HEICOBACTER FELIS PLASMID: A GOOD CANDIDATE FOR A SHUTTLE VECTOR?
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Aim: The Helicobacter felis-mouse model has been shown to be a useful and convenient animal model to study the pathogenesis of helicobacter infection. Since H. felis is a relatively new organism, there is an urgent need to learn more about its molecular biology. Hence the aim of this study is to construct a H. felis shuttle vector to stably introduce genes into Helicobacter sp.

Methods: Plasmids were extracted from a number of H. felis isolates originating from cats and dogs including the type strain ATCC 49179 (CS1). In addition, CS1 was re-isolated from colonised Specific Pathogen Free rodent. Plasmid profiles of CS1 from these animals were compared with those of stock cultures and a plasmid (S-1) was chosen for further characterisation. S-1 was purified from a gel and separately digested with 12 restriction enzymes.

Results: We have shown that all H. felis isolates examined possess more than one plasmid as well as unique plasmid profiles. Close examination of the plasmid profile of CS1 stock cultures indicated the stability of its plasmids in vitro. However, one 6.5 kb plasmid (S-1) was absent from the CS1 that was re-isolated from colonised animals. Of the 12 restriction enzymes, only four were able to digest S-1. The inability of some restriction enzymes to digest S-1 could either be due to the absence of specific recognition sequences or the presence of inhibitors, e.g. methylation, at these sites. Clal was the only enzyme of the 12, that digested S-1 at a unique site.

Conclusion: This is the first study to demonstrate the presence of plasmids in H. felis. The isolation of S-1, a 6.5kb plasmid from CS1, is a major step towards the construction of a H. felis shuttle vector. Once the H. felis shuttle vector is constructed, it will be useful for in vitro and in vivo manipulation of genes in Helicobacter sp.

The Role of Helicobacter pylori in Neutrophil Toxic Oxygen Radical Release
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In the mouse model of H. pylori infection, neutrophils are recruited to the stomach. The mechanism by which this occurs is not well understood. Helicobacter pylori is a Gram-negative, spiral-shaped bacterium that colonizes the gastric mucosa and is the major cause of peptic ulcer disease and chronic gastritis. It is established that infected gastric mucosa is characterized by chronic inflammation and persistent neutrophil accumulation. In this study, we investigated the role of H. pylori in the pathogenesis of peptic ulcer disease and inflammatory bowel disease. We have investigated the interaction of H. pylori with the human immune system.

Eighteen patients with H. pylori infection confirmed by histology and culture of antral mucosal biopsies were included in the study. H. pylori were cultured on chocolate agar plates at 35°C under microaerophilic conditions. H. pylori proteins were prepared by sonication of pure cultures of all 18 isolates. Neutrophils were isolated from peripheral blood and release of T.orr's were measured in a luminol enhanced chemiluminescence assay by stimulation with a reference strain (CH-20249) and the patients own strain. Control experiments with cross stimulation of neutrophils from healthy donors were performed. All 18 isolates were unable to stimulate the homologous neutrophils to release T.orr's. The reference strain and patient strains were comparable in response when neutrophils from healthy donors were used.

The fact, that all the 18 strains have the ability to stimulate neutrophils from a healthy donor but not neutrophils from the homologous donor, is strongly indicating that a specific selective immunomodulatory activity is present in H. pylori that is not previously described for other bacterial infections.
European H pylori Study Group

SPECIFIC Helicobacter pylori vacA GENOTYPES ARE ASSOCIATED WITH PRESENCE OF DUODENAL AND GASTRIC ULCERATION, AND DEGREE OF GASTRIC INFLAMMATION. J.C. Ahlén, K.T. Tham, R.M. Peak, M.J. Blaser, T.L. Cover, Division of Infectious Diseases, Vanderbilt University School of Medicine, Vanderbilt University Medical School and Veterans Affairs Medical Center, Nashville, TN.

