Infect Immun 1998). Although C. difficle has an IRS:ISS ratio of greater than one, its ability to cause infection is predicated on toxin formation. Interestingly, probiotic bacteria have low IRS:ISS ratios suggesting their ability to modulate the immune response is mediated through a different mechanism.

S1648

Modulation of Pellino 1 by *Helicobacter pylori* Lipopolysaccharide Enhances Toll-Like Receptor 2-Mediated Nuclear Factor-Kappa B Activation and Chemokine Induction

Sinead M. Smith, Anthony P. Moran, Paul N. Moynagh, Dermot P. Kelleher

Background & Aims: Toll-like receptors (TLRs) play a key role in host defense against invading pathogens by functioning as primary sensors for conserved microbial structures known as pathogen-associated molecular patterns (PAMPs). Recognition of PAMPs by a particular TLR results in the activation of transcription factors such as NF-κB, AP-1 and interferon regulatory factors, culminating in the induction of gene expression necessary for the co-ordination of the innate immune response. This study set out to identify novel regulators of TLR signaling in response to the gastric pathogen *Helicobacter pylori*. We investigated the role of the interacting protein Pellino 1 (PELI1) during the TLR2-mediated response to *H. pylori* lipopolysaccharide (LPS). PELI1 has recently been suggested to interact with components of the TLR signaling pathway (IRAKs 1 & 4, TRAF6), thereby regulating NF-κB activation. Methods: PELI1 expression in response to *H. pylori* infection or *H. pylori* LPS was monitored by quantitative real-time PCR in HEK-TLR2 cells and MKN45 gastric epithelial cells. In order to determine the involvement of PELI1 during TLR2-mediated signaling, HEK-TLR2 cells were co-transfected with increasing quantities of an expression vector for PELI1, and either an NF-κB-reporter construct or promoter-reporter constructs for interleukin 8 (IL-8) and chemokine (C-C) motif ligand 20 (CCL20). Forty-eight hours post-transfection, cells were stimulated with either *H. pylori* LPS or the synthetic TLR2 ligand Pam_2CSK_4 for 8 hours. Additionally, co-transfection experiments were carried out using small interfering RNA (siRNA) for PELI1. Results: Stimulation of HEK-TLR2 and MKN45 cells with both intact *H. pylori* or LPS resulted in a significant increase in PELI1 mRNA expression. PELI1 over-expression in HEK-TLR2 cells resulted in a dose-dependent increase in NF-κB activity in response to both Pam_2CSK_4 and *H. pylori* LPS. Additionally, increased PELI1 expression led to transcriptional activation of the IL-8 and CCL20 promoters in *H. pylori* LPS-treated cells. Furthermore, PELI1 knock-down using siRNA inhibited the TLR2-mediated activating properties of Pam_2CSK_4 and *H. pylori* LPS. Conclusions: PELI1 expression positively regulates TLR2-mediated signaling in response to *H. pylori* LPS, resulting in increased NF-κB activation and pro-inflammatory chemokine induction. Thus, modulation of PELI1 by *H. pylori* and/or its products may be an important mechanism during *H. pylori*-associated pathogenesis.

S1649

Activation of Proteinase Activated Receptor-2 (PAR-2) and IL-8 Release in *H. pylori*-Infected Patients

Arne Kandulski, Thomas Wex, Klaus Monkemuller, Doerthe Kuester, Lucia C. Fry, Peter Malfertheiner

Background: Epithelial cells are activated by serine proteases via protease-activated receptors (PARs) in response to inflammatory challenges. In *H. pylori*-infected human gastric epithelial cells PAR-2 acivation mediates *H. pylori*-induced IL-8 secretion. Aim: To investigate the PAR-2 expression in gastric mucosa of *H. pylori*-infected and non-infected patients related to IL-8 secretion. Methods: 28 *H. pylori*-infected patients [male: 16/female: 12; 47.7±2.73 ys.] and 69 *H. pylori*-negative subjects [male: 46/female: 23, 47.9±1.83ys.] underwent upper GI endoscopy. In mucosal biopsies obtained from the antrum, PAR-2 and IL-8 expression was analyzed by RT-PCR. Histological evaluation of mucosal samples was performed according to the updated Sydney classification. Results: IL-8 gene expression was 4-fold increased in the mucosa of *H. pylori*-infected patients compared to non-infected ($P<0.0001$) and a positive coorelation between IL-8 and PAR-2 gene expression (r: 0.47, P=0.011) implicates a functional role of this pathway *In Vivo*. For PAR-2 gene expression, no differences were observed between both groups. Furthermore, a significant difference of the IL-8/PAR-2 ratio was identified between *H. pylori*-infected and non-infected ($P<0.0001$) implying additional mechanisms contributing to IL-8 release in course of *H. pylori* infection. Conclusions: PAR-2 is expressed in antral mucosa of *H. pylori*-infected and non-infected patients representing a positive correlation to IL-8 release and a novel pathway for the regulation of IL-8 release and inflammation in *H. pylori* induced gastritis *In Vivo*.

