ORIGINAL ARTICLE

Effects of *Helicobacter pylori* on the cadherin–catenin complex

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Background: The cadherin–catenin complex is the key component of the adherens junction in epithelial cells, and changes in this complex are implicated in gastric adenocarcinoma. Germline mutations in E-cadherin have been described in diffuse-type gastric adenocarcinoma. *Helicobacter pylori* infection is the first stage in gastric carcinogenesis.
Aims: To determine whether *H pylori* was associated with changes in the complex, and whether this was affected by virulence of the strain.
Methods: Epithelial cell lines were cultured with *H pylori* using the wild-type pathogenic and non-pathogenic strains and *CagE* null and *VacA* null isogenic mutants. Gastric biopsy specimens at endoscopy were obtained from patients with (n = 17) and without (n = 15) *H pylori* infection, and E-cadherin and β–catenin expression was assessed by immunohistochemistry. *H pylori* was typed by polymerase chain reaction from these patients for *CagE* and *VacA*.
Results: In vitro studies showed that coculture with a pathogenic strain of *H pylori* led to disruption of epithelial junctional β-catenin expression, but without evidence of nuclear translocation or signalling. This effect was independent of a functional *Cag* pathogenicity island and vacuolating activity, but dependent on live bacteria. No marked differences in β-catenin or E-cadherin expression were seen in gastric biopsy specimens in patients with and without *H pylori* infection.
Conclusion: Acute *H pylori* infection disrupts junctional β-catenin in vitro, but chronic infection by *H pylori* has no effect on E-cadherin and β-catenin expression, as seen in gastric biopsy specimens at the initial gastritis stage of the proposed Correa pathway of gastric carcinogenesis. A later effect at the later stages of atrophy or intestinal metaplasia cannot be ruled out.

Helicobacter pylori is the main cause of peptic ulceration and gastric mucosa-associated lymphoid tissue lymphoma,[1 2] and the strongest risk factor for the development of distal gastric adenocarcinoma.[3] The outcome of *H pylori* infection is dependent on the host[4] and environmental[5] and bacterial factors.[6 7] Strains possessing the Cag pathogenicity island, encoding a type IV bacterial protein secretion system,[8–11] are more strongly associated with increased levels of inflammation and disease[6] as are those producing an active form of VacA, a pore-forming toxin that induces cytoplasmic vacuolation in vitro.[12–15]

The cadherin–catenin complex is the key component of the adherens junction between epithelial cells. It is important in providing structural support to cells by linkage with the actin cytoskeleton, and also in promoting epithelial cross-talk. Changes in expression of proteins in this complex are implicated in several epithelial cancers including gastric adenocarcinoma,[16–21] and germline mutations in E-cadherin have been described in diffuse-type gastric adenocarcinoma.[22 23] β-catenin is also an important cell signalling molecule (part of the *wnt* pathway) and has an important role in cell proliferation and oncogenesis.

Few data are available at present on expression of the cadherins and catenins in normal and inflamed gastric epithelial cells. A few reports suggest that *H pylori* disrupts E-cadherin[24–26] and stimulates β-catenin signalling,[27] and also that these mechanisms underlie gastric carcinogenesis. We assessed whether *H pylori* disrupted E-cadherin and β-catenin localisation in relevant cell lines, whether this resulted in β-catenin-mediated cell signalling and whether *H pylori* infection was associated with changes in E-cadherin and β-catenin immunolocalisation in vivo.

METHODS

In vitro studies

H pylori strains and epithelial cells

The following strains were used:

- 60190 (ATCC 49503, *cagPaI*+, *VacA* s1m1 (vacuolating)[13])
- Tx30a (ATCC 51392, *cagPaI*−, *VacA* s2m2 (non-vacuolating)[13])
- 60190CagE− (*cagE*− insertion isogenic mutant of 60190[28])
- 60190VacA− (disrupted *VacA*− insertion mutant of 60190[29])

AGS, RK13, HeLa and HT29 cell lines were used for coculture[28] and grown in Lab-Tek chamber slides (Nunc, Roskilde, Denmark) and 75-cm^2 tissue culture flasks. After 48 h of incubation, *H pylori* was added to subconfluent HT29 cells such that the final ratio of viable bacteria to epithelial cells ranged from 100:1 to 1:1. Cells and strains were grown in a 5% carbon dioxide incubator at 37°C for 24 h.

