Modulation of host cell signal transduction pathways by *Helicobacter pylori* infection

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Bacterial pathogens modulate host cell signal transduction responses to establish infection and cause disease. The purpose of the present summary, first presented at the Canadian Helicobacter Study Group meeting, is to discuss current knowledge of specific *Helicobacter pylori* factors, including the vacuolating cytotoxin, cytotoxin-associated gene A and the type four secretion system encoded by the cytotoxin-associated gene pathogenicity island and review the host cell signal transduction cascades that they modulate.

Key Words: *Epithelium;* H pylori*; Signal transduction*

La modulation des voies de transduction du signal des cellules hôtes par l'infection à *Helicobacter pylori*

Les pathogènes bactériens modulent les réponses de transduction du signal des cellules hôtes pour établir une infection et provoquer une maladie. Le présent résumé, présenté pour la première fois à la réunion du groupe d'étude canadien de l'*Helicobacter*, vise à aborder les connaissances actuelles sur des facteurs précis de l'*Helicobacter pylori*, y compris la cytotoxine vacuolante, le gène A associé à la cytotoxine et le système de sécrétion de type quatre codé par l'îlot de pouvoir pathogène du gène associé à la cytotoxine, et à examiner les cascades de transduction du signal des cellules hôtes qu'elles modulent.

MODULATION OF HOST CELL SIGNALLING BY BACTERIAL PATHOGENS

Current evidence suggests that bacterial pathogens establish infection and cause disease by modulating host cell signal transduction responses. Indeed, disrupting the ability of pathogens to modulate host signalling cascades can alter the sequelae of infection. For example, treating mice with Gram-positive lipoprotein leads to cytokine production and induces shock; effects that are ameliorated by cotreatment with a monoclonal antibody directed against the host cell lipoprotein receptor, Toll-like receptor (TLR)-2 (1). Also, humans suffering from traveller's diarrhea treated with a calmodulin kinase inhibitor experience a decreased severity and duration of the disease (2). In addition, pharmacological inhibition of the mitogen-activated protein kinases (MAPKs) p38 and c-jun N-terminal kinase proved safe and effective for treatment of Crohn's disease in humans (3). Such observations indicate that knowledge of signalling cascades can facilitate therapeutic intervention; however, a sound understanding of host cell signalling pathways modulated by specific bacterial factors is first required. The purpose of the present summary, first presented at the Canadian Helicobacter Study Group meeting, is to discuss current knowledge of specific *Helicobacter pylori* factors and the host cell signal transduction cascades they modulate. Putative host cell signal transduction pathways modulated by *H pylori* infection are shown in Figure 1.

ADHESION TO HOST EPITHELIAL CELLS

Bacterial adhesion to host cells is often a critical step for microbial infections as a prerequisite to colonization, internalization or the delivery of toxins (4). In vivo studies (5) to delineate tissue tropism of *H pylori* have shown that the organism binds specifically to gastric epithelial cells and in areas of gastric metaplasia, with multiple, redundant bacterial adhesins likely participating in adhesion (6).

Several laboratories have delineated bacterial adhesins and their respective host cell receptors (Table 1). Although adhesin-receptor binding at the host cell surface probably initiates host cell signalling casacades, the precise events triggered by such binding are not characterized. For example, *H pylori* heat shock protein 60 binds host cell sulfogalactosylceramide (7), but whether this is involved in heat shock protein 60-mediated activation of nuclear factor-kappa B (NF-κB) and interleukin (IL)-6 secretion through TLR-2 (8) is unknown.

Delineation of host receptors for bacterial adhesins remains a priority. For example, it was recently discovered that expression of trefoil factor-1 in the mucous layer of the stomach mirrors the tissue tropism of *H pylori* (9). Determination of the bacterial adhesin involved is an important future goal.

Of note, *H pylori* mainly reside in the mucous layer of the human stomach (10). Thus, bacterial adhesion to multiple host cell receptors, either secreted in the mucous or attached to the plasma membrane, in either healthy or inflamed gastric tissues, likely serves to mediate the chronic colonization observed following *H pylori* infection.

