1B: Immunological aspects leading to vaccine against *H. pylori*

### 1B:01 ORAL IMMUNIZATION WITH RECOMBINANT UREA SEPECIFIC ANTIGENS IN CHILDREN INFECTED WITH *H. pylori*

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Oral immunization with *H. pylori* urease induces the cure of *Helicobacter* infection in animal models, when administered with an adjuvant. As a first step to use therapeutic immunization in humans, we studied the safety and the immunogenicity of recombinant, enzymatically inactive urease in *H. pylori*-infected adults. Methods: 12 healthy adults (21-39 yrs.) with documented asymptomatic *H. pylori* infection, participated in a double-blind, placebo-controlled phase 1 study and were randomized to receive 60 mg recombinant urease po or placebo once weekly for 4 weeks. Clinical examination and safety laboratory tests were performed at regular intervals. Gastric biopsies obtained at baseline and one month after the last dose were scored for inflammation, mucosal damage, and *H. pylori* density according to a modified Sydney system and were analysed by immunocytchemistry. Urease-specific antibody-secreting cells (ASC) were measured in peripheral blood by ELISPOT and antibodies to urease were determined in saliva and serum. Results: The administration of urease was well tolerated and no serious adverse events occurred. No clinically relevant abnormalities in blood count or chemistry were observed. All baseline gastrocopies were normal except for antral non-erosive gastritis. At the end of the study, isolated erosions were noted in 2 volunteers having received placebo and in 3 having received urease. No change in the total gastritis score for corpus and antrum was observed. All subjects remained infected throughout the study (14C-urea breath test, culture, and histology). ASC were found in gastric mucosa of most subjects and there was no evidence that immunization with urease enhanced the local antibody response. No urease-specific ASC were detected in peripheral blood throughout the study. Antibodies to urease were detected in saliva and serum of all subjects. Serum IgG and IgA anti-urease titers remained unchanged in all volunteers. One of six subjects having received the test product developed a borderline increase (3.4 fold) in salivary anti-urease IgG. No change in salivary anti-urease IgG was noted in other volunteers. Conclusion: The administration of recombinant urease in *H. pylori* infected, asymptomatic adults is well tolerated. As expected, urease is not immunogenic in humans in the absence of an adjuvant. The safety of oral immunization with urease and an adjuvant is currently being tested in humans. (Supported by SNF 32-34369.92 and by a grant from Oravax, Inc. Cambridge, MA)

### 1B:02 IMMUNE RESPONSE TO *HELICOBACTER PYLORI* ANTIGENS IN INFECTED CHILDREN AND ADULTS

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*H. pylori* is clearly associated with gastritis, ulcers and gastric cancer. Few studies have investigated the immune response to the natural infection in children. The aim of this work was to study the serum IgA and IgG antibody response to different antigens of *H. pylori* in children infected with *H. pylori* and with chronic abdominal pain (CAP) and compared with the response observed in infected adults with duodenal ulcers (DU). Infection was assessed by biopsy, culture, and histology in all cases. Thirty one infected children, mean age 10.3 ± 4.1, and 35 infected adults, mean age 48 ± 17.5, were studied. ELISA was performed using as antigens: a pool of sonicated whole cells from three Mexican strains, a recombinant urease and a recombinant CagA protein (kindly supplied by Oravax, Inc.); all three assays were validated with known negative and positive sera to establish cut off values. Seropositivity in children with CAP, and adults with DU, respectively, were as follow: 64.5%, and 100% for IgG sonicated extract; 9.7%, and 53.6% for IgG urease; 25.8%, and 71.4% for IgA urease; and 48.4%, and 88.6% for IgG CagA. Among seropositive patients, adults had a significantly stronger response for IgG sonicate and IgA urease (mean of 7.0 and 3.3 ELISA units, respectively) than children (mean of 4.3 and 1.7 units, respectively). However, children had a significantly stronger response for IgG CagA (mean of 6.4 units) than adults (mean of 4.3 units). CagA+ strains seem to be more frequent in DU adults than in CAP children. The response to urease is inconsistent in both children and adults infected with *H. pylori*.

### 1B:03 THE SYDNEY STRAIN OF *H. pylori*: A NEW STANDARD FOR VACCINE STUDIES IN MICE?

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**Aim:** A number of *H. pylori* strains capable of colonising mice have now been isolated, however these have not been well characterised and some strains show poor colonisation. Clearly there is a need for a better *H. pylori* mouse model. The new ‘Sydney strain’ isolated by our group has been shown to colonise mice with high infection levels, specific adhesion to gastric epithelial cells and pathology similar to that seen in humans. This study investigates the potential of this new, well characterised model as a tool for protective and therapeutic immunisation studies.

**Method:** Mice: SPF BALB/c. Vaccine: whole cell sonicate of *H. pylori* (1 mg/dose) with cholera toxin (CT) (10 µg/dose). Infection/Challenge: *H. pylori* (SS1) in liquid culture, 3 doses over 5 days (≈ 10^7 organisms/dose). Protective immunisation: mice were immunised (IG) on days 0, 7, 14, and 21. 3 weeks post immunisation, animals were challenged with SS1. After a further 3 weeks infection was assessed. Therapeutic immunisation: mice were infected with SS1 for 2 months and then immunised (IG) on days 1, 15, 17 & 20. 1 month post immunisation colonisation levels were assessed.

**Results:**

<table>
<thead>
<tr>
<th>Immunisation</th>
<th>Vaccine</th>
<th>% <em>H. pylori</em> infection (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urease assay</td>
<td>Histology</td>
</tr>
<tr>
<td>Protective</td>
<td><em>H. pylori</em> + CT</td>
<td>10% (1/10)</td>
</tr>
<tr>
<td>Protective</td>
<td>Saline</td>
<td>85% (8/10)</td>
</tr>
<tr>
<td>Therapeutic</td>
<td><em>H. pylori</em></td>
<td>57% (9/24)</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>Saline</td>
<td>88% (22/25)**</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.001 (Chi-square test)

**Conclusion:** Both protective and therapeutic immunisation were successful against *H. pylori* SS1, a strain known to colonise mice better than any other reported to date. This strain of *H. pylori* is now available to all those involved in vaccine studies. Hopefully its use will help standardise results between groups and facilitate selection of the final human vaccine.

