THE EFFECT OF EXOGENOUS CO-FACTORS ON ULCEROGENESIS BY *H. HELMANNII*-LIKE BACTERIA IN MICE

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We have shown that chronic infection by *H. helmannii*-like bacteria results in gastric ulceration in Balb/c mice. The purpose of this study was to determine the effect of bedding ingestion and fasting stress on ulcerogenesis in *H. helmannii*-infected mice.

Mice were inoculated with either gastric homogenate containing *H. helmannii*, uninfected gastric homogenate, or sterile Brucella broth, and housed in corn cob bedding. Eight months after inoculation, mice were randomly divided into 3 groups. Group A was fed on bedding for 24 hours prior to sacrifice. Group B was removed from the corn cob bedding, placed in cages with wire inserts and no bedding, and fasted for 24 hours. Group C was not fasted and not removed from the bedding. At sacrifice, the stomachs were scored for the presence of *H. helmannii* and gastritis, and for the number and severity of ulcers.

All mice given infected gastric homogenate became infected with *H. helmannii*, and all developed gastritis and lymphofollicular and epithelial hyperplasia. The stomachs of group A mice contained corn cob bedding, the stomachs of Group C mice contained lab chow, and the stomachs of group B mice were empty. Of the infected mice, gastric ulcers were grossly apparent in 4/8 Group A mice, 2/8 group B mice, and 1/8 group C mice, but were not present in any uninfected mice. Histologically, ulcers or erosions were detected in 11/24 of the infected mice: 5/8 in Group A (ulcer score, 1.375), 4/8 in Group B (ulcer score, 0.875), and 2/8 in Group C (ulcer score, 0.125). Gastric erosions were present in 6/14 uninfected mice: 2/16 in Group A and 4/16 in Group B (ulcer score, 0.145). Ulcers were more common and severe in infected fasted mice than in uninfected mice or uninfected mice and were more severe in mice fasted on bedding than in mice fasted on wire. Mechanical trauma due to ingestion of bedding, stress due to fasting, or both may exacerbate gastric ulcerogenesis in mice infected with *H. helmannii*.

CHARACTERIZATION OF FELINE HELICOBACTER PYLORI STRAINS AND ASSOCIATED GASTRITIS IN A COLONY OF DOMESTIC CATS

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Background: Twenty four young adult domestic cats from a commercial vendor were found to be infected with Helicobacter pylori. Methods: Histopathologic analysis, selected electron microscopy and urease mapping were performed on mucosal samples collected from the cardia and fundus, body and antrum of these cats' stomachs. Results: *H. pylori* organisms were abundant in all areas of the stomach, based on histologic evaluation and urease mapping. *H. pylori* infection was associated with a moderate to severe, lymphomucosal gastritis in 21 of 24 cats (88%). The gastritis was most pronounced in the antral region and consisted mainly of multifocal lymphoplasmyocytic follicular infiltrates in the deep mucosa. The severity of gastritis in the antrum corresponded to high numbers of *H. pylori* there, based on use of the urease assay as an indicator of *H. pylori* colonization. Ten of 24 cats (42%) also had small to moderate numbers of eosinophils in the gastric mucosa. All twenty four cats had gastric lymphoid follicles, with follicles being most prevalent in the antrum. Electron microscopy of gastric tissue revealed numerous *H. pylori*, some of which were closely adhered to the mucosal epithelium. Human *H. pylori* gene specific primers to ureA and ureB amplified similar sized products from *H. pylori* cat isolates. Digestion of the products with restriction enzymes resulted in fragments characteristic of RFLP patterns of *H. pylori* human isolates.

Conclusion: In the domestic cat, *H. pylori* infection is associated with a lymphomucosal gastritis, consisting of lymphocytic and plasmyocytic infiltration into the lamina propria, and the organism appears to provide chronic antigenic stimulation resulting in the formation of gastric lymphoid follicles.

PROTECTIVE IMMUNISATION AGAINST HELICOBACTER SPP STIMULATES LONG TERM IMMUNITY

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Aim This study was designed to establish whether immunisation against *H. felis* provides long-term protection.

