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Helicobacter pylori infection produces expression of a secretory component in gastric mucous cells

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Abstract *Helicobacter pylori* infection induces the expression of a secretory component (SC) in gastric epithelial cells. We investigated the cell lineage of the SC- and immunoglobulin (Ig) A-expressing epithelial cells in *H. pylori*-infected gastric mucosa. Materials were obtained by means of gastric biopsy from *H. pylori*-infected patients (24 cases) before and after the eradication of *H. pylori*, from five normal uninfected volunteers, and from three gastrectomy cases. Acetic acid–ethanol-fixed and paraffin-embedded specimens were examined using histochemical staining for gastric mucins (periodic acid oxidation-thionine Schiff reaction-concanavalin A-horse radish peroxidase staining) by means of immunostaining for gastric mucins (45M1 and HIK1083), intestinal cells (MUC2 and CD10), Ki67, *H. pylori*, SC, and IgA. The SC and IgA were not found in normal gastric mucosa. The expressions of the SC and IgA in gastric surface mucous cells and mucous neck cells in the generating zone of the gastric mucosa of *H. pylori*-infected patients were significantly higher before eradication of *H. pylori* than after the eradication. These mucous cells have the potential for SC-mediated translocation of IgA into the gastric lumen, and this may act as part of the antibacterial defense system against *H. pylori* infection in the gastric generating zone.

Keywords *Helicobacter pylori* · Gastric mucosa · Secretory component · Immunoglobulin A · Immunohistochemistry

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Introduction

Infection with *Helicobacter pylori* induces *H. pylori*-specific immunoglobulin (Ig) A-producing plasma cells in the gastric mucosa [13, 21], and this specific IgA can be detected in the gastric aspirate and in the serum of *H. pylori*-infected individuals [7]. In mucosal tissues, IgA is an important mediator of humoral immunity and transport of IgA to the lumen is facilitated by a secretory component (SC) expressed in epithelial cells [3]. Thus, dimeric IgA, secreted by mucosal plasma cells, is attached to the SC and then transported as a SC-IgA complex through the epithelial cells to the mucosal surface [3].

The association of *H. pylori* infection with increased expression of the SC by gastric epithelial cells has been repeatedly noted [1, 8, 10, 12, 22]. Recently, it was reported that the expression of the SC by gastric epithelial cells in *H. pylori*-infected subjects was reduced after successful eradication of the infection [6]. However, the cell lineage-specificity of the SC-expressing epithelial cells in the *H. pylori*-infected gastric mucosa has not been established. For this reason, we decided to examine immunohistochemically the epithelial expression of the SC and IgA in the gastric mucosa from normal volunteers and from *H. pylori*-infected subjects before and after eradication. This was done in combination with a battery of histochemical and immunohistochemical techniques designed to characterize gastrointestinal epithelial cells.

Materials and methods

Study subjects

Gastric mucosal biopsy specimens from two groups of individuals were studied using microscopy. The first group consisted of non-infected volunteers (three men and two women; age range 22–65 years; mean 45.0 years) with no subjective or objective evidence of gastrointestinal disease, with negative serology for anti-*H. pylori* antibody, and with no visible organisms in any of the biopsy specimens examined. The second group included 24 patients with active *H. pylori* infection (16 men and 8 women; age range 28–65 years; mean 51.6 years; 10 with gastric ulcer, 10 with duodenal ulcer, and 4 with chronic active gastritis; all were positive for *H. pylori* according to both histological examination and culture). These *H. pylori*-infected patients were evaluated before and after successful treatment with a 1-week course involving oral administration of lansoprazole, amoxicillin, and clarithromycin. Status of *H. pylori* infection was determined according to both histological examination and culture. In addition, we carried out a histological study of non-neoplastic mucosa with *H. pylori* infection in three stomachs surgically resected for gastric cancer.

H. pylori-infected patients who were to undergo endoscopic examination and gastric cancer patients who had undergone a gastrectomy gave informed consent to their enrollment in this study. The tissues were used with the approval of the ethics committee of Shinshu University, Japan and only after obtaining written consent from the patients.

