis in gastric epithelial cells is induced by *Helicobacter pylori* and accompanied by increased expression of Bak. Biochem. Biophys. Res. Commun. **239:**626–632.
6. **Cohn, S. M., S. Schloemann, T. Tessner, K. Seibert, and W. F. Stenson.** 1997. Crypt stem cell survival in the mouse intestinal epithelium is regulated by prostaglandins synthesized through cyclooxygenase-1. J. Clin. Investig. **99:** 1367–1379.
7. **Correa, P., and M. J. S. Miller.** 1998. Carcinogenesis, apoptosis and cell proliferation. Br. Med. Bull. **54:**151–162.
8. **Crabtree, J. E., S. M. Farmery, I. J. Lindley, N. Figura, P. Peichl, and D. S. Tompkins.** 1994. CagA/cytotoxic strains of *Helicobacter pylori* and interleukin-8 in gastric epithelial cell lines. J. Clin. Pathol. **47:**945–950.
9. **Dixon, M. F., R. M. Genta, J. H. Yardley, and P. Correa.** 1996. Classification and grading of gastritis. Am. J. Surg. Pathol. **20:**1161–1181.
10. **Eberhart, C. E., R. J. Coffey, A. Radhika, F. M. Giardiello, S. Ferrenbach, and R. N. Dubois.** 1994. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology **107:** 1183–1188.
11. **Emery, P., and M. Samon.** 1991. Systemic mediators of inflammation. Br. J. Hosp. Med. **45:**164–168.
12. **Fraser, A. G., R. Sim, E. A. Sankey, A. P. Dhillon, and R. E. Pounder.** 1994. Effect of eradication of *Helicobacter pylori* on gastric epithelial cell proliferation. Aliment. Pharmacol. Ther. **8:**167–173.
13. **Fu, S., K. S. Ramanujam, A. Wong, G. T. Fantry, C. B. Drachenberg, S. P. James, S. J. Meltzer, and K. T. Wilson.** 1999. Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in *Helicobacter pylori* gastritis. Gastroenterology **116:**1319–1329.
14. **Futaki, N., I. Arai, Y. Hamasaka, S. Takahashi, S. Higushi, and S. Otomo.** 1993. Selective inhibition of NS-398 on prostanoid production in inflamed tissue in rat carrageenan-air-pouch inflammation. J. Pharm. Pharmacol. **45:** 753–755.
15. **Graham, D. Y., J. L. Smith, H. J. Spjut, and E. Torres.** 1988. Gastric adaptation: studies in humans during continuous aspirin administration. Gastroenterology **95:**327–333.
16. **Graham, D. Y., N. M. Agrawal, and S. Roth.** 1988. Prevention of nonsteroidal antiinflammatory drug-induced gastric ulceration with misoprostol: multicenter, double blind, placebo-controlled trial. Lancet **ii:**1277–1280.
17. **Gridley, G., J. K. McLaughlin, A. Ekbom, L. Klareskog, H. O. Adami, D. G. Hacker, R. Hoover, and J. F. Fraumeni, Jr.** 1993. Incidence of cancer among patients with rheumatoid arthritis. J. Natl. Cancer Inst. **85:**307–311.
18. **Hawkey, C. J.** 1999. Personal review: *Helicobacter pylori*, NSAIDs and cognitive dissonance. Aliment. Pharmacol. Ther. **13:**695–702.
19. **Hawkey, C. J., Z. Tulassay, L. Szczepanski, C. J. van Rensburg, A. Filipowicz-Sosnowska, A. Lanas, C. M. Wason, R. A. Peacock, and K. R. W. Gillon.** 1998. Randomised controlled trial of *Helicobacter pylori* eradication in patients on non-steroidal anti-inflammatory drugs: HELP NSAIDs study. *Helicobacter* Eradication for Lesion Prevention. Lancet **352:**1016–1021.
20. **Hessey, S. J., J. Spencer, J. I. Wyatt, G. Sobala, B. J. Rathbone, A. T. Axon, and M. F. Dixon.** 1990. Bacterial adhesion and disease activity in *Helicobacter* associated chronic gastritis. Gut **31:**134–138.