S1650

***Helicobacter pylori* Induce Distinct Cytokine Profiles and Phagocytic Activity in Suspended and Attached Macrophages**

Hua Ding, Tom G. Blanchard, Steven J. Czinn

Aims: Macrophages play important roles in bacterial infections and are capable generating multiple types of responses. The role macrophages play in the host response to *H. pylori* infection however remains largely undefined. The goal of this study is to elaborate on the influence of macrophages on the host response to *H. pylori*, and to compare the activities of macrophages in different physiologic states with regard to inflammation and regulation. Methods: Tissue macrophages were identified using confocal microscopy by examining sections of gastric mucosa from Helicobacter infected mice injected with Texas Red-labeled Dextran 24 hours prior to sacrifice, or by direct staining of sections for F4/80 antigen. For *In Vitro* studies with *H. pylori* SS1 antigen, bone marrow derived macrophages (BMMac) were stimulated with lysate antigen for 24 hours and the supernatant were assayed for IL-10 and TNFα by quantitative ELISA. For studies with live *H. pylori*, bacteria were incubated with BMMac for 30 minutes and then the BMMac supernatants were evaluated after 24 hours as described above. The response was compared for BMMac growing in suspension, and attached to tissue culture plates. The efficiency of phagocytosis was also compared by quantifying intracellular *H. pylori* by direct enumeration of Hematoxylin stained cells or by CFU determination. Results: Confocal microscopy revealed the presence of Texas Red Dextran labeled macrophages in the gastric submucosa and serosa of H. felis infected mice. Staining for F4/80 showed a wider distribution including the epithelium and lamina propria. *In Vitro*, plate bound BMMac responded to soluble *H. pylori* antigen by producing significantly greater amounts of TNFα compared to BMMac in suspension ($P < 0.041$) whereas BMMac in suspension produced significantly greater amounts of IL-10 ($P < 0.02$). Plate bound BMMac cells also produced more TNFα than BMMac in suspension when co-cultured with live *H. pylori* ($P < 0.05$). BMMac in suspension also were better at phagocytosis of *H. pylori* as indicated by the uniform presence of intracellular *H. pylori* after 30 minutes of co-culture. Conclusions: Macrophages are widely disseminated throughout the gastric mucosa in response to proinflammatory Helicobacter infection. *In Vitro*, attached macrophages preferentially produce TNFα in response to *H. pylori* bacteria or soluble antigen compared to suspended macrophages, whereas macrophages in suspension preferentially produce IL-10. The character and morphology of gastric macrophages during Helicobacter infection therefore may determine whether they contribute to inflammation or regulation.

S1651

Genotypic Features of *Helicobacter pylori* Isolated From Residents of Aklavik, Northwest Territories, Canada

Maysoon A. Mahmoud, Monika M. Keelan, Megan Burlet, Sander Veldhuyzen van Zanten, Karen J. Goodman