vacA, the gene encoding the vacuolating cytotoxin of H. pylori, has a mosaic structure consisting of one of 3 signal sequence types (s1a, s1b and s2, the first 2 being closely related), and one of 2 mid-region types (m1 and m2). Type 1 vacA is closely related to possession of cagA. In this study we assessed the relationship between vacA genotype and peptic ulceration, gastric inflammation, and density of H. pylori colonisation. Methods. H. pylori was isolated from gastric antral biopsies from 65 dyspeptic US subjects, median age 58, range 23-80, for whom past and present endoscopic findings were recorded. The vacA type of isolates was determined by PCR. For 31 of the subjects, further antral biopsies were processed for histology (graded 0-3 for neutrophil and lymphocyte density) and quantitative culture. Peptic ulcer data were analysed using z' or Fisher's exact tests, histological data using Wilcoxon's rank sum test and quantitative culture data using t tests. Results. 25/41 (61%) type 1 strains were associated with past or present duodenal ulceration (DU), more than 2/10 (90%) type 2 strains (p<0.001). Thus 25/27 (93%) DU isolates were type 1. Within the 31 group, 12/17 (72%) s1a strains were associated with DU, more than 8/19 (42%) s1b strains (p<0.05). All 5 subjects with past or present ulceration harboured type s1b strains (p<0.01). Type 1 strains were associated with greater neutrophil density in antral biopsies than type 2 strains (p<0.05), greater lymphocyte density (p<0.05), and more dense colonisation (1.5 x10^8 vs 6.8 x10^7 CFU/mg wet weight, p<0.05). Type s1a strains were associated with greater neutrophil density than type s1b strains (p<0.05) and greater lymphocyte density (p<0.05). VacA mid-region typing was not associated independently with ulcer status, inflammation, or bacterial density. Conclusion. Specific vacA s1 gene sequence types are associated with the ability of H. pylori strains to cause duodenal and gastric ulceration, and with the degree of gastric inflammation these strains induce.

EXPRESSON OF cagA GENE AND NONOPSONIC NEUTROPHIL ACTIVATION BY HELICOBACTER PYLORI. J.E. Crabtree, S.M. Farney, S. Perry, B. Blomberg, D. Denison, Division of Medicine, St. James's Hospital, Leeds, UK and Dept. of Clin Microbiol & Immunol, Örebro Medical Centre Hospital, Örebro, Sweden.

Purpose. Activity of H. pylori (Hp) associated gastroduodenal disease is determined by the infiltration of neutrophils into the gastric mucosa and epithelial layer which may be an important factor for tissue damage and ulceration. CagA is a putative marker for pro-inflammatory Hp strains which induce expression of IL-8 in gastric epithelial cells. Particular strains of Hp also have nonopsonic neutrophil activating capacity (NA). Both CagA expression and NA are associated with peptic ulcer disease (PUD). The aims of this study were to examine the incidence of cagA gene and NA in Hp from patients with PUD and chronic gastritis alone. Material and Methods. Wild type Hp strains were isolated from 54 nonselected patients undergoing upper gastrointestinal endoscopy. PUD was diagnosed in 24 patients. 30 patients had only active chronic gastritis. The presence of the cagA gene in Hp was determined by PCR and NA by chemiluminescence. Results. The cagA gene was present in 39 (72.2%) strains, 20 of which were from patients with PUD. NA was present in 29 (53.2%) of strains, 18 of which were from patients with PUD. NA was more frequently expressed in cagA+ (59%) than cagA- (40%) strains. Whilst 4 of the 15 cagA+ strains and 8 of the 25 NA+ strains were from patients with PUD, only 2 of 24 (8%) PUD strains expressed neither cagA nor NA. Conclusions. NA expression is expressed in both type I cagA+ and type II cagA- strains. The ability of Hp to induce pro-inflammatory epithelial IL-8 responses may act in concert with neutrophil activating properties to recruit and activate neutrophils and induce tissue damage and ulceration.


H. pylori-Adhesion to gastric epithelial cells and cytotoxin-production are essential virulence factors. Aim of this study was to investigate differences in the adhesive and cytotoxic properties of strains from patients with chronic gastritis (CG) and duodenal ulcer (DU) and the ability to stimulate IL-8 release from epithelial cells in vitro. Methods: 6 HP-strains from patients with CG and 6 HP strains from DU patients were investigated. Adhesion and cytotoxicity of the strains were tested in vitro on human tumour surface mucosa cells (TSMC). Cytotoxic activity of bacterial sonicate (3mg/ml - 1mg/ml) was tested photometrically with the neutral-red cytotoxicity assay and by light microscopy after an incubation time of 24 hours. Cytotoxicity was graded in the cytophactic effect (CPE) and vacuolisation of cells (VAC). IL-8 release from TSMC was measured after 8 hours of incubation. The adhesion-rate of HP to TSMC was determined after 5 hours of incubation by counting adherent bacteria using the acidin-orange-staining (100 counted cells). Results: Adhesion-rate of HP from patients with CG was 12 (+/-7) cell compared to HP from patients with DU 37 (+/-10) cell (p<0.01). All HP strains tested showed a CPE but only strains from patients with DU additionally exerted VAC. To achieve a similar damage rate of TSMC the required sonicate concentration was significantly (10 times) higher from CG strains than from DU strains IL-8 release was similar in both groups [DU 174 µg/ml (+/- 135) CG 252 µg/ml (+/- 91) not significant]. Conclusion: H. pylori strains from patients with DU are more adhesive and cytotoxic than strains from patients with CG. However no difference was found in IL-8 release between strains from the 2 groups.

