Importance of changes in epithelial cell turnover during *Helicobacter pylori* infection in gastric carcinogenesis

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Summary

The role of *Helicobacter pylori* in gastric carcinogenesis is supported almost exclusively by epidemiological data and prospective histopathological studies. From biological and molecular points of view, there is no evidence that *H pylori* or its cytotoxic products have any mutagenic effects. Nevertheless, this infection is associated with profound changes in the pattern of epithelial cell turnover in gastric glands, though the importance of these changes in gastric carcinogenesis is still controversial. *H pylori* infection increases cell proliferation and alters the distribution of cycling cells within these glands, but these changes can be reversed by successful eradication of the infection. Apoptosis seems to be increased in gastric epithelial cells during *H pylori* infection, as shown by in vitro studies. There is some, though no conclusive, evidence that this finding also occurs in *H pylori* positive subjects. It seems that *cagA* status influences the effect of *H pylori* on epithelial apoptosis in infected patients. An association of in vitro *H pylori* induced apoptosis with changes in the expression of pro- and anti-apoptotic genes is reported in the literature, but further study is necessary to clarify the effect of *H pylori* infection on the molecular events of the apoptotic pathway.

Introduction

The suspected link between *Helicobacter pylori* infection and gastric cancer is supported by histopathological,¹⁻⁴ and epidemiological studies.⁵⁻⁹ Some of these data led the International Agency for Research on Cancer to classify *H pylori* as a class I carcinogen in 1994.¹⁰ Some authors feel, however, that this conclusion has been based on simple association rather than a true causal link, and they dispute the quality of epidemiological evidence in its favour.¹¹ One of the main criticisms that has been raised concerns the paradoxically low gastric cancer rates found in some populations with very high rates of infection.¹² Another arises from the low incidence of gastric cancer among patients with duodenal ulcer, a disease known to be caused by *H pylori* infection.¹³⁻¹⁴ Many hypotheses have been advanced to explain these discrepancies. The genetic makeup of the host, diet, immunological status, differences between various strains of *H pylori*,¹²⁻¹⁵⁻¹⁹ and age at the time of infection²⁰ have all been proposed as factors potentially capable of influencing the outcome of *H pylori* infection in different geographical areas and in different individuals. From the biological point

of view, malignancy is known to be the result of an accumulation of genetic alterations and mutations that are responsible for the different phenotypes within the carcinogenic cascade. Up to now there has been no evidence that *H pylori* induces any sort of irreversible DNA damage, and there are no experimental models of *H pylori* related gastric carcinogenesis. However, it is clear that the organism is capable of modifying epithelial cell turnover within gastric glands. Disturbances in cell turnover in the gastrointestinal tract are believed to predispose to cancer development, and, until recently, these changes were considered to be a marker of increased cancer risk.²¹ The purpose of this article is to review current thinking on the importance of these changes.

Helicobacter pylori infection and epithelial cell turnover in gastric glands

There is strong evidence that *H pylori* infection alters the kinetic pattern of gastric glandular epithelium. In the glands of the gastric mucosa, as in the other segments of the digestive tract, cell renewal is rapid. Thus a delicate balance among all factors influencing this process is needed to maintain a normal tissue volume in healthy people. The key points of this homeostatic process are cell proliferation and programmed cell death, or apoptosis. Both are highly regulated phenomena that are essential for remodelling tissues during development, maintaining tissue homeostasis and repair following injury, and both can be modulated (that is, promoted or inhibited) by several factors. Cell proliferation and apoptosis may be reversible phenomena, though in some cases—that is, neoplastic tissues, they are not. The proliferative zone of the gastric gland—that is, the site of stem cells, is located in the neck and isthmus (fig 1). As they mature, these cells normally migrate towards the two extremities of the gland: mucus cells moving upward into the foveolar zone; parietal cells, together with a limited number of mucus cells, shifting downwards into the base of the gland. During this bi-directional migration, the cells differentiate, gradually lose their replicative capacity and eventually die. In fact, proliferating cells are rarely found in the intermediate zone and apex of the pits or at the bottom of glands.²²⁻²³ Many investigators have demonstrated increased cell proliferation in gastric glands associated with *H pylori* infection.²⁴⁻²⁹ The kinetic pattern usually observed in these cases is represented by an actual increase in the total number of proliferating epithelial cells within the gland, together