Endoscopy and sampling

At the initial endoscopy, during which the diagnosis of *H. pylori* infection was established, one biopsy specimen was taken from a site in the antral lesser curvature close to the pylorus and another from a site in the lesser curvature in the region of the upper middle corpus. At the same examination, two biopsy specimens were obtained from a site in the antral greater curvature close to the pylorus and two from a site in the greater curvature in the region of the upper middle corpus. One specimen from the antral greater curvature and one from the greater curvature of the corpus were used for the culture of *H. pylori*. The remaining specimens were used for histological examination. After treatment for the infection, *H. pylori* status was evaluated at intervals of from 1 month to 12 months, with a biopsy protocol identical to that described above being followed each time.

Histological assessment and immunohistochemical staining

Biopsy specimens for histological examination and gastrectomy specimens were immediately fixed in acetic acid–ethanol solution (a 99:1 mixture of 95% ethanol and acetic acid on a volume basis) for 24 h at 4°C, and then dehydrated in 100% ethyl alcohol, cleared in xylene, and embedded in paraffin. In our preliminary experiments, the cold acetic acid ethanol fixation method was found to be superior to formalin fixation in preserving the antigenicity of epithelial SC and IgA, good enough for immunostaining for the other epithelial markers used in this study, and superior in terms of tissue preservation to ethanol fixation which has been reported to be good for immunostaining for the SC and IgA [19].

Hematoxylin and eosin staining was used for histological examination. Immunohistochemical staining was performed using the indirect immunoperoxidase staining method. Briefly, sections were dewaxed and rehydrated, and endogenous peroxidase activity was blocked with hydrogen peroxide/methanol. Prior to immunostaining, antigen retrieval was carried out using a steamer (Black and Decker HS90, Shelton, Conn.) for 30 min in 0.01 mol/l citrate buffer (pH 6.0) for M-GGMC-1 (HIK1083; Kanto Chemical, Japan), or in 1 mM ethylene diamine tetraacetic acid (EDTA; pH 8.0) for HGM-45M1 (M1) (Novocastra, UK), MUC2 (Novocastra), Ki67 (Dako, Carpenteria, Calif.), and CD10 (Novocastra). For immunostaining with anti-*H. pylori* (Dako), sections were refixed with 20% buffered formalin for 30 min and then pretreated with a 0.1% solution of trypsin (Sigma Chemical Co., St. Louis, Mo.) in Tris-HCl buffer (0.05 M, pH 7.6) containing 0.1% $CaCl_2$ at 37°C for 10 min. For immunostaining with anti-IgA (Dako) and anti-SC (Dako), antigen retrieval was not carried out. The tissue sections were blocked with 1:20 normal bovine serum albumin in Tris-buffered saline (TBS; 140 mmol/l NaCl, 50 mmol/l Tris/HCl, pH 7.6) and incubated either for 2 h with primary antibodies (in the case of anti-IgA and anti-SC) or overnight (in the case of HIK1083, M1, MUC2, Ki67, CD10, and *H. pylori*). After washing in TBS, slides were then incubated with horseradish peroxidase-labeled second antibody for 30 min. Visualization of immunostaining was performed using diaminobenzidine as a substrate, and the tissue sections were counterstained with hematoxylin, dehydrated, and mounted. To clarify the cell lineage of the gastric epithelial cells expressing the SC, immunostaining for the SC was followed by histochemical staining for gastric mucins performed on pyloric and fundic mucosa specimens obtained at gastrectomy. Briefly, after immunostaining for the SC using the indirect immuno-alkaline phosphatase method with new fuchsin as a coupler, tissue sections were treated with 1% periodic acid solution, immersed in cold thionine Schiff reagent [15], and then stained with concanavalin A-horse radish peroxidase [9] (PA-TS-ConA-HRP staining). This method stains the SC red, gastric surface mucous cells blue, and gastric gland mucous cells (mucous neck cells and pyloric gland cells) brown.

Negative controls were obtained by omitting the primary antibody. Surface mucous cells and pyloric gland cells in the specimens were used as internal positive controls for M1 and HIK1083, respectively. Intestinal metaplasia in the stomach was used to provide internal positive controls for MUC2 and CD10.

