Helicobacter pylori W9–W16

W9

COMPARISON OF H. PYLORI STRAINS FROM INFECTED PARENTS AND OFFSPRING USING DNA TYPING

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The source of infection and transmission mode of H. Pylori is unclear. A high prevalence of infection within families has been reported using breath testing or serological techniques. The aim of this study was to analyse the strains of H. pylori present within a series of cohabiting family groups. All 8 parents of 4 infected children who had presented to a paediatric clinic with dyspepsia underwent endoscopy, gastric antral biopsy and H. pylori culture. Although the parents were asymptomatic at time of investigation, one male had reflux oesophagitis at endoscopy. Biopsies were cultured on Columbia-based blood agar under microaerophilic conditions for 4 days. Cultures from parents and children underwent DNA extraction and the electrophoretic patterns were compared after digestion with restriction endonucleases and the use of a biotinylated cDNA probe prepared from 16S and 23S rRNA of H. pylori NCTC strain 11638. Seven parents were H. pylori positive on rapid urease testing and histology, and cultures were obtained. DNA typing showed the same strain present in at least two members in 3 of the 4 families. In family 1, mother, father and child had the same strain; in family 2, father and son had the same strain while mother was culture negative; father and mother had the same strain in family 3; and all strains were unique in family 4. These findings suggest, for the first time, that there is intra-familial spread or a common source of infection within families.

W10

DEFECTIVE ANTIGEN-SPECIFIC RESPONSES IN PATIENTS WITH GASTRIC H. PYLORI COLONIZATION.

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Helicobacter pylori (HP) is capable of colonizing the gastric mucosa, despite a significant humoral response, in 30-60% of healthy adults. In this study we have examined the cellular response to HP by measuring *in vitro* proliferative response of peripheral blood mononuclear cells (PBMC) to inactivated whole cell HP antigens, purified protein derivative (PPD), whole cell inactivated E Coli antigens and phythaemagglutinin (PHA) in 37 dyspeptic patients undergoing upper gastrointestinal endoscopy. A standard five day thymidine incorporation assay using an optimal dose of HP antigen preparation (as determined in preliminary experiments) of 3μg/ml was utilized. HP status was determined by culture, histology and the rapid urease test on antral biopsies.

Patients with HP colonisation (HP+) had significantly lower proliferative responses relative to HP negative (HP-) individuals [1610±555 (n=21) vs 3946±778 (n=16) cpm 3H-Tdr incorporation, x±SEM, p<.01]. There was no significant difference in proliferative responses to PPD [4771±1613 vs 8109±2466, x±SEM, p. ns] or to PHA [10417±2156 vs 9510±1552]. We could not detect proliferative responses to E coli antigen at significant levels in patients or controls. We also examined the secretion of T-cell cytokine γ-interferon (IFN) in these patients by ELISA. IFN secretion by PBMC in response to HP antigen was also lower in HP+ individuals relative to HP- controls [5.1±0.98 (n=14) vs 12.3±3.2 (n=14) U/ml, p<.02]. However, neither spontaneous production nor responses to PHA were significantly different in the two groups (3.5±1.1 vs 4.8±0.96, 79.8±36 vs 71.9±24).

These findings suggest a defective peripheral blood T cell response to HP antigens in HP+ individuals. This might reflect an imbalance between mucosal and systemic immune responses in these individuals. Alternatively, these data might suggest prior encounter with and elimination of HP in HP- individuals due to recognition of specific epitopes. Finally, these findings could also reflect antigen-specific suppression of T-cell proliferative responses in the peripheral circulation. These possibilities are under investigation.

W11

HOW QUICKLY DOES *HELICOBACTER PYLORI (H.PYLORI)* RECUR AFTER TREATMENT?

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Duodenal ulcer recurrence can be prevented by eradication of *H.pylori*: no evidence of infection at least one month after finishing treatment. The non-invasive ^{13}C-urea breath test, free of sampling error and able to detect lower levels of active infection than endoscopic biopsy based methods or serology, is ideal for follow up after treatment. This study determines how soon *H.pylori* recurs after therapy thus providing a rational timing for assessment of eradication.

Patients needing *H.pylori* eradication were invited to enter the study. Before starting treatment *H.pylori* status was assessed by antral histology (H&E, and Gimenez stains), culture (micoraerophobic conditions for up to 10 days) and ^{13}C-UBT (European standard protocol, positive result = excess $\delta^{13}CO_2$ > 5 per mil). The ^{13}C-UBT was repeated immediately after finishing treatment and then at weekly intervals for 1 month (or until positive) and subsequently at 3, 6, and 12 months.

