Cell proliferation in type C gastritis affecting the intact stomach

J E Mac Dowall, P Willis, R Prescott, S Lamonby, D A F Lynch

Abstract

***Aims*—Type C gastritis caused by bile reflux has a characteristic appearance, similar to that seen in other forms of chemical gastritis, such as those associated with NSAIDs or alcohol. An increase in mucosal cell proliferation increases the likelihood of a neoplastic clone of epithelial cells emerging, particularly where there is chronic epithelial injury associated with bile reflux. It has been shown previously that type C gastritis is associated with increased cell proliferation in the postsurgical stomach. The aim of this study was to determine cell proliferation in type C gastritis caused by bile reflux affecting the intact stomach.**

***Methods*—Specimens from 15 patients with a histological diagnosis of type C gastritis on antral biopsy were obtained from the pathology archives between 1994 and 1997. A control group of nine normal antral biopsies was also selected and all underwent MIB-1 immunostaining. The gastric glands were divided into three zones (zone 1, gastric pit; zone 2, isthmus; and zone 3, gland base) and the numbers of positively staining nuclei for 500 epithelial cell nuclei were counted in each zone to determine the percentage labelling index (LI%).**

***Results*—Cell proliferation was significantly higher in all three zones of the gastric glands with type C gastritis compared with controls as follows: zone 1, median LI% in type C gastritis 64.7 (range, 7.8–99.2), controls 4.7 (range, 2.0–11.3); zone 2, median LI% in type C gastritis 94.7 (range, 28.8–98.7), controls 40.2 (range, 23.1–70.3); and zone 3, median LI% in type C gastritis 20.0 (range, 1.3–96.0), controls 2.6 (range, 0.9–8.7).**

***Conclusions*—Bile reflux is thought to act as a promoter of gastric carcinogenesis in the postsurgical stomach. The same may be true in the intact stomach.**

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Keywords: cell proliferation; epithelial kinetics; chemical gastritis

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Correa's hypothesis of gastric carcinogenesis proposes a series of sequential steps towards malignant transformation, namely gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and invasive type adenocarcinoma. Various aetiological factors are thought to be important in this process such as excessive salt intake, *N*-nitroso compounds, antioxidant deficiency, and *Helicobacter pylori* infection.[1]

Increased cell proliferation in the gastric glands could increase the statistical probability of transformation from normal mucosa to gastric carcinoma; thus, factors that cause persistent hyperproliferation might promote carcinogenesis.[2] *Helicobacter pylori* is a well documented cause of increased cell proliferation in both the intact and postsurgical stomach.[3–5]

Bile has been shown to cause increased cell proliferation and carcinoma in animal models.[6] Bile reflux has also been implicated as a causal factor in the increase in carcinoma rates seen after gastric resection in humans. Studies have shown an increase in epithelial cell kinetics with bile reflux in the postsurgical stomach,[7] and it has been suggested that bile might act synergistically with *H pylori* to cause a greater degree of cell proliferation.[8] The severity of duodenogastric reflux has been shown to correlate with histological changes of atrophic chronic gastritis, metaplasia, and foveolar hyperplasia in patients undergoing gastric resection.[9]

Gastritis caused by bile reflux is recognised as a distinct histopathological entity. It is characterised by foveolar hyperplasia, congestion of capillaries, focal lamina propria oedema, increased numbers of vertically orientated smooth muscle fibres with respect to the surface epithelium, and a paucity of acute and chronic inflammatory cells, sometimes associated with intestinal metaplasia.[10 11] Similar features can also be seen in non-steroidal anti-inflammatory drug (NSAID) induced gastropathy.

There is a paucity of literature concerning the effects of bile on the intact stomach. Concentrations of bile acid have been shown to be positively associated with intestinal metaplasia.[12 13] Gastric foveolar hyperplasia, a histological marker of bile reflux, has also been shown to be significantly increased in patients with cancer of the intact stomach.[14]

It could be postulated that if bile is indeed a promoter of gastric carcinogenesis then cell proliferation will be increased in type C gastritis. Our study aims to investigate the above hypothesis by determining cell proliferation in type C gastritis caused by bile reflux in the intact stomach.

