ORIGINAL ARTICLE

Suppressed Gastric Mucosal TGF-β1 Increases Susceptibility to *H. pylori*-Induced Gastric Inflammation and Ulceration: A Stupid Host Defense Response

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Background/Aims: Loss of transforming growth factor β1 (TGF-β1) exhibits a similar pathology to that seen in a subset of individuals infected with *Helicobacter pylori*, including propagated gastric inflammation, oxidative stress, and autoimmune features. We thus hypothesized that gastric mucosal TGF-β1 levels could be used to determine the outcome after *H. pylori* infection. **Methods:** Northern blot for the TGF-β1 transcript, staining of TGF-β1 expression, luciferase reporter assay, and enzyme-linked immunosorbent assay for TGF-β1 levels were performed at different times after *H. pylori* infection. **Results:** The TGF-β1 level was markedly lower in patients with *H. pylori*-induced gastritis than in patients with a similar degree of gastritis induced by nonsteroidal anti-inflammatory drugs. There was a significant negative correlation between the severity of inflammation and gastric mucosal TGF-β1 levels. SNU-16 cells showing intact TGF-β signaling exhibited a marked decrease in TGF-β1 expression, whereas SNU-638 cells defective in TGF-β signaling exhibited no such decrease after *H. pylori* infection. The decreased expressions of TGF-β1 in SNU-16 cells recovered to normal after 24 hr of *H. pylori* infection, but lasted very spatial times, suggesting that attenuated expression of TGF-β1 is a host defense mechanism to avoid attachment of *H. pylori*. **Conclusions:** *H. pylori* infection was associated with depressed gastric mucosal TGF-β1 for up to 24 hr, but this apparent strategy for rescuing cells from *H. pylori* attachment exacerbated the gastric inflammation. **(Gut Liver 2010;4:43-53)**

Key Words: *Helicobacter pylori*; TGF-β; Inflammation; Ulcer; Host defense

INTRODUCTION

With astonishing success in culturing *H. pylori* by Warren and Marshall,[1] the bugs have become recognized as one of the most common bacterial infections in humans, presenting in almost half of world population and key etiology of diverse gastric diseases in addition to extra-gastric manifestations. The presence of *H. pylori* in gastric mucosa is associated with chronic active gastritis and implicated in more severe gastric diseases, such as chronic atrophic gastritis and intestinal metaplasia (a precursor of gastric carcinogenesis), peptic ulcer, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. However, the fact that peptic ulcer disease and gastric carcinoma occur in only a small portion of individuals chronically infected with *H. pylori* stressed that the role of the host defense factor is postulated to be an important determinant in the clinical outcome of *H. pylori* infection,[2] further supported with outstanding publication of Mimuro H *et al*.[3] that *H. pylori* dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach.

Even though the success of *H. pylori* as a gastric pathogen is known to be dependent on either strain-associated virulence factors or host related mechanisms, the virulence factors of *H. pylori* are features that allow it to survive in the hostile environment of the gastric lumen, such as its spiral shape, motility, adaptive enzymes, alterations in mucin synthesis, and ability to adhere to gastric mucosal cells.[4] On the other hand, regarding host factor as the critical determinant of clinical outcomes, particularly the role of the host defense system which may prevent or exacerbate *H. pylori*-induced gastroduodenal injury, little is known. That is, host factors known to increase an individual risk for *H. pylori*-associated diseases are as follows; host genetics,[5] gender,[6] HLA genotypes,[7] blood group antigens,[8] and gastric acid secretory capability,[9] but thriving efforts of host to rescue from either *H. pylori*-induced cytotoxicity or relieving the burden of colonization has never been documented.

Transforming growth factor-$\beta 1$ (TGF-$\beta 1$) plays a pivotal role in modulating inflammatory responses by inhibiting the proliferation of B- and T-lymphocytes and suppressing macrophages and natural killer cell activity.[10-12] In addition, TGF-$\beta 1$ acts as a chemoattractant and activator of monocyte functions and regulates cytokine production by different cell types.[13] TGF-$\beta 1$ also inhibits the respiratory burst of macrophages and neutrophils.[14] The importance of TGF-$\beta 1$ in modulating inflammation is exemplified in TGF-$\beta 1$ gene knockout mice, in which surviving animals appear normal at birth, but develop progressive inflammatory disease dying within 3 to 4 weeks. They also exhibit significant autoimmune responses in the form of severe gastric inflammation and ulceration as well as dysplastic lesions in the stomach.[15,16] Interestingly, the gastric mucosal alterations observed in the TGF-$\beta 1$ null mice are very similar to changes observed in *H. pylori*-associated gastritis.

Based on these backgrounds, we hypothesized that the degree of down-regulation of endogenous TGF-$\beta 1$ expression by *H. pylori* might be the host defense response to cope with harmful pathogens based on our finding that TGF-$\beta 1$ or intact signaling system was prerequisite for continuing the pathogenic signaling of *H. pylori*. In the current study, we examined *H. pylori*-induced suppression of gastric mucosal TGF-$\beta 1$ expression and attempted to correlate these results with the various clinical gastroduodenal diseases. *In vitro* study was added to document the reason of decreased TGF-$\beta 1$ expression after *H. pylori* infection.

