Pathophysiological investigation of the gastric surface mucous gel layer of patients with *Helicobacter pylori* infection by using immunoassays for trefoil factor family 2 and gastric gland mucous cell-type mucin in gastric juice

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Abstract

Background The trefoil factor family (TFF) 2 protein is produced by gastric gland mucous cells (GMCs), and the secreted TFF2 shares a mucosal barrier function with GMC-type mucin. Recently, we presented an enzyme-linked immunosorbent assay (ELISA) method for measurement of GMC-type mucin in the gastric juice.

Aims We aimed to develop an ELISA for TFF2 and to assess pathophysiological changes in the gastric surface mucous gel layer (SMGL) of patients with *Helicobacter pylori* infection.

Methods The distribution of TFF2 and GMC-type mucin in the SMGL was immunohistochemically determined. The ELISA for TFF2 was based on a polyclonal goat antibody. Recombinant TFF2 was employed to prepare the calibrators. TFF2 and GMC-type mucin in the gastric juice in healthy individuals (n = 33) and patients with gastritis (n = 37), gastric ulcer (n = 16), and duodenal ulcer (n = 10) were assayed using ELISA.

Results TFF2 and GMC-type mucin were immunohistochemically co-localized in the gastric SMGL and GMCs. The TFF2 levels in the patients were significantly higher than those in the healthy individuals. Further, the TFF2 levels in the *H. pylori*-positive patients were significantly higher than those in the *H. pylori*-negative patients, and decreased after the eradication of the infection. GMC-type mucin levels showed a tendency similar to that of TFF2 levels.

Conclusion The upregulation of TFF2 and GMC-type mucin secretion may reflect the response of the gastric mucosa to *H. pylori*-induced injuries. TFF2 and GMC-type mucin secreted into the SMGL may protect the gastric mucosa against *H. pylori*.

Key words: ELISA, gastric juice, *Helicobacter pylori*, mucin, TFF2

Introduction

In the gastric mucosa, 2 types of mucous cells are present, namely, surface mucous cells (SMCs) lining the gastric pits and gland mucous cells (GMCs) in the gastric glands (cardiac gland cells, mucous neck cells, and pyloric gland cells) [1]. Mucins secreted from these mucous cells form the surface mucous gel layer (SMGL) covering the luminal surface of the gastric mucosa [2]. The SMGL shows a laminated structure consisting of mucous layers of the SMC-type mucin and those of the GMC-type mucin [2, 3].

In addition to mucin, gastric mucus cells secrete the trefoil factor family (TFF) proteins; TFF1 (formerly pS2) is produced primarily by gastric SMCs [4, 5] and TFF2 (formerly human spamolytic peptide: HSP) is produced by gastric GMCs [4, 6]. TFFs are mucin-associated peptides containing 1 or 2 characteristic trefoil domains [7]. TFFs along with mucin are involved in mucosal barrier functions and in the healing and repair of mucosal damage as luminal surveillance proteins [8-11]. TFF1 and TFF2 are present in the SMGL and in the gastric mucous cells of both humans [12, 13] and rodents [14]. Interestingly, the secretion of TFF2 and GMC-type mucins has been reported to increase in gastric mucosal lesions [14]; furthermore, the secreted TFF2 has been shown to be co-localized with secreted GMC-type mucin in the SMGL of normal gastric mucosa and in the mucoid cap covering gastric mucosal lesions [12].

Helicobacter pylori (*H. pylori*) is a primary causative of gastrointestinal diseases, such as chronic gastritis, gastroduodenal ulcer, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [15, 16]. Our previous histopathological studies clearly showed that *H. pylori* attaches to SMCs and colonizes the gel layers of SMC-type mucin in the gastric mucosa, but does not attach to GMCs and rarely colonizes the gel layer of GMC-type mucin [17, 18]. In addition, GMC-type mucin was reported to have a natural antibiotic function against *H. pylori* [19]. In our previous study, we presented an enzyme-linked immunosorbent assay (ELISA) and reported that *H. pylori* infection causes the up-regulation of GMC-type mucin in human gastric juice [20].

GMC-type mucin and TFF2 have a common mucosal barrier function in addition to participating in the repair and healing of the damaged mucosa by mucosal restitution. Therefore, measurement of TFF2 and GMC-type mucin levels in the gastric juice can be used to estimate the pathophysiological changes in the SMGL.

In this study, we developed an ELISA for TFF2 and assessed pathophysiological changes in the gastric SMGL of patients with *H. pylori* infection by using ELISAs for TFF2 and GMC-type mucin in gastric juice.

Methods

Collection of samples

To histologically examine the SMGL covering normal gastric mucosa, a 2 × 2 cm section was excised from a macroscopically normal corpus and antrum of freshly resected stomachs (n = 5) for early gastric cancer.

