

Host epithelial interactions with *Helicobacter pylori*: A role for disrupted gastric barrier function in the clinical outcome of infection?

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Infection of the human stomach with *Helicobacter pylori* may develop into gastritis, ulceration, adenocarcinoma and mucosal lymphomas. The pathogenic mechanisms that determine the clinical outcome from this microbial-epithelial interaction remain poorly understood. An increasing number of reports suggests that disruptions of epithelial barrier function may contribute to pathology and postinfectious complications in a variety of gastrointestinal infections. The aim of this review is to critically discuss the implications of *H pylori* persistence on gastric disease, with emphasis on the role of myosin light chain kinase, claudins and matrix metalloproteinases in gastric permeability defects, and their contribution to the development of cancer. These mechanisms and the associated signalling events may represent novel therapeutic targets to control disease processes induced by *H pylori*, a microbial pathogen that colonizes the stomach of over 50% of the human population.

Key Words: Epithelial; Gastric cancer; Gastritis; *Helicobacter*; Permeability; Tight junctions

Helicobacter pylori persistently colonizes the stomach of over one-half of the human population and, in the majority of cases, causes chronic gastritis. However, the microbial epithelial interactions and gastritis associated with infection may also cause gastroduodenal ulcers, gastric adenocarcinoma and mucosal lymphomas (1-3). An American study (4) found that the lifetime risk for development of gastric cancer in those infected with *H pylori* ranges between 1% and 3%. Despite intensive study, the host-microbial interactions that determine the clinical outcome of infection remain unknown. The main *H pylori* virulence factors that have been associated with pathogenesis to date include the pathogenicity island-encoded protein cytotoxin-associated antigen (CagA) and the vacuolating (VacA) cytotoxin (2,3,5-7). Indeed, CagA is the factor that has most often been linked with the development of adenocarcinoma (2,3,7). *H pylori* CagA and VacA have been shown to disrupt epithelial barrier function, and the focus of the present review is to discuss how this phenomenon may participate in disease pathogenesis. Insights into the operating principles that regulate the interactions between *H pylori* and host epithelia may improve our understanding of the initiation of the diverse disease processes associated with the infection, and help identify new therapeutic targets. The present review critically discusses the current knowledge of the effect of

Les interactions épithéliales de l'hôte avec *Helicobacter pylori* : Un rôle pour le dérèglement de la fonction de la barrière gastrique dans l'issue clinique de l'infection ?

L'infection de l'estomac humain à *Helicobacter pylori* peut se transformer en gastrite, en ulcération, en adénocarcinome et en lymphomes muqueux. Les mécanismes pathogènes qui déterminent l'issue clinique de cette interaction épithéliale microbienne demeurent mal compris. Un nombre croissant de comptes rendus laissent supposer que des dérèglements de la fonction de la barrière épithéliale contribueraient à la pathologie et aux complications postinfectieuses de diverses infections gastro-intestinales. La présente analyse vise à exposer d'un point de vue critique les répercussions de la persistance du *H pylori* sur la maladie gastrique, en s'attardant sur le rôle de la myosine kinase, des claudines et des métalloprotéinases matricielles dans les anomalies de la perméabilité gastrique, et leur contribution à l'apparition du cancer. Ces mécanismes et les événements de signalisation connexes peuvent représenter de nouvelles cibles thérapeutiques pour contrôler les processus de la maladie induits par le *H pylori*, un pathogène microbien qui colonise l'estomac de plus de 50 % de la population humaine.

H pylori on epithelial permeability and explores its possible clinical significance, with particular emphasis on how it may be linked to the development of gastric cancer.

H PYLORI PERSISTENCE AND DISEASE

H pylori infection of the human stomach is most commonly acquired in childhood. Although this microaerophilic bacterium remains in the gastric environment of its host for decades, consequences of colonization are usually benign, marked by a persistent mucosal inflammatory reaction (7,8). However, in a subset of hosts, this gastritis may lead to severe pathology. In addition to its well-established role in peptic ulcer disease and noncardiac gastric adenocarcinoma, *H pylori* infection has also been implicated in the development of extragastroenteric diseases (1-3,7,9-13). The possible implication of altered epithelial permeability in the etiology of any of these abnormalities remains a topic of ardent controversy.

The ability of *H pylori* to chronically colonize the human stomach and to cause disease is the focus of intense research and is the topic of recent reviews (5-8). The scope of host-microbial interactions and their influence on disease development are briefly reviewed in the following section. The difficulty of infecting animal models with human *H pylori* isolates should be noted. Similarly, nontransformed confluent human gastric

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cell lines with functional tight junctions have only been established very recently (14). This has made research difficult, and forced scientists to use models of epithelial cells belonging to phenotypes that may normally not be infected by *H pylori*. In view of these limitations, while highlighting observations based on human gastric cells, the present review critically addresses research findings that are most relevant to the topic, regardless of the model system being used.

