HT29-MTX-E12 cell adherent mucus layer following infection and in gastric biopsies from infected humans.
Culture of HT29-MTX-E12 cells in the presence of copper, which increases TFF1 dimer formation, resulted in a significant increase in colonisation by *H. pylori*.
Isogenic mutants of *H. pylori* with truncated LPS core structures were produced and their binding to TFF1 and ability to colonise adherent mucus determined. One of these isogenic mutants of *H. pylori* was unable to interact with TFF1, and colonization of HT29-MTX-E12 cells was reduced 100-fold as compared to the wild-type strain ($p < .05$).
Using the HT29-MTX-E12 cell model system results indicate that the interaction of *H. pylori* with TFF1 promotes colonization of gastric mucus and that the core oligosaccharide of *H. pylori* LPS is the critical adhesin in this interaction.

Abstract no.: P03.07

CHARACTERIZATION OF NEW HUMAN GASTRIC EPITHELIAL CELL LINES DERIVED FROM NCI-N87 CELLS AFTER OVER-EXPRESSION OF HUMAN TELOMERASE CATALYTIC SUBUNIT

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The lack of a cellular model which correctly mimics the natural niche of the pathogen *Helicobacter pylori* is still limitative for the study of this infection. Aiming to overcome this limitation, we have previously isolated clones of a subpopulation of the widely used heterogenic NCI-N87 (ATCC CRL-5822) gastric cell line[1], those presenting typical epithelial markers and a progenitor-like phenotype (simultaneous synthesis of mucus and zymogens). For that, we stably-transduced the NCI-N87 cells with human telomerase reverse-transcriptase (hTERT) catalytic subunit (pGRN145 plasmid, ATCC MBA-141), using the FuGENE-HD reagent (Roche). The two most promising NCI-N87-derived clones (C5 and C6) were shown to be composed of cells with homogenous phenotype with ability to grow in adherent monolayers, to produce gastric zymogens (hematoxylin staining) and to produce and secrete neutral mucins (Periodic-Acid-Schiff staining). Preliminary results have also shown that they are able to generate transepithelial electrical resistance and the ability of C5 to produce and secrete acidic mucins (Alcian-Blue staining). We are now clarifying the identity of the mucin species C5 and C6 produce by immunohistochemical analysis and zymogens (Pepsinogen) by western-blot. Moreover, the subcellular localization (immunocytochemistry) of adherens and tight-junctions' proteins (*E*-cadherin and ZO-1) and the polarization status of both clones is now under evaluation. Due to their improved properties, compared to the heterogeneous parental line, these NCI-N87-derived clones are promising models of the human gastric epithelium.
1. Chailler, P. and D. Ménard. *J.Cell.Physiol.,* 202:263–274, 2005.
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Abstract no.: P03.08

HELICOBACTER PYLORI HTRA VIRULENCE FACTOR IS CONSERVED AMONG CLINICAL ISOLATES

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Helicobacter pylori virulence factor HtrA, encoded by the *htrA* gene, is a secreted serine protease. It has recently been shown that HtrA cleaves the cell-cell adhesion protein E-cadherin, possibly allowing *H. pylori* to access the intercellular space of epithelial cells.
Our aim was to elucidate whether HtrA is conserved among clinical isolates. For that, we fully sequenced *htrA* in 36 clinical isolates and two reference strains (*H. pylori* 26695 and G27), using primers designed to cover the whole gene.
Using this strategy, we were able to sequence *htrA* in all clinical isolates. All sequences gave rise to open reading frames of 1431 bp (476 amino-acids). Neither insertions nor deletions were observed along the gene. The phylogenetic relationship between *htrA* sequences was analysed using the MEGA 4 software, applying the Neighbour-Joining method. The mean similarity between *htrA* sequences was 96.5% ± 0.3 (mean ± SE), and the mean molecular distance was 0.034 ± 0.003. The nucleotide substitutions were 0.158 ± 0.013 and 0.003 ± 0.001, for the synonymous (Ks) and the non-synonymous (Ka) rates, respectively. The Ka/Ks ratio was 0.019, implying that these sequences are under stabilizing selection. After translation of the nucleotide sequences and using strain 26695 as reference, 17 amino-acid substitutions were found, mainly concentrated at the N- and C-terminus. No mutations were found at the active site (Ser205), suggesting that all strains have an active HtrA.
Our results show that HtrA is highly conserved among clinical isolates, reinforcing its essentiality for *H. pylori* survival.
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Abstract no.: P03.09