Mononuclear cell infiltration, neutrophil infiltration, and *H. pylori* colonization in the biopsy specimens were each scored as 0 (normal), 1 (mild), 2 (moderate), or 3 (marked) according to the Updated Sydney System [4]. The epithelial expressions of the SC and IgA in biopsy specimens of gastric mucosa showing no expression of intestinal markers (MUC2 for intestinal goblet cells and CD10 for intestinal absorptive cells) were scored semiquantitatively as 0 (negative), 1 (less than one third of the epithelium of the foveola-isthmus zone), 2 (more than one third, but less than two thirds), or 3 (more than two thirds). In addition, the foveola-isthmus zone was divided into upper and lower parts, and the degree of *H. pylori* colonization and the epithelial expressions of the SC and IgA were each estimated semiquantitatively in a given part as 0 (negative), 1 (less than one third of the epithelium of a given compartment), 2 (more than one third, but less than two thirds), or

Fig. 1 Normal fundic mucosa of a normal volunteer. Hematoxylin and eosin staining (**A**). Gastric surface mucous cells show immunoreactivity for M1 (**B**), while mucous neck cells show immunoreactivity for HIK1083 (**C**). Immunoperoxidase method; original magnification ×200

Fig. 2 Intestinal metaplasia. Hematoxylin and eosin staining (**A**). Metaplastic goblet cells stain with anti-MUC2 (**B**) and brush-bordered cells stain with anti-CD10 (**C**). Immunoperoxidase method; original magnification ×200

Fig. 3 Intestinal metaplasia. Gastric surface mucous cells stain with M1 (**A**), but not with MUC2 (**B**). Some metaplastic goblet cells reveal immunoreactivity with both M1 (**A**) and anti-MUC2 (**B**). Immunoperoxidase method; original magnification ×200

Fig. 4 Intestinal metaplasia. Epithelial expression of the secretory component (SC; **A**) and immunoglobulin (Ig) A (**B**) are found in the apical cytoplasm of the absorptive cell-type metaplastic cells. The epithelial distribution of IgA corresponds to that of the SC. IgA is also present in plasma cells and extracellularly in the lamina propria (**B**). Immunoperoxidase method; original magnification ×200