H pylori is well-adapted to the hostile environment of the human stomach. The bacterium is likely transmitted via the fecal-oral route and, once established, can persist for the lifetime of the host. *H pylori* demonstrates a marked tropism for gastric mucus-producing epithelial cells (15). The bacterium expresses a number of adhesins that allow tight attachment to epithelial cells; this interaction is ultimately the cause of the chronic inflammatory response in the host (5,7,16-18). These adhesins also direct *H pylori* colonization to areas of the stomach containing only few parietal cells, further allowing the bacterium to circumvent gastroprotective acid production (19). Facilitation of this process appears to be aided by the fact that longstanding *H pylori*-induced gastritis leads to the loss of specific cell types in the stomach, and a shift in the types of glycans expressed on the surface of gastric epithelial cells (16,19). Remarkably, therefore, *H pylori* can modulate its adhesin expression pattern to colonize a variety of gastric niches, and to selectively avoid areas of active inflammation that may be capable of clearing the infection. *H pylori* is an incredibly adaptable micro-organism that is able to persist in the changing and hostile environment of the stomach, even in the presence of a chronic inflammatory response.

IMMUNOPATHOLOGY OF *H PYLORI* INFECTIONS

There is growing evidence that, in addition to being unable to clear the infection, the T helper cell (Th)-1 polarity of the host response to *H pylori* infection contributes to the development of disease in the host. Production of Th-1 cytokines, including interferon gamma (IFN γ), tumour necrosis factor alpha (TNF α) and interleukin-1beta (IL-1 β) are increased during *H pylori* infection, which in turn amplifies the inflammatory response (20). IL-1 β is also a potent inhibitor of gastric acid secretion (21). Interestingly, polymorphisms in the human IL-1 loci, which are implicated in the increased IL-1 β production, are also associated with hypochlorhydria and gastric cancer in patients infected with *H pylori* (22). Several reports (23-26) have suggested that the host Th-1 response to the infection may contribute to the carcinogenic effects of the bacterium. Similarly, *H pylori*-stimulated upregulation of inducible nitric oxide synthase promotes apoptosis and induces carcinogenesis during the infection, via mechanisms that remain unclear (27-30). In addition, though still incompletely understood, the events leading to *H pylori*-induced B cell proliferation and mucosa-associated lymphoid tissue lymphoma appear to involve cytokines of both the Th-1 and the Th-2 subsets (31-33).

The principles governing the host microbial interactions during an infection with *H pylori* result from a complex multifactorial process. For example, microbial factors may include adaptive point mutations that allow variants to emerge after selective pressure (eg, antibiotic therapy) (34), restriction barriers to genetic transformation that favour maintenance of diversity (35), and other factors that add to genetic diversity,

such as local selection due to ligand specificity for local receptors (16,36). Conversely, host factors that contribute to the chronicity of infection include determinants of bacterial tropism to various gastric niches that circumvent gastroprotective barriers like acid-producing parietal cells (19), and factors that polarize the primary adaptive immunity to *H pylori* to a Th-1 response (20).

Th-1 immunity also appears to compromise parietal cell responses and induce proliferation of epithelial glycan receptors for *H pylori* adhesins (37). Interestingly, concurrent intestinal helminth infections that drive a polarized Th-2 immune response reduce *H pylori*-induced gastritis and premalignant gastric atrophy (38). This phenomenon has been linked to the 'African enigma', which refers to the surprisingly low rates of gastric cancers in African countries despite equally high prevalence rates of *H pylori* infection (38). Low-grade mucosa-associated lymphoid tissue lymphomas often regress on elimination of *H pylori* and, conversely, they may relapse after *H pylori* reinfection (39-41). Persistent host-microbe interactions with *H pylori* also increase the risk for gastric adenocarcinoma in humans, the second leading cause of cancer-related death worldwide (8,10,42). As a result, *H pylori* is now considered to be a class I carcinogen, and this risk is most commonly associated with the ability of some strains to inject CagA into the host cell (43,44). The development of *H pylori*-induced 'intestinal type' adenocarcinoma occurs in a well-defined sequence of events. First, the normal gastric mucosa progresses through chronic superficial gastritis, which then leads to atrophic gastritis, and the still poorly understood formation of gastric ulcers. Atrophic gastritis can progress into metaplasia, dysplasia and, ultimately, adenocarcinoma (3,8,10,42,45). Yet, for reasons that remain unclear, only a relatively small number of *H pylori*-infected patients ever proceeds from gastritis to neoplasia formation (4). Together, these observations are consistent with the hypothesis that the oncogenic potential of *H pylori* may be strain- and/or host-dependent, and related to specific signalling events of the epithelial-microbial interaction. Little is known about how these events may be causally related, and the role played by determinants of epithelial barrier function in carcinogenesis remains obscure. As discussed below, such determinants of epithelial barrier may include tight junctional proteins including claudins. Figure 1 illustrates the main components of gastric tight junctional and adherens proteins discussed in the present review.

Loss of epithelial barrier function: The role of claudins and matrix metalloproteinases

While the majority of *H pylori* micro-organisms swim in the mucus layer coating the gastric epithelium, approximately 10% eventually adhere to the epithelial cells (46). *H pylori* attachment to epithelial cells alters the outcome of infection. Using a murine model of infection, a recent study (47) observed that in the gastric mucosa, the mucus cell exhibits the greatest transcriptional response to *H pylori*. Subsequent cellular alterations implicate genes that broadly regulate inflammation, angiogenesis, iron metabolism and tumour suppression (47). Loss of cell-to-cell adhesion is a well-established precursor of gastric adenocarcinomas, and abnormalities of the adherens junctional protein E-cadherin have been found to contribute to this phenomenon as well as to the migratory potential of tumour cells (48,49). In keeping with these findings, functional inhibition of E-cadherin through mutations initiates

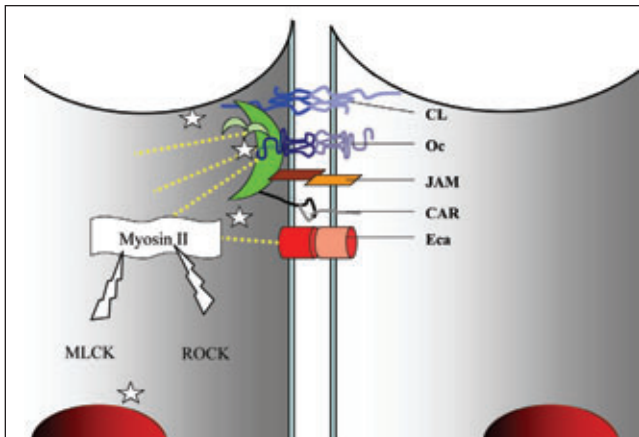


Figure 1) Schematic illustration of the junctional complexes linking polarized epithelial cells. Comprehensive listings of tight junctional elements are available in a number of reviews (68-75). The various components of the intercellular junctions have been assigned to three different groups, and their main representatives are listed below, giving particular emphasis to those discussed in the text. 1) Intercellular junctional proteins: these include tight junctional proteins per se such as claudins (CL), occludin (Oc), junctional adhesion molecules (JAM), and coxsackie virus and adenovirus receptor (CAR), as well as proteins of the adherens junctions such as E-cadherin (Eca) which helps initiate tight junctional assembly. Any of these may be directly modified by microbial products to increase paracellular permeability. 2) Junctional plaque proteins (crescent-shaped in the figure): these include scaffolding protein interaction domain proteins such as those belonging to the zonula occludens family, cingulin or those representing partitioning-defective proteins (PAR3 and PAR6). The main function of these proteins is to help crosslink tight junctional proteins to the actin cytoskeleton (dotted lines). 3) Regulatory factors (star-shaped in the figure): these products may be cytosolic or linked to tight junctional proteins, to the plasma membrane, to cellular actin filaments or to the nucleus. They include atypical protein kinase C, zonula occludens-1-associated nucleic acid binding protein and a number of G proteins, as well as a long list of other factors. Their principal function is to signal and regulate, at the transcriptional or post-transcriptional levels, the assembly and/or function of tight junctions. The myosin II-actin complex may be modulated by myosin-light chain kinase (MLCK) or Rho kinases (ROCK), which allows the opening of tight junctions and increases paracellular permeability

gastric tumourigenesis (50). A recent study (51) has now established a possible link between *H pylori* and these processes. Indeed, it was found that *H pylori* modulates the migration of human gastric epithelial cells by destabilizing E-cadherin in a Rho-GTP-dependent fashion. Consistent with these observations, *H pylori* is capable of altering epithelial permeability, a topic that will be discussed in the following paragraphs. Among the various tight junctional proteins that are affected by *H pylori*, the authors recently observed that the bacterium was capable of disrupting tight junctional claudin-4 and -5 in a strain-dependent manner (52) (Figure 2). In addition to cell adhesion, polarity may also be lost during carcinogenesis, and a role for disruption of specific claudins in these events has been recently suggested. Indeed, while its role in epithelial barrier is well-established, claudin-4 expression is also inversely correlated with the metastatic potential of pancreatic cancer cells (53,54). Intriguingly in the context of the present article, a recent report (55) indicates that loss of tight junctional claudin-4 correlates with poor differentiation in advanced gastric adenocarcinomas. This observation offers further support