H PYLORI-INDUCED NF-κB ACTIVATION

H pylori infection induces secretion of IL-8 from human gastric epithelial cells (11,12), a process requiring NF-κB activation.

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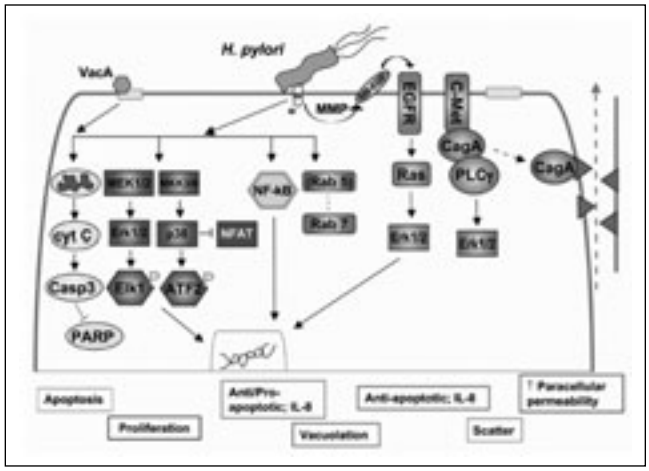

Figure 1) *Putative host cell signal transduction pathways modulated by* Helicobacter pylori *infection. Select examples of specific bacterial factors that activate host cell signalling cascades to elicit functional effects (outlined beneath the pathways). From left to right:* H pylori *vacuolating cytotoxin (VacA) (hexagon) induces mitochondrial membrane damage, ultimately leading to caspase-3 (Casp3) activation, inhibition of poly (ADP-ribose) polymerase (PARP) and apoptosis. Furthermore, VacA interacts with host cell plasma membrane microdomains enriched with cholesterol to exert functional effects. Next, activation of mitogen-activated protein kinase cascades, including extracellular signal-regulated kinase 1/2 (Erk1/2) and p38, may be involved in causing proliferation of host cells, while nuclear factor-kappa B (NF-κB) activity may exert both pro- and antiapoptotic activity, and is involved with induction of interleukin (IL)-8 secretion. Host cell vacuolation is achieved mainly through activation of the small GTPase Rab7. In addition,* H pylori *injects cytotoxin-associated gene A (CagA) into host cells via a type IV secretion system. CagA is then tyrosine phosphorylated and induces multiple signalling cascades by affecting host cell surface receptors, including the epidermal growth factor receptor (EGFR) and c-Met receptor, to ultimately cause IL-8 secretion and cell scattering, respectively. CagA also binds to Src homology-2-containing phosphatase and to the tight junction proteins zonula occludens 1 and junctional adhesion molecule to cause cytoskeletal changes and increased paracellular permeability, respectively. cyt C Cytochrome C; HB-EGF Heparin-binding EGF; MMP Metalloproteinase; NFAT Nuclear factor of activated T cells; PLCγ Phospholipase C gamma*

The proximal aspect of this pathway begins with functional tumour necrosis factor receptor-associated factor-2 and factor-6 adapter proteins, which are necessary to activate NF-κB-inducing kinase. NF-κB-inducing kinase subsequently phosphorylates inhibitory (I)κB kinase-α and -β which, when activated, phosphorylate IκBα, leading to degradation of IκBα by the proteosome. NF-κB is then released from its complex with IκBα, enabling the transcription factor to translocate from the cytoplasm to the nucleus and bind DNA (13).

Proinflammatory mediators, including bacteria and their products, can lead to the activation of NF-κB. This recognition of common microbial components, or pathogen-associated molecular patterns, is achieved through either cell surface expressed TLRs or intracellular nucleotide-binding oligomerization domain protein (Nod)1 and Nod2 receptors (14). Subsequent signal transduction initiated by the different TLR or Nod ligands can overlap, involving recruitment of IL-1 receptor-associated kinase-1 and -4 via an adaptor protein, such as myeloid differentiation factor-88, with subsequent activation of MAPKs followed by activation and nuclear translocation of NF-κB (15). However, the signalling pathways can vary based on the TLR or Nod activated, and on the adaptor molecules expressed in a particular cell type, leading to distinct gene expression effects.