Gastric juice samples were obtained at gastroendoscopic examination after an overnight fast from healthy individuals, who underwent a health screening and were verified to be *H. pylori*-negative and without any disease (n = 33) and from patients with gastroduodenal diseases (n = 63) (Table 1). Serological examination was performed to test for *H. pylori* infection, and serum samples and gastric juice samples were obtained on the same day from all subjects enrolled in this study. The collected gastric juice samples were centrifuged at 3,000 rpm for 10 minutes at 4°C to remove debris, and the supernatants were stored at -80°C prior to use.

This study was approved by the ethics committee of Shinshu University, Japan (approval No. 051104). Patients and healthy individuals gave informed consent before participation.

Histological examination

To preserve the SMGL, the excised tissue was laid flat without rinsing on a supporting board with the mucosa surface facing upward, pinned, and immersed in formalin solution containing saturated picric acid (formalin/ picric acid) (a 3:1 mixture of saturated picric acid and 40% formalin solution, v/v) for 2 h at 4°C as previously described [12]. These specimens were cut into 5-mm segments, dehydrated, and embedded in paraffin wax. Tissue sections were stained using hematoxylin and eosin (H&E) for morphological observation and with dual stain consisting of galactose oxidase-cold thionine Schiff reaction (GOTS) for SMC-type mucin, and paradoxical Concanavalin A staining (PCS) for GMC-type mucin [1]. Mouse monoclonal anti-gastric GMC antibody (M-GGMC-1, clone HIK1083, 10-fold dilution) (Kanto Chemical, Tokyo, Japan) and mouse monoclonal anti-human TFF2 antibody (20-fold dilution) (NovoCastra, Newcastle-upon-Tyne, UK) were used to localize GMC-type mucin and TFF2, respectively. Immunohistochemical staining was performed using the

immuno-enzyme polymer method (Histofine Simple Stain MAX PO MULTI, Nichirei Biosciences, Tokyo, Japan) using 3, 3'-diaminobenzidone as chromogen.

TFF2 and GMC-type mucin-ELISA protocols

TFF2 levels in human gastric juice samples were determined using ELISA according to the method developed by Ohmoto *et al*. (unpublished data) with a minor modification. Briefly, commercially available polystyrene immunoplates (Nunc, Roskilde, Denmark) were coated with goat polyclonal anti-human TFF2 antibody (2,000-fold dilution) (R&D systems, Minneapolis, MN) in 0.1 mol/l sodium bicarbonate, pH9.6 (1.0 mg protein/l) for 24 h at 4°C. Plates were washed four times with PBS-Tween after each of the subsequent incubation steps. Unoccupied sites were blocked with the blocking buffer (NOF Corp., Tokyo, Japan) for 24 h at 4°C. The prepared calibrators and samples were then added at 100 µl/well and incubated for 1 h at room temperature. Biotinylated anti-human TFF2 antibody (2,000-fold dilution) was added at 100 µl/well and incubated for 1 h at room temperature. The biotinylated anti-human TFF2 antibody was prepared by using the goat polyclonal anti-human TFF2 antibody (R&D systems) and the EZ-Link Sulfo-NHS-LC- Biotinylation kit (Pierce, Rockford, IL) according to the manufacturer's instructions. Horseradish peroxidase-conjugated streptavidin (10,000-fold dilution) (Vector Laboratoies, Burlingame, CA) was then added at 100 µl/well and incubated for 1 h at room temperature. After the final washing, the color reaction was developed using 100 µl/well of 5 g/l t-methylbenzidine dihydrochloride and hydrogen peroxide. After 30 min incubation at room temperature, the reaction was stopped by adding 50 µl of 0.4 mol/l sulfuric acid, and the absorbance at 450 nm was measured on a Personal LAB (Biochem Immuno Systems, Allentown, PA). A calibration curve was generated, and the TFF2 concentration in serum or gastric juice was calculated from the curve. We used PBS-Tween BSA as dilution buffer for all antibodies used in this study. Calibrators were prepared by diluting the recombinant TFF2 (Peprotech, London, UK) to 0, 0.063, 0.125, 0.25, 0.50, and 1.00 ng/ml using phosphate-buffered saline (PBS), pH7.4, containing 5 g/l bovine serum albumin (BSA) and 5 g/l Tween 20 (PBS-Tween BSA). The assay specificity was investigated by measuring the dilution series of recombinant TFF2, diluted by solutions of recombinant TFF1 (Abnova, Taipei, Taiwan), recombinant TFF3 (Protein Tech Group, Chicago, IL), or PBS-Tween BSA. To assess the effect of the heterogeneity of TFF2 molecule in

gastric juice on this TFF2-ELISA, TFF2 levels were measured in serial dilution of mixed-gastric juice samples showing a different pattern of immunoblot analysis of TFF2.

The GMC-type mucin levels in gastric juice were determined by ELISA using a mouse monoclonal anti-gastric gland mucous cell antibody (Kanto Chemical) as previously described [20].

Because volumes of gastric juice varied among the patients and the total volume of gastric juice could not always be measured during endoscopic examination, we measured the concentrations of TFF2 and GMC-type mucin in gastric juices.

