Correlation between epithelial cell proliferation and histological grading in gastric mucosa

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Abstract

Aim—To determine if there is a correlation between the histological findings in the gastric mucosa and the degree of cell proliferation in gastric antral biopsies.

Methods—Cell proliferation in gastric antral biopsies was determined by *in vitro* bromodeoxyuridine labelling. Histological sections were assessed using the Sydney System.

Results—There was a positive correlation between antral mucosal cell proliferation and the acute inflammatory cell infiltrate ($r = 0.29$; $p = 0.03$). There was a stronger correlation with the chronic inflammatory cell infiltrate ($r = 0.53$; $p < 0.0001$) and the density of *H pylori* colonisation ($r = 0.54$; $p < 0.0001$). There was no correlation between gastric epithelial proliferation and the degree of atrophy. Stepwise multiple regression indicates that the only independent predictor of epithelial cell proliferation is the density of *H pylori* colonisation ($p < 0.0001$).

Conclusions—*H pylori* increases gastric epithelial cell proliferation through the mucosal inflammatory response and probably by other means. The strong correlation between epithelial proliferation, the chronic inflammatory cell infiltrate, and the density of *H pylori* colonisation may have implications for gastric carcinogenesis.

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We have shown previously that epithelial proliferation is increased in *H pylori* gastritis affecting antral and corpus mucosa.^{1,2} Eradication of the organism results in antral cell proliferation returning to normal, in contrast to persistent infection where cell proliferation remains increased on long term follow up.¹ The increased cellular proliferation may be caused by the inflammatory response stimulated by *H pylori* infection. In order to establish whether there is a relation between the two, we have studied the relation of both the density of *H pylori* colonisation of the gastric epithelium and the histological changes within the gastric mucosa with the degree of cell proliferation. We have also explored the relation between antral and corpus epithelial kinetics, and the effect of patient age, sex, and cigarette smoking.

Methods

Patients undergoing routine diagnostic endoscopy were recruited following informed consent. Those taking non-steroidal anti-inflammatory drugs, antibiotics, or bismuth salts were excluded from the study. At endoscopy, biopsies were taken from the antrum (2) and corpus (2) for *in vitro* bromodeoxyuridine labelling and routine histological processing. This study was approved by the hospital ethics committee.

BROMODEOXYURIDINE LABELLING

Two antral biopsies for immunostaining were put immediately into RPMI medium without L-glutamine (Gibco) containing bromodeoxyuridine (5 mg/10 ml) and placed in a water bath for 60 minutes at 37°C. They were then placed on filter paper and fixed in formalin. Using a three step immunoperoxidase technique, sections were stained with anti-bromodeoxyuridine (Dakopatt) antibody (1:20 dilution) for 60 minutes.

DETERMINATION OF LABELLING INDEX

Only sections that were complete and oriented were counted. For the purpose of counting, the gastric mucosa was divided into three zones: zone 1, surface and gastric pit; zone 2, isthmus; zone 3, glandular layer. The number of cells to

Table 1 Details the number, male to female ratio, number of smokers, and age distribution in the different patient groups studied†

Group	n	M:F	Smokers	Age (years) *
Normal	13	6:7	3/13	42 (21 to 57)
<i>H pylori</i> negative gastritis	10	6:4	2/9	38 (20 to 55)
<i>H pylori</i> positive gastritis	34	23:11	14/32	41.5 (18 to 63)

*Values are median (range).

†Details regarding the age and gender of one patient with *H pylori* gastritis were not obtained and therefore this patient was only included in the correlation studies and regression analysis.

Table 2 Mucosal cell proliferation in antrum and corpus

Group	n	Labelling index (%)		p Value
		Antrum	Corpus	
Normal	7	15.7 (4.0 to 18.5)	10.8 (5.3 to 15.2)	0.22
<i>H pylori</i> gastritis	18	19.1 (10.4 to 28.5)	14.4 (7.6 to 34.4)	0.07
All groups	28	17.0 (4.0 to 28.5)	13.6 (1.0 to 34.4)	0.02

Values are median (range). There is no difference in labelling index (LI%) between the antrum and corpus in subjects with normal gastric mucosa and patients with *H pylori* gastritis. When all groups are combined antral LI% is significantly greater than corpus LI% ($p = 0.02$).

