

Helicobacter pylori gastritis: glandular proliferation and homeostasis differ between gastric antrum and corpus

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Background. The aim of the study was to assess the effect of *H. pylori* on epithelial cell proliferation and on the expression of the apoptosis protector oncoprotein bcl-2 at different grades of chronic inflammation and atrophy in the antrum and corpus mucosa, and on the density of gastrin (G), somatostatin (D) cells and β -H+, K+ ATP-ase immunoreactive cells.

Materials and Methods. antrum and corpus biopsies from 59 consecutive patients (34 men, 25 women, median age 47) were analysed for *H. pylori*, presence of chronic inflammation and atrophy. Immunostaining for PCNA (APAAP method), Ki-67, bcl-2, G-, and D-cells, as well as for β -H+, K+ ATP-ase (Avidin-Biotin method) was performed on paraffin embedded biopsy specimens.

Results. A significantly higher proliferation activity was established in cases of atrophy in the corpus mucosa ($p = 0.007$), while for the antrum the proliferation indices did not reveal a significant difference in the cases of normal, non-atrophic and atrophic mucosa. The proliferation activity was inversely related to higher *H. pylori* score in the antrum but not in the corpus ($p = 0.03$). In the antrum, positive staining for bcl-2 positive epithelial cells was observed predominantly in the proliferating zone (PZ) of atrophic mucosa, whereas in the corpus it was observed in normal and non-atrophic mucosa. In corpus mucosa, the density of bcl-2 positive interstitial lymphocytes in PZ and glandular part (GP) was significantly higher in atrophic than in non-atrophic mucosa ($p = 0.01$; $p = 0.002$), but was not correlated with *H. pylori* density. In the antrum, bcl-2 positivity of interstitial lymphocytes was higher in *H. pylori* positive cases ($p = 0.02$). The densities of G and D cells in the PZ of the antral glands and of β -H+, K+ ATP-ase-positive parietal cells in the corpus revealed a significant negative correlation with the severity of atrophic alterations ($p = 0.02$; $p = 0.0005$; $p = 0.0004$), but not with *H. pylori* density ($p = 0.29$).
Conclusion. Severity of gastritis and presence of *H. pylori* affect gastric epithelial cell proliferation and the expression of bcl-2 protein in gastric epithelial cells and in interstitial lymphocytes differently in antrum and corpus mucosa. Homeostasis of antral G and D cells as well as the loss of β -H+, K+ ATP-ase-reactive cells in the corpus was shown to be a function of the degree of atrophic alterations rather than the impact of *H. pylori*.

Key words: *H. pylori* gastritis, antrum and corpus mucosa, PCNA, Ki-67, bcl-2, gastrin and somatostatin cells, β -H+, K+ ATP-ase

BACKGROUND

Helicobacter pylori (*H. pylori*) is the main environmental factor contributing to the development of chronic

gastritis and is associated with an enhanced risk of developing peptic ulcer and gastric cancer (1–4). In addition to the direct immunological role (5), there is also evidence that *H. pylori* is associated with changes in epithelial cell turnover, including the influence on programmed cell death or apoptosis (6–13). Upregulation of apoptosis protectors may supply cells with a survival advantage and hence play a role in carcinogenic pathway (14, 15). The expression of the apoptosis protector, bcl-2 protein, is enhanced in the case

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of gastric carcinoma as well as in the proliferative zone of gastric glands in presence of chronic atrophic gastritis with intestinal metaplasia (14, 16, 17).

Several earlier studies have shown that *H. pylori* can affect gastrin (G) cells as well as somatostatin (D) cell density, in particular with selective reduction and suppression of D-cells, which results in reduced inhibition of G-cell secretion by somatostatin (18–20). In contrast, the influence of *H. pylori* on the density of parietal cells, expressing the β subunit of H⁺, K⁺ ATP-ase, is not so widely described. However, recent studies have demonstrated an association between *H. pylori* and development of autoimmune reactions against canalicular structures within human parietal cells (21, 22). Gastric H⁺, K⁺ ATP-ase represents a major autoantigen in the canaliculi of parietal cells, and an association of anticanalicular autoantibodies with gastric corpus atrophy has been confirmed (21–24). The significance of homology between the β subunit of H⁺, K⁺ ATP-ase of parietal cells and *H. pylori* urease has also been shown (25). These relationships suggest a role of *H. pylori* in development of gastric corpus autoimmunity (21, 26).

