EXPERIMENTAL INFECTION OF HUMAN MONKEYS WITH H. PYLORI: A MODEL OF GASTRIC COLONIZATION AND DISEASE

A.  EXPERIMENTAL

INTRODUCTION

Helicobacter pylori is the most common bacterial pathogen in the human gastrointestinal tract. Infection with this bacterium is associated with a wide range of gastrointestinal conditions, including chronic gastritis, gastric ulcer, and gastric cancer. The development of H. pylori colonization is a complex process that involves various factors, including the host immune response and the bacterial virulence factors. In this study, we investigated the colonization of human monkeys with H. pylori and evaluated the success of colonization and disease outcomes.

METHODS

A total of 100 human monkeys were challenged with H. pylori in a controlled environment. The monkeys were divided into two groups: group A and group B. Group A monkeys were infected with a high dose of H. pylori bacteria, while group B monkeys were infected with a low dose. The colonization process was monitored by periodic endoscopy and biopsy sampling. The colonization status of each monkey was determined by histological examination of the biopsy samples.

RESULTS

In group A, 70% of the monkeys became colonized with H. pylori, whereas in group B, 40% of the monkeys became colonized. The colonization was persistent in all infected monkeys, as confirmed by histological examination and DNA fingerprinting. A bacterial strain that was isolated from the initial colonization was used to infect a second group of 20 monkeys. All of these monkeys became colonized with H. pylori, indicating a successful colonization.

CONCLUSIONS

The results of this study demonstrate that human monkeys can be successfully colonized with H. pylori, providing a valuable model for studying the colonization process and related diseases. This model may be useful for developing new therapeutic strategies against H. pylori infection and for understanding the role of the host immune response in the colonization process.

DEVELOPMENT OF A MOUSE MODEL OF GASTRIC COLONIZATION WITH HELICOBACTER PYLORI

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GASTRIC MUCOUS MEMBRANE PROTEASE ACTIVITY FROM HELICOBACTER PYLORI WHICH IS IDENTICAL TO THE PROTEASE OF H. PYLORI: A MODEL OF GASTRIC COLONIZATION AND DISEASE

H. pylori infection is associated with various gastrointestinal conditions, including chronic gastritis, gastric ulcer, and gastric cancer. The colonization process involves the mucosal lining of the stomach, and the ability of H. pylori to colonize the stomach is a crucial factor in the development of these conditions. In this study, we investigated the protease activity of H. pylori and its role in gastric colonization.

METHODS

A total of 100 human monkeys were infected with H. pylori in a controlled environment. The colonization process was monitored by periodic endoscopy and biopsy sampling. The colonization status of each monkey was determined by histological examination of the biopsy samples.

RESULTS

In all infected monkeys, the mucosal lining of the stomach was colonized with H. pylori. The colonization was persistent in all infected monkeys, as confirmed by histological examination. A bacterial strain that was isolated from the initial colonization was used to infect a second group of 20 monkeys. All of these monkeys became colonized with H. pylori, indicating a successful colonization.

CONCLUSIONS

The results of this study demonstrate that human monkeys can be successfully colonized with H. pylori, providing a valuable model for studying the colonization process and related diseases. This model may be useful for developing new therapeutic strategies against H. pylori infection and for understanding the role of the host immune response in the colonization process.
ARE AUTOIMMUNITY AND ENDOXIN SENSITIVITY PART OF THE SPECTRUM OF HELICOBACTER-INDUCED DISEASE?


Aim: Previously we have shown that the severity of gastritis induced following long term infection with Helicobacter felis is dependent on the strain of mouse utilised. The goal of the current study was to identify host factors responsible for these differences. The availability of the C3H/He strain of mice, which is unresponsive to LPS, allowed investigation of the role of sensitivity to endotoxin. These animals were compared with the parent strain and other mouse strains.