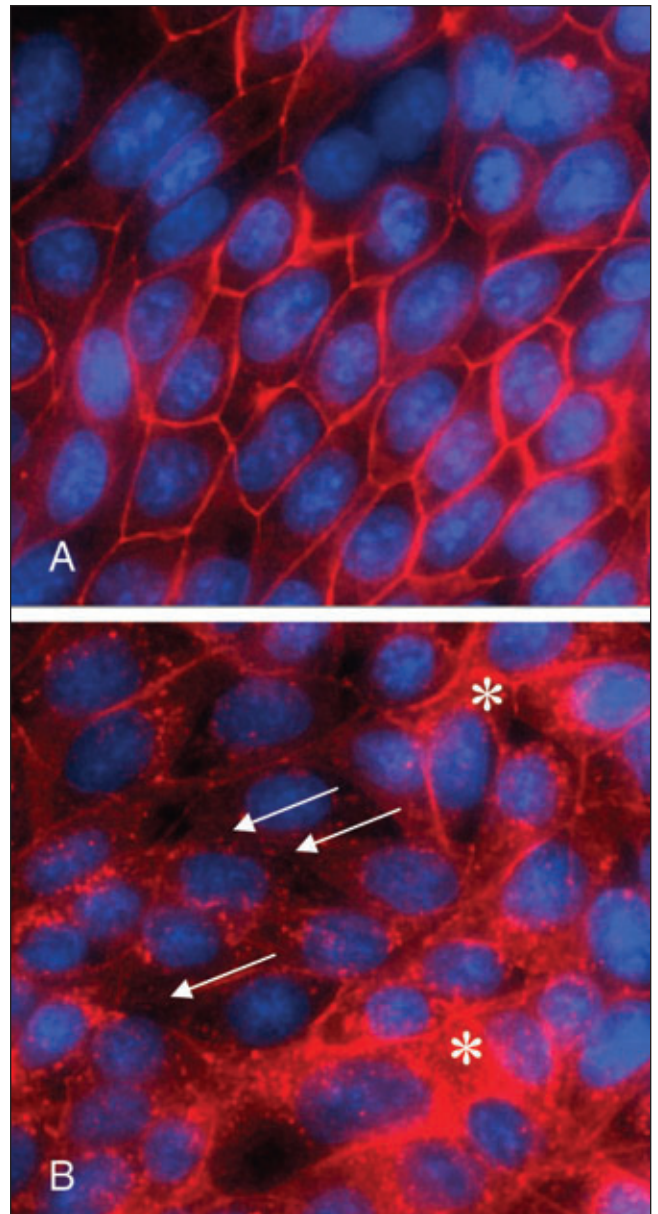


Figure 2) *Helicobacter pylori* disrupts tight junctional claudin-4. Shown are representative micrographs illustrating claudin-4 (red) and cell nuclei (blue) in epithelial monolayers after 6 h incubation with *H pylori* SS1, a human clinical isolate passaged through mice. *Abnormalities include focal disruptions of claudin-4 along the pericellular junctions (arrows) and punctate redistribution of claudin-4 into cytosolic compartments. Original magnification $\times 400$

to the hypothesis that disruption in gastric epithelial barrier function may influence the clinical outcome of infection. Similarly, loss of claudin-7 has recently been implicated in the ability of breast cancer cells to disseminate (56). Therefore, the tight junction acts as a scaffold for signalling proteins involved in cell growth and differentiation, and loss of normal tight junctional structure and function, possibly via disruptions of claudins, may represent an important precursor to the development of human carcinomas (57).

Matrix metalloproteinases (MMP), a family of enzymes associated with inflammatory processes and capable of degrading

components of the extracellular matrix, have become intriguing candidates on the list of potential markers of oncogenesis. For example, MMP-7, also called matrilysin, is found in high concentrations within premalignant gastric ulcers as well as in pancreatic intraepithelial neoplasia (58-60) implying that, in addition to its role in host defense and tissue remodelling, this enzyme may also be an early marker of carcinogenesis. Clearly, additional research is needed to establish a cause-to-effect relationship in this interaction. While MMP-7 expression is known to increase in response to *H pylori* infection (60), a potential relationship with defects in epithelial barrier structure and function has yet to be established. Studies are needed to assess whether and how elevated MMP-7 and altered claudin-4 (and possibly claudin-7) may coincide with events leading to increased gastric permeability, which may then help link the loss of epithelial barrier with lesions of premalignant significance.