The aim of the present study was to assess the peculiarities of the effect of *H. pylori* on epithelial cell proliferation and on the expression of the apoptosis protector oncoprotein bcl-2 in the antrum and corpus mucosa by different grades of chronic inflammation and atrophy. Furthermore, using the same biopsy specimens, we aimed to assess the effect of *H. pylori* and the severity of gastritis on the density of G and D cells and β -H⁺, K⁺ ATP-ase immunoreactive cells.

In this study, we have tested the hypothesis that *H. pylori* enhances epithelial cell proliferation and affects the expression of the bcl-2 protein as well as the density of specific glandular cells at advanced grades of chronic inflammation and in the case of atrophy, differently in the antrum and the corpus.

MATERIALS AND METHODS

A total of 59 patients (34 men, 25 women, median age 47), consecutively investigated by routine endoscopy at the Gastrointestinal Unit of the University Hospital, Bern, were studied. The group of patients included 20 persons with peptic ulcer (gastric ulcer 4 cases; duodenal ulcer 16 cases); 18 with erosion in the antrum or/and corpus mucosa and duodenitis; one with gastric corpus adenocarcinoma. In 20 patients no pathological gastroduodenoscopic findings were established.

In 40 patients, a minimum of two biopsies were sampled both from the antrum and the corpus, in 16 patients only from the antrum, and in 3 patients only from the corpus. The 56 antrum biopsies and 43 corpus biopsies were fixed in 4% formalin and embedded in paraffin.

Histopathologic evaluation

The state of the gastric mucosa and the presence of *H. pylori* were examined in haematoxylin and eosin-stained sections and graded by the criteria in accordance with the Sydney system: normal mucosa – no loss of glands and no chronic inflammation (score 0); non-atrophic gastritis – chronic inflammation present without atrophy and scored as mild (1), moderate (2) and severe (3); atrophic gastritis in three stages – mild, moderate or severe loss of mucosal glands (score numbers 1, 2 and 3 respectively). The severity of *H. pylori* colonization was evaluated as follows: absence of bacteria (grade 0), mild (grade 1), moderate (grade 2) or severe (grade 3) colonization (27–29).

Immunohistochemistry

Gastric epithelial cell proliferation was assessed by employing both the Proliferating Cell Nuclear Antigen (PCNA) and Ki-67 (MIB 1) labelling. Since PCNA is increasingly expressed through the G₁ phase, reaching its maximum in the early S phase and persisting in the G₂ phase (30), while the Ki-67 antigen is expressed in all phases except G₀ and early G₁ (31), we used both antibodies. PCNA immunostaining was performed using alkaline phosphatase anti-alkaline phosphatase (APAAP) procedure. After deparaffinisation and pre-treatment with 20% acetic acid (15 s, 4 °C) and 3% BSA (Merck) in TRIS-NaCl buffer (20 min., 20 °C), the tissue sections were incubated overnight (4 °C) with a primary mouse monoclonal antibody against PCNA (PCNA PC10, DakoCytomation AS, Glostrup, Denmark, dilution 1:50). Rabbit anti-mouse immunoglobulin (DakoCytomation AS, Glostrup, Denmark, dilution 1:30) was used as the secondary antibody (incubation 30 min, at 20 °C). The APAAP mouse monoclonal antibody complex (DakoCytomation AS, Glostrup, Denmark, dilution 1:50) was then applied for 60 min. To enhance the intensity of final staining, these two steps were repeated. New fuchsin (Merck)-naphthol AS-Bi phosphate (Sigma, St. Louis, USA), in TRIS-HCl buffer, pH 8.7, containing 2.1 mg/ml levamisole (Sigma, St. Louis, USA) was used as the alkaline phosphatase substrate for 20 min at room temperature. The reaction was stopped by rinsing the sections in cold water. The sections were counterstained with haematoxylin (Merck) and mounted with Aquatex (Merck).

Immunostaining for Ki-67, bcl-2 oncoprotein, as well as for gastrin, somatostatin, and β -H⁺, K⁺ ATPase immunoreactive cells was performed using avidin-biotin procedure. After deparaffinisation and rehydration, the sections were treated in the microwave oven in 10 mM citrate buffer, pH 6.0 at 190 W for 5 min and twice at 140 W for 5 min. before staining for Ki-67, bcl-2 and gastrin cells. Further, the plastic jar was allowed to cool for 15 min at

room temperature and was then washed for 10 min in TRIS-NaCl buffer, pH 7.5.