Methods: SPF C3H/He mice (Non LPS responders), the congenic C3H/He mice (LPS responders), SJL and CBA mice were infected with H. felis. At 2 & 6 months post infection gastric pathology was assessed using a standard three point scale for activity (Act) and atrophy (Atr) and bacterial density (Bact). Uninfected mice of the same age were included as controls.

Results: The main findings were i) severe atrophy in the body of C3H/He and SJL mice yet H. felis only colonises the antrum, ii) loss of bacteria in both the body and antrum of mouse strains demonstrating atrophy, iii) loss of sensitivity to lipopolysaccharide in C3H/He mice resulting in virtually no helicobacter-associated gastritis despite good colonisation. Conclusion: Bacterial endotoxin (LPS) plays a major role in the induction of destructive Helicobacter-induced gastritis. Atrrophic gastritis with destruction of parietal cells in some strains of mice may result from the development of autoimmunity.

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 Hsps 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 orally once a week for 4 wks with 50 μg antigen and 50 μg cholera toxin. On week 6, the animals were challenged once with 10^8 H. felis bacteria and then sacrificed 2 wks 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. pylori 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 IMICS 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 cytotoxin-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 and 14 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 CFU 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.
A COMPARISON OF CULTURE, HISTOLOGY, AND 13C-
UREA BREATH TESTS FOR DETECTION OF H. PYLORI.
H. Stephens, H. McIlhenny, S. G. Olson. Abbott Laboratories, Abbott Park, IL, USA.
Three diagnostic techniques for detecting H. pylori were employed in four large, well-controlled, treatment studies. 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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<tr>
<td>History</td>
<td>UBT</td>
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<td>neg</td>
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<tr>
<td>negative</td>
<td>47</td>
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<td>positive</td>
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The sensitivities for UBT and Cx using Hx as the gold standard were 91% and 83% at pretreatment and 89% and 62% at 4-6 weeks respectively.

The US and European results were similar when comparing Cx versus Hx; however, the UBT was considerably more sensitive in the European studies than in the US (98% compared to 89% 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 indicating the need for standardization.

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 the disease should be a IgA 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 Helicobacter were cloned in E. coli, and the multimeric urease apoenzyme expressed and purified as a soluble protein of the hexameric urease (550kDa) having 12 nm 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 apoenzyme 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 atonic LT mutant, which was effective at doses of ≤ 500 ng.

Neglected: 657

SALIVARY SPECIFIC IGG IN THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION IN DYSPEPTIC PATIENTS. F. Luzz, M. Maletta, M. Immeno, L. Blancone & F. Palleone. Dept Medicina Sperimentale, University of R. Calabria, Catenanuova, 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 antral and body biopsies were taken. Patients were classified as H. pylori positive when the urease quick test and histology (Giemsma staining) were positive for H. pylori. Serum and unstimulated saliva were collected from each patient before endoscopy and assayed for H. pylori IgG 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 OD±SD.

Results: H. pylori was found by histology in 131 (86%) patients: 66/67 DU (98%) and 65/85 (76%) non-DU patients, respectively (OR=20, p=0002). As expected serum H. pylori IgG were significantly higher in patients who were positive for H. pylori than in those who were negative (1.100±0.316 vs 0.437±0.176, p<0.01). Salivary H. pylori IgG were higher in H. pylori positive than negative patients (0.800±0.906 vs 0.307±0.305, p<0.01). Serum H. pylori IgA were also raised in H. pylori positive patients (0.670±0.436 vs 0.401±0.2.37, 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 45%, and the accuracy 81%. The corresponding figures for serum H. pylori IgA were 67% and 91%, 56% and 93%, and 89%, those for serum H. pylori IgG were 80% and 52%, 91% and 30%, and 76%. The sensitivity of salivary H. pylori IgA in 4-weekly detecting DU was 83% (65/79), that of serum H. pylori IgG 97% (65/67) (OR=15, CI:0.02-0.8, p<0.02). Conclusions: 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.

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 (1.5x10^10 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 focal lymphocytic aggregates in the body submucosa, whereas the second cat had normal gastric mucosa. All 10 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 body of the antrum. 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.