EXPERIMENTAL INFECTION OF RHESUS MONKEYS WITH H. PYLORI A. Dubois, N. Fiala, E.T. Incicci, 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 specificity 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 the "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 fewRAPD 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 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.

DEVELOPMENT OF A MOUSE MODEL OF GASTRIC COLONISATION WITH HELICOBACTER PYLORI AA McCollom, J Bagshaw, C O'Malley and A McLaren Glaxo Research and Development Limited Glaxo Medicines Research Centre, Stevenage, Herts SG1 2NY

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) One 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, lead to starch 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.

THE REGULATION OF ACTIVATED HELPER T CELLS IN THE GASTRIC MUCOSA DURING INFECTION WITH H. PYLORI. H. Häberle, M. Kubin, G. Trinchieri, R. Luthra, W.K. Goulafy, R. Garafalo, S.E. Crowe, V.E. Reyes, D.Y. Graham, R. Karttunen and E.B. Ernst. Dept. of Pediatrics and Internal Medicine, University of Texas Medical Branch, Galveston, TX. The Wistar Institute, Philadelphia, PA and the Department of Gastroenterology, V.A. Medical Center, Houston TX. Recent work by recent work by Karttunen et al suggests that Th1 subset of helper T (Th) cells is preferentially increased in the stomach during gastritis. The objective of these experiments was to characterize the cytokines which may select for the Th cell response during infection with H. pylori. Gastric biopsies were collected from over 160 patients who were categorized with respect to the presence of H. pylori, gastric disease as well as their age, gender, medications and other factors. Analysis of T cell subsets in individuals infected with H. pylori and not regularly taking NSAIDS showed a marked increase in CD54R0 CD4 Th cells in the lamina propria and epithelium compared to uninfected controls. CD4 and CD45RO are normally co-expressed on activated or memory Th cells. Since this subset of T cells can be recruited by the chemokine RANTES, we measured the levels of immunoreactive RANTES in solubilized biopsies collected from the antrum of infected individuals. RANTES was significantly increased (P < 0.05, Wilcoxon Rank Sum test) in the gastric mucosa of H. pylori infected patients. As Th1 and Th2 cells are selected by IL-12 and IL-10, respectively, we determined whether H. pylori stimulates IL-10 or IL-12 production. Peripheral blood leukocytes stimulated with live or killed H. pylori released IL-12 and IL-10, however, IL-12 was found in higher levels in the gastric tissue while IL-10 levels were negligible. In addition, the interferon-γ-inducible class II MHC and invariant chain molecules were expressed by epithelial cells adjacent to activated CD4+ cells. These results suggest H. pylori stimulates induction of RANTES and IL-12 leading to the selection of activated Th1 cells that produce interferon-γ and other cytokines. In turn, these cytokines may activate local antigen presenting cells and facilitate the induction of cell-mediated immune reactions and IgG production. This model may help explain some of the changes in the gastric mucosa associated with H. pylori infection.

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

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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 secretory protein 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 stable when expressed 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 gastroduodenal disease as well as a means of evading the hosts immune response to the pathogen.
IMMUNISATION WITH H. PYLORI HEAT-SHOCK PROTEIN A (HspA) AND UREASE SUBUNIT B (UreB) AFFORDS TOTAL PROTECTION AGAINST H. FELIS INFECTION IN MICE.

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H. pylori expresses heat-shock proteins (HSPs) that share homologies with the GroES and GroEL class of proteins from other organisms. The aims of the study were: 1) to test H. pylori HspA as vaccine candidates in the H. felis mouse model; and 2) to identify a combined recombinant antigen preparation capable of inducing a level of protection in mice equivalent to that provided by whole cell preparations of H. felis.

Mice were immunised orogastrically once a week for 6 weeks with 50 μg antigen containing 5 μg cholera toxin. On week 6, the animals were challenged with 10^9 H. felis bacteria and then sacrificed 2 weeks later. Bacterial colonisation was determined by "blind" histological analysis of Giemsa-stained gastric tissue sections. Orogastic immunisation of mice with MalE fused H. pylori HspA and HspB antigens protected 80% (n=20) and 70% (n=10), respectively, from gastric Helicobacter infection. Immunisation with recombinant H. pylori urease subunit B protected 86% of mice (n=21), whilst administration of this antigen together with H. pylori MalE-HspA achieved 100% protection (n=19). In comparison, 94% of mice (n=17) that had been immunised with whole cell sonicates of H. felis were protected against H. felis infection. Analysis of antibody isotypes by ELISA suggested that the protection was mediated by a Th-2 type cell response.