Immunoblots

Cell lysates were obtained by scraping cells into radioimmuno precipitation buffer (7.5 ml 1 M sodium chloride, 5 ml 0.5 M Tris, 0.5 ml NP40, 0.05 g sodium dodecyl sulphate, 0.25 g sodium deoxycholate made up to 50 ml with sterile distilled water and broad-spectrum protease inhibitor cocktail (Sigma-Aldrich, Gillingham, UK)) after washes in phosphate-buffered saline. Immunoblots were carried out on epithelial cell lysates[28] using monoclonal antibodies to E-cadherin (R&D systems, Abingdon, UK), α-catenin (Transduction Laboratories, BD Biosciences, San Jose, California, USA), β-catenin (Sigma-Aldrich) and Cyclin D1

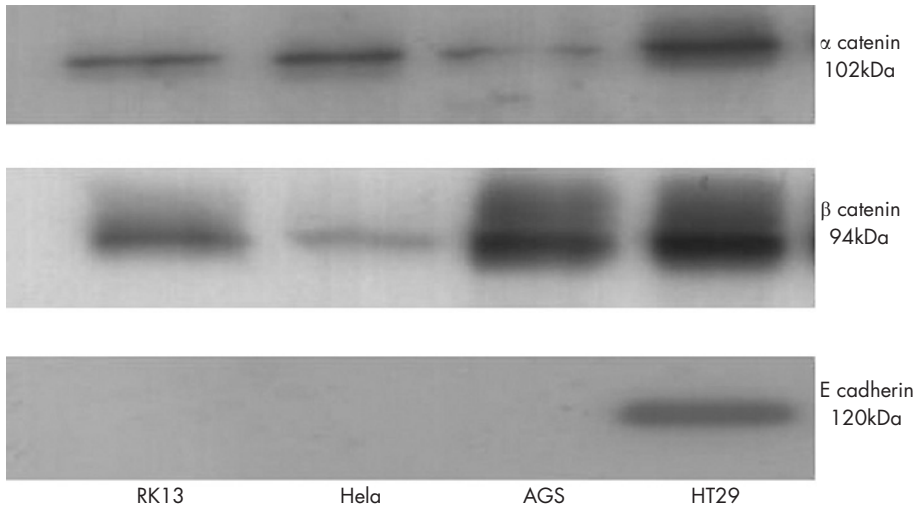

Figure 1 Western blots of cell lysates from RK13, HeLa, AGS and HT29 cells. Cells were grown in 75-cm^2 flasks before lysis with radioimmuno precipitation buffer and protease inhibitors. Lysates were standardised for protein concentration before equal loading on sodium dodecyl sulphate–polyacrylamide gel electrophoresis and immunoblotting to monoclonal antibodies for catenins and E-cadherin. Size in kDa is the size of the human form of catenin or E-cadherin.

(Santa Cruz Biotechnology, Wembley, UK) at titres of 1:1000, 1:250, 1:1000 and 1:1000, respectively.

Cell fractionation

Epithelial cells were separated into Triton-X soluble (cytosolic) and Triton-X insoluble (membranous) fractions for immunoblotting by the addition of cytoskeletal extraction buffer (250 mM 2-(N-morpholino) ethanesulfonic acid, 125 mM ethylene glycol bis (β-aminoethylether)-NNN′N′-tetra-acetic acid, 25 mM magnesium chloride, 2.5% Triton-X) to cells in tissue culture flasks. Fractions were microcentrifuged and pellets resuspended in sodium dodecyl sulphate running buffer before immunoblotting.