Immunoblot analysis of TFF2 in gastric juice

Gastric juices were treated by the method of Laemmli [21] and then loaded onto 4–20% gradient polyacrylamide gels. The separated proteins were electrophoretically transferred from the gels to nitrocellulose membrane (pore size 0.45 μm; Advantek, Tokyo, Japan) using a semi dry transfer apparatus (GE Healthcare, Buckinghamshire, UK), and the membranes were then incubated in a blocking buffer (PBS containing 5 g/l Tween 20 and 20 g/l skim milk) for 30 min at room temperature. We then incubated the membranes with anti-human TFF2 antibody (1,000-fold dilution) (R&D systems), in PBS-Tween BSA for 1 h at room temperature. After three more times washes, the membranes were incubated for a further 1 h at room temperature with horseradish peroxidase-conjugated anti-goat IgG (2,000-fold dilution) (MBL, Nagoya, Japan) in PBS-Tween BSA. After washing, the bands were visualized using the ECL Plus system (GE Healthcare) according to the manufacturer's instructions.

Examination of *H. pylori* infection

Before eradication of *H. pylori*, the status of *H. pylori* infection was determined by an assay for *H. pylori* IgG antibodies using the GAP-IgG kit (Biomerica, Newport Beach, CA) according to the manufacturer's instructions. After eradication of *H. pylori*, the status of *H. pylori* infection was determined by *H. pylori* culture or ^{13}C-urea breath test at intervals ranging from 1 month to 12 months according to the guidelines for the management of *H. pylori* infections in Japan [22].

Statistical analysis

Each experiment was performed at least 3 times, and the results are expressed as mean ± standard deviation (SD) or standard error (SE). The statistical significance was assessed by the Student's or Welch's t test, with $P < 0.05$ being considered significant.

Results

1. Histology of the SMGL

The SMGL was preserved and appeared as an eosiophilic band covering the gastric mucosa (Fig. 1A). The dual stain (GOTS-PCS procedure) stained SMCs blue and GMCs brown (Fig. 1B). The SMGL had an alternating laminated structure consisting of gel layers of SMC-type mucin (stained blue with GOTS in Fig. 1B) and gel layers of GMC-type mucin (stained brown with PCS in Fig. 1B and positive with M-GGMC-1 in Fig. 1C). In the SMGL, TFF2 was detected in the gel layers (Fig. 1D) corresponding to those reactive with PCS (Fig. 1B) and M-GGMC-1 (Fig. 1C). TFF2 were also detected in mucous granules of GMCs (Fig. 1D).

2. Assay characteristics

2.1 *Dilution linearity of the ELISA for TFF2*

The minimum detection limit, calculated from the mean absorbance of the 0 calibrator ± 2.6 SD, was 0.05 ng/ml. The upper limit was 1.0 ng/ml.

2.2 *Precision and accuracy of the ELISA for TFF2*

The within-run reproducibility was determined by making 20 replicate measurements of 3 diluted gastric juices samples on the same plate (CV, 4.0–6.5%, Table 2). The between-run reproducibility was determined by making duplicate measurements of 3 samples on each of 10 consecutive days (CV, 6.3–9.0%, Table 2). The accuracy of the assay was also confirmed by analytical recovery studies. The recovery rates from gastric juices to which 0.5, 0.25 or 0.125 ng/ml of recombinant TFF2 had been added were 93.0, 87.4, and 120.1%, respectively. The TFF2 levels in serum (mean ± SE) obtained from healthy individuals was 0.742 ± 0.064 ng/ml (61.8 ± 5.3 pmol/l).

2.3 *Specificity of the ELISA for TFF2*

We investigated the assay specificity by measuring the dilution series of recombinant TFF2, which was diluted by using solutions of recombinant TFF1, recombinant TFF3, or PBS-Tween BSA. The absorbance of each dilution series was correlated with the dilution ratios (data not shown).

3. Heterogeneity of the trefoil factor family 2 molecule in gastric juice and its influence on the ELISA for TFF2.

The immunoblot patterns indicated that TFF2 existed as heterogeneous protein in human gastric juices as described previously (Fig. 2A) [23]; that is, we could observe not only the mature TFF2 protein with a molecular weight (Mr) of 12 kDa, but also several bands of glycosylated TFF2 protein with Mr higher than mature one. The main band of the glycosylated TFF2 protein had a Mr of 19 kDa, although several higher Mr bands were detected, this band was more intense than that of mature TFF2 protein. To assess the effect of the heterogeneity of TFF2 protein on this assay method, we measured TFF2 levels in serial dilution of mixed-gastric juice samples prepared by using 2 gastric juices (lane 2 and 4 in Fig. 2A). The TFF2 concentrations in the mixed-gastric juice samples were correlated with the calculated ratios (Fig. 2B).