Background: *Helicobacter pylori* infection and gastric cancer have an elevated occurrence in aboriginal communities of northern Canada. Several virulence factors are associated with distinct manifestations of *H. pylori* infection. The aim of this study is to genotype *H. pylori* isolated from residents of Aklavik (a remote aboriginal community in the NWT, population ~600), with respect to the cytotoxin-associated gene pathogenicity island (*cag* PAI), the EPIYA motifs of the *cagA* gene (tyrosine phosphorylation sites of CagA), and the regions of the vacuolating cytotoxin gene A (*vacA*). Methods: Genomic DNA was isolated from 117 *H. pylori* isolates cultured from gastric biopsies obtained from residents of Aklavik, NWT (biopsies were taken from 194 participants in a community *H. pylori* study that revealed 58% of 313 study participants were *H. pylori* positive). Bacterial genotypes were determined by polymerase chain reaction analysis of the *cagA*, *cagE* and *vacA* genes. The *cagA* and *cagE* genes were used as markers of the *cagA* PAI. All *cagA* positive isolates were further characterized for the number and the type of EPIYA motifs. The signal (s), intermediate (i) and middle (m) regions of the *vacA* gene were typed and correlated with the presence of *cagA*. Results: The *cagA* gene was detected in 33% (39/117) of the *H. pylori* isolates. 87% (34/39) of *cagA* positive isolates were also *cagE* positive. The *vacA* gene was detected in 96% (112/117) of *H. pylori* isolates. All *vacA* negative isolates (5/117, 4%) were also *cagA* negative. The presence of more than one *vacA* type indicated mixed infections (11/112, 10%) and all but one were *cagA* positive. The *vacA s1/i1/m1* type (26/101) was significantly associated with the presence of *cagA* (25/26, $p<0.001$). Most *cagA* positive mixed infections (9/10) contained *s1/i1/m1* among their vacA types. *H. pylori* isolates that were negative for *cagA* were associated with *vacA* types *s2/i2/m2* (29/101), *s1/i2/m2* (43/101), *s1/i1/m2* (1/101), *s1/i2/m1* (1/101), and *s1/i(untypeable)/m2* (1/101). 30 *cagA* positive isolates contained the ABCC EPIYA motif. Additional EPIYA types (ABC, ABCCC) were present in the mixed infections. Conclusions: The *vacA s1/i1/m1* type was almost exclusively present in *cagA* positive *H. pylori* isolates from Aklavik which also had multiple EPIYA-C sites. Only the *vacA i2* intermediate region was highly associated with *cagA* negative isolates. The presence of *cagA*, *cagE*, *vacA s1/i1/m1* and multiple EPIYA-C type in *H. pylori* isolates may contribute to the reported high prevalence of moderate and severe infections in residents of Aklavik.

S1652

Longitudinal Analysis of Serological Responses of Adults to *Helicobacter pylori* Antigens

Guillermo I. Perez Perez, Anna MAw, Lani Feingold-Link, Jennifer Gunn, Andrea L. Bowers, Cecilia Minano, Hilpi Rautelin, Timo U. Kosunen, Martin J. Blaser

Since *Helicobacter pylori* persist for decades in the human stomach, the aim of this study was to examine the long-term course in *H. pylori*-specific serum IgG responses with respect to subclass and antigenic target. We studied paired serum samples obtained in 1973 and in 1994 in Vammala, Finland from 64 healthy *H. pylori*-positive adults and from other healthy controls. *H. pylori* serum IgA, IgG, and IgG subclass responses were determined by antigen-specific ELISAs. *H. pylori*-specific IgG1 and IgG4 subtype responses from 47 subjects were similar in 1973 and 1994, but not when compared to unrelated persons. *H. pylori*-specific IgG1/IgG4 ratios amongst the participants varied > 1000-fold ; however, 89.4% had an IgG1/IgG4 ratio >1.0, consitent with a predominant IgG1 (Th1) response. Furthermore, ratios in individual hosts were stable over the 21-year period ($r=0.56$, $p< 0.001$). HspA status was unchanged in 49 (77%) of the 64 subjects tested; of the 15 who changed serostatus, all seroconverted and were significantly younger than those who did not change status. These findings indicate that *H. pylori*-specific antibody responses are host-specific with IgG1/IgG4 ratios stable over 21 years, IgG1 responses predominating, and HspA seroconversion trending with aging.

S1653

Targeted Disruption of Heat Shock Protein 70 Facilitates Cancer Progression in the Corpus of *Helicobacter pylori* Infected Mice

Susan J. Hagen, JiHye Seo, Nancy S. Taylor, Sureshkumar Muthupalani, James G. Fox

Despite that infection with *Helicobacter pylori*(HP) is an important risk factor for the development of human gastric cancer, little is known about bacterial or host factors that facilitate cancer progression. Substantial evidence indicates that the induced expression of heat shock protein 70 (HSP70), an important chaperone protein that facilitates mucosal protection, is down-regulated during HP infection, *In Vivo*. Thus, our aim was to determine whether the attenuation of HSP70 facilitates cancer progression in HP-infected mice. **Methods**: Mice