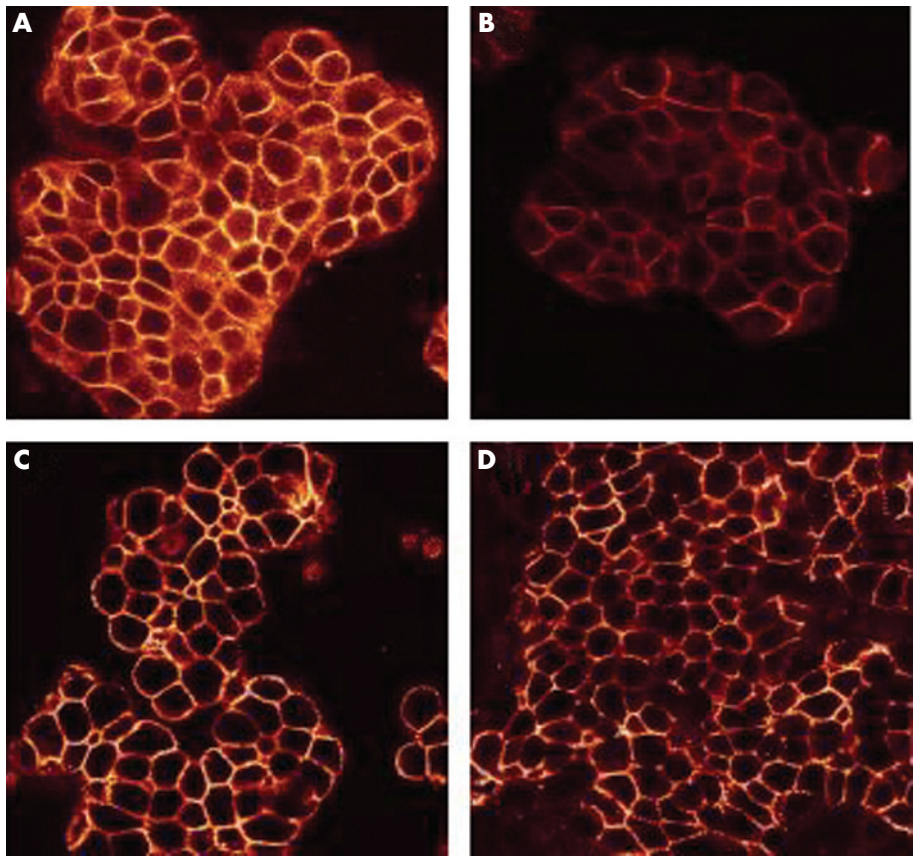

Figure 2 Immunofluorescent images of HT29 cells on confocal microscopy. (A,B) Stained for β-catenin. (A) Untreated cells, (B) after coculture for 24 h with *Helicobacter pylori* strain 60190. Decreased membranous staining intensity is seen in (B). (C,D) Stained for E-cadherin. (C) Untreated cells, (D) after coculture with *H pylori* strain 60190. No changes can be seen in the staining pattern or intensity.

Table 1 Immunoreactivity grades

Grade	Immunoreactive epithelial cells (%)
0	0–5
1	5–25
2	25–50
3	50–75
4	75–100

Immunohistochemical analysis

Immunohistochemical analysis was carried out[29] using the following primary monoclonal antibodies: E-cadherin (1:200), β-catenin, 1:200). Slides were examined by confocal microscopy (Leica SP2 confocal microscope, 488-nm laser line, Leica, Milton Keynes, UK). Assessment and scoring was described in Results.

In vivo studies

Patients and biopsy samples

Patients and biopsy samples were acquired as described previously[28] with full ethical approval from the University Hospital Nottingham Ethics Committee. *H pylori* strains were cultured and typed by polymerase chain reaction for *VacA* and *CagE*.[28]

Immunohistochemical analysis was carried out on paraffin-wax-embedded sections of antral and corpus biopsy specimens,[28] with staining using specific monoclonal antibodies to E-cadherin and β-catenin (Affiniti, Biomol International, Exeter, UK) at dilutions of 1:800 and 1:500, respectively. Criteria for *H pylori* infection were as stated previously.[28]

Assessment and grading of biopsy staining

Table 1 describes a validated method[30] used to grade immunoreactivity for E-cadherin and particularly for β-catenin.

Scoring was carried out by two observers blinded to the patient's *H pylori* status (JRB and AZ), and epithelial cells were scored in three zones (superficial, proliferative and deep).[28]

Statistical analysis

All data were analysed using the GraphPad Prism package, San Diego, California, USA. Mann–Whitney U tests were used to compare median scores for histological grading in vivo, and t test was used to compare in vitro grading.

RESULTS

Characterisation of cadherin–catenin expression in epithelial cell lines

Western blot confirmed the expression of E-cadherin, α-catenin and β-catenin in the positive control cell line HT29. No bands were seen for E-cadherin in AGS, HeLa or RK13 cells (fig 1). Immunohistochemical analysis and fluorescent microscopy confirmed the absence of expression of E-cadherin in AGS cells and diffuse cytoplasmic or junctional staining with β-catenin. In contrast, HT29 cells stained predominantly at cell–cell contact regions for both E-cadherin and β-catenin.

We therefore used the colon-derived HT29 cell line as our model cell line to study the effects of *H pylori*, as it expressed all four proteins at the correct predicted size.

Response of HT29 cell line to coculture with two different *H pylori* strains

Coculture of *H pylori* strain 60190 with HT29 cells led to altered distribution of β-catenin, with decreased intensity of junctional immunoreactivity and without any increase in cytoplasmic or nuclear immunostaining (fig 2).

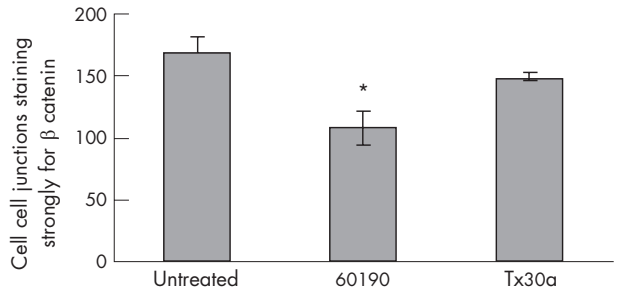

Figure 3 Number of cell–cell junctions staining strongly for β-catenin (out of 200 counted), for HT29 cells untreated and cocultured with *Helicobacter pylori* strains 60190 (pathogenic) and Tx30a (non-pathogenic). Coculture was carried out for 24 h with a bacteria:cell ratio of 100:1. Bar shows mean (SEM) of 5 experiment. *60190 versus untreated, $p<0.05$, t test.

