tenuate ghrelin production in the stomach and may consequently reduce plasma ghrelin concentrations in *H. pylori*-positive subjects. To examine this hypothesis, we next examined the specific changes of gastric ghrelin production in association with *H. pylori* infection.

Ghrelin mRNA in gastric mucosa is lower in H. pylori-positive subjects

In an effort to examine the effects of *H. pylori* infection on ghrelin production in the gastric mucosa, we compared gastric ghrelin mRNA expression levels between *H. pylori*-positive and -negative subjects using real-time quantitative RT-PCR using corpus mucosa because gastric ghrelin is predominantly produced in the corpus rather than in the antrum (10). As shown in Fig. 2, gastric ghrelin mRNA levels of corpus mucosa were significantly lower in *H. pylori*-positive patients than *H. pylori*-negative controls. A similar difference was also significantly observed between patients with chronic gastritis alone and *H. pylori*-negative controls (data not shown). These results suggest that the expression of ghrelin mRNA in the gastric mucosa is markedly decreased in association with *H. pylori* infection. It is important to note that the average of gastric ghrelin mRNA expression levels in *H. pylori*-positive subjects was less than one 45th of that in *H. pylori*-negative controls. Moreover, as shown in Fig. 3, plasma ghrelin concentrations were in parallel with the ghrelin mRNA expression levels in *H. pylori*-positive subjects. Taken together, these results suggest that the attenuation of the ghrelin production in the gastric mucosa accounts for the decrease in the plasma ghrelin concentrations in *H. pylori*-positive individuals.

Ghrelin-producing cells in the gastric mucosa are lower in H. pylori-positive subjects

As an independent test to examine the effect of *H. pylori* infection on gastric ghrelin production and its relation with plasma ghrelin concentrations, we next investigated the numbers of ghrelin-producing cells of the corpus mucosa in *H. pylori*-positive and -negative subjects. For this purpose, biopsy samples taken from gastric mucosa were immunostained using an antighrelin polyclonal antibody. Immunoreactive cells were seen in the lower half of fundic epithelial glands as described previously (10). No immunoreactivity was detected in the tissue when control serum was used for staining (data not shown). Immunoreactivity was concentrated in the basal cytoplasm of the positive cells as shown in Fig. 4, A and B. As shown in Fig. 4C, the number of ghrelin-positive cells in the gastric mucosa of *H. pylori*-positive individuals was significantly lower than those of *H. pylori*-negative individuals. Furthermore, the numbers of ghrelin-positive cells in the gastric mucosa fell significantly in accompaniment to the decrease in plasma ghrelin concentrations in *H. pylori*-positive subjects (Fig. 5). These results reinforce that the attenuation of the gastric ghrelin production caused by *H. pylori* infection accounts for the decrease in the plasma ghrelin concentrations in *H. pylori*-positive individuals.

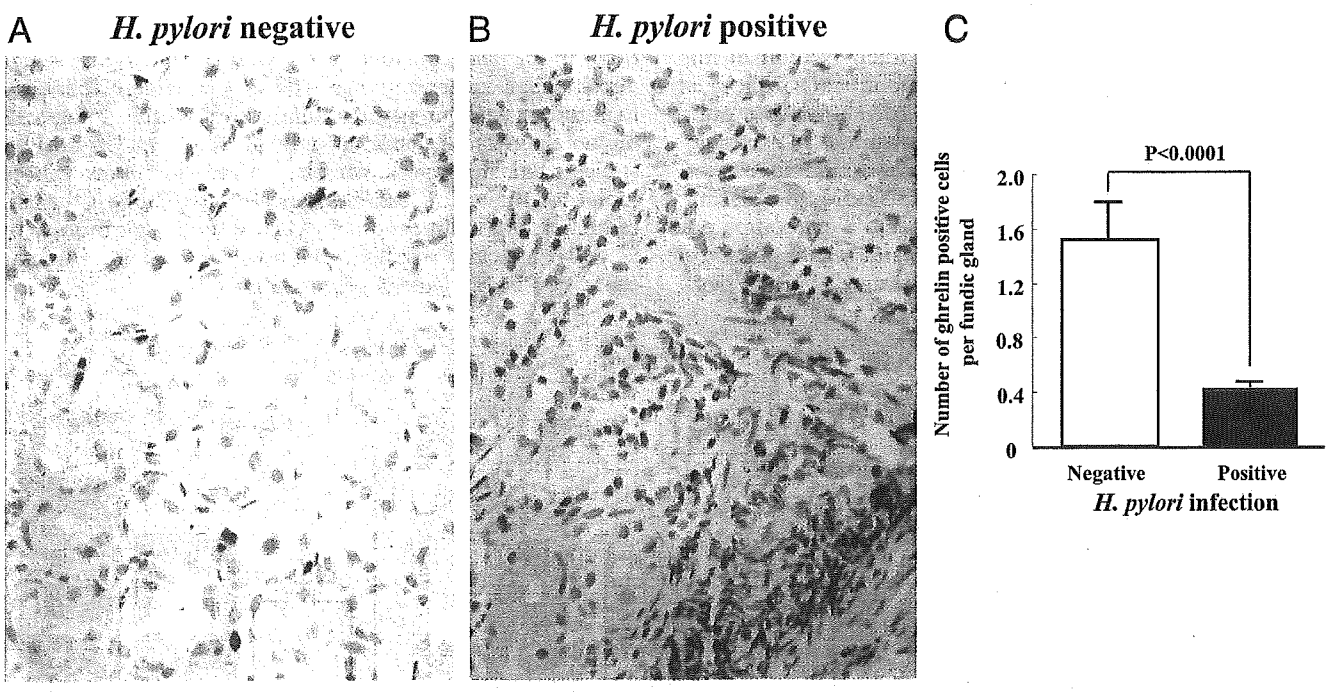

FIG. 4. Immunostaining of ghrelin in the fundic glands. A, Normal epithelium without *H. pylori* infection. B, Inflammatory gastric mucosa with *H. pylori* infection. Ghrelin-producing cells were abundant in the mucosa from *H. pylori*-negative subject, whereas they were seldom observed the mucosa from *H. pylori*-positive subject (magnification, ×200). C, Numbers of the immunoreactive cells in *H. pylori*-positive and -negative subjects. Immunoreactive cells in the gastric mucosa were counted and presented as the number of positive cells per branch of oxyntic glands. Values are expressed as the mean ± SE. The numbers of the immunoreactive cells in the gastric mucosa were significantly lower in the *H. pylori*-positive subjects than the *H. pylori*-negative controls ($P < 0.0001$ by unpaired two-tailed t test).

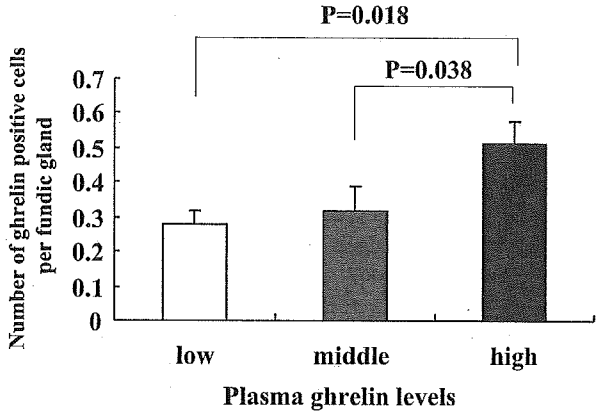

FIG. 5. Comparison of the frequencies of the immunoreactive cells in *H. pylori*-positive subjects among different levels of plasma ghrelin groups. Values are expressed as the mean ± SE. There was a correlation between plasma ghrelin concentrations and the frequencies of the immunoreactive cells with a correlation coefficient value of 0.41 ($P < 0.0001$). The numbers of the positive cells were significantly lower in the low and middle ghrelin groups than those in the high ghrelin group (low *vs.* high: $P = 0.018$; middle *vs.* high: $P = 0.038$ by variance (ANOVA) based on Fisher's protected least significant difference test).

Plasma ghrelin concentrations are associated with serum pepsinogen concentrations in H. pylori-positive subjects

In the last sets of examinations, we further attempted to demonstrate the association between *H. pylori* infection and plasma ghrelin concentrations. Because *H. pylori* infection first induces gastric atrophy in its pathological course, we compared plasma ghrelin concentration with serum pepsinogen concentrations in *H. pylori*-positive patients. Pepsinogen I and pepsinogen II differ in their location in the stomach. Both are located in the chief and mucous neck cells of the oxyntic gland mucosa in the gastric corpus, but only pepsinogen II is present in the gastric antrum. A pepsinogen I to II ratio less than 3 is considered to be a reliable marker for severe atrophic gastritis (19, 20). Therefore, the plasma ghrelin levels in *H. pylori*-positive patients were compared with serum pepsinogen concentrations and serum pepsinogen I to II ratios. As shown in Fig. 6, serum levels of pepsinogen I and the ratio of pepsinogen I to II fell significantly as plasma ghrelin concentrations decreased, indicating the positive association between plasma ghrelin and pepsinogen I concentrations as well as pepsinogen I to II ratios in *H. pylori*-positive patients. Collectively, these results reveal that plasma ghrelin concentrations are associated with the progression of gastric atrophy.

Discussion

Plasma ghrelin levels have been associated with several clinical factors including BMI, food intake, and serum insulin levels (9, 21, 22). Although ghrelin-producing endocrine cells have been found mainly in the oxyntic mucosa of the stomach, ghrelin is also released from other tissues including small and large intestines, lung, kidney, the nucleus of the hypothalamus, and A cells of the pancreatic islet (23, 24). In fact, plasma ghrelin concentrations in gastrectomized patients still remain about one third of those in normal subjects (6). Thus, it is important to clarify which organ primarily influences changes in plasma ghrelin concentrations in each disease. In this study, we have demonstrated that plasma ghrelin concentrations are influenced by *H. pylori* infection. In particular, we focused on the gastric mucosa to better understand the effects of *H. pylori* infection on the alteration of ghrelin expression. The expression levels of ghrelin mRNA and the numbers of ghrelin-producing cells in the gastric mucosa were much lower in patients with *H. pylori* infection. Plasma ghrelin concentrations correlated with the gastric ghrelin mRNA as well as the frequency of ghrelin-immunoreactive cells in the gastric mucosa. Finally, we compared plasma ghrelin concentrations with serum pepsinogen levels, a marker for gastric atrophy. Plasma ghrelin concentrations in *H. pylori*-positive patients correlated with serum pepsinogen I concentration as well as pepsinogen I to II ratio. In addition, we demonstrated that groups with histologically higher degrees of gastric atrophy in the *H. pylori*-positive subjects tend to have lower plasma ghrelin concentrations (data not shown). These findings strongly suggest that the reduction of ghrelin-producing cells in the gastric mucosa by *H. pylori* infection results in the lower plasma ghrelin concentration in *H. pylori*-positive patients.

