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***Helicobacter pylori*: Expect the Unexpected**

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An unexpected finding. Akin to the lone majestic drive that lures golfers back to the links after a forgettable round, seasoned investigators often point to these as being most influential in altering research directions. *Helicobacter pylori* is an organism that epitomizes unexpected outcomes as its very discovery challenged centuries of dogma that the stomach was a sterile sanctuary, inhospitable to bacterial colonization. Subsequent studies have determined that chronic *H. pylori* infection not only causes certain diseases such as peptic ulceration and distal gastric adenocarcinoma but, paradoxically, is inversely associated with other pathologic outcomes including complications of gastroesophageal reflux disease and atopic disorders.

Due to its ability to infect humans for decades, *H. pylori* harbors effectors that interact differently with hosts than the respective counterparts in other mucosal pathogens. Unlike FlaA of *Salmonella* or other Gram-negative organisms, *H. pylori* FlaA is a non-inflammatory molecule, which may contribute to long-term persistence (Lee *et al.*, 2003, Peek *et al.*, 2010, Gewirtz *et al.*, 2004). Another conserved constituent expressed by *H. pylori* that induces an attenuated host response is lipopolysaccharide (LPS), and *H. pylori* LPS is ~1000 fold less immunogenic than *E. coli* LPS (Perez-Perez *et al.*, 1995, Muotiala *et al.*, 1992, Ogawa *et al.*, 2003). LPS cloaks Gram-negative bacteria and performs multiple functions, including protection against complement-mediated attack and evasion of the immune response. Small amounts of LPS are liberated under homeostatic conditions, such as bacterial replication; however, antibiotic therapy, phagocytosis, complement-mediated attack, or treatment with divalent cation chelators can augment LPS release (Matsuura, 2013, Cullen *et al.*, 2011, Needham & Trent, 2013).

LPS is composed of a hydrophobic membrane anchor termed lipid A, a core oligosaccharide, and a distal polysaccharide chain or O-antigen that extends into the extracellular milieu (Raetz *et al.*, 2007). O-antigens consist of up to 50 repeated oligosaccharides, each harboring between 2–8 units of monosaccharide. Due to their variability in structure, even within the same bacterial species, O-antigens are frequently used as tools for taxonomic classification. In pathogenic bacteria, although O-antigen is not essential for immunostimulatory responses associated with LPS, it is an important virulence determinant and can protect microbes from lysis due to complement, antibiotics, and/or antibacterial peptides.

H. pylori can evade the host immune response by producing O-antigen structures that mimic human glycosphingolipids (Moran *et al.*, 1996). This occurs via the addition of fucose residues to O-antigens to generate Lewis antigens, which are normally expressed on human gastric epithelium. Specifically, *H. pylori* modifies its O-antigen by synthesizing Lewis X

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(Le^x), which is generated by adding fucose to the *N*-acetyl- β -lactosamine, and Lewis Y (Le^y), which is generated by adding a second fucose residue to the Le^x antigen (Matsuura, 2013). *H. pylori* strains harvested from patients with gastritis alone typically harbor LPS that is either non-glycosylated or glycosylated with either Le^x or Le^y but not both. In contrast, *H. pylori* isolates associated with diseases such as ulceration or cancer more often harbor highly glycosylated LPS moieties consisting of both Le^x and Le^y (Skoglund *et al.*, 2009).

Lipid A or endotoxin represents the bioactive anchored portion of LPS and is recognized by the host innate immune receptor TLR4/MD2. In an infected host, liberated LPS forms a complex with LPS binding protein (LBP) and soluble CD14. The LPS is then delivered to membrane-associated CD14 and then to TLR4/MD2, which initiates a proinflammatory signaling cascade (Schumann *et al.*, 1990, Tobias *et al.*, 1995, Wright *et al.*, 1990, Haziot *et al.*, 1998). *E. coli* lipid A is a glucosamine disaccharide that is phosphorylated and acylated at six sites, and this hexa-acylated lipid A structure induces robust activation of TLR4/MD2 (Raetz & Whitfield, 2002). Conversely, variations in the lipid A structure can antagonize TLR4/MD2 activation, thereby limiting the pro-inflammatory response (Fujimoto *et al.*, 2013).