To quantify differences with *H pylori* and assess any differences between pathogenic and non-pathogenic strains, a series of images was acquired by confocal microscopy of HT29 cells untreated or cocultured with either *H pylori* strain 60190 (pathogenic), or Tx30a (non-pathogenic). In each case, five images (randomly selected fields) were acquired at least 5 μm from the bottom or top of the field of cells and at least 15 μm apart. Both microscopists (JRB and SA) were blinded to *H pylori* status.

For slide analysis, 200 cell–cell junctions were counted and the intensity of immunostaining was graded as strong, absent or weak. For each field, the strongest immunoreactivity pattern was scored, in case sections had been taken from too near the top or bottom of the monolayer. This was carried out five times for each treatment and results were expressed as number of junctions per 200 counted sections expressing strong, weak or no immunoreactivity (fig 3).

Untreated HT29 cells stained predominantly in a junctional fashion, and most cell–cell junctions showed strong immunoreactivity for β-catenin (mean (standard error (SEM)), 168 (SEM13)). Cells treated with the pathogenic strain 60190 stained less strongly (mean 108 (SEM 13); $p<0.05$ *v* untreated) as did those treated with the non-pathogenic strain Tx30a, (mean 149 (SEM 3) cells).

Western immunoblots showed that 60190-treated cells showed less intense bands for β-catenin, whereas no changes were seen for E-cadherin (fig 4A,B).

Coculture experiments using serial 10-fold dilutions of bacteria showed that increased bacterial dilution led to abrogation of the effect on β-catenin levels (fig 4C).

Cell fractionation studies confirmed that in the Triton-insoluble fraction (membrane-bound), there was less total β-catenin (fig 5), whereas there was no change in the Triton-soluble (cytoplasmic) fraction.

Role of bacterial factors in inducing β-catenin changes

We analysed the role of bacterial factors using *VacA* and *CagE* insertion mutants of 60190 that we have previously shown do not express VacA or a type IV secretion system, respectively.[29]

Coculture experiments and subsequent immunoblots showed similar decreases with both VacA null and CagE null mutant strains, implying that neither VacA nor the *Cag*PaI-encoded T4SS contributed to the effect (fig 6); however, live bacteria were required (fig 7).

Functional significance of in vitro changes

We examined whether a known β-catenin target gene product, cyclin D1, was up regulated as we had not observed

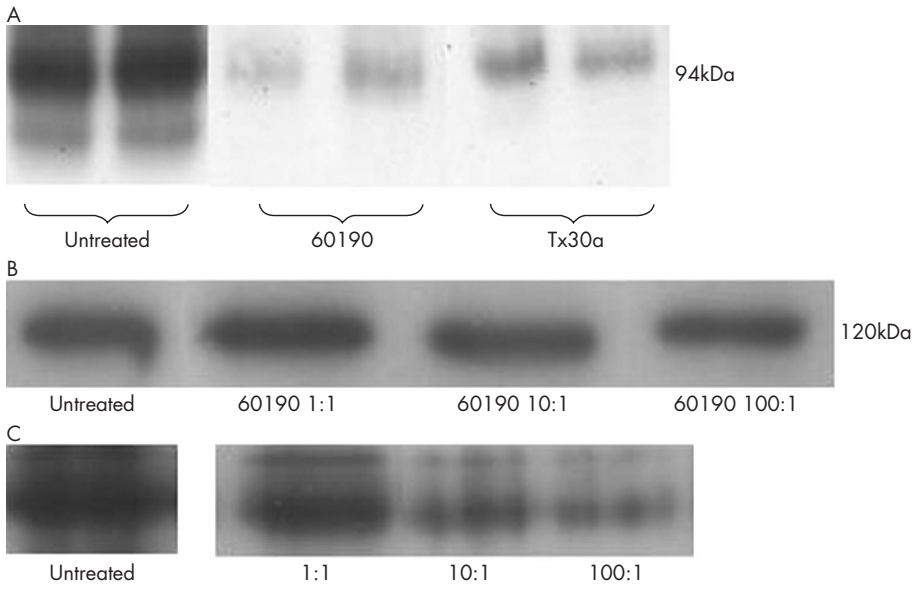

Figure 4 (A) Cell lysates or untreated cocultured with *Helicobacter pylori* strains 60190 or Tx30a, blotted to β-catenin. Bands are fainter with coculture, particularly for 60190. Duplicate experiments were run in parallel are shown. (B) HT29 lysates blotted to E-cadherin. Three different ratios of bacteria:cell have no effect on the amount of total E-cadherin. (C) HT29 lysates blotted to β-catenin. The total amount of β-catenin is seen to reduce as bacterial density increases. Total protein loading was equal in each lane in (A), (B) and (C).

nuclear immunolocalisation (with presumed β-catenin-induced transcription of genes), but no differences were observed (fig 8).