### 1B:04 TOPICAL DUODENAL AND GASTRIC MUCOSAL PRODUCTION OF INTERLEUKIN-1B, INTERLEUKIN-6, INTERLEUKIN-8, TUMOR NECROSIS FACTOR ALFA AND INTERLEUKIN-2-SOLUBLE RECEPTOR IN *HELICOBACTER PYLORI* POSITIVE AND NEGATIVE PATIENTS. A PILOT STUDY

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**Purpose of the study** was to evaluate topical differences of mucosal cytokine production in *H. pylori* (HP) positive and negative patients. **Methods.** Seventeen patients [6 men, 11 women, aged 21-74] entered the study. Five biopsy specimens for in vitro culture were taken from each person during routine gastrosopy: from duodenal bulb [DB], distal antrum, proximal gastric corpus and fundus [GF]. HP positive status (7 patients) had both histology and CLO-testing positive, and vice versa in HP negative one (10 patients). Biopsy specimens were cultivated in RPMI medium for 24 hours. Cytokines were measured in homogenate supernatants by means of "sandwich" ELIA using Quantikine kits (R&D Systems): interleukin-1β [IL-1β], interleukin-6 [IL 6], interleukin-8 [IL-8], tumor necrosis factor-α [TNF-α] and interleukin-2-soluble receptor [sIL-2R]. Data were statistically treated (*-test, Mann Whitney, Student Newman-Keuls tests, PM ANOVA and Pearson Correlation) using Jandel Scientific.

**Results** are given as median (in pg/ml, except sIL-2R in pm, *significant = p < 0.015*).

There was a correlation between antral IL-1β and IL-6 (p = 0.0001), IL-1β and TNF-α (p = 0.0001), IL-8 and IL-8 (p = 0.0076). There was a correlation between fudnal IL-6 and IL-8 (p = 0.0009). Duodenal sIL-2R production was significantly higher than antral one (p = 0.0038).
Conclusions. A great deviation of values (both personal and topical difference) suggests the significance of several factors (including HP) influencing the consequent local inflammatory reaction.

1B:05 HELICOBACTER PYLORI (Hp) CagA Protein induces a lymphocyte proliferative response in infected subjects

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T lymphocytes play a pivotal role in producing chemotactic factors, activating cytokines, driving a healing reaction and, overall, in determining the outcome of Hp infection. Aim of our study was to investigate if the lymphocytes obtained from peripheral blood (PBL) and those from gastric vein blood (GVBL) were able to proliferate to Hp CagA protein and if this assay had any specificity in Hp infected patients. Patients supposed to undergo abdominal surgery (not for neoplastic or gastric diseases) underwent a thorough study for Hp infection. During the surgical intervention blood samples were obtained by puncture of the gastric draining veins and the antecubital vein. Lymphocytes were purified and cultured in the presence of several mitogenic stimuli (anti-CD3, anti-CD28, PHA, HA CagA protein) and the proliferative response was measured by means of triitated thymidine uptake. Hp CagA protein induced lymphocyte proliferative response in a high percentage of Hp infected subjects (71.5% GVBL and 57% PBL) while it did not in the Hp negative patients. Comparing the Hp positive and negative groups the mean lymphocyte proliferative response to CagA was significantly higher in the GVBL (p < 0.05) while no difference was detected for the other mitogenic stimuli. Analyzing the patients as a whole, GVBL showed a significantly higher response to PHA than PBL. It is known that the Hp CagA protein can induce a humoral immune response. We have demonstrated that T lymphocytes from Hp infected subjects specifically proliferate in response to this antigen. Furthermore we detected a different functional behaviour of GVBL compared to PBL.

1B:06 platelet enzyme activities before and after H. pylori infection in asymptomatic volunteers

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Platelet cytochrome c oxidase (CCO) and superoxide dismutase (SOD) activities are copper status and antioxidant markers. CCO (nmol/min/mg protein) and SOD (units/min/mg protein) activities were measured in washed platelets taken from asymptomatic volunteers before and after eradication of H. pylori, if present (as assessed by carbon-13 urea breath test, BSIA, UK). Results are shown in the Table.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>H. pylori n</th>
<th>Eradication Therapy</th>
<th>P Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCO</td>
<td>Positive</td>
<td>8 5.7 (5.0-6.5)</td>
<td>9.3 (5.9-12.8) 0.02</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>7 7.8 (6.7-9.2)</td>
<td>8.3 (7.7-9.0) NS</td>
<td>0.07</td>
</tr>
<tr>
<td>SOD</td>
<td>Positive</td>
<td>6 65 (35-95)</td>
<td>225 (88-362) 0.04</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>7 244 (210-279)</td>
<td>212 (187-237) NS</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Volunteers with H. pylori infection had decreased CCO and SOD activities which normalized on treatment. This indicates that the decreased copper and antioxidant status associated with H. pylori infection can be corrected by successful eradication.

1B:07 Mucosal interleukin-8, platelet-activating factor, endothelin-1, leukotriene B4 and leukotriene C4 production in patients with Helicobacter Pylori infection

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H. pylori synthesizes or causes very different cells to release a variety of inflammatory mediators (IM) which have been involved in pathophysiology of chronic gastric inflammation. We investigated whether interleukin-8 (IL-8), platelet-activating factor (PAF), endothelin-1 (ET-1), and leukotrienes B4 and C4 (LTB4 and LTC4) are involved in the inflammatory reaction of H. pylori infection. Also, we investigated the effect of triple therapy on eradication of H. pylori and IM production. In 16 duodenal ulcer (DU) patients with H. pylori infection and 15 patients with negative urease test, serology and normal antral mucusa, the mucosal production of IL-8, PAF, ET-1, LTB4, and LTC4 was measured in antral biopsy specimens after incubation in special conditions. The levels of IM appeared to be significantly higher in H. pylori-infected patients (p < 0.05). IL-8, PAF, ET-1, LTB4, and LTC4 production was significantly decreased one month after eradication therapy (CBS 120 x 4 x 14, AMO 500 x 4 x 14, MET 250 x 4 x 14, eradication rate <90%). A strong correlation were found between the production of IL-8 and PAF, PAF and LTB4, ET-1 and LTC4, correspondingly. The biological effects of these IM may explain recruitment, influx, and activation inflammatory cells in the gastric mucusa during H. pylori infection. Release of PAF and ET-1 might lead to occlusion in the microcirculation and affect epithelial integrity by ischaemic damage.

1B:08 Increased levels of soluble tumour necrosis factor receptor I (sTNF RI) in serum of Helicobacter pylori-positive ischemic heart disease patients

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Recent data suggest that Helicobacter pylori (Hp) infection is an independent risk factor for Ischemic Heart Disease (IHD; Mendall et al; BHJ 1994; Patel et al, BMJ 1995). In this context, a possible role of a chronic systemic inflammatory response has been suggested, possibly mediated by cytokines such as TNF. This cytokine has been proposed as the physiologic stimulus for the release of its soluble receptors (sTNF RI and sTNF RI); in addition, sTNF RI has been demonstrated to circulate during experimental and clinical inflammation. Moreover, Hp induces the release and the expression of TNF in the gastric mucusa of infected patients (Moss et al, Gut 1994). Aim of the present study was to investigate the potential mechanisms of this association by evaluating serum concentrations of sTNF RI in Hp positive and Hp negative patients with IHD in comparison to a control population. Methods: 29 male patients with IHD scheduled for coronary by-pass surgery (mean age 61-65, range 38-85) were age and sex matched to a control population of 11 subjects without IHD (mean age 46 ± 8 range 40-65) after controlling for other risk factors (smoke, diabetes, hypertension, serum cholesterol). In patients and subjects the presence of Hp infection was assessed by means of serum anti-Hp IgG and 125I urea breath test and considered Hp negative when both tests were negative. sTNF RI levels were assayed by ELISA using a commercial kit. Results: Data are shown in the following table:

<table>
<thead>
<tr>
<th>H. pylori positive</th>
<th>H. pylori negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHD</td>
<td>1877 + 138 (n = 19)</td>
<td>1274 + 125 (n = 10)</td>
</tr>
<tr>
<td>Controls</td>
<td>1196 + 152 (n = 5)</td>
<td>961 + 74 (n = 6)</td>
</tr>
</tbody>
</table>

*p < 0.01 vs controls; t-test; p < 0.01 vs H. pylori negative IHD and controls (ANOVA with Tukey’s test).