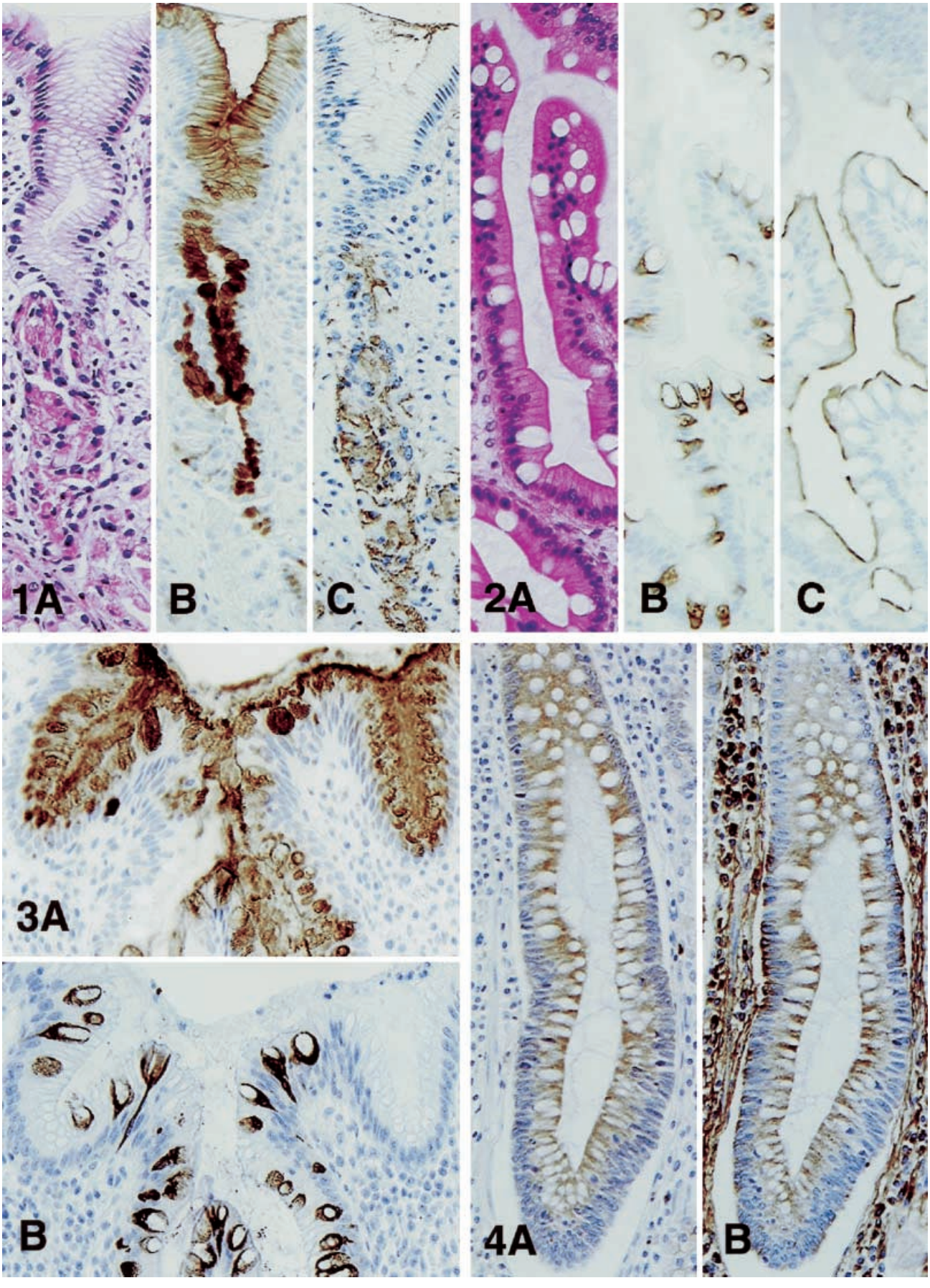
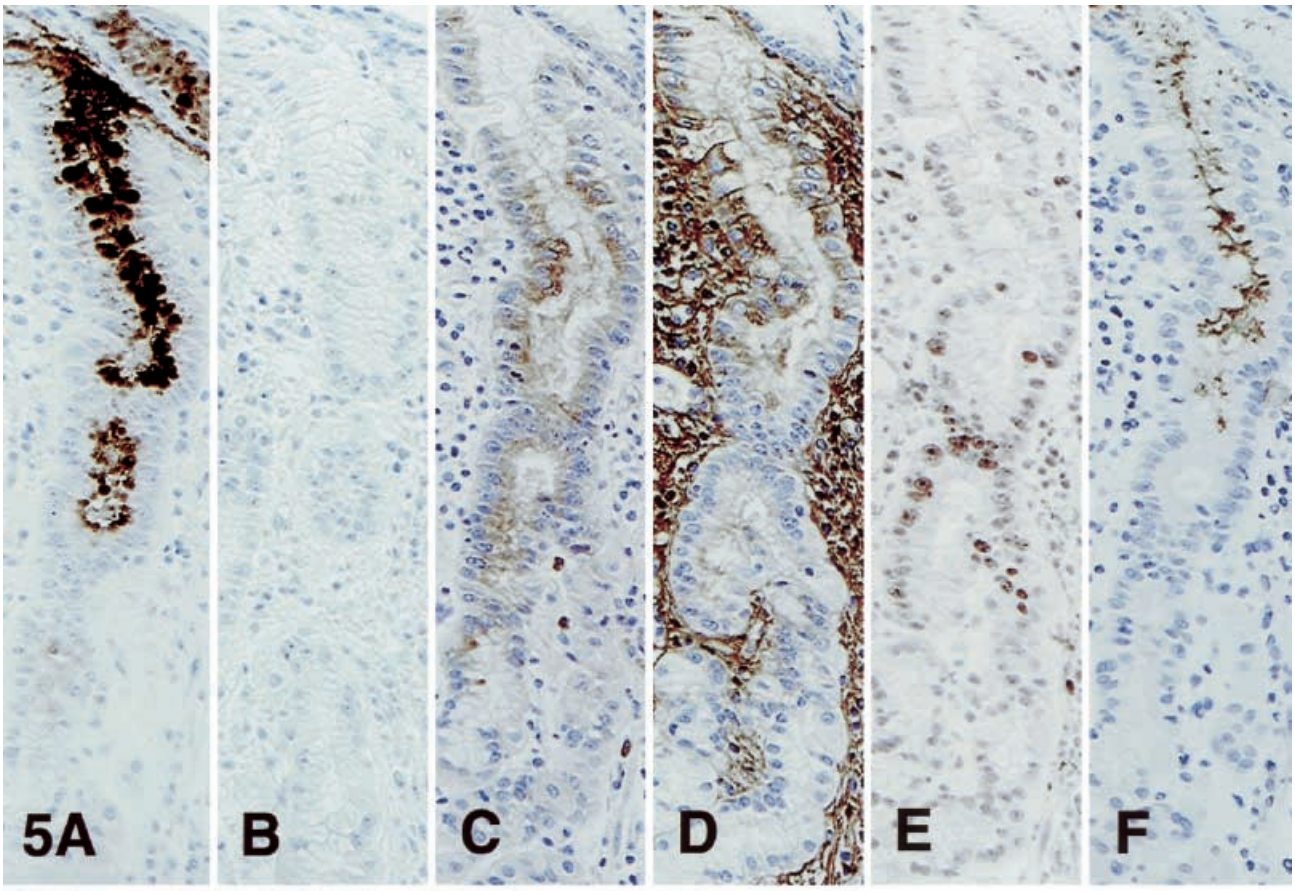