H pylori and the cadherin–catenin complex in vivo

To examine whether changes in vitro applied in vivo, we used immunohistochemical analysis to examine gastric biopsy specimens obtained from patients with and without *H. pylori* infection. We stained biopsy specimens (antrum and corpus) from 32 patients (17 with and 15 without *H pylori* infection) for E-cadherin and β-catenin, and conventionally assessed for the presence of gastritis, atrophy, intestinal metaplasia and malignancy. *H pylori* strains were cultured and typed by polymerase chain reaction for *CagE* and *VacA* genotypes. Of the 17 strains cultured, 11 were *CagE*+, 6 were *VacA* s1m1 and 7 were *VacA* s1m2.

Slides were examined and graded for the amount and pattern (junctional, cytoplasmic or nuclear) of staining. As we were interested in proliferation and β-catenin-mediated oncogenesis, we focused on the proliferative zone containing pluripotent stem cells. Thus we divided gastric pits into three zones: superficial, proliferative and deep.

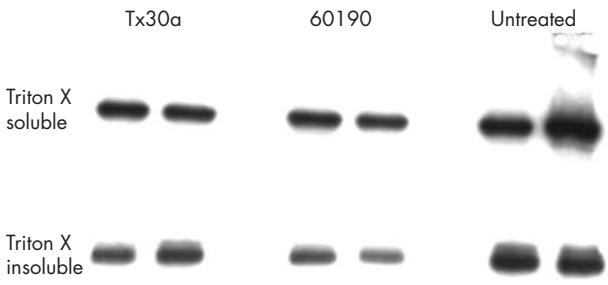

Figure 5 Immunoblots for β-catenin on Triton-soluble and Triton-insoluble fractions of HT29 cells cocultured with *Helicobacter pylori*. A reduction in band intensity is seen for the Triton-insoluble (membrane-bound) fraction for *H pylori* strain 60190. This is consistent with the findings of immunohistochemical analysis. Duplicate experiments were run in parallel.

Gastric epithelial cells stained clearly for E-cadherin in a predominantly junctional pattern (fig 9). Staining was present in most cells in the epithelial cell layer. Quantification of staining showed no differences between biopsy specimens of patients with and without *H pylori* infection, or any differences associated with gastric atrophy or intestinal metaplasia. We found no association between bacterial strain type (*Cag* status, *VacA* genotype) and E-cadherin staining intensity.

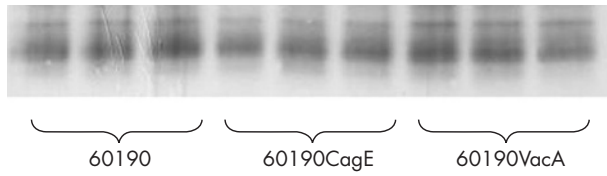

Figure 6 Immunoblots to β-catenin for cell lysates treated with *Helicobacter pylori* wild-type strain 60190, and its isogenic cagE− and vacA− mutant strains. No differences were seen in β-catenin band intensity (checked by densitometry). Cocultures were carried out in triplicate.

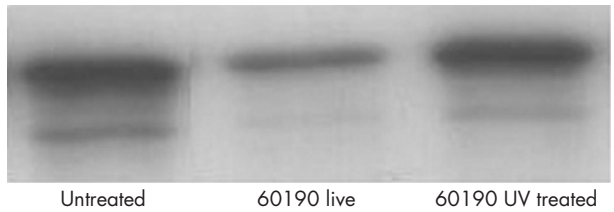

Figure 7 A representative immunoblot of HT29 cell lysates after coculture with *Helicobacter pylori*, which had either been pretreated with ultraviolet (UV) irradiation for 2 min (thereby killing the bacteria, confirmed by viable count) or not pretreated with UV light. HT29 cells not treated with *H pylori* are shown for comparison. Intensity bands are decreased for ''live'' bacteria compared with UV-treated *H pylori*. This suggests that the changes seen in levels of β-catenin are due to a bacteria-specific effect and that live bacteria are required for the effect.

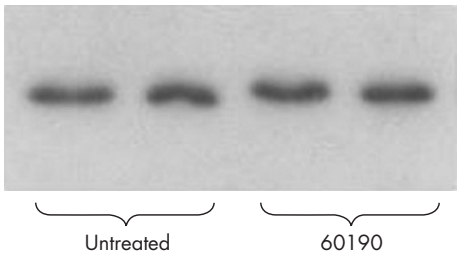

Figure 8 Immunoblot of fresh HT29 cell lysates to cyclin D1 monoclonal antibody. No differences were seen between untreated cells and cells treated with *Helicobacter pylori* strain 60190. Equal protein concentrations were loaded in each well. Representative blot, n = 4 per treatment.