Summary: Hp infection is associated to a systemic release of the RI soluble receptor for TNF. IHD patients showed significantly increased serum levels of sTNF RI with respect to controls. sTNF RI levels were significantly increased in Hp positive IHD patients with respect to Hp negative IHD patients and both control groups. Conclusion: Our data suggest that the previously observed association between Hp infection and IHD might be explained through a TNF-mediated inflammatory mechanism elicited by chronic bacterial infection.
Introduction: It has been described that Helicobacter pylori (Hp) positive patients have reduced gastric histamine concentrations compared to Hp negative controls. It was proposed that this was due to an increased liberation of histamine from mast cells in the Hp infected gastric mucosa.

Materials and methods: Sixty consecutive patients were included in the study. Thirty three were Hp + assessed by culture. The 33 strains were isolated and bacterial sonic extracts were made. Basophil histamine release was determined spectrophotometrically and expressed in percent of total histamine content. A release less than 10% was considered to be negative.

Results: When stimulating basophils from the 33 Hp + patients the mean peak histamine release was (13 ± 1)% when stimulating with the homologous strain, compared to (6 ± 1)% with the heterologous strain. Comparing the Hp + vs Hp − when stimulating basophil histamine release with the homologous strain mean peaks were (6 ± 1)% vs (5 ± 1)%.

Conclusions: There is a type 1 hypersensitivity reaction against homologous Hp strain. The basophil histamine release was significantly higher when stimulating with the homologous strain than the heterologous strain (p < 0.0001). The histamine release was IgE-mediated. There were no differences in histamine release between the Hp + and Hp − group of patients when stimulating with the heterologous strain.

A11

CHEMIULUMINESCENT IMAGING OF INDUCIBLE NITRIC OXIDE SYNTHASE IN THE GASTRIC EPITHELIUM IN H. PYLORI INFECTION AND GASTRIC CANCER

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Purpose. Increases in inducible nitric oxide synthase (iNOS) are associated with intestinal inflammatory conditions. Products of NO, such as peroxynitrite, may be important mediators of mucosal damage but NO may also contribute to mucosal defence. This study investigates quantitatively iNOS protein and tyrosine nitrosylated proteins in the gastric epithelium of patients with chronic gastritis and gastric cancer by immunochemiluminescence.

Methods. Cryosections of antral biopsies (n = 23) were incubated with rabbit anti-iNOS antibody, normal rabbit serum and anti-nitrotyrosine monoclonal antibody. Bound antibodies were detected using a low light imaging lumigrafollowing incubation with peroxidase and phosphatase labelled second antibodies and substrate.

Results. 14/18 (78%) Hp + patients with chronic gastritis, 1/5 (20%) Hp − with normal histology and 7/9 (78%) of the cancer patients had a positive chemiluminescent signal for iNOS in the epithelium. No signal was detected in epithelial cells with control rabbit sera. Median (IQR) iNOS levels were respectively 42.6 (10.8–66.4) photons/sec/unit area (Hp +), 0 (0–22.1) (Hp −; p < 0.05 versus Hp +) and 32.1 (11–64) in cancer patients. In Hp + patients 7/10 CagA seronegatives and 7/10 CagA seropositives were iNOS positive. Median iNOS values were 48.1 (21.7–72.9) (CagA neg) and 41.3 (0–63) (CagA pos). Focal areas of nitrotyrosine staining in epithelial cells were observed in 3/18 Hp +, 29 cancer patients but in no Hp − patients.

Conclusions: Sensitive chemiluminescence techniques can quantitatively detect iNOS in gastric epithelial cells. Infection with CagA pos and CagA neg Hp pylori strains is associated with increased iNOS in the epithelium which in some cases results in peroxynitrite formation.

A12

ACTIVATION OF T CELL SUBSETS IN THE PERIPHERAL BLOOD AND THE GASTRIC MUCOSA IN H. PYLORI (HP) INFECTION


In this study we investigated the activation of mucosal T lymphocytes in Hp infection in comparison to lymphocytes of uninfected mucosa. To determine the influence of Hp antigens on the activation of peripheral mucosa and mucosa-associated mononuclear cells (PBMC/MAMC), isolated lymphocyte populations were stimulated with Hp antigens in vitro. Methods: PBMC of Hp positive blood donors were isolated by ficoll density gradient. MAMC were isolated from macroscopically unaffected antral mucosa of gastric carcinoma patients who underwent gastric resection. MAMC and HP stimulated MAMC were characterised by three colour flow cytometry. Hp specific activation was determined by proliferation tests. Results: More CD3+ mucosal lymphocytes of Hp+ patients were CD4+ (CD4/CD8 ratio: Hp + 1.2, Hp − 0.3) and expressed CD25 marker in a higher percentage compared to Hp− patients (Hp+ 8.5%, Hp− 3.3%). CD25 was expressed by CD4+ as well as CD8+ cells but the CD4/CD8 ratio of activated cells showed a shift in favour of CD8+ T cells (Hp− 4.5, Hp+ 2.6). No differences were found between the two patient groups concerning the expression of HLADR. PBMC as well as MAMC of Hp+ patients proliferated well after stimulation with PHA, tuberkulin, and IL-2. MAMC of only two out of six patients proliferated after Hp stimulation. In contrast PBMC in five out of six patients proliferated after Hp stimulation. Both CD4+ and CD8+ T cells in PBMC expressed activation markers after antigen stimulation (table, median %). Conclusion: The finding of a poor proliferation response of MAMC indicates differences in responsiveness of peripheral and mucosal T cells. Although more mucosal CD4+ T cells in Hp+ were observed, both CD4+ and CD8+ T cells were activated. There was even an increase in the relative number of activated CD8+ T cells.
1B:13  H. PYLORI-SPECIFIC TH1 EFFECTOR CELLS IN THE GASTRIC ANTRUM OF PATIENTS WITH PEPTIC ULCER DISEASE

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Infection of the gastric antrum by H. pylori (Hp) is characterized by a cellular inflammatory infiltrate which is thought to play a role in the pathogenesis of peptic ulcer. The nature of cytokines produced during the immune response to Hp may represent a host-dependent factor able to influence the outcome of the infection. To analyze the pattern of cytokines produced by the immunologically active cells within the gastric antrum, cytokine mRNA expression was studied in antral biopsies from 5 Hp-infected patients with duodenal ulcer and 3 Hp-negative dyspeptic controls. T-cell clones were also generated from parallel samples of antral mucosa of the same Hp-infected patients and assessed for their reactivity to Hp antigens, profile of cytokine secretion, and effector functions. Antigenic biopsies from all Hp-infected patients showed IFN-γ, TNF-α, IL-12, but not IL-4, mRNA expression, whereas no cytokine mRNA signal was found in the mucosa of 3 Hp-negative controls. When assayed for their responsiveness to a Hp lysate, 24 out of the 163 CD4+ T-cell clones (15%) derived from Hp-infected patients proliferated in response to Hp lysate. Eleven clones (46%) reacted with Cag-A, 2 with Vac-A, and 1 with Urease.