In addition to their effects on epithelial barrier function, the upregulation of host proteinases may also contribute to the mitogenic response of epithelial cells. A number of growth factors and their receptors are upregulated in response to *H pylori* exposure. Specifically, epidermal growth factor (EGF) receptor expression is increased during *H pylori*-induced gastritis in humans and this micro-organism can activate the EGF receptor on gastric epithelial cells (61,62). *H pylori* infection also leads to an increase in the EGF receptor ligand, heparin-binding EGF (HB-EGF) (62,63). Current research data (64) suggest that *H pylori* activates a G-protein coupled receptor, leading to MMP release and cleavage of HB-EGF. The proteinases responsible for the cleavage of HB-EGF remain unknown. In addition to stimulating MMP-7, *H pylori* increases MMP-1 and MMP-3 release and activity in transformed adenocarcinoma gastric stomach epithelial cancer cell lines and the micro-organism may itself have MMP-3 activity (65). Cag-positive strains activate nuclear factor-kappa B and induce the expression of MMP-9 in MKN28 and MKN45 gastric epithelial cells, and increased levels of MMP-9 are found in biopsies from *H pylori*-infected patients (66). Overall, altered expression of host growth factors in response to *H pylori* signalling has been implicated in gastric adenocarcinomas, and MMPs may play an important role in this process. It has been recently demonstrated that the *H pylori*-induced EGF receptor transactivation required metalloproteinase cleavage of HB-EGF (63). However, the exact molecular, biochemical and physiological mechanisms involved in this chain of events remain unclear.

Loss of epithelial barrier function: The role of myosin light chain kinase

The gastric epithelium acts as a selective barrier that prevents potentially harmful luminal agents (eg, microbial products, food antigens, toxins) from penetrating underlying tissues, while allowing for exchanges of ions and small molecules (67). Tight junctions and adherens junctions between gastric epithelial cells play a key role in this barrier function, and consist of a complex interaction among several protein families. Tight junctions can easily be recognized under transmission electron microscopy (68,69). Figure 1 offers an overview of the main tight junctional structures discussed in the present review. For comprehensive articles on the composition and function of these structures, interested readers are directed to recent reviews on this topic (68-75). Tight junctions comprise:

- transmembrane proteins belonging to several families including occludin, claudins and the immunoglobulin

superfamily members: junctional adhesion molecules (JAM) and coxsackie virus and adenovirus receptor;

- an ever-increasing number of cytosolic proteins, including zonula-occludens (ZO)-1, -2 and -3, and partitioning defective proteins (eg, PAR3 and PAR6), that form complex junctional plaques; and
- a mixed group of cytosolic, membrane-bound or nuclear-associated proteins that interact with the former two groups to regulate epithelial permeability, polarity and proliferation.

Transmembrane proteins are thought to directly regulate paracellular diffusion. Proteins of the tight junctional plaques may act as adaptors (eg, ZO proteins), and the cytosolic and nuclear components function as signalling and regulatory proteins. Adaptor proteins help connect the transmembrane components to cytosolic factors such as GTPases and protein kinases. Many tight junctional proteins are directly or indirectly attached to actin filaments. For example, claudins, occludin and junctional adhesion molecules are anchored to actin filaments and myosin light chain (MLC) of the perijunctional actinomyosin ring by the linker proteins of the ZO family (76-78). Paracellular permeability may be increased by phosphorylation or degradation of transmembrane tight junctional proteins (79). In addition, dephosphorylation of MLC by MLC kinase (MLCK) or by Rho kinases may also physiologically regulate paracellular permeability by placing tension on the tight junctional complexes (80). The molecular mechanisms responsible for this process are topics of intensive research efforts.

It has been well-established that the paracellular permeability offered by tight junctions could be altered in response to physiological and pathological stimuli (81). Recent reports (80,82,83) indicate that a variety of pathogens may actively disrupt epithelial barrier function by subverting cellular pathways, including those that lead to the activation of MLCK. As illustrated in Table 1, these effects are commonly mediated via microbial toxins and/or proteases. Interestingly, a recent study (84,85) has found that a membrane permeant inhibitor of MLCK was able to inhibit the tight junctional disruptions induced by enteropathogenic *Escherichia coli* (EPEC) or TNF α and IFN γ (84,85). In view of the implication of TNF α , a Th-1 cytokine, in the modulation of barrier function, and the significance of Th-1-mediated pathogenesis during *H pylori* infections, these findings may provide fertile ground from which to develop a new class of therapeutic agents targeting the loss of gastrointestinal barrier function.

H pylori preferentially adheres near the tight junctions of gastric mucous cells (15,47,87). Strains possessing the Cag pathogenicity island translocate the CagA protein into host cells via a type IV secretory system (88,89). Findings from recent studies (90) using Madin-Darby canine kidney cells or gastric adenocarcinoma cells indicate that translocated CagA increases paracellular permeability by recruiting ZO-1 and JAM to sites of bacterial attachment. Other reports (91) have shown that *H pylori* strains producing VacA modulate the integrity of the epithelium by increasing tight junction permeability to small ions and molecules. Indeed, adherence of VacA-producing strains reduced transepithelial electrical resistance in cell monolayers in vitro, while isogenic VacA $-/-$ mutants lost this ability. Acid-activated VacA on its own was also found to increase paracellular permeability for small molecules in vitro,

TABLE 1
Examples of pathogen-epithelial interactions known to modulate epithelial permeability