Similar staining patterns were seen for β-catenin as for E-cadherin, with predominantly junctional staining (fig 10). Differences in staining were seen between the different zones of epithelial cells, with stronger staining patterns in deeper zones. Neither marked differences nor any relationship between staining patterns and bacterial strain type was seen between biopsy specimens of patients with and without *H pyrori* infection.

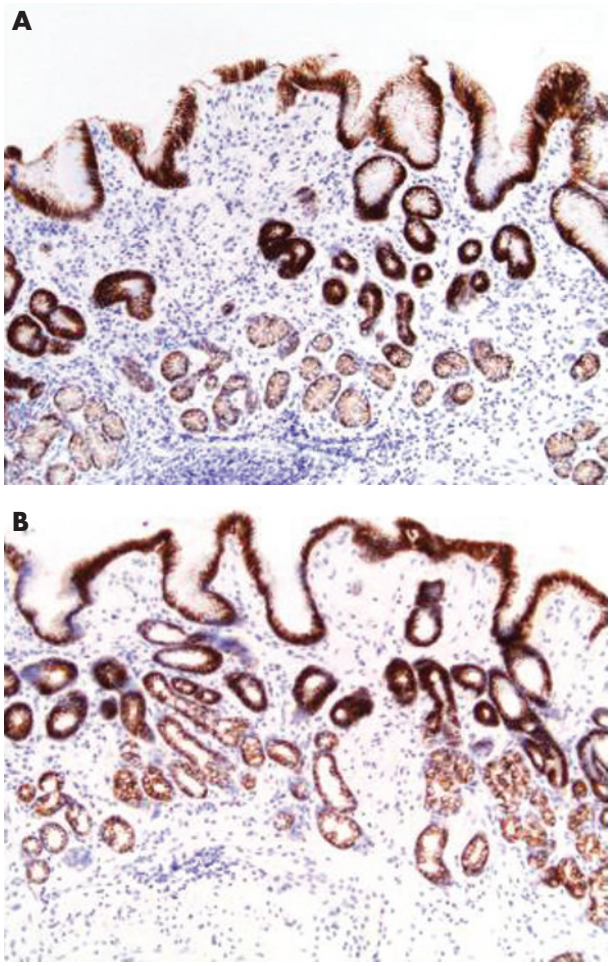

Figure 9 Gastric antral biopsy specimens stained for E-cadherin from a patient (A) with and (B) with out *Helicobacter pylori*-infection. Almost all epithelial cells are stained in both biopsy specimens, independent of *H pylori* status and inflammation (inflammatory infiltrate seen in (A)).

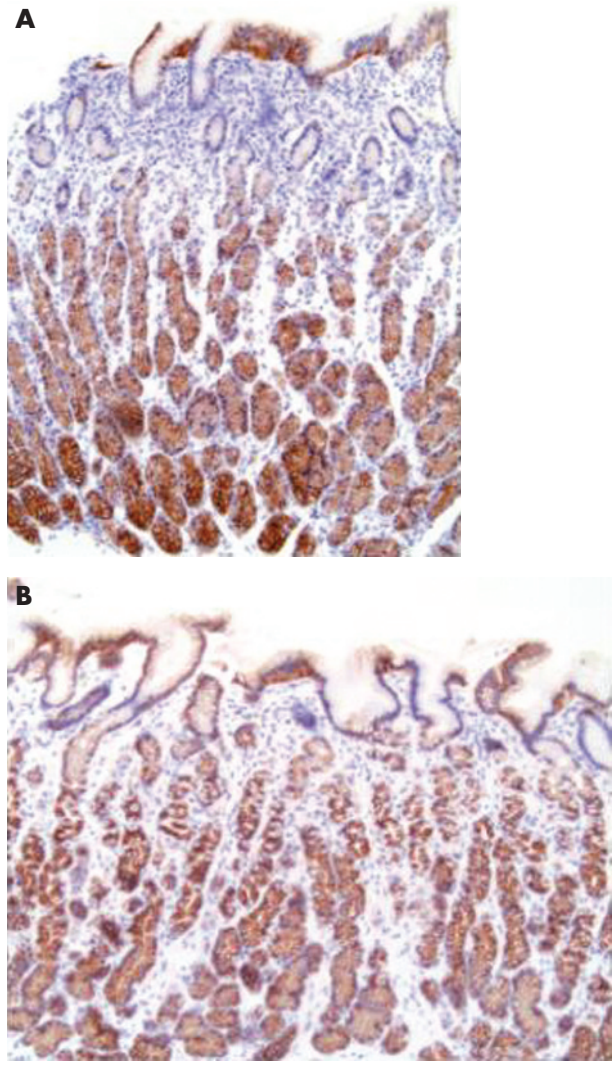

Figure 10 Gastric corpus biopsy specimens stained for β-catenin (A) with *Helicobacter pylori* infection; (B) without *Helicobacter pylori* infection.

