Effect of *Helicobacter pylori* infection and its eradication on cell proliferation, DNA status, and oncogene expression in patients with chronic gastritis

G Nardone, S Staibano, A Rocco, E Mezza, F P D’Armiento, L Insabato, A Coppola, G Salvatore, A Lucariello, N Figura, G De Rosa, G Budillon

**Abstract**

**Background**—*Helicobacter pylori*, the main cause of chronic gastritis, is a class I gastric carcinogen. Chronic gastritis progresses to cancer through atrophy, metaplasia, and dysplasia. Precancerous phenotypic expression is generally associated with acquired genomic instability.

**Aim**—To evaluate the effect of *H pylori* infection and its eradication on gastric histology, cell proliferation, DNA status, and oncogene expression.

**Methods/Subjects**—Morphometric and immunohistochemical techniques were used to examine gastric mucosal biopsy specimens from eight controls, 10 patients with *H pylori* negative chronic gastritis, 53 with *H pylori* positive chronic gastritis, and 11 with gastric cancer.

**Results**—All patients with chronic gastritis were in a hyperproliferative state related to mucosal inflammation, regardless of *H pylori* infection. Atrophy was present in three of 10 patients with *H pylori* negative chronic gastritis and in 26 of 53 with *H pylori* positive chronic gastritis, associated in 18 with intestinal metaplasia. DNA content was abnormal in only 11 patients with atrophy and *H pylori* infection; eight of these also had c-Myc expression, associated in six cases with p53 expression. Fifty three patients with *H pylori* positive chronic gastritis were monitored for 12 months after antibiotic treatment: three dropped out; infection was eradicated in 45, in whom cell proliferation decreased in parallel with the reduction in gastritis activity; atrophy previously detected in 21/45 disappeared in five, regressed from moderate to mild in nine, and remained unchanged in seven; complete metaplasia disappeared in 4/14, and markers of genomic instability disappeared where previously present. In the five patients in whom *H pylori* persisted, atrophy, metaplasia, dysplasia, and markers of genomic instability remained unchanged.

**Conclusions**—Chronic *H pylori* infection seems to be responsible for genomic instability in a subset of cases of *H pylori* positive chronic atrophic gastritis; eradication of *H pylori* infection can reverse inflammation and the related atrophy, metaplasia, and genomic instability.

**Keywords:** *H pylori* infection; atrophic gastritis; genomic instability; eradication therapy

*Helicobacter pylori*, the main cause of chronic gastric disorders, has been defined as a class I gastric carcinogen. However, only a minority of *H pylori* positive patients with chronic gastritis develop gastric cancer, and the link between *H pylori* and gastric carcinoma is unclear.

Gastric carcinogenesis is a multistep process progressing from chronic gastritis through glandular atrophy, metaplasia, and dysplasia. Atrophic gastritis and intestinal metaplasia may be the long term consequences of *H pylori* infection, but it is debatable whether or not bacterial eradication reverses these lesions. Acquired genomic instability, which is typical of the phenotypic expression of precancerous lesions, generally precedes neoplastic clonal expansion.

Chronic *H pylori* infection damages gastric barrier function and stimulates gastric cell proliferation, which leads to mucosal repair, but which can also induce cellular DNA damage. The most frequent epigenetic phenomenon of DNA alteration is activation of oncogenes and/or mutation of oncosuppressor genes. The role of these genes has been studied in colon carcinogenesis and, to a lesser extent, in gastric carcinogenesis, but their interrelation with *H pylori* infection has yet to be defined. The aim of our study was to detect the relation between *H pylori* infection and gastric carcinogenesis evaluated as the appearance of genomic instability and the associated phenotypic expression. To this aim we investigated gastric histology, cell proliferation, DNA content, and bel-2, p53, and c-Myc expression in patients with chronic gastritis with and without *H pylori* infection and after *H pylori* eradication therapy.

**Materials and methods**

**SUBJECTS**

The study population consisted of 82 subjects (52 men and 30 women, age range 24–75 years). They were consecutively recruited from 1 January 1995 to 30 June 1995 from a population of 680 subjects referred to our department for dyspepsia according to the following criteria for *H pylori* infection

**Abbreviations used in this paper:** PCNA, proliferating cell nuclear antigen; AgNOR, nucleolar organiser region.
Values are percentages except for age which is mean (SD). nCG, H pylori negative chronic gastritis; pCG, H pylori positive chronic gastritis; GC, gastric cancer; PMN, polymorphonuclear leucocytes; IM, intestinal metaplasia (*total number 18: 16 complete and two incomplete).

Table 1  Frequency of clinical, histological and immunohistochemical variables in the population studied

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disappeared in four subjects.

Three patients dropped out; intestinal metaplasia after H pylori eradication. 

Figure 2 Immunohistochemical expression of proliferating cell nuclear antigen (PCNA) in gastric crypt sections (as indicated on the figure; a mean of five well oriented crypts for each patient) of patients with functional dyspepsia, H pylori negative chronic gastritis, or H pylori positive chronic gastritis, with or without complete intestinal metaplasia (IM), before and after H pylori eradication. LI, labelling index, expressed as percentage of PCNA positive nuclei; C, controls with functional dyspepsia; nCG, H pylori negative chronic gastritis; pCG IM+, H pylori positive chronic gastritis without intestinal metaplasia; IM+, chronic gastritis without intestinal metaplasia after H pylori eradication; IM+, chronic gastritis with intestinal metaplasia after H pylori eradication. /Three patients dropped out; intestinal metaplasia disappeared in four subjects.