Upon Hp antigen stimulation, the great majority of Hp-reactive clones (2B1) produced IFN-γ, TNF-α, but not IL-4 or IL-5 (Th1-like), whereas prolifera-
ted both IFN-γ, and IL-4 and IL-5 (Th0-like). In addition, all Hp-specific clones secreted high amounts of TNF-α. At low T/B cell ratio, Hp-specific clones expressed antigen-dependent helper function for B-cell proliferation and immunoglobulin production, whereas at higher T/B cell ratio, 15 Th1 and 2 Th0 clones lysed antigen-pulsed autologous E. coli-transformed B cells.

These results demonstrate the presence of Hp-specific Th1 effector cells in the gastric antral mucosa of Hp-infected patients. These cells may play a role in the pathogenesis of both peptic ulcer and gastric B-cell lymphoma associated with Hp infection.

1B:14  VACCINATION OF GNOTOBOTIC PIGLETS AGAINST H. PYLORI

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Parenteral vaccination with bacterial antigen fails to protect piglets against challenge with H. pylori, but results in increased severity of gastritis. In contrast, oral vaccination with bacterial antigen and choler toxin protects mice against challenge with H. felis. To determine the effect of oral adjuvant in piglets, we vaccinated piglets parenterally and orally and evaluated their response to oral challenge with live H. pylori.

Four vaccination regimens were used: Subcutaneous vaccination with bacterial antigen in Freund’s adjuvant; oral vaccination with bacterial antigen and cholera toxin; oral vaccination with bacterial antigen and E. coli labile toxin (LT); and oral administration of sterile brucella broth (unvaccinated controls). Piglets were vaccinated 3 times at weekly intervals, challenged with live H. pylori 1 week after the last vaccination, and killed 1 or 2 weeks after challenge. Bacterial colonization was quantitated, tissues were examined histologically, and serum and gastric secretions were collected for antibody determination.

All groups of piglets became infected with H. pylori. Groups vaccinated either parenterally or orally with LT had lower levels of colonization than unvaccinated controls or piglets vaccinated without adjuvant, but the rate of colonization varied widely (10^3 cfug to 10^6 cfug) and group differences were not statistically significant. All piglets developed IgA and lymphocytic inflammation, but only piglets vaccinated parenterally and piglets vaccinated orally with LT had IgG and neutrophilic inflammation. 

Parenteral vaccination, oral vaccination failed to protect piglets against challenge with live H. pylori, even when LT is used as an adjuvant. Both parenteral and adjuvant-assisted oral vaccination appear to diminish colonization, but both induce neutrophilic inflammation in some piglets.

1B:15  IL-10 INHIBITS H. PYLORI INDUCED NEUTROPHIL BUT NOT EPITHELIAL CHEMOKINE SECRETION

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Purpose: In H. pylori infection, gastric mucosal expression of proinflamma-
tory cytokines (IL-1, TNF-α, IL-8) and down-regulatory cytokines (IL-10) is increased. H. pylori directly induces neutrophils and gastric epithelial cells to secrete IL-8 in vitro. IL-8 inhibits the neutrophil chemokine production induced by cytokines or LPS stimulation. This study investi-
gates the inhibitory effects of IL-10 on H. pylori induced neutrophil and epithelial IL-8 secretion.

Methods. Peripheral blood neutrophils were cultured at 2 x 10^6/ml for 24 hours with 5 x 10^-6 heat killed H. pylori (NCTC 11637) with or without IL-10 or IL-13 (1–100 ng/ml). Kato-3 gastric epithelial cell 5 x 10^6/ml were cultured with viable CagA positive NCTC 11637 (bacteria:cell 100:1) for 24 hours with or without IL-10 or IL-13. Cell supernatants were assayed for secreted IL-8 by ELISA.

Results. Neutrophil IL-8 secretion was strongly induced by co-culture with H. pylori (mean ± SE 13.7 ± 0.51 ng/ml, control 0.56 ± 0.41, n = 3). Co-culture in the presence of IL-10 decreased IL-8 secretion in a dose dependent fashion; reduction at 1 ng/ml (14%), 10 ng/ml (53%), 50 ng/ml (74%), 100 ng/ml (80%). In contrast to IL-10, IL-13 had no effect on H. pylori stimulated neutrophil IL-8 secretion. The gastric epithelial cell line Kato-3 secreted IL-8 in response to H. pylori, 5.8 ± 2.1 ng/ml (n = 3). Co-culture with IL-10 or IL-13 over a 1 to 100 ng/ml range had no effect H. pylori induced epithelial IL-8 secretion.

Conclusions: Whilst direct stimulation of neutrophil chemokine secre-
tion by H. pylori may amplify mucosal neutrophil recruitment, IL-10 can down regulate this inflammatory cascade. In contrast to the effect of IL-10 on neutrophils, the induction of IL-8 in gastric epithelial cells by H. pylori could not be inhibited by IL-10.

1B:16  OXIDATIVE BURST AND RELEASE OF CONSTITUENTS OF PRIMARY AND SECONDARY GRANULES FROM NEUTROPHILS ACTIVATED BY HELICOBACTER PYLORI. SURVIVAL OF PHAGOCYTOSIS

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Background and objectives: Chemotaxis by H. pylori and Il-8 produced by gastric epithelial cells in infected and inflamed areas attracts large numbers of neutrophils to the gastric mucosa, a crucial part of active chronic gastritis. The aims of the present investigations were to study the oxidative burst (chemiluminescence, CL), and the release of constituents of primary (myeloperoxidase, MPO) and secondary (lactoferrin, LF) granules from neutrophils during phagocytosis of cytotoxic (ct) and noncytotoxic (nct) producing H. pylori strains, nonopsonized (nonops) or opsonized (ops).

Methods: H. pylori NCTC 11637 (ct with rapid and strong CL when nonops), and C-7050 (nct with weak and slow CL when nonops) were used. Neutrophils isolated by Ficol-Hypaque were stimulated by nonops or ops organisms of H. pylori, the oxidative burst measured by CL the release of MPO by radioimmune assay, and LF by ELISA.