Patients and methods

A retrospective review of the pathology archives was performed from 1994 to 1997 and all patients with a histological diagnosis of type C gastritis were identified. Biopsy samples of antral mucosa with normal macroscopic appearances obtained at upper gastrointestinal endoscopy from these patients were assessed

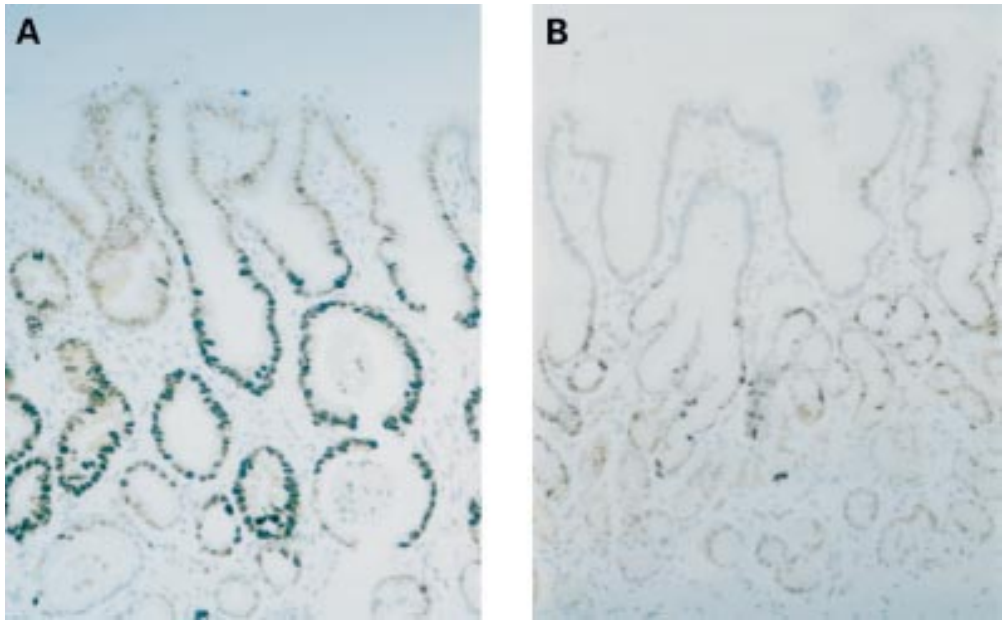

Figure 1 MIB-1 immunostaining of gastric glands in (A) type C gastritis (B) normal mucosa.

for the characteristic features of type C gastritis, as described previously. All samples were reviewed by a histopathologist before inclusion in our study.

A control group of subjects with histologically normal antral mucosa was also identified from the archives. All patients with a history of NSAID ingestion, alcohol abuse, acid suppression medication, antibiotic ingestion, or previous gastric surgery were excluded from the study. A modified Giemsa stain was used to detect the presence of *H pylori* and any positive samples were excluded.

MIB-1 IMMUNOSTAINING

Sections were dewaxed with xylene, rinsed in absolute alcohol, treated with 0.5% hydrogen peroxide in methanol (8 ml 100 vol 30% wt/vol hydrogen peroxide in 400 ml of methanol) for 30 minutes, then rinsed with water. Sections for labelling with MIB-1 were placed in citrate buffer and incubated twice for five minutes (650 W) in a microwave oven (Miele M696). After this, sections were placed in prewarmed (37°C) distilled water containing 0.1% trypsin in 0.1% calcium chloride, with the pH adjusted to 7.8 using 1% sodium hydroxide. The sections were incubated for eight minutes, then rinsed in cold running water for five minutes to prevent further digestion. After the slides were rinsed in Tris buffered saline, pH 7.6 (TBS), excess buffer was removed, and the sections were placed in a humidity chamber. The sections were then covered by two to three drops of horse serum and incubated for 10 minutes. After this, the excess serum was removed by wiping and the sections covered with optimally diluted primary antibody. The sections were stained with the MIB-1 antibody (1/50; Binding Site, Birmingham, UK) for 60 minutes. The antibodies were diluted using Tris/Tween (0.01% Tween in TBS). After washing in TBS, the sections were incubated with a biotinylated rabbit antimouse immunoglobulin (1/200 dilution; Dakopatts) for 30 minutes. The sections were then incubated in streptavidin–biotin–horseradish peroxidase complex (HRP; comprising 20 µl avidin, 20 µl biotinylated HRP, and 1 ml Tris/Tween) for 30 minutes, developed in DAB solution for at least 10 minutes, and finally incubated in copper sulphate solution for five minutes. The sections were washed in water before being counterstained. The sections were then dried in alcohol, cleared in xylene, and mounted.