HISTOPATHOLOGICAL DIAGNOSIS

Haematoxylin and eosin stains were used for the histopathological diagnosis; the modified Giemsa stain was used for H pylori identification. Presence of H pylori, degree of inflammatory reaction and glandular atrophy, intestinal metaplasia, and cellular dysplasia were diagnosed and classified according to the updated Sydney system in each biopsy sample. The diagnosis of atrophy, defined as loss of glandular tissue, was based on agreement between the pathologists on at least two samples for each site investigated.

IMMUNOHISTOCHEMISTRY

For each case, 4 µm thick serial sections were cut from paraffin wax blocks, mounted on acid-cleaned glass slides, and heated at 55°C for 60 minutes. Slides were dewaxed and rehydrated,
then the endogenous peroxidase activity was inhibited by incubation with 3% H$_2$O$_2$ in methanol (20 minutes at room temperature). To reduce non-specific background staining, slides were incubated with 5% goat serum (15 minutes at room temperature). To enhance immunostaining, sections were treated with an antigen retrieval solution (10 mM citric acid monohydrate, pH 6.0, adjusted with 2 M NaOH) and heated three times in a microwave oven at high

![Image](https://example.com/image1)

**Figure 3** Proliferating cell nuclear antigen (PCNA) immunostaining of the gastric mucosa. (A) H pylori negative chronic gastritis with expression of PCNA in the nuclei of the cells of the glandular neck; (B) H pylori positive chronic gastritis; (C) H pylori positive chronic gastritis with intestinal metaplasia; (D) H pylori positive chronic gastritis after successful eradication treatment; (E) invasive gastric cancer showing high levels of PCNA positivity. Note the low labelling index of PCNA in the adjacent normal mucosa. Original magnifications: A, ×250; B, ×150; C, ×400; D, ×250; E, ×150.

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<td>Area</td>
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<td>4.0 (0.07)</td>
<td>&lt;0.05</td>
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<td>4.3 (0.10)</td>
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<td>1.1 (0.02)</td>
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<td>1.1 (0.01)</td>
<td>4.8 (0.08)</td>
<td>&lt;0.05</td>
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<td>Gastric cancer</td>
<td>11</td>
<td>3 (7.8)</td>
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</table>

A mean number of five well oriented crypts was examined for each patient. Results are expressed as mean (SD) of positive cells. p Value was calculated using the Mann-Whitney U test. Gastric cancer values (total rate) are reported but they were not considered in the statistical evaluation.
power for five minutes. Finally, slides were incubated with the appropriate primary antisera in a moist chamber overnight at 4°C. The monoclonal primary antibodies used were: anti-proliferating cell nuclear antigen (anti-PCNA, PC10; DBA, Milan, Italy; dilution 1:500); anti-bcl-2 protein (Dako, Milan, Italy; dilution 1:400); anti-c-Myc protein (Onco-gene Science, San Diego, California, USA; c-Myc p62, dilution 1:50); anti-p53 protein (NCL-CM1, YLEM, Rome, Italy; dilution 1:150).

Immunohistochemical staining reactions for each of the antibodies tested were compared within corresponding areas of the tissue sections from the consecutive slides. The avidin-biotin-peroxidase complex procedure (ABC standard; Vector Laboratories, Burlingame, California, USA) was then performed as described by Hsu et al. Peroxidase activity was detected with diaminobenzidine as substrate. Finally, sections were weakly counterstained with Harris’s haematoxylin and coverslipped with a synthetic mounting medium.

Negative controls with normal human serum replacing specific primary antibodies were included in each run. Positive controls were a case of colon adenocarcinoma for p53 protein, a normal lymph node for c-Myc and PCNA, and a case of a low grade follicular lymphoma for bcl-2 protein. Sections were considered positively stained only in cases of unequivocal nuclear staining for p53, c-Myc, and PCNA, and cytoplasmic staining for bcl-2.

The degree of immunopositivity was evaluated semiquantitatively. A total of 300 cells was counted in random fields from representative areas of the lesions, and the immunoreactive cells were roughly assessed and expressed as percentages. The scoring system for all the antibodies tested was: 0–5% (negative); 5–25% (low positivity); 25–50% (moderate positivity); >50% (high positivity).

STAINING AND COUNTING OF NUCLEOLAR ORGANISER REGIONS (AgNORS)

For each case, a 4 µm section was dewaxed in xylene and rehydrated through graded ethanol to deionised distilled water. AgNOR staining was performed as described by Egan et al. The final working solution was obtained by dissolving gelatin in 1% aqueous formic acid at a concentration of 2%, and mixing it with a 60% aqueous silver nitrate solution (1:2, v/v). This silver colloid solution was applied to the sections (60 minutes at room temperature, shielded from daylight). The sections were then washed with deionised distilled water, and counterstained with Mayer’s haematoxylin, dehydrated, and mounted with a synthetic medium. The usual controls were performed as described by Crocker and Nar.

Figure 4  Silver stained nucleolar organiser regions (AgNORS) in gastric mucosa. (A) H pylori negative chronic gastritis: small to medium-sized AgNORs in the nuclei. (B) H pylori positive chronic gastritis: medium-sized irregular AgNORS in the nuclei. (C) H pylori positive chronic gastritis, after eradication treatment. (D) Gastric cancer: large, irregular and sometimes unusually shaped AgNORS in neoplastic cells. Original magnification: A, B, C and D, ×1000, oil immersion.
MORPHOMETRIC ANALYSIS
A Leica Quantimet 500C-Image analyser and processing system was used for morphometric analysis. Images were recorded by a JVC TK-1280E videocamera, connected to a Leitz Orthoplan light microscope. A QWIN V01.00 software package elaborated the data. Within the selected fields, a final number of 200 consecutive nuclei of cells from each case were measured with a × 40 lens. Image acquisition and colour detection were performed in “RGB” (an option that allows the selection of red, green, and blue). Artefacts and image overlapping were corrected in Binary Edit. The AgNOR number for each nuclear area, the area of nucleus and each AgNOR, and the total area of AgNOR were calculated automatically, as were AgNOR length, breadth, perimeter, roundness, and the aspect ratio of each nucleus. The perimeter was rectified using a correction factor of 1.064. Values are expressed in µm.

DNA PLOIDY
The Feulgen (sulphuric acid-fucsin) technique was used for nuclear DNA staining of a section from each case, deparaffinised in xylene and rehydrated through decreasing concentrations of ethanol. Cellular DNA was quantified using a microprocessor-controlled image analysis system (Leica-Quantimet 500C analyser, a Sony Ccd camera, and a Leitz Orthoplan microscope). Leica-QWIN V0200A software was used to analyse the data. At least 200 cells from each patient were examined in non-consecutive random fields of representative areas of the lesions. The following indexes were determined for each

Figure 5  bcl-2 protein expression. (A) H pylori negative chronic gastritis: moderate bcl-2 expression. (B) H pylori positive chronic gastritis. (C) H pylori positive chronic gastritis with dysplasia: low bcl-2 expression. (D) H pylori positive chronic gastritis: moderate expression of bcl-2 protein in areas of intestinal metaplasia. (E) Nodal metastasis from gastric cancer: negative for bcl-2 protein in gastric cancer cells. Original magnifications: A, × 250; B, × 250; C, × 400; D, × 250; E, × 100.
measurement: normal diploid DNA content (2c); 2c deviation index (2cDI), defined as the variance in DNA content of single cells around the normal diploid (2c) DNA peak; 5c exceeding events (5cEE), defined as the percentage of cells with DNA content higher than 5c; DNA malignancy grade (DNA-MG), a logarithmic transformation of the 2cDI value to produce a continuous scale ranging from 0 to 3.0 DNA content.

The methodology described here has been fully validated in gastric biopsy samples.

**ANTI-CagA SERUM**

The *H pylori* strain CCUG 17874 (CagA+) was denatured in Laemmli buffer at 100°C for five minutes and electrophoresed in a 10% polyacrylamide gel with sodium dodecyl sulphate. Proteins were transferred to nitrocellulose sheets which were saturated with 3% defatted milk in phosphate buffered saline and 0.1% Triton X (Blotto). Strips were cut out, and serum samples were assayed at a dilution of 1:200 in Blotto. After overnight incubation at room temperature, strips were washed with Blotto, and then incubated with anti-human IgG conjugated with peroxidase at room temperature for 90 minutes. After washing, the reaction was visualised by addition of the substrate (H2O2 in a solution of 0.3% 4-chloro-I-naphthol in 0.05 M Tris/HCl buffer, pH 6.8).

An anti-CagA antibody raised in rabbit against a recombinant CagA fragment served as a positive control.

**STATISTICAL ANALYSIS**

We set the power to 0.8, with type I error = 0.05 and control to case ratio 1:1. The aim was to detect a minimal difference of 30% between nCG and pCG groups. Data were analysed with the SPSS package for Windows. The categorical variables (*H pylori* infection, cell infiltration, atrophy, intestinal metaplasia, dysplasia, DNA ploidy, p53, and c-Myc) were analysed using the χ² test or Fisher’s exact test. Correlation between these variables was assessed using the Spearman correlation coefficient. The non-parametric Mann-Whitney U test was used to compare the DNA values (2c, 2cDI, 5cEE, and MG), AgNOR area, and PCNA expression in gastric crypt sections of patients with functional dyspepsia and gastritis with and without *H pylori* infection. The Wilcoxon matched pair test was used to evaluate PCNA expression in gastric crypt sections of patients with gastritis with *H pylori* infection before and after eradication therapy. The analysis of the “before” and “after” data in the *H pylori* positive group given eradication treatment was evaluated with the McNemar test.

**Results**

Table 1 shows the clinical, histological, and immunohistochemical variables of the population studied. Mean age increased in rank order from normal controls to patients with chronic gastritis and to those with gastric cancer; male sex was prevalent in *H pylori* related disease.
and gastric cancer. A gastric and/or duodenal erosive/ulcerative disease was detected in 30% of nCG cases and 55% of pCG cases. The inflammatory infiltrate consisted of neutrophils in pCG patients and mainly lymphomonocytes in nCG. Gastric atrophy was mild in 30% of nCG subjects and was generally restricted to the antrum. Atrophy was detected in 49% (26/53) of pCG patients; it was mild and restricted to the antrum in five patients, and moderate, extending to the angulus, in 21 patients. Complete intestinal metaplasia, associated with atrophy, was found in 1/10 nCG and 16/53 pCG patients (restricted to the antrum in 10 of the latter). Incomplete metaplasia with atrophy was found in antral biopsy specimens from only 2/53 pCG patients. These two patients also had an abnormal DNA content and p53 and c-Myc overexpression. Gastric dysplasia, detected in the antrum of 3/53 pCG patients (one mild and two moderate), was associated with intestinal metaplasia (one complete and one incomplete), c-Myc and/or p53 overexpression in two cases, and abnormal DNA content in all three. Figure 1 gives the histological and immunohistochemical features and DNA content in pCG patients.

Cell proliferation, assessed by evaluation of PCNA expression (figs 2 and 3) and AgNOR silver staining, number, and morphometric variables, was increased (table 2 and fig 4) with respect to healthy controls in patients with gastritis regardless of H pylori infection, and associated with the inflammation. AgNOR expression was higher (about 3- to 5-fold) in patients with gastric cancer than in the other groups (table 2). PCNA expression was 2-fold higher in the lower basal portion of the gland in pCG patients with intestinal metaplasia compared with pCG patients without intestinal metaplasia (fig 2).