4. TFF2 and GMC-type mucin concentration in gastric juices

TFF2 levels were higher in patients with gastritis (24.8 ± 0.7 μg/ml, $P < 0.01$), gastric ulcer (GU) (21.3 ± 1.2 μg /ml, $P < 0.01$), or duodenal ulcer (DU) (18.4 ±1.5 μg /ml, $P < 0.05$) than those of healthy individual group (1.9 ± 0.4 μg /ml) (Fig. 3A). Similarly, the GMC-type mucin levels in patients with gastroduodenal diseases (gastritis, 33.0 ± 1.0 U/ml, $P < 0.01$; GU, 29.9 ± 1.7 U/ml, $P = 0.15$; DU, 29.5 ± 2.0 U/ml, $P = 0.21$) tended to be higher than those in the healthy individual group (11.6 ± 0.7 U/ml) (Fig. 4A). The levels of TFF2 in gastric juices showed no correlation with those of GMC-type mucin in gastric juices ($r = 0.220$, data not shown).

5. Effects of *H. pylori* infection on TFF2 and GMC-type mucin levels in gastric juices

TFF2 levels in *H. pylori*-positive patients were significantly higher (26.5± 0.7 μg/ml, $P <0.01$) than those in healthy individuals (1.9 ± 0.4 μg /ml) and in *H. pylori*-negative patients (8.9 ± 0.9μg /ml) (Fig. 3B). The GMC-type mucin levels in *H. pylori* positive patients (29.9 ± 0.8 U/ml) were significantly higher than those in healthy individuals (11.6 ± 0.7 U/ml, $P <0.01$) (Fig. 4B); however, they were not significantly higher than those in *H. pylori*-negative patients (26.6 ± 1.8 U/ml) (Fig. 4B).

After eradication of *H. pylori*, the TFF2 levels significantly decreased (before

eradication, 22.6 ± 1.3 μg/ml; after eradication, 7.6 ± 0.9 μg /ml, $P < 0.05$) (Fig. 3C) and GMC-type mucin levels also decreased (before eradication, 24.9 ± 1.7 U/ml; after eradication, 18.2 ± 1.4 U/ml), although their decrease was not statistically significant (Fig. 4C).

Discussion

In the present study, we report an ELISA method for measurement of TFF2 in gastric juice, which in combination with the previously published ELISA for GMC-type mucin [20] shows reversible upregulation of TFF2 and GMC-type mucin in the gastric juice of patients with *H. pylori* infection.

Our assay for TFF2 had adequate sensitivity, specificity, and accuracy, similar to that of the assay for serum TFF2 reported by Vestergaard *et al.* [23]. Although the CV% of our assay of the gastric juice samples appeared to be lower than that of the method reported by Vestergaard *et al.* [23], the CV% calculated from the measurements of the serum samples (data not shown) were consistent with those reported by them.

TFF2 has been shown to exist in glycosylated and non-glycosylated forms in human gastric juice [24]. In the immunoblot analysis, we mainly detected 19- and 12-kDa bands, corresponding to glycosylated and non-glycosylated TFF2, respectively. In addition to these 2 bands, we detected several bands with higher Mr reactive with the anti-human TFF2 antibody, and the immunoblot patterns differed among the subjects. These findings indicate that TFF2 in the gastric juice is a heterogeneous molecule, and this heterogeneity will affect the stoichiometry, which is the most important requirement for quantitative methods. However, the results of our experiments using the mixed-gastric juice samples strongly indicate that the stoichiometry of our method is sufficient.

In this study, we immunohistochemically confirmed that TFF2 and GMC-type mucin were colocalized in both the gastric GMCs and gastric SMGL. This result is consistent with our previous results [12, 14]. Interestingly, TFF2 has been reported to be trapped in the mucoid cap adhered to erosion together with GMC-type mucin in both human gastric mucosa [12] and *H. pylori*-infected Mongolian gerbil gastric mucosa [14].

In the present study, the level of TFF2 secreted in gastric juice samples was significantly high in patients with gastritis, GU, and DU. This upregulation of TFF2 levels in gastric juices was closely related with *H. pylori* infection, and the elevated TFF2 levels in gastric juices returned to approximately the normal levels after successful eradication of *H. pylori*. Interestingly, a previous report showed that *H. pylori* infection upregulated the expression of TFF2 mRNA in gastric cancer cells [25]. On the other hand, immunohistochemical examination showed that the expression of TFF2 in GMCs was decreased in *H. pylori*-infected gastric mucosa [26]. These observations

suggest that in addition to a possible increase in the synthesis of TFF2 in the *H. pylori*-infected mucosa, there may be an increase in the release of TFF2. Importantly, secreted TFF2 is thought to share a mucosal barrier function with mucins and participate as "luminal surveillance peptides" in the repair and healing of the damaged mucosa [8, 9, 11]. Taking into account the mucosal protective function of TFF2, the upregulation of TFF2 secretion may promote the healing of gastric mucosal injuries induced by *H. pylori* infection.