DETERMINATION OF LABELLING INDEX

Only those sections with a full thickness of mucosa (epithelium intermuscular mucosae) and orientated perpendicularly to the epithelial surface were counted. For the purpose of counting, the gastric glands were divided into three zones: zone 1, gastric pit; zone 2, isthmus; and zone 3, gland base. The number of positively staining nuclei in each 500 epithelial cell nuclei (or whole section when less than 500 cells present) was counted in each zone and expressed as a percentage. This value corresponds to the labelling index (LI%). The number of cells counted was determined by counting consecutive high power fields until the continuous mean varied by less than 5%. In pilot studies, which preceded several studies that we have undertaken in this area, the optimal sample size was found to be 500 cells because at that number the coefficient of variation is $< 5\%$.[15 16] Only unequivocally stained cells were counted as positive. All sections were counted by one person.

STATISTICAL ANALYSIS

The Mann-Whitney U test was used for the analysis of non-parametric data. A p value of > 0.05 was regarded as significant.

Results

Thirteen patients with a histological diagnosis of type C gastritis were recruited (four men, nine women) with a median age of 71 years

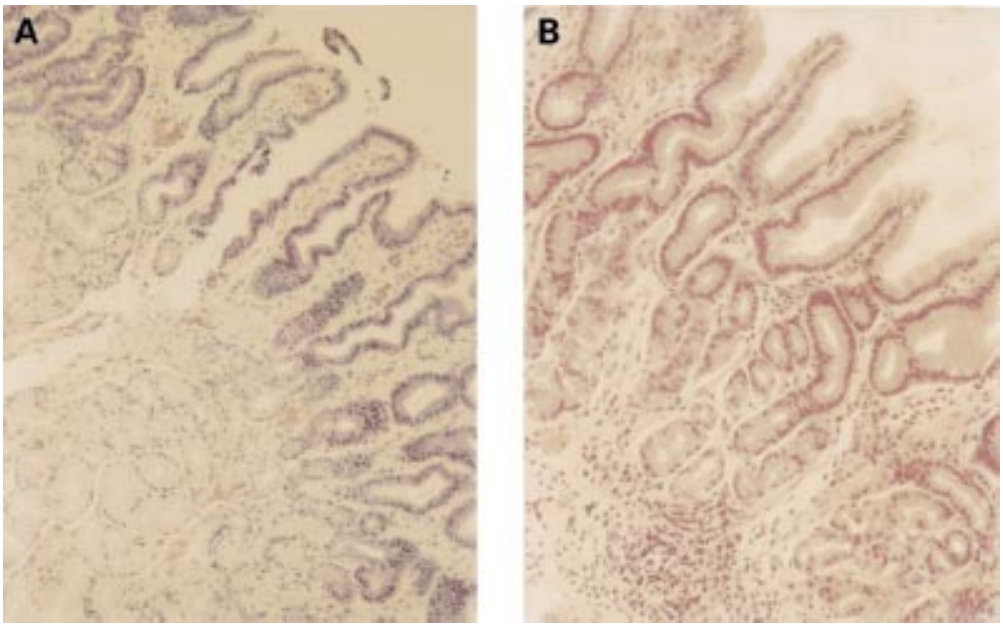

Figure 2 Haematoxylin and eosin staining of gastric glands in (A) type C gastritis (B) normal mucosa.