Expression of bcl-2 was preserved in controls and the gastritis groups, regardless of H pylori infection, intestinal metaplasia, and dysplasia (fig 5A–D). In gastric cancer, bcl-2 was expressed at very low levels in three early cancers and was not detectable in the remaining eight invasive cancers (fig 5E).

Image analysis of DNA content showed aneuploidy in 11/53 pCG patients, confined to biopsy specimens in which atrophy was present, and in all gastric cancer patients (fig 6). The DNA values (DNA indexes, 2cDI, 5cEE, and MG) were higher in gastric cancer than in pCG with aneuploidy (table 3). There were no significant differences in DNA values between the remaining groups (nCG with euploidy versus pCG with euploidy; table 3) or with regard to sex and age of patients (data not shown). Aneuploidy was significantly associated with atrophy (11/11) and expression of p53 (6/11) and c-Myc (8/11) oncoproteins (fig 1 and table 4).

The expression of c-Myc was low to moderate in 8/53 pCG patients (fig 7A, B); all these

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<th>PMN</th>
<th>Lymphocytes/monocytes</th>
<th>Atrophy</th>
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PMN, polymorphonuclear leucocytes; IM, complete intestinal metaplasia; PCNA, proliferating cell nuclear antigen.
patients also had an abnormal DNA content, and two had glandular dysplasia (fig 1). A moderate to strong c-Myc positivity was detected in 4/11 gastric cancers (fig 7C), while c-Myc was undetectable in control subjects and nCG patients.

Finally, p53 was overexpressed in 63% (7/11) of gastric cancers (fig 8C) and in 15% (8/53) of pCG (fig 8A, B); six of the latter also had abnormal DNA content and gastric metaplasia (fig 1). No control subject nor nCG patient showed p53 expression.

The frequency of DNA aneuploidy, and p53 and c-Myc expression in relation to histological findings shows that aneuploidy and p53 and c-Myc expression were invariably associated with atrophy and were more pronounced in patients with intestinal metaplasia (fig 1).

The *H pylori* CagA status, investigated in 25/53 patients, was positive in 22 and negative in three. Atrophy was present in 54% of the CagA positive patients, intestinal metaplasia in 36%, dysplasia in 4%, DNA aneuploidy in 22%, and c-Myc and p53 expression in 22% and 18% respectively. However, the small number of subjects with CagA negative *H pylori* prevented statistical evaluation.

One year after antibiotic treatment, the study protocol was repeated in the 53 pCG patients (table 5). Three patients dropped out. *H pylori* was eradicated in 90% of patients (45/50), in whom cell proliferation, PCNA expression (fig 3E) and AgNOR staining (fig 4C) decreased in parallel with the reduction in inflammatory infiltrate, whereas no significant difference was detected in patients with intestinal metaplasia (fig 2). The intensity of neutrophil infiltration was directly related to the rate of mucosal cell proliferation in the patients who were responsive to *H pylori* eradication treatment (p<0.02). Atrophy disappeared completely in five patients (all with mild atrophy restricted to the antrum); it regressed from moderate to mild only in the antrum in nine and remained unchanged in seven. Complete metaplasia associated with atrophy disappeared in four of 14 patients. Finally, DNA aneuploidy and p53 and c-Myc expression, previously detected in eight, six, and five of the 45 patients respectively, disappeared.

In the five subjects in whom *H pylori* infection persisted after eradication therapy, atrophy, metaplasia, dysplasia, and markers of genomic instability remained unchanged.

**Discussion**

Concurrent or previous *H pylori* infection is associated with a 2.7- to 12-fold risk of gastric cancer.2 Carcinogenesis invariably starts with cell hyperproliferation.2 13 52 The relation between *H pylori* infection, gastric mucosal damage, and the cell proliferation rate is a matter of debate. *H pylori* urease activity and leucocyte infiltration have a mitogenic effect,13 53-55 but gastric cell hyperproliferation also occurs independently of *H pylori* infection.36 37 In our study, the gastric epithelial proliferative rate, evaluated using PCNA and AgNOR analyses, was increased in gastric cancer and chronic gastritis, regardless of *H pylori* infection (figs 2–4 and table 2). Therefore mucosal cell hyperproliferation appears to be a constant finding of chronic gastric damage whatever the aetiology, but it is related to inflammatory infiltrates which were prevalently lympho-monocytic in nCG and made up of

<table>
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<td>Dysplasia</td>
<td>3 (6)</td>
<td>0</td>
</tr>
<tr>
<td>DNA aneuploidy</td>
<td>11 (21)</td>
<td>0</td>
</tr>
<tr>
<td>p53</td>
<td>8 (15)</td>
<td>0</td>
</tr>
<tr>
<td>c-Myc</td>
<td>8 (15)</td>
<td>0</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. p Value was calculated using the McNemar test comparing before and after therapy regardless of the outcome of treatment. The eradication treatment consisted of 15 days on omeprazole 20 mg twice a day, clarithromycin 500 mg twice a day, and tinidazole 500 mg twice a day. Three subjects dropped out during follow up.

*16 complete and two incomplete intestinal metaplasias; †two complete plus two incomplete intestinal metaplasias.
polymorphonuclear leucocytes in pCG (table 1). Mucosal hyperproliferation is linked to apoptosis, and there is mounting evidence that *H pylori* infection induces apoptosis. The bcl-2 proto-oncogen is involved in controlling apoptosis and is related to the initial phase of cancer. The expression of the bcl-2 gene was unaltered in our patients with chronic gastritis, regardless of *H pylori* infection, which supports the idea that *H pylori* induced apoptosis occurs through a mechanism that is independent of bcl-2 gene expression. bcl-2 gene expression was also preserved in our patients with early gastric cancer, but was undetectable in advanced cancer. This finding contrasts with reports of bcl-2 overexpression in intestinal type gastric cancer. It is conceivable that the discrepancy is related to differences in populations studied and in the type, staging, and grading of cancer.