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Figure 1 Bi-directional migration of gastric epithelial cells during the proliferation/differentiation process.

with an abnormal distribution of the cycling cells along the length of the gland. This situation translates into an unusually large number of proliferating (and therefore, undifferentiated) cells at the apex of the pits.^{26 28 29} Table 1 shows the results of a proliferation study we conducted using bromodeoxyuridine immunohistochemical analysis of antral biopsy specimens from patients with *H pylori* related gastritis and non-infected controls. The total labelling indexes (LI) and the LIs for each of the five compartments into which gastric pits were divided confirmed that in the *H pylori* infected patients there is an overall increase in epithelial cell proliferation within the gastric foveolae, with an upward shift of proliferating cells toward the surface.³⁰ These changes are thought to be caused by an increase in the mucosal content of ammonia, known to be a strong stimulus of cell proliferation,^{31 32 33} provoked by the bacterium itself. *H pylori* infection associated hypergastrinaemia is also believed to play a role in increasing epithelial cell turnover.³¹ Whether the altered cell proliferation is also related to the inflammatory infiltrate is controversial. Using semi-quantitative scoring systems to estimate the intensity of inflammation, various investigators have reached different conclusions.^{23 26 34} However, when cell proliferation figures were compared

with mucosal inflammatory cell counts using computer aided image analysis,^{35 36} a positive correlation emerged between total labelling indexes and both polymorphonuclear and mononuclear cell densities (fig 2).³⁷ This finding seems to support a role for inflammation in determining the alterations in foveolar cell kinetics seen during gastric *H pylori* infection. The inflammatory mechanism underlying the bacterium's enhancement of epithelial cell turnover may include mucosal production of tumour necrosis factor α , interleukin 1 and interleukin 6, all of which have been shown to influence epithelial cell proliferation.³⁸ It has also been shown that increased generation of free radicals occurs during *H pylori* infection,^{38 39} and these are believed to interact with the cells in the proliferative compartment to increase cell proliferation.²³ Moreover, inflammation during *H pylori* infection is accompanied by a decrease in the mucosal content of vitamin C,⁴⁰ which is an important chemical defence against oxidative DNA damage. Epithelial hyperproliferation in *H pylori* associated chronic superficial gastritis returns to normal after the infection has been eradicated^{27 41 42} and remains normal unless reinfection occurs.²³ The hyperproliferative state provoked by the infection thus seems to be reversible.

Epithelial kinetic patterns of glands along the gastrointestinal tract can be easily modulated by several factors. Hyperproliferation in the colonic mucosa has—for example, been successfully diminished by modifying the mucosal content and quality of polyunsaturated fatty acids.^{43 44} Similar improvement has been achieved in the gastric mucosa with beta-carotene supplementation.⁴⁵ During progression from chronic superficial gastritis to subsequent phases of Correa's cascade (chronic atrophic gastritis, intestinal metaplasia, dysplasia, cancer),⁴⁶ this reversibility seems to be lost. In the pre-helicobacter era, it was already known that epithelial hyperproliferation occurs in both atrophic and metaplastic glands,^{47 48} and this finding was later confirmed in association with *H pylori* infection.^{28 29} It has been shown recently that the development of atrophic gastritis and intestinal metaplasia are strongly related to *H pylori* infection,⁴⁹ but the proliferative pattern of metaplastic epithelium does not seem to be related to *H pylori* status.²⁹ Recently Ierardi *et al* observed that in patients with chronic active *H pylori* gastritis,⁴² proliferation in the gastric foveolae returns to normal after the infection has been eliminated, but normalisation is not observed in metaplastic (complete or incomplete) cells. This finding indicates that control of cell proliferation is somehow lost during the progression from chronic gastritis to intermediate steps of gastric carcinogenesis. Intestinal metaplasia might thus represent the phenotypic expression of the true initiating phase of the carcinogenic process. The genetic mutations underlying these crucial changes in the regulation of proliferation are still unknown. Progression from chronic active *H pylori* gastritis to chronic atrophic gastritis seems to be related to *cagA* positivity.¹⁹ Very few studies have focused on