Results: Release from neutrophils of 60–100% of LF but < 7% of MPO occurred within 30–60 min after they were activated with nonops H. pylori but not C-7050. Corresponding experiments with C-7050 gave a release of 14–47% of LF and < 7% of MPO. When nonops were opsonized a strong and rapid release of 100% of LF within 15–30 min and 15–20% of MPO. Nonops 11637 were rapidly phagocytosed (within 5 min) whereas C-7050 with a weak and slow CL were not. When ops both strains activated neutrophils to a strong and rapid burst and were rapidly phagocytosed and killed. Neutrophils 11637 survived phagocytosis for > 24 hours.

Conclusions: The rapid and strong activation of neutrophils by nonops H. pylori leads to phagocytosis and complete release of LF but very little of MPO which may explain the survival of nonops H. pylori.

1B:17  HELICOBACTER PYLORI SPECIFIC B-CELLS IN GASTRIC MUCOSA


Purpose: The aim of this study was to evaluate the presence of H. pylori specific antibody secreting cells (ASCs) in gastric mucosa of asymptomatic H. pylori carriers and non-carriers in symptomatic H. pylori patients.

Methods: Five H. pylori infected patients with DU, five asymptomatic H. pylori carriers and nine healthy, non-infected subjects were gastroscoped and 10 biopsies were taken from the antrum and corpus, respectively, of each subject. Mononuclear cells were isolated by means of enzymatic dispersion and tested for specificity against different H. pylori antigens, i.e. membrane proteins (MP), flagellin and urease, by the ELISPOT-assay.

Results: None of the non-infected subjects had ASCs that were specific for any of the antigens tested. On the contrary, all of the infected subjects had high numbers of ASCs against flagellin and most of them also had urease and MP specific ASCs. Furthermore, the infected subjects had approximately 20 times more total IgA secreting cells than non-infected subjects while the frequency of IgG and IgM secreting cells were similar between the two groups. No difference in antigen specificities has been noted between symptomatic and asymptomatic H. pylori carriers so far.

Conclusions: H. pylori induces strong antibody responses locally in the stomach, especially against flagellin and urease, in symptomatic as well as asymptomatic individuals.
Aim: To identify and localize the cytokines produced in *H. pylori*-infected.

Methods: An indirect immunohistochemical technique was used to determine the production and localization of IL-1β, IL-6, IL-8, IFN-γ, TNF-α and TGF-β in the single-cell level in cryopreserved antral biopsies from *H. pylori*-positive duodenal ulcer patients (*n* = 6) and healthy controls (*n* = 6).

Results: All infected individuals had moderate to severe chronic antral gastritis whereas the biopsy subjects from the healthy controls were normal. IL-6, IL-8 and IFN-γ production was detected in all *H. pylori*-positive subjects and in 5 of 6, 1 of 6 and 2 of 6, respectively, of the controls. IL-6 was intensely stained in the infected individuals whereas there was a very weak reaction in the controls. TGF-β and IL-1β was found in 5 of 6 of as well the controls as the *H. pylori*-positive subjects. 5 of 6 infected showed positive staining for TNF-α as compared to 2 of 6 controls. IL-6, IFN-γ and TGF-β were detected in surface epithelial cells, gastric pits, intrapithelial lymphocytes and mononuclear cells in the lamina propria whereas IL-1β, IL-8 and TNF-α showed immunoreactivity for the 3 latter cases only.

Conclusions: The inflammatory response to *H. pylori*-infection is characterised by an increased antral production of IL-8, IFN-γ and TNF-α.

Cytokine Production in the Peripheral and Gastrointestinal Tracts

The cytokine production in the peripheral and gastrointestinal tracts of *H. pylori*-infected individuals was measured in serum and tissue samples. IL-1β, IL-6, IL-8, IFN-γ and TNF-α were detected in serum and tissue samples from *H. pylori*-infected individuals. IL-1β, IL-6 and IFN-γ production was detected in serum and tissue samples from healthy individuals. IL-8 and TNF-α were detected in serum and tissue samples from *H. pylori*-infected individuals.

Conclusions: The cytokine production in the peripheral and gastrointestinal tracts of *H. pylori*-infected individuals was measured in serum and tissue samples. IL-1β, IL-6, IL-8, IFN-γ and TNF-α were detected in serum and tissue samples from *H. pylori*-infected individuals. IL-1β, IL-6 and IFN-γ production was detected in serum and tissue samples from healthy individuals. IL-8 and TNF-α were detected in serum and tissue samples from *H. pylori*-infected individuals.

Gastric inflammation in the presence of *H. pylori*-infection was measured in tissue samples from *H. pylori*-infected individuals. IL-1β, IL-6 and IFN-γ production was detected in tissue samples from healthy individuals. IL-8 and TNF-α were detected in tissue samples from *H. pylori*-infected individuals.

Conclusions: The cytokine production in the peripheral and gastrointestinal tracts of *H. pylori*-infected individuals was measured in serum and tissue samples. IL-1β, IL-6, IL-8, IFN-γ and TNF-α were detected in serum and tissue samples from *H. pylori*-infected individuals. IL-1β, IL-6 and IFN-γ production was detected in serum and tissue samples from healthy individuals. IL-8 and TNF-α were detected in serum and tissue samples from *H. pylori*-infected individuals.

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Results:

<table>
<thead>
<tr>
<th>Plasmid DNA immunization</th>
<th>Number of animals</th>
<th>Urease test IgG levels (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tested</td>
<td>protected</td>
</tr>
<tr>
<td>Non imm.</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>pUreA</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>pUreB</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pUreC</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mann Whitney U-test: p = 0.0007 vs non immunized, p = 0.02 vs pUreA; *Mann Whitney U-test: p < 0.03 vs non immunized

Summary: DNA immunization with Hp urease subunits leads to the appearance of anti-urease seric antibodies but does not prevent bacterial colonization of the host. However, UreB-DNA vaccinated mice present a lower degree of infection as compared to non immunized animals.

Conclusions: DNA immunization might represent a complement to mucosal/furoral immunization. Supported by SNF 32 36349.92

1B:23 PROTECTIVE POTENTIAL OF UREASE B POLYPEPTIDES IN THE MURINE MODEL


The urease B subunit (569 amino acids, 66 kDa) of Helicobacter pylori (Hp) protects mice against Helicobacter felis (Hf) infection. In this study, we have examined whether urease B polypeptides were as effective as purified non active urease (apoenzyme) in conferring protection against infection.

Methods: UreB DNA sequences were cloned into an E. coli expression vector to produce proteins containing a 1-569, aa 226-569, aa 340-569, and aa 220-345, respectively. Specific pathogen-free BALB/c female mice were orally immunized with Hp urease/fragments at days 0, 7, 14, and 21 in presence of cholera toxin (CT) and challenged at day 28 with Hp. Presence of Hp in gastric biopsies was assessed one month later by rapid urease test (OD at 550 mm).