MATERIALS AND METHODS

1. Patients

Thirty patients with endoscopically proved gastric and/or duodenal ulcers associated with *H. pylori* infection were recruited from the Gastroenterology outpatient clinic and informed consent was obtained. They were aged 18-78 years (mean 36.5 years). None had a history of gastric surgery, had taken antibiotics or bismuth preparations in the preceding six months or histamine-2 receptor antagonists, omeprazole, or non-steroidal anti-inflammatory drugs the 14 days before their initial endoscopy. At the time of the initial endoscopy, gastric antral biopsy specimens were taken with Olympus biopsy forceps. The patients were then treated with triple eradication regimen including 40 mg pantoprazole (AmorePacific Pharma., Seoul, Korea), 1 gm amoxicillin (ChongKeunDang Pharma., Seoul, Korea) and 0.5 gm clarithromycin (Abott Pharma., Seoul, Korea) *b.i.d*, for a week. Two months after the initiation of treatment, the patients were re-endoscoped and biopsy specimens were taken from antral mucosa. Successful eradication of *H. pylori* was presumed to have occurred if all the tests like hematoxylin and eosin staining and Warthin-Starry silver stain, CLO test and ^{13}C urea breath test were converted to be negative at follow up endoscopy. We also selected the same numbers of patients with nonsteroidal anti-inflammatory drug (NSAID), whose biopsied tissues were retrospectively reviewed and the patients, who showed similar degree of gastritis as with *H. pylori*-associated gastritis were chosen, for which pathologist (prof. YB Kim, Ajou University Hospital) tried to select the patients whose degree of gastritis was similar to those with *H. pylori*-associated gastritis. This is due the reason that the degree of gastritis can affect the levels of TGF-β. Also, 30 patients were recruited from Health Check Center, who showed very mild degree of superficial gastritis, of course, *H. pylori* were proven to be negative. The mean ages were 53.5 years of *H. pylori* negative, NSAID-associated gastritis and 34.1 years of normal controls.

2. Bacterial strain and cell culture

SNU-16 and SNU-638 gastric cancer cell lines were grown in minimal essential media (MEM), supplemented with 10% certified fetal bovine serum (GIBCO-BRL, Gaithesberg, MD), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified 37°C, 5% CO_2 atmosphere. *H. pylori* strain (*CagA*$^+$ strain ATCC 43504) was purchased from American Tissue Culture Collection microbiology and this strain is positive for the cytotoxin-associated gene, *CagA*. Through reactivation by applica-

tion of tryptic soy media, *H. pylori* were cultured on brucellar agar plates containing 10% sheep blood, Skirrow selective supplement. They were grown in a microaerobic, humidified atmosphere at 37°C and passed every 72 hours. Bacteria were gently scraped off the plates with a sterile swab and suspended in phosphate buffered saline. Concentrations of *H. pylori* were estimated, using OD_{600} of 1 as 1×10^8 bacteria/mL. For experiment, the cells were seeded at a density of 5×10^4/mL on twenty four well plate or 75-cm^2 flask (15 mL media volume) and stimulated the following day with the various numbers of bacterial preparations.

3. Immunohistochemistry of TGF-β1

Sections were stained immunohistochemically using a diaminobensidine peroxidase-antiperoxidase technique incorporating a streptavidine-biotin technique essentially described by Hsu *et al.*[17] Briefly, sections were fixed in 95% ethanol and incubated overnight with TGF-β1 antibody named as "LC antibody" (kind gift from Kathleen Flander, NCI, Bethesda, MD, USA) at a dilution of 1:200 in a humid chamber. A goat biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) was then applied for 60 minutes. For signal amplification, an immunoperoxidase ABC kit (Vector Laboratories) was used. Diaminobenzidine (Sigma, Saint Louis, MO, USA) was used as a chromogen. TGF-β1 immunohistochemical stainings were also performed in slides prepared by the cytospin centrifugation of cultured gastric cancer cells treated with different numbers of *H. pylori*. The intensity of immunostaining for TGF-β1, relative to normal, in each case was scored on the following scale; −5, no staining; −4, 0-25%; −3, 26-50%; −2, 51-75%; −1, 76-99%; 0, normal; 1, 101-125%; 2, 126-150%; 3, 151-175%; 4, 176-200; 5, over 200%. This scoring system shows high reproducibility. All scorings were done by two observers (Dr. T. Parks and Dr. YB Kim) blinded to the specimen identity.

4. Grading of gastritis and density of *H. pylori*

Two pieces of the biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, and sectioned. Tissue slides were stained for routine histology and Warthin-Starry silver stain, which was used for scoring density of *H. pylori*. Grading of the chronic gastritis, neutrophilic infiltration, and the density of *H. pylori* were conducted as suggested by the Updated Sydney System (1996).[18] For degree of inflammation, 0, absent; 1, mild; 2, moderate; and 3, severe. For density of *H. pylori*, 0, absence of *H. pylori*; 1, focal presence of small amounts of *H. pylori*; 2, intermediate situation between score 1 and

score 3; 3, diffuse presence of large amounts of *H. pylori*. All scorings were done by two pathologists.

5. TGF-β1 levels

TGF-β levels of supernatants obtained from cell culture media treated with different numbers of *H. pylori* were measured by the quantitative sandwich enzyme immunoassay techniques (R&D, Mineapolis, MN, USA). Briefly, an affinity purified polyclonal antibody specific for human TGF-β1 has been pre-coated onto a microtiter plate. Standards, controls, and samples were pippetted into the wells and any human TGF-β1 was bounded by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody was added to the wells.

6. Preparation of RNA and Northern blotting

Total RNA was isolated from cells treated with 1×10^4 to 1×10^8 *H. pylori*/mL according to the guanidium isothiocyanate/phenol/chloroform method. Twenty μg aliquots of RNA were electrophoresed on 1% agarose gel containing 0.66 M formaldehyde, transferred to Nytron membrane, and cross-linked with a UV Strata-linker (Stratagene, La Jolla, CA, USA) Blots were prehybridized and hybridized in a 1% bovine serum albumin, 7% (w/v) SDS, 0.5 M sodium phosphate, 1 mM EDTA at 65°C. RNA blots were hybridized with ^{32}P-labelled cDNA probes for TGF-β1.

7. Luciferase reporter assay for TGF-β1 activity

For transient transfection, cells were seeded in six-well plates at a density of 3×10^5 cells/well. For assays involving TGF-β1 stimulation, each plate was transfected with the reporter of interest, 3TP-lux or SBE or empty vector (pcDNA3.1) and pRSV-Gal (Promega, Madison, WI, USA). Cells were incubated for 24 hours prior to treatment with 5 ng/mL TGF-β1 and treated for 12 hours before harvesting. Luciferase activities were normalized for galactosidase activity as determined using a galactosidase assay system (Promega). Luciferase assays were performed with commercially available reagents and normalized to protein concentration as determined by the Bradford assay kit (Bio-Rad, Hercules, CA, USA). All experiments were repeated at least three times with similar results.