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Figure 1 Relation between degree of atrophy and antral mucosal cell proliferation (labelling index, LI%). There was no significant correlation.

Figure 2 Significant correlation between polymorphonuclear cell infiltration and antral mucosal cell proliferation (labelling index, LI%).

Figure 3 Relation between lymphocyte infiltration and antral mucosal cell proliferation (labelling index, LI%). The correlation was stronger than for polymorphonuclear cell infiltration (see fig 2).

be counted in each zone was determined by counting consecutive high power fields until the continuous mean varied by less than 5%. It was found necessary to count 500 cells. The number of positively staining nuclei per 500 epithelial cell nuclei (or whole section when less than 500 cells per zone were present) was counted in each zone and expressed as a percentage. This value corresponds to the labelling index (LI%). The total LI% for the gastric glands was calculated from the sum of zones 1 to 3. Only unequivocally stained cells were counted as positive. All sections were counted by one person who was unaware of the *H pylori* status.

HISTOLOGICAL GRADING

Sections were stained with haematoxylin and eosin and periodic acid Schiff/Alcian blue. A modified Giemsa stain was used to detect *H pylori*. Each section was graded according to the Sydney system.³ The variables assessed were the presence and degree of lymphocyte/mononuclear cell (0–3) and neutrophil polymorph (0–3) infiltration, atrophy (0–3) and intestinal metaplasia (0–3), and density of colonisation of the mucosa by *H pylori* (0–3). All sections were examined by one histopathologist with a special interest in gastro-intestinal histopathology who was unaware of the endoscopic diagnosis or cell proliferation results.

STATISTICAL ANALYSIS

Pearson correlation analysis was used to determine if there was any relation between patient age and gastric antral LI%. Spearman rank analyses were used for the correlation studies between antral LI% and the gastric mucosal histological variables as well as density of *H pylori* colonisation. The Mann-Whitney U test analysis was used for comparing non-parametric data. Stepwise multiple regression was used to determine independent predictors of cell proliferation. Statistical analyses were performed using SSPS version 6.1.3. A p value of less than 0.05 was regarded as significant.

Results

PATIENTS

Fifty eight patients were enrolled in the study. Thirteen had normal endoscopic appearances and normal gastric histology. Ten had normal gastric endoscopic appearances but *H pylori* negative chronic gastritis on histological examination. Thirty five had *H pylori* gastritis and normal endoscopic appearances. Details of the three groups are provided in table 1. Antral biopsies were suitable for analysis in all 58 patients. Paired antral and corpus biopsies were obtained from 28 patients (seven control subjects with histologically normal gastric mucosa, three subjects with *H pylori* negative chronic gastritis, and 18 patients with *H pylori* gastritis). For the purposes of this study the labelling index (LI%) of zone 2, or isthmus, of the gastric glands (corresponding to the proliferative compartment) was used.^{1 2}

Figure 4 Relation between *H pylori* colonisation and antral mucosal cell proliferation (labelling index, LI%). The correlation was stronger than for polymorphonuclear cell infiltration (see fig 2).

GASTRIC MUCOSAL CELL PROLIFERATION, AGE, SEX, AND SMOKING

There was no difference in antral epithelial proliferation between males and females ($p = 0.8$: males, median (range) LI% = 15.7 (1.4 to 28.5); females, 15.6 (4 to 26.8)). We found no correlation between patient age and antral labelling index ($r = 0.13$; $p = 0.35$). When patients with *H pylori* gastritis alone were analysed there was still no correlation ($r = -0.19$; $p = 0.29$). There was no difference in antral cell proliferation between smokers and non-smokers in all groups combined ($p = 0.95$): smokers LI% = 14 (7.6 to 28.5); non-smokers LI% = 16.2 (1.4 to 26.8), or in patients with *H pylori* gastritis ($p = 0.67$): smokers LI% = 16.8 (10.4 to 16.8); non-smokers LI% = 17.6 (17.6 to 26.8).