Fig. 1–4 Legend see page 515



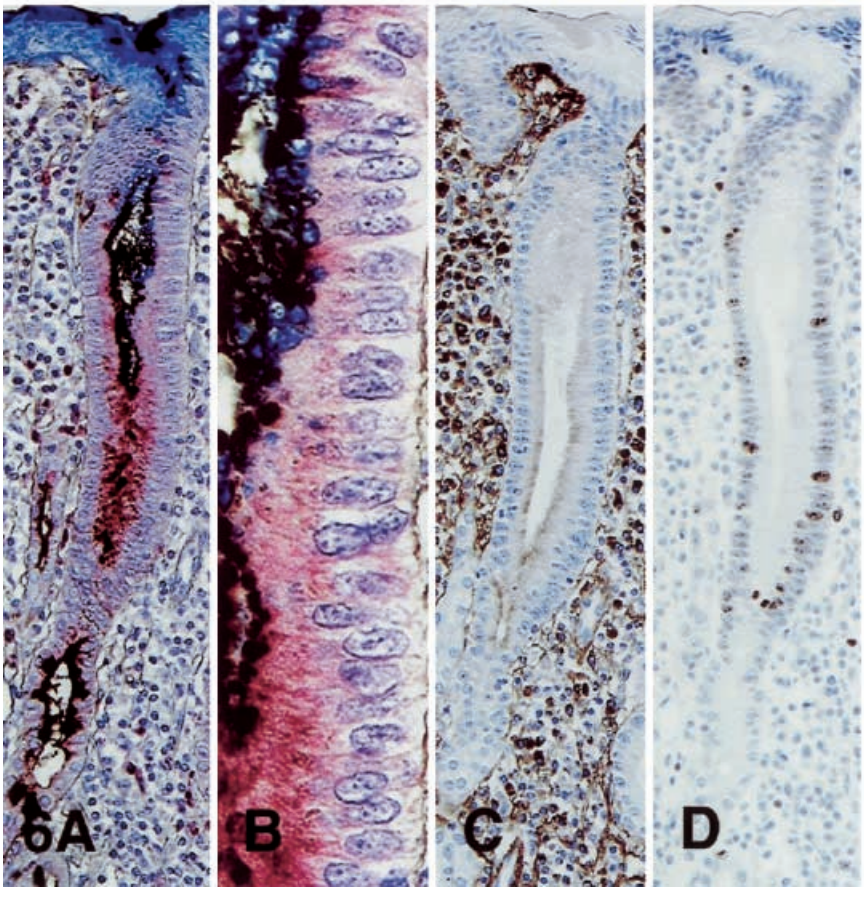


Fig. 5 Gastric fundic mucosa of a patient before treatment of *Helicobacter pylori*. In the gastric mucosa, which shows immunoreactivity with M1 (**A**) but not with MUC2 (**B**), the secretory component (SC; **C**) and immunoglobulin (Ig)A (**D**) are expressed in epithelial cells around the proliferating zone in which Ki-67-reactive proliferating cells are found (**E**). The density of *H. pylori* is higher in the upper region of the crypt than in the lower region (**F**). Immunoperoxidase method; original magnification ×200

Fig. 6 Gastric fundic mucosa with *Helicobacter pylori* infection of a stomach surgically resected for gastric cancer. Epithelial expression of the secretory component (SC; **A, B**) and immunoglobulin (Ig)A (**C**) are found in the foveola-isthmus region around the proliferative zone (**D**). With periodic acid-cold thionine Schiff reaction–concanavalin A–horseradish peroxidase staining, surface mucous cells stain blue and mucous neck cells stain brown (**A, B**). Localization of the SC is visualized by means of the immuno-alkaline phosphatase method, and the SC stains red (**A, B**). Immature surface mucous cells and mucous neck cells around the foveola-isthmus region show immunoreactivity for the SC (**A, B**). **A**, **B** original magnification ×200. **C**, **D** immunoperoxidase method; original magnification ×200

3 (more than two thirds). The grading of *H. pylori* colonization, inflammation, and immunoreactivity was performed by a single observer.