8. Statistics

All data were represented as mean±SD. SPSS version 6.1 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Analysis of paired observations before and after treatment was performed using a paired t test for nor-

mally distributed data and a Wilcoxon signed rank test for non-parametric data.

RESULTS

1. Immmunostaining of gastric mucosal TGF-β1 in patients with *H. pylori*-associated various gastric diseases including peptic ulcer diseases

TGF-β1 levels were markedly decreased in gastric antral tissues of patients with *H. pylori*-associated gastritis compared with both normal control and non-*H. pylori*-associated gastritis, that is, NSAID-induced gastritis even though their scores of gastritis were similar (Fig. 1A). In normal gastric tissues, immunoreactive TGF-β1 was mainly localized in epithelial cells beneath the proliferative zone in the gastric glands (Fig. 1Aa), but in gastric antral mucosa of the *H. pylori*-associated duodenal ulcer patients, there was only scarce immunostaining in the gastric glands, superficial and glandular mucosa. Only infiltrated inflammatory cells and lymphoid follicles formed
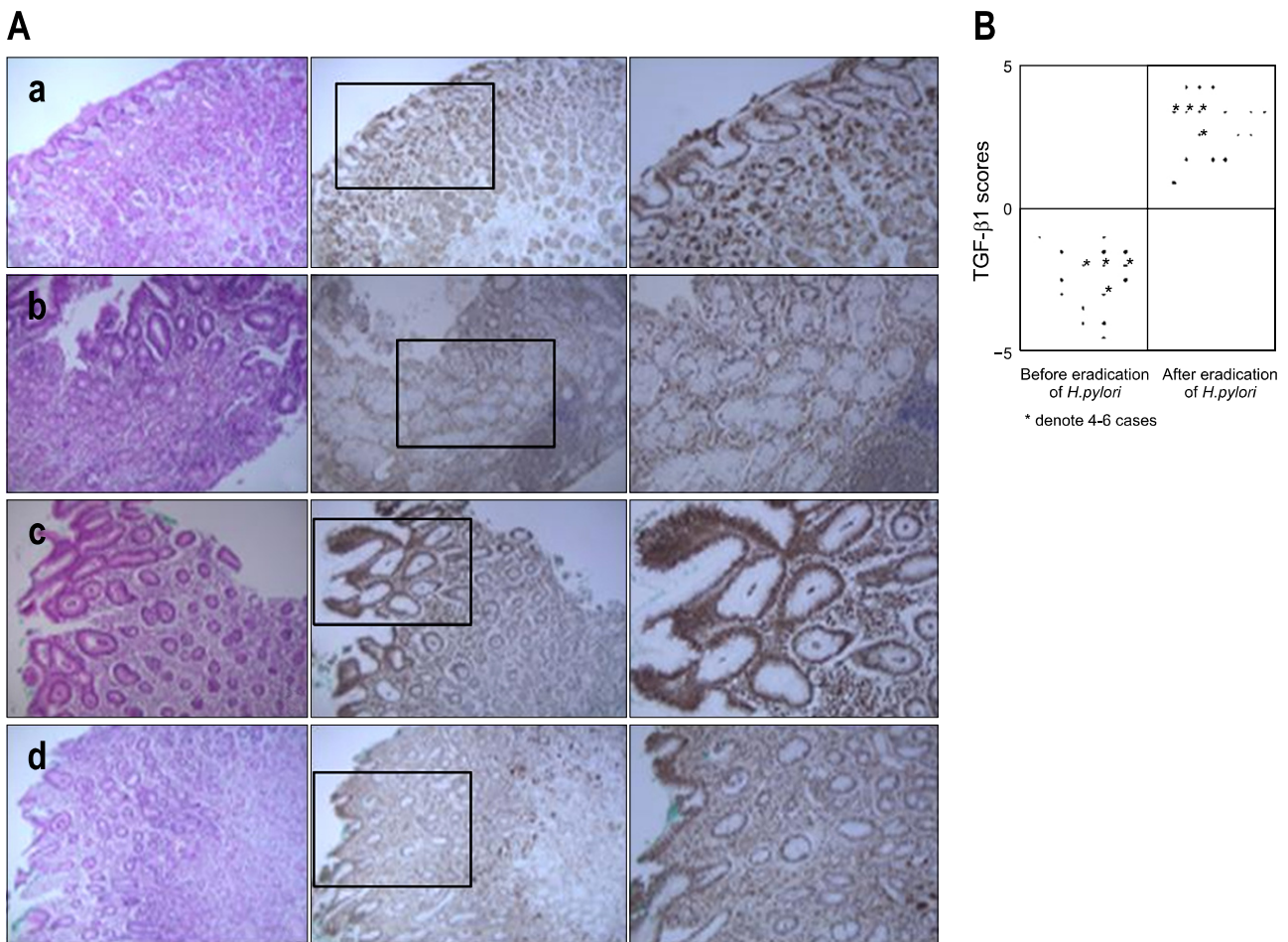

Fig. 1. (A) Immunohistochemical staining of TGF-β1 in the antral mucosa of a normal control (a), the antral mucosa of an *H. pylori*-associated duodenal ulcer patient (before eradication; b), the antral mucosa of a duodenal ulcer patient after eradication of *H. pylori* (same patient as in b; c), and the antral mucosa of a patient with nonsteroidal anti-inflammatory drug (NSAID)-induced gastritis as a control for *H. pylori*-negative gastritis (d). The intensity of TGF-β1 immunostaining in the antral mucosa was similar in the normal control (a) and NSAID-induced gastritis (d), whereas it was decreased in patients with *H. pylori*-associated duodenal ulcer. However, immunostaining of TGF-β1 in the gastric antral mucosa was profoundly increased in group (c) patients, in whom *H. pylori* infection has been successfully eradicated. Left, H&E staining; middle, immunostaining with TGF-β1 (LC antibody), $\times100$ magnification; right, immunostaining with TGF-β1 (LC antibody), $\times200$ magnification. (B) Serial changes in immunostaining score for TGF-β1 and degree of inflammation according to the presence or absence of *H. pylori* in gastric biopsy samples. Immunostaining scores for TGF-β1 were significantly elevated after eradication of *H. pylori*, whereas the degree of inflammation was significantly decreased. The immunostaining intensity was scored based on the intensity of normal controls, where a minus score indicates that the TGF-β expression was lower than in the normal control. The intensity of immunostaining for TGF-β1, relative to normal, in each case was scored on the following scale: -5, no staining; -4, 0-25%; -3, 26-50%; -2, 51-75%; -1, 76-99%; 0, normal; 1, 101-125%; 2, 126-150%; 3, 151-175%; 4, 176-200; 5, over 200%.