As the primary antibodies, monoclonal anti Ki-67 (MIB-1, Dianova, Hamburg, dilution 1:50), the anti-human bcl-2 oncoprotein (clone 124, DakoCytomation AS, Glostrup, Denmark, dilution 1:50), the anti- β subunit of H⁺, K⁺ ATP-ase (AB 2611 kindly donated by Dr. J. G. Forte, Berkeley, USA; dilution 1:200), polyclonal anti-gastrin (DakoCytomation AS, Glostrup, Denmark; dilution 1:300) and anti-somatostatin (DakoCytomation AS, Glostrup, Denmark; dilution 1:1000) were used. Anti- β -H⁺, K⁺ ATPase and anti-somatostatin antibodies were applied on the tissue sections without microwave pre-treatment of the latter. All primary antibodies were diluted in the TRIS-NaCl buffer with 0.1% casein and 0.1% NaN₃ (pH 7.5). The preparations were incubated with the primary antibodies for 60 min at 20 °C. Subsequently, the sections were incubated with rabbit anti-mouse, biotinylated immunoglobulins (DakoCytomation AS, Glostrup, Denmark) for monoclonal primary antibodies, or with swine anti-rabbit immunoglobulins, biotinylated immunoglobulins (DakoCytomation AS, Glostrup, Denmark) for polyclonal primary antibodies diluted to 1:200 for 45 min. The Avidin-Biotin Complex (DakoCytomation AS, Glostrup, Denmark) was applied for 45 min at room temperature. Alkaline phosphatase reaction was developed for 30 min in the New Fuchsin substrate solution. The specimens were then washed in tap water, counterstained with haematoxylin and mounted with Aquatex (Merck). Negative controls were obtained by replacing the primary antibodies by the TRIS-NaCl buffer.

Evaluation of immunostained sections

The sections were examined using an objective $\times 40$ and an eyepiece $\times 10$. The antral and corpus mucosa glands were divided into the foveolar cell compartment (FC), the proliferating zone (PZ; neck-isthmus) and the glandular part (GP). In each of them five fields were examined.

Only bright red-stained nuclei were considered positive either for PCNA or for Ki-67. PCNA and Ki-67 labelling indices (LI) were defined as the proportion of bright red-stained nuclei in relation to 100 nuclei counted per field. Bcl-2 positive staining for interstitial lymphocytes was scored as 0 to 5. Bcl-2 staining of epithelial cells in the antrum and corpus mucosa was considered positive (red staining of cytoplasm) or negative.

The densities of G, D and β -H⁺, K⁺ ATP-ase-positive cells were expressed as the mean values of the cells counted in five fields of each gland part. The slides were analysed by one investigator (T.V.) who was not aware of the data of histological alterations. The intraobserver variability was $12.6 \pm 7.0\%$.

A typical Ki-67 labelling pattern in the PZ and FC compartments of the corpus mucosa is shown in Fig. 1. Bcl-2 positive staining of interstitial lymphocytes in the antrum mucosa is shown in Fig. 2. Staining of G cells, D cells and β -H⁺, K⁺ ATP-ase-reactive cells is presented in Figs. 3, 4 and in Fig. 5.

Statistics

The values of LI were indicated as mean \pm SD. Statistical analysis was done with Student's t test, χ^2 , Spearman rank correlation analysis as well as with multiple regression analysis (Statistika, Statsoft 99). The p-values < 0.05 were considered significant.

The study protocol was approved by the Ethics Committee of the University of Bern, Switzerland.

RESULTS

The state of gastric mucosa as revealed by histological data is presented in Table 1. There was a significant positive correlation between the density of *H. pylori* and the density of chronic inflammation in the antrum mucosa ($r = 0.67$, $p = 0.00009$), but not in the corpus mucosa ($r = 0.13$; $p = 0.37$).

Epithelial cell proliferation

Overall mean PCNA LI was significantly higher in the antrum mucosa than in the corpus mucosa (12.0 ± 7.4 versus 8.7 ± 6.9 ; $p = 0.02$ for PZ).

PCNA LI in the FC and GP of corpus glands was significantly higher in presence of atrophic alterations (10.0 ± 10.9) as compared to the non-atrophic (13.2 ± 2.3) and normal mucosa (2.2 ± 2.0) ($p = 0.007$ and $p = 0.03$, respectively).

