Digestive Diseases and Sciences, Vol. 49, Nos. 7/8 (August 2004), pp. 1116–1122 (© 2004)

The Association Between cagA+ *H. pylori* Infection and Distal Gastric Cancer: A Proposed Model

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Cytotoxin-associated gene A (cagA)+ infection is associated with an increased risk of distal gastric cancer. The aim was to determine the effect of *Helicobacter pylori* (HP) on gastric mucus thickness, hydrophobicity, and PGE_2 and their relation to colonization density. Ninety-nine patients were recruited (69 HP− and 30 HP+: 10 cagA+, 18 cagA−, 2 undetermined) and six biopsies were obtained from each patient. Mucus thickness, hydrophobicity, PGE_2, and colonization density were determined. HP status was assessed by histology and culture; cagA+ was determined by PCR. In age- and sex-matched patients, PGE_2 was greater in PH+ than HP− ($P = 0.04$), with cagA+ having higher PGE_2 than HP− patients ($P = 0.031$). No differences were observed in mucus thickness ($P = 0.717$) or hydrophobicity ($P = 0.27$) between HP+ and HP− patients. However, cagA+ showed a nonsignificant trend of increase in mucus thickness ($P = 0.784$) and hydrophobicity ($P = 0.30$) compared to cagA− and HP− patients. cagA+ colonization density was weakly correlated with increased thickness ($r = 0.333$, $P = 0.381$), whereas cagA− density was inversely correlated with thickness ($r = -0.805$, $P = 0.0001$). A model suggesting the possible changes induced by cagA+ infection is proposed which explains the high association of cagA+ with distal gastric cancer. If supported by large multicenter studies, this could form the basis for the development of new therapies directed at the mucous layer to eradicate HP and thus reduce the risk of gastric cancer.

KEY WORDS: *H. pylori*; cagA+; cagA−; cancer; gastric; model.

The stomach is the site of a number of important common gastric diseases in humans including gastritis (1), peptic ulcer disease (2), gastric lymphoma (3), and distal gastric adenocarcinoma (4). Gastric cancer is the world's third most common cancer, after lung and breast cancers, and the second common cause of cancer-related deaths, after lung cancer (5). The incidence and mortality rate of gastric cancer have, however been decreasing in recent years (6). In contrast to the trend for an overall decrease in gastric cancer rates, in developed countries there has been a rapid increase in the incidence of gastric cancer localized to the cardia (7). Distal gastric cancer is most common in the 50–70 age range, with a male-to-female ratio of 2:1 (8). The intestinal-type gastric adenocarcinoma is more common in males and in older age groups (9).

Helicobacter pylori (HP) has been designated a group 1 (definitive) carcinogen by the World Health Organization (WHO) (10). Evidence for the association between HP infection and distal gastric cancer came from (a) epidemiological studies (11), (b) cross-sectional studies (12), (c) long-term prospective studies (13), (d) animal studies on Mongolian gerbils (14), and (e) HP eradication studies

Manuscript accepted April 23, 2004.

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in both animals (15) and humans (16). Although the prevention of gastric cancer by HP eradication in humans presents an interesting option, this issue is still controversial (17) and further research is clearly required.

Individuals who carry cytotoxin-associated gene A (cagA)+ HP strains exhibit severe forms of gastric disease compared with those carrying cagA− strains (18). In Western populations cagA+ strains are associated with an increased risk of developing peptic ulcer disease and adenocarcinoma of the distal stomach (19). However, in Asian populations most HP-positive individuals carry cagA+ strains and the association with gastric diseases is not readily apparent; nevertheless, parts of the world with high rates of cagA+ seropositivity are also areas in which the incidence of distal gastric cancer is high (20, 21).

HP colonizes the stomach (22) and uses its mucous layer as a protective barrier (23). The close association of HP with the gastric mucous layer and epithelium (23) has promoted research into its effect on gastric mucus thickness, hydrophobicity, and PGE_2. Unfortunately, most of the studies addressing these issues have not differentiated between HP cagA+ and cagA− strains and have, therefore, produced contradictory results (Table 1).

METHODS

Subjects. One hundred patients were randomly recruited from endoscopy clinics in the North of England without prior knowledge of their HP status. The study was approved by both the Leeds Health Authority clinical research ethics committee and the York research ethics committee. Written consent was obtained from and information sheets given to each patient. Patients were included in the study if they were not taking acid suppressive drugs or NSAIDs or receiving treatment for HP but excluded if they were known or found to have peptic ulcer disease or gastric cancer. Following recruitment into the study one patient was subsequently excluded due to the presence of duodenal ulcer. Of the remaining 99 patients, 42 (42%) were male and 57 (58%) female, with mean ages of 46 ± 11 and 51 ± 15 years, respectively. Six antral gastric biopsies were taken from each patient for the assessment of gastric mucus thickness, contact angle, prostaglandin E_2 (PGE_2) levels, polymerase chain reaction (PCR), culture of HP, and histology.