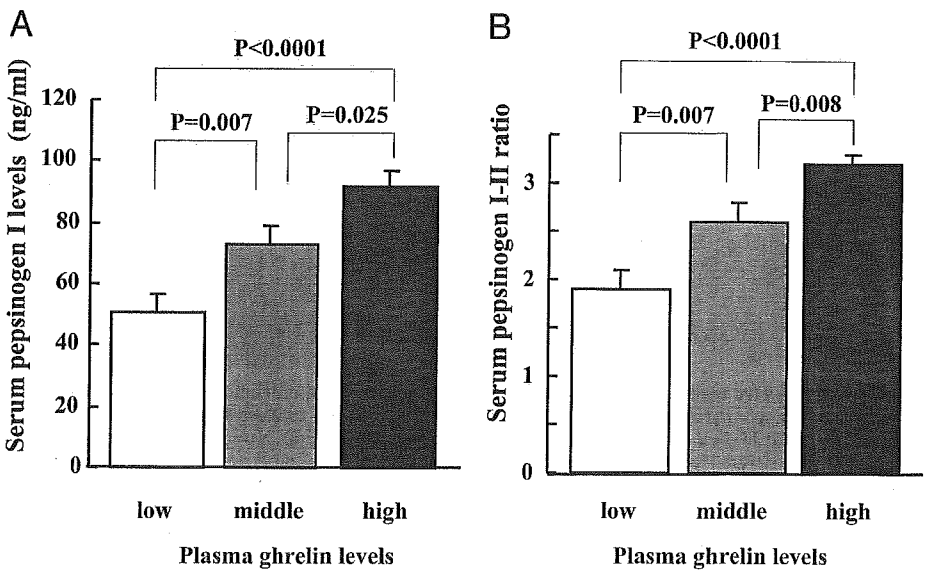

FIG. 6. Comparison of plasma ghrelin levels and serum pepsinogen levels in *H. pylori*-infected subjects. Values are expressed as the mean ± SE. Both serum pepsinogen I levels and pepsinogen I to II ratio significantly fell with the decrease of plasma ghrelin levels (A and B). An ANOVA based on Fisher's protected least significant difference test was used.

Our current data are consistent with the report of Nwokolo's group (17) that plasma ghrelin concentrations increased after *H. pylori* eradication. In their study, however, gastric ghrelin before and after *H. pylori* eradication was not measured. Moreover, effects of a change in BMI before and after *H. pylori* eradication on plasma ghrelin concentrations could not be excluded. In addition, the number of the enrolled subjects in their study was relatively small (10 subjects). Therefore, our present study expanded their observations by enrolling many more subjects with comparable BMI and showing a direct association between *H. pylori* infection and lower gastric ghrelin production.

On the other hand, Gokcel's group compared plasma ghrelin concentrations between *H. pylori*-positive and -negative subjects, and, opposed to our results, they found no differences. Although their study design was similar to ours, they did not provide any data on the gastric atrophy or gastric ghrelin production in their subjects. It is, therefore, only a speculation, but the discrepancy of the results may be due to the different features of gastric atrophy between Western and Japanese populations including disease frequency and severity. The earlier age of acquiring *H. pylori* infection in Japan, compared with Western countries, may also explain the high incidence of atrophic gastritis in Japanese adults and lower concentrations of plasma ghrelin concentrations as well.

It would be intriguing to clarify how a persistent decrease in plasma ghrelin concentration influences human growth and body weight. Recently several reports demonstrated that *H. pylori*-positive children have a high incidence of growth retardation (25, 26). *H. pylori* is acquired early in life in most of the developing world. Together with our results, the decrease of plasma ghrelin levels accompanied by *H. pylori* gastritis may have considerable influences on growth retardation in childhood. In our study, the plasma ghrelin levels in *H. pylori*-positive subjects were lower than *H. pylori*-negative subjects, even in patients with mild atrophic changes, implying that even mild gastric inflammation by *H. pylori* infection in children may reduce the production of gastric ghrelin. Further study in children including plasma ghrelin levels, degree of atrophy in the stomach, and presence of *H. pylori* infection may clarify these relationships.

In conclusion, our study indicates that plasma ghrelin concentrations are influenced by the presence of chronic gastritis in association with *H. pylori* infection. Decreases in gastric ghrelin production may account for lower concentrations of plasma ghrelin in *H. pylori*-positive individuals. These observations provide novel insights for understanding the physiological function of ghrelin and its relation to various diseases.

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References

1. **Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S** 2001 Ghrelin acts in the central nervous system to stimulate gastric acid secretion. Biochem Biophys Res Commun 280:904–907
2. **Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K** 2000 Ghrelin stimulates gastric acid secretion and motility in rats. Biochem Biophys Res Commun 276:905–908
3. **Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S** 2001 A role for ghrelin in the central regulation of feeding. Nature 409:194–198
4. **Tschop M, Smiley DL, Heiman ML** 2000 Ghrelin induces adiposity in rodents. Nature 407:908–913
5. **Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M** 2001 Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology 120:337–345
6. **Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ** 2002 Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 346:1623–1630
7. **Bellone S, Rapa A, Vivenza D, Vercellotti A, Petri A, Radetti G, Bellone J, Broglio F, Ghigo E, Bona G** 2003 Circulating ghrelin levels in newborns are not associated to gender, body weight and hormonal parameters but depend on the type of delivery. J Endocrinol Invest 26:RC9–RC11
8. **Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, LaFranchi SH, Purnell JQ** 2003 Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab 88:174–178
9. **Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S** 2002 Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 87:240–244
10. **Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M** 2000 Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 141: 4255–4261
11. **Marshall BJ, Goodwin CS, Warren JR, Murray R, Blincow ED, Blackbourn SJ, Phillips M, Waters TE, Sanderson CR** 1988 Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. Lancet 2:1437–1442
12. **Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ** 2001 *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 345: 784–789
13. **Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG** 1993 Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 342:575–577
14. **Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ** 1991 *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 325:1132–1136
15. **Parsonnet J, Friedman GD, Orentreich N, Vogelman H** 1997 Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. Gut 40:297–301
16. **Goodwin CS, Mendall MM, Northfield TC** 1997 *Helicobacter pylori* infection. Lancet 349:265–269
17. **Nwokolo CU, Freshwater DA, O'Hare P, Randeva HS** 2003 Plasma ghrelin following cure of *Helicobacter pylori*. Gut 52:637–640
18. **Gokcel A, Gumurdulu Y, Kayaselcuk F, Serin E, Ozer B, Ozsahin AK, Guvener N** 2003 *Helicobacter pylori* has no effect on plasma ghrelin levels. Eur J Endocrinol 148:423–426
19. **Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI** 1982 Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterology 83:204–209
20. **Karnes Jr WE, Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SW, Walsh JH** 1991 Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. Gastroenterology 101:167–174

21. **Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S** 2002 Ghrelin is present in pancreatic α-cells of humans and rats and stimulates insulin secretion. Diabetes 51:124–129
22. **Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML** 2001 Circulating ghrelin levels are decreased in human obesity. Diabetes 50: 707–709
23. **Mori K, Yoshimoto A, Takaya K, Hosoda K, Ariyasu H, Yahata K, Mukoyama M, Sugawara A, Hosoda H, Kojima M, Kangawa K, Nakao K** 2000 Kidney produces a novel acylated peptide, ghrelin. FEBS Lett 486: 213–216
24. **Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K** 2001 Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 86: 4753–4758
25. **Demir H, Saltik IN, Kocak N, Yuce A, Ozen H, Gurakan F** 2001 Subnormal growth in children with *Helicobacter pylori* infection. Arch Dis Child 84:89–90
26. **Patel P, Mendall MA, Khulusi S, Northfield TC, Strachan DP** 1994 *Helicobacter pylori* infection in childhood: risk factors and effect on growth. BMJ 309:1119–1123

宿主サイドからのアプローチ(宿主側因子)

H. pylori 感染における血漿グレリン濃度と胃粘膜グレリン発現量

Plasma and gastric ghrelin levels in subjects with *Helicobacter pylori* infection

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Key words：血漿グレリン，胃粘膜グレリン，*H. pylori* 感染，萎縮性胃炎

はじめに

成長ホルモン(GH)の分泌は，これまで視床下部ホルモンである成長ホルモン放出ホルモン(GHRH)およびソマトスタチンによりそれぞれ促進性，抑制性に制御されていると考えられていた．1996年にヒト，ラット，ブタの視床下部と下垂体でのGHS-Rの存在と1次構造が明らかにされ[1]，それまで存在が疑問視されていた内在性リガンドの探索が，国内外で競って行われた．1999年に児島，寒川ら[2]は新しい成長ホルモン分泌促進ペプチド，グレリンを発見・構造決定に成功した．彼らはGHS-Rを安定発現する培養細胞を調製し，細胞内カルシウムイオン濃度の上昇を指標とするアッセイ系を確立した．これを用いて胃抽出物中にGHS-Rに対する非常に強い活性分画を見いだし，グレリン(ghrelin)と名づけた．現在では，哺乳類以外の動物でもその構造が決定され，グレリンが進化上，保存されてきた機能的に重要なホルモンであることがうかがえる．グレリンは成長ホルモン分泌促進作用，強力な摂食促進作用[3]，消化管運動促進作用[4]，胃酸分泌促進作用[5]を有し，また視床下部に作用して体重増加を促す[3]．血漿グレリン濃度は食前に上昇し，食後に低下する．このペプチドは成長や脂肪組織量の制御に関与し，短期の食欲促進や長期的な体重の制御物質でもある[4,6,7]．

1. グレリンの構造

単離されたグレリンのペプチドは28アミノ酸残基よりなり，興味深いことに3番目のセリン残基の側鎖は炭素数8個の脂肪酸，オクタン酸によってエステル化されていた．このオクタン酸によるアシル化修飾はグレリンの生物活性発現に重要であり，アシル化修飾のないペプチド結合鎖のみでは全くGH分泌作用は示さないと報告されてきた．しかしながら，最近では非アシル化グレリンは，アシル化グレリンと反対の作用を示すという内容が我が国の学会で報告され，今後の詳細な報告を待ちたい．

2. グレリンの摂食促進作用

グレリンは脳内の視床下部弓状核のニューロンでも産生され，グレリン受容体は脳の様々な部位で発現しているので，種々の中枢生理機能に関与していると考えられていた．グレリンをラットの脳室内に投与すると，摂食が促進されて体重増加をもたらし，この効果は，遺伝的に成長ホルモンを欠くラットにもみられる．逆に

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0047-1852/05/¥40/頁/JCLS

グレリン抗体を投与すると，摂食が強く抑制される．グレリンの脳室内投与後に，ニューロン活性化の指標となるFos蛋白質の発現が，神経ペプチドY(NPY)産生ニューロンやアグーチ関連蛋白質(AGRP)産生ニューロンを含む摂食制御に重要な複数の脳領域で起こる．NPYやAGRPに対する抗体を投与すると，グレリン誘発性の摂食行動は消失する[3]．グレリンはNPY遺伝子の発現を増強し，レプチンで誘発される摂食低下を抑えることから，グレリンとレプチンは摂食行動に関して拮抗的に作用するといえる．このように，グレリンは摂食行動の生理的信号物質であり，成長ホルモンの分泌と摂食を促進して成長を制御する機能をもつ．