Results:

<table>
<thead>
<tr>
<th>Immunization:</th>
<th>Tested</th>
<th>Protected</th>
<th>Urease mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoenzyme + CT</td>
<td>10</td>
<td>9*</td>
<td>0.02 ± 0.05</td>
</tr>
<tr>
<td>aa 1-569 + CT</td>
<td>8</td>
<td>8*</td>
<td>0.06 ± 0.15</td>
</tr>
<tr>
<td>aa 226-569 + CT</td>
<td>10</td>
<td>9*</td>
<td>0.003 ± 0.008</td>
</tr>
<tr>
<td>aa 340-569 + CT</td>
<td>10</td>
<td>2</td>
<td>0.22 ± 0.17</td>
</tr>
<tr>
<td>aa 220-345 + CT</td>
<td>19</td>
<td>1</td>
<td>0.40 ± 0.17</td>
</tr>
</tbody>
</table>

*p = 0.00002, **p = 0.026 (Fisher's exact test) compared to CT.

Summary: Deletion of the first 220 amino acids of UreB does not affect the ability of the recombinant protein to confer protection against infection. However, aa 220 to 345 given orally in presence of CT do not protect from infection. Conclusions: Our data suggest that the 120 amino acids located between amino acids 220 and 340 of the B subunit are necessary but not sufficient for protection. Supported by SNF 32 36349.92

1B:24 ORAL IMMUNIZATION WITH RECOMBINANT UREASE CONFRAMES LONG-LASTING IMMUNITY


Urease has been shown to confer protection against challenge with H. felis in mice. The purpose of the present study was to examine duration of the immune response and long-term protective efficacy of recombinant urease (rUre).

Swiss Webster mice (n = 200) were orally immunized four times at weekly intervals with 100 mg rUre and 5 mg heat-labile enterotoxin of Escherichia coli (LT), or with LT alone. At time intervals of 4, 10, 20, or 40 weeks post immunization, 25 rUre-immunized mice and 25 control mice were challenged with H. felis and sacrificed at 2 or 10 weeks post challenge. H. felis infection was assessed by quantitative gastric urease assay and by histology. Anti-urease antibody levels were measured in serum and saliva both pre- and post-challenge. Over the 40 week time period, the infection rates in rUre-immunized mice were significantly lower than those in controls (p < 0.05) as assessed by gastric urease activity, with protection levels ranging from 79-100% at 2 weeks post-challenge and 63-78% at 10 weeks post-challenge. Anti-urease antibody levels remained elevated in the serum and mucosal compartments at 39 weeks following immunization. This study shows that immunization with rUre and LT results in long-lasting protective immunity against challenge with H. felis.

1B:25 LOCAL IMMUNOGLOBULIN G ANTIBODIES, AND NOT IgA, CONTRIBUTE TO PROTECTIVE RESPONSE AGAINST GASTRIC H. FELIS INFECTION IN MICE

R.L. Ferrero, J.-M. Thieme, A. Labigne. Unité de Pathogénie Bactérienne des Maquises (INSERM U389), Institut Pasteur, Paris, France

Orogastric immunization of mice with Helicobacter antigens, together with mucosal adjuvants, has been shown to confer immunity in the H. felis infection model. The aim of the study was to investigate the immune responses associated with immunity and to compare these with responses in H. felis-infected mice. To do this, antibody-secreting cells and antibodies present at mucosal and systemic sites in mice were characterized by enzyme-linked immunosorbent assays. It was found that mice preferentially induced the recruitment of plasma cells committed to immunoglobulin A (IgA) synthesis in salivary gland and gastric tissues. Antigen-specific IgA was the major antibody class detected, representing between 9 and 50% of total IgA in mucosal secretions recovered from these tissues. In contrast, immunization of mice against gastric H. felis infection induced the proliferation of large numbers of immunoglobulin G (IgG)-secreting cells at mucosal tissue sites. Anti-H. felis IgG antibodies (accounting for between 3 and 9% of total IgG), but not specific IgA antibodies, were present in the gastric secretions of immunized animals. Protective immune response patterns were different between sera of asymptomatic population antibodies in the sera. In conclusion, immunization against gastric H. felis infection was shown to induce local IgG responses. It is proposed that locally synthesized specific IgG antibodies contribute to immunity against gastric Helicobacter infection.

1B:26 IMMUNOBLOT ANALYSIS OF HUMORAL RESPONSE AMONG HELICOBACTER PYLORI STRAINS ISOLATED FROM DUODENAL ULCER PATIENTS LIVING IN VENEZUELA

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The purpose of the study was to screen sera of patients with duodenal ulcer (DU) and asymptomatic population using the immunoblot technique to define immunoreactive patterns characteristic of H. pylori infection. Methods: Endogastrudoosendoscopy was done on 15 patients with dyspeptic symptoms and the biopsy specimens obtained were cultured under microaerophilic conditions. The pathological examination came from 15 patients with DU, 11 healthy children under 10 years old and 61 teenagers from an endemic gastric cancer state of Venezuela. The soluble antigen material was prepared by sonication of whole cells. Samples containing about 200 μg of protein were subjected to SDS-PAGE 10% acrylamide running gel. The proteins were transferred to nitrocellulose support. Results: The immunoblots of the patients sera infected with H. pylori, showed a characteristic pattern of reactivity to protein bands of: 83, 67, 63, 47 and 17 kDa for IgG antibodies and 74, 65, 58 kDa for IgA antibodies. The same reactivity patterns were observed in 3 (27.2%) of sera from healthy children. The reactivity of protein bands was different in the teenager group. The 110-120 kDa bands reactive on the IgG blot of two individuals (7.6%) but on the IgA blots, these proteins did not show reactivity with any sera tested. The percentage of positive results increased with the band proteins of 83 kDa for IgG and IgA bands (38.46%). Only five sera (19.23%) were negative in the immunoblot assay. Conclusions: Immunoblot analysis showed that the reactivity patterns were different between sera of asymptomatic population of endemic gastric cancer, healthy children and patients infected with H. pylori who live in Metropolitan areas. The presence of immunoreactivity against H. pylori antigens would be a good marker for the follow-up of the H. pylori infection specially in high risk population living in endemic gastric districts.

1B:27 HLA-DQA1 ALLELES AND H. PYLORI INFECTION

J. Karhuokori, I. Kähärä, S. Silvennoinen-Kassinen, A. Tiilikainen, R. Karttunen. University of Oulu, Oulu, Finland

HLA molecules play an important role in antigen presentation in immune defence against infectious agents. HLA-DQA1*0102 allele was suggested to protect against and/or to delay response to H. pylori-caused disease in a recently published study involving Japanese patients. To analyze this possible association, we tested DQA1 alleles in infected and non-infected subjects, mostly staff from the medical faculty.
**Methods:** H. pylori-specific IgG antibodies were determined by ELISA (Pylostat, EIA-G, Orion Diagnostica, Espoo, Finland). HLA-DQA1 typing was done with PCR-SSP. Statistical comparisons were made by Fisher’s exact test.