TABLE 1. PREVIOUS STUDIES OF THE EFFECT OF *H. pylori* INFECTION ON THE HUMAN GASTRIC MUCUS THICKNESS, MUCOSAL HYDROPHOBICITY, AND PGE_2

Decreased	n	*No effect*	n
Studies on gastric mucus thickness			
Sarosiek *et al*. (46)	32	Newton *et al*. (47)	40
Studies on mucosal hydrophobicity			
Goggin *et al*. (48)	124	Day *et al*. (50)*	32 mice
Spychal *et al*. (49)	114		
Increased		*Decreased* (↓) *or no change* (→)	
Studies on gastric PGE_2 levels			
Oderda *et al*. (51)	104	Goren (53) (↓)	64
Ahmed *et al*. (28)	28	Taha *et al*. (54) (→)	48
Avunduk *et al*. (52)	30		

Note. n, total number of patients studied including *H. pylori*-infected and control subjects.
*Animal study, on mice.

HP Status. HP infection was assessed by histology, culture, and PCR. Patients were considered to be HP positive if two or more of the above tests were positive.

Polymerase Chain Reaction. Identification of HP cagA+ strains was carried out by PCR using two pairs of primers, cagA (product size, 130 bp) and urease C (product size, 120 bp). DNA extraction and PCR were performed using standard protocols.

HP Culture. HP culture was performed as described by Goodwin (24). The bacterial viable count (colonization density) was assessed by direct counting of the bacterial colonies on the culture plates that were inoculated with the serial dilutions prepared from the bacterial homogenates using the method described by Collins and Lyne (25). The bacterial viable count is expressed as colony forming units (CFU) per milligram of biopsy weight.

Mucus Thickness Measurement. The biopsy for mucus thickness measurement was sandwiched in pig's liver (1×1.5 cm) for support, wrapped in aluminium foil, and then immediately frozen in liquid nitrogen for transport. For each biopsy 18-μm sections were prepared and stained using the modified PAS/AB (periodic acid Schiff's/alcian blue) technique described by Jordan *et al*. (26). The measurements, in micrometers, were performed using a light microscope (Leitz Wetzlar 512583, Dialux 20 EM, Germany) at 100× magnification with the help of specific computer software (TAS version 2.09).

Contact Angle (Hydrophobicity) Measurement. The biospy was collected in a 10-ml saline glass bottle and transported at room temperature. Contact angle measurements were performed as described by Spychal *et al*. (27).

PGE_2 Level Measurement. The gastric biopsy obtained for PGE_2 measurement was immediately placed in a cryogenic tube (Nalgene; 1.2 ml) containing 0.5 ml ice-cold 0.9% saline and transported on ice. The biopsy incubation procedure was performed as described by Ahmed *et al*. (28). PGE_2 levels were measured using the PGE_2 Biotrak enzyme immunoassay system protocol 1 (RPN 222; Amersham Biosciences, UK).

Statistical Analysis of Data. Results were analyzed using SPSS software version 10.1 and are expressed as mean ± SE (standard error of the mean). Statistical significance was tested using analysis of variance (ANOVA). Data transformation (log) was used when required. The level of statistical significance was taken at P values <0.05. Spearman's correlation coefficient (r) was used in the determination of associations between various parameters.

RESULTS

Of the 99 patients studied 69 were *H. pylori* negative (HP−) and 30 *H. pylori* positive (HP+): 10 cagA+, 18 cagA−, 2 undetermined. In age- and sex-matched patients the PGE_2 level was significantly higher in HP-infected patients (37.2 ± 1.2 pg/mg/20 min; $n = 30$)

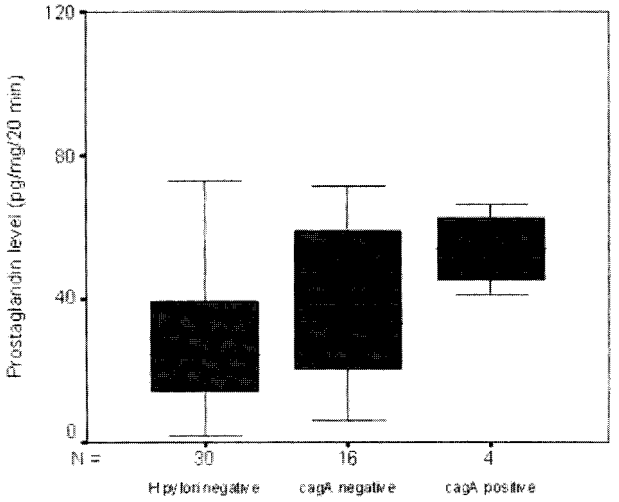

Fig 1. PGE_2 levels in patients without atrophy. Patients infected with cagA+ had significantly ($P = 0.031$) higher levels of PGE_2 than uninfected patients.