in response to *H. pylori* infection showed positive TGF-*β* 1 immunostaining (Fig. 1Ab). Cells exhibiting positive staining were mostly mononuclear cells. However, levels of TGF-*β*1 expressions in the same patient following the eradication of *H. pylori* were markedly increased in gastric surface epithelial cells and glandular mucosa (Fig. 1Ac) and could be still seen in the remaining inflammatory cells. TGF-*β*1 immunostaining in *H. pylori* negative pa-
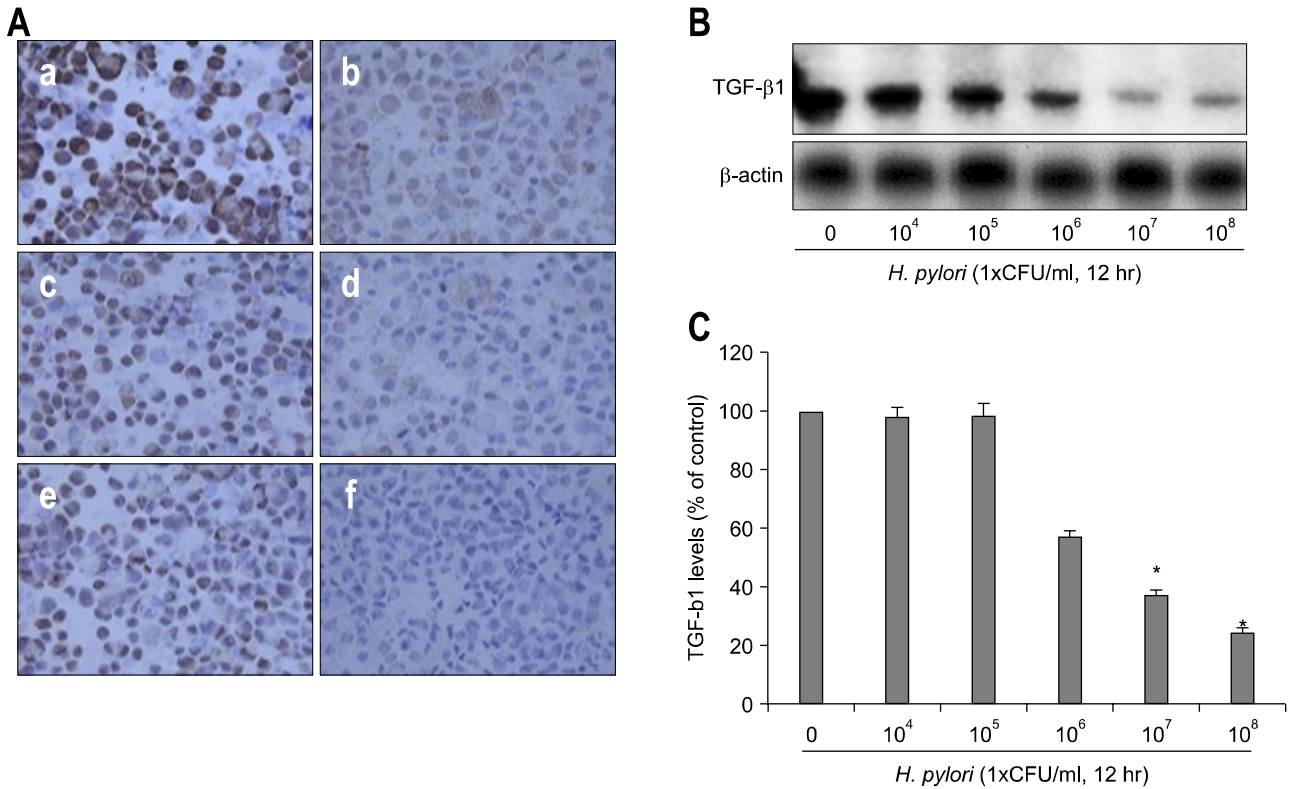

Fig. 2. (A) Immunohistochemical TGF-*β*1 results in a cytospin smear of gastric cancer cells (SNU-16) treated as follows: (a) no infection control; (b) after 1×10^5 *H. pylori*/mL; (c) after 1×10^6 *H. pylori*/mL; (d) after 1×10^7 *H. pylori*/mL; (e) after 1×10^8 *H. pylori*/mL; (f) after 1×10^9 *H. pylori*/mL. Control cells showed intense positive immunostaining over 80% of dispersed cells, and average immunostaining intensities with TGF-*β*1 LC antibody were similar in (b) and (c). However, as the numbers of *H. pylori*-treated cells increased, the percentages of those showing positive immunostaining for TGF-*β*1 decreased significantly. In cells treated with 1×10^8 or 1×10^9 *H. pylori*/mL (f), only very few cells were immunopositive for TGF-*β*1. (B) TGF-*β*1 mRNA levels in SNU-16 cells treated with different numbers of *H. pylori*. Total RNA (10 μg) from SNU-16 cells treated with 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 *H. pylori*/mL for 5 days was probed with either the TGF-*β*1 cDNA or a *β*-actin probe. There was an inverse reduction in the intensities of TGF-*β*1 documented with Northern blot with increasing multiplicity of infection. (C) TGF-*β*1 levels secreted by SNU-16 cells as determined by enzyme-linked immunosorbent assay. As the numbers of *H. pylori* increased, there were significant decreases in TGF-*β*1 levels. There was little change in TGF-*β*1 levels in cell supernatants treated with 1×10^6 *H. pylori*/mL, but the TGF-*β*1 levels for treatment with higher than 1×10^6 *H. pylori*/mL decreased markedly in a bacterial number-dependent manner. On the other hand, TGF-*β*1 levels measured in supernatants of SNU-16 cells treated with *Escherichia coli* (1×10^8 and 1×10^9 *E. coli*/mL) did not change, with all levels being higher than that of the control.