Ki-67 LI, too, was significantly higher in the case of atrophic corpus glands compared with the normal mucosa (0.9 ± 1.2 versus 0.1 ± 0.3 ; $p = 0.04$). However, in the antrum the mean values of PCNA LI and Ki-67 LI did not differ significantly in the cases of the normal mucosa, non-atrophic gastritis and atrophic gastritis ($p > 0.05$). Correlation analysis showed also a significant correlation of PCNA LI in corpus glands with the severity of chronic inflammation and atrophy ($r = 0.34$; $p = 0.03$ for FC and $r = 0.46$; $p = 0.001$ for GP).

In the antrum glands, in the case of non-atrophic gastritis there was a significant negative correlation of Ki-67 LI with *H. pylori* density ($r = -0.41$; $p = 0.03$). Also, the mean value of Ki-67 in absence of *H. pylori* showed a significantly higher value (0.9 ± 1.6) as compared with moderate *H. pylori* colonization (0.05 ± 0.1 ; $p = 0.03$). In antrum atrophy, PCNA LI in PZ and FC were negatively correlated with *H. pylori* density ($r = -0.42$; $p = 0.04$). The mean value of PCNA LI in the FC of the antrum mucosa in absence of *H. pylori* was higher (4.6 ± 3.9) as compared with mild (2.2 ± 1.1 ; $p = 0.03$), moderate (1.8 ± 2.0 ; $p = 0.04$) and severe (1.4 ± 0.8 ; $p = 0.01$) grade of

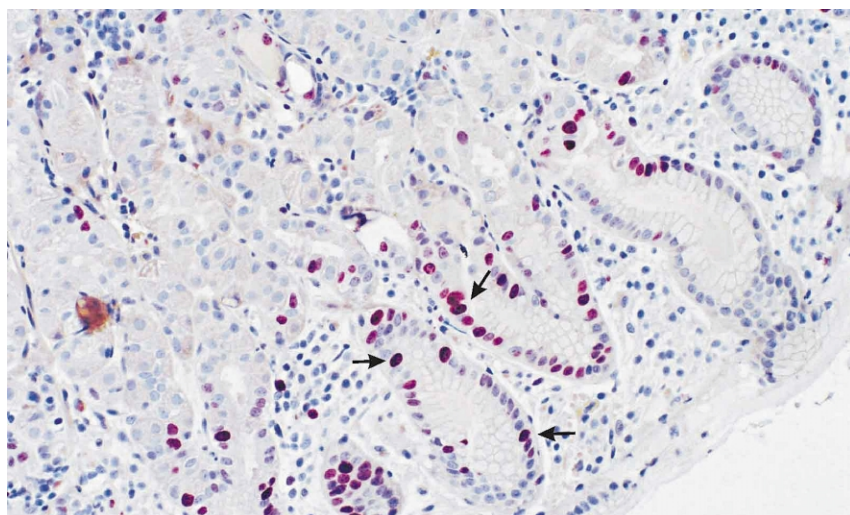


Fig. 1. Ki-67 labelling pattern in the proliferating zone and in the foveolar cell compartment of the corpus mucosa (intensive red staining, arrow; $\times 20$ objective, $\times 10$ eyepiece). Avidin-Biotin method

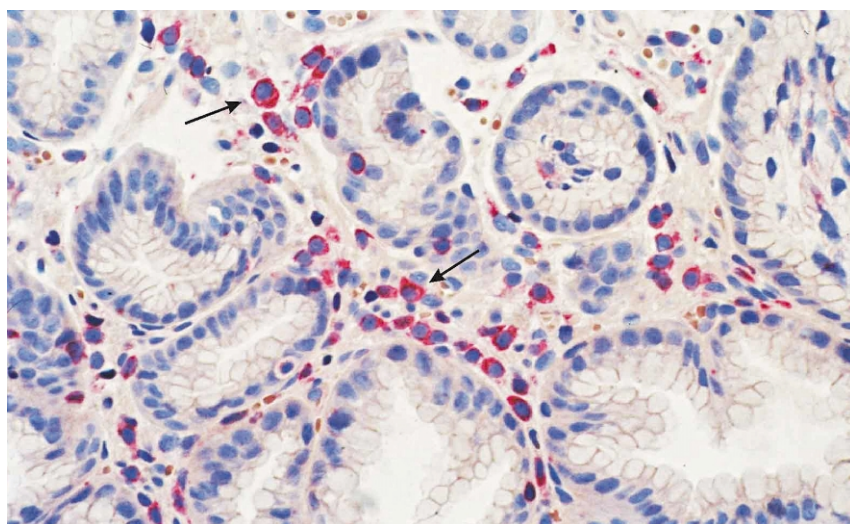


Fig. 2. Bcl-2 positive staining for interstitial lymphocytes in the antrum mucosa (intensive red staining, arrow; $\times 40$ objective, $\times 10$ eyepiece). Avidin-Biotin method