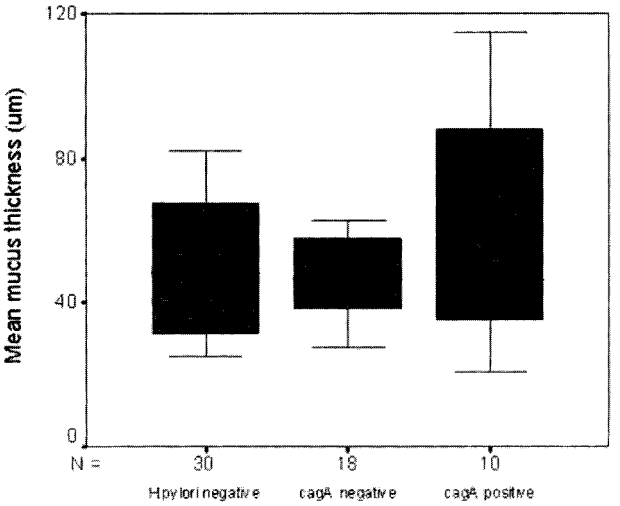

Fig 2. Box plot comparing the mean mucus thickness between age- and sex-matched *H. pylori*-negative, cagA−, and cagA+ patients. Although cagA+ patients have higher mean mucus thickness than cagA−, the difference was not statistically significant ($P = 0.784$). *N*, number of patients.

than uninfected patients (22.6 ± 1.2 pg/mg/20 min; $n = 30$), $P = 0.04$. In matched patients without atrophy, cagA+ patients had significantly higher PGE_2 (53 ± 1.1 pg/mg/20 min; $n = 4$) than HP− patients (22.6 ± 1.1 pg/mg/20 min; $n = 30$), $P = 0.031$. However, there was no significant difference in mean PGE_2 between cagA− (35 ± 1.3 pg/mg/20 min; $n = 16$) and HP− (22.6 ± 1.1 pg/mg/20 min; $n = 30$), $P = 0.414$, or between cagA+ and cagA− patients, $P = 0.292$ (Figure 1).

In age- and sex-matched patients there was no significant difference ($P = 0.717$) in mean mucus thickness between HP− (51.3 ± 1.1 μm; $n = 30$) and HP+ (48.8 ± 1.1 μm; $n = 30$) patients; also, there was no significant difference in mean contact angle between HP+ ($61 \pm 2.8°$; $n = 30$) and HP− patients ($65.5 \pm 3°$; $n = 30$), $P = 0.27$. However, cagA+ patients had a nonsignificant ($P = 0.784$) trend of increased mean mucus thickness in comparison to cagA− and HP− patients (52.7 ± 1.2 μm [$n = 10$], 46.6 ± 1.1 μm [$n = 18$], and 51.3 ± 1.1 μm [$n = 30$], respectively) (Figure 2). Also, cagA+ patients had a similar nonsignificant ($P = 0.30$) trend of increased mean contact angles in comparison to cagA− and HP− patients ($71.1 \pm 8°$ [$n = 4$], $59.6 \pm 4°$ [$n = 16$], and $66 \pm 2°$ [$n = 69$], respectively) (Figure 3).

In cagA+ patients there was a weak but nonsignificant positive association between mean mucus thickness and HP colonization density (Spearman's $r = 0.333$, $P = 0.381$) (Figure 4). However, in cagA− patients, the mean mucus thickness was significantly reduced with increased HP colonization density ($r = -0.805$, $P < 0.0001$) (Figure 5).

Based on the above results a model is proposed to explain the high association of cagA+ infection with distal gastric cancer (Figure 6).

DISCUSSION

A sequence of events leading from gastritis to gastric cancer has been proposed by Correa *et al.* (29). Correa (30) later proposed that HP infection is one of the triggering

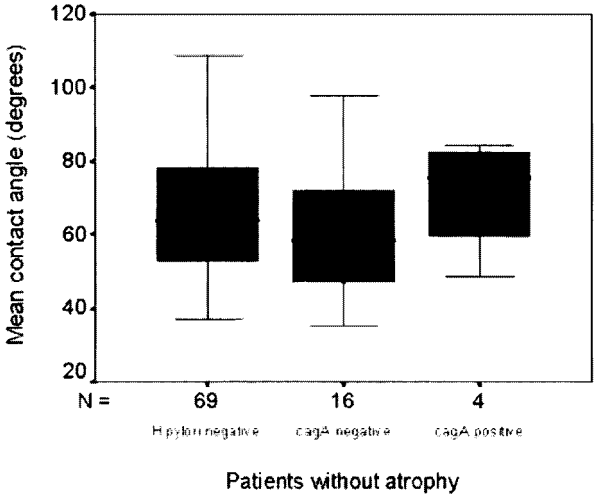

Fig 3. Categorization of the mean contact angle according to *H. pylori* infection in patients without atrophy. Patients with cagA+ had a higher mean contact angle (but not statistically significant) than cagA− and HP− patients.

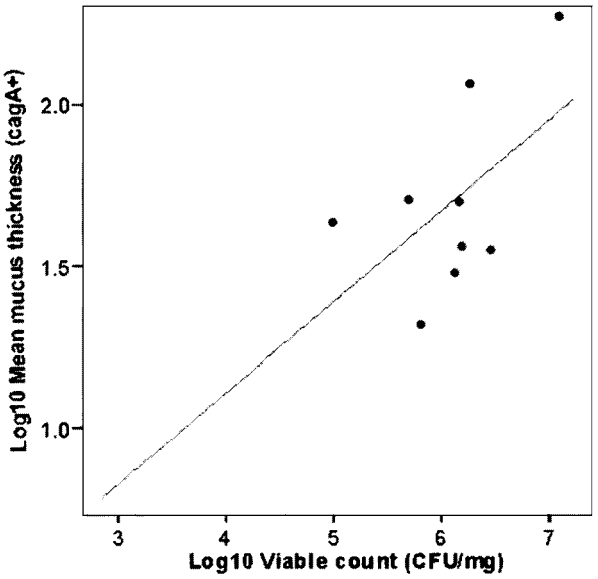

Fig 4. Correlation between mean mucus thickness in cagA+ infected patients and *H. pylori* colonization density. Positive association but not significant ($r = 0.333$, $P = 0.381$).

factors in the progressive sequence of gastric lesions beginning with superficial to chronic gastritis, followed by the development of atrophy, intestinal metaplasia, dysplasia, and, finally, gastric cancer.

From this study a model is proposed to explain the high association of cagA+ infection with distal gastric cancer

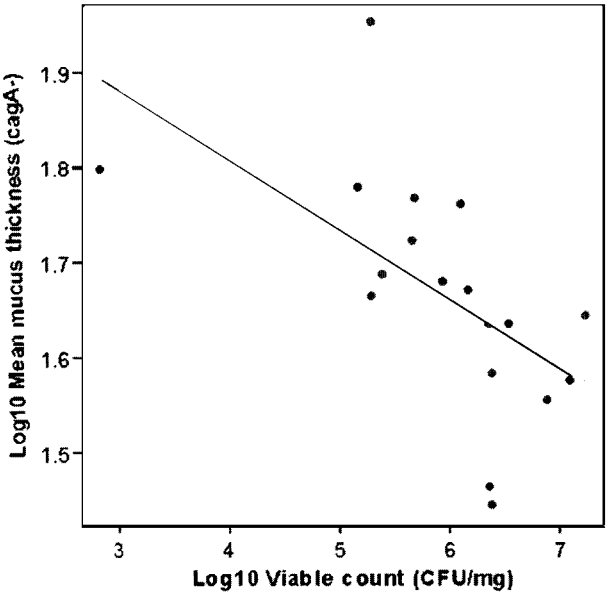

Fig 5. Correlation between mean mucus thickness in cagA− infected patients and *H. pylori* colonization density. Significant ($P < 0.0001$) inverse association ($r = -0.805$).

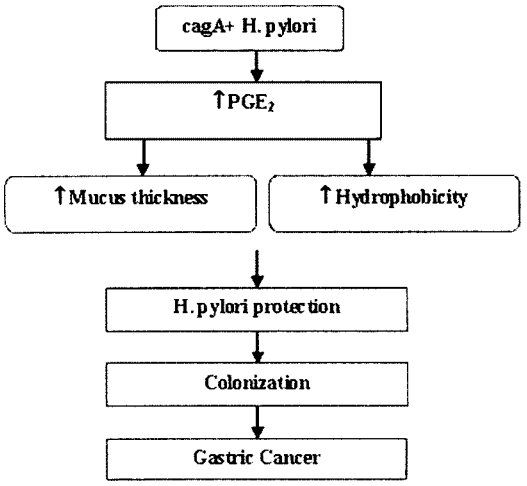

Fig 6. A proposed model for the association of cagA+ infection with distal gatric cancer. ↑, increase.

(Figure 6), which proposes that within the human stomach cagA+ is protected by an increased gastric mucous layer thickness and hydrophobicity, mediated by PGE_2 stimulation, leading to established colonization and gastritis. This may then increase the risk of gastric cancer in susceptible patients.