In addition to the increase in the level of secreted TFF2, the level of GMC-type mucin secreted in the gastric juices of *H. pylori*-infected patients has been reported to increase [20, 27]. Taking into account the finding that GMC-type mucin has a natural antibiotic function against *H. pylori* [19], our findings led us to the hypothesis that the upregulation of secretion of GMC-type mucin may be a part of the defense mechanisms of the gastric mucosa against *H. pylori* infection.

TFF2 has been reported to stabilize the gastric mucus gel [28], leading to a strengthening of the physical barrier function of the SMGL at the luminal surface [28]. Compared to the addition of TFF1 and TFF3, the addition of TFF2 to gastric mucin solutions was reported to result in a significant increase in viscosity and elasticity [28]. In addition, the rat model showed TFF2 interacted with gastric mucin in a manner that inhibited proton permeation through the mucus gel layer [29]). Interestingly, previous reports have also shown that secreted TFF2 was trapped in the mucoid cap covering the damaged mucosa together with gland mucous cell mucin and adhered to the sites of mucosal damage [12, 14]. TFF2 has been reported to promote epithelial restitution [30, 31]; this finding suggests that secreted TFF2 may play a role in healing and repairing gastric mucosal lesion. The increase in TFF2 and gland mucous cell mucin in *H. pylori*-infected gastric mucosa may operate in a synergistic manner to heal and repair the gastric mucosal lesions.

Results from the following studies suggest the mechanism underlying the induction of the upregulation of TFF2 and GMC-type mucins in the *H. pylori*-infected gastric mucosa. Hypoxia inducible factor-1 (HIF1)-mediated induction of TFF1, TFF2 and TFF3 has been reported in gastric damage induced by non-steroidal anti-inflammatory drugs, which compromise mucosal microcirculation [32]. Interestingly, the HIF1 levels have been reported to show a dose-dependent increase in *H. pylori*-infected gastric

epithelia [33]. Thus, HIF1 induced in *H. pylori*-infected gastric epithelia may mediate the induction of TFF2. Upregulation of the expression of TFF2 and GMC-type mucin may also be related to the inflammatory processes in the gastric mucosa. Treatment of cultured chondrocytes with tumor necrosis factor (TNF)-α or interleukin (IL)-1β was reported to increase TFF3 expression [34]. On the other hand**,** the expression of MUC6 (a core protein of GMC-type mucin) and the secretion of mucins were reported to be stimulated by TNF-α and IL-6 in the human colonic carcinoma-derived cell line LS180 [35]. The levels of TNF-α, IL-6, and IL-1β levels were reported to be elevated in the gastric mucosa in *H. pylori*-associated gastritis [36], and thus cytokine-mediated mechanisms may be involved in the upregulation of the expression of TFF2 and GMC-type mucin in the gastric mucosa in *H. pylori*-associated gastritis.

In conclusion, we used ELISA for measuring the levels of TFF2 and GMC-type mucin in gastric juice samples of *H. pylori*-infected patients and showed a reversible increase in their levels, which suggested that this elevation may reflect the response of the gastric mucosa to injuries induced by *H. pylori* infection. The upregulation of secretion of TFF2 and GMC-type mucin may be involved in the healing of gastric mucosal injuries induced by *H. pylori* infection and also in the protection of gastric mucosa against *H. pylori* infection.

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Table 1 Clinical data of healthy individuals and patients

Clinical data	Healthy individuals	Patient with gastroduodenal diseases			*Helicobacter pylori* infection		*Helicobacter pylori* eradication	
		Gastritis	Gastric ulcer	Duodenal ulcer	Positive	Negative	Before	After
n	33	37	16	10	50	13	11	
Male / Female	14 / 19	11 / 26	9 / 7	8 / 2	20 / 30	8 / 5	6 / 5	
Age Years (mean ± SD)	50.4 (±12.5)	67.5 (±11.8)	68.2 (±8.6)	56.2 (±23.9)	65.6 (±14.6)	67.0 (±12.5)	60.4 (±14.7)	61.1 (±14.9)

Table 2 Reproducibility within-run and between-run.

	Sample†	Concentration*	CV (%)
Within-run (n = 20)	L	0.231 (0.009)	4.0
	M	0.465 (0.024)	5.2
	H	0.743 (0.048)	6.5
Between-run (n = 10)	L	0.233 (0.020)	8.7
	M	0.456 (0.041)	9.0
	H	0.716 (0.045)	6.3

† patients' gastric juices: L, M, and H indicate low, medium, and high levels of trefoil factor family 2, respectively.

*Mean (±standard deviation), ng/ml

Figure Legends

Figure 1

Histological examination of the distribution of trefoil factor family 2 and gland mucous cell-type mucin in the human gastric pyloric mucosa.

(A) The surface mucous gel layer (SMGL) appears as an eosiophilic band covering the gastric mucosa. (B) The dual stain (galactose-oxidase/thionine Schiff reaction (GOTS)-paradoxical concanavalin A staining (PCS) procedure) stains surface mucous cells (SMCs) blue and gland mucous cells (GMCs) brown. The SMGL shows an alternating laminated structure consisting of gel layers of SMC-type mucin (blue with GOTS) and gel layers of GMC-type mucin (brown with PCS). (C) Mouse monoclonal anti-gastric gland mucous cell antibody (M-GGMC-1)-positive GMC-type mucin is present in the mucous granules of GMCs and also in the gel layers corresponding to those reactive with PCS. (D) Trefoil factor family 2 is found in the mucous granules of GMCs and also in the gel layers corresponding to those reactive with PCS and M-GGMC-1.

Figure 2

Heterogeneity of the trefoil factor family 2 molecule in gastric juice, and its influence on the present assay method.

(A) Recombinant trefoil factor family 2 (TFF2) (lane Ag) and patients' gastric juices (lane 1–4), treated with Laemmli buffer (without 2-mercaptoethanol), were loaded onto 4–20% gradient polyacrylamide gels. After electrophoresis, separated proteins were transferred electrophoretically onto a nitrocellulose membrane. The bands containing TFF2 were visualized by immunoblot analysis using anti-TFF2 antibody. (B) To investigate the effect of heterogeneity of the TFF2 molecule, we measured TFF2 levels in the serial dilutions of mixed-gastric juice samples that were prepared using 2 gastric juices (lane 2 and 4 in Fig. 2A). Data are expressed as mean ± standard deviation (SD) derived from triplicate determinations in each of the 2 separate experiments.

Figure 3

TFF2 concentration in gastric juices from healthy individuals and patients.

(A) TFF2 concentration in gastric juices from patients with gastritis, gastric ulcer (GU),

duodenal ulcer (DU) and healthy individuals (control). (B) TFF2 concentration in gastric juices from patients with *H. pylori* infection (HP (+)) and in *H. pylori*-negative patients (HP (-)). (C) TFF2 concentration in gastric juices from patients before and after the *H. pylori* eradication.

*$P < 0.05$; **$P < 0.01$; NS, not significant

Figure 4

Gland mucous cell (GMC)-type mucin concentration in gastric juices from healthy individuals and patients.

(A) GMC-type mucin concentration in gastric juices from patients with gastritis, gastric ulcer (GU), duodenal ulcer (DU) and healthy individuals (control). (B) GMC-type mucin concentration in gastric juices from patients with *H. pylori* infection (HP (+)) and in *H. pylori*-negative patients (HP (-)). (C) GMC-type mucin concentration in gastric juices from patients before and after the *H. pylori* eradication.