CYTOTOXIN PRODUCTION AND NEUTROPHIL ACTIVATION BY HELICOBACTER PYLORI IN PATIENTS WITH PEPTIC ULCERATION AND CHRONIC GASTRITIS. QB Zhang, IM Nakshabendi, MS Mokhashi, JB Dawodu, CG Gemmell, RJ Russell. Departments of Gastroenterology and Bacteriology, Royal Infirmary, Glasgow G31 2ER.

Reactive oxygen radicals have been implicated in the pathogenesis of peptic ulceration. Helicobacter pylori (H. pylori) cytotoxin may be important in this respect. The aim of this study is to investigate whether cytotoxin-producing strains of H. pylori are associated with the generation of an oxidative burst of polymorphonuclear neutrophils (PMNs). Results: 65 clinical isolates of H. pylori from 37 patients with peptic ulcer and 28 with chronic gastritis only were cultured in Brucella broth containing 5% foetal bovine serum. After 48 h, bacteria were harvested by centrifugation and a bacterial suspension (5x10^7) prepared and used to trigger PMN oxidative burst using luminol-dependent chemiluminescence (CL). PMNs were prepared from healthy blood donors. To test the ability of strains to produce cytotoxin, culture supernatants of each were concentrated (15-fold) by polyethylene glycol and were tested on cultured Vero cells for intracellular vaculuation. Results: Two distinct patterns of oxidative burst were seen; 37 strains induced a rapid, strong CL response and 22 induced a slow, weak response. Of those strains which evoked the former, 64.9% (24/37) were cytotoxic positive, while only 18% (4/22) of the latter were toxin positive (p<0.01). 6 strains gave inconclusive results. Most of the strains (78.4%, 29/37) from peptic ulcer patients were of high activity compared to only 28.6% (6/21) of those from patients with chronic gastritis only (p<0.01). Toxinogenicity was similarly distributed (67.7% versus 32.6% (p<0.01)). Conclusion: Toxinogenicity of H. pylori strains appears to be correlated with oxygen radical formation by PMNs and disease activity. It is possible that some association between cytotoxin production and CagA gene expression may be important in this respect.
SPECTRUM OF H. PYLO RII GASTRITIS IN DUODENAL ULCER PATIENTS FROM DIFFERENT COUNTRIES

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Background: It is commonly accepted that some types of gastritis are more prevalent in certain regions of the world than in others. However, histopathologic studies performed in different areas may not be comparable, because many features of gastritis lack well defined reproducible criteria.

Objectives: To have the same group of pathologists compare type, prevalence and topographic distribution of H. pylori-gastritis associated with duodenal ulcer in several groups of young patients from different areas of the world.

Methods: Patients aged 20 to 40 with documented duodenal ulcer were recruited in institutions in South Africa, Jordan, Korea, India, and United States. From each patient 6 to 8 biopsy specimens were obtained according to our mapping protocol, sent to our laboratory in Houston where they were stained with the Giemsa stain. The following features were evaluated using a 6-point visual analog scale: density of H. pylori, neutrophils, mononuclear cells, atrophy, and intestinal metaplasia. All specimens with intestinal metaplasia were subsequently stained with an Alcian blue/High Iron Diamine stain.

Results: The prevalence of intestinal metaplasia in these patients is presented in the following table:

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of Subjects</th>
<th>Subjects with Intestinal Metaplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.A. (Texas)</td>
<td>44</td>
<td>11</td>
</tr>
<tr>
<td>South Africa</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Jordan</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td>India</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Korea</td>
<td>132</td>
<td>76</td>
</tr>
</tbody>
</table>

Conclusions: The results of this study suggest that even an apparently universal manifestation of H. pylori infection such as duodenal ulcer may be accompanied by a wide variety of expressions of gastritis. It is also interesting to note that the only group of patients with a significantly higher prevalence of intestinal metaplasia were patients from Korea, a country where the incidence of gastric cancer is much higher than in any of the other four populations included in the study.

H. PYLORI, GASTRITIS AND SERUM PEPSEINOGEN A IN A MALE NON-PATIENT POPULATION.

J. I. Wyatt, T. Knight, A. Wilson, S. Greaves, D. Newell, K. Hegels, M. Corlett, D. Fornan, J. Elder. Dept. of Pathology, St. James's Hospital, Leeds, UK, and other contributors in the Stoke Stomach Project, Stoke on Trent, UK.

Aim - to explore the prevalence and pattern of gastritis in asymptomatic male volunteers from a UK area with relatively high gastric cancer prevalence.

Methods: As part of the 'Stoke Stomach Project' we determined serum pepsinogen A (PGA) levels and H. pylori serology in 505 male volunteers aged 18-63 yrs. A 10% subsample, representing the range of observed PGA levels underwent endoscopy and biopsy with gastritis graded by the Sydney System.