This model is supported by the results of the present study. (a) The PGE_2 levels in age- and sex-matched patients were significantly higher in HP-infected patients than uninfected patients. In matched patients without atrophy cagA+ patients had significantly higher PGE_2 than HP− patients, whereas cagA− patients were not significantly different from HP− patients. In addition, cagA+ patients had higher but nonsignificant PGE_2 levels than cagA− patients. (b) There was no significant difference in mean mucus thickness between HP-infected and age- and sex-matched uninfected patients, however, cagA+ patients showed a trend of increased mucus thicknesses compared with cagA− patients that did not reach statistical significance. (c) No significant difference was observed in gastric mucus hydrophobicity between HP-infected and age- and sex-matched uninfected patients, however, cagA+ patients showed a trend of increased hydrophobicity compared with cagA− and HP− patients that did not reach statistical significance. (d) Colonization of the stomach with cagA+ strains is associated with severe forms of gastritis, as most of the patients with atrophic gastritis were infected with cagA+. The lack of significance observed is probably related to the small number of cagA+ patients involved. Nevertheless, this does not invalidate the proposed model.

There are questions that need to be answered from the present study in relation to the model of cagA+

association with distal gastric cancer. First, although there was no significant difference in mucus thickness in the present study between cagA+ and cagA− infection, what is the reason for the opposing trends in mucus thickness in cagA+ and cagA− strains of HP? One possible explanation relates to the role of the 120-kDa CagA protein (20) in inducing the secretion of host signaling factors when the cagA+ attaches to the gastric epithelial cells and the subsequent internalization and phosphorylation of the CagA protein (31).

Second, how does HP increase PGE_2 production? It is believed that HP increases gastric prostaglandin concentration through increased COX-2 (cyclooxygenase-2) expression (32). This is supported by the finding that HP infection induces the expression of COX-2 in the gastric mucosa (33), whereas eradication of HP results in reduced gastric antral mucosal COX-2 expression (34).

Third, how does PGE_2 increase mucus thickness and hydrophobicity? Lichtenberger *et al*. (35) have shown that rats injected with PGE_2 have higher gastric mucosal concentrations of phospholipids and surfactants compared to controls, indicating the importance of PGE_2 in enhancing gastric hydrophobicity. Kerss *et al*. (36) reported a significant increase in gastric mucus thickness in both rats and frogs in response to topical administration of 16,16-dimethylprostaglandin E_2 to the stomach of these animals by oral intubation.

Fourth, how can HP cause gastric cancer? HP may cause gastric carcinomas by the converging effects of two main types of events (37): (a) collateral damage of inflammatory by-products causing mutational events in gastric epithelial cells, for example, production of reactive oxygen intermediates that can induce DNA damage with DNA mutations contributing to the pathogenesis of gastric cancer (38) by Farinati *et al*. (39) showed that cagA+ patients had higher oxidative DNA damage than cagA− and HP− patients as assessed by the tissue concentrations of 8-hydroxydeoxyguanosine (8OHdG) levels, which is responsible for DNA base mutation induced by reactive oxygen metabolites; and (b) direct effects on gastric epithelial cells by H. pylori organisms or released bacterial products. The latter include (i) direct toxic effects on epithelial cells. HP is known to produce damaging enzymes, such as phospholipase A_2, and cytotoxins, such as vacA; and (ii) alterations in the balance between apoptosis and proliferation (40). HP produces an apoptosis-inducing protein that was found to have γ-glutamyl transpeptidase activity (41).

Finally, why is distal gastric cancer more common in males than in females infected with cagA+ strains? Fox *et al*. (42), in a study of INS-GAS mice, have demonstrated a greater susceptibility of male gastric tissue to HP infection, as males had more severe hyperplasia, dysplasia and epithelial cell proliferation than females. In addition, the development of gastric cancer over the 7-month observation period was seen only in INS-GAS HP-infected male mice (four of six) on the low-salt diet. The underlying mechanisms responsible for this gender difference in susceptibility are not known. This raises the question whether the combination of cagA+ infection and male sex increases the risk of gastric cancer. This needs further investigation in humans.

The model postulated and tested in this study has several limitations: (a) the study on which the model is based was carried out in a geographical area of low HP prevalence, therefore the number of cagA+ patients ($n = 10$) and cagA− patients ($n = 18$) was small; (b) the model does not explain the presence of gastric cancer in association with cagA− infection; and (c) the early changes of gastric mucus thickness and hydrophobicity in relation to cagA+ infection occurring at an early phase of HP colonization in the human stomach are not known. This is because (i) the acquisition of HP infection occurs in childhood (43) and (ii) the initial acute phase of HP infection is subclinical and rarely encountered in gastric biopsies (44).