REGULATION OF MDM2 ONCOGENE BY *HELICOBACTER PYLORI* LIPOPOLYSACCHARIDE IN GASTRIC EPITHELIAL CELLS

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Purpose: Mdm2 is critical regulators of the p53 protein which plays a crucial role in maintaining genomic integrity and tumor prevention. *Helicobacter pylori* is reportedly involved in the development of gastric cancer. We investigated the mechanisms between *H. pylori* and MDM2, focusing on *H. pylori*-derived lipopolysaccharide (LPS).
Experimental Design: *H. pylori*-LPS and two gastric cancer cell lines (AGS and MKN28) were used. We examined whether the expression of MDM2 in a dose and time-dependent manner of Gastric epithelial cells, when they are exposed to *H. pylori*-LPS. We also examined if PI3k/Akt/mTOR signaling pathway mediated this expression. Western blotting was employed to evaluate the expressions of MDM2, pAkt-S473 and Akt, and the functionality of the MDM2 promoter is examined by luciferase assay.
Results: Gastric epithelial cells express more MDM2 in a dose- and time-dependent manner when they are stimulated with *H. pylori*-LPS. Treatment of Gastric epithelial cells application of LY294002 and Rapamycin caused a dramatic reduction of *H. pylori*-LPS induced MDM2. In addition, *H. pylori*-LPS stimulation increased the MDM2 promoter activity.
Conclusion: *H. pylori*-LPS induced MDM2 over expression is mediated by PI3K/Akt/mTOR.

Abstract no.: P03.10

DISRUPTION OF TIGHT JUNCTIONS OF GASTRIC EPITHELIAL CELLS INDUCED BY *HELICOBACTER PYLORI* AS ANALYSED USING REAL-TIME PHASE CONTRAST MICROSCOPY

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Helicobacter pylori cytotoxin-associated gene A (CagA) has been regarded as a major player in the disruption of tight junctions. However, the exact mechanism of tight junction disruption induced by *H. pylori* is still not well-established. This study uses a high resolution imaging system that is able to maintain perfect focus and optimal growth conditions for cells to follow live cell observations. Using MKN28 cells, which form functional tight junctions, these cells were infected with *H. pylori* 26695 wildtype or Δ*cag*A separately. The real-time event of tight junction disruption of the gastric cells induced by *H. pylori* was recorded over a period of 44 hours and the images were then analyzed quantitatively using ImageJ software. The images show that the tight junctions of uninfected MKN28 cells remained intact for the entire recording period. Interestingly, tight junction disruption as observed in wildtype-infected and Δ*cag*A-infected host cells began at 4 hours post-infection. The process of tight junction disruption as shown by the real-time microscopy observations is further supported by results obtained from barrier function test. Taken together, our findings show that real-time phase contrast microscopy can provide a highly supportive role on the mechanistic events occurring during host-pathogen interactions.