Table 1 Antral epithelial cell proliferation of gastric pits, evaluated by bromodeoxyuridine immunohistochemistry performed on endoscopic biopsy specimens, in *H pylori* positive and negative subjects. *H pylori* status was determined by histology and the ¹³C-urea breath test

	<i>H pylori</i> positive (n=20)	<i>H pylori</i> negative (n=17)
LI% total	5.4 (1.0–11.7)*	3.3 (1.5–5.6)
LI%/compartment (1–5)		
LI% 1 (apex)	1.3 (0–9.6)	0 (0–3.4)
LI% 2	2.9 (0–14.5)†	0 (0–3.0)
LI% 3	4.9 (0–21.2)	3.7 (0–6.7)
LI% 4	4.8 (0–21.6)	5.6 (0–10.9)
LI% 5 (base)	9.2 (2.5–15.3)	7.2 (0–16.2)

All results are expressed as median (range). Subjects with *H pylori* positive gastritis had a significantly higher total labelling index (LI) compared with *H pylori* negative controls (* $p < 0.01$, Mann-Whitney U test). Moreover, when gastric pits were divided into five longitudinal compartments (base compartment 5 to apex compartment 1), cell proliferation was significantly increased in the upper part of the pits in *H pylori* positive subjects († $p < 0.005$, Mann-Whitney U test).

Figure 2 Relation between antral epithelial cell proliferation and mucosal inflammatory cell infiltrate. Computer aided image analysis was used to evaluate acute (polymorphonuclear cells) and chronic (mononuclear cells) inflammation in gastric antral lamina propria from biopsy specimens. Thirty seven subjects, 20 *H pylori* positive and 17 *H pylori* negative, were included in the analysis. Consecutive sections (stained with haematoxylin and eosin) from those examined for cell proliferation were evaluated in each case. Mononuclear cells were automatically evaluated using microdensitometric software (Microscience Phoenix Inc.), whereas polymorphonuclear cells were evaluated using software for linear morphometry (Microscience Phoenix Inc.). The number of inflammatory cells counted was expressed as density (cells/mm²). A significant relation was found between total labelling index and both polymorphonuclear and mononuclear cell densities. (Spearman rank test $R=0.42$; $p<0.05$).

the influence of different *H pylori* strains on gastric epithelial cell proliferation, but the results that have emerged seem to be somewhat contradictory.⁵⁰⁻⁵¹ This issue clearly requires further investigation.