ANTRAL AND CORPUS MUCOSAL CELL PROLIFERATION

These results are shown in table 2. Cell proliferation tended to be higher in the antrum than in the corpus in normal gastric mucosa and *H pylori* gastritis but this did not reach statistical significance. However, when all groups combined were analysed (including three subjects with *H pylori* negative gastritis), antral epithelial proliferation was significantly greater than that present in the corpus. This trend may have been accounted for by the difference in density of colonisation of the different sites by the organism, being greater in the non-acid-secreting antrum than in the acid producing corpus. In nine patients in whom the density of colonisation between antral and corpus biopsies was similar there was no difference in the labelling indices.

ANTRAL MUCOSAL CELL PROLIFERATION AND HISTOLOGICAL GRADING

These results are shown in figs 1 to 4. Biopsies from 58 patients were analysed. There was a significant positive correlation between antral cell proliferation and the acute inflammatory infiltrate (fig 2; $r = 0.29$; $p = 0.03$). There was a stronger correlation between antral mucosal cell proliferation and the chronic inflammatory

cell infiltrate (fig 3; $r = 0.53$; $p < 0.0001$), and also the density of *H pylori* colonisation (fig 4; $r = 0.54$; $p < 0.0001$). There was no correlation with the degree of mucosal atrophy (fig 1; $r = 0.17$), though mucosal atrophy was strongly associated with the presence of a chronic inflammatory infiltrate ($r = 0.65$; $p < 0.0001$). There were insufficient biopsies with intestinal metaplasia ($n = 4$) to draw any valid statistical conclusions. Sex ($r = 0.01$) and age ($r = 0.04$) were not correlated with the degree of cell proliferation. Using stepwise multiple regression the only independent predictor of cell proliferation in the model was the density of *H pylori* infection (regression coefficient 7.2; $p < 0.0001$).

Discussion

The finding that there is no relation between gastric epithelial kinetics and a subject's age and sex is not surprising. Other factors such as the mucosal morphology appear to be more important.⁴⁻⁶ Though cigarette smoking is believed to be important in gastric carcinogenesis,⁷⁻¹⁰ it does not appear to have any effect on antral cell proliferation as determined using bromodeoxyuridine labelling. It may act through the production of mutagenic agents within the gastric lumen.¹¹

In contrast to previous studies, using similar techniques, we have not found any difference between antral and corpus cell proliferation in subjects with normal gastric mucosa or those with *H pylori* gastritis. Cell proliferation in the antrum has been shown to be greater than in the corpus^{6,12} and has been attributed to the duodenogastric reflux of bile. Our findings may be attributable to the different patient groups studied. Previous biliary surgery, such as cholecystectomy, is believed to be associated with increased bile reflux into the stomach. None of the patients in our study had biliary surgery. This point was not addressed in the previous studies.^{6,12} Thus some patients may have undergone cholecystectomy, which causes increased duodenogastric bile reflux and may have affected antral epithelial kinetics. An alternative explanation for the difference in results is the possible effect of drugs. Subjects taking bismuth, aspirin, non-steroidal anti-inflammatory drugs, or gastric acid suppressants, which may affect gastric cell proliferation, were specifically excluded from our study. Drug ingestion by those patients studied previously was not specifically addressed.

Damage to an epithelium, in the context of chronic gastritis, results in accelerated exfoliation and cell death. The gastric mucosa responds to this insult by increasing the number of cells produced in the proliferative compartment, which then migrate luminally to replace the lost cells. The finding that eradication of *H pylori* does not lead to an immediate decline in epithelial proliferation argues against a direct effect. Indeed recent in vitro studies indicate that *H pylori* itself has a suppressant effect on epithelial proliferation.¹³ It seems more likely that the chronic inflammatory response, which only slowly regresses following eradication, provides the stimulus to cell

proliferation. The strong correlation between the density of *H pylori* colonisation and epithelial proliferation suggests that *H pylori* promotes cell proliferation through the inflammatory response but possibly also through independent mechanisms. The organism may increase cell exfoliation from the mucosal surface by direct injury, or through production of ammonia, other products of metabolism, or toxins, with compensatory hyperproliferation. Alternatively, *H pylori* has an effect on cell proliferation through an independent mechanism mediated by mucosal lymphocytes.¹⁴ *H pylori* produce ammonia which increases gastric epithelial cell proliferation.^{15, 16} There are other factors which influence gastric epithelial proliferation that were not included in our model. Salt intake¹⁷ and bile reflux¹⁸ are likely candidates, although interestingly, salt intake correlates with cell proliferation only in *H pylori* positive patients.¹⁹

