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Bacterial factors and immune pathogenesis in *Helicobacter pylori* infection

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Summary
Virulent *Helicobacter pylori* strains which have been clinically associated with severe outcome induce increased gastric mucosal immune responses. Although several bacterial pathogenic factors have been shown to have a considerable role in *H pylori* infection, variability in host immune responses may also contribute to mucosal damage in *H pylori* associated gastritis.

Introduction

Since the discovery of *H pylori*, many studies have implicated infection with this bacterium in the pathogenesis of gastric and duodenal diseases. However, most patients with *H pylori* infection have chronic gastritis only and few develop peptic ulcers or gastric tumours. To date, the role of bacterial virulence factors such as *vacA*, *cagA* and lipopolysaccharide (LPS) in the pathogenesis of *H pylori* infection has been extensively studied. The host's mucosal immune response, which includes activation of neutrophils, T cells and complement, has also been the subject of recent investigation. Both bacterial and host factors are likely to play a critical role in the clinical outcome of *H pylori* infection.

Bacterial virulence factors

Increasing evidence has shown that *H pylori* strains are highly diverse and that strain diversity is associated with greater gastric mucosal inflammation and the clinical outcome of infected patients.

CYTOTOXIN

The vacuolating cytotoxin (VacA) which induces cytoplasmic vacuolation in eukaryotic cells was first described by Leunk *et al*.[1] The cytotoxin is produced by approximately 50% of *H pylori* strains[2] and infection with toxin producing strains is more common in patients with peptic ulcer disease.[3] Bernard *et al* showed that biological activity of VacA protein was increased by exposure to acidic pH.[4] The acid activated VacA is currently considered to be an important factor of increased mucosal damage in *H pylori* infection.

The vacuolating cytotoxin gene A (*vacA*), which encodes VacA, is possessed by all *H pylori* strains. Atherton *et al* showed that there are two divergent regions in *vacA*.[5] One is in the second half of the signal sequence (s1a/s1b and s2) and the other is in the mid-region of the gene (m1 and m2). Recent studies demonstrate the association between diversity in this gene and cