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ORIGINAL ARTICLE

Cholesterol glucosylation by *Helicobacter pylori* delays internalization and arrests phagosome maturation in macrophages

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Background/Purpose: Helicobacter pylori colonizes the human stomach and contributes to chronic inflammation of the gastric mucosa. *H. pylori* persistence occurs because of insufficient eradication by phagocytic cells. A key factor of *H. pylori*, cholesterol-α-glucosyltransferase encoded by *capJ* that extracts host cholesterol and converts it to cholesteryl glucosides, is important to evade host immunity. Here, we examined whether phagocytic trafficking in macrophages was perturbed by *capJ*-carrying *H. pylori*.
Methods: J774A.1 cells were infected with *H. pylori* at a multiplicity of infection of 50. Live-cell imaging and confocal microscopic analysis were applied to monitor the phagocytic trafficking events. The viability of *H. pylori* inside macrophages was determined by using gentamicin colony-forming unit assay. The phagocytic routes were characterized by using trafficking-intervention compounds.
Results: Wild type (WT) *H. pylori* exhibited more delayed entry into macrophages and also arrested phagosome maturation more than did *capJ* knockout mutant. Pretreatment of genistein and LY294002 prior to *H. pylori* infection reduced the internalization of WT but not *capJ*-knockout *H. pylori* in macrophages.

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Conclusion: Cholesterol glucosylation by *H. pylori* interferes with phagosome trafficking via a lipid-raft and PI3K-dependent manner, which retards engulfment of bacteria for prolonged intracellular survival of *H. pylori*.

Introduction

Helicobacter pylori colonizes the human stomach in approximately half the population over a lifetime. If not treated, *H. pylori* is an exceptionally successful pathogen. Persistent infection with *H. pylori* causes chronic inflammation, which may lead to cellular atrophy and gastric malignances.[1,2,3,4] In 1994, this bacterium was categorized as a Group I carcinogen by the World Health Organization's International Agency for Research on Cancer.[5] Using the mongolian gerbil, a model of gastric carcinogenesis was established,[6,7] starting with *H. pylori* infection that progresses to gastritis, intestinal metaplasia, and dysplasia to cancer as originally proposed by Correa.[8]

Virulent *H. pylori* strains, or type I strains, carry *vacA* and the *cag* pathogenicity island (*cagPAI*), which encodes a type IV secretion system (TFSS) and a key virulence factor called CagA that plays an important role in oncogenesis.[9,10,11] CagA is currently the only bacterial protein known to be delivered into the host cell by TFSS. TFSS-mediated CagA translocation and phosphorylation, as well as CagA-induced responses during *H. pylori* infection, are cholesterol dependent.[12,13] Interestingly, the delivery of CagA into epithelial cells is also influenced by cholesteryl glucosides (CGs) present in the *H. pylori* cell wall.[13,14] Cholesterol-α-glucosyltransferase (CGT) is an important enzyme in this regard, and it is encoded by *capJ* and synthesizes cholesterol-α-D-glucopyranoside (αCG) from host cholesterol. αCG can be further modified to cholesteryl-6′-O-tetradecaboyl-α-D-glucopyranoside (αCAG) and cholesteryl-6′-O-phosphatidyl-α-D-glucopyranoside (αCGP).[15] Because the catalytic product of CGT is a starting material for other cholesteryl derivatives, the loss of this enzyme in Δ*capJ H. pylori* leads to complete deficiency in CGs in the bacterial cell wall as opposed to the large amount that is present in wild type (WT) *H. pylori* (25% of the total lipids).[16] The Δ*capJ H. pylori* exhibits a greatly reduced ability to inject CagA,[14] it also has a substantially lower ability to escape phagocytosis by macrophages and to reduce antigen-specific T-cell responses in antigen-presenting cells, evading clearance by the host immune system.[17]

Although phagocytosis is frequently considered a useful means to eliminate invading microbes,[18,19] *H. pylori* has developed a way to evade attack by macrophages and causes chronic inflammation. Phagocytosed *H. pylori*, particularly type I strains, can survive even though efficient engulfment by macrophages occurs.[20] Delayed phagocytosis resulting from the need to reorganize the membrane architecture and cytoskeleton has been noted for type I strains[21] in which the type IV secretion apparatus functions as an adhesin-like molecule.[14] Whether any other effectors are involved in this process remains unknown. In this investigation, we defined the role of CGT in influencing bacterial engulfment and phagosome maturation processes.

Materials and methods

Bacteria, cell culture, and construction of *H. pylori* mutants

The reference strain *H. pylori* 26695 (ATCC 700392) was used in this study. *H. pylori* was cultured on *Brucella* agar plates (Becton Dickinson, Franklin Lakes, NJ, USA) supplemented with 2.8% *Brucella* broth powder, 0.2% β-cyclodextrin, 0.1% yeast extract, 1.5% agar, 1% isovitalex, and 10% sheep blood at 37°C in a microaerophilic atmosphere (10% CO_2, 5% O_2, and 85% N_2) for 2 days. The murine macrophage cell line J774A.1 (TIB-67; American Type Culture Collection) was cultured in Dulbecco Modified Eagle Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT, USA) at 37°C in 5% CO_2. The isogenic *H. pylori* mutants Δ*cagA*, Δ*capJ*, and *ΔcapJ*-in strain were constructed as described.[12,14]

Live-cell imaging of J774A.1 cells infected with *H. pylori*

J774A.1 cells were plated on a glass-bottom 35-mm dish at a concentration of 2×10^5 cells per dish and cultured overnight to obtain 50% confluency. Cells were infected with PKH67 (Sigma-Aldrich, St. Louis, MO, USA)-stained *H. pylori* at a multiplicity of infection (MOI) of 50. Infected live cells were then incubated at 37°C in 5% CO_2 for 5 hours and visualized with an LSM780 confocal laser scanning microscope (Carl Zeiss, Gottingen, Germany). Images of serial optical sections (512 × 512 pixels) were obtained using a Plan-Apochromat 63 × /1.4 differential interference contrast (DIC) objective. Confocal laser (488 nm) and phase-contrast images were collected at 1-minute intervals for 5 hours.

Gentamicin colony-forming unit assay

Bacterial survival inside macrophages was determined with a gentamicin colony-forming unit (CFU) assay.[22] J774A.1 cells (1×10^5) were seeded in 24-well plates and cultured overnight to obtain 80% confluency. Cells were infected with *H. pylori* at an MOI of 50 and synchronized by centrifugation at 600*g* for 5 minutes. After incubation for 1 hour at 37°C, noninternalized bacteria were killed by addition of 100 μg/mL gentamicin to the culture medium for 1 hour. Macrophages were then washed three times with phosphate