DISCUSSION

Our study shows that *H pylori* infection leads to a reduction in junctional expression of β-catenin, and this effect is induced more markedly by a candidate pathogenic than a non-pathogenic strain and is mediated by live bacteria. Experiments using cell-free extracts and isogenic mutants suggest that the effect was not dependent on secreted proteins such as the toxin VacA or the *Cag*-encoded type IV secretion system. Immunohistochemistry showed that in vivo β-catenin distribution was not different between gastric biopsy specimens of patients with and without *H pyrori* infection.

The functional relevance of the changes we observed in β-catenin distribution is not clear; we saw no effect on cyclin D1, which is induced by β-catenin, and no nuclear translocation of β-catenin in vitro or in vivo. Previous work[31] showing that *H pylori* transactivates cyclin D1 in AGS cells in a manner dependent on time, dose and partly *Cag*PaI was not conducted on a biologically relevant cell line.

The other important cell adhesion molecule we examined in this study was E-cadherin, for which both germline[22 23] and somatic mutations[18] with prognostic implications[19] have been described in gastric cancer.[22 23] *H pylori* has been associated with changes in E-cadherin expression,[24 32] although in the

first study[24] no images were shown and their grading system was not as rigorous as ours. Another study[32] has shown promoter E-cadherin methylation to be strongly associated with *H pylori* infection and also to be increasingly common in the Correa progression to gastric cancer. Our study showed no changes in E-cadherin expression with *H pylori* infection either in vitro or in vivo.

One limitation of this study relates to the choice of the HT29 cell line, which our preliminary characterisation suggested to be suitable. However, the HT29 cell line possesses a truncated Adenomatosis polyposis coli gene product,[33] and another study has shown β-catenin phosphorylation patterns[34] leading to accumulation of non-phosphorylated β-catenin. However, β-catenin trafficking is clearly under multiple and complex control,[35] and changes in antigen-presenting cell cannot explain the differences we showed in membranous localisation and are unlikely to explain our observed lack of nuclear translocation. Besides this, we showed a lack of nuclear translocation in vivo, supporting our cell line data.

We carried out a careful and rigorous analysis of the effects of *H pylori* infection on the cadherin–catenin complex. The effects seen in cell lines may be due to acute *H pylori* infection, but once infection is chronically established (in vivo), no differences are observed between biopsy specimens of patients with and without infection. Therefore, we conclude that there is no effect of chronic *H pylori* infection on β-catenin, although of course we cannot exclude an effect later in the Correa model of gastric cancer at the level of intestinal metaplasia or atrophy.