Future research is needed to substantiate the model presented in this study and to overcome the limitations outlined above. There are a number of ways to go forward: (a) a multicenter study of a large number of patients needs to be undertaken in areas of high HP prevalence; (b) long-term prospective studies to clarify the importance of male cagA+ infection in the risk of gastric cancer; and (c) human and animal model studies to investigate the effects of HP on gastric mucus thickness, hydrophobicity, and PGE_2 occurring at the early stage of HP colonization are required. Sugiyama *et al*. (45) studied the early changes (at 4 and 12 weeks) of HP infection occurring in the gastric mucosa of Mongolian gerbils and found that HP infection caused chronic active gastritis, gastric erosions, hypersecretion of mucins from gland mucous cells, and an increase in intramucosal PGE_2 in infected animals compared to uninfected animals. They also reported an increase in gastric mucous layer thickness in HP-infected gerbils at 4 and 12 weeks compared to an unifected control group.

In summary, the work described in this study has shown that cagA+ infection has the potential to increase the gastric mucus layer thickness and hydrophobicity by increasing PGE_2 production. This facilitates its protection and enhances its chronic colonization, leading to the progression of gastritis to more severe forms, which may increase the risk of distal gastric cancer. However, it could be argued that mucous gel thickness and surface hydrophobicity may increase in cagA+ patients as a compensatory

mechanism to limit mucosal injury and inflammation as opposed to being a trigger in the development of distal gastric cancer. The small number of cagA+ patients in this study may have been a factor in the nonsignificance of some of the results. Consequently, large multicenter studies need to be conducted before firm conclusions can be drawn. If appropriate, the conclusions may then form the basis for the development of further HP therapies that concentrate on mechanisms to decrease mucous gel layer thickness as an additional method for eradicating HP in infected subjects, who fail to respond to antibiotic therapy without compromising the mucous layer protective function to the underlying gastric epithelial cells. Moreover, further research is needed in order to explain the apparent opposing relation of cagA+ infection to the risk of distal gastric cancer compared to proximal gastric cardia cancer.

ACKNOWLEDGMENTS

This work was funded by Sultan Qaboos University (Sultanate of Oman) and supported by the University of Leeds (UK). From the University of Leeds we thank Professor Stephen Evans (Physics and Astronomy), Dr. Fraser Lewis (Pathology), Dr. Richard Rodway (Biology), Dr. David Roberts (Biomedical Sciences), Mr. Andy West (Microbiology), Mr. Paul Drake (Biomedical Sciences), and Mr. Steve Paxton (Biomedical Sciences). From the clinical side we thank Dr. Mark Denyer (Senior Clinical Lecturer in Medicine, Seacroft University Hospital, UK), Dr. Sean Kelly (Consultant in Gastroenterology, York District Hospital, UK), and Sister Andrea Reilly (Nurse Endoscopist, St. James's University Hospital, UK) for providing the gastric biopsies.