Method Specific Pathogen Free BALB/c mice were immunised oegroagistically with whole cell sonicates of *H. felis* + cholera toxin adjuvant on days 3, 6, 10 and 15. Groups of mice were challenged with *H. felis* 1, 3, 6 and 15 months after the last immunising dose. Three weeks after challenge antral biopsies were taken from the mice for detection of *H. felis* by urease reaction and histology. A small group of mice from the 15 month time point were challenged, then re-challenged several weeks later. Clearance of *H. felis* was compared with previously unchallenged immunised mice from the same time point 2 and 4 days after challenge.

Results:

<table>
<thead>
<tr>
<th>TIME AFTER IMMUNISATION</th>
<th>H. FELIS INFECTION (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMMUNISED</td>
</tr>
<tr>
<td>1 Month</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>3 Months</td>
<td>2/14 (14)</td>
</tr>
<tr>
<td>6 Months</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td>15 Months</td>
<td>0/10 (0)</td>
</tr>
</tbody>
</table>

Fifteen months after immunisation all immunised mice remained protected from *H. felis* challenge, whilst all control mice were infected.

TIME AFTER CHALLENGE

Challenged days 1 & 44

<table>
<thead>
<tr>
<th>IMMUNISED</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>4 days</td>
<td>0/5 (0)</td>
</tr>
</tbody>
</table>

Challenged day 44

<table>
<thead>
<tr>
<th>IMMUNISED</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>4 days</td>
<td>0/5 (0)</td>
</tr>
</tbody>
</table>

Immunised mice which had been challenged on 3 occasions rapidly mounted a response which totally cleared *H. felis* after 2 days. However, immunised mice which received one challenge dose took 4 days to clear *H. felis*.

Conclusions Orogastric immunisation with *H. felis* + CT induced complete protection against *H. felis* challenge for nearly a lifetime in mice. The ability of recently challenged mice to quickly clear a second *H. felis* challenge also indicates that the local mucosal immune response can very effectively prevent infection.

INABILITY OF A UREASE-NEGATIVE ISOCENIC MUTANT OF HELICOBACTER MUSTELAE TO COLONIZE THE FERRET STOMACH

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Eight ferrets (Mustela putorius fur), specific pathogen free for Helicobacter mustelae, were administered 4.5 X 10^7 CFU of either the parent strain of *H. mustelae* (NCTC 12032) (2 ferrets), the urease-negative isogenic mutant strain of *H. mustelae* (Hm10:TsKm) (4 ferrets), or the sterile culture broth as a negative control (2 ferrets). Infection status was monitored by serology and endoscopic gastric biopsy for urease activity, histopathology and culture at 3, 6, 10 and 21 weeks. All ferrets were necropsied at 25 weeks. Both negative control ferrets remained uninfected throughout the study. Both ferrets receiving the *H. mustelae* parent strain became infected after two doses of the organism as evidenced by culture, serology and urease assay. All four ferrets administered the urease-negative isogenic mutant *H. mustelae* remained uninfected throughout the study. Histopathology correlated with infection status. *H. mustelae*-infected ferrets exhibited diffuse mononuclear inflammation in the submucosal portion and the lamina propria of the gastric mucosa while uninfected ferrets showed no or minimal inflammation. These results suggest that urease activity is essential for colonization of the ferret stomach by *H. mustelae*.

Phenotypic and molecular characteristics of a "new" Helicobacter sp. isolated from gastric biopsies of dogs are described.

Ten isolates studied were 4 to 8 \( \mu \)m long spiral-shaped organisms without periplasmic fibrils and with sheathed flagella at both ends. All were catalase, oxidase and urease positive and they were positive in alkaline phosphatase, gamma-glutamyl transferase, DNAase and nitrite reductase tests. They differed from \( H. \) felis by being positive in indoxyl acetate test. They were resistant to nalidixic acid and sensitive to cephalexin.

The bacteria had a rather homogenous SDS-PAGE protein profiles of whole cell proteins and their protein profiles were clearly different from those of \( H. \) felis. DNA-DNA hybridization confirmed that the organisms were closely related and they differed from \( H. \) felis, \( H. \) pylori and \( P. \) raskii. Sequencing of 16S rRNA is in progress.