Statistics

Spearman's correlation coefficient by rank test was used to analyze the correlation between the scores given for the immunoreactivity of the SC and IgA on the one hand, and *H. pylori* colonization and inflammation on the other, before eradication of *H. pylori*. The Mann-Whitney U test was used to compare the scores given for non-infected volunteers to *H. pylori*-infected patients before eradication of *H. pylori*. The Wilcoxon signed-rank test was used to compare the scores given to *H. pylori*-infected patients before and after eradication of *H. pylori* and to compare the scores given to upper and lower parts of the foveola-isthmus zone.

Results

Before treatment for *H. pylori*, each and every biopsy specimen and gastrectomy specimen from the *H. pylori*-infected patients showed chronic active gastritis with various degrees of neutrophil infiltration in addition to mononuclear cell infiltration. After treatment for *H. pylori*, neutrophils and *H. pylori* disappeared completely, but a less dense mononuclear cell infiltration remained in the biopsy specimens.

Gastric mucous cells could be distinguished from intestinalized cells by immunostaining. Thus, gastric surface mucous cells were positive with M1 and negative with HIK1083 (Fig. 1), while gastric gland mucous cells were positive with HIK1083 and negative with M1 (Fig. 1). Both types of cells were negative with MUC2 and CD10. However, intestinalized cells were positive with MUC2 (goblet cells; Fig. 2A) and CD10 (brush bordered cells; Fig. 2B). Intestinalized cells were negative with HIK1083. Some M1-positive cells also showed a positive reaction with MUC2 (Fig. 3). These hybrid cells, showing characteristics of both gastric surface mucous cells and intestinal cells [16], are referred to as intestinalized cells in this paper. No intestinalized cells, including these hybrid cells, were found in the gastric mucosa from non-infected volunteers. In intestinal metaplasia, epithelial expression of the SC and IgA were found in the apical cytoplasm of the absorptive cell-type metaplastic cells (Fig. 4). Neither the SC nor IgA was expressed in the gastric mucosa from non-infected volunteers.

Before treatment for *H. pylori*, the SC was expressed in gastric epithelial cells, especially around the gastric generating zone defined by its positive immunostaining for Ki67 (Fig. 5 and Fig. 6) and in intestinalized cells. Immunostaining for the SC followed by PA-TS-ConA-HRP staining in gastrectomy specimens revealed gastric surface mucous cells and mucous neck cells (especially in the foveola close to the isthmus and in the isthmus zone) exhibiting immunoreactivity for the SC (Fig. 6). In biopsy specimens from the antrum and the corpus, the degree of SC expression in gastric mucous cells showed a good correlation with the degree of mononuclear cell infiltration ($P<0.01$) but no correlation with the degree of neutrophil infiltration or *H. pylori* colonization.

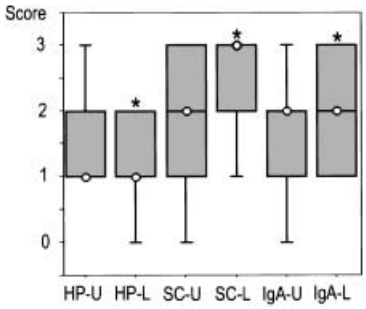


Fig. 7 Degree of *Helicobacter pylori* colonization and epithelial expression of secretory component (SC) and immunoglobulin (Ig)A in the foveola-isthmus zone of the gastric mucosa. The 10th, 25th, 50th (median), 75th, and 90th percentiles of the scores for *H. pylori* colonization and the epithelial expressions of IgA and SC are displayed. The differences between parameters were analyzed using the Wilcoxon signed-ranks test (upper vs lower: *; $P<0.01$). Degree of *H. pylori* colonization did not show significant correlation with the degree of the epithelial expression of SC and IgA