(range, 33–82). Nine matched controls were recruited (five men, four women) with a median age of 48 years (range, 35–77). Most positively staining nuclei were situated in zone 2, corresponding to the proliferative compartment of the gastric glands (figs 1 and 2). Cell proliferation was found to be significantly increased in all three zones of the gastric glands with type C gastritis compared with controls ($p = 0.0001$, $p = 0.0005$, $p = 0.0002$, respectively). Zone 1: median LI% in type C gastritis, 64.7 (range, 7.8–99.2), controls 4.7 (range 2.0–11.3); zone 2: median LI% in type C gastritis 94.7 (range, 28.8–98.7), controls 40.2 (range, 23.1–70.3); and zone 3: median LI% in type C gastritis, 20.0 (range 1.3–96.0), controls 2.6 (range, 0.9–8.7) (table 1).

Discussion

MIB-1 immunostaining has been shown to provide an accurate assessment of cell proliferation,[15] and using this technique we have demonstrated an increase in mucosal cell proliferation in the gastric antrum in patients with a histological diagnosis of type C gastritis, presumed to be caused by bile reflux. This supports the hypothesis that bile might be a promoter of gastric hyperproliferation and possibly carcinogenesis in the intact stomach.

Data regarding the postsurgical stomach already support this theory. The high degrees of bile reflux found postsurgery have been implicated as a causal factor in the higher incidence of carcinoma in the gastric remnant.[4 7 8] Increased cell proliferation in the gastric corpus of the postsurgical stomach has been demonstrated and is thought to be a result of high bile acid concentrations. Our findings suggest that the same might be true in the intact stomach. Bile reflux has also been shown to result in an increase in intestinal metaplasia, both in the intact and the postsurgical stomach,[12] and it is known that incomplete intestinal metaplasia (type III) is associated with the dysplasia–carcinoma sequence.

However, little is known of the mechanism by which bile causes damage to the gastric epithelium. Concentrations of putrescine, which reflect the degree of cell proliferation, have been shown to be increased in humans after cholecystectomy, an operation that increases duodenogastric reflux. It is thought that this might be a repair response to the cytotoxic effects of bile on the antral mucosa.[17] The effect of bile acids on the colon have been studied more extensively, with deoxycholic acid being shown to cause increased cell proliferation in both animal and human models, thus increasing the risk of carcinoma. Alterations in prostaglandin synthesis and bile induced apoptosis have been suggested as possible mechanisms.[18 19]

The role of *H pylori* in this type of chemical gastritis is unclear. Bile is thought to be inhibitory to the presence of *H pylori*; however, the two can coexist and have been shown to increase intestinal metaplasia when present together.[12] In addition, cell proliferation in the postsurgical stomach has been shown to be greater in patients positive for *H pylori*.[4] It is possible that the two factors act synergistically to produce greater damage to the gastric epithelium, with a corresponding increased cell proliferation response. It has also been postulated that the presence of *H pylori* might itself result in increased duodenogastric reflux, possibly because of raised serum gastrin affecting antroduodenal motility.[20]

Table 1 Cell proliferation in the gastric antrum in type C gastritis and normal mucosa

		Median LI (range)					
Group	*n*	*Zone 1*		*Zone 2*		*Zone 3*	
TCG	13	64.7%	(7.8–99.2%)	94.7%	(28.8–98.7%)	20.0%	(1.3–96.0%)
Controls	9	4.7%	(2.0–11.3%)	40.2%	(23.1–70.3%)	2.6%	(0.9–8.7%)
p Value		0.0001		0.0005		0.0002	

LI, labelling index; TCG, type C gastritis.

A limitation of our study is that although patients had histological features consistent with type C gastritis caused by bile, the concentrations of bile acid in the stomach were not assayed. However, by using strict exclusion criteria it can be reasonably assumed that bile reflux was the main aetiological factor. A positive association between degrees of foveolar hyperplasia, chronic inflammation, lamina propria oedema, glandular atrophy, and intestinal metaplasia has already been demonstrated in both the intact and the postsurgical stomach.[12 13] However, in our study we cannot confirm that the histological findings of type C gastritis were in fact accompanied by higher than normal bile acid concentrations. At endoscopy, some patients had been noted to have pronounced amounts of bile present in the stomach, but most were reported as macroscopically normal. Further work to clarify this would be of interest.

In conclusion, we have demonstrated increased cell proliferation associated with type C gastritis in the intact stomach. Bile reflux is thought to act as a promoter of gastric hyperproliferation and possibly carcinogenesis in the postsurgical stomach. Our findings suggest that the same might be true in the intact stomach.

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