**$P < 0.01$; NS, not significant

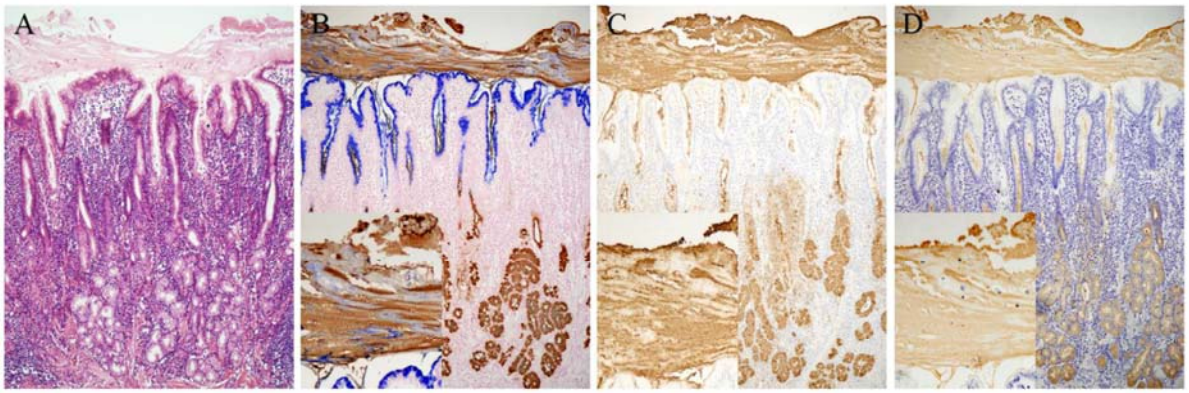

Figure 1

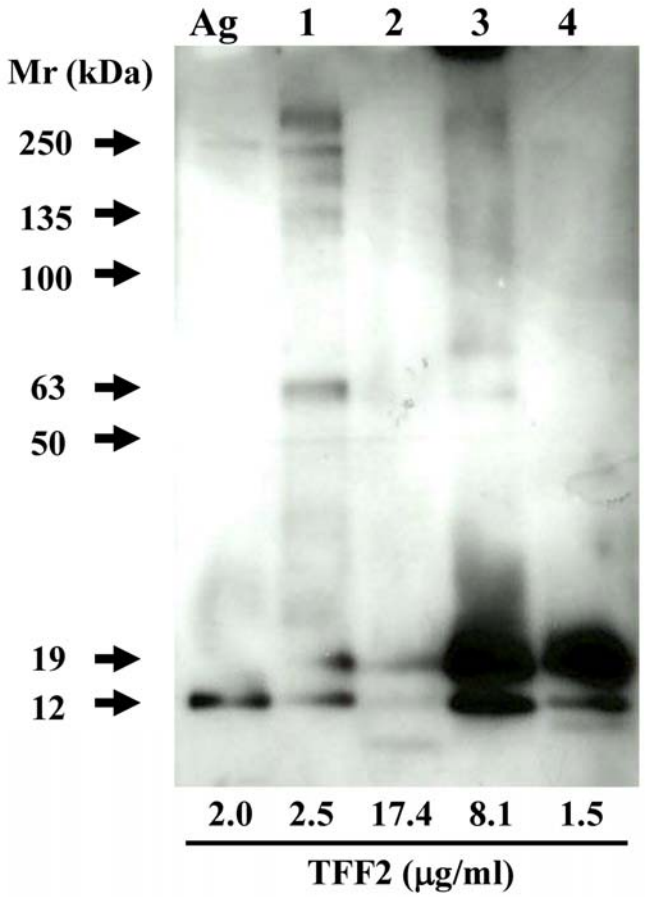

Figure 2A

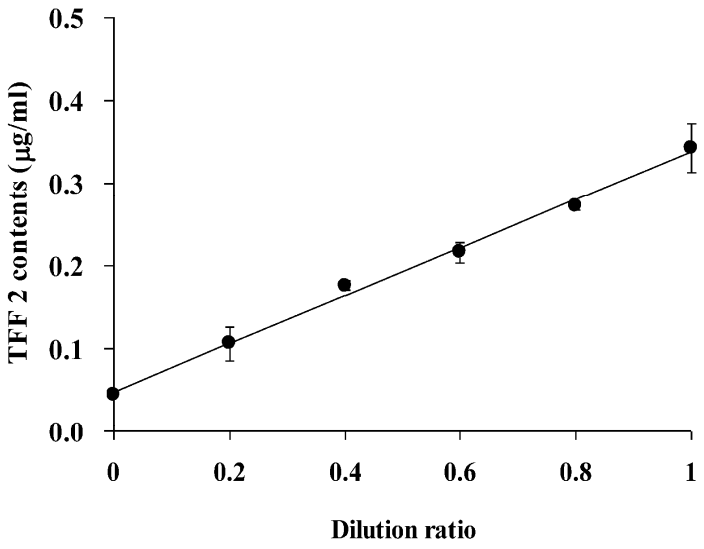

Figure 2B

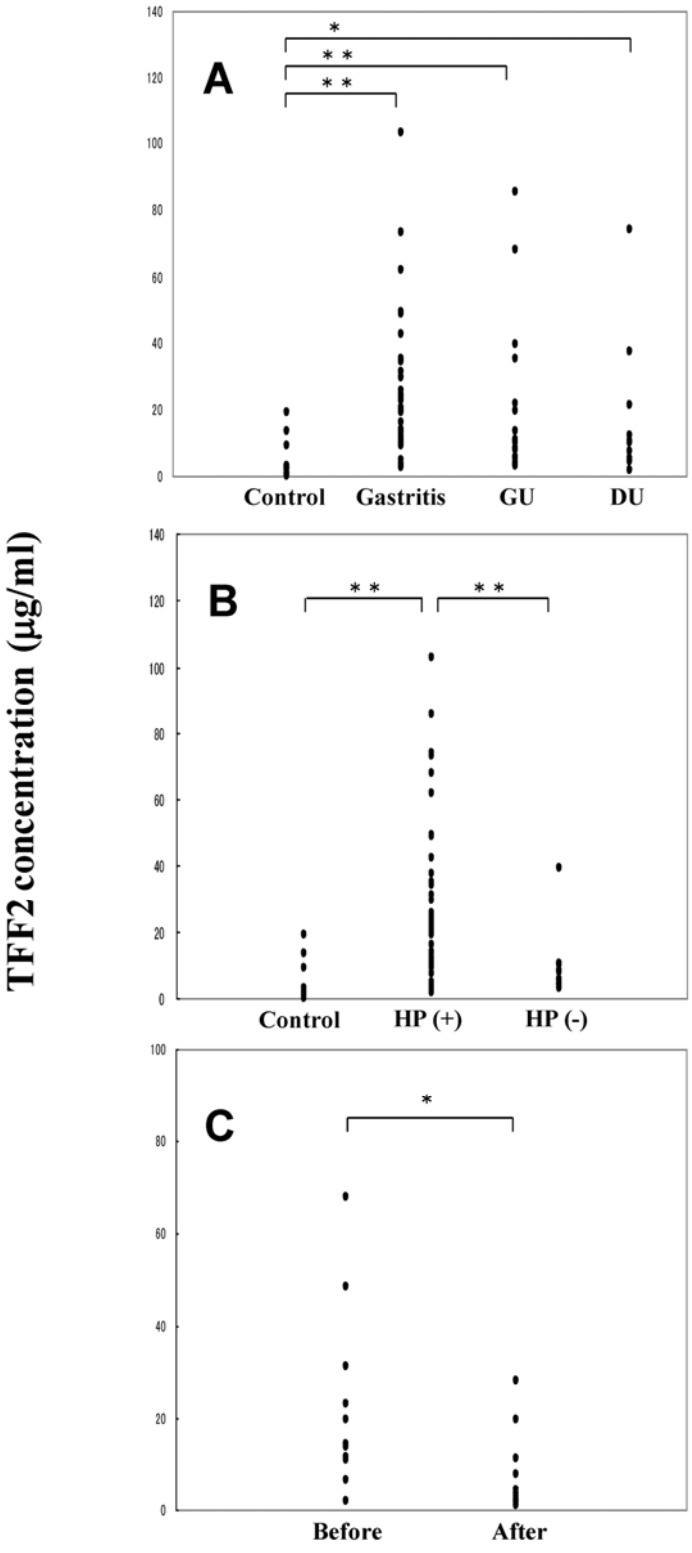
TFF2 concentration (μg/ml)
A
B
C
Control
Gastritis
GU
DU
HP (+)
HP (-)
Before
After