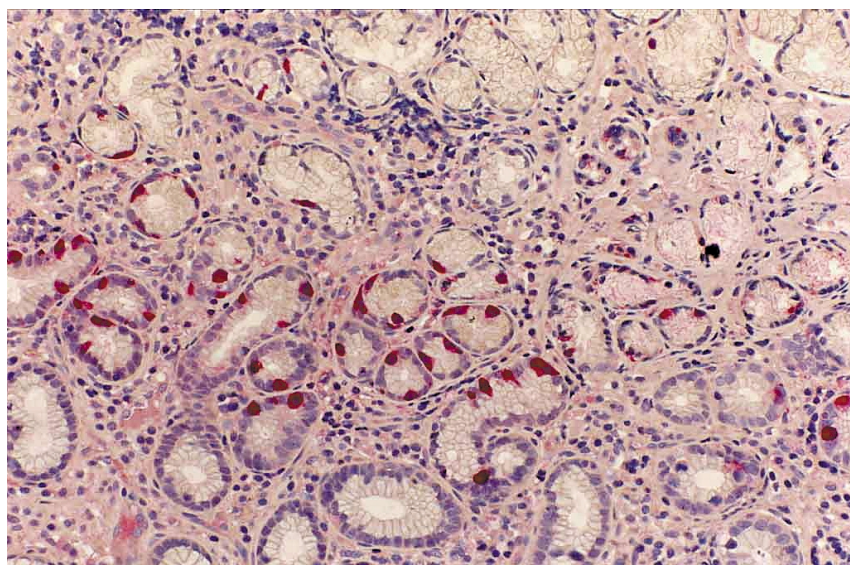


Fig. 3. Positive staining for gastrin cells in the glandular part of the antrum mucosa (intensive red staining; $\times 40$ objective, $\times 10$ eyepiece). Avidin-Biotin method

H. pylori colonization. In the corpus mucosa, PCNA LI and Ki-67 LI did not differ significantly in *H. pylori* positive and *H. pylori* negative cases ($p > 0.05$).

Multiple regression analysis showed that proliferation activity in the antrum mucosa depended significantly on *H. pylori* density but not on the grade of atrophy ($\beta = -0.42$; $p = 0.04$ for PCNA LI in PZ).

***Bcl-2* protein in epithelial cells and interstitial lymphocytes**

Bcl-2 positive staining was found in epithelial cells, *i.e.* parietal cells in the corpus mucosa and neck cells in the antral mucosa. In the antrum, positive staining of bcl-2 positive epithelial cells was observed predominantly in the atrophic mucosa (5/23 cases *versus* 2/28 in the non-atrophic mucosa), whereas in the corpus it was seen predominantly in the normal and non-atrophic mucosa (3/11 and 4/25 cases, respectively) and in only one case in the atrophic mucosa.

The mean value of the bcl-2 positive staining score of interstitial lymphocytes was significantly higher in atrophic corpus gastritis (2.1 ± 1.6 for FC, 2.8 ± 1.5 for PZ, 3.2 ± 1.7 for GP) than in non-atrophic gastritis (0.9 ± 0.9 for FC, 1.6 ± 1.1 for PZ and 0.9 ± 1.0 for GP) in all compartments studied ($p = 0.01$, $p = 0.02$ and $p = 0.002$, respectively).

In the antrum, in non-atrophic gastritis the positive staining of interstitial lymphocytes for detecting bcl-2 in PZ was higher in *H. pylori* positive cases (2.7 ± 0.8 *versus* 1.9 ± 1.3 ; $p = 0.02$).

The overall mean bcl-2 positive staining score of interstitial lymphocytes for the PZ of the antrum mucosa was significantly higher compared with the respective score for the PZ of the corpus mucosa (2.3 ± 1.2 *versus* 1.7 ± 1.7 ; $p = 0.02$).