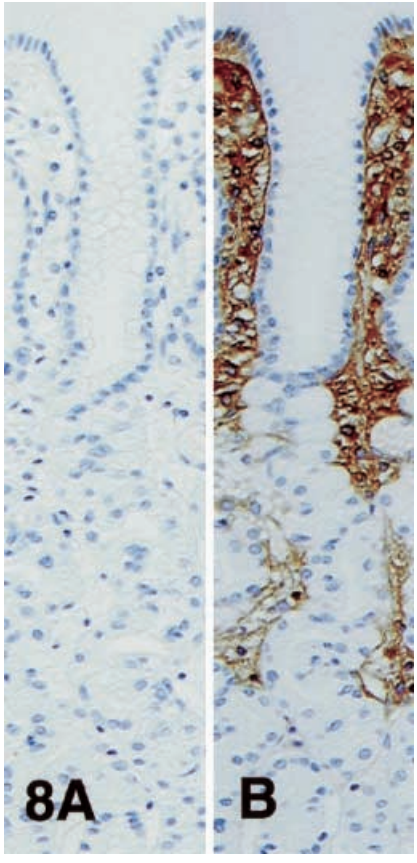


Fig. 8 Gastric fundic mucosa of a *Helicobacter pylori*-eradicated patient. In the gastric mucosa of this patient, gastric epithelial cells reveal no expression of both the secretory component (SC; **A**) and immunoglobulin (Ig)A (**B**), although IgA is present in plasma cells and extracellularly in the lamina propria. Immunoperoxidase method; original magnification ×200

Before treatment for *H. pylori*, IgA was expressed in both gastric epithelial cells and intestinalized cells, and

Table 1 Degree of epithelial expression of the secretory component (SC) and immunoglobulin (Ig)A and inflammation and *Helicobacter pylori* colonization in the gastric mucosa. Scores were expressed as median score with the interquartile range in parentheses. *Mono* mononuclear cell infiltration; *Neut* neutrophil infiltration

	SC	IgA	Mono	Neut	*H. pylori*
Antrum					
Normal volunteers	0.0 (0.0)	0.0 (0.0)	0.5 (0.0–1.0)	0.0 (0.0)	0.0 (0.0)
H. pylori-positive patients					
Pretreatment	3.0[a] (2.0–3.0)	2.0[a] (2.0–3.0)	2.0[a] (2.0)	1.0[a] (1.0)	1.0[a] (1.0–2.0)
Posttreatment	0.0[b] (0.0–1.0)	0.0[b] (0.0–1.0)	1.0[b] (1.0)	0.0[b] (0.0)	0.0[b] (0.0)
Corpus					
Normal volunteers	0.0 (0.0)	0.0 (0.0)	0.0 (0.0–1.0)	0.0 (0.0)	0.0 (0.0)
H. pylori-positive patients					
Pretreatment	3.0[a] (1.0–3.0)	2.0[a] (1.0–3.0)	2.0[a] (1.0–2.0)	1.0[a] (0.0–1.0)	2.0[a] (1.0–2.0)
Posttreatment	0.0[b] (0.0)	0.0[b] (0.0)	1.0[b] (1.0)	0.0[b] (0.0)	0.0[b] (0.0)

[a] Scores were analyzed using the Mann-Whitney U-Test (control vs pretreatment; $P<0.01$)
[b] Scores were analyzed using the Wilcoxon signed-ranks test (pretreatment vs posttreatment; $P<0.01$)

in plasma cells in the lamina propria of the gastric mucosa (Fig. 5D). Gastric epithelial cells showing IgA immunoreactivity also exhibited the SC immunoreactivity, although IgA-immunoreactive cells were less frequent than SC-immunoreactive cells (Fig. 5C, D). The degree of IgA expression in gastric mucous cells in biopsy specimens (whether from the antrum or the corpus) showed a good correlation with the degree of mononuclear cell infiltration and the SC expression ($P<0.01$).

The expressions of the SC and IgA in gastric mucous cells were both stronger in the lower part of the foveolaisthmus zone than in the upper part ($P<0.01$) (Fig. 5C, D and Fig. 7). In contrast, the *H. pylori* colonization was less dense in the lower part than in the upper part ($P<0.01$; Fig. 5F and Fig. 7). However, there was no significant correlation between the degree of SC expression and that of *H. pylori* colonization.