REFERENCES

1. Dixon MF: Helicobacter pylori and chronic gastritis. *In* Helicobacter pylori and Gastroduodenal Disease. BJ Rathbone, RV Heatley (eds). Oxford, Blackwell Scientific, 1992.
2. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R: Helicobacter pylori virulence and genetic geography. Science 284:1328–1333, 1999
3. Bayerdorffer E, Neubauer A, Rudolph B, Thiede C, Lehn N, Eidt S, Stolte, M: Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of Helicobacter pylori infection. MALT Lymphoma Study Group. Lancet 345:1591–1594, 1995
4. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas, F: Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. BMJ 302:1302–1305, 1991
5. Parkin DM, Bray FI, Devesa SS: Cancer burden in the year 2000. The global picture [Review]. Eur J Cancer 37(Suppl 8): S4–S66, 2001
6. Parkin DM, Pisani P, Ferlay J: Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 54:594–606, 1993
7. Powell J, McConkey CC: The rising trend in oesophageal adenocarcinoma and gastric cardia. Eur J Cancer Prev 1:265–269, 1992
8. Kelly JR, Duggan JM: Gastric cancer epidemiology and risk factors. J Clin Epidemiol 56:1–9, 2003
9. Lauren P: The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. Acta Path Microbiol Scand 64:31–49, 1965
10. International agency for research on cancer. Schistosomes, liver flukes and Helicobacter pylori: IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum 61:1–241, 1994
11. Danesh J: Helicobacter pylori infection and gastric cancer: systematic review of the epidemiological studies. Aliment Pharmacol Ther 13:851–856, 1999
12. Parsonnet J, Vandersteen D, Goates J, Sibley RK, Pritikin J, Chang Y: Helicobacter pylori infection in intestinal- and diffuse-type gastric adenocarcinomas. J Natl Cancer Inst 83:640–643, 1991
13. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ: Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 325:1132–1136, 1991
14. Hirayama F, Takagi S, Iwao E, Yokoyama Y, Haga K, Hanada S: Development of poorly differentiated adenocarcinoma and carcinoid due to long-term Helicobacter pylori colonization in Mongolian gerbils. J Gastroenterol 34:450–454, 1999
15. Shimizu N, Inada K, Nakanishi H, Tsukamoto T, Ikehara Y, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M: Helicobacter pylori infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens. Carcinogenesis 20:669–676, 1999
16. Uemura N, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, Sasaki N, Haruma K, Sumii K, Kajiyama G: Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. Cancer Epidemiol Biomarkers Prev 6:639–642, 1997
17. Schandl L, Malfertheiner P, Ebert MP: Prevention of gastric cancer by Helicobacter pylori eradication? Results from clinical intervention studies. Dig Dis 20:18–22, 2002
18. Covacci A, Rappuoli R: Helicobacter pylori: Molecular evolution of a bacterial quasi-species. Curr Opin Microbiol 1:96–102, 1998
19. Rudi J, Kolb C, Maiwald M, Zuna I, von Herbay A, Galle PR, Stremmel W: Serum antibodies against Helicobacter pylori proteins VacA and CagA are associated with increased risk for gastric adenocarcinoma. Dig Dis Sci 42:1652–1659, 1997
20. Crabtree JE, Taylor JD, Wyatt JI, Heatley RV, Shallcross TM, Tompkins DS, Rathbone BJ: Mucosal IgA recognition of Helicobacter pylori 120 kDa protein, peptic ulceration, and gastric pathology. Lancet 338:332–335, 1991
21. Blaser MJ: The interaction of cag+ Helicobacter pylori strains with their hosts. *In* Helicobacter pylori: Basic Mechanisms to Clinical Cure 1998. RH Hunt, GNJ Tytgat (eds). Dordrecht, Kluwer Academic, 1998
22. Warren JR, Marshall BJ: Unidentified curved bacilli on gastric epithelium in chronic active gastritis. Lancet 1:1273–1275, 1983
23. Marshall BJ: Helicobacter pylori. Am J Gastroenterol 89:S116–S128, 1994
24. Goodwin S: Detection of H. pylori by biopsy urease, histology, and culture. *In* Helicobacter pylori Protocols. CL Clayton, HL Mobley (eds). Totowa, NJ, Human Press, 1997
25. Collins CH, Lyne PM: Counting micro-organisms. *In* Microbiological Methods. CH Collins, PM Lyne (eds). London, Boston, Butterworths, 1984