H pylori flagellin is 1000-fold less potent at activating gastric epithelial cell TLR-5 than is flagellin of *Salmonella typhimurium* (16). In addition, blockade of TLR-4 using a monoclonal antibody did not prevent *H pylori*-induced IL-8 secretion from gastric epithelial cells (17). However, the absence of cytotoxin-associated gene (*cag*) E abolishes the ability of *H pylori* to induce NF-κB activity and IL-8 secretion from epithelial cells, but not from monocytes (18,19). Moreover, a *cag* pathogenicity island (PAI)-positive type I *H pylori* strain modulates expression of many genes in epithelial cells, as determined by microarray analysis, while gene expression in epithelial cells infected with an isogenic mutant of *H pylori* lacking the *cag* PAI more closely resembled uninfected cells (20). Taken together, this evidence suggests that *H pylori* does not activate NF-κB through ligation of TLRs on epithelial cells, but that the *cag* PAI is required.

VACUOLATING CYTOTOXIN

Vacuolating cytoxin (VacA) activity is produced by approximately 50% of *H pylori* isolates, but the *vacA* gene is encoded in the genome of all *H pylori* strains (21). Activity of the protein toxin is dependent on allelic variations in the gene, in which *vacA* alleles posses either signal region s1 or s2, and

TABLE 1
Adhesins of *Helicobacter pylori* and their host cell receptors

Reference	Bacterial adhesin	Host cell receptor
Gold et al (83)	Not characterized (employed whole, live *H pylori*)	Phosphatidylethanolamine and the glycolipid gangliotetraosylceramide
Valkonen et al (84)	~25 kDa outer membrane protein	Sialic residues in the basement membrane protein laminin
Huesca et al (7)	Bacterial heat shock protein 60	Sulfogalactosylceramide
Ilver et al (85)	Outer membrane protein: blood-group antigen-binding adhesin	Lewis B-type antigen (on red blood cells, gastric epithelia)
Linden et al (86) Van de Bovenkamp et al (87)	Blood-group antigen-binding adhesin	Mucin 5AC harbouring Lewis B-type antigen
Mahdavi et al (88)	Outer membrane protein: sialic acid-binding adhesin	Sialylated glycoconjugates expressed by inflamed gastric mucosa
Su et al (17)	Not characterized (employed whole, live *H pylori*)	Toll-like receptor-4
Clyne et al (9)	Not characterized (employed whole, live *H pylori*)	Trefoil factor-1

midregion m1 or m2 (in all possible combinations), and where s1/m1 represents the most active toxin (21,22). Clinical studies have associated higher VacA activity with more severe disease (23). However, this observation remains controversial, with variability depending on the human populations under study (24). Furthermore, in most studies (25) involving the pediatric population, there has been a lack of association between *vac*A alleles and disease outcome.

Nevertheless, production of the VacA toxin elicits an antibody response in infected humans, and these antibodies neutralize toxin activity in vitro (26). Moreover, a murine model of *H pylori* infection suggests that VacA production is advantageous to colonization of the stomach (27), and gastric administration of recombinant VacA to mice induced gastric ulcerations and lesions similar to those seen in infected humans (28). This damage occurs through VacA-mediated activation of its putative receptor, protein tyrosine phosphatase type-Z, and this damage was mimicked by addition of the natural ligand, pleiotrophin (29). Other putative VacA receptors include receptor-like protein tyrosine phosphatase-alpha and -beta (30), but their functional role in vivo is unknown. Together, this evidence suggests an in vivo role for VacA during pathogenesis.