Figure 3

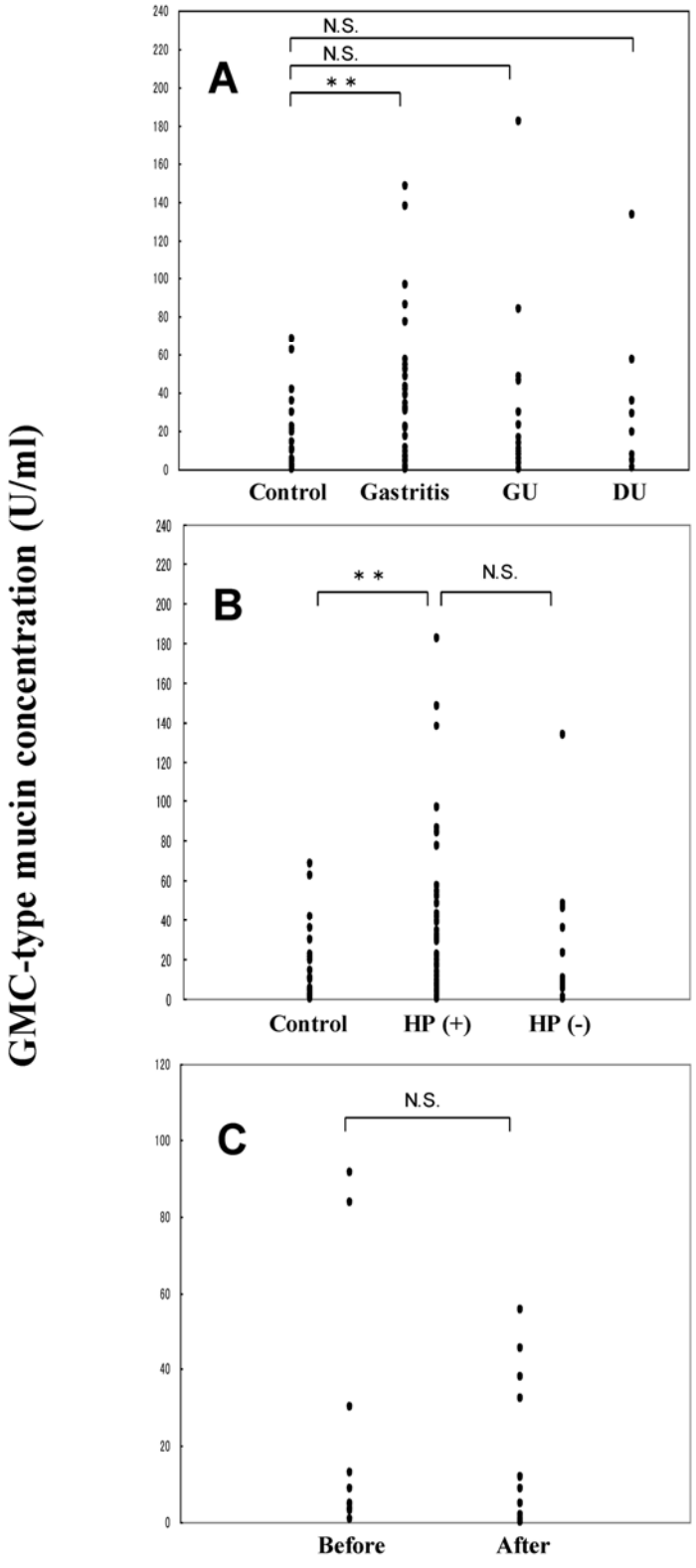
GMC-type mucin concentration (U/ml)
A
N.S.
N.S.
* *
Control
Gastritis
GU
DU
B
* *
N.S.
Control
HP (+)
HP (-)
C
N.S.
Before
After

Figure 4