Abstract no.: P03.11

ULCEROGENIC PROFILE OF *HELICOBACTER PYLORI* PEDIATRIC STRAINS: A CONTRIBUTION TO GET INSIGHT INTO THE VIRULENCE OF THE BACTERIA

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Helicobacter pylori infection is the major cause for the development of peptic ulcer disease (PUD). In addition to patient genetic susceptibility, PUD occurrence in

children, with no other ethiology for the disease, presumes the involvement of more virulent strains.
Indeed, our in vitro infection assays showed the marked ability of a pool of five *H. pylori* strains isolated from PUD pediatric patients to induce a decrease in host-cells' viability, severe damages in cytoskeleton and impairment in the production/secretion of mucins in NCI-N87 cells, when compared with a pool of five other isolated from non-ulcer dyspepsia (NUD) pediatric patients. Subsequently proteomic comparison of these two groups of *H. pylori* strains revealed 27 differentially expressed proteins between them. In addition to the presence of genes encoding well established virulence factors (*cagA, vacAs1, oipA* ''on'' status, *homB* and *jhp562*), these ulcerogenic strains shared a proteome profile characterized by changes in the abundance of: motility-associated proteins, accounting for higher motility; antioxidant proteins, which may confer increased resistance to inflammation; key enzymes in the metabolism of glucose, amino acids and urea, which may be advantageous to face nutrient fluctuations. Therefore, during infection the pediatric ulcerogenic *H. pylori* strains may take advantage of a synergy between their natural ability to better adapt to their hostile niche and the expression of those virulence factors. We are now characterizing the interaction of these strains with human gastric epithelial cells and mucins.
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Abstract no.: P03.12

PREVALENCE OF *CAGA* AND *VACA* GENES IN *HELICOBACTER PYLORI* FROM THE GAMBIA IN RELATION TO DISEASE PHENOTYPE

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Helicobacter pylori is common in Africa, whereas *H. pylori*-associated disease is less common than in developed countries. In this study we investigated the prevalence of virulence-related *H. pylori* genotypes and disease phenotype in The Gambia.
Hundred and twenty-one of 169 patients with abdominal pain or dyspepsia, tested for *H. pylori* by PCR of DNA from gastric biopsies, were found to be *H. pylori* positive. The *cagA* gene, *s1*, *m1* alleles of the *vacA* gene were found in 61.2%, 76.9% and 45.5% respectively. The less toxigenic *s2 vacA* gene allele was found in 19.0% of the patients. *cagA* positive strains were found more frequently in patients with overt gastric diseases compared to patients with non-ulcerative disease (NUD); 85.7% in those with duodenal ulcers, 71.4%, in patients with gastric erosions, 72.7% in those with gastric ulcers and 56.4% in patients with NUD. There was no link between *vacA* allele and disease phenotype. However, we found that the co-existence of mixed *cagA* positive and *cagA* negative strains was more common in patients with non-ulcerative diseases compared to patients with gastric disease (24.5% versus 0%; $p = .002$).
This study indicates that the prevalence of *H. pylori* is high in dyspeptic patients in The Gambia and showed that *cagA+, s1* and *m1* are common genotypes. Carriage of *cagA* positive strain was associated with an increased risk of overt gastric disease. In addition, patients who were infected with mixed *cagA* positive and *cagA* negative strains were less likely to have gastro-duodenal diseases than those infected with pure strains.

Abstract no.: P03.13

NEUTRALIZING MONOCLONAL ANTIBODIES ARE EFFECTIVE AGAINST *HELICOBACTER PYLORI* Γ-GLUTAMYL TRANSPEPTIDASE ACTIVITY

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Helicobacter pylori γ-glutamyl transpeptidase (GGT) has been reported to be an important colonizing and apoptosis-inducing factor. Recently, we have also shown that GGT induces reactive oxygen species production, interleukin-8 upregulation and DNA damage in gastric cells. The aim of this study was to investigate if monoclonal antibodies raised against *H. pylori* GGT were able to inhibit its activity. Using recombinant GGT protein purified by affinity chromatography as an immunogen, monoclonal antibodies (MAbs) were generated in mice. Specificity of MAbs was analyzed by immunofluorescence staining of *H. pylori* and Western blot analysis. The MAbs were tested for their ability to neutralize GGT activity of various *H. pylori* strains using GGT assay. One of the MAbs generated was found to inhibit and neutralize GGT activity by 46–95%. Further characterization of this MAb is in progress to understand the underlying mode of inhibition.