21. **Jackson, L. M., K. C. Wu, Y. R. Mahida, D. Jenkins, and C. J. Hawkey.** 2000. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. Gut **47:**762–770.
22. **Jones, M. K., H. Wang, B. M. Peskar, E. Levin, R. M. Itani, I. J. Sarfeh, and A. S. Tarnawski.** 1999. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. Nat. Med. **5:**1418–1423.
23. **Jones, N. L., P. T. Shannon, E. Cutz, H. Yeger, and P. M. Sherman.** 1997. Increase in proliferation and apoptosis of gastric epithelial cells early in the natural history of *Helicobacter pylori* infection. Am. J. Pathol. **151:**1695–1703.
24. **Kim, J. G., D. Y. Graham, and The Misoprostol Study Group.** 1994. *Helicobacter pylori* infection and the development of gastric or duodenal ulcer in arthritic patients receiving chronic NSAID therapy. Am. J. Gastroenterol. **89:**203–207.
25. **Konturek, J. W., A. Dembinski, S. J. Konturek, J. Stachura, and W. Domschke.** 1998. Infection of *Helicobacter pylori* in gastric adaptation to continued administration of aspirin in humans. Gastroenterology **114:** 245–255.
26. **Konturek, J. W., S. J. Konturek, J. Stachura, and W. Domschke.** 1998. *Helicobacter pylori*-positive peptic ulcer patients do not adapt to aspirin. Aliment. Pharmacol. Ther. **12:**857–864.
27. **Laine, L., S. Harper, T. Simon, R. Bath, J. Johanson, H. Schwartz, S. Stern, H. Quan, and J. Bolognese.** 1999. A randomised trial comparing the effect of rofecoxib, a cyclooxygenase 2-specific inhibitor, with that of ibuprofen on the gastroduodenal mucosa of patients with osteoarthritis. Gastroenterology **117:**776–783.
28. **Lee, A., J. O'Rourke, M. C. De Ungria, B. Robertson, G. Daskalopoulos, and M. F. Dixon.** 1997. A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. Gastroenterology **112:**1386–1397.
29. **Leung, W. K., K. F. To, F. K. L. Chan, T. L. Lee, S. C. Chung, and J. J. Sung.** 2000. Interaction of *Helicobacter pylori* eradication and non-steroidal antiinflammatory drugs on gastric epithelial apoptosis and proliferation: implications on ulcerogenesis. Aliment. Pharmacol. Ther. **14:**879–885.
30. **Levi, M.** 1974. Aspirin use in patients with major gastrointestinal bleeding and peptic ulceration. N. Engl. J. Med. **290:**1158–1162.
31. **Levi, S., R. A. Goodlad, C. Y. Lee, M. J. Walport, N. A. Wright, and H. J. F. Hodgeson.** 1992. Effects of nonsteroidal anti-inflammatory drugs and misoprostal on gastroduodenal epithelial proliferation in arthritis. Gastroenterology **102:**1605–1611.
32. **Lunec, J.** 1990. Free radicals: their involvement in disease processes. Ann. Clin. Biochem. **27:**173–182.
33. **Lynch, D. A. F., and A. T. R. Axon.** 1995. *Helicobacter pylori*, gastric cancer and gastric epithelial kinetics: a review. Eur. J. Gastroenterol. Hepatol. **7**(Suppl.)**:**s17–s23.
34. **Mannick, E. E., L. E. Bravo, G. Zarama, J. L. Realpe, X. J. Zhang, B. Ruiz, E. T. Fontham, R. Mera, M. J. Miller, and P. Correa.** 1996. Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in *Helicobacter pylori* gastritis: effect of antibiotics and antioxidants. Cancer Res. **56:**3238–3243.
35. **Meikrantz, W., and R. Schlegel.** 1995. Apoptosis and the cell cycle. J. Cell. Biochem. **58:**160–174.
36. **Miller, M. J. S., and M. B. Grisham.** 1995. Nitric oxide as a mediator of inflammation?—You had better believe it. Mediators inflamm. **4:**387–396.