Apart from cell proliferation studies, attention has recently been focused on the role of cell death in the development and progression of tumours. In the gastrointestinal tract (as elsewhere), homeostasis during the renewal of tissues is a balance between cell production and cell loss. There seem to be two distinct types of cell death: necrosis and apoptosis.⁵² While the former is a pathological event resulting from acute cellular injury, apoptosis is a genetically encoded, physiological, active and irreversible process that plays a major role in tissue development and renewal, and alterations in this process are believed to be involved in the pathogenesis of many human diseases.⁵³ Apoptosis is, in fact, regulated by a variety of intra- and extracellular signals that act either to suppress or enhance the expression of specific cellular genes.⁵³ The genetic programmes involved in regulation of apoptosis seem to occur in distinctly different stages,⁵⁴ each modulated by a specific set of genes identified as *ced* (cell death defective), as seen in studies of the nematode *Caenorhabditis elegans*.⁵⁵⁻⁵⁶ *bcl-2*, a human proto-oncogene, which is highly homologous to *ced-9* of *C. elegans*,⁵⁷ is known to play an important role in regulation of apoptosis; it encodes a 26 kDa protein and is localised to mitochondria, endoplasmic reticulum and the perinuclear membrane. Bcl-2 protein is a part of a large family of proteins encoded by specific genes belonging to the so called *bcl-2* family. Some of these proteins (*bcl-2* and *bcl-xL*) support survival,⁵⁸ whereas others (*bax*, *bad*, *bcl-xS*) are apoptosis inducers.⁵⁹⁻⁶¹ Recently, overexpression of *bcl-2* with abnormal distribution of apoptotic cells along the glands, which are usually found at the extremities of normal gastric glands,⁶² has been described in both intestinal metaplasia and gastric dysplasia.⁶³⁻⁶⁴ Altered distribution of apoptotic cells, particularly their unusual presence in the regenerative zone of gastric glands,

has also been confirmed by using a specific immunohistochemical technique (TUNEL, see later).⁶⁵⁻⁶⁶ These findings indicate that defective control of the process of apoptosis already occurs in the early stages of gastric carcinogenesis.⁶⁷ The p53 gene is also known to be heavily implicated in the life and death processes of human cells. It is currently believed that the function of p53 takes place through two main mechanisms: cell cycle arrest and stimulation of apoptosis.⁶⁸⁻⁶⁹ This stimulation is demonstrated by the fact that the apoptotic response to radiation and other DNA damaging agents is reduced in the absence of p53.⁷⁰⁻⁷³ The current opinion is that in physiological conditions p53 is activated when DNA damage occurs. At this point different factors induce cells to decide to enter p53 mediated cell cycle arrest or the apoptotic pathway. The factors influencing the decision of a cell to select one of these two pathways are not fully known. It seems that in the case of unstable genomes, activation of oncogenes forcing the cells into a replicative cycle or when survival factors of cells are limited, the p53 mediated apoptosis prevails.⁶⁹ The p53 gene is mutated in a large percentage of cancers. Immunohistochemically demonstrable overexpression of p53 is regarded as a marker of the accumulation of a stable mutant p53 protein within the cells and it has been found in a high percentage of advanced gastric cancers.⁷⁴⁻⁷⁵ Detectable overexpression of p53 protein⁷⁶ as well as p53 gene mutations⁷⁷ have been also found in early gastric carcinomas. Furthermore, alterations in the p53 gene have been described in both intestinal metaplasia and gastric dysplasia.⁷⁸ As far as the influence of *H pylori* infection on gastric epithelial apoptosis is concerned, different groups have investigated this phenomenon both in experimental models and in vivo studies. Moss *et al.*,⁷⁹ using the TdT-mediated dUTP-biotin nick end labelling (TUNEL) technique, found that gastric epithelial apoptosis was clearly enhanced in duodenal ulcer patients with *H pylori* infection compared with *H pylori* negative controls. Similar findings have been reported by

Figure 3 Apoptotic indexes in 20 *H. pylori* positive and 17 *H. pylori* negative dyspeptic patients. Epithelial apoptosis was assessed using the TUNEL method on antral glands from endoscopic biopsy specimens. The apoptotic index was expressed as the percentage of labelled cells to total cells in each case. Median values are shown by horizontal bars. No significant differences were found between the two groups (Mann-Whitney U test; $p=0.07$).