Abstract no.: P03.14

NO ASSOCIATION OF THE *H. PYLORI* VACA, DUPA AND OIPA GENES WITH ATROPHIC GASTRITIS IN DYSPEPTIC PATIENTS FROM A POPULATION AT HIGH RISK OF GASTRIC CANCER IN COSTA RICA

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Background and Aim: Costa Rica is one of the countries with the highest incidence and mortality rates from gastric cancer. Gastric cancer prevalence varies among different regions. However, the prevalence of *Helicobacter pylori* infection is high in the whole country. We have previously shown that *H. pylori* CagA+, as defined by a combination of PCR analisis and serology, is significantly associated with atrophic gastritis of the antrum in a dyspeptic population. The aim of this study was to determine if other *H. pylori* virulence factors are associated with atrophic gastritis.
Methods: Seven biopsies and a blood sample were obtained from 501 patients referred to endoscopy for dyspeptic symptoms. In each case, a histopathological examination was performed. The presence of the *vac*A, *dup*A and *oip*A genes was analyzed by PCR in 88 cultured strains. Odds ratio and 95% confidence intervals for atrophic gastritis patients versus non-atrophic gastritis were calculated.
Results:

Table 1 Association of *H. pylori dupA*, *oipA*, *vacA s1m1* genes with atrophic gastritis

Gene (n)	OR (AG vs NAG)	95% CI	*p*
dupA$^+$ (59)	0.74	(0.27–2.06)	.425
oipA$^+$ (77)	1.40	(0.15–13.31)	.584
vacA s1m1 (57)	2.04	(0.66–6.24)	.296

OR, odds ratio; CI, confidence intervals; AG, atrophic gastritis; NAG, non atrophic gastritis.

Conclusion: Infection with *Helicobacter pylori* strains carrying the *dupA, oipA, vac*A *s1m1* genes is not significantly associated with atrophic gastritis risk in this dyspeptic population.

Abstract no.: P03.15

HELICOBACTER PYLORI INFECTION AND PATHOGENESIS OF PEPTIC ULCER IN EXTREME COLD CLIMATE

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Objective: To investigate the relationship between *Helicobacter pylori* (*H. pylori*, Hp) infection and Pathogenesis of peptic ulcer (PU) in extreme cold climate.
Methods: Collected gastric mucosa and juice of peptic ulcer patients who were taking endoscopy examination in our hospital and didn't take any stomach-related drugs for a month in extreme cold weather (temperature <10 °C). PH values of gastric juice were obtained on site by precise PH dipstick. Hp infection were determined by modified Giemsa staining. Tregs, macrophages infiltrating and Occludin, HSP70, NOS, EGF and EGFR expression in gastric mucosa were detected by immunohistochemical stain.
Results: 1, 82(80.4%) PU were Hp+, PH value of gastric juice in Hp+ PU was significantly lower than that in Hp- (1.00 ± 0.699 vs 1.88 ± 1.193, $p < .01$). 2, There were more Tregs and macrophages infiltrated in Hp+ gastric mucosa than those in Hp- (26.6 ± 10.0 vs 39.3 ± 24.0, $t = -3.567$, $p = .001$; 12.7 ± 11.1 vs 23.4 ± 14.4, $t = -2.932$, $p = .004$). 3, EGFR expression in gastric antrum mucosa of Hp+ was significantly lower than that of Hp- (*H* value:61.44 vs 48.10, $U = -2.101$, $p < .05$). 4, Occludin, HSP70, NOS and EGF expression in gastric mucosa were not significantly associated with Hp infection ($p > .05$).
Conclusion: Promoting gastric acid secretion and increasing Tregs and macrophages infiltration might be one of the pathogenesis of Hp associated peptic ulcer in extreme cold conditions.