Morphologically these bacteria resemble human \"H. heilmanii\" which has not been cultured in vitro. We believe that these two bacteria are very closely related. A name Helicobacter bizzozeroi is proposed according to Bizzozero, who was one of the first scientists who found spiral organisms from dogs.

METRONIDAZOLE RESISTANT MUTANT ISOLATED FROM \( H.PyLORI \) INFECTED EUTHYMIC MICE MODEL S.Matsutomo, Y.Washizuka, Y.Matsubuki, S.Tawara, M.Kurita. New Drug Research Lab. Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan and Vanderbilt University School of Medicine, Nashville, TN, USA

Both clinical monotherapy and in vitro studies have shown that \( H. \) pylori can easily acquire resistance to antibiotics resulting in a serious issue in the treatment of \( H. \) pylori infection. The purpose of this study was to screen for resistance in \( H. \) pylori infected mice treated with different antibiotics.

[Method] \( H. \) pylori CPY802, grown in Brucella broth with 10\% fetal bovine serum at 37° C under 10%CO\(_2\), was orally infected into 4 weeks old male ICR mice. After 4 days, the mice were treated with Metronidazole (MNZ), Clarithromycin (CM) or Aminoglycosides (AMPc) for 4 days. 2 weeks after the final treatment, the mice were sacrificed and the gastric mucosa homogenated in phosphate buffered saline. 0.1ml aliquots were inoculated onto selective Brucella agar plates which were then incubated at 37° C under 10%CO\(_2\) for 4 days. The MIC of MNZ, CAM and AMPC for isolated strains from each treated groups and control mice gastric mucosa were determined. [Result]

<table>
<thead>
<tr>
<th>drug</th>
<th>dose (mg/ml)</th>
<th>MIC (( \mu )g/ml)</th>
<th>frequency</th>
<th>MIC (( \mu )g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>3.2</td>
<td>0.8</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>10</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.1</td>
<td>10</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>3.2</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>1</td>
<td>58</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>98</td>
<td>not isolated</td>
<td>not isolated</td>
</tr>
</tbody>
</table>

[Conclusion] Here, we report for the first time about a \( H. \) pylori resistant mutant isolated from mice treated with MNZ for 4 days. This result suggests that \( H. \) pylori rapidly acquires resistance to MNZ in vivo as well as in vitro and the mice model allows us to examine whether \( H. \) pylori tends to acquire resistance to drugs in vivo.

High pH is unfavourable for growth of \( H. \) felis in the rat gastric antrum

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Aim: To study the effect of decreased gastric acid production by means of surgical corpectomy (CP) or treatment with high dose of omeprazole (Omg) on the growth of \( H. \) felis in the rat gastric antrum.

Methods: In the first experiment, fifteen female Sprague-Dawley rats (SPF) were infected with \( H. \) felis. Of these, nine were subjected to macroscopically complete CP. Operated animals and controls were sacrificed 6-8 weeks after surgery. In the second experiment, twelve rats were infected with \( H. \) felis and of these, six were treated with Omg (400 \( \mu \)mol/kg/day, orally) for seven days and, together with their controls, sacrificed immediately thereafter. All stomachs were prepared for histological examination. Scoring of degree of infection was done using microscopic evaluation on a 0-5 scale.

Results: Of the nine operated rats two were excluded because of incomplete CP. In the first experiment the control animals were found to have a mean antral scoring of 4.1. In the CP rats however, the mean antral scoring was only 1.6, and 3/7 were negative for \( H. \) felis at this location. In most CP rats some remaining oxyntic glands were found, and such glands were often colonised with \( H. \) felis, even in animals devoid of \( H. \) felis in the antrum. In the second experiment the control animals had a mean antral scoring of 3.5 and the Omg treated animals a mean antral scoring of 2.0.

Conclusion: \( H. \) felis is highly adapted to its normal habitat in the mucus layer of the acid producing stomach. Removal of most of the acid secretion, irrespective of whether this is done by surgery or by pharmacological treatment, creates unfavourable growth conditions for \( H. \) felis. The most likely explanation for this is the resulting high pH in the antrum. Thus, our results may explain the decrease in antral colonisation of \( H. \) pylori seen in man during Omg treatment, as caused by an increase in gastric pH.