Table 1. TGF-*β*1 Expression and Gastric Inflammation according to *H. pylori* in Peptic Ulcer Disease

	Before treatment (*H. pylori* +)			After treatment (*H. pylori* −)		
	TGF-*β*1 scores	Degree of inflammation	Correlation coefficient	TGF-*β*1 scores	Degree of inflammation	Correlation coefficient
PUD (n=30)	−1.57+/−2.10	2.77+/−1.01	r=0.15	3.93+/−5.30	1.67+/−0.48	r=−0.31
DU (n=18)	−2.83+/−1.10	2.78+/−0.94	r=0.64*	4.56+/−0.85	1.78+/−0.43	r=−0.13
GU (n=12)	−0.33+/−1.78	2.75+/−1.14	r=0.88*	3.00+/−0.00	1.50+/−0.52	r=−0.54

PUD denotes peptic ulcer diseases, DU duodenal ulcer, and GU gastric ulcer.
*p<1.001.

tients of NSAID-associated gastritis in Fig. 1Ad, showed similar immunostaining in surface and deeper glands, as compared to that of normal control. A summary of the immunostaining data scored as -5 to 5 point is shown in Table 1. The mean scores and standard deviation for TGF-β1 levels, density of *H. pylori,* and degree of inflammation in patients with *H. pylori*-associated peptic ulcer disease were -1.57 ± 2.10, 3.70 ± 0.53, and 3.93 ± 5.30, respectively, whereas those measured after the eradication of *H. pylori* were 2.77 ± 1.01, 0.07 ± 0.37, and 1.67 ± 0.48. Comparison of the respective mean scores for pre-eradication and post-eradication of *H. pylori* in these patients revealed a statistically significant difference ($p<0.05$) (Fig. 1B). The mean score for TGF-β1 levels in patients with *H. pylori*-associated duodenal ulcer was -2.83 ± 1.10, which was found to be statistically different from the mean score of -0.33 ± 1.78 for patients with *H. pylori*-associated gastric ulcer. The degree of inflammation was also significantly different ($p<0.01$) between *H. pylori* associated duodenal ulcer (4.56 ± 0.85) and gastric ulcer (3.00 ± 0.00). More apparent decreases in gastric mucosal TGF-β1 levels and increases in the degree of inflammation were noted in patients with *H. pylori*-associated duodenal ulcer than in *H. pylori*-associated gastric ulcer patients. The correlation between the decrement in gastric mucosal TGF-β1 levels and the degree of inflammation in duodenal ulcer patients was also statistically significant. These results suggest that the attenuated levels of gastric antral mucosal TGF-β1 associated with *H. pylori* infection could be an important determinant for the degree of inflammation provoked by *H. pylori* and the associated clinical outcomes.

2. Down-regulation of TGF-β1 expression in cultured human gastric cells after *H. pylori* infection

To validate the finding obtained from the above clinical samples that *H. pylori* infection is associated with decreased levels of TGF-β and of which decreased levels were associated with increased chance of inflammation and ulceration, we progressed experiment to determine whether *H. pylori* infection suppress expression of TGF-β1 in cells and immunohistochemical staining of TGF-β1 was performed on cytospin smears of SNU-16 human gastric cancer cell lines after 12 hours treatment with different concentrations of *H. pylori* from 1×10^5 to 1×10^9 *H. pylori/* mL. SNU-16 cells are growth inhibited by TGF-β1 and express functional TGF-β1 RI and RII receptors.[19] The number of cells staining positive for TGF-β1 decreased in a CFU-dependent manner after *H. pylori*. In cells treated with 1×10^8 or 1×10^9 *H. pylori*/mL TGF-β

1-positive cells were scarcely observed, whereas TGF-β1 positive cells were over 80% of both control cells (Fig. 2A) and cells treated with 1×10^5 *H. pylori*/mL showed positive TGF-β1 immunoreactivity. Apoptosis was observed in SNU-16 cells treated with 1×10^5 to 1×10^8 *H. pylori*/mL for 24 hours by DAPI staining and DNA gel electrophoresis. Northern blot analysis after 12 hours treatment of *H. pylori* indicated that levels of steady-state mRNA for TGF-β1 were significantly decreased upon treatment with increasing doses of *H. pylori* ($p<0.001$; Fig. 2B). There was 80-90% decrease in TGF-β1 mRNA
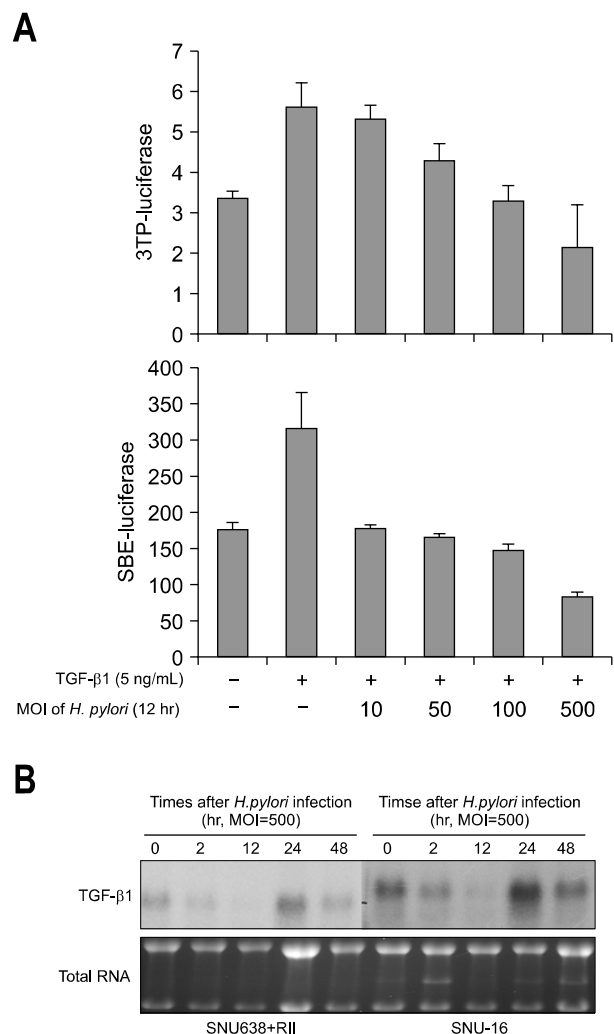