Forty-six patients (26 men, median age 45 y, range 19-67 y) with either active (n=10) or previous / recurrent (n=36) DU were studied. All patients had a positive ^{13}C-UBT (mean (±sem) excess $\delta^{13}CO_2$ excretion = 26.8 (±12.4) per mil) and either positive histology (n=42), or culture (n=40) before starting treatment. *H.pylori* was cleared in all patients: ^{13}C-UBT negative immediately after finishing treatment (double / triple therapy for 1 or 2 weeks). In 17/46 patients eradication therapy failed: *H.pylori* recurred in 15/17 without symptom recurrence, (mean (±sem) excess $\delta^{13}CO_2$ excretion = 12.4 (±6.7) per mil) at median 8 days (range 6-18d). In all 29 patients in whom *H.pylori* was successfully eradicated at one month mean (±sem) excess $\delta^{13}CO_2$ excretion = 2.4 (±0.9) per mil) the breath test was negative at 2 weeks and thereafter (median f/u = 4.6 months, range 1.0 - 7.2)

These results show that the ^{13}C-UBT can detect recurrent *H.pylori* within days of finishing anti-*H.pylori* therapy, and suggest that eradication can be accurately diagnosed 2 weeks after the end of treatment.

W12

TWENTY-FOUR HOUR HYPERPEPSINOGENAEMIA IN *HELICOBACTER PYLORI*-POSITIVE SUBJECTS IS ABOLISHED BY ERADICATION OF THE INFECTION

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H.pylori infection is associated with hyperpepsinogenaemia, but the effect of eradication on 24-h profiles of plasma pepsinogen I (PG I) and pepsinogen II (PG II) is not known.
METHODS: 24-h plasma PG I and PG II concentrations were determined in 8 healthy subjects with antibody to *H.pylori*, before and 4-6 weeks and 20-24 weeks after treatment with tripotassium dicitrato bismuthate, amoxycillin and metronidazole.
RESULTS: Therapy was successful in the 5 subjects with active infection. Antral chronic active gastritis, which was moderate to severe before treatment, had resolved by the time of follow-up biopsy 20-24 weeks post-treatment.

Median integrated 24-h plasma PG I and PG II (μg.h/L).

HP +ve (n=5)	Before	4-6 wks	20-24wks
PG I	2288	1811*	1643*
PG II	357	171*	150*
HP -ve (n=3)			
PG I	1450	1517	1515
PG II	157	155	152

* p < 0.05 compared with before treatment.

In the 5 subjects with active *H.pylori* infection, PG I and PG II fell significantly by 4-6 weeks after successful eradication. The decrease in PG II was proportionally greater than the decrease in PG I (58 vs. 27%) and the median fasting (0800h) PG I:PG II ratio rose from 7.05 to 11.1. There was little variation of hourly median PG I concentration throughout the 24-h period, although some individuals show a delayed meal-associated increase. In the 3 subjects without active *H.pylori* infection, pretreatment plasma PG I and PG II were similar to values found in the *H.pylori*-infected subjects after successful treatment, and did not change in response to therapy. **CONCLUSION:** *H.pylori* infection is associated with reversible 24-h hyperpepsinogaemia.

W13

EXPRESSION OF 120,000 M_r PROTEIN AND CYTOTOXICITY IN HELICOBACTER PYLORI

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Two characteristics of Helicobacter pylori which have been associated with peptic ulceration are immune recognition by mucosal IgA of a 120,000 M_r protein (p120) and the production of a vacuolating cytotoxin. Only some strains of H.pylori express p120, suggesting that p120 may be involved in pathogenicity. To determine the relationship between p120 expression and host mucosal immune defences, in vitro culture supernatants from endoscopic antral biopsies from 27 patients with H.pylori-associated gastritis were studied. Immune recognition was determined by immunoblotting against H.pylori strains of defined cytotoxin potential (cytotoxin-positive strain CCUG 17874, cytotoxin-negative strains G-21 and G-17-91).

All 27 patients showed a positive mucosal IgA response against all three strains of H.pylori. p120 was recognised only in the cytotoxin-positive CCUG 17874 strain, but not in the two cytotoxin-negative strains. In total, culture supernatants from 14 patients recognised p120. In the other 13 patients (and 4 controls with histologically normal mucosae) there was no recognition of p120 by immunoblotting of both the cytotoxin-positive and cytotoxin-negative strains.

We suggest that the previously demonstrated association between mucosal recognition of p120 and peptic ulceration is a factor of bacterial strain rather than the host immune response, and may be directly related to cytotoxin production.

W14

H.PYLORI LECTINS DETECTED BY NEOGLYCOPROTEINS.

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Gastric epithelial surface glycoconjugates were found previously to bind Peanut agglutinin (PNA, gal-galNAc binding), Vicia villosa agglutinin (VVA, galNAc binding) Ulex europaeus I (fucose binding), Griffonia simplicifolia 2 (glcNAc binding) and concanavalin A (mannose binding) lectins. We have used 5 neoglycoproteins (NG), each bearing one of the carbohydrate epitopes, to detect H.pylori surface lectins in this study.