3. グレリンの分布

ヒトのグレリンは，ペプチドとmRNAともに胃に最も多く，腸，膵臓，視床下部，胎盤，腎臓などでも産生される．胃グレリンは胃体部の内分泌細胞で主に産生される．胃体部には，ヒスタミンとウログアニリンを産生するenterochromaffin-like(ECL)細胞，ソマトスタチンを産生するD細胞，セロトニンを産生するenterochromaffin(EC)細胞に加え，膵臓でグルカゴンを産生するA細胞と形態が類似しているがグルカゴンを含まず，また産生物質が不明なことからA-like細胞(またはX細胞)と呼ばれていた内分泌細胞の4種類がある．これらの既知の細胞が産生する物質とグレリンとの二重免疫染色の結果，グレリン産生細胞は前三者の内分泌細胞とは異なっていた．グレリン抗体を用いた免疫電顕により，グレリンは直径120nmでほぼ均一なサイズの電子密度の高い分子顆粒に貯蔵されていることが明らかになった[8]．グレリン細胞は過去の消化管内分泌細胞の命名法に準じ，Gr細胞と呼ばれている．

4. 血漿グレリン濃度

新生児では血漿グレリン濃度は性差や体重によって影響されないが[9]，小児や成人の肥満者では正常体重者や痩せ型の人より低下している[10]．血漿グレリンの大半は胃で産生されるので，慢性胃粘膜障害では，その産生が障害される可能性がある．*H. pylori*感染は胃十二指腸潰瘍，慢性萎縮性胃炎に関連しているので，この菌が胃グレリン産生だけでなく血漿グレリン濃度に影響を及ぼすかどうか検討することは重要である．

欧州ではNwokoloら[11]は，血漿グレリン濃度は*H. pylori*除菌後に上昇すると報告し，一方，Gokcelら[12]は*H. pylori*感染の有無は血漿グレリン濃度に影響しないと報告した．しかし，これまでの報告では，*H. pylori*感染胃粘膜のグレリン発現について検討されてはこなかった．

5. *H. pylori*感染による胃グレリンの産生障害

日本人では*H. pylori*感染に伴う重度の萎縮性胃炎がみられることが多い．著者らは，内視鏡生検材料を用いて*H. pylori*感染胃粘膜のグレリンmRNA量とペプチド発現，更に血漿グレリン濃度を比較検討した[13]．体重の影響を最小限にするため，正常肥満指数(18.5-25)を有する*H. pylori*陽性者110人と陰性者50人において空腹時血漿グレリン濃度を測定し，更に胃体部大彎粘膜からRNAを抽出してreal-time RT-PCR法にて胃グレリンmRNA量を測定した．また，*H. pylori*陽性者の血漿グレリン濃度を高グレリン群(≧150fmol/ml，n=40)，中グレリン群(70-150fmol/ml，n=36)，低グレリン群(<70fmol/ml，n=34)に分類し，それらを慢性胃炎の萎縮の程度，胃グレリンmRNA量，胃グレリン陽性細胞数と比較した．

*H. pylori*陽性者の血漿グレリン濃度および胃グレリンmRNA量は*H. pylori*陰性者より低下していた(**図1，2**)．*H. pylori*陽性者では血漿グレリン濃度が低い症例ほど胃グレリンmRNA量の低下(**図3**)と組織学的に強い萎縮を認め，血清ペプシノゲンIおよびI-II比(**図4**)も有意に低下していた．また，グレリン陽性細胞数は*H. pylori*陽性粘膜で減少し(**図5**)，血漿グレリン濃度が低い症例ほどその低下は顕著となった．

胃グレリンとの関連でCummingsら[7]は，胃切除後の血漿グレリン濃度が約1/3に低下すると報告した．著者らの検討では*H. pylori*慢性胃炎

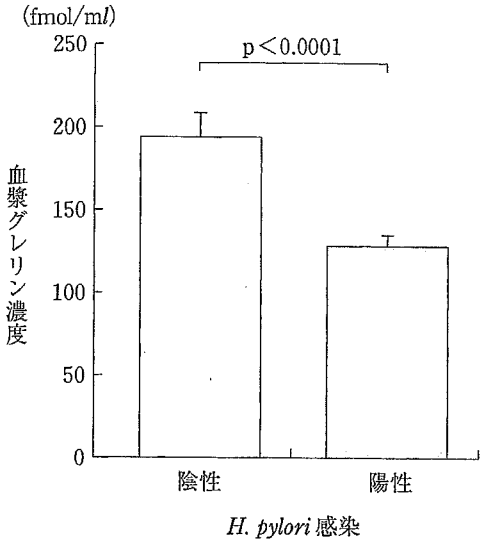

図1 血漿グレリン濃度[13]

H. pylori 陽性者の血漿グレリン濃度は *H. pylori* 陰性者と比較して有意に低下していた(128±8 vs 194±15 fmol/ml).

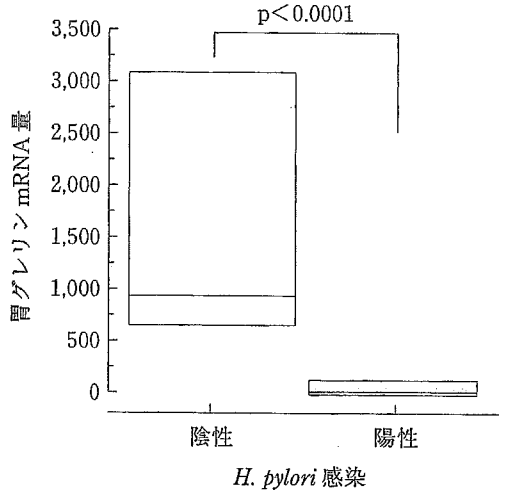

図2 胃グレリン mRNA 量[13]

real-time RT-PCR法にて胃体部粘膜内グレリン mRNA を定量した. *H. pylori* 陽性者の胃粘膜内グレリン mRNA 量は *H. pylori* 陰性者と比較して著明に低下していた.

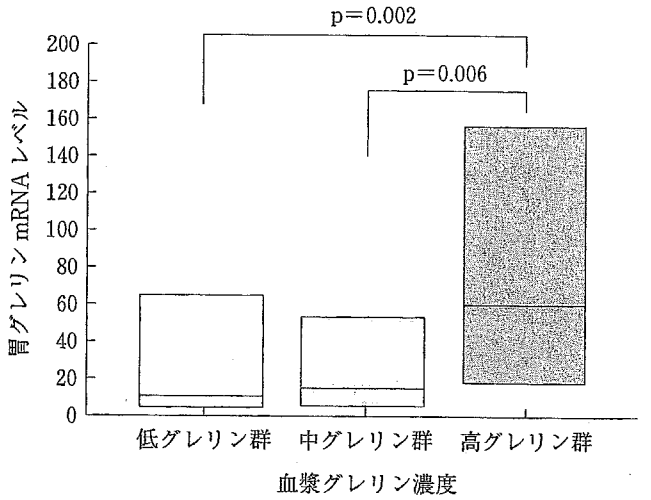

図3 *H. pylori* 感染胃粘膜グレリン mRNA 量[13]

H. pylori 陽性者では血漿グレリン濃度が低い症例ほど胃粘膜グレリン mRNA 量は低下していた.

では血漿グレリン濃度は *H. pylori* 陰性者に比べて約2/3に低下していた. これは胃グレリン産生が *H. pylori* 感染による胃粘膜の炎症や萎縮と関連して血漿グレリン濃度に大きく影響することを示している. 逆に, 除菌治療によって胃グレリン産生が回復するのかもしれない. 萎縮性変化との検討では血清ペプシノゲンの低下と血漿グレリン濃度および胃グレリン mRNA 産生の低下が相関しており, 胃体部粘膜の組織学的な萎縮の程度とも相関していた. 更に萎縮のない *H. pylori* 陽性症例と *H. pylori* 陰性症例の比較では, 陽性症例で血漿グレリン濃度の有意な低下がみられ, 炎症だけでも胃グレリン産生は低下すると考えられた.

欧米では *H. pylori* 感染は血漿グレリン濃度に影響しないとの報告[12]もみられる. しかし, 研

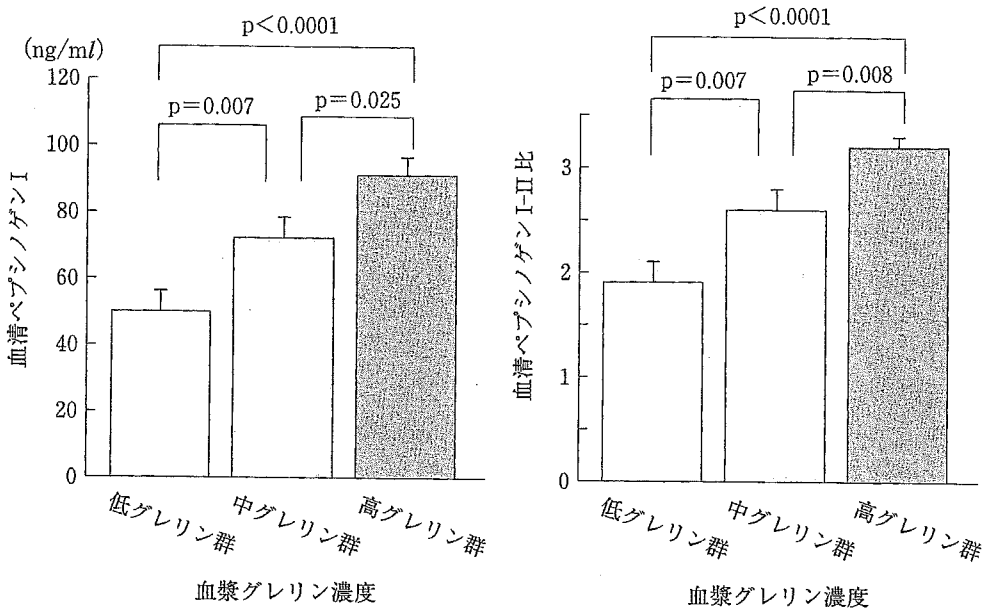

図4 血清ペプシノゲンと血漿グレリン濃度[13]

H. pylori 陽性者では血漿グレリン濃度が低い症例ほど血清ペプシノゲンIおよびI-II比も有意に低下していた.

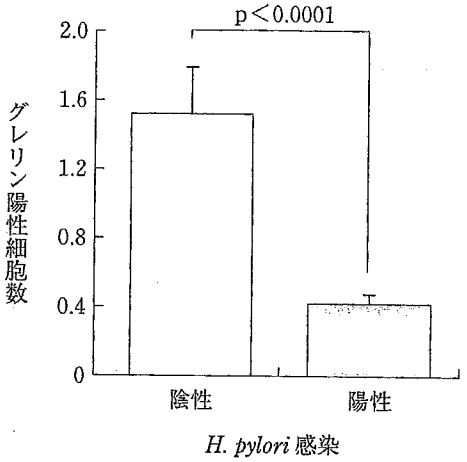

図5 グレリン陽性細胞[13]

グレリン陽性細胞数(1腺管あたり)は *H. pylori* 陽性粘膜で有意に減少していた.

究対象症例が少なすぎる点，肥満指数によるグレリン濃度の変化を考慮していない点，*H. pylori* 感染の胃粘膜への影響を検討していない点において，その結果には疑問が残る.