Results: 187/505 (37%) subjects were seropositive for H. pylori; the mean PGA was 84.5 ng/ml in the seropositives and 63ng/ml in seronegatives. Of the 29 seropositives endoscoped, 26 had gastritis and 3 had normal histology and were H. pylori negative (serological false positives) as were all 25 seronegative subjects. Nine subjects had antrum or antrum predominant gastritis, all with PGA >100ng/ml one with DU. Ten had pangastritis, which was mild in 7 with PGA >300ng/ml (one showed mild corpus atrophy) and moderate in 3 with PGA <80ng/ml all with some corpus atrophy. Seven had corpus predominant gastritis with corpus atrophy, all with PGA <50ng/ml. Results for serum gastr, pepsinogen C, and gastric juice pH supported the histological assessment of corpus atrophy.

Conclusions: H. pylori gastritis is associated with elevated PGA unless this effect is counteracted by corpus atrophy. By extrapolating the subgroup results to the whole group we would estimate about half the 187 seropositives to have gastritis with some corpus atrophy, and about 14% corpus predominant gastritis.

THE MACH 1 STUDY: OPTIMAL ONE-WEEK TREATMENT FOR H. PYLORI DEFINED?


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We define optimal Helicobacter pylori treatment as eradication rate > 90%, easy to take and with few side effects.

Methods: International multi-centre, double-blind randomised placebo controlled study including 787 patients with proven duodenal ulcer disease either active or in remission. All patients received omeprazole 20mg (O) twice daily in combination with either placebo (P) or two of the following antimicrobials twice daily: Metronidazole 400 mg (M), Amoxicillin 1000 mg (A), Clarithromycin 250 or 500 mg (C250, C500) to eradicate H. pylori. Treatments were given over one week. H. pylori status was assessed by 13C-aurea breath test (UBT) before and four weeks after cessation of therapy.

Results: The following patients were excluded: 48 H. pylori negative and 16 UBT test failures at entry, 12 adverse event discontinuations and 27 miscellaneous.

The eradication rates and 95% CI according to the APT analysis:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of pat</th>
<th>Eradication</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA500C</td>
<td>106/110</td>
<td>96%</td>
</tr>
<tr>
<td>OMC250</td>
<td>106/115</td>
<td>95%</td>
</tr>
<tr>
<td>OMC500</td>
<td>106/118</td>
<td>90%</td>
</tr>
<tr>
<td>OAC250</td>
<td>103/111</td>
<td>84%</td>
</tr>
<tr>
<td>OAM</td>
<td>94/119</td>
<td>79%</td>
</tr>
</tbody>
</table>

All treatments were well tolerated. The most common side effects were diarrhea and taste disturbances. Diarrhea seemed to be more related to A and taste disturbances more related to C and M. Only twelve discontinued due to adverse events.

Conclusion: Two of the tested combinations fulfilled our criteria for optimal treatment of H. pylori.

CLARITHROMYCIN (CL) IN COMBINATION WITH OMEPRAZOLE (OM) FOR HEALING OF DUODENAL ULCERS (DU), PREVENTION OF DU RECURRENCE, AND ERADICATION OF H. PYLORI (HP) IN TWO EUROPEAN STUDIES.


Meath Hospital, Dublin, Ire, BHURG study, St. Mary’s Hospital, London, UK

Patients with HP and DU were enrolled in two well-controlled, randomized, double-blind, multi-center studies. Patients received for two weeks either CL 500 mg TID and OM 40 mg QD or OM 40 mg QD alone; all patients received an additional two weeks of OM (40 mg QD in one study and 20 mg QD in the other). Patients were followed for 6 months. Ulcer status was assessed by endoscopy and HP status was assessed by culture, histology, and 13C-UBT at 4-6 weeks post-Rx. 356 patients with DU and HP pretreatment (mean age 47 yrs, mean DU size 16 mm) were enrolled.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DU Healing</th>
<th>Hp Eradication</th>
<th>Ulcer Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL+OM99%</td>
<td>76% (123/162)</td>
<td>8% (10/121)</td>
<td>5% (9/162)</td>
</tr>
<tr>
<td>OM 97%</td>
<td>3% (5/171)</td>
<td>51% (77/150)</td>
<td>3% (5/162)</td>
</tr>
</tbody>
</table>

Table includes all patients with both DU and HP pretreatment who had the appropriate post-Rx visit.

5% (5/92) of Hp negative CL+OM patients and 13% (5/39) of Hp positive CL+OM patients had recurrence of ulcer while 53% of Hp positive OM patients had recurrence of ulcer at the end of the 6 months follow-up.

Both CL+OM and OM alone were well tolerated. Only 3% of CL+OM patients 2% of OM patients discontinued Rx due to adverse events.