A chronic hyperproliferative state may also favour cellular DNA damage. We detected a relation between chronic hyperproliferation and cellular DNA damage only in *H pylori* positive patients with active gastritis. Therefore the cytotoxic effect of *H pylori* infection and the related polymorphonuclear leucocyte infiltration may play important roles in the development and progression of mucosal damage. Aberrant DNA is a prognostic indicator for cancer and seems to be independent of other clinical pathological factors. We detected aneuploidy in all our cancer patients, but we also identified a novel subset of pCG patients with aneuploidy (1/53) (table 3). Aneuploidy was significantly associated with c-Myc and/or p53 expression (fig 1 and table 4), which are the most widely used markers of genomic instability. The c-Myc oncogene is implicated in the transformation and progression of mutated cells. In this study it was expressed in 36% of gastric cancer (4/11) and 15% of pCG (8/53) patients. This is a novel finding because the expression of c-Myc has not previously been investigated in chronic gastritis or in relation to *H pylori* status. c-Myc expression is more frequent in gastric adenocarcinoma than in adenoma and has also been proposed as an aid to differentiate between the two conditions. Half of the patients with c-Myc expression and aneuploidy had p53 overexpression, which was also detected in 63% of gastric cancer patients. The mutation of the p53 gene, which we found in 15% of pCG patients, is a common occurrence in colorectal carcinoma where it is related to a poor prognosis and distant metastasis. It has been reported in gastric cancer and precancerous gastric lesions but never in relation to *H pylori* infection.

Interestingly, aneuploidy and c-Myc and p53 expression were not detected in the absence of *H pylori* contamination or in patients with *H pylori* infection but without gastric atrophy. Therefore, in our population, the appearance of genomic instability required the presence of both gastric atrophy and chronic *H pylori* infection (fig 1).

Several studies have shown that patients with peptic ulcer and neoplastic gastric epithelial lesions are more likely to be infected by CagA positive strains which possess a genomic insertion called “cag pathogenicity island” which includes genes involved in virulence. Infection by CagA positive *H pylori* strains increases the risk of developing atrophy and intestinal metaplasia possibly because of the enhanced inflammatory potential shown by these strains. Although investigated only in about 50% of pCG patients, the high prevalence of positivity for CagA (23 of 25 subjects studied) may help to explain the high incidence of atrophy (49%) and intestinal metaplasia (34%) observed in our patients. Furthermore, the enhanced prevalence of DNA aneuploidy and p53 and c-Myc expression in our patients is in agreement with the observation of an increased cancer risk in individuals infected by CagA positive *H pylori* strains compared with individuals infected by Cag negative *H pylori* strains and, more in general, in individuals who live in areas with a high rate of CagA positive *H pylori* strains. Unfortunately, we were not able to verify this hypothesis directly because of the small number of CagA negative patients in this study.

The effects of *H pylori* eradication on atrophy and related intestinal metaplasia are controversial and there is no consensus among gastrointestinal pathologists as to the identification and grading of these lesions. In a recent study, no changes in intestinal metaplasia and atrophy were detected after *H pylori* eradication irrespective of *H pylori* CagA status. In contrast, we found that *H pylori* eradication may be followed, one year after treatment, by the disappearance of complete metaplasia and/or reduction of atrophy (table 5), when both are present in the antrum. Other groups have reported similar results. The patchy nature of the lesions and the subjective nature of the interpretation may account for these controversial findings. However, according to a recent stringent definition of atrophy, “true irreversible” atrophy is differentiated from “apparent” atrophy which is reversed by removal of inflammation. Our finding of disappearance or regression of atrophy, mainly from the antrum, after *H pylori* eradication suggests that inflammatory infiltration plays a role in this controversial histological finding, even though we cannot rule out the involvement of other factors such as age, sex, diet, genetic makeup of the host, nature of the lesions, biopsy sampling, *H pylori* strains, and environmental toxic factors. Long term studies and a larger number of stratified patients in relation to age of infection are needed to establish definitely the reversibility of *H pylori* related lesions and the predictive value of genetic markers for *H pylori* eradication.

Our data confirm the significant link between such precancerous features as incomplete metaplasia and dysplasia and genomic instability. However, we found genomic instability in four cases not showing these morphological aspects (fig 1). It is noteworthy that in Barrett’s oesophagus, p53 overexpression...
precedes dysplasia in patients followed for about three years.76 77

In conclusion, chronic H pylori infection seems to be responsible for genomic instability in a subset of cases of H pylori positive chronic atrophic gastritis and also in the absence of notorious precancerous lesions like metaplasia and dysplasia; eradication of H pylori infection can reverse inflammation and related atrophy, metaplasia, and genomic instability. Finally, we suggest that pCG patients with atrophy should receive eradication treatment for H pylori, while pCG patients with H pylori infection who do not respond to antibiotic treatment should be examined for markers of genomic instability and closely monitored.

We would like to thank Rosa Napolano and Antonella Coppeto for help and assistance with endoscopy and biopsy specimens and we are indebted to Jean Gilder for editing and revising the text. Preliminary data from this study were presented at the American Gastroenterological Association Meeting held on 10–16 May, 1997. This research was supported by a grant from MURST (40 and 60%) Rome, Italy.