There are multiple lines of in vitro evidence showing that VacA modulates host cell signal transduction and function. VacA is associated with the bacterial outer membrane and its secretion allows monomers of the toxin to assemble into hexamers or heptamers (31) in a process requiring acid activation (32). In the presence of host cells, VacA associates with plasma membrane microdomains, or lipid rafts, which are enriched in cholesterol and sphingolipids (33), and are becoming increasingly recognized as platforms for multiple pathogenic microbes to modulate host cell signalling (34). Whether putative VacA receptors also localize to membrane microdomains is unknown. Regardless, the toxin is internalized by cells in an actin-dependent process (35), where it is targeted to late endosomes (36), the Golgi apparatus and mitochondria (37). The toxin also inserts into the host cell plasma membrane to form anion-specific channels (38) that allow water to accumulate in the cell, contributing to the swelling phenotype observed during vacuolation (39). An increase in paracellular permeability of epithelial monolayers also results from treatment with VacA, which may provide a strategy for the bacterium to release nutrients from the host (40).

Phenotypically, VacA induces formation of numerous cytoplasmic vacuoles in host cells (41). These vacuoles exhibit an acidic microenvironment and express specific markers of late endosomes and lysosomes, the development of which are coordinated by the small GTPase Rab7 and, to a lesser extent, Rab5 (42). Functionally, in antigen-presenting cells, these vacuoles exhibit decreased proteolytic-dependent antigen processing capability (43). VacA binds to the intermediate filament VIP54, but the role of this interaction in vacuolation is not established (44).

In addition to vacuolation, VacA expression allows the bacterium to avoid endosomal fusion with the lysosome after phagocytosis (45). Toxin activity plays a role during induction of apoptosis in both epithelial cells (37) and macrophages (46). VacA also exerts effects on cells of the adaptive immune system, as VacA-induced p38 MAPK activity inhibits T-cell activation by suppressing nuclear factor of activated T-cell nuclear translocation (47). Furthermore, VacA inhibits epidermal growth factor-mediated signal transduction responses in KATO III gastric epithelial cells (48). Taken together, these observations indicate that this toxin exerts multiple effects on the host, including the ability to both activate and suppress host cell signal transduction responses.

CAG PAI

Sequencing of the *H pylori* genome in 1997 (49) facilitated a closer look at the *cag* PAI, an approximately 40 kb segment of DNA integrated into the *H pylori* chromosome that encodes about 30 genes (50). This PAI likely was acquired by horizontal transfer, as indicated by a guanine and cytosine content that is different from the remainder of the bacterial genome (35% versus 39% [49]) and by the transposable elements that flank its sequence (50). It is present only in some *H pylori* strains and, thus, serves as a marker to classify strains, whereby type I strains carry the *cag* PAI and type II strains do not. Geographical variability exists in expression of the *cag* PAI by *H pylori* strains. Nonetheless, studies in both Mongolian gerbils (51) and humans (21,52,53) suggest that the presence of the *cag* PAI correlates with more severe disease outcome in some populations. Although many functional responses by host cells to *cag* PAI-positive *H pylori* have been documented in vitro (below), a lack of association between these responses and gastroduodenal disease in humans could be made (54). Whether this represents a true lack of association or, rather, an incomplete understanding of the interplay between bacterial, host and environmental factors, is unclear (54).

The precise functions of genes encoded on the *cag* PAI remains incompletely characterized. Deletion of the *cag* PAI reduces the ability of *H pylori* to induce IL-8 secretion from host cells (55). Some genes on the *cag* PAI also show similarity to known genes in *Agrobacterium tumefaciens* that encode a type IV secretion system (56), which is a filamentous bacterial surface appendage that can breach the host cell membrane to inject bacterial proteins (57). For example, *cagE* (formerly known as picB) shows homology to genes from other bacteria that code for a type IV secretion system ATPase, which likely provides energy for the secretion of bacterial proteins. Functionally, CagE expression is required to induce IL-8 secretion from host cells (18,50,58). CagE also may be involved in the recruitment of Arp3 during *H pylori*-induced cytoskeletal rearrangements resulting in epithelial cell elongation (17).