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REFERENCES

1 **Nomura A**, Stemmermann GN, Chyou PH, *et al*. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii [see comments]. *N Engl J Med* 1991;**325**:1132–6.
2 **Parsonnet J**, Friedman GD, Vandersteen DP, *et al.* Helicobacter pylori infection and the risk of gastric carcinoma [see comments]. *NEngl J Med* 1991;**325**:1127–31.
3 **Nomura A**, Stemmermann GN, Chyou PH, *et al*. Helicobacter pylori infection and the risk for duodenal and gastric ulceration [see comments]. *Ann Int Med* 1994;**120**:977–81.
4 **El-Omar EM**, Carrington M, Chow WH, *et al.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000;**404**:398–402.
5 **Martin DF**, Montgomery E, Dobek AS, *et al*. Campylobacter pylori, NSAIDS, and smoking: risk factors for peptic ulcer disease. *Am J Gastroenterol* 1989;**84**:1268–72.
6 **Atherton JC**. H. pylori virulence factors [review]. *Br Med Bulletin* 1998;**54**:105–20.
7 **Blaser MJ**, Berg DE. Helicobacter pylori genetic diversity and risk of human disease [review]. *J Clin Invest* 2001;**107**:767–73.
8 **Asahi M**, Azuma T, Ito S, *et al.* Helicobacter pylori CagA protein can be tyrosine phosphorylated in gastric epithelial cells [see comments]. *J Exp Med* 2000;**191**:593–602.
9 **Odenbreit S**, Puls J, Sedlmaier B, *et al.* Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000;**287**:1497–500.
10 **Segal ED**, Cha J, Lo J, *et al.* Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. *Proc Natl Acad Sci USA* 1999;**96**:14559–64.
11 **Stein M**, Rappuoli R, Covacci A. Tyrosine phosphorylation of the Helicobacter pylori CagA antigen after cag-driven host cell translocation. *Proc Natl Acad Sci USA* 2000;**97**:1263–8.
12 **Atherton JC**. The clinical relevance of strain types of Helicobacter pylori [review]. *Gut* 1997;**40**:701–3.
13 **Leunk RD**, Johnson PT, David BC, *et al.* Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. *J Med Microbiol* 1988;**26**:93–9.
14 **Papini E**, de Bernard M, Milia E, *et al.* Cellular vacuoles induced by Helicobacter pylori originate from late endosomal compartments. *Proc Natl Acad Sci USA* 1994;**91**:9720–4.
15 **Szabo I**, Brutsche S, Tombola F, *et al.* Formation of anion-selective channels in the cell plasma membrane by the toxin VacA of Helicobacter pylori is required for its biological activity. *EMBO J* 1999;**18**:5517–27.
16 **Matsuura K**, Kawanishi J, Fujii S, *et al.* Altered expression of E-cadherin in gastric cancer tissues and carcinomatous fluid. *Br J Cancer* 1992;**66**:1122–30.
17 **Matsui S**, Shiozaki H, Inoue M, *et al.* Immunohistochemical evaluation of alpha-catenin expression in human gastric cancer. *Virchows Arch* 1994;**424**:375–81.
18 **Becker KF**, Atkinson MJ, Reich U, *et al.* E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res* 1994;**54**:3845–52.
19 **Jawhari A**, Jordan S, Poole S, *et al.* Abnormal immunoreactivity of the E-cadherin-catenin complex in gastric carcinoma: relationship with patient survival. *Gastroenterology* 1997;**112**:46–54.
20 **Ramesh S**, Nash J, McCulloch PG. Reduction in membranous expression of beta-catenin and increased cytoplasmic E-cadherin expression predict poor survival in gastric cancer. *Br J Cancer* 1999;**81**:1392–7.
21 **Yu J**, Ebert MP, Miehlke S, *et al.* Alpha-catenin expression is decreased in human gastric cancers and in the gastric mucosa of first degree relatives. *Gut* 2000;**46**:639–44.
22 **Gayther SA**, Gorringe KL, Ramus SJ, *et al.* Identification of germ-line E-cadherin mutations in gastric cancer families of European origin. *Cancer Res* 1998;**58**:4086–9.
23 **Guilford P**, Hopkins J, Harraway J, *et al.* E-cadherin germline mutations in familial gastric cancer. *Nature* 1998;**392**:402–5.
24 **Terres AM**, Pajares JM, O'Toole D, *et al.* H pylori infection is associated with downregulation of E-cadherin, a molecule involved in epithelial cell adhesion and proliferation control. *J Clin Pathol* 1998;**51**:410–12.
25 **Blok P**, Craanen ME, Offerhaus GJ, *et al.* Molecular alterations in early gastric carcinomas. No apparent correlation with Helicobacter pylori status. *Am J Clin Pathol* 1999;**111**:241–7.
26 **Shun CT**, Wu MS, Lin MT, *et al.* Immunohistochemical evaluation of cadherin and catenin expression in early gastric carcinomas: correlation with clinicopathologic characteristics and Helicobacter pylori infection. *Oncology* 2001;**60**:339–45.
27 **Franco A**, Krishna U, Peek R. Helicobacter pylori induces aberrant beta-catenin signalling in gastric epithelial cells. *Gastroenterology* 2004;**126**(Suppl 2):848.
28 **Bebb JR**, Letley DP, Thomas RJ, *et al.* Helicobacter pylori upregulates matrilysin (MMP-7) in epithelial cells in vivo and in vitro in a Cag dependent manner. *Gut* 2003;**52**:1408–13.
29 **Bebb JR**, Letley DP, Rhead JL, *et al.* Helicobacter pylori supernatants cause epithelial cytoskeletal disruption that is bacterial strain and epithelial cell line dependent but not toxin VacA dependent. *Infect Immun* 2003;**71**:3623–7.
30 **Kapiteijn E**, Liefers GJ, Los LC, *et al.* Mechanisms of oncogenesis in colon versus rectal cancer. *J Pathol* 2001;**195**:171–8.
31 **Hirata Y**, Maeda S, Mitsuno Y, *et al.* Helicobacter pylori activates the cyclin D1 gene through mitogen-activated protein kinase pathway in gastric cancer cells. *Infect Immun* 2001;**69**:3965–71.
32 **Chan AO**, Lam SK, Wong BC, *et al.* Promoter methylation of E-cadherin gene in gastric mucosa associated with Helicobacter pylori infection and in gastric cancer. *Gut* 2003;**52**:502–6.
33 **Hsi LC**, Angerman-Stewart J, Eling TE. Introduction of full-length APC modulates cyclooxygenase-2 expression in HT-29 human colorectal carcinoma cells at the translational level. *Carcinogenesis* 1999;**20**:2045–9.
34 **Sadot E**, Conacci-Sorrell M, Zhurinsky J, *et al.* Regulation of S33/S37 phosphorylated beta-catenin in normal and transformed cells. *J Cell Sci* 2002;**115**(Pt 13):2771–80.
35 **Kucerova D**, Sloncova E, Tuhackova Z, *et al.* Expression and interaction of different catenins in colorectal carcinoma cells. *Int J Mol Med* 2001;**8**:695–8.