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

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

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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 activation 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: 16females: 12, 47.7±7.3 ys.] and 28 H. pylori-negative subjects [male: 16females: 12, 47.9±7.8ys.] underwent endoscopy. Genotypic and sero logical analysis was carried out using real-time 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). There was a positive correlation between IL-8 and PAR-2 gene expression (r=0.47, P=0.01) implicating 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-1β/PAR-2 ratio was determined between H. pylori-infected and non-infected patients (P=0.0001) implying additional mechan-isms 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 infected gastritis in vivo.

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

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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 evaluate 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 infection and regulation. Methods: Tissue macrophages were identified using confocal microscopy by examining sections of gastric mucosa and Helicobacter infected mice infected with Texas Red 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 lipase antigen for 24 hours and the supernatants were assayed for IL-10 and TNFα by 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 efficacy 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. fells 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<0.001) whereas BMMac in suspension produced significantly greater amounts of IL-10 (P<0.02). Plate bound BMMac infected more TNFα and IL-10 compared to BMMac co-transfected 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 culture. Conclusions: Macrophages are widely disseminated throughout the gastric mucosa in response to portal inflammatory Helicobacter infection. In vitro, attached macrophages therefore may determine whether they contribute to inflammation or regulation.

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

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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 presence of the cagA gene and the expression of the vacuolating cytotoxin gene A (vacA).

Results: The cagA gene was present in 117 H. pylori isolates cultured from gastric biopsies obtained from residents of Aklavik. (biopsies were taken from 194 participants in a community H. pylori study that revealed 58% (114/194) 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 cag PAI. All cagA-positive isolates were further characterized for the number and the type of EPIYA motifs. The signal (s), intermediate (i) and multiple (m) regions of the vacA gene were typed and correlated with the presence of cagA.

Conclusions: The cagA gene was detected in 33% (39/117) of the H. pylori isolates. 87% (31/36) 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 indicating mixed infections (11/12, 10%) and all but one were cagA positive. The vacA s1i1m1 type (26/101) was significantly associated with the presence of cagA (25/26, p<0.001). Most cagA-positive mixed infections (9/101) contained s1i1m1 among their vacA types. H. pylori isolates that were negative for cagA were associated with vacA types s1i1m2 (5/101), s1i2m2 (4/101), s1i1m2/s1i2m1 (3/101), s1i2m1 (1/101), and s1i1/m1 (9/101). 30 cagA positive isolates contained the ABCCC EPITAMOTY genes. Additional EPITAMOTY genes (ABC, ABCC) were present in the mixed infections. Conclusions: The vacA s1i1m1 type was almost exclusively present in cagA-positive H. pylori isolates from Aklavik which also had multiple EPITAMOTY-C sites. Only the vacA s2i2m2i2 interme-diate region was highly associated with cagA negative isolates. The presence of cagA, cagE, vacA s1i1m1 and multiple EPITAMOTY-C type in H. pylori isolates may contribute to the reported high prevalence of moderate and severe infections in residents of Aklavik.

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Longitudinal Analysis of Serological Responses of Adults to Helicobacter pylori Antigens

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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 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 IgM subclass responses were determined by antigen-specific ELISAs. H. pylori-specific IgG1 and IgA subtype responses from 47 subjects were similar in 1973 and 1994, but not when compared to uninfected persons. H. pylori-specific IgG1/ IgG4 ratios amongst the participants varied > 1000-fold ; however, 89% had an IgG1/IgG4 ratio >1.0, consistent with a predominant IgG1 (Th1) response. Furthermore, rapid or gradual subclass changes in the samples from the same individual were generally not observed over the 21-year period. 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.

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

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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, our aim was to determine whether the attenuation of HSP70 facilitates cancer progression in HP-infected mice.

Methods: Mice...