**Results:** DQA1 allele frequencies were similar in H. pylori antibody positive (n = 50) and antibody negative (n = 138) individuals. Frequencies of allele 0102 were 15% vs. 18% and those of 0301 24% vs. 20% (antibody-positive vs. antibody-negative, p = NS).

HLA-DQA1 homozygosity was, however, increased in the former (24% vs. 12%, p = 0.03).

**Conclusions:** We could not confirm the possible association between H. pylori infection and certain HLA-DQA1 alleles. Our finding of the increased DQA1 homozygosity in subjects with H. pylori antibodies fits well with the hypothesis that heterozygosity in MHC genes favors resistance to microbial infections.

**1B:28 ORALLY IMMUNIZED IG A DEFICIENT MICE ARE PROTECTED AGAINST H. FELIS INFECTION**

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Oral immunization with a variety of antigens plus cholera toxin adjuvant can protect mice from H. felis infection. Although oral immunization induces mucosal immune responses including IgA in gastric secretions, the mechanism(s) of protection remain ill-defined. To address this issue, IgA deficient mice (produced by gene-targeting) were orally immunized 4x weekly with 2 mg H. felis sonicate plus 10 μg cholera toxin. Mice were challenged one week after immunization with 5 x 106 viable H. felis and sacrificed two weeks later. Both wild type immunized mice (1/10 urease positive) and IgA deficient mice (1/10 urease positive) were protected from infection when compared to unimmunized controls (68% and 10/11 positive). Both groups of protected mice had comparable levels of H. felis-specific serum and gastric IgG. IgA deficient mice did not develop any IgA antibodies but had much higher titers of IgM antibodies that wild type mice (table).

<table>
<thead>
<tr>
<th>Mice</th>
<th>Serum Ab (log 10)</th>
<th>Gastric Ab (log 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>IgM</td>
<td>IgA</td>
</tr>
<tr>
<td>IgA +/+</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>IgA +/-</td>
<td>&gt; 1.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

In conclusion, orally-immunized IgA deficient mice develop high levels of H. felis-specific IgM in gastric secretions and are protected from H. felis infection.

**1B:29 ADOPTIVE TRANSFER OF HELICOBACTER-SPECIFIC TH1 OR TH2 CELLS EXACERBATES HELICOBACTER-ASSOCIATED GASTRITIS, BUT GASTRITIS CELLS REDUCE THE MAGNITUDE OF INFECTION**

M. Mohammadi, S. Czinn, R. Redline, J. Nedrud. Case Western Reserve University, Cleveland, OH, USA

Previous findings in the H. felis-mouse model suggest that TH1 cellular immune responses may contribute to Helicobacter-associated gastritis. To further investigate this issue, a series of adoptive transfer experiments were performed.

**Methods:** Antibiotic-specific spleen cells, isolated from immunized/challenged or non-immunized/infected mice, or tissue culture-derived CD4+ or CD8+ T cell lines were adoptively transferred into naive recipients before live bacterial challenge. Recipient mice were sacrificed at various times after challenge and evaluated for gastritis, bacterial load, cellular proliferation, cytokine production, and serum antibody levels.

**Results:** Transfer of cells from both groups of donors as well as TH1 and TH2 cells elicited a similar response (p < 0.05). However, when the magnitude of infection was determined for various groups of recipients, only transfer of cells from immunized/challenged (protected) mice and a TH2 cell line (but not cells from infected mice or TH1 cell lines) led to a significant reduction in the bacterial load. The reduction of bacterial load in the recipients of cells from immunized/challenged mice correlated with raised serum IgG1 antibodies in the mice.

**Conclusion:** These data suggest two different roles for cell-mediated immune responses in Helicobacter infection, one associated with the pathogenesis of disease (TH1 phenotype), and the other associated with protection from or control of infection (TH2 phenotype).

**1B:30 ANTIBODY RESPONSE TO SPECIFIC H. PYLORI-ANTIGENS IN NONULCER DYSPEPSIA, PERSISTENT ULCER AND HEALTH: PREDICTORS OF DISEASE OUTCOME?**

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H. pylori is linked to the pathogenesis of duodenal ulcers (DU) and may play a role in the development of nonulcer dyspepsia (NUD). However, whether specific H. pylori antigens are of clinical importance is unclear. We aimed to investigate whether patterns of antibody response differ in infected individuals with DU and NUD. Methods: Studied were H. pylori positive patients with DU (n = 50), NUD (n = 34) and healthy blood donors without dyspeptic symptoms (n = 92). Western blot analysis was used to identify immune responses to specific H. pylori antigens in serum. The proportion of H. pylori-specific immune responses was similar among antibody-deficient mice as compared using non-parametric tests. Factor analysis and logistic regression analysis were done to detect specific patterns of H. pylori antibodies associated with specific disorders. Results: Overall, Western blot analyses yielded significantly more antibody responses in DU and NUD patients compared with 4 weekly doses of 100 μg recombinant urease (p < 0.001). Univariate, antibodies against 120 (p < 0.001), 81 (p < 0.001), 60 (p < 0.02) and 50 kDa antigens (p < 0.05) were significantly associated with DU. In contrast, the 22 kDa antigen was significantly associated with NUD and there was a negative association between NUD and the 55 kDa antigen (p < 0.05). Factor analysis revealed a two-factor structure with all antigens except the 22 kDa antigen loading on factor one. Conclusions: The pathophysiologic role of H. pylori in DU and NUD cannot be attributed to a single antigen expressed by H. pylori. While there is no specific pathogenic pattern of antigens, the risk of DU increases with the number of antigens. Lack of the 55 kDa antigens appears to be a risk factor for the development of NUD.

**1B:31 RESOLUTION OF GASTRITIS AND DURATION OF PROTECTION AGAINST H. FELIS INFECTION AFTER ORAL IMMUNIZATION WITH RECOMBINANT UREASE**


Several groups including our own have reported an increase in leukocytic infiltration in the gastric corpus of mice immunized with Helicobacter antigens and protected against challenge with H. felis. We report here studies on the characteristics of the inflammatory response and its mechanism. Swiss Webster mice were orally immunized with 4 weekly doses of 100 μg recombinant urease (rUre) plus either 10 μg cholera toxin (CT) or 5 μg heat-labile toxin (LT), or adjuvant alone, and challenged 2 weeks later with 107 live H. felis. At various intervals postchallenge, gastric tissues were examined for H. felis infection, leukocytic infiltration, and epithelial alterations. By gastric urease activity, protection after rUre-immunization remained between 70-95% from 2 weeks through 57 weeks postchallenge. Significantly higher densities of infiltrating leukocytes were found in the corpus of rUre-immunized mice within the first 8 months postchallenge in comparison to LT-controls (p < 0.03). Phenotypic analysis of T cells showed that rUre-immunized mice also had increased total number of CD4+ and CD8+ T cells. CD8+ cells were rare in the gastric mucosa of infected LT-controls and unchallenged control mice. 20% (21/108) of mice immunized with rUre plus LT but only 5% (6/116) of LT-control mice had epithelial changes consisting of parietal cell loss, hypertrophy of surface epithelium, and microbodies. When mice were treated with antibiotic triple therapy, at 7-9 wk postchallenge, gastritis and epithelial changes in the corpus (12 wk postchallenge) were reduced, suggesting that gastritis was caused by undetected residual bacteria and persistent antigenic stimulation in the mucosa of rUre-immunized mice. These results indicate that oral immunization of mice with rUre produces a long lasting protection against H. felis, accompanied by the infiltration of immunoregulatory T cells, but that sterilizing immunity may not be achieved. Further studies are underway to determine whether these phenomena occur in non-murine hosts.