26. Jordan N, Newton J, Pearson J, Allen A: A novel method for the visualization of the in situ mucus layer in rat and man. Clin Sci 95:97–106, 1998
27. Spychal RT, Marrero JM, Saverymuttu SH, Northfield TC: Measurement of the surface hydrophobicity of human gastrointestinal mucosa. Gastroenterology 97:104–111, 1989
28. Ahmed A, Holton J, Vaira D, Smith SK, Hoult JR: Eicosanoid synthesis and Helicobacter pylori associated gastritis: increase in leukotriene C4 generation associated with H. pylori colonization. Prostaglandins 44:75–86, 1992
29. Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M: A model for gastric cancer epidemiology. Lancet 2:58–60, 1975
30. Correa P: Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 52:6735–6740, 1992
31. Testerman TL, McGee DJ, Mobley HL: Adherence and colonization. *In* Helicobacter pylori: Physiology and Genetics. HL Mobley, GL Mendz, SL Hazell (eds). Washington DC, ASM Press, 2001
32. Cryer B: Mucosal defense and repair. Role of prostaglandins in the stomach and duodenum. Gastroenterol Clin North Am 30:877–894, 2001
33. Chan FK, To KF, Ng YP, Lee TL, Cheng AS, Leung WK, Sung JJ: Expression and cellular localization of COX-1 and -2 in Helicobacter pylori gastritis. Aliment Pharmacol Ther 15:187–193, 2001
34. McCarthy CJ, Crofford LJ, Greenson J, Scheiman JM: Cyclooxygenase-2 expression in gastric antral mucosa before and after eradication of Helicobacter pylori infection. Am J Gastroenterol 94:1218–1223, 1999
35. Lichtenberger LM, Graziani LA, Dial EJ, Butler BD, Hills BA: Role of surface active phospholipids in gastric cytoprotection. Science 219:1327–1329, 1983
36. Kerss S, Allen A, Garner A: A simple method for measuring thickness of the mucus gel layer adherent to rat, frog and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents. Clin Sci 63:187–195, 1982
37. Asaka M, Sepulveda AR, Sugiyama T, Graham DY: Gastric Cancer. *In* Helicobacter pylori: Physiology and Genetics. HL Mobley, GL Mendz, SL Hazell (eds). Washington DC, ASM Press, 2001
38. Grisham MB: Review article: chronic inflammation and reactive oxygen and nitrogen metabolism—Implications in DNA damage and mutagenesis. Aliment Pharmacol Ther Supplement 14:3–9, 2000
39. Farinati F, Cardin R, Russo VM, Busatto G, Franco M, Rugge M: Helicobacter pylori CagA status, mucosal oxidative damage and gastritis phenotype: A potential pathway to cancer? Helicobacter 8:227–234, 2003
40. Rokkas T, Ladas S, Liatsos C, Petridou E, Papatheodorou G, Theocharis S, Karameris A, Raptis S: Relationship of Helicobacter pylori CagA status to gastric cell proliferation and apoptosis. Dig Dis Sci 44:487–493, 1999
41. Shibayama K, Kamachi K, Yagi T, Yamane K, Doi Y, Shibata N, Kato H, and Arakawa Y: A novel apoptosis-inducing protein from Helicobacter pylori. Helicobacter 8(4):354, 2003
42. Fox JG, Rogers AB, Ihrig M, Taylor NS, Whary MT, Dockray G, Varro A, Wang TC: Helicobacter pylori-associated gastric cancer in INS-GAS mice is gender specific. Cancer Res 63:942–950, 2003
43. Mitchell HM: Epidemiology of Infection. *In* Helicobacter pylori: Physiology and Genetics. HL Mobley, GL Mendz, SL Hazell (eds). Washington DC, ASM Press, 2001
44. Day DW, Dixon MF: Acute and Chronic Gastritis. *In* Biopsy Pathology of the Oesophagus, Stomach and Duodenum. DW Day, MF Dixon (eds). Hong Kong, Chapman & Hall Medical, 1995
45. Sugiyama A, Ikeno T, Ishida K, Maruta F, Murakami M, Sato T, Saito H, Ishizone S, Kawasaki S, Ota H, Katsuyama T: Paradoxical role of Helicobacter pylori infection: protective effect against ethanol-induced gastric mucosal injury in Mongolian gerbils. Dig Dis Sci 46:2433–2439, 2001
46. Sarosiek J, Marshall BJ, Peura DA, Hoffman S, Feng T, McCallum RW: Gastroduodenal mucus gel thickness in patients with Helicobacter pylori: a method for assessment of biopsy specimens. Am J Gastroenterol 86:729–734, 1991
47. Newton JL, Jordan N, Oliver L, Strugala V, Pearson J, James OF, Allen A: Helicobacter pylori in vivo causes structural changes in the adherent gastric mucus layer but barrier thickness is not compromised. Gut 43:470–475, 1998
48. Goggin PM, Marrero JM, Spychal RT, Jackson PA, Corbishley CM, Northfield TC: Surface hydrophobicity of gastric mucosa in Helicobacter pylori infection: effect of clearance and eradication. Gastroenterology 103:1486–1490, 1992
49. Spychal RT, Goggin PM, Marrero JM, Saverymuttu SH, Yu, CW, Corbishley CM, Maxwell JD, Northfield TC: Surface hydrophobicity of gastric mucosa in peptic ulcer disease. Relationship to gastritis and Campylobacter pylori infection. Gastroenterology 98:1250–1254, 1990
50. Day AS, Jones NL, Policova Z, Jennings HA, Yau EK, Shannon P, Neumann AW, Sherman PM: Characterization of virulence factors of mouse-adapted Helicobacter pylori strain SS1 and effects on gastric hydrophobicity. Dig Dis Sci 46:1943–1951, 2001
51. Oderda G, D'Alessandro M, Mariani P, Lionetti P, Bonamico M, Dell'Olio D, Ansaldi N: Prostaglandin E2 in gastric mucosa of children with Helicobacter pylori gastritis: relation to thickness of mucus gel layer. J Clin Pathol 46:836–839, 1993
52. Avunduk C, Suliman M, Gang D, Polakowski N, Eastwood GL: Gastroduodenal mucosal prostaglandin generation in patients with Helicobacter pylori before and after treatment with bismuth subsalicylate. Dig Dis Sci 36:431–434, 1991
53. Goren A: Campylobacter pylori and acid secretion. Lancet 2:212, 1989
54. Taha AS, Boothman P, Holland P, McKinlay A, Upadhyay R, Kelly RW, Lee F, Russell RI: Gastric mucosal prostaglandin synthesis in the presence of Campylobacter pylori in patients with gastric ulcers and non-ulcer dyspepsia. Am J Gastroenterol 85:47–50, 1990