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.

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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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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.

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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