After the eradication of *H. pylori*, distributions of the SC and IgA were similar to those found before the eradication; however, after the eradication of *H. pylori*, gastric mucosa revealed no or less dense epithelial expressions of IgA and the SC in the gastric mucous cells than before the eradication (Fig. 8). After the eradication of *H. pylori*, the staining scores for epithelial expressions of IgA and the SC and the degree of inflammation decreased significantly ($P<0.01$; Table 1).

Discussion

In this study, we assessed the expressions of the SC and IgA in epithelial cells in gastric biopsy specimens from *H. pylori*-infected patients before and after *H. pylori* eradication, surgically resected stomachs with *H. pylori* infection, and gastric biopsy from normal volunteers. In addition, histochemical and immunohistochemical staining procedures were used to identify gastrointestinal epithelial cells. The data obtained indicates that the SC and IgA are both expressed in the gastric mucous cells around the generating zone of the gastric mucosa in *H. pylori*-associated gastritis.

Epithelial expressions of the SC and IgA in *H. pylori*-associated gastritis have been reported before [1, 6, 8, 10, 12, 22]. However, those studies relied on morphology to define gastric epithelial cells. In this study, we investigated the cell-lineage specificity of the expressions of the SC and IgA in gastric epithelial cells using a battery of immunostaining and histochemical staining techniques to label gastrointestinal cells. By doing this, we were able to confirm the epithelial expressions of the SC and IgA in *H. pylori*-associated gastritis and to extend the analysis. As reported previously and confirmed in this study, the SC and IgA were expressed in intestinal metaplastic cells that were positive for the intestinal cell markers CD10 or MUC2. In addition, we found that the SC and IgA were not expressed in epithelial cells in normal gastric mucosa, although they were expressed in the mucous cells localized in the generating zone [were positive for gastric mucous cell markers (PA-TS-Con A-HRP, M1, and HIK1083), but not for intestinal cell markers (MUC2 and CD10)] in *H. pylori*-associated gastritis. It remains to be clarified whether this epithelial expression of the SC in the gastric mucous cells around the generating zone would be the beginning of the intestinalization. Its clarification would be important, because within the gastric cancer risk index, the presence of intestinal metaplasia was reported to be the only criteria associated with the development of intestinal-type gastric cancer in Japan [20].

The epithelial expressions of the SC and IgA were significantly greater before the eradication of *H. pylori* than after the eradication of *H. pylori*. Furthermore, we confirmed that the epithelial expressions of the SC and IgA showed a significant correlation with the degree of mononuclear cell infiltration in the gastric lamina propria [6]. This indicates that the epithelial expression of these antigens may be reversible. Similarly, reversible changes in gastric mucins also have been reported in *H. pylori*-associated gastritis [2, 17].

The expressions of the SC and IgA in gastric epithelial cells have been reported not to be specific for *H. pylori* infection, but rather to reflect the presence of in-

flammatory processes in the gastric mucosa [5]. In the human colonic carcinoma-derived cell line HT-29, the expression of the SC has been reported to be stimulated by interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-4 [14]. In the gastric mucosa in *H. pylori*-associated gastritis, IFN-γ and TNF-α have been reported to be elevated to a degree that correlates well with that of mononuclear cell infiltration in the gastric lamina propria [11, 18, 23]. In addition, Ahlstedt et al. found that the expression of the SC in the antral mucosa of *H. pylori*-associated gastritis correlated well with the occurrence of IFN-γ- or TNF-α-positive lymphocytes in the gastric mucosa, and that IFN-γ-positive lamina propria lymphocytes were detected in the greatest number in the region of the gastric glands, where there was also an intense epithelial expression of the SC [1]. Possibly, gastric mucous cells in the generating zone may be susceptible to IFN-γ or TNF-α, which are putative factors for the up-regulation of SC expression in the gastric mucosa.

The numbers of cells expressing the SC and IgA decreased towards the gastric surface, while the degree of *H. pylori* colonization increased towards the gastric surface, although the epithelial expressions of the SC and IgA did not show a significant inverse correlation with *H. pylori* density. Possibly, this may indicate that the local immune response to *H. pylori*, although not good enough to achieve the bacterial clearance, acts as a part of the antibacterial defense system by which cells in the gastric generating zone act against *H. pylori* infection.

In conclusion, this study has shown that gastric surface mucous cells and gland mucous cells in the gastric generating zone have the potential for SC-mediated translocation of IgA into the gastric lumen, a process that may form part of the antibacterial defense system of these cells against *H. pylori* infection.

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