Organism	Virulence factor	Mechanism	Reference
Viral			
Reovirus		Binds JAM, effect on permeability unknown	124
Rotavirus		Metabolic interference	103
Adenovirus	Fibre protein	Binds coxsackie adenovirus receptor	122
Bacterial			
<i>Vibrio cholerae</i>	ZOT	PKC activation	102,148,149
<i>Escherichia coli</i>	CNF-1	Rho-dependent disruption of occludin/ZO-1	114
Enteropathogenic <i>E coli</i>	T3SS	Occludin dephosphorylation	79,150
		MLCK activation	83
Enterohemorrhagic <i>E coli</i>	EspF/T3SS	Unclear	151
		T3SS	E-cadherin disruption, rac-1 and ezrin activation
<i>Bacteroides fragilis</i>	Fragilysin	E-cadherin disruption	153
<i>Clostridium difficile</i>	Toxin A/B	Rho-GTPase and PKC activation	154
<i>Clostridium perfringens</i>	CPE	Disruption of claudin-3 and -4	155
<i>Shigella flexneri</i>	Unclear	Disruption of ZO-1, occluding, and claudin-1	104
<i>Helicobacter pylori</i>	CagA, T4SS	Recruits ZO-1/JAM, disrupts SHP-2, Grb2 and c-met	44,90,156,157
	VacA	TJ penetration	91,92
Parasitic			
<i>Giardia</i> species	Unclear	Caspase-3-dependent ZO-1 disruption, MLCK	82,109
Other			
<i>Aspergillus, Penicillium</i>	Ochratoxin A	Disruption of claudin-3 and -4	158

CagA Cytotoxin-associated antigen; Grb2 Growth factor receptor bound protein 2; GTPase Guanosine triphosphatase; JAM Junctional adhesion molecules; MLCK Myosin light chain kinase; PKC Protein kinase C; SHP-2 Src homology 2 domain-containing protein-tyrosine phosphatase; T3SS Type 3 secretion system; T4SS Type 4 secretion system; TJ Tight junction; VacA Vacuolating cytotoxin A; ZO-1 Zonula occludens; ZOT Zonula occludens toxin

without affecting the transcellular fluxes of 5 kDa or 47 kDa probes (92). In addition to a direct effect of the bacterium, transmigration of inflammatory cells in response to infection may also contribute to a disruption of epithelial barrier function (93). The degree of *H pylori*-induced gastritis has been associated with permeability changes (94), consistent with the findings that TNF α and IFN γ are able to disrupt epithelial junctions by activating MLCK (85,86). However, studies (95,96) involving human subjects have argued for and against the ability of the bacterium to disrupt tight junctions of the gastric epithelium. Yet, *H pylori* has the ability to increase the passage of food antigens across human gastric biopsies (97), as well as in animal model systems (98). These findings are consistent with the observations that the infection may be associated with the development of food allergy (99,100). Intestinal pathogens such as *Vibrio cholerae*, *Salmonella* species, *E coli*, Rotavirus, *Shigella* species and *Giardia* species all have been found to directly alter tight junction permeability, a change suggested to contribute to disease symptoms (82,83,101-104). Therefore, whether and how *H pylori*-induced epithelial permeability defects may influence the clinical outcome of this infection represents an important topic for future research.

Loss of epithelial barrier function: Signalling events

Molecular signalling of epithelial permeability is a fast-growing field of investigation. Studies (105) have found that integrin-mediated cell migration was mediated by MLCK, following a cascade of events in which Ras, extracellular signal-regulated kinase (ERK), and mitogen-activated protein kinase (MAPK)/ERK serve as essential downstream effectors. However, signalling events implicated in the MLC-dependent regulation

of epithelial tight junction and barrier function, particularly in the gastric mucosa, are less clear. A variety of cellular events are associated with the epithelial abnormalities caused by *H pylori*, including apoptosis, cytoskeletal and tight junctional alterations, and loss of barrier function. The complex signalling events that may be responsible for *H pylori*-induced disruptions of gastric epithelial barrier function remain unclear. Figure 3 illustrates possible mechanisms suggested by the scientific literature currently available. *H pylori* has proapoptotic effects (106-108), and immune or microbially induced enterocyte apoptosis may directly increase epithelial permeability (109-111). Any of these alterations can be linked to MLCK, which further underscores the gatekeeper role played by phosphorylated MLC in gastrointestinal barrier function. Moreover, activation of PAR1, which results in caspase-3-dependent loss of barrier function (112), microbially induced loss of epithelial barrier (Table 1) and EGF signalling (113), may each implicate phosphorylation of MLC in their epithelial signalling cascade. Downstream from Rho, Rho-associated kinase (ROCK) serine/threonine kinase isoenzymes are known to modulate cytoskeletal arrangements and cellular contractility via MLC, as well as tight junctional function (114-116). Furthermore, intracellular caspases can cleave ROCK and its constitutively active cleavage products lead to the activation of MLC, and ultimately to the formation of apoptotic membrane blebbing and the chromatin condensation characteristic of programmed cell death (116-119). Interestingly, MAPK activation is also implicated in tumour progression (120). While levels of Rho-GTP are increased during the *H pylori*-induced translocation of E-cadherin from membrane to cytosolic vesicles (51), the implication of ROCK in *H pylori*-induced epithelial cell signalling and subsequent injury is less clear.