Infection by *H pylori* is invariably associated with an inflammatory response in the gastric mucosa²⁰. The inflammatory response correlates closely with the density of *H pylori* colonisation of the gastric mucosa, and eradication of the organism is followed, in the long term, by a return to normal mucosal morphology.²¹ It is not unreasonable to suggest that the mucosal inflammatory response to *H pylori* infection, rather than the organism itself, is responsible for the increased epithelial cell proliferation in *H pylori* gastritis. In order to answer this question we sought to determine whether there was any correlation between epithelial cell proliferation and the morphology of antral mucosa, using the Sydney system for the grading of gastritis.

The relation between the acute inflammatory cell infiltrate and the degree of mucosal cell proliferation is consistent with the presence of a high density of polymorphs in the region of the proliferative compartment of the gastric glands.²⁰ It is likely that free oxygen radicals and other factors including cytokines released by the inflammatory response to *H pylori* interact with cells in the proliferative zone to increase cell proliferation.^{5, 22}

In this study, the strong correlation between the lymphocytic infiltrate and gastric epithelial proliferation is of interest. It is possible that factors unique to the chronic inflammatory cell infiltrate are involved in the promotion of cell proliferation,^{5, 6} for example the increased expression of epidermal growth factor (EGF) in the proliferative zone.⁶ EGF receptor (EGFr) is strongly expressed in the proliferative compartment and EGF expression is increased in gastritis.⁶ EGF and transforming growth factor α (TGF α), a ligand to EGFr, also have increased expression in gastric carcinoma.^{23, 24}

The finding that there is no correlation between glandular atrophy and reduced epithelial cell proliferation is of particular interest. Histologically, gastric atrophy is usually found in association with an increase in the mucosal chronic inflammatory infiltrate, so called atrophic gastritis. Atrophic gastritis has persistently been shown to be associated with

increased cell proliferation but of a similar level to that found in *H pylori* gastritis alone.²⁵⁻²⁷ This suggests that while atrophic gastritis is a hyperproliferative state, the presence of atrophy does not itself confer a greater degree of epithelial proliferation than that present in *H pylori* gastritis, and indeed atrophy in itself may be associated with a decline in cell proliferation. It is likely that the greater degree of cell proliferation may be accounted for by the mucosal inflammatory response and, if present, by *H pylori*. Glandular loss is accompanied by elongation of the proliferative compartment so that the atrophic gastric mucosa is part of a hyperproliferative state which will be more susceptible than normal mucosa to the effect of luminal mutagens present in the altered gastric environment.

Although it is well recognised that atrophic gastritis is associated with a reduction in cell cycle time and the presence of mitoses in gastric surface epithelial cells,^{26, 28} indicating an upward expansion of the proliferative compartment, the development of atrophy may be a manifestation of a failure of the gastric mucosa to respond adequately to the injurious luminal agents. It is noteworthy that in two patients with gastric atrophy alone, the levels of cell proliferation were no different from in control subjects with normal mucosa.²⁵ The development of intestinal metaplasia, while not stimulating a greater degree of cell proliferation,²⁷ may confer some protective effect. The development of gastric intestinal metaplasia is an enigma and, depending on the subtype, is believed to be premalignant.²⁹⁻³⁴ It could be argued that the development of a certain subtype of intestinal metaplasia is a failed adaptive gastric mucosal response which leads to an increased risk of developing gastric carcinoma.

Our findings suggest that while cigarette smoking does not influence gastric epithelial kinetics, *H pylori* infection does promote gastric epithelial proliferation through the mucosal inflammatory response and by other mechanisms. Other factors such as dietary salt and bile reflux probably also play important roles in promoting gastric epithelial cell proliferation. The strong correlation between epithelial proliferation, the chronic inflammatory cell infiltrate, and the density of colonisation by *H pylori* may have implications for gastric carcinogenesis.

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