Multiple regression analysis demonstrated that bcl-2 positive staining of interstitial lymphocytes in the corpus mucosa depended significantly on the severity of gastri-

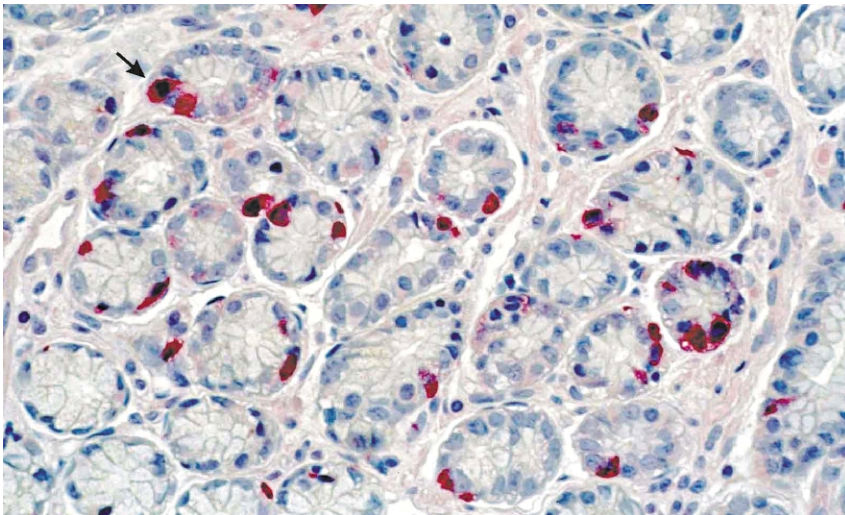


Fig. 4. Positive staining for somatostatin cells in the glandular part of the antrum mucosa ($\times 40$ objective, $\times 10$ eyepiece). Avidin-Biotin method



Fig. 5. Positive staining for β -H⁺, K⁺ ATP-ase in the canaliculi of parietal cells (intensive red staining, $\times 100$ objective, $\times 10$ eyepiece). Avidin-Biotin method

tis but not on the *H. pylori* density score (for FC $\beta = 0.45$; $p = 0.002$; for PZ $\beta = 0.47$; $p = 0.001$; for GP $\beta = 0.58$; $p = 0.00003$). In the antral mucosa, positive staining of interstitial lymphocytes for bcl-2 was more associated with the *H. pylori* score (in PZ $\beta = 0.24$; $p = 0.08$).

Density of G, D and β -H⁺, K⁺ ATP-ase positive cells

G and D cells were predominantly found in the PZ of the antral glands, and the mean density of G and D cells was significantly lower in atrophic and in non-atrophic gastritis compared with the normal mucosa (21.6 ± 20.8 ; 26.5 ± 20.4 and 54.2 ± 46.5 , respectively; $p = 0.04$; $p = 0.02$ for G cells and 4.1 ± 3.1 ; 7.2 ± 4.4 and 14.1 ± 10.5 respectively; $p = 0.02$ and $p = 0.0005$ for D cells). The density of the G cells of the antral glands revealed a significant negative correlation with atrophic alterations ($r = -0.49$; $p = 0.01$). The density of D cells was signifi-

cantly lower with a higher grade of chronic inflammation in the antrum mucosa ($r = -0.39$, $p = 0.03$). In the atrophic antrum mucosa there was a tendency to a negative correlation between D cell and *H. pylori* densities ($r = -0.35$; $p = 0.06$).

The density of β -H⁺, K⁺ ATP-ase-positive parietal cells in the atrophic corpus mucosa (22.8 ± 23.7) was significantly lower than in the normal mucosa (100.6 ± 26.7 ; $p = 0.0004$) and non-atrophic gastritis (84.7 ± 27.8 ; $p = 0.0001$). There were no significant differences between the mean densities of β -H⁺, K⁺ ATP-ase-positive parietal cells in the *H. pylori* positive and *H. pylori* negative mucosa (89.7 ± 30.5 versus 76.8 ± 37.1 ; $p = 0.29$). Also, multiple regression analysis showed that the density of β -H⁺, K⁺ ATP-ase-positive cells was significantly dependent on the grade of atrophy in the corpus ($\beta = -0.65$; $p = 0.000007$) but not on *H. pylori* score ($\beta = 0.053$; $p = 0.66$).

A comparison of the antrum and corpus with regard to proliferation activity, expression of bcl-2 and density of specific cells in association with the severity of gastritis and *H. pylori* colonization is summarized in Table 2.