37. **Mizuno, H., C. Sakamoto, K. Matsuda, K. Wada, T. Uchida, H. Noguchi, T. Akamatsu, and M. Kasuga.** 1997. Induction of cyclooxygenase-2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. Gastroenterology **112:**387–397.
38. **Moss, S. F.** 1998. Cellular markers in the gastric precancerous process. Aliment. Pharmacol. Ther. **12**(Suppl. 1)**:**91–109.
39. **Moss, S. F., J. Calam, B. Agarwal, S. Wang, and P. R. Holt.** 1996. Induction of gastric epithelial apoptosis by *Helicobacter pylori*. Gut **38:**498–501.
40. **Parsonnet, J., G. D. Friedman, D. P. Vandersteen, Y. Chang, J. H. Vogelman, N. Orentreich, and R. K. Sibley.** 1991. *Helicobacter pylori* infection and the risk of gastric carcinoma. N. Engl. J. Med. **325:**1127–1131.
41. **Peek, R. M. Jr., H. Wirth, S. F. Moss, M. Yang, A. M. Abdalla, K. T. Tham, T. Zhang, L. H. Tang, I. M. Modlin, and M. J. Blaser.** 2000. *Helicobacter pylori* alters gastric epithelial cell cycle events and gastrin secretion in mongolian gerbils. Gastroenterology **118:**48–59.
42. **Peek, R. M. Jr., S. F. Moss, K. T. Tham, G. I. Perez-Perez, S. Wang, G. G. Miller, J. C. Atherton, P. R. Holt, and M. J. Blaser.** 1997. *Helicobacter pylori* cagA+ strains and dissociation of gastric epithelial cell proliferation from apoptosis. J. Natl. Cancer Inst. **89:**863–868.
43. **Piazza, G. A., A. L. Rahm, M. Krutzsch, G. Sperl, N. S. Paranka, P. H. Gross, K. Brendel, R. W. Burt, D. S. Albert, R. Pamukcu, and D. J. Ahnen.** 1995. Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. Cancer Res. **55:**3110–3116.
44. **Que, F. G., and G. J. Gores.** 1996. Cell death by apoptosis: basic concepts and disease relevance for the gastroenterologist. Gastroenterology **110:**1238–1243.
45. **Ricci, V., C. Ciacci, R. Zarrilli, P. Sommi, M. K. Tummuru, C. Del Vecchio Blanco, C. B. Bruni, T. L. Cover, M. J. Blaser, and M. Romano.** 1996. Effect of *Helicobacter pylori* on gastric epithelial cell migration and proliferation in vitro: role of VacA and CagA. Infect. Immun. **64:**2829–2833.
46. **Romano, M., V. Ricci, A. Memoli, C. Tuccillo, A. DiPopolo, P. Sommi, A. M. Acquaviva, C. Del Vecchio Blanco, C. B. Bruni, and R. Zamilli.** 1998. *Helicobacter pylori* up-regulates cyclooxygenase-2 mRNA expression and prostaglandin E_2 synthesis in MKN 28 gastric mucosal cells in vitro. J. Biol. Chem. **273:**28560–28563.

47. **Rudi, J., D. Kuck, S. Strand, A. von Herbay, S. M. Mariana, P. H. Krammer, P. R. Galle, W. Stremmel.** 1998. Involvement of the CD95 (APO-1/Fas) receptor and ligand system in *Helicobacter pylori*-induced gastric epithelial apoptosis. J. Clin. Investig. **102:**1506–1514.
48. **Sant, S. M., R. J. Cahill, J. Gilvarry, and C. A. O'Morain.** 1995. Do nonsteroidal anti-inflammatory drugs have an effect on gastric cell turnover? Aliment. Pharmacol. Ther. **9:**575–579.
49. **Sawaoka, H., S. Kawano, S. Tsuji, M. Tsujii, E. S. Gunawan, Y. Takei, K. Nagano, and M. Hori.** 1998. Cyclooxygenase-2 inhibitors suppress the growth of gastric xenografts via induction of apoptosis in nude mice. Am. J. Physiol. **274:**G1061–G1067.