CagA, the product of another gene on the PAI, is the first and only substrate to date that has been shown to be injected into host cells by the type IV secretion system (59,60). CagA is a 128 kDa to 145 kDa protein translocated into host cells where it is phosphorylated on tyrosine residues (60-63) by host cell Src kinase (64). Interestingly, the number of tyrosine residues on which CagA can be phosphorylated shows variation among strains, and more phosphorylation sites lead to increased CagA biological activity and association with disease (65). Functionally, CagA is implicated in cytoskeletal rearrangements (61) through binding Src homology-2-containing phosphatase (66) and dephosphorylation of host cell cytoskeletal proteins (67). CagA also binds to tight junction proteins, including zonula occludens 1 and junctional adhesion molecule to increase paracellular permeability of an epithelial monolayer (68). In addition, CagA affects host cell plasma membrane receptor activity. For instance, CagA is required to activate host cell heparin-binding epidermal growth factor-like growth

factor by inducing matrix metalloproteinase activity, ultimately leading to activation of epidermal growth factor receptor signalling (69). Furthermore, CagA attaches to the hepatocyte growth factor receptor c-Met to induce epithelial cell scattering through activation of phospholipase C gamma and extracellular signal-regulated kinase 1/2 MAPK (70).

While understanding of CagA is improving, our knowledge of other Cag proteins is minimal. For example, CagF associates with the bacterial outer membrane and induces an antibody response from infected humans, but its precise function remains unknown (71). Isogenic mutants lacking CagG, CagL, CagH, CagI or CagM all show a reduced capacity to stimulate DNA-binding activity of the host cell transcription factor AP-1 compared with the wild-type strain (72). Furthermore, CagG could be involved in bacterial adherence and the induction of IL-8 secretion (73), while CagP may be involved in adherence (74). Whether these Cag proteins are injected into the host cell and the precise signalling pathways they target remain to be defined.

In addition to characterizing the *cag* PAI, sequencing of genomes from two different *H pylori* strains showed that much of the genome was shared. However, a segment of DNA termed the plasticity region contains genes that are unique among strains (75). Similar to the *cag* PAI, this region displays a lower guanine and cytosine content than the rest of the genome, suggesting this region may also contain horizontally acquired PAIs (75). Knowledge of the genes encoded within the plasticity region is limited but growing. For example, genes coding for proteins with homology to type IV secretion system components unique from those encoded on the *cag* PAI have been localized to the plasticity region (76). Furthermore, a gene of unknown function found in the plasticity region, named *JHP947*, may be associated with duodenal ulcer and gastric cancer (77,78). Thus, a more thorough examination of gene products from the plasticity region, their effects on signal transduction and their association with disease is warranted in future studies.

SUMMARY

H pylori infection modulates signal transduction pathways in multiple host cell types, and activation of certain cascades can be attributed to specific bacterial factors. Indeed, some redundancy exists whereby multiple *H pylori* factors cause similar effects, such as induction of macrophage apoptosis by both CagA and VacA (46). On the other hand, infection can exert opposing effects, such as bacterial-induced NF-κB activity that is antiapoptotic (79). There are also instances whereby functional effects have yet to be associated with a specific bacterial factor, such as suppression of IL-4-induced signal transduction by *H pylori* infection (80), indicating a need for more work in the signal transduction area.

Future directions should employ multiple experimental techniques to characterize signalling pathways and functional outcomes modulated by *H pylori* infection. For example, signature tagged mutagenesis could reveal which *H pylori* genes are important during infection in vivo (81). Also, a proteomic analysis of host cell protein expression induced during *H pylori* infection, as was determined for enteropathogenic *Escherichia coli* (82), would be valuable in establishing functional outcomes of infection. Furthermore, future experiments should aim to determine the link between particular bacterial factors and the activation of specific signalling pathways and, ultimately, to clinical outcomes in infected individuals. Overall, such research provides a better understanding of the molecular mechanisms underlying chronic infectious disease, and may provide insight toward the development of therapeutic interventions based on knowledge of signal transduction.

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