Effect of *Helicobacter pylori* infection and its eradication on cell proliferation, DNA status, and oncogene expression in patients with chronic gastritis

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*Gut* 1999 44: 789-799
doi: 10.1136/gut.44.6.789

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Towards immunotherapy for pancreatic cancer

Editor,—McKenzie and Apostolopoulos’ recent article on immunotherapy for pancreatic carcinoma (Gut 1999;44:767–769) gave an excellent overview. We agree that the poor prognosis of this disease makes it imperative that new agents and novel therapeutic strategies are investigated. However, although this paper discusses classical immunotherapy (where immune competent cells are stimulated to attack pancreatic cancer cells directly), the induction of antibodies directed against growth factors by immunisation (where the immunogen stimulates the immune system to inhibit the growth of tumour cells indirectly) is not mentioned. We are currently undertaking a phase II clinical trial for inoperable pancreatic cancer using one such immunogen, Gastrimmune, which induces neutralising antibodies against amidated gastrin-17 and its precursor glycine extended gastrin-17 (this immunogen is also undergoing a phase II trial for gastric cancer at the University Department of Surgery, Nottingham, UK).

Gastrin has been shown to be a growth factor in a variety of malignancies including colorectal, gastric, and hepatocellular cancers both in vitro and in vivo studies; precursor forms such as progastrin and glycine extended gastrin also have a trophic effect. More recently the autocrine/paracrine pathway, in which tumour cells produce and respond to gastrin, has been shown to be increasingly important. In vivo and in vitro studies have also shown the trophic effect of gastrin and the inhibitory effect of both gastrin receptor antagonists and anti-gastrin antibodies,1 2 3 and further studies have confirmed gastrin expression in human pancreatic cancer cell lines and resection specimens.4 Thus, there is good evidence to suggest that immunisation against gastrin may be beneficial in the prevention of pancreatic cancer.

We have shown that Gastrimmune induced antibodies inhibit the growth of human pancreatic cancer cell lines,5 and they have previously been shown to inhibit the growth of gastric, colon, and hepatocellular cancer cell lines in vitro and in vivo.6 Over 150 patients have now received Gastrimmune in several trials. The side effect profile has been extremely good and the early efficacy data in colorectal cancer has been encouraging7 8 phase III studies are currently being designed for both pancreatic and colorectal cancers.

Pancrastic cancer has an appalling prognosis. New molecular insights provide encouragement that novel therapeutic strategies may improve the outlook. However, these strategies cannot be employed directly and indirectly to target pancreatic cancer cells, and we hope the promise of these new strategies is fulfilled in the next decade.

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Reply

Editor,—Brett and Caplin have highlighted that our paper was biased towards cellular immunotherapy. We specifically excluded references to antibodies, but welcome the opportunity to mention these in the context of immunotherapy on pancreatic cancer.

In the early 1980s, murine monoclonal antibodies offered great hope for the diagnosis and cure of cancer, but by the end of the decade, the outlook was pessimistic. Used alone, murine monoclonal antibodies had little effect, mostly because of the occurrence of HAMA (human anti-mouse response) which curtailed the life of the monoclonal antibody in patients by forming immune complexes; furthermore, most of the pieces of immunoglobin are particularly poor at marshaling human defence mechanisms to cause inflammation and tumour eradication and thus, these antibodies proved ineffective in the treatment of most types of human cancer. More recently, however, an effort has been made to “humanise” the antibodies, either by making them as chimeras (essentially murine Fc to bind the antigen, coupled with human Fc) or by retaining only the critical murine amino acids so that the rest of the molecule is human. These techniques are achieved by genetic engineering and sophisticated computer modellers. Ironically, such humanised antibodies are less immunogenic than their murine counterpart, but this is not always so, and they should be more active with the human Fc piece, by activating complement and macrophages. However, at present such antibodies and are in phase I/II trials, and with several exceptions (see later), trials have not been particularly rewarding. We are particularly pessimistic about the use of antibodies, be they humanised or not, against solid tumours in humans, as we are experienced in rejecting grafts and with tumour grafts using antibody and complement.4 For mucin 1, we have now been able to cause rejection of human MUC1+ tumours in mice by using large amounts of monoclonal antibodies and additional complement, under circumstances which lead to rapid destruction of lymphoid cells.

However, total pessimism now seems unwarranted. Firstly, murine antibodies in lymphoma and leukaemia have been found to be particularly useful in the treatment of these diseases. The antibodies need to be “arm’d”, especially with isotypes, and using antibodies to CD19 and CD20 “labeled antibodies in patients effectively treat B cell NHL and therefore could be treated successfully.

Antibodies that do not act primarily via their Fc piece by activating complement but have a direct effect on cell surface molecules are another exception. A well known antibody, Her2/neu, reacts with molecules in 20–30% of patients with breast cancer. Her2/neu antibodies have a growth effect, function and if they can be blocked on the cell surface by the antibody, which inhibits the binding of the ligand (growth factor), the cells die. This has been further illustrated by the use of Gastrimmune against amidated gastrin-17 by Brett and Caplin. Antibodies to other growth factor receptors have also been described, which are proving to be extremely useful in certain trials. Thus, selective antibodies against growth factor receptors may be useful in the treatment of diseases like pancreatic cancer. It is possible that the antibodies will be immunogenic and a HAMA or HAMA (human anti-human antibody) response will occur, but this can only be shown by a clinical trial. There may also be problems with antibodies obtaining access to tumours not expressing the appropriate molecules as they de-differentiate. However, these are problems of any cellular or humoral immune response, and are no longer regarded as being peculiar to antibodies. Nevertheless, it is appropriate to consider special antibodies and growth factors to be part of immunotherapy for pancreatic cancer.