Fig. 3. (A) 3TP-luciferase (3-TP-Lux) and Smad binding element (SBE) luciferase activities TGF-β increased the transcription of the SBE or 3-TP-Lux luciferase reporter. (B) Northern blot of TGF-β1 according to times of *H. pylori* infection. Repeated experiments performed to examine the expression of TGF-β1 at different times after *H. pylori* infection revealed a significant restoration of TGF-β1 after 24 hours.
MOI, multiplicity of infection.

in cells treated with 1×10^7 or 1×10^8 *H. pylori*/mL compared with both untreated control cells and cells treated with 1×10^5 *H. pylori*/ mL. TGF-β1 secreted from cells treated with *H. pylori* was assayed using a Quantikine human TGF-β1 immunoassay system of R & D System. Data for TGF-β1 concentrations in conditioned medium from the SNU16 cells treated with varying numbers of *H. pylori* were shown in Fig. 2C. Total TGF-β1 levels in the acid-activated conditioned medium decreased following *H. pylori* infection (12 hours) in SNU-16 cells, with maximal decrease of TGF-β1 secretion in the 1×10^9 *H. pylori*/mL treatment. Treatment of SNU-16 cells with *E. coli* had no significant change on TGF-β1 production (data not shown). All of these data suggested that significant reductions in TGF-β1 were induced after *H. pylori* infection. Next, 3TP-luciferase and SBE-luciferase activities were measured after the administration of 5 ng/mL TGF-β in the presence of different multiplicity of infection (MOI) of *H. pylori* to look at whether the decrement in TGF-β1 after *H. pylori* infection was transcriptionally associated with TGF-β1 promoter. As shown in Fig. 3A as MOI is increased, the promoter activities of TGF-β1 were proportionally decreased.

3. Restoration of TGF-β1 expression after 24 hours in cultured human cancer cells infected with *H. pylori*, but not lasted longer

The continued exposure to *H. pylori* after 24 hours rendered the restoration of TGF-β1 expression as shown in Fig. 3B. After 2 hours and 12 hours of *H. pylori* infection, the TGF-β1 expressions were markedly decreased, but of which levels were apparently increased after 24 hours. However, these incremental increases in TGF-β1 did not last longer, reverted to preinfection levels at 48 hours. The current result suggested that the decrement of TGF-β1 expression and restoration was occurred spatially. Therefore we have another question why the TGF-β1 expression was attenuated after *H. pylori* infection and restoration did not last long.

4. Hindrance of *H. pylori* attachment in gastric cells defective in TGF-β signaling and restoration after resuming TGF-β signaling

In order to find the biologic significance of TGF-β in *H. pylori* infection, we traced the cell morphologic changes after *H. pylori* infection. When we compare the cell morphology after *H. pylori* infection between SNU-16 and SNU-638 cells, we found that *H. pylori* attachment was not noted in SNU-638 compared to SNU-16 as shown in Fig. 4A. The marked adhesions of *H. pylori* were noted in SNU-16, whereas no attachment was noted in SNU-638

in both 6 hours and 24 hours after *H. pylori* infection (Fig. 4A), suggesting that attenuated expression of TGF-β1 after *H. pylori* infection could be the host defense for avoiding cells from *H. pylori* colonization and imposing the hindrance to provoking signaling from *H. pylori*. As far as the attachment of *H. pylori* is concerned according to TGF-β status, the fact that prominent attachment of *H. pylori* was noted in SNU-638 transfected with type II receptor enabling normal TGF-β signaling compared SNU-638 transfected with mock vector signifies that the TGF-β levels or signaling might be plausible factor determining the adherence of *H. pylori* to targeted epithelium. Taken together, we speculated that this attenuation of TGF-β ligands persists up to 24 hours as part of host defense against *H. pylori* infection. However, restoration of TGF-β1 occurred with the advancement of *H. pylori* attachment, vacuolization, and apoptosis after 24 hours. Therefore, we inferred that if the host defense against *H. pylori* infection did not occur, active attachment of *H. pylori*, signals to vacuolization, and subsequent accumulation of apoptosis will commence immediately. The massive occurrence of these pathogenic signaling after *H. pylori* infection can lead to inflammation and consequent ulceration, resulting in either active gastritis or gastroduodenal ulcer. Conclusively, unwise suppression of gastric mucosal TGF-β1 expression as part of host defense against *H. pylori* leads to *H. pylori*-associated gastritis or ulceration (Fig. 4B).