DISCUSSION

In the present study we have revealed that severity of gastritis and presence of *H. pylori* have different impacts on gastric epithelial cell proliferation in the antrum and corpus. A significantly higher proliferation activity was established in the corpus mucosa in atrophy, while in the antrum the proliferation indices did not reveal a significant difference between the normal, non-atrophic and atrophic mucosa. These findings are in accordance with the data of Bechi et al. (32), which suggest differences in the proliferative activity of epithelial cells between the gastric antrum mucosa and corpus mucosa. There are data of other authors, which show enhancement of proliferation index with increasing degrees of gastritis both in the antrum and in corpus mucosa (33, 34). Our data are in concordance with the study of Lynch et al. who have shown also a strong correlation between epithelial proliferation and chronic inflammatory cell infiltrate. However, these authors

Table 1. State of antral and corpus mucosa and grade of *H. pylori* colonization in the studied patients

State of gastric mucosa	Antrum					Corpus				
	<i>H. pylori</i> colonization					<i>H. pylori</i> colonization				
	n	Absence	Mild	Moderate	Severe	n	Absence	Mild	Moderate	Severe
Normal (grade 0) (no chronic inflammation, no atrophy)	4	4	0	0	0	11	10	1	0	0
Non-atrophic gastritis (grade of chronic inflammation):										
mild (1)	9	9	0	0	0	15	8	2	4	1
moderate (2)	11	6	3	1	1	6	3	0	0	3
severe (3)	8	1	3	3	1	5	2	0	3	0
Atrophic gastritis (grade of atrophy):										
mild (1)	15	7	4	3	1	0	0	0	0	0
moderate (2)	2	0	0	0	2	2	2	0	0	0
severe (3)	7	4	1	2	0	4	4	0	0	0
Total	56	31	11	9	5	43	29	3	7	4

found no correlation between the degree of atrophy and cell proliferation, and only an independent predictor of cell proliferation in the mentioned study was the density of *H. pylori* (7).

In our study, the proliferation rates in the corpus glandular epithelium were not related to *H. pylori* status, while in the antrum glands proliferation activity was inversely related to a higher *H. pylori* score. This is in accordance with the study of Chow et al. (35) and the study of Unger et al. (36), where *H. pylori* also failed to increase cell proliferation in the antrum. Increased proliferative activity in the atrophic gastric corpus mucosa, especially in FC and GP, suggests that the corpus gland cell populations maintain an active cell turnover despite reduction in cellular mass as established through grading criteria. Extension of the proliferative compartment with a consequent displacement of proliferating cells to the epithelial surface has indeed been reported in advanced gastritis (37). Moreover, corpus gastritis can be compared with antrum gastritis in an earlier phase of inflammatory evolution, while the former still retains its proliferative repertoire: there is evidence that lesions in the antrum can be considered older than lesions in the corpus (33). Indeed, in the group of patients studied, atrophic antrum alterations prevailed over atrophic corpus alterations.

One of the aims of the present study was to test whether cell protection from apoptosis is related to gastritis and *H. pylori* colonization. Immunoreactivity for the apoptosis protector, bcl-2 protein, was detected in a subset of gastric epithelial cells, mainly in the glandular neck region. The noteworthy finding of our study was that both glandular proliferation

and bcl-2 protein reactivity were differently expressed already in the normal (*i.e.* without chronic inflammation or atrophy of the glands) corpus and antrum mucosa. In normal corpus glands, the balance between cell proliferation and programmed cell death was manifested as a lower PCNA and Ki-67 LI's and higher bcl-2 protein staining of epithelial cells in GP as compared with the atrophic mucosa. However, in the normal antrum mucosa bcl-2 protein staining of epithelial cells was reduced. In our study, the overall mean PCNA LI was significantly higher in the antrum than in the corpus. The overall mean bcl-2 positive staining score was higher in the antrum, particularly for *H. pylori* positive cases, than in the corpus, which is in accordance with the study of Loogna et al. 2002 (38). This finding may support the evidence of presence of different environments in the antrum and in the corpus mucosa (39), as well as dissimilarities between the antrum and the corpus regarding the interaction of the bacterium and the host (40, 41).

For the atrophic corpus mucosa, we established a higher proliferation index as compared with the respective index for normal corpus glands, and a simultaneous increase in bcl-2 positivity of the interstitial lymphocytes. While upregulation of the bcl-2 as the apoptosis protector factor suggests reduced apoptosis, its association with prolonged higher proliferation activity can be regarded as a factor contributing to development of MALT lymphoma and carcinogenesis (14, 42). A positive correlation of positive staining of bcl-2 in the interstitial lymphocytes of the corpus with the grade of gastritis may also indicate that these lymphocytes are protected from

Table 2. Comparison of antrum and corpus in relation to proliferation activity, expression of bcl-2 and density of specific cells in association with gastritis and *H. pylori* colonization