In conclusion, we have demonstrated that two unrelated, yet highly conserved proteins could achieve a protective efficacy comparable to that of a whole cell Helicobacter preparation. H. pylori HspA is the first homologue of the GroES class of HSPs to have been shown to induce a protective immune response. This protein differs from other GroES homologues in that it possesses a unique nickel-binding domain at its C-terminal region. The unique character of H. pylori HspA, together with the fact that this protein can be easily purified by metal affinity chromatography, makes HspA, an ideal component of a future H. pylori subunit vaccine.

INFECTION BY HELICOBACTER PYLORI IN A MOUSE MODEL THAT MIMICS HUMAN DISEASE: PROTECTION BY ORAL VACCINATION.


The human pathogen Helicobacter pylori is associated with gastritis, peptic ulcer disease and gastric cancer. To study the pathogenesis of H. pylori infection in vivo, fresh clinical isolates of both cytotoxic (Type I) and non-cytotoxic (Type II) strains were adapted to colonize the stomach of CD1/SPF and conventional BALB/c and CD1 mice, through in vivo passages. Gastric pathology resembling the human disease was detected two months after infection. Cell infiltration and epithelial erosions were observed in infections with cytotoxic-producing strains inflammatory, while non-cytotoxic strains only caused a mild inflammatory reaction. One month after the onset of infection serum antibodies against the colonizing strain were detectable in infected mice. To assess the feasibility of developing vaccines against H. pylori, mice were immunized orally at days 0, 7, 14 and 21 with H. pylori whole cell extracts or purified antigens (urease and VacA) plus LT. At days 21,23 and 25 mice were challenged orally with 10^8 CFUs of H. pylori, and colonization was then assessed 2 weeks later. Immunization with whole cell extracts or urease protected mice from infection by both cytotoxic and non-cytotoxic bacteria. As expected, immunization with VacA gave protection only against infection by cytotoxic strains of H. pylori. Finally, genetically detoxified mutants of LT, suitable for use in humans, seem to be good adjuvants in immunizations against H. pylori. These results provide the rationale to move into human clinical trials. This mouse model will allow to better study the pathogenesis of H. pylori infection and the development of vaccines.

Vaccination is a potentially cost-effective approach to the prevention and treatment of Helicobacter pylori-induced chronic disease. Since Helicobacter pylori causes a superficial infection of the gastric epithelium, the principal mediator of effective immunity should be a UG antibody stimulated by mucosal presentation of antigen and secreted into the mucus gel. A strong rationale exists for selection of urease as a vaccine candidate. The ureA and ureB genes of H. pylori were cloned in E. coli, and the multicmeric urease aepoynzyme expressed and purified as a soluble protein of heat-stable urease (55kDa), having 12-14 particulate structure. Manufacturing, quality control, stability and toxicological tests were performed in accordance with regulatory guidelines for clinical products. Given by the oral or intragastric route (but not by the parenteral route) to mice, 4 weekly doses of > 5 μg aepoynzyme protected up to 100% of mice against challenge with virulent H. felis. Protection was antigen dose-dependent and required coadministration of a mucosal adjuvant, such as LT or an atoxic LT mutant, which was effective at doses of < 500 ng. or stimulated by mucosal presentation of antigen and secreted into the mucus and anti-urease ASC into gastric infected Balb/c mice, 4 weeks after challenge. In treated mice, the recruited IgA+ ASC were shown to be specific for the antigen and to be a determinant of response, whereas oral or intragastric immunization of untreated mice failed to develop serum protein-specific mechanism response, whereas oral immunization of untreated mice failed to develop serum protein-specific mechanism response.

The oral or intragastric route of administration of a urease vaccine urease would mimic the oral or intragastric route of action of a urease vaccine urease in humans. The vaccine was administered orally in mice. Immunized mice became infected with a urease vaccine urease and failed to develop serum IgG in response. The IgA+ ASC responses were shown to be specific for the antigen and to be a determinant of response, whereas oral or intragastric immunization of untreated mice failed to develop serum protein-specific mechanism response.

HELICOBACTER PYLORI INDUCED GASTRITIS IN THE DOMESTIC CAT. J.G. Fox, M. Batchelder, R.P. Marin, L. Yan, L. Handt, X. Li, B. Shames, A. Hayward, J. Campbell, J.C. Murphy. Division of Comparative Medicine, MIT, Cambridge, MA, USA