DISCUSSION

Within a selected population after the exposure to similar strains of *H. pylori*, some individuals developed peptic ulcer disease or gastric cancer, while others remained asymptomatic. As probability, more than 80% of individuals harboring *H. pylori* remains to be mild chronic gastritis or asymptomatic. Our study proposes the possible explanation about these discrepancies that the initial, spatial attenuation of TGF-β1 expression could be the host defense reaction to avoid the attachment of *H. pylori* and to prevent subsequent injuries including apoptosis or inflammation. Therefore, we put hypothesis that gastric mucosal TGF-β1 expression can predict susceptibility to the diversity of *H. pylori*-associated gastroduodenal diseases. Even though the suppression of the gastric mucosal TGF-β1 level by *H. pylori* infection can hinder the attachment of bacteria to gastric epithelial cells, paradoxically suppressed TGF-β1 results in rather increased gastric inflammation,[20] potentiation of oxidative damage,[21] progression to atrophic gastritis,[22] autoimmune disease,[23] delay of ulcer healing, and increased ulcer recurrence,[24]
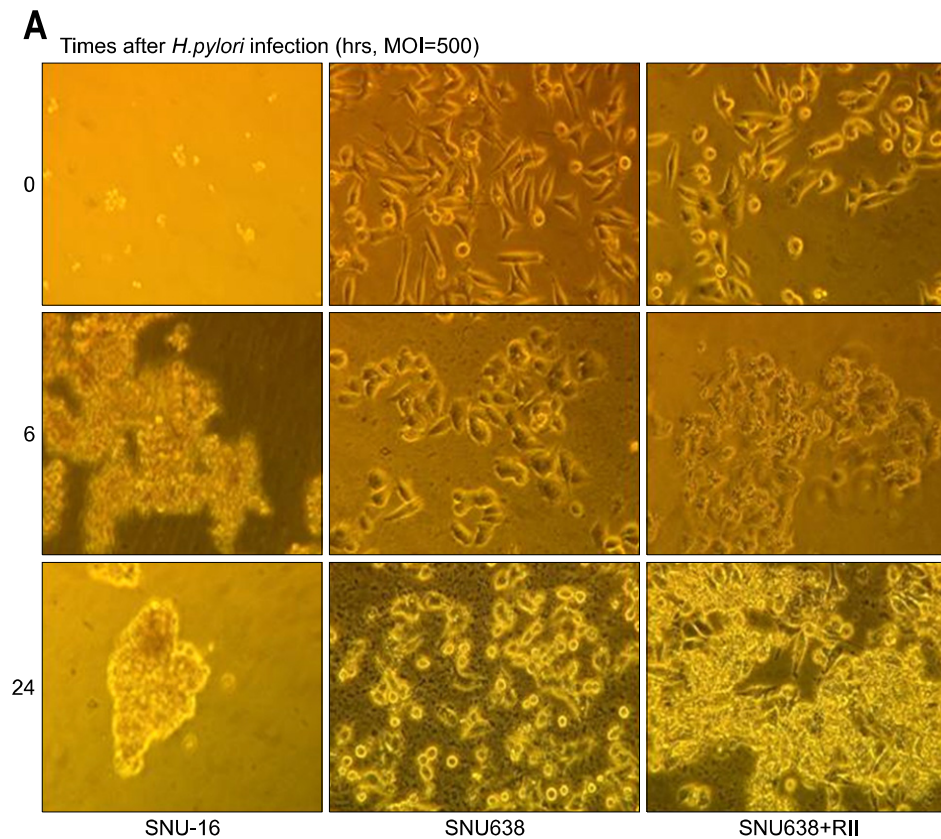
A

Times after *H.pylori* infection (hrs, MOI=500)

SNU-16   SNU638   SNU638+RII

B

H.pylori infection →

"Stupid host defense" strategy

"*Decreased TGF-β1 expression*",
for hindrance of *H.pylori attachment*

Oxidative stress ↓ Inflammation

Peptic ulcer disease
(GU & DU)

Cancelled stupid host defense

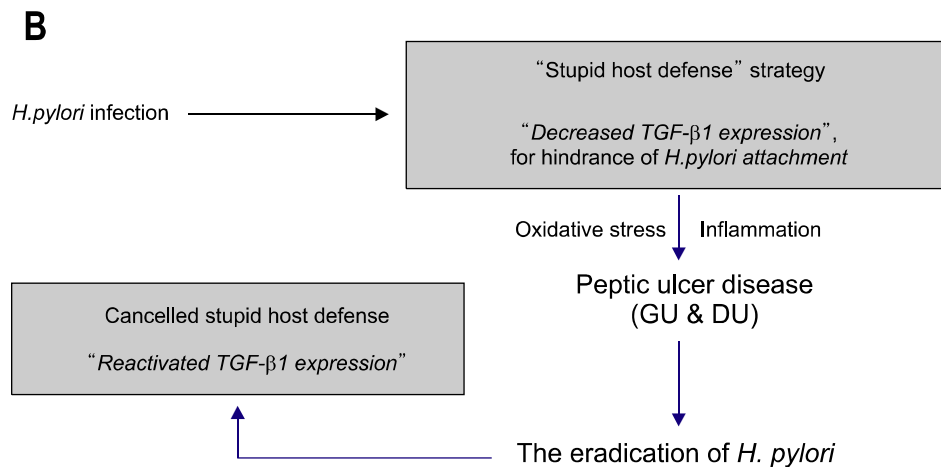"*Reactivated TGF-β1 expression*"

The eradication of *H. pylori*

Fig. 4. (A) Cell morphology after *H. pylori* infection in SNU-638 and SNU-16 cells. The attachment of *H. pylori* was lower in SNU-638 cells than in SNU-16 cells, suggesting that a normal TGF-β signaling or ligand level is one of the determining factors for *H. pylori* attachment. When SNU-638 cells were transfected with functioning TGF-β type II receptors, designated as SNU638+RII, highly active attachment of *H. pylori* was noted. (B) Plausible mechanisms to explain the difference in outcomes after *H. pylori* infection. Even though attenuated levels of TGF-β1 might be a host defense mechanism to rescue the stomach from the attachment of *H. pylori* to the gastric epithelial cells and consequent apoptotic cell loss and cytotoxicity, subsequent emergence of inflammatory bouts and oxidative stress lead to worsened clinical outcomes, termed "*decreased TGF-β 1 expression as an unwise, stupid, foolish host defense against H. pylori infection*".
GU, gastric ulcer; DU, duodenal ulcer.

pathological findings typically observed in *H. pylori*-associated gastritis/peptic ulceration. Therefore, we concluded that even though attenuated levels of TGF-β1 might be the host defense to rescue stomach from the attachment of *H. pylori* to gastric epithelial cells, consequent apoptotic cell loss, cytotoxicity, subsequent emergence of inflammatory bouts, and oxidative stress lead to worsen clinical outcomes, termed as "decreased TGF-β1 expression as unwise, stupid, foolish host defense against *H. pylori* infection" (Fig. 4B).

There might be possibility of the reverse interpretation like that decreased expression of TGF-β1 within 24 hours after *H. pylori* infection was induced by the attack of bacteria and the restoration of TGF-β1 after 24 hours was rendered by the reaction of host. However, the findings that i) proportional decrement of TGF-β1 according to incremental load of *H. pylori* (Figs 2 and 3A), ii) restoration of decreased TGF-β1 after 24 hours, but very spatial time (Fig. 3B), iii) dependence of *H. pylori* attachment on TGF-β signaling status (Fig. 4A), iv) reverse association

between TGF-β levels and gastric inflammation (Table 1), v) transactivation of TGF-β signaling with *H. pylori* and TGF-β ligand, and vi) publication[3] showing that *H. pylori* dampen gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach, supported our conclusion that attenuated TGF-β response could be stupid host response against clever *H. pylori* infection.