	Antrum	Corpus
Chr. inflamm. and <i>H. pylori</i>	Correlation with <i>H. pylori</i> score (p = 0.00009)	No correlation with <i>H. pylori</i> score (p = 0.42)
Atrophic gastritis	More cases of atrophic gastritis in comparison with corpus (24/56; 43%); p = 0.002	Less cases of atrophic gastritis in comparison with antrum (6/43; 14%)
Proliferation activity: PCNA LI	Overall mean PCNA LI higher than in corpus (p = 0.02) No correlation with chr. infl. and atrophy (p > 0.05)	Overall mean PCNA LI lower than in antrum Significant correlation with severity of chr. inflammation and atrophy (r = 0.46; p = 0.001)
Ki-67 LI	Mean score higher in <i>H. pylori</i> negative cases (p = 0.03) No correlation with gastritis score (p > 0.05) Negative correlation with <i>H. pylori</i> score (p = 0.03)	No correlation with <i>H. pylori</i> score (p > 0.05) Higher in atrophy (p = 0.04) No correlation with <i>H. pylori</i> score
Bcl-2 positive interstitial lymphocytes	No correlation with gastritis score, Mean score higher by <i>H. pylori</i> positive cases (p = 0.02)	Mean score higher in atrophy (p = 0.02) No correlation with <i>H. pylori</i> score
Bcl-2 positive epithelial cells	Overall mean bcl-2 positive staining score higher than in corpus (p = 0.02) 4/23 cases of positive staining localized in atrophic proliferating zone	Overall mean bcl-2 positive staining score lower than in antrum 5/36 cases of positive staining localized in normal and non-atrophic glandular part
G and D cell density	Negative correlation with gastritis score (r = - 0.49; p = 0.01) Tendency for negative correlation with <i>H. pylori</i> score in atrophy (r = - 0.35; p = 0.06)	
β -H+, K+, ATP-ase positive cells		Negative correlation with gastritis score (r = - 0.44; p = 0.02) No correlation with <i>H. pylori</i> score

programmed cell death and may hence be responsible for an increase in chronic inflammation.

The atrophic antral mucosa was associated with a more frequent bcl-2 protein staining of epithelial cells in the neck part of glands (PZ) than in the normal mucosa, which indicates a possible protection from cell loss and may thus reflect an important mechanism of retaining the potential for renewal of the antrum glands (14).

Concerning specific gastric cells, the present investigation has shown that antral G and D cell densities decrease significantly already in the stage of non-atrophic gastritis and reach a minimum in atrophic gastritis. This finding confirms previous data demonstrating a decrease in G cells, particularly in atrophic gastritis (43, 44). In our study, the presence

of *H. pylori* tended to be associated with a decrease in G and D cell density in the antrum, however, this decrease was significantly affected by the advanced grade of atrophic gastritis. Our results are consistent with the observation of Wyatt et al. (45) who also found significantly fewer gastrin cells in *H. pylori* positive patients, while this decrease was positively correlated with the degree of antral atrophic gastritis. The lower density of D-cells at a higher grade of chronic inflammation in the *H. pylori* positive antrum mucosa found in our study is in agreement with the evidence that *H. pylori* induces reduction of somatostatin cells and is correlated with inflammation severity and inflammatory mediators (19, 20, 46, 47).

The density of β -H+, K+ ATP-ase-reactive parietal cells was dependent on the grade of atrophic

gastritis rather than on the presence of *H. pylori*. The role of H⁺, K⁺ ATP-ase β -subunit in the epithelial cell development and the development of mature parietal cells was demonstrated by Franic et al. (48). This finding could imply that the decrease of H⁺, K⁺ ATPase-reactive parietal cells is associated predominantly with an advanced stage of mucosal atrophy rather than with *H. pylori* status.

In conclusion, the severity of gastritis and *H. pylori* colonization influences gastric epithelial cell proliferation and the expression of bcl-2 differently in the antrum and in the corpus mucosa. In the corpus mucosa, proliferation activity and the expression of bcl-2 in the interstitial lymphocytes was significantly higher in atrophic than in non-atrophic gastritis, but did not depend on *H. pylori* density. In contrast, antral mucosa proliferative activity showed no association with the severity of gastritis, while the expression of bcl-2 positive interstitial lymphocytes was more associated with *H. pylori* density. Homeostasis of antral G and D cells as well as the loss of β -H⁺, K⁺ ATP-ase-reactive cells in the corpus have been shown to be a function of the degree of atrophic alterations rather than of the impact of *H. pylori*.

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