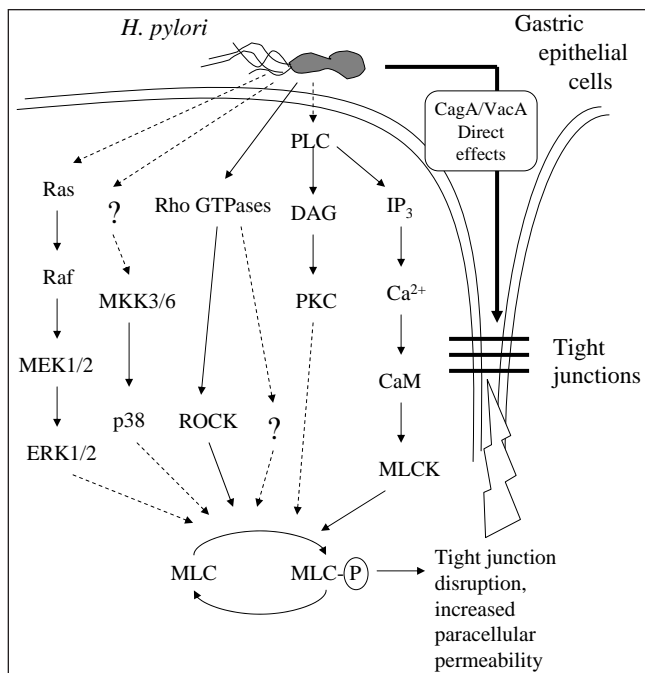


Figure 3 Illustration of the various signaling pathways through which *H. pylori* may disrupt epithelial permeability. References to specific reports for each of these steps are discussed in the text. Dotted lines illustrate links that have yet to be established. CagA Cytotoxin-associated antigen; CaM Calcium-calmodulin; DAG Diacylglycerol; ERK Extracellular signal-regulated kinase; GTPase Guanosine triphosphatase; IP₃ Inositol-triphosphate; MEK Mitogen-activated protein kinase/ERK kinase; MLCK Myosin light chain kinase; PLC Phospholipase C; PKC Protein kinase C; ROCK Rho-associated kinase; VacA Vacuolating cytotoxin

Similarly, the significance of cell signalling events such as Rho-kinase-mediated events to functional parameters of epithelial barrier require further investigation. Unquestionably, microbes have evolved elaborate strategies to bypass the tight junction, including mechanisms independent of MLCK. Direct binding of microbes to tight junctional elements for example may disrupt the tight junctions and increase permeability. Examples include coxsackie B virus and adenovirus, which bind to their tight junctional receptor (coxsackie virus and adenovirus receptor), which is located between other tight junctional proteins and E-cadherin (121,122). Similarly, *Clostridium perfringens* toxin attaches to claudin-3 via its carboxyl terminus, which in turn leads to junctional disruptions via signalling events that have yet to be uncovered (123). Epithelial attachment of reovirus is also associated with tight junctional disruptions by activating nuclear factor-kappa B, which in turn causes apoptosis (124). In addition, microbial enzymes may break down tight junctional proteins to allow the passage of antigenic material. For example, peptidases from the house dust mite *Dermatophagoides pteronyssinus*, a potent allergen, cleaves occludin and ZO-1, which in turn allows the penetration of antigenic material (125). Intriguingly, other observations have found that these serine and cysteine proteases were able to activate PAR (126), a phenomenon recently linked to the capability of PAR-agonists to disrupt epithelial permeability via the induction of apoptosis and the activation of MLCK (112,127). Clearly, much remains to be learned about

the mechanisms leading to tight junctional disruptions, and their significance for the development of novel therapeutic strategies.