Finally, the colon cancer trial in which patients with Duke’s C disease had improved prognoses after receiving 171A antibody, is of interest, although it has still to be established whether the specific or non-specific nature of the antibody was responsible for the improvement. Nevertheless, phase III trials are now in progress to assess this. It is easier to treat disease by immunotherapy if treatment starts at an early stage. Unfortunately, early diagnosis of pancreatic cancer remains difficult—how can a disease with a relatively low frequency be diagnosed in the absence of symptoms? When the symptoms finally appear it may already be too late for immunotherapy.


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Gut 2000;46:582–584
Ring-like elevations in the large bowel: endoscopic signs to distinguish the artefact from true neoplastic lesions

**Editor,—**We read with great interest the article by Martin et al (Gut 1999;45:147) on normal histological findings in small depressed lesions of the large bowel. They described three patients with <7 mm deep round lesions, similar to small flat or depressed neoplastic lesions, during sigmoidoscopy (two cases) and total colonoscopy (one case). Specimens taken by extensive biopsy or removed by endoscopic mucosectomy were histologically normal with no evidence of neoplasia. Two weeks later, colonoscopy with chromoscopy in one patient failed to locate the lesion. In contrast to true flat adenomas characterised by rough, reddened and mucosal and an irregular outline, the lesions had normal central mucosa and a regular circular elevation. The authors therefore concluded that flat lesions with a regular circular shape and normal central mucosa are likely to be of little significance, and recommended diagnostic cold biopsy in these cases.

Despite their claim of the first report of normal histological findings in small depressed lesions, we had described similar lesions as ring-like elevations.1 Histological assessment of biopsy and endoscopic mucosectomy specimens of the elevations revealed slight oedema within the rectal mucosa. Whereas we observed such elevations predominantly in the ascending colon, their lesions were seen in the rectum. Because cleansing preparation fluid tends to be retained often in the ascending colon and rectum, requiring frequent aspiration, we suspect that their lesions are also pseudolesions caused by suction of the mucosa into a colonic polyp forming ring-like elevations.1 Although they mentioned that suction had not been applied to the mucosa, experienced endoscopists usually aspirate retained fluid unconsciously during colonoscopy. Although histological findings of their lesions showed normal mucosa, we suggest that slight oedema was probably present within the lamina propria of their ring-like lesions. We believe their lesions are the same as ring-like elevations.

Some diminutive polyps disappear during maximal colonscopic insufflation. This phenomenon is invariably associated with hyperplastic polyps, but not with adenomas.2 Dieye-spraying techniques readily visualise the innumerable fine grooves, the so-called in-nominate grooves, which remain visible in non-neoplastic lesions and in normal colorectal mucosa, but not in neoplastic lesions.2 Because ring-like elevations usually disappear after vigorous air insufflation and the innominate grooves are already visualised in the elevations, the disappearing phenomenon and the presence of innominate grooves in the lesions serve to differentiate this artefact from true neoplastic lesions.3 With these endoscopic signs, we can avoid biopsy or removal, and thus the cost of pathological examinations.

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**Reply**

**Editor,—**We value the comments made by Matsushita et al and are grateful to them for bringing to our attention their earlier letter which reports ring-like elevations of the colon thought to be suction artefacts.1 Although we agree with their points regarding the use of suction by experienced endoscopists, we believe it is unlikely that the lesions we reported were suction related. All of our lesions were initially visualised in the distance, away from the colonoscopy tip, and showed no signs of mucosal trauma such as spotted haemorrhage, on close inspection. In addition, there was no histological evidence of increased mucosal oedema to suggest suction trauma. Maximum air insufflation and observation of these lesions over several minutes was performed routinely, and a striking feature noted was their “fixed nature”. We agree that if a normal groove pattern is seen following dye spray and the lesion disappears after air insufflation, then biopsy is unnecessary. However, if lesions fail to disappear, as in the cases we reported, some doubt must remain regarding their nature. In this situation biopsy seems a safe precaution, particularly given the relatively high incidence of advanced neoplasia in true flat adenomas.

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J E PAINTER Department of Gastroenterology, University of Manchester

**Liver biopsy under ultrasound control: implications for training**

**Editor,—**As a gastroenterologist/hepatologist, I appreciate the anxiety expressed by Shah et al (Gut 1999;48:628-629) about having to surrender liver biopsy samples to radiologists as a result of a reduced training opportunities. The desire of an overwhelming number of gastroenterology trainees in the United Kingdom, such as in Europe or the USA, the questions raised by Shah et al would have been partially solved, albeit indirectly.

The purpose of ultrasound guidance in liver biopsy is threefold: (i) to target the liver, (ii) to target the lesion, and (iii) to avoid the gall bladder.1 In this day and age, it would be unthinkable to perform a blind liver biopsy on a patient who has a discrete liver lesion. However, when it comes to diffuse or generalised disease, “X marks the spot” or ultrasound assisted technique should suffice. Although the liver is a large and superficial organ, targeting it, even for diffuse or generalised disease, should not be left completely to chance. In the ultrasound assisted technique, the procedural aspect of liver biopsy is essentially blind subsequent to the initial ultrasound to mark the spot. Therefore becoming proficient in this technique would prevent loss of expertise in the blind approach, and yet the medicolegal position remains sound.

Inadvertent biopsy of the gall bladder can be accurately avoided by allowing the patient to have a light breakfast, as the gall bladder becomes contracted following a meal.2 However, the more cautious would prefer patients to be fasted, in case they develop a complication requiring operative intervention.

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**Reply**

**Editor,—**Dr Chua makes some valuable points about the problems of training in gastroenterology in relation to ultrasound. One of the key messages of our article was to differentiate the “X marks the spot” ultrasound technique from the real time ultrasound guided method, which is in standard use within our hospitals. Using this technique, the needle is continuously visualised throughout its time within the liver and therefore there is minimal risk of biopsy of gall bladder or intrahepatic vessels. We consider this to be the safest technique to use but it is still the most difficult method in which to become proficient. Most training schemes for specialist registrars in gastroenterology will have difficulty in accommodating the additional time required to learn this technique.

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S SHAH J F MAYBERRY
BOOK REVIEW


A comprehensive study of vitamin D, this book starts with a brief consideration of the evolutionary aspects of vitamin D and the essential role of photosynthesis of the vitamin in the conservation of calcium in aquatic and land animals. Cutaneous synthesis is the principal source of vitamin D for most healthy people but dietary intake becomes increasingly important in the very young and the elderly. Adequate intakes (formerly called recommended daily allowances) for all age groups, and for pregnant or lactating women, are provided and the central question of how to define vitamin D deficiency is revisited; based on serum parathyroid hormone responses to vitamin D supplementation, a threshold level of intake of 20 ng/ml (50 nmol/l) is suggested.

Vitamin D deficiency is a common side effect of hepatic and gastrointestinal diseases and often results in bone disease; gastroenterologists should, therefore, have some knowledge of the causes, consequences, and treatment of vitamin D related bone disorders. It also has a wide range of actions which are unrelated to its effects on calcium metabolism; receptors for its active metabolite, 1,25-dihydroxyvitamin D, are found in many places including the stomach, thymus, immune system, gonads, and some cancer cells. The antiproliferative and predifferentiation effects of vitamin D have already been exploited in the development of treatment for psoriasis and other skin disorders and the exciting potential applications of vitamin D in some malignant diseases are discussed towards the end of the book.

Since the pivotal research in the 1960s on the metabolism of vitamin D there has been intense research activity in a number of related areas, including the synthesis and metabolism of vitamin D metabolites and analogues, the molecular biology of the vitamin D receptor, and the mechanisms by which 1,25-dihydroxyvitamin D affects the renal, intestinal, and skeletal transport of calcium. These aspects are covered in considerable detail and occupy about one half of the book; there is also a detailed chapter on the methodology for assays of vitamin D, although the authors do not discuss the usefulness of these assays in clinical practice. The latter part of the book is devoted to clinical issues—for example, rickets and osteomalacia, osteoporosis, inherited defects of vitamin D metabolism, and the pathophysiology of hypercalcaemia associated with the extrarenal production of 1,25-dihydroxyvitamin D, which occurs in conditions such as sarcoidosis and lymphoproliferative disorders. There is also an interesting chapter on the epidemiology of cancer risk and vitamin D. Disappointingly, at least for the gastroenterologist, there is very little coverage of vitamin D deficiency associated with hepatic and gastrointestinal disorders.

The book is well produced and has many illustrations and diagrams; it provides an excellent and comprehensive account of the substantial advances occurring in this area. Furthermore, the chapters are well referenced, many containing over 100 references. This book is not for the gastroenterologist who wishes to extract information about the diagnosis and management of vitamin D deficiency in clinical practice, but will be highly valued by those with a close interest in following the fascinating progress of this hormone.

J E COMPSTON

CORRECTIONS

An error occurred in the keys to figures 4 and 5 of the paper by Yamaoka et al (Gut 1999;45:804–11). Gastritis should be represented by open circles and duodenal ulcer by closed circles. We apologise for any confusion this error may have caused.

The authors of Nardone et al (Gut 1999;44:789–99) have conceded an error. Figure 3(B) was an inverted image of figure 3(A) at a different magnification. The correct figure is published below. The authors regret any confusion this may have caused.

NOTE

The Wellcome Institute for the History of Medicine with the 20th Century History of Medicine Group present A Witness Seminar—Peptic Ulcers: rise and fall in the twentieth century

This seminar will be held on 12 May 2000 in London. Registration is £15 (Students/Friends £10) and the closing date is 5 May 2000. For registration/further information: Ms Frieda House, The Wellcome Institute for the History of Medicine, 183 Euston Road, London NW1 2BE, UK. Tel: +44 (0)20 7611 8619/8888.

Falk Symposia and Workshops

The Symposium on Hepatology 2000 will be held in Munich, Germany, on 4 and 5 May 2000.

The Workshop on Hepatobiliary Diseases: Cholestasis and Gallstones will be held in Cluj Napoca, Romania, on 9 and 10 June 2000.

The Symposium on Non-Neoplastic Diseases of the Anorectum—An Interdisciplinary Approach on 1 and 2 October 2000, and the Symposium on Immunosuppression in Inflammatory Bowel Diseases—Standards, News, Future Trends on 3 and 4 October 2000 will be held at Gastroenterology 2000 in Freiburg, Germany.

The Symposium on Biology of Bile Acids in Health and Disease will be held at the XVI International Bile Acid Meeting in Den Haag, The Netherlands on 12 and 13 October, 2000.

The Symposium on Steatohepatitis (NASH and ASH) will be held in Den Haag, The Netherlands, on 14 and 15 October.

The Symposium on Chronic Inflammatory Bowel Diseases—Progress and Controversies at the End of the Century will be held in Bucharest, Romania, on 4 November 2000.

For further information on any of these symposia or workshops, please contact: Falk Foundation e.V.—Congress Division, Lennéweg 85, 30250 Hanover, Germany. Fax: +49 761 15140; email: symposia@falkfoundation.de

Digestive Disease Week

The Digestive Disease Week will be held at the San Diego Convention Centre, San Diego, California, USA, on 21–24 May 2000. Further information from: DDW Administration, 7910 Woodmont Avenue, 7th Floor, Bethesda, Maryland 20814, USA. Tel: +1 301 272 0022; fax: +1 301 654 3978; website: www.ddw.org