**1B:32 UREASE IMMUNIZATION PROTECTS AGAINST REINFECTION BY HELICOBACTER PYLORI IN RHEUS MONKEYS**

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Rhesus monkeys (Macaca mulatta) determined to be naturally colonized with H. pylori by culture and histology were immunized with either 40
mg urease administered orally with 25 micrograms LT or sham-immunized with LT alone. A total of 6 doses were administered over an 8 week period. Urease-specific antibodies were generated in the serum in 5 of 6 animals and in the saliva in 3 of 6 animals. The 6 animals receiving LT only and 5 animals receiving urease + LT were treated with a quadruple therapy regimen consisting of 40 mg metronidazole, 24 mg clarithromycin and 44 mg omeprazole BID, and 2 mg omeprazole QD for 10 days. H. pylori was eradicated in all 11 animals as determined by culture and histology of antral biopsies obtained 5 weeks and 4 months after treatment. The animals were then boosted with either urease + LT or LT alone and challenged with low passage cultures of an H. pylori strain originally isolated from one of the animals in this study. Three inoculations, each consisting of 2 x 10^9 colony forming units, were delivered to the monkeys by combined oral and intragastic routes every other day. Biopsies taken from the gastric antrum and corpus 3 weeks after inoculation showed a decrease in the level of H. pylori colonization in animals receiving urease + LT (a median value of 15 CFUs ranging from 0 to 1,500) compared to animals receiving LT alone (a median value of 1068 CFUs ranging from 57 to 39,000). This difference was statistically significant by the Wilcoxon rank sum test (p = 0.047). Later time points will be examined and local (gastric) immune responses will be defined. Studies are also underway to improve vaccine efficacy by combining urease with other antigens, and by the use of alternative routes and adjuvants. This study provides the first evidence for effective immunization of non-human primates against H. pylori.

18:33 ORAL IMMUNIZATION OF BALB/c MICE AGAINST HELICOBACTER PYLORI: USE OF HEPARAN SULFATE BINDING PROTEINS COUPLE TO MUCOSAL ADJUVANTS

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Gastrointestinal disorders have long plagued mankind, and their pathology has changed since they were associated with H. pylori was (gastroduodenal ulcers, gastric cancer). Antibiotic therapies do not eradicate the bacteria since there is a high percentage of recurrence in the gastric tissue. Therefore, it is important to develop new means to eradicate H. pylori by stimulating the host's own immune system to produce high titers of antibodies against the putative virulence factors of the bacterium. Here, we assess the use of bacterial proteins with Heparan Sulfate-proteoglycan affinity (HSBP) coupled to different mucosal adjuvants, to stimulate a local and systemic immune response of BALB/c mice when orally immunized with these antigens. We observed a significant increase in antibody titers, mainly slgA and seric IgG, which recognized the HSBP in animals immunized with the antigen covalently coupled to the subunit of cholea toxin (HSBP-CTB) as revealed by ELISA. Under the same conditions, we also observed high titers of antibody producing cells (SPF) of the Iga isotype among cells in the gastric tissue. Our findings suggest that HSBP-CTB is a putative candidate for stimulating the immune system, thereby preventing the organism from colonizing the gastric mucosa during present and future exposure to this pathogen.

18:34 HELICOBACTER PYLORI CATALASE: A NOVEL ANTIGEN FOR VACCINATION

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Aim: To examine whether immunisation with recombinant catalase can stimulate protection from H. pylori challenge.

Methods: A genomic DNA library was constructed from H. pylori, strain R11, in a ZAP-Express vector. A catalase containing clone was identified by probing the library with a 710 bp fragment of the H. pylori catalase gene. The positive clone was excised and the resulting plasmids were introduced into E. coli strain XLORL. A full length catalase clone was identified by placing the clones into 30% hydrogen peroxide and looking for the formation of bubbles as a measure of catalase activity. The recombinant H. pylori catalase was purified on the basis of size and isoelectric point. Specific Pathogen Free BALB/c mice were immunised on days 0, 7, 14 & 21 with 200 μg purified catalase + 10 μg of cholea toxin (CT) adjuvant per dose. Control animals were immunised with 1 mg of H. pylori sonicate + CT; 1 mg of XLORL E. coli sonicate + CT; or saline alone. After 3 weeks, the animals were challenged 3 times with the ‘sydney Strain’ of H. pylori, a mouse colonising strain isolated by our group. Two weeks after challenge the animals were killed and the stomach removed for assessment of infection by urease assay and histology.

### 18:35 ROLE OF HLA-DR AND HLA-DQ ANTIGENS IN THE PATHOGENESIS OF CHRONIC SUPERFICIAL GASTRITIS AND IN THE H. PYLORI INFECTION

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Individuals with different HLA types differ in their susceptibility or resistance to infectious pathogens. In the present study we have analyzed 61 Italian patients with superficial chronic gastritis, 36 H. pylori+ and 25 H. pylori-, and 126 healthy controls by PCR-SSO typing for DQA1, DQB1, and DR alleles. In contrast with the results in a Japanese population, the distribution of the phenotypic frequencies of DQA1 alleles did not show any significant statistical difference either comparing the two groups with gastritis (H. pylori+ and H. pylori-) or comparing the patients H. pylori+ with healthy controls. The distribution of the phenotypic frequencies of the DQB1 alleles showed a no difference in the DQB1 allele distribution between patients H. pylori+ and H. pylori-: a statistical significant difference in the frequency of the allele DQB1*0501 between patients H. pylori+ and the healthy controls group (36% vs 15%) or between patients H. pylori- and the healthy group (40% vs 15.9%). When we have considered the whole group of gastritic patients (n = 61) compared with the control group (n = 126) we obtained a significant difference for the DQB1*0501 allele (p = 0.0014, pc = 0.022, RR = 3.2). No difference were observed in the DR allele distribution between patients H. pylori+ and H. pylori-. When we join the two groups, we observed a significal difference between the phenotypic frequency of the DR1 allele comparing all gastritic patients (n = 61) with healthy controls typed for DR (n = 120) (p = 0.0005, pc = 0.006, RR = 3.6). This last statistical significance resulted higher than that observed for the DQ-B1*0501 allele in linkage disequilibrium with DR1, suggesting a possible association of the disease with the DR antigen.