6. 今後の課題

以前から *H. pylori* 感染児の発育遅延が報告されている[14]. *H. pylori* は発展途上国では幼児期に感染している. グレリンは強力な成長ホルモン分泌促進作用を有しており，小児における胃グレリン産生が *H. pylori* 感染によって影響を受けている可能性が高く，今後の検討課題である. また，最近グレリンの粘膜防御作用が報告されている[15]. 逆に *H. pylori* 感染によるグレリン産生低下が胃粘膜防御機能低下に関連している可能性も否定できない. 更にグレリンは消化管運動とも関連しており，運動機能異常を来す消化管疾患についても検討が必要である.

おわりに

グレリンの発見により胃が消化機能だけでなく，成長ホルモンの分泌調節や摂食調節にも機能していることが明らかになった. 更に *H. pylori* 感染は胃グレリン産生を低下させ，最終的に血漿グレリン濃度を低下させる. 血漿グレリン濃度は，胃粘膜内グレリンmRNA量と正の相関性を示しており，また組織学的および血清学的検討から慢性胃炎，特に萎縮の程度を反映していた.

文　献

1) Howard AD, et al: A receptor in pituitary and hypothalamus that functions in growth hormone release. Science **273**: 974-977, 1996.
2) Kojima M, et al: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature **402**: 656-660, 1999.
3) Nakazato M, et al: A role for ghrelin in the central regulation of feeding. Nature **409**: 194-198, 2001.
4) Asakawa A, et al: Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology **120**: 337-345, 2001.
5) Masuda Y, et al: Ghrelin stimulates gastric acid secretion and motility in rats. Biochem Biophys Res Commun **276**: 905-908, 2000.
6) Tschop M, et al: Ghrelin induces adiposity in rodents. Nature **407**: 908-913, 2000.
7) Cummings DE, et al: Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med **346**: 1623-1630, 2002.
8) Date Y, et al: Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology **141**: 4255-4261, 2000.
9) Bellone S, et al: Circulating ghrelin levels in newborns are not associated to gender, body weight and hormonal parameters but depend on the type of delivery. J Endocrinol Invest **26**: RC9-11, 2003.
10) Shiiya T, et al: Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab **87**: 240-244, 2002.
11) Nwokolo CU, et al: Plasma ghrelin following cure of *Helicobacter pylori*. Gut **52**: 637-640, 2003.
12) Gokcel A, et al: *Helicobacter pylori* has no effect on plasma ghrelin levels. Eur J Endocrinol **148**: 423-426, 2003.
13) Osawa H, et al: Impaired production of gastric ghrelin in chronic gastritis associated with *Helicobacter pylori*. J Clin Endocrinol Metab **90**: 10-16, 2005.
14) Demir H, et al: Subnormal growth in children with *Helicobacter pylori* infection. Arch Dis Child **84**: 89-90, 2001.
15) Konturek PC, et al: Ghrelin-a new gastroprotective factor in gastric mucosa. J Physiol Pharmacol **55**: 325-336, 2004.

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Histamine-2 receptor expression in gastric mucosa before and after Helicobacter pylori *cure*

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SUMMARY

Background: *Helicobacter pylori* infection prevents the occurrence of the tolerance phenomenon of Histamine-2 (H2) receptor antagonists. Gastro-esophageal reflux disease develops in some cases with the restoration of acid secretion after *H. pylori* eradication therapy.
Aim: To clarify the mechanisms of H2 receptor restoration after the eradication of *H. pylori* on parietal cells.
Methods: We enrolled 80 consecutive asymptomatic male patients with *H. pylori* infection, having chronic gastritis with or without the presence of peptic ulcers. Biopsy specimens from the greater curvatures at the mid-corpus of the stomach were obtained endoscopically from all subjects before and 12 weeks after the eradication of *H. pylori*. Degrees of gastric atrophy were evaluated by serum pepsinogen levels. The amounts of mRNA expression of H2 receptor were evaluated in each subject's gastric mucosa by real time reverse transcriptase-polymerase chain reaction (RT-PCR).
Results: H2 receptor mRNA expression levels significantly correlated with serum pepsinogens I and II ratios. The expression level of H2 receptor mRNA was lower in subjects with hypergastrinemia. The median expression level of H2 receptor after *H. pylori* eradication was threefold greater than prior to treatment. In addition, its restoration became more pronounced in subjects with severe gastric atrophy. However, a comparatively low restoration of H2 receptor mRNA was found in subjects with hypergastrinemia.
Conclusions: H2 receptor mRNA levels decrease with the progression of gastric atrophy induced by *H. pylori* infection, and are restored after *H. pylori* eradication. Such expression levels of H2 receptor may explain a part of the tolerance phenomenon to H2 receptor antagonists.

INTRODUCTION

Histamine-2 receptor antagonists (H2RAs) show a potent and quick acid-suppressing effect, especially during the nocturnal period.[1–3] However, the antisecretory activity of H2RAs was reported to decrease during continuous administration, leading to rebound hypersecretion of gastric acid after withdrawal of H2RAs.[3–11] This attenuation of the antisecretory activity of H2RAs has been described as tolerance. In patients with gastro-oesophageal reflux disease (GERD), such rebound hypersecretion may increase acid reflux, making it difficult to stop treatment.[12,13]

Helicobacter pylori infection directly and indirectly influences the physiological functions of gastric parietal cells.[14] Treatment with proton pump inhibitors induces tolerance to H2RAs in *H. pylori*-negative patients.[15] On the other hand, *H. pylori* infection prevents the tolerance phenomenon of H2RAs.[16] Therefore, *H. pylori* infection has a strong effect on tolerance to H2RAs. However, the

Abbreviations: RT-PCR, reverse transcriptase-polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GERD, gastro-oesophageal reflux disease; H2, Histamine-2; H2RAs, Histamine-2 receptor antagonists..

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effect of H2 receptor expression on the restoration of acid secretion after *H. pylori* eradication has not been clearly identified. We therefore conducted this study to investigate the relationship between expression levels of H2 receptor in the oxyntic gland before and after eradication therapy. To this end, we evaluated H2 receptor mRNA expression levels in detail using quantitative real time reverse transcriptase-polymerase chain reaction (RT-PCR) methods. We report here that the expression levels of H2 receptor mRNA decreases with the progression of gastric atrophy in *H. pylori*-infected subjects, and restoration after *H. pylori* cure is threefold greater than the levels prior to treatment.

MATERIALS AND METHODS

Patients

Of those who participated in the gastric cancer surveillance program in Tochigi, Japan, 85 consecutive men with *H. pylori* infection had received eradication therapy; treatment was successful in 80 subjects (mean age ± S.E.; 49.2 ± 0.5 years) enrolled in the present study. Indications of the eradication therapy in these patients included chronic gastritis alone (four patients), chronic gastritis accompanied by either adenoma, a family history of gastric cancer, hyperplastic polyp, severe atrophic gastritis, chronic urticaria, or gastric ulcer (66 patients), duodenal ulcer (10 patients). All subjects were clinically stable at the time of evaluation and had no history of eradication therapy before the study. Written informed consent was obtained from the participants in accordance with the Declaration of Helsinki and its later revision. The Ethics Committee of the Jichi Medical School, Japan, approved this study.

Eradication therapy for H. pylori*-infected subjects*

A triple regimen, composed of lansoprazole 30 mg twice daily, clarithromycin 200 mg twice daily, and amoxicillin 750 mg twice daily, was given for 7 days following endoscopic examination. No other medications were given during the course of the study.

Specimens

Five adjacent biopsy specimens from the greater curvatures at the mid-corpus of the stomach as well as five from the antrum were obtained endoscopically from all subjects. One biopsy specimen from the corpus of the stomach and one from the antrum were cultured individually to assess the presence of *H. pylori* infection. Three biopsy specimens from the corpus and three from the antrum were immediately snap-frozen and stored in liquid nitrogen for later use. The remaining corpus and antral specimens were fixed and stained with hematoxylin and eosin, and Giemsa. Histological assessments were performed by a single observer (H.Os.). The presence of *H. pylori* infection was diagnosed by either positive bacterial culture or positive Giemsa staining.

Blood sample

Before and 12 weeks after the eradication of *H. pylori*, venous blood samples after fasting overnight were obtained for the determination of serum gastrin and serum pepsinogens I and II levels.

RNA extraction and RT-PCR

Total RNA was isolated from biopsy specimens with ISOGEN (Nippon Gene, Tokyo, Japan), and 2 μg of total RNA was reverse-transcribed with random nanomers and reverse transcriptase (TOYOBO, Osaka, Japan), which includes a DNase incubation step. The expression level of H2 receptor mRNA was evaluated using a real-time quantitative RT-PCR method with an ABI 7700 sequence detector system (PE Applied Biosystems, Foster City, CA, USA). The sense primer for H2 receptor was 5′-CCACCATCAGGGAGCACAA-3′ and the antisense primer was 5′-AGGGAAACCAGCAGATGATGAA-3′. The reaction mixture was prepared according to the manufacturer's protocol using TaqMan PCR kits (PE Applied Biosystems). The reactions also contained target hybridization H2 receptor probe labeled with a reporter fluorescent dye, 6-carboxyfluorescein, at the 5′ end (5′-CACAGTGACACTGGCCGCCGTC-3′). Thermal cycling conditions for all reactions included 50 °C for 2 minutes and 95 °C for 10 minutes, followed by 40 cycles of 15 seconds. of denaturation at 95 °C and 1 minute of annealing and extension at 60 °C.

As a control, the mRNA was also subjected to real-time quantitative RT-PCR for the measurement of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using TaqMan GAPDH control reagents (PE Applied Biosystems). For relative quantification of H2 receptor

marker expression, calibration curves were constructed using the mRNA obtained from *H. pylori*-negative gastric mucosa. The levels of H2 receptor mRNA were normalized to that of GAPDH.

Statistical analyses

The level of H2 receptor mRNA was expressed as the median (first quartile to third quartile). The Wilcoxon rank sum test was used to compare H2 receptor mRNA levels before and after *H. pylori* eradication. Differences at $P < 0.05$ were considered significant.

RESULTS

H2 receptor mRNA levels decrease with the progression of gastric atrophy in H. pylori*-infected subjects*

Since the relationship between H2 receptor mRNA and *H. pylori* infection has not yet been adequately elucidated, we first attempted to assess the effect of *H. pylori* infection on H2 receptor mRNA expression. To this end, we compared H2 receptor mRNA expression in oxyntic glands with serum pepsinogen levels. Pepsinogens I and II differ in their location in the stomach. Both are located in the chief and mucous neck cells of the oxyntic gland mucosa in the gastric corpus, but only pepsinogen II is present in the gastric antrum. Serum pepsinogen I significantly correlate with peak acid output.[17] A pepsinogen I/II ratio of <3 is considered to be a reliable marker for severe atrophic gastritis.[18,19] As shown in Figure 1a and b, expression levels of H2 receptor mRNA significantly correlated with serum pepsinogen I/II ratios, but did not correlate with serum pepsinogen I levels. These results reveal that expression levels of H2 receptor mRNA were associated with the progression of gastric atrophy.

The lower expression levels of H2 receptor mRNA in subjects with hypergastrinemia

Reduced gastric acidity induces hypergastrinemia. Therefore, in an additional effort to examine the relationship between gastric atrophy and the expression levels of H2 receptor mRNA, we further attempted to investigate its levels in subjects with hypergastrinemia (>200 pg/mL) who were considered to have severe gastric atrophy.[20,21] As shown in Figure 2, there was a slightly negative correlation of H2 receptor mRNA and serum gastrin levels before *H. pylori* eradication, and extremely lower expression levels of H2 receptor mRNA were found in subjects with hypergastrinemia.

H2 receptor mRNA restore significantly after H. pylori *eradication*

In the next series of examinations, we investigated the restoration of H2 receptor mRNA after *H. pylori* eradication. As shown in Figure 3, the median expression levels after *H. pylori* treatment increased to 2.9 times levels in comparison with the prior levels.

Restoration of H2 receptor mRNA after H. pylori *eradication is enhanced in most subjects, especially with a lower serum pepsinogens I and II ratios*

In an additional effort to examine the relationship between gastric atrophy and restoration of H2 receptor mRNA expression, we investigated the restoration of H2 receptor mRNA in association with the degree of gastric atrophy that *H. pylori*-infection induces in its pathological course. Therefore, H2 receptor mRNA expression levels before and after *H. pylori* cure were compared relative to serum pepsinogen I/II ratios. As shown in Figure 4, H2 receptor mRNA expression levels rose significantly despite the serum pepsinogen I/II ratio

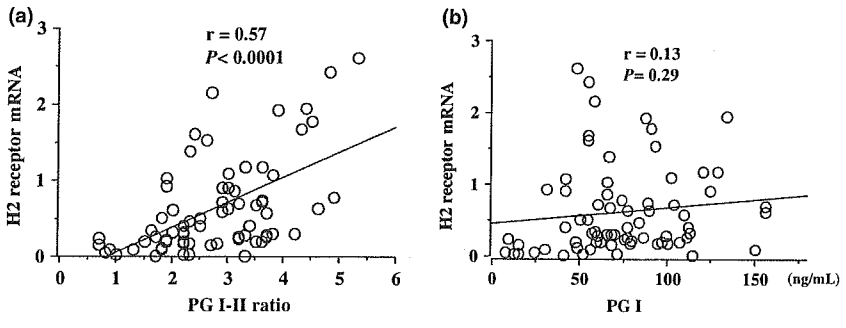

Figure 1. Relationship between H2 receptor mRNA expression in the oxyntic mucosa and serum pepsinogen levels. H2 receptor mRNA expression levels significantly correlate with serum pepsinogen I/II ratios ($n = 80$) (a), but not with serum pepsinogen-I levels ($n = 80$) (b).

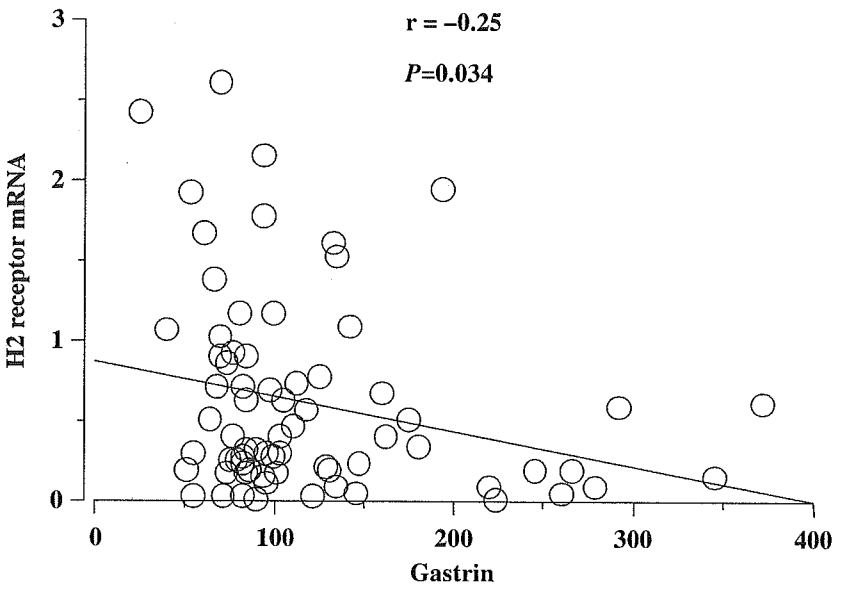

Figure 2. Relationship between H2 receptor mRNA expression in the oxyntic mucosa and serum gastrin levels. There is a slightly negative correlation of H2 receptor mRNA and serum gastrin levels ($n = 80$), and extremely lower expression levels of H2 receptor mRNA are found in subjects with hypergastrinemia (>200 pg/mL).

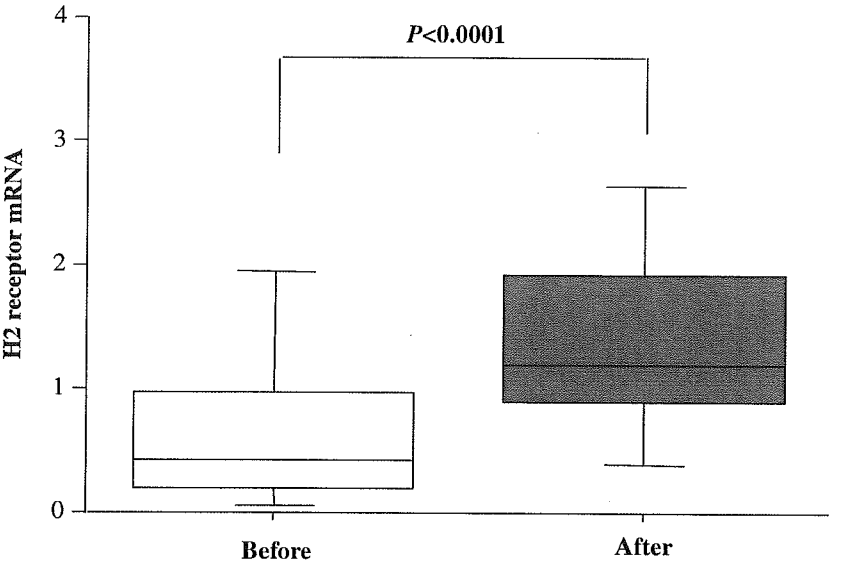

Figure 3. Comparison of H2 receptor mRNA expression levels in the oxyntic mucosa before and after *H. pylori* eradication. The median expression levels after *H. pylori* treatment increased to 2.9 times levels in comparison with prior levels [median (first quartile to third quartile); 0.42 (0.20–0.97) and 1.19 (0.88–1.91), $n = 80$; $P < 0.0001$ by Wilcoxon rank sum test].

levels, in particular showing an increase of 4.2 times in subjects with a lower pepsinogen I/II ratios.

Restoration of H2 receptor mRNA after H. pylori *eradication is less enhanced in subjects with hypergastrinemia*

In the last sets of examinations, we further attempted to investigate H2 receptor mRNA restoration in subjects with hypergastrinemia (>200 pg/mL). As shown in Figure 5, a relatively low restoration of H2 receptor mRNA was found in such subjects, compared with those having normal serum gastrin levels.

DISCUSSION

The present study shows that H2 receptor mRNA levels correlated positively with pepsinogens I and II ratios, suggesting that its levels decreased with the progression of gastric atrophy induced by *H. pylori* infection. Moreover, we found that the restoration of H2 receptor expression after *H. pylori* eradication was threefold

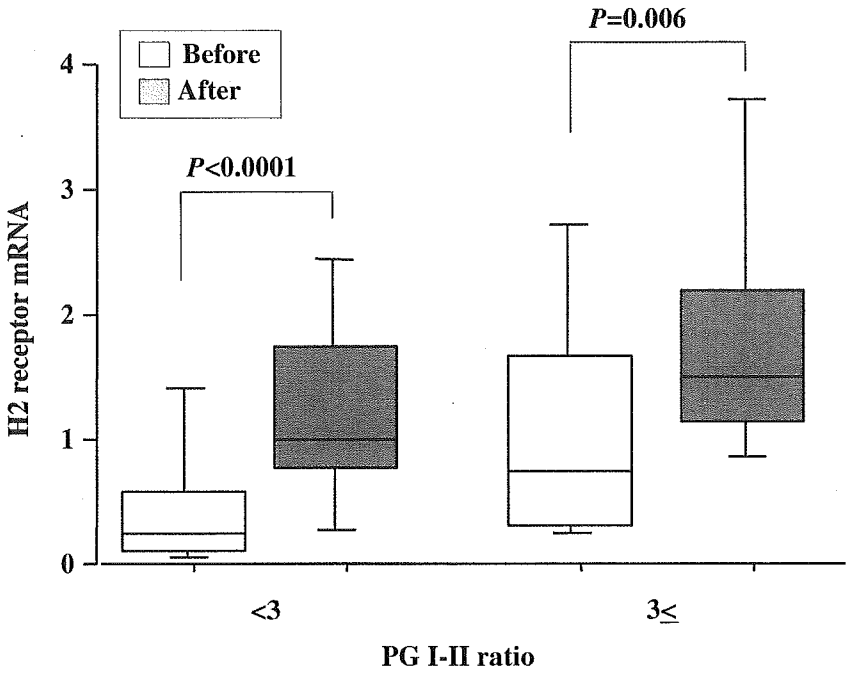

Figure 4. Comparison of H2 receptor mRNA expression levels in the oxyntic mucosa and serum pepsinogen levels before and after *H. pylori* eradication. H2 receptor mRNA expression levels rose despite the levels of serum pepsinogen I/II ratios, especially showing an increase of 4.2 times in subjects with a lower pepsinogen I/II ratios [median (first quartile to third quartile); 0.24 (0.11–0.57) and 1.00 (0.77–1.76) in 43 subjects with pepsinogen I/II ratios <3; $P < 0.0001$, 0.75 (0.31–1.68) and 1.51 (1.13–2.20) in 37 subjects with I/II ratio >3; $P < 0.0001$ by Wilcoxon rank sum test].

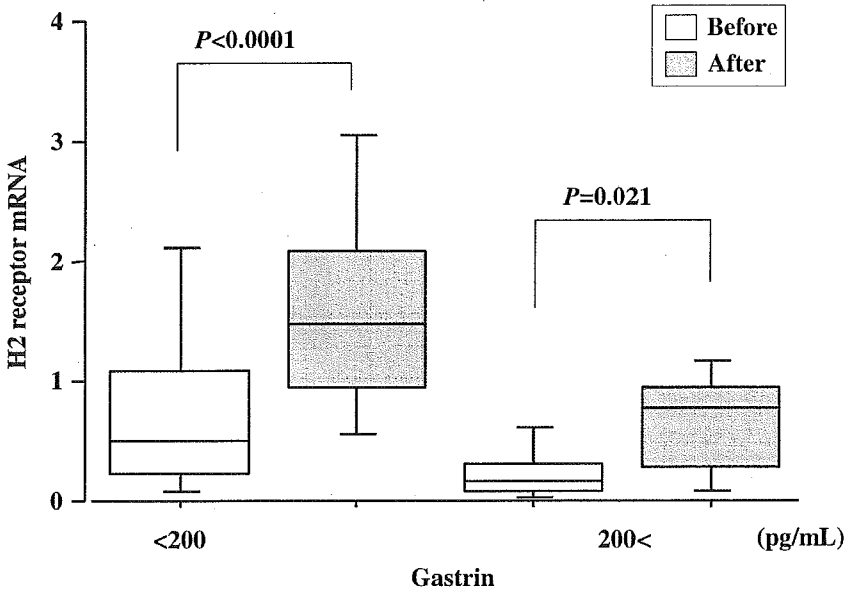

Figure 5. Comparison of H2 receptor mRNA expression levels in the oxyntic mucosa and serum gastrin levels before and after *H. pylori* eradication. A weak restoration of H2 receptor mRNA was found in subjects with hypergastrinemia, compared with subjects having normal serum gastrin levels [median (first quartile to third quartile); 0.50 (0.23–1.09) and 1.48 (0.94–2.09) in 71 subjects with serum gastrin <200 pg/mL; $P < 0.0001$, 0.17 (0.89–0.31) and 0.76 (0.29–0.95) in nine subjects with serum gastrin >200 pg/mL; $P = 0.021$ by Wilcoxon rank sum test].

greater than prior levels in most subjects with successful eradication therapy. This restoration was enhanced in subjects with more severe gastric atrophy showing lower serum pepsinogens I and II ratios.

To date, the evaluation of H2 receptor mRNA expression levels on parietal cells was difficult and contentious because RT-PCR studies using non-intron spanning primers were unable to differentiate between genomic DNA and mRNA. The present study found that gastric atrophy induced by *H. pylori*-infection affected strongly the expression levels of H2 receptor mRNA on parietal cells, because there was a positive correlation between pepsinogens I and II ratios and H2 receptor mRNA. These results suggested that decreased mRNA expression in atrophic mucosa was partially associated with the hyposecretion of gastric acid, accompanied by an attenuation of parietal cell mass.

H. pylori infection is associated with various disturbances of gastric secretory function, ranging from a marked increase to marked decrease in acid output. El-Omar *et al.* reported that the degree of recovery acid secretion was variable, with some regaining normal or near-normal values while others remained significantly hypochlorhydric over 18 months after eradication.[22] In this study, the degree of recovery H2 receptor mRNA was stronger in subjects with severe gastric atrophy showing lower serum pepsinogens I and II ratios, consistent with a previous report, in which the increase in acid secretion after *H. pylori* eradication was greatest in subjects with severe gastritis.[23] However, subjects with hypergastrinemia were observed to have only a mild recovery of H2 receptor mRNA. These subjects may have had persisting hypochlorhydria, which may not resolve the atrophy and intestinal metaplasia after *H. pylori* eradication, resulting in an irreversible stage of hyposecretion of gastric acid.[22, 24]

The exact mechanism by which the eradication of *H. pylori* increases gastric acid secretion remains unclear. El-Omar *et al.* reported that both the inflammation and subsequent inhibition of acid secretion are readily reversed by the eradication of *H. pylori.*[22] Another investigator reported that atrophic gastritis induced by chronic infection of *H. pylori* decreases gastric acid secretion, simply by reducing the number and mass of acid-secreting parietal cells and oxyntic glands of the stomach.[25] The restoration of H2 receptor mRNA expression on parietal cells in the present study may be partially associated with the increase of gastric acid secretion.

Several previous studies have demonstrated that long-term therapeutic effects of H2RAs are inferior to those of proton pump inhibitors, particularly when these drugs are administered to patients with GERD whose infection rate of *H. pylori* is low.[2,26–29] Whether this effect is simply due to an increase in parietal cell mass, an increase in H2 receptor sensitivity, or a true biological H2 receptor up-regulation is unclear. This tolerance phenomenon is not likely to occur in *H. pylori*-positive subjects.[16] The precise mechanism as to why *H. pylori*-infection prevents the occurrence of the tolerance phenomenon was not clarified. The attenuated expression of H2 receptor mRNA on parietal cells in *H. pylori*-infected mucosa in the present study may explain the phenomenon of lack of tolerance to H2RAs. Furthermore, higher levels of H2 receptor on parietal cells after *H. pylori* cure may explain the tolerance phenomenon in *H. pylori*-negative subjects.

In summary, H2 receptor mRNA levels decrease with the progression of gastric atrophy induced by *H. pylori* infection. The restoration of H2 receptor expression after *H. pylori* eradication was threefold greater than the previous levels, which occurred in most subjects with successful eradication therapy. These expression levels of H2 receptor may explain a part of the tolerance phenomenon of H2RAs.

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REFERENCES

1 Hurlimann S, Abbuhl B, Inauen W, Halter F. Comparison of acid inhibition by either oral high-dose ranitidine or omeprazole. Aliment Pharmacol Ther 1994; 8: 193–201.
2 Jansen JB, Van Oene JC. Standard-dose lansoprazole is more effective than high-dose ranitidine in achieving endoscopic healing and symptom relief in patients with moderately severe reflux oesophagitis. The Dutch Lansoprazole Study Group. Aliment Pharmacol Ther 1999; 13: 1611–20.
3 Komazawa Y, Adachi K, Mihara T, *et al.* Tolerance to famotidine and ranitidine treatment after 14 days of administration in healthy subjects without Helicobacter pylori infection. J Gastroenterol Hepatol 2003; 18: 678–82.
4 Lachman L, Howden CW. Twenty-four-hour intragastric pH: tolerance within 5 days of continuous ranitidine administration. Am J Gastroenterol 2000; 95: 57–61.
5 Labenz J, Peitz U, Leusing C, Tillenburg B, Blum AL, Borsch G. Efficacy of primed infusions with high dose ranitidine and omeprazole to maintain high intragastric pH in patients with peptic ulcer bleeding: a prospective randomised controlled study. Gut 1997; 40: 36–41.
6 Merki HS, Wilder-Smith CH. Do continuous infusions of omeprazole and ranitidine retain their effect with prolonged dosing? Gastroenterology 1994; 106: 60–4.
7 Netzer P, Gaia C, Sandoz M, *et al.* Effect of repeated injection and continuous infusion of omeprazole and ranitidine on intragastric pH over 72 hours. Am J Gastroenterol 1999; 94: 351–7.
8 Nwokolo CU, Smith JT, Gavey C, Sawyerr A, Pounder RE. Tolerance during 29 days of conventional dosing with cimetidine, nizatidine, famotidine or ranitidine. Aliment Pharmacol Ther 1990; 4(Suppl. 1): 29–45.
9 Sandvik AK, Brenna E, Waldum HL. Review article: the pharmacological inhibition of gastric acid secretion–tolerance and rebound. Aliment Pharmacol Ther 1997; 11: 1013–8.

10 Smith JT, Gavey C, Nwokolo CU, Pounder RE. Tolerance during 8 days of high-dose H2-blockade: placebo-controlled studies of 24-hour acidity and gastrin. Aliment Pharmacol Ther 1990; 4(Suppl. 1): 47–63.
11 Wilder-Smith C, Halter F, Ernst T, *et al.* Loss of acid suppression during dosing with H2-receptor antagonists. Aliment Pharmacol Ther 1990; 4(Suppl. 1): 15–27.
12 Nwokolo CU, Smith JT, Sawyerr AM, Pounder RE. Rebound intragastric hyperacidity after abrupt withdrawal of histamine H2 receptor blockade. Gut 1991; 32: 1455–60.
13 Viljakka M, Luostarinen M, Isolauri J. Incidence of antireflux surgery in Finland 1988–1993. Influence of proton-pump inhibitors and laparoscopic technique. Scand J Gastroenterol 1997; 32: 415–8.
14 Calam J, Gibbons A, Healey ZV, Bliss P, Arebi N. How does Helicobacter pylori cause mucosal damage? Its effect on acid and gastrin physiology. Gastroenterology 1997; 113: S43–9; discussion S50.
15 Qvigstad G, Arnestad JS, Brenna E, Waldum HL. Treatment with proton pump inhibitors induces tolerance to histamine-2 receptor antagonists in Helicobacter pylori-negative patients. Scand J Gastroenterol 1998; 33: 1244–8.
16 Fujisawa T, Adachi K, Komazawa Y, *et al.* Helicobacter pylori infection prevents the occurrence of the tolerance phenomenon of histamine H2 receptor antagonists. Aliment Pharmacol Ther 2004; 20: 559–65.
17 Samloff IM, Secrist DM, Passaro E Jr. A study of the relationship between serum group I pepsinogen levels and gastric acid secretion. Gastroenterology 1975; 69: 1196–200.
18 Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterology 1982; 83: 204–9.
19 Karnes WE Jr, Samloff IM, Siurala M, *et al.* Positive serum antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. Gastroenterology 1991; 101: 167–74.
20 Mulholland G, Ardill JE, Fillmore D, Chittajallu RS, Fullarton GM, McColl KE. Helicobacter pylori related hypergastrinaemia is the result of a selective increase in gastrin 17. Gut 1993; 34: 757–61.
21 Katelaris PH, Seow F, Lin BP, Napoli J, Ngu MC, Jones DB. Effect of age, Helicobacter pylori infection, and gastritis with atrophy on serum gastrin and gastric acid secretion in healthy men. Gut 1993; 34: 1032–7.
22 El-Omar EM, Oien K, El-Nujumi A, *et al.* Helicobacter pylori infection and chronic gastric acid hyposecretion. Gastroenterology 1997; 113: 15–24.
23 Yasunaga Y, Shinomura Y, Kanayama S, *et al.* Improved fold width and increased acid secretion after eradication of the organism in Helicobacter pylori associated enlarged fold gastritis. Gut 1994; 35: 1571–4.
24 Gutierrez O, Melo M, Segura AM, Angel A, Genta RM, Graham DY. Cure of Helicobacter pylori infection improves gastric acid secretion in patients with corpus gastritis. Scand J Gastroenterol 1997; 32: 664–8.
25 Ihamaki T, Varis K, Siurala M. Morphological, functional and immunological state of the gastric mucosa in gastric carcinoma families. Comparison with a computer-matched family sample. Scand J Gastroenterol 1979; 14: 801–12.
26 Kawano S, Murata H, Tsuji S, *et al.* Randomized comparative study of omeprazole and famotidine in reflux esophagitis. J Gastroenterol Hepatol 2002; 17: 955–9.
27 Shirota T, Kusano M, Kawamura O, Horikoshi T, Mori M, Sekiguchi T. Helicobacter pylori infection correlates with severity of reflux esophagitis: with manometry findings. J Gastroenterol 1999; 34: 553–9.
28 Koike T, Ohara S, Sekine H, *et al.* Helicobacter pylori infection inhibits reflux esophagitis by inducing atrophic gastritis. Am J Gastroenterol 1999; 94: 3468–72.
29 Loffeld RJ, Werdmuller BF, Kuster JG, Perez-Perez GI, Blaser MJ, Kuipers EJ. Colonization with cagA-positive Helicobacter pylori strains inversely associated with reflux esophagitis and Barrett's esophagus. Digestion 2000; 62: 95–9.

STOMACH

Pericryptal fibroblast sheath in intestinal metaplasia and gastric carcinoma

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Background and aims: In the progression of chronic gastritis, gastric mucosal cells deviate from the normal pathway of gastric differentiation to an intestinal phenotype which is closely related to gastric carcinoma. However, to date, it has not been elucidated whether the intestinal metaplasia is merely a change in the epithelium or whether the underlying mesenchyme also changes from gastric type to intestinal type. We have investigated the relationship between intestinal metaplasia and the pericryptal fibroblast sheath (PCFS) in the mesenchyme. In addition, we also examined PCFS in gastric carcinoma.
Methods: We determined the existence of PCFS in the intestinal metaplastic mucosa and carcinoma of both human and Cdx2 transgenic mouse stomach. PCFS was determined using the antibody against α-smooth muscle actin and electron microscopic observations.
Results: PCFS formed an almost complete layer around the small and large intestinal crypts while it did not exist around the normal gastric glands in both mice and humans. PCFS was seen around the glands of intestinal metaplastic mucosa in both Cdx2 transgenic mouse and human stomachs. However, PCFS was virtually absent in the intestinal-type gastric adenocarcinoma area.
Conclusion: We successfully demonstrated that the epithelium as well as the mesenchyme changed from the gastric type to the intestinal type in intestinal metaplasia and that PCFS disappeared in intestinal-type gastric carcinoma.

Human intestinal-type gastric carcinoma is associated with gastric atrophy and intestinal metaplasia that are caused mainly by *Helicobacter pylori* infection. Correa presented a hypothesis with respect to the mechanism of gastric carcinogenesis due to *H pylori* infection.[1] *H pylori* infection is involved in the process of progression from normal gastric mucosa to superficial gastritis, chronic active gastritis, atrophic gastritis, and finally to intestinal metaplasia.[1] The terminal stage of this process is gastric carcinoma.

We and others have reported that the intestinal specific transcription factor Cdx2 is expressed in human gastric intestinal metaplastic mucosa.[2–6] Furthermore, we established Cdx2 transgenic mice expressing the transcription factor Cdx2 exclusively in the gastric epithelium.[7] The gastric fundic mucosa of the Cdx2 transgenic mouse was completely changed into intestinal metaplastic mucosa. However, whether intestinal metaplasia is limited to the epithelium or influences the underlying mesenchyme has not been elucidated as it is difficult to discriminate intestinal mesenchyme from gastric mesenchyme.

The normal intestinal crypt of Lieberkühn is invested by a mesenchymal sheath, a specialised part of the lamina propria consisting of fibroblasts tightly surrounding the epithelium and of collagen fibres oriented circumferentially to the crypt.[8] The mesenchymal sheath is a distinct and highly organised system of fibroblasts immediately subjacent to the epithelial basement membrane located at the epithelial-mesenchymal interface. The highly specialised fibroblasts, that were reported as pericryptal fibroblasts by Kay and colleagues,[9] form the pericryptal fibroblast sheath (PCFS). PCFS consists of a network of fibroblast cells and extracellular matrix immediately subjacent to the crypt epithelial cells.[8 10] Such pericryptal fibroblasts under the basement membrane envelop the glands of the intestine, are seen as elongated cells tightly appliquéd to the base of the epithelial cells of the crypt, have a fusiform appearance, and follow the contours of the wall of the crypt. Fibroblasts of the PCFS have an important role in colonic fluid absorption.[11] Colonic absorptive function depends not only on crypt luminal cells but also on the fibroblast cells of the surrounding pericryptal sheath.[11]

There is a close interactive epithelial-mesenchymal relationship between the epithelial cell system and the underlying pericryptal fibroblast system to maintain the normal structure and function of the crypts of Lieberkühn.[8 9] Pericryptal fibroblasts have been shown to play a fundamental role in epithelial differentiation via epithelial-mesenchymal cell interactions during both fetal and adult life.[12 13] The PCFS is a self renewing population of mesenchymal cells in close contact with the intestinal epithelium; its cells maintain a parallel relationship in replication, migration, and differentiation with the overlying epithelium suggesting that it is involved in the maintenance of the normal structure and function of the intestinal mucosa.[8 9] Autoradiographic studies in rabbit colon after ^{3}H-thymidine injection demonstrate steady state renewal of pericryptal fibroblasts and migration to upper portions of the synchrony with epithelial migration. The fibroblast progenitor population, like that of the epithelium, is in the deep one third of the crypt.[8] The kinetics of this portion of the normal intestinal mucosa suggest that the pericryptal fibroblasts and the epithelium act as a unit to maintain the normal structure, maturation, and function of the crypt of Lieberkühn.[8]

These findings prompted us to investigate (1) whether intestinal metaplasia influences the epithelium as well as the formation of the PCFS and (2) the relationship between PCFS and intestinal-type gastric carcinoma.

Abbreviations: PCFS, pericryptal fibroblast sheath; α-SMA, α-smooth muscle actin; PBS, phosphate buffered saline

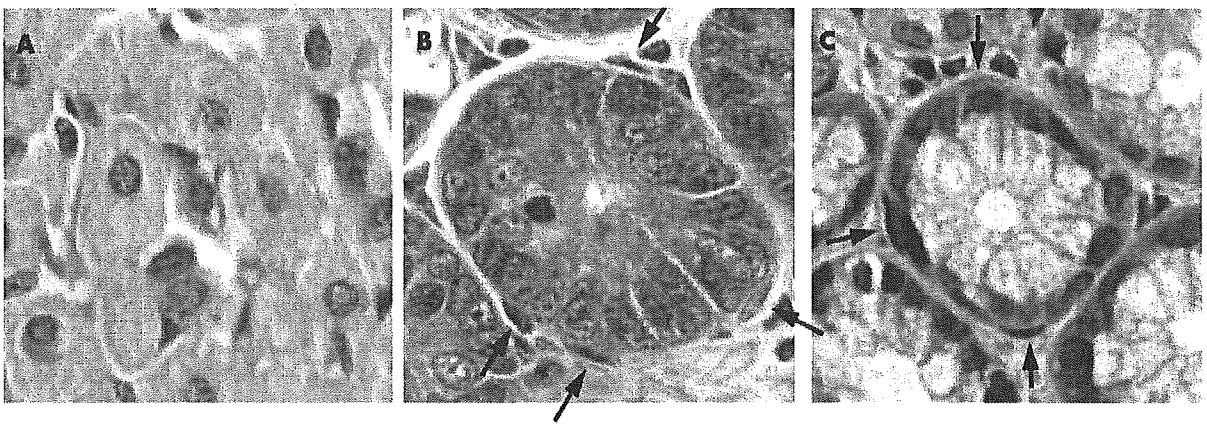

Figure 1 Mouse gastric glands (A), small intestinal crypts (B), and large intestinal crypts (C) shown in cross section. Cross sections of crypts of mouse small (B) and large (C) intestines show pericryptal fibroblast nuclei (arrows) immediately subjacent to the epithelial cells. The long axis of each pericryptal fibroblast is perpendicular to that of the crypt. Pericryptal fibroblast nuclei are not seen forming a sheath round the glands of mouse gastric mucosa (A). Magnification ×400.

MATERIALS AND METHODS

Cdx2 transgenic mice

We used Cdx2 transgenic mice with stomach specific expression of Cdx2 using the β-subunit gene promoter of rat H^+/K^+-ATPase.[7] The gastric mucosa of Cdx2 transgenic mice was completely changed to intestinal metaplastic mucosa.[7]

Histology

Stomach tissue specimens were fixed in neutral buffered 10% formalin for 12–24 hours, washed in 70% ethanol, processed by standard methods, embedded in paraffin, sectioned at 3 μm, and stained with haematoxylin and eosin for histological evaluation.

Immunohistochemistry

Thick sections (3 μm) were cut, deparaffinised, rehydrated in phosphate buffered saline (PBS), placed in 10 mM citrate buffer (pH 6.0), and heated in an 850 W microwave for 15 minutes to recover antigenicity. Endogenous peroxidase activity was blocked by incubation for 30 minutes in methanol containing 0.3% H_2O_2. After washing twice with PBS, including 0.1% Triton X-100, sections were preincubated with blocking buffer (Dako, Carpinteria, California, USA) for 15 minutes at room temperature. Primary antisera, anti-α-smooth muscle actin (α-SMA) (1:100; Dako), or anti-Cdx2 (1:100; BioGenex, San Ramon, California, USA) were diluted in PBS and incubated overnight at 4°C. Slides were then washed in PBS and incubated with Envision (Dako). After

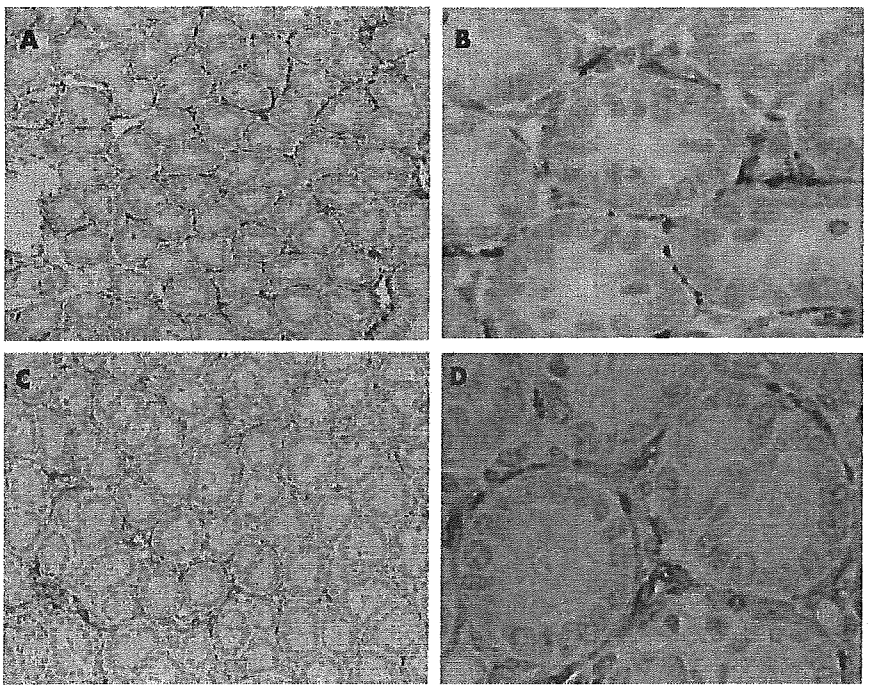

Figure 2 Normal mouse small (A, B) and large (C, D) intestinal mucosa. Immunohistochemical stain for α-smooth muscle actin. The pericryptal fibroblast sheath formed by pericryptal fibroblasts is closely embracing epithelial cells of normal intestinal crypts. Magnification ×100 (A, C); ×400 (B, D).

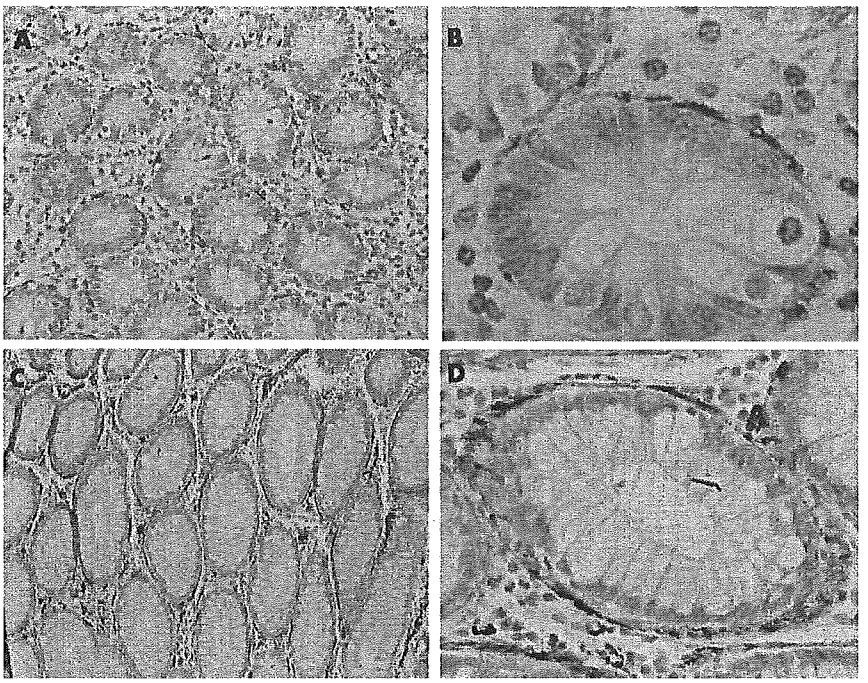

Figure 3 Normal human small (A, B) and large (C, D) intestinal mucosa. Immunohistochemical stain for α-smooth muscle actin. Pericryptal fibroblast sheath formed by pericryptal fibroblasts is closely embracing the epithelial cells of normal intestinal crypts. Magnification ×100 (A, C); ×400 (B, D).

development with 3,3'-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, Osaka, Japan), slides were counterstained with haematoxylin and viewed under a light microscope.

Fixation and preparation of tissue for electron microscopy

Intestinal metaplastic mucosa from Cdx2 transgenic mouse stomach was fixed at 4℃ in 2% glutaraldehyde in PBS, followed by six washes in PBS with post fixation in 1% osmium tetroxide. Sections were examined under a Hitachi H-7500 scanning electron microscope.

RESULTS

To examine whether intestinal metaplasia is merely epithelial cell metaplasia or influences the underlying mesenchyme, we focused our attention on the PCFS in the mesenchyme. Light microscopic examination of normal mouse small and large intestine revealed fibroblasts subtending the epithelial basement membrane in the crypt (fig 1B, 1C). In contrast, fibroblasts were not seen forming a sheath round the glands of normal mouse gastric mucosa (fig 1A). These fibroblasts subjacent to the epithelium were easily distinguished from the rest of the mesenchymal elements of the lamina propria, which consist of a loose meshwork of collagen, fibroblasts, haematogenous cells, and capillaries.

α-SMA is present in pericryptal fibroblasts and is used as a marker for pericryptal fibroblast cells. Pericryptal fibroblasts labelled by α-SMA formed single cell layers that embraced the whole length of the crypts in the normal small and large intestinal mucosa of the mouse (fig 2) and humans (fig 3). α-SMA positive PCFS was seen around many glands. Areas of abutment were broad and the sheath appeared continuous.

We previously generated Cdx2 transgenic mice expressing intestine specific transcription factor *Cdx2* gene exclusively in the gastric epithelium under the control of the β-subunit gene promoter of rat H^+/K^+-ATPase.[7] Cdx2 transgenic mice developed normally into superficially healthy adults and showed intestinal metaplasia in the stomach up to 12 weeks of age. The gastric mucosa of Cdx2 transgenic mouse was completely replaced by intestinal metaplastic mucosa. Cdx2 induced intestinal metaplastic mucosa consisted of terminally differentiated intestinal epithelial cells, including absorptive enterocytes, goblet cells, and enteroendocrine cells.[7] To clarify whether Cdx2 expression in gastric epithelium affects the underlying mesenchyme in vivo, the intestinal metaplastic mucosa of Cdx2 transgenic mouse stomach was stained for α-SMA. The PCFS expressing α-SMA was not seen around the glands of the normal gastric mucosa (fig 4A, B) whereas PCFS was easily recognised around the crypts of intestinal metaplastic mucosa of Cdx2 transgenic mouse stomach (fig 4C, 4D). In addition to mouse intestinal metaplastic mucosa, PCFS was also recognised around the glands of human intestinal metaplastic mucosa (fig 5C, D) while it was not seen around normal human gastric glands (fig 5A, B).

Electron microscopy revealed an even closer association between pericryptal fibroblasts and the epithelium than was revealed by light microscopy. In intestinal metaplastic mucosa of Cdx2 transgenic mouse stomach, the PCFS was in intimate contact with the epithelial basal lamina (fig 6). Fibroblasts were seen surrounding the base of the crypts in the intestinal metaplastic mucosa. These cells had large areas of contact with the epithelial basal lamina and had a plum fusiform shape (fig 6B, C).

As it is reported that the PCFS is significantly reduced in colorectal epithelial neoplasms, we examined the relationship between PCFS and gastric adenocarcinoma. We observed Cdx2 transgenic mice periodically without carcinogens or *H pylori* infection. Cdx2 transgenic mice at 50 weeks of age indicated preservation of intestinal metaplasia and no gastric polyp formation, similar to those at 12 weeks (unpublished data). Cdx2 transgenic mice developed gastric polyps in the intestinal metaplastic lesion at two years after birth. Gastric polyps developed from intestinal metaplastic mucosa in all stomachs of 10 Cdx2 transgenic mice examined. The polyps consisted of adenocarcinoma that invaded the submucosa or beyond (unpublished data). Using the adenocarcinoma, we examined the relationship between PCFS and adenocarcinoma. PCFS detected by immunohistochemical stain for α-SMA was virtually absent in the area of gastric

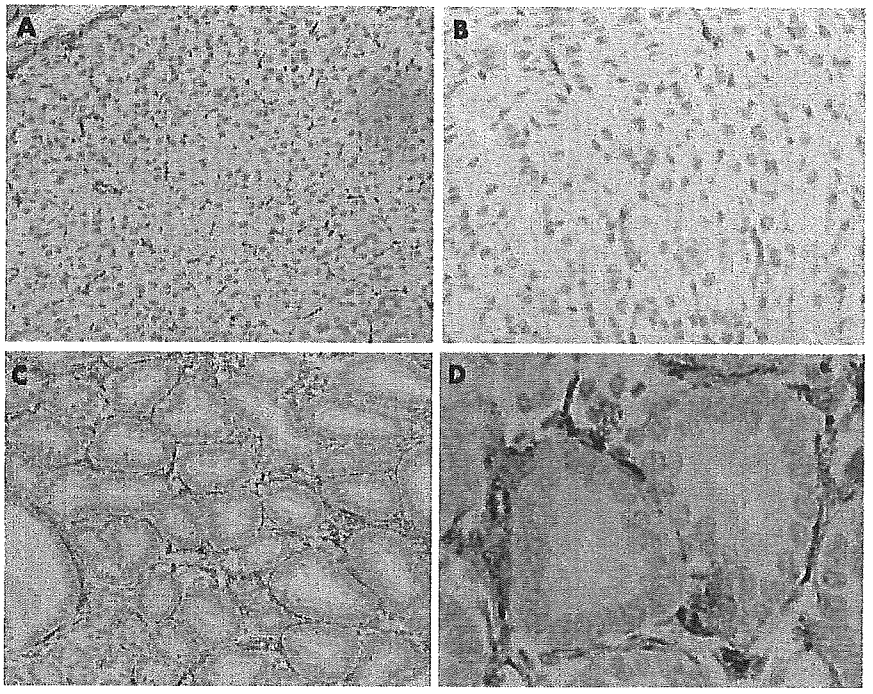

Figure 4 Normal and intestinal metaplastic mucosa of the mouse stomach. Immunohistochemical stain for α-smooth muscle actin. Pericryptal fibroblast sheath (PCFS) formed by pericryptal fibroblasts is closely embracing the epithelial cells of the intestinal metaplastic mucosa (C, D) while PCFS is not seen in the normal gastric mucosa (A, B). Magnification ×100 (A, C); ×400 (B, D).

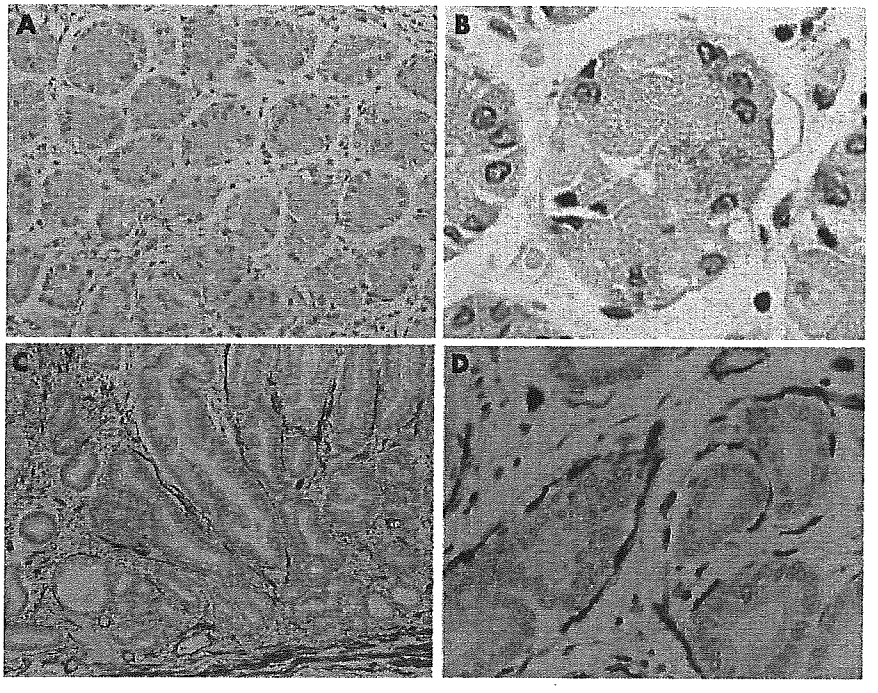

Figure 5 Normal and intestinal metaplastic mucosa of the human stomach. Immunohistochemical stain for α-smooth muscle actin. Pericryptal fibroblast sheath (PCFS) formed by pericryptal fibroblasts is closely embracing the epithelial cells of the intestinal metaplastic mucosa (C, D) while PCFS is not seen in the normal gastric mucosa (A, B). Magnification ×100 (A, C); ×400 (B, D).

adenocarcinoma of both humans and mice (fig 7C, G) while PCFS was easily recognised in the intestinal metaplastic areas (fig 7A, E). PCFS was absent in all 10 Cdx2 transgenic mouse and 10 human intestinal-type adenocarcinomas. Both human and murine gastric carcinomas were classified as intestinal-type according to the criteria of Lauren[14] and as category 5.2 according to the five categories of the Vienna classification.[15 16] We examined expression of Cdx2 in intestinal metaplasia and adenocarcinoma. Cdx2 staining for the adenocarcinoma lesion (fig 7D, H) was extremely weak compared with the intestinal metaplastic lesion (fig 7, B and F). The decrease in Cdx2 may explain, in part, the cause of the disappearance of PCFS in the gastric adenocarcinoma. There was no difference in immunoreactivities for Cdx2 and α-SMA in intestinal metaplastic mucosa between 12 and 50 week old Cdx2 transgenic mice (data not shown).

DISCUSSION

Our results demonstrate that intestinal metaplasia is not merely epithelial metaplasia but also affects the underlying