The NG gal-galNAc-Human serum albumin (HSA) and galNAc-HSA were conjugated to biotin and then purified by gel filtration. They were subsequently confirmed to bind PNA and VVA by ELISA. Gold labelled Fucose-Bovine serum albumin (BSA), glcNAc-BSA and mannose-BSA NG were obtained commercially. These 5 NG were used in the ELISA. H.pylori surface proteins coated micro-ELISA wells were incubated with 100 µl (20 µg/ml) conjugated NG. The unbound NG were then washed off and the bound biotin-labelled NG were identified by using Avidin-Biotin complex/peroxidase and O'phenylene diamine in citric acid buffer containing H_2O_2. The bound gold-labelled NG were identified by silver enhancement technique. The controls were performed by replacing the NG by unconjugated HSA or BSA followed by washings and then further incubation with peroxidase-labelled anti-HSA or anti-BSA antibody. The peroxidase activity was similarly identified as before.

Gal-galNAc-HSA, galNAc-HSA and mannose-BSA bound to H.pylori surface proteins but not the fucose-BSA or glcNAc-BSA. The controls were negative. These results indicate that H.pylori possess surface lectins that bind to gal-galNAc, galNAc and mannose. These lectins probably enhance their colonisation in the stomach and account for their gastric tropism.

W15

HELICOBACTER PYLORI AND REACTIVE OXYGEN METABOLITE PRODUCTION IN DUODENAL ULCER DISEASE

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We have previously shown that production of reactive oxygen metabolites (ROMs) correlates with macroscopic and microscopic grading of duodenal inflammation, and is enhanced in H.pylori positive, compared to negative, mild antral gastritis. The aim of this study was to assess the influence of gastric H.pylori infection on the production of ROMs in human duodenal ulcer disease.

Methods Endoscopic biopsies were taken from the edge of duodenal ulcers (DU), areas of maximal inflammation in patients with severe erosive duodenitis (ED), and from controls with macroscopically normal duodenal mucosa. ROM production was assessed using 75 µM luminol-amplified chemiluminescence (CL). H.pylori were detected in antral biopsies by commercial urease ('CLO') test and culture.

Results CL was enhanced in biopsies from DU (median CL after subtraction of background 15.9 x 10^3 photons/min/mg (95% confidence interval 4.9 to 47.9), n = 24), and ED (7.0 (0.0 to 88.4), n = 13) compared to control (-0.4 (-0.9 to 1.1), n = 21) (p = 0.0001, Kruskal-Wallis). There was no difference in CL between the ED and DU groups. In the 20% of patients with DU and ED who were H.pylori negative, CL (-0.1 (-8.9 to 7.2), (n = 8)) although not different to control, was much lower than in patients with the same macroscopic lesions who were H.pylori positive (24.2 (6.7 to 62.9), n = 29) (p = 0.004). In the control group, duodenal CL was not affected by antral H.pylori status (HP positive -0.5 (-1.1 to 1.5) n = 10); HP negative -0.4 (-1.7 to 1.7) n = 11) (p = NS).

Conclusions 1) Duodenal mucosa from patients with erosive duodenitis or duodenal ulcer produces excess reactive oxygen metabolites only in the presence of antral H.pylori infection. 2) A pathogenic role for reactive oxygen metabolite production in duodenal ulcer disease is likely to be restricted to H.pylori-related cases. 3) In the minority of patients with antral H.pylori infection who develop duodenal ulceration, the infection is likely to act synergistically with other pathogenic factors, stimulating reactive oxygen metabolite production in duodenal mucosa.

W16

PREVALENCE OF HELICOBACTER PYLORI (HP) INFECTION AMONGST ATTENDANTS AT THE AUTUMN BSG MEETING 1991: "BARE HANDED" ENDOSCOPY A RISK FACTOR FOR HP ACQUISITION.

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Diagnostic testing for Hp infection by ^{13}C breath test was offered by a pharmaceutical company at the Autumn 1991 meeting of the BSG. 144 attendants were tested and 115 returned an anonymously completed questionnaire giving details of their test result, age, sex, professional status, endoscopy service, use of gloves and past medical history of dyspepsia and proven upper GI disease.

Respondents were 63 doctors (61 male, (mean age ± SD) 43.0 ± 8.9), 37 nurses (all female, 44.2 ± 8.6) and 15 non-clinical respondents (7 male, 42.4 ± 12.3). Positive tests for Hp infection were found in 25 of 63 doctors, 13 of 37 nurses and only 1 of 15 non-clinical respondents ($P<0.05$ cf doctors and nurses, Fisher's exact test). Life table analysis did not show a significant increase in probability of infection with age, length of clinical or endoscopic service. However, Endoscopy Unit staff who always wore gloves had a lower rate of infection (8 Hp +ve of 34) than did staff who had at some time worked "bare-handed" (27 Hp +ve of 57) $P<0.05$ (Chi square test). Overall, Hp +ve respondents were more likely to suffer from dyspepsia (21 of 39) than Hp -ve respondents (21 of 76) $P<0.01$ (Chi square test). Three Hp +ve respondents and one Hp -ve respondent had a past medical history of DU (NS, Fisher's exact test).

In this small survey both doctors and nurses have a greater prevalence of Hp infection than the non-clinical group. Acquisition is probably early in the career as prevalence does not increase with length of service. Always wearing gloves is associated with a lower rate of infection. Infection with Hp is associated with a greater rate of dyspepsia but not in proven cases of ulcer disease, but the survey may have been too small to show this.