Helicobacter pylori has been cultured from the inflamed gastric mucosa of naturally infected cats, the lesions in the H. pylori infected cat stomach mimic many of the features seen in humans. To determine whether H. pylori negative specific pathogen free cats with normal gastric mucosa were susceptible to colonization and whether gastritis developed after infections, four H. pylori negative cats treated with cimetidine were orally dosed 3 times with 3 ml (1.5x10⁸ CFU/ml) of H. pylori on alternate days. All 4 cats became persistently colonized as determined by gastric culture and PCR during serial gastric biopsies and necropsy at 7 months p.i. The two control cats did not have H. pylori isolated, nor was gastric tissue positive by PCR, one cat had a few foci lymphocytic aggregates in the body submucosa, whereas the second cat had normal gastric mucosa. All 4 H. pylori infected cats had multifocal gastritis, consisting of lymphoid aggregates plus multiple large lymphoid nodules, which were most noticeable in the antral mucosa. In addition, one H. pylori infected cat had a moderate diffuse infiltration of polymorphonuclear leukocytes in the antral region. H. pylori like organisms were focally distributed in glandular crypts of the antrum. H. pylori infected cats had an increase in IgG H. pylori serum antibody over baseline. The H. pylori isolated from the 4 experimentally infected cats had identical RFLP patterns specific for the fla A gene to that of the inoculating strain. H. pylori readily colonizes the cat stomach and produces a persistent gastritis.


Three diagnostic techniques for detecting H. pylori were employed in four large, well-controlled, treatment studies. Clinical Cultures (Cx), histology (Hx), and 13C-urea breath (UBT) tests were used to assess H. pylori status in multicenter US and European studies conducted in 13 countries in patients with duodenal ulcers and Hp infection. The pretreatment and 4-6 week follow-up results are displayed below:

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>4-6 Week Follow-Up</th>
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<tbody>
<tr>
<td>UBT</td>
<td>History</td>
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<tr>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>neg</td>
<td>47</td>
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<tr>
<td>pos</td>
<td>77</td>
</tr>
<tr>
<td>total</td>
<td>124</td>
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The sensitivities for UBT and Cx using Hx as the gold standard were 91% and 83% at pretreatment and 99% and 82% at 4-6 weeks respectively.

The US and European results were similar when comparing Cx versus Hx; however, the UBT was considered more sensitive in the European studies than in the US (98% compared to 86% in the US pretreatment).

The results from these studies suggest Hx was the most sensitive test for detecting H. pylori. Cx is useful for verifying positive results but is limited because of the number of false-negatives in the US studies. The UBT provides useful information in the European studies but a number of false-negatives in the US studies indicate for standardization.

SALIVARY SPECIFIC IgG IN THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION IN DYSPEPTIC PATIENTS. F. Luzz, M. Maletta, M. Imenco, L. Biancone & F. Pallone. Dept Medicine Experimental, University of R. Calabria, Catanzaro, Italy

Background & Aim: Specific H. pylori IgG and IgA antibodies have been shown in the saliva of H. pylori infected patients. The aim of our study was to assess the accuracy of salivary diagnosis of H. pylori infection and to compare the performance of salivary specific IgG and IgA in the diagnosis of H. pylori infection. Methods: A total of 152 dyspeptic patients who underwent gastroscopy were available for the study: 67 had duodenal ulcer (DU), 85 had no lesion (non-DU). Five salivary and oral biopsies were taken. Patients were classified as H. pylori positive when the urease test and/or histology (Giemsa staining) were positive for H. pylori. Serum and unstimulated saliva were collected from each patient before endoscopy and assayed for H. pylori IgG and IgA by an in-house ELISA using a crude H. pylori sonicate as antigen. Working dilution were 1:100 for sera and 1:4 for saliva. Results were expressed as mean ODs/SD. Results: H. pylori was found by histology in 131 (86%) patients: 65/67 DU (96%) and 65/85 (76%) non-DU patients, respectively (OR:20.9, p=0.0002). As expected serum H. pylori IgG were significantly higher in patients who were positive for H. pylori than in those who were negative (1.10x10⁸±0.316 vs 0.437±0.176, p=0.01). Salivary H. pylori IgG were higher in H. pylori positive than negative patients (0.80±0.908 vs 0.307±0.305, p=0.01). Serum H. pylori IgA were also higher in H. pylori positive patients (0.67±0.436 vs 0.40±0.23, p=0.01). The sensitivity and specificity of salivary H. pylori IgG were 82% and 71% with positive and negative predictive values of 95% and 49%, and the accuracy 81%. The corresponding figures for serum H. pylori IgG were 67% and 91%, 38% and 95%, and those for serum H. pylori IgA were 80% and 52%, 91% and 30%, and 76%. The sensitivity of salivary H. pylori IgG and IgA detecting DU was 83% (65/78), that of serum H. pylori IgG 97% (65/67) (OR:15, Cl:0.02-0.8, p=0.02). Conclusion: Salivary H. pylori IgG was a fairly sensitive and accurate indicator of gastric H. pylori colonization with a high positive predictive value in our population. Data, however, suggest that salivary H. pylori IgG measurement do not compare favourably with serology and do not encourage at present the use of salivary H. pylori IgG in screening dyspepsia.