Signalling pathways of *H. pylori*-induced loss of barrier function

H. pylori activates a broad range of signalling molecules, including protein kinase C (PKC), ERK and p38 (128-131). These kinases have been shown to contribute to barrier mal-function and/or MLC phosphorylation in several in vitro models (83,132-134), and the fact that MAPKs may also be implicated in cancer progression (120) further underscores the possible links between these abnormalities. Figure 3 illustrates possible signalling pathways through which *H. pylori* may disrupt tight junctional function via MLC. It is not clear whether *H. pylori* may increase gastric permeability via signalling cascades associated with PKC, ERK or p38. In addition, while *H. pylori* is known to activate the small Rho-GTPase (51,135), it is not known whether this leads to MLC phosphorylation and impaired epithelial barrier function. Findings from a recent study (136) suggest a key role for Src homology 2 domain-containing protein-tyrosine phosphatase (SHP-2) in *H. pylori* CagA-mediated signalling in adenocarcinoma gastric stomach cells. The role of ERK in the epithelial abnormalities caused by this micro-organism needs to be further investigated. *H. pylori* also activates the other two members of the Rho family of small GTPases, Rac and cell division control protein 42 (Cdc42) (128,137), which are known to influence tight junction assembly and epithelial permeability (90,138). Indeed, it has been established that Rho-A and Rac1 are directly involved in the regulation of tight junctional structure and function (139). However, no direct link has yet been established between *H. pylori*-mediated activation of Rac or Cdc42 and epithelial barrier dysfunction, nor with other cytoskeletal rearrangements known to occur in response to this bacterium (140). More research is needed to determine if strain-specific signalling causing gastric barrier disruptions may explain, at least in part, the variable clinical outcomes seen in response to *H. pylori* infection.

In addition to acting as a selective barrier, tight junctions serve as a scaffold for a number of signalling molecules, and it is becoming increasingly apparent that the tight junction is an important component of the pathways that regulate cell proliferation and differentiation. JAM attached to ZO-1 is capable of recruiting PAR3 (141). In turn, PAR3 recruits and assembles a protein complex containing atypical PKC (aPKC)-PAR3-PAR6 at the tight junction (142,143). PAR6 of this complex binds Cdc42, and this Rho-family GTPase can activate aPKC, a cascade of events that are crucial for the assembly of the epithelial tight junctions (142-144). Furthermore, Cdc42 inhibits protein trafficking to the basolateral membrane and controls the development of epithelial cell polarity (144). *H. pylori*, through the injection of CagA, production of VacA, and possibly through the expression of an outer membrane protein alters a number of cell signalling and trafficking pathways in epithelial cells (43,44,90-92,145), a number of which have detrimental effects on epithelial barrier structure and function. The additional insult of tight junctional disruption may have effects on epithelial cell adhesion and polarity.

H pylori is known to have proapoptotic properties (106,107). While physiological sloughing of epithelial cells via apoptosis does not alter epithelial permeability (146,147), microbially or immunologically activated apoptosis does have the ability to cause a loss of barrier function (109-112). Therefore, research findings available to date offer solid support to the hypothesis that, independently of the chronic inflammatory response to the infection, at least some strains of *H pylori* may express virulence factors that can directly alter normal epithelial cell functioning and predispose these cells to malignant transformation.

CONCLUSION

A growing body of evidence in the scientific literature supports the hypothesis that *H pylori* may alter epithelial tight junctional components and disrupt gastric barrier function, and that these effects may be strain-dependent. While studies (95,96) in human subjects have yielded controversial findings on this topic, *H pylori* has the ability to increase the passage of food antigens across the gastric epithelium (97,98). This mechanism may represent a common route towards the development of postinfectious food allergies, which have been reported in association with *H pylori* as well as other enteric pathogens (99,100). *H pylori* is considered a class 1 carcinogen. Cell adhesion and polarity are often lost during carcinogenesis, and a role for the

loss of specific claudins in these events has been suggested. Indeed, in pancreatic cells, claudin-4 expression inversely correlates with metastatic potential (53,54), and it was recently observed that loss of claudin-4 may be implicated in the development of gastric adenocarcinoma (55). In addition, cell adhesion is sensitive to a number of host factors, including MMP. One of these, MMP-7 (matrilysin), which is secreted by epithelial cells, was recently identified as a precursor of *H pylori*-induced gastric adenocarcinoma (58-60). The exact involvement of disrupted claudins and MMP-7 in the production of *H pylori*-induced loss of gastric barrier function remains unclear. Nevertheless, the loss of claudin-4 (and possibly that of other tight junctional proteins), perhaps in association with increased MMP-7, could contribute to phenotypic changes induced by *H pylori*, and thereby increase the metastatic potential of gastric epithelial cells, consistent with the hypothesis that *H pylori*-induced abnormalities at epithelial tight junctions contribute at least in part to the clinical outcome of the infection.

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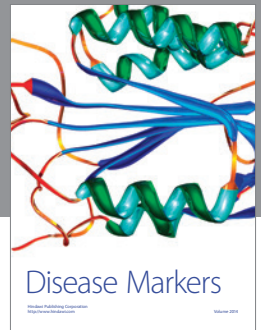
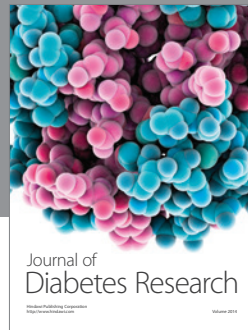
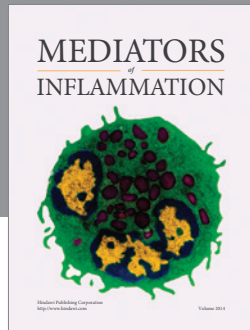
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