Compared to previous publications showing that decreased epithelial cytokine responses including TGF-β1 were noted in the duodenal mucosa of *H. pylori*-infected duodenal ulcer patients,[25] the superiority of our study lied on that we could add more plausible explanation why TGF-β ligand/signaling was decreased after *H. pylori* infection, unwise host defense mechanism. TGF-β1 is a potent inhibitor of the macrophage respiratory burst, as it suppresses H_2O_2 release by activated macrophages.[14] It is conceivable that, in the absence of TGF-β1, exposure of the recruited cells to the uninhibited respiratory-burst capacity of macrophages could be detrimental to the surrounding cells. Although *H. pylori* colonize the epithelial surface of gastric mucosa, they damage the mucosa both directly through either urease-catalyzed synthesis of ammonia or production of cytotoxins and indirectly through the stimulation of local and systemic immune responses. Among them, oxidative stress including generation of superoxide, hydroxyl radicals and nitric oxide (NO) is associated with severe gastritis, atrophic gastritis, intestinal metaplasia, and even gastric cancer.[26,27] Down-regulation of TGF-β1 after *H. pylori* infection can contribute to the promotion of oxidative stress by un-inhibiting respiratory bursts of macrophages and inducing prominent *i*NOS generations.

Persistent *H. pylori* infection results in delayed ulcer healing and ulcer recurrence.[28] Therefore, an NIH consensus meeting in 1994 recommended that therapy aimed at eradicating *H. pylori* should be reserved for patients who present a relapse of duodenal ulcer.[29] While it appears that *H. pylori* interfere with ulcer healing, the exact cellular and molecular targets and their underlying mechanisms have not fully been elucidated. Based on our findings reported here, we propose that there is high possibility that decreased gastric mucosal TGF-β1 levels could be the mechanistic cause of delayed ulcer healing in *H. pylori* infection. The process of ulcer/wound healing is known to involve cell proliferation, re-epithelialization, reconstruction of glandular epithelial structure, and development of granulation tissue. TGF-β1 regulates cell growth and differentiation, and has critical regulatory roles in the process of repair and remodeling.[30,31] During inflammation and post-inflammation, TGF-β1 induces

both angiogenesis and extracellular matrix (ECM) accumulation, which continue through the remodeling phase of repair. ECM production results from the effects of TGF-β1 on fibroblastic cells, which include chemotaxis, proliferation, and induction of the synthesis and release of matrix proteins, fibronectin, collagens, proteoglycans, and hyaluronic acid. The effects of exogenous TGF-β isoforms on wound/ulcer healing have been evaluated by numerous investigators who have shown that both local and systemic application stimulates and accelerates wound/ulcer healing in laboratory animals.[32] Therefore, down-regulation of gastric mucosal TGF-β1 by *H. pylori* infection can provide the basis to explain delayed ulcer healing and recurrence of ulcer frequently encountered in *H. pylori* infection.[25,28,33,34] Moreover, according to the publications[19,35,36] revealing inhibitory effect of *H. pylori* on the regenerating events mediated by epidermal growth factor-related growth factor might also intervene in this event provoked by the down-regulation of TGF-β1.

A study of old heterozygous TGF-β1 mice revealed a high incidence of abnormal hyperplastic lesions in the gastric mucosa of mice expressing only a single TGF-β1 allele, whereas abnormal lesions were not identified in any other organ system studied[36] and another recent studies performed in our laboratory showed that levels of TGF-β1 expression in tissues of TGF-β1 heterozygous mice may be severely depressed in a manner disproportional to the expression of TGF-β1 mRNA. The presence of potentially preneoplastic lesions in the stomach alone in association with allelic loss of TGF-β1, severe gastric inflammation as seen in TGF-β1 knock-out mice, and the current observations suggest that the stomach may be uniquely vulnerable to the development of disease and malignancy in response to defects in the TGF-β signaling pathway. Moreover, the fact that mRNA coding for histamine, muscarine, gastrin, and dopamine receptors in the stomach are expressed at the inflammatory cells of the lamina propria[37] suggests that the regulation of increased acid output and possibly peptic ulcer development is associated with intense inflammation within the lamina propria as seen in the TGF-β1 knockout mice. Our immunostaining and *in vitro* data suggest that TGF-β1 is selectively down-regulated in *H. pylori*-associated gastritis and decreased gastric mucosal TGF-β1 may exacerbate the gastric inflammation.

Over-expression of MHC I and II molecules in TGF-β1 null mice is known to lead to autoimmune disease.[15] Recognizing that the β-chain of parietal H^+/K^+-proton pump has Lewis *y* epitope in common with a majority of *H. pylori* strains generates the obvious question as to whether *H. pylori* infection can also play a role in the on-

set of autoimmune atrophic gastritis marked by the presence of anti-parietal cell antibodies. *H. pylori* infection stimulates formation of antibodies that cross-react with human antral gastric antigens.[38] These observations suggest that decreased gastric mucosal TGF-β1 response by *H. pylori* infection might trigger an autoimmune response, and can explain the pathogenic link between *H. pylori* infection and autoimmune-associated gastritis. This hide-away may eventually fail and can induce gastric mucosal atrophy and clearance of the bacteria at the same time.[39]

Based on our data that suppressed expression of TGF-β after *H. pylori* can hinder the attachment of *H. pylori* to lessen the colonization, stupid host defense paradoxically increased the chance of inflammation propagation and ulceration, suggesting that the gastric mucosal TGF-β1 level could be an important determinant predicting the clinical outcome of *H. pylori* infection. Although variation in strain as well as environment converges in the gastro-duodenal milieu can influence the final outcome of infection, the host TGF-β1 level seems to be an important determining element in the pathogenesis of *H. pylori*-associated gastric disease.

ACKNOWLEDGEMENTS

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