50. **Sharma, S. A., M. K. Tummuru, G. G. Miller, and M. J. Blaser.** 1995. Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. Infect. Immun. **63:**1681–1687.
51. **Shiff, S. J., L. Qiao, L. L. Tsai, and B. Rigas.** 1995. Sulindac sulfide, an aspirin-like compound, inhibits proliferation, causes cell cycle quiescence, and induces apoptosis in HT-29 colon adenocarcinoma cells. J. Clin. Investig. **96:**491–503.
52. **Shigeta, J., S. Takahashi, and S. Okabe.** 1998. Role of cyclooxygenase-2 in the healing of gastric ulcers in rats. J. Pharm. Exp. Ther. **286:**1383–1390.
53. **Spyridon, L., N. Akira, K. Hiromasa, G. Katsutoshi, M. Takao, O. Yoshiki, S. Hideo, and F. Hisayuki.** 1994. The development of the endothelin-1-induced gastric ulcer: time sequence analysis of morphologic changes. Int. J. Exp. Pathol. **75:**345–355.
54. **Subbaramaiah, K, D. Zakim, B. B. Weksler, and A. J. Dannenberg.** 1997. Inhibition of cyclooxygenase: a novel approach to cancer prevention. Proc. Soc. Exp. Biol. Med. **216:**201–210.
55. **Tanaka, K., H. Tanaka., Y. Kanemoto, and I. Tsuboi.** 1998. The effects of nonsteroidal anti-inflammatory drugs on immune functions of human peripheral blood mononuclear cells. Immunopharmacology **40:**209–217.
56. **The Eurogast Study Group.** 1993. An international association between *Helicobacter pylori* infection and gastric cancer. Lancet **314:**1359–1362.
57. **Thun, M. J., M. M. Namboodiri, E. E. Calle, W. D. Flanders, and C. W. Heath, Jr.** 1993. Aspirin use and risk of fatal cancer. Cancer Res. **53:**1322–1327.
58. **Tsujii, M., and R. N. DuBois.** 1995. Alteration in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell **83:**493–501.
59. **Wagner, S., W. Beil, J. Westermann, R. P. Logan, C. T. Bock, C. Trautwein, J. S. Bleck, and M. P. Manns.** 1997. Regulation of gastric epithelial cell growth by *Helicobacter pylori*: evidence for a major role of apoptosis. Gastroenterology **113:**1836–1847.
60. **Wallace, J. L., A. Bak, W. McKnight, S. Asfaha, K. A. Sharkey, and W. K. MacNaughton.** 1998. Cyclooxygenase 1 contributes to inflammatory responses in rats and mice: implications for gastrointestinal toxicity. Gastroenterology **115:**101–109.
61. **Wallace, J. L., W. McKnight, B. K. Reuter, and N. Vergnolle.** 2000. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. Gastroenterology **119:**706–714.
62. **Watson, A. J.** 1998. Chemopreventive effects of NSAIDs against colorectal cancer: regulation of apoptosis and mitosis by COX-1 and COX-2. Histol. Histopathol. **13:**591–597.
63. **Weitzman, S. A., and L. I. Gordon.** 1990. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. Blood **76:**655–663.
64. **Wolfe, M., D. Lichtenstein, and G. Singh.** 1999. Gastroduodenal toxicity of nonsteroidal antiinflammatory drugs. N. Engl. J. Med. **340:**1888–1899.
65. **Yang, V. W., J. M. Shields, S. R. Hamilton, E. W. Spannhake, W. C. Hubbard, L. M. Hylind, C. R. Robinson, and F. M. Giardiello.** 1998. Size-dependent increase in prostanoid levels in adenomas of patients with familial adenomatous polyposis. Cancer Res. **58:**1750–1753.
66. **Zhu, G. H., X. L. Yang, K. C. Lai, C. K. Ching, B. C. Wong, S. T. Yuen, J. Ho, and S. K. Lam.** 1998. Nonsteroidal antiinflmmatory drugs could reverse *Helicobacter pylori*-induced apoptosis and proliferation in gastric epithelial cells. Dig. Dis. Sci. **43:**1957–1963.

Editor: J. D. Clements