LONG-TERM INFECTION OF HELICOBACTER PYLORI PROMOTES GASTRIC MUCOSAL ATROPHY IN CYMOMOLUS MONKEY


It has been reported that there is close relationship between Helicobacter pylori infection and development of gastric mucosal atrophy. However, it has not been well known about the cell proliferation of gastric mucosa infected with \( H. \) pylori . Thus, the aim of this study is to clarify the change of gastric mucosa after infection with \( H. \) pylori in animal model.

METHODS: Four \( H. \) pylori -free monkeys were inoculated with \( H. \) pylori of monkey origin at a dose of 10\(^6\)CFU. Gastroscopic examination with gastric biopsy was done every 3 to 6 months after infection over 2 years. Biopsy samples were taken from both gastric antrum and body for culture and histological examination. Samples were stained by concavalin A paradox (con A) and Ki-67 (MIB-1). The ratio of con A positive zone in gastric mucosa were measured for evaluation of gastric glandular portion. The ratio of MIB-1 positive cells in gastric mucosal cells were counted for evaluation of cell proliferation and expressed as labeling index (L1%).

RESULTS: 1. \( H. \) pylori could colonize on the gastric mucosa and gave rise to gastritis. This infection and gastritis were continued over 2 years. 2. The ratio of con A positive zone increased in \( H. \) pylori infected gastric mucosa. 3. MIB-1 positive cells were detected at lower tubular portion. 4. 1.1% increased in \( H. \) pylori infected gastric mucosa.

CONCLUSION: Gastric glandular cells changed to mucous neck cells in gastric mucosa and the cell proliferation was stimulated with \( H. \) pylori infection through two years. Long term infection of \( H. \) pylori promotes gastric mucosal atrophy in Cynomolgus monkey.
EXPERIMENTAL INFECTION OF RHEUS MONKEYS WITH H. PYLORI, A. Dubois, N. Fiala, E.T. Inceci, G. Perez-Perez, M.J. Blaser, D.E. Berg. USUHS and AFRRI, Bethesda, MD; Washington University, St. Louis, MO; Vanderbilt University, Nashville, TN.

Analyses of H. pylori colonization and virulence factors require models that closely match humans in terms of anatomy, physiology and genetics. Rhesus monkeys may meet this need. We report here the successful experimental infection of these monkeys by H. pylori strains of human as well as of monkey origin, and indications of differences among H. pylori strains in species for individual hosts. Five animals that were H. pylori negative by culture and histology, and a sixth animal that was later found to have a low grade H. pylori infection, were challenged with a mixture of 2 laboratory-passaged human strains and 1 monkey strain. Two of the five H. pylori-free animals, and "monkey 6" became persistently colonized after challenge, but the other 3 monkeys were not. Arbitrarily primed PCR DNA fingerprinting showed that all strains recovered from the first two monkeys, and most of those from "monkey 6" were of monkey origin. The exceptional isolates from "monkey 6" were closely related to its resident strain, but nevertheless distinguishable by a few RAPD bands. The 3 monkeys that had resisted the initial challenge were again challenged this time with two fresh human isolates, and each became colonized. In each case, DNA fingerprinting showed that colonization had been by only 1 of the 2 human strains.

The success strain of monkeys that had previously resisted colonization by a "monkey" strain suggests that individual H. pylori strains may be better suited for certain individual hosts, and that other strains may be best suited for other hosts. In this context, the special isolates from "monkey 6" that seemed to have diverged from the resident strain may have arisen by recombination between this resident strain and the input strain(s). Those new bacterial genotype(s) that were now better adapted to this individual host would then have been selected in the gastric mucosa. In conclusion, the present results show that monkeys can, indeed, be infected by H. pylori of human origin, and suggest that these animals will be very useful for understanding how bacterial genotype and host genotype, physiology and history of past disease, all affect whether a given exposure to H. pylori results in colonization and disease.