others.⁸⁰⁻⁸¹ We have recently investigated epithelial apoptosis in antral gastric mucosa of patients with non-ulcer dyspepsia with and without *H. pylori* infection, using the same technical approach.⁸² In our study the presence of *H. pylori* infection was not associated with any significant changes in gastric epithelial apoptosis and there was wide variability in the apoptotic indexes of both *H. pylori* positive and *H. pylori* negative groups (fig 3). This discrepancy may be explained by additional data recently published by Peek *et al*,⁸³ who found that patients infected with *cagA* positive *H. pylori* strains had apoptotic indexes significantly lower than patients with *cagA* negative strains. When these subgroups were combined and compared with a non-infected control group, no difference in the mean apoptotic indexes was observed. So it is possible that the similarity of the apoptotic indexes found in *H. pylori* positive and *H. pylori* negative patients in our study might reflect the *cagA*+/*cagA*-makeup of the former group (we did not attempt to classify the strains in our patients). Conversely, the results of in vitro studies seem to be more consistent, indicating that apoptosis is indeed stimulated by *H. pylori* regardless of whether the strain is cytotoxic.⁸⁴⁻⁹⁰ However, it must be emphasised that data on apoptosis in cultured cells may not always be applicable to the in vivo situation.⁹¹ A decrease in apoptosis has been described after eradication of *H. pylori* infection.⁷⁹⁻⁹²⁻⁹³ As for the mechanisms underlying the possible effects of *H. pylori* on gastric epithelial apoptosis, several recent findings may be relevant. For example, Kuck *et al* have reported that *H. pylori* up-regulates the expression of the CD95 (or apo-1/FAS) receptor,⁸⁷ which plays an important role in initiating apoptosis. In fact, the enhanced CD95 expression observed in this study was associated with increased rates of apoptosis in gastric epithelial cell lines. The inhibitory effect on human gastric apoptosis provoked by cytotoxic strains of *H. pylori* has recently been attributed to the activation of NF- κ B, a transcription factor whose enhancement seems to reduce the rate of apoptosis.⁸³⁻⁹⁴⁻⁹⁵ However, there is also evidence that apoptosis in AGS gastric epithelial cells is an NF- κ B independent phenomenon.⁹⁰

Future studies on the genes that regulate apoptosis in humans, such as p53 and *bcl-2*,⁵³

will probably provide more information on the actual effect of *H. pylori* infection on epithelial apoptosis. Recently, overexpression of bax and bak proteins encoded by the two pro-apoptotic members of the *bcl-2* gene family, together with moderate suppression of *bcl-2* itself, has been associated with *H. pylori* induced apoptosis in the AGS gastric epithelial cell line.⁸⁸

Conclusions

Epithelial cell proliferation and altered distribution of cycling cells within gastric glands are a common feature of chronic superficial gastritis caused by *H. pylori* infection. This phenomenon seems to be reversible as successful eradication of the infection is followed by persistent normalisation of cell kinetics. Because of its reversibility, gastric epithelial cell hyperproliferation in chronic superficial gastritis cannot be regarded as a marker of tumour initiation. Certainly the persistence of this altered cell turnover pattern, with proliferating cells located in the superficial zone of the gastric pits, increases the risk of DNA damage by endogenous and exogenous carcinogens. In this sense the enhanced cell proliferation in chronic active gastritis represents only a very generic increase in the risk of developing cancer. Other co-factors are needed to induce the genetic changes underlying premalignant and malignant phenotypes. Thus, the hyperproliferation associated with chronic active *H. pylori* gastritis represents a sort of "driver" for carcinogens.¹² Hyperproliferation within atrophic and metaplastic glands seems to have a different significance and to express a different level of risk. In this case the phenomenon seems to have escaped the reach of normal control mechanisms, persisting even after the apparent stimulus (that is, the infection) has been eradicated. Apoptosis of gastric epithelial cells seems to be enhanced by *H. pylori* as shown by in vitro experiments, whereas in vivo studies have not produced conclusive results so far. It seems that *cagA* status may influence the in vivo effect of *H. pylori* infection on epithelial apoptosis in gastric glands.⁸³ There is some evidence that in vitro *H. pylori* induced apoptosis is associated with different expressions of pro- and anti-apoptotic genes of the *bcl-2* family, but further study is needed to clarify how the molecular events of the apoptotic pathway of gastric epithelial cells can be affected by *H. pylori* infection.

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