Synthesis of lipid A in Gram-negative bacteria progresses through a constitutive conserved cascade known as the Raetz pathway, which involves the activity of nine enzymes (Raetz *et al.*, 2007, Raetz & Whitfield, 2002). Modification of lipid A can vary between species and the requisite enzymes responsible for such modifications are primarily located in the periplasm, in the periplasmic leaflet of the inner membrane, or in the outer membrane. Lipid A modifications may involve charge alteration through the addition of polar groups including phosphoethanolamine or aminoarabinose and/or removal of 3-deoxy-D-mannooctulosonic acid (Kdo) sugars or phosphate groups; such modifications, which confer resistance to cationic antimicrobial peptides. Lipid A structure can also be altered by removing or changing the length of acyl chains, hence affecting recognition by TLR4/MD2 dimers (Gunn *et al.*, 1998, Cullen *et al.*, 2011, Tran *et al.*, 2005, Stead *et al.*, 2010).

H. pylori lipid A is similarly synthesized by the Raetz pathway, which generates a hexa-acylated molecule. However there are several additional modifications. Hp0021, a homolog of *E. coli* LpxE, removes the lipid A 1'-phosphate group, and the remaining free hydroxyl group facilitates the addition of phosphoethanolamine by Hp0022 (an EptA homolog), which alters the charge status of lipid A. This is augmented by the action of another phosphatase, Hp1580 (a homolog of *E. coli* LpxF), which removes the lipid A 4'-phosphate group. Removal of the outer Kdo by Hp0579 and Hp0580 (KdoH1/KdoH2 homologs) is important for promoting resistance to antibiotics. Hexa-acylated *H. pylori* lipid A then becomes tetra-acylated by the activity of Hp0694, a homolog of LpxR, which removes 3'-O-linked acyl chains. This five-step modification (1'-dephosphorylation, addition of phosphoethanolamine, removal of a Kdo subunit, 4'-dephosphorylation, and deacylation) renders *H. pylori* lipid A a weak stimulator of TLR4/MD2 (Stead *et al.*, 2010).

Although the mechanisms underlying *H. pylori* lipid A synthesis have been well-delineated, detailed understanding of this cascade remained incomplete as only 8 of the 9 enzymes in the Raetz pathway had been identified (Figure 1). In *E. coli* lipid A synthesis, the presence of Kdo sugars is required for two of the late acyltransferases, LpxL and LpxM, to acylate lipid IV_A in a step-wise manner. In *H. pylori*, no obvious homolog of *E. coli* LpxM exists based purely on sequence homology searches. In this issue of *Molecular Microbiology*, Rubin *et al.* (Rubin *et al.*, 2014) have now identified and characterized an *H. pylori* gene encoding this missing enzyme. Using the Pfam database, which segregates proteins by predicted functional domains, the authors cleverly identified the locus jhp0255 of *H. pylori* strain J99 (similar to HP0270 in *H. pylori* strain 26695) as an ORF encoding a product that

shares homology only within the catalytic domain of *E. coli* LpxM. This putative enzyme, which was renamed LpxJ due to limited overall sequence homology with *E. coli* LpxM, was then shown to catalyze the addition of a secondary acyl chain to the 3'-linked acyl chain of *H. pylori* lipid A (Figure 1). Unexpectedly, the function of this enzyme showed substantial flexibility in terms of substrate. LpxJ can acylate Kdo₂-lipid A that is either tetra- or penta-acylated, in contradistinction to *E. coli* LpxM, which can only acylate penta-acylated Kdo₂-lipid A and only after LpxL acylation. Such versatility in LpxJ substrates and order broadens the repertoire of end products that can be generated in the late stages of *H. pylori* lipid A biosynthesis. These results were subsequently extended into two related organisms, *Campylobacter jejuni* and *Wolinella succinogenes*, which were also found to harbor LpxJ homologs that function in a similar non-ordered manner (Rubin *et al.*, 2014).

What are the biological ramifications of this work? *H. pylori* is one of the most common bacterial infections worldwide, and virtually all infected persons develop co-existing gastritis, a signature feature of which is the capacity to persist for decades (Wroblewski *et al.*, 2010). In support of its lifestyle, *H. pylori* has evolved to express an array of diverse phenotypes, including enzyme functional diversity, that help to subvert obstacles presented by the human host, which permits long-term microbial colonization. The versatility of LpxJ may also allow *H. pylori* to quickly adapt to dynamic and hostile conditions present within its cognate gastric niche. Finally, *H. pylori* eradication rates are often less than 80% in regions of the world where diseases such as cancer are most prevalent, necessitating the development of new treatment regimens. The elucidation and careful study of enzymes involved in LPS biosynthesis offers the possibility to develop new therapeutic tools for more effective elimination of *H. pylori* infection.

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Figure 1.
Role of LpxJ in the synthesis of *H. pylori* lipid A.