THE REGULATION OF ACTIVATED HELPER T CELLS IN THE GASTRIC MUCOSA DURING INFECTION WITH H. PYLORI

H. pylori infection may meet this need. We report the successful infection by H. pylori strains of human as well as of monkey origin, and indications of differences among H. pylori strains in species for individual hosts. Five animals that were H. pylori negative by culture and histology, and a sixth animal that was later found to have a low grade H. pylori infection, were challenged with a mixture of 2 laboratory-passaged human strains and 1 monkey strain. Two of the five H. pylori-free animals, and "monkey 6" became persistently colonized after challenge, but the other 3 monkeys were not. Arbitrarily primed PCR DNA fingerprinting showed that all strains recovered from the first two monkeys, and most of those from "monkey 6" were of monkey origin. The exceptional isolates from "monkey 6" were closely related to its resident strain, but nevertheless distinguishable by a few RAPD bands. The 3 monkeys that had resisted the initial challenge were again challenged this time with two fresh human isolates, and each became colonized. In each case, DNA fingerprinting showed that colonization had been by only 1 of the 2 human strains.

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IDENTIFICATION AND CHARACTERIZATION OF A METALLO-PROTEASE ACTIVITY FROM HELICOBACTER PYLORI WHICH IS CAPABLE OF DEGRADING IMMUNOGLLOBULINS

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INTRODUCTION Helicobacter pylori infections persist despite the specific IgA and IgG responses generated by the host against the bacterium. One mechanism whereby H. pylori could evade the immune response would be provided by a secretion protease whose substrate specificity includes immunoglobulins, as has been described for other pathogenic gram negative bacteria.

RESULTS We have identified a protease activity in both clinical isolates and reference strains of agar-grown H. pylori. The protease activity is associated with the outer membrane fraction of the bacterium and is also secreted by the bacterium when grown in broth medium. The protease has been partially purified by ion exchange and metal chelate chromatography. The protease does not copurify with urease activity.

The protease activity was inhibited by EDTA, phosphoramidon and 1,10-phenanthroline, but not by E-64. It was thus considered to be a metalloprotease. Protease activity was stimulated in the presence of the metal ions Zn > Cu > Mg > Co. The activity had a pH optimum of 8.1 ± 0.1. The protease is shown to be inducible as it remains active even after repeated subculture. In vitro, the metalloprotease degrades both polyclonal IgG and IgA1 in addition to the chromogenic substrate, azocasein.

CONCLUSIONS Although the enzyme proteolyses IgA and IgG in vitro the possibility that other non-immunoglobulin proteins may act as natural substrates remains to be explored. This protease represents a potential virulence determinant in the pathogenesis of H. pylori induced gastrroduodenal disease as well as a means of evading the hosts immune response to the pathogen.

DEVELOPMENT OF A MOUSE MODEL OF GASTRIC COLONISATION WITH HELICOBACTER PYLORI

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Experimental studies on H. pylori (Hp) in vivo would be significantly advanced by development of a reliable small animal model of gastric colonisation. Initial studies using a cocktail of 15 freshly isolated strains of Hp administered on 3 successive days indicated that prolonged colonisation (>5 months) occurred in approximately 95% of germ free (GF) CD-1 mice, 61% of conventional (CV) mice (HSD-ICR strain) and 82% of CV mice antibiotic treated to partially decontaminate the gut. No requirement for prior acid block or immunosuppression appeared necessary. Minimal or no gastritis was seen in these animals. All mouse strains tested (approx 20) supported at least some colonisation. Similarly, most strains of Hp evaluated colonised to some degree. Subsequent and ongoing experiments aimed at identifying the necessary criteria for successful colonisation have shown that high and persisting colonisation rates (~100% for at least 6 weeks) can be reproducibly achieved with individual Hp strains in CV HSD-ICR mice as follows:

a) Oral challenge on 2 sequential days with 1ml of a 24hr broth culture containing 10^7-10^8 bacteria.

b) Tryptose soya broth is preferable to brain heart infusion broth or brocclia broth.

c) Bacteria grown for <1 month in vitro and exposed to urea did not. It is postulated to stimulate urease activity will colonise better than older cultures.

Our studies indicate that quality of the challenge inoculum appears to be more important than strain of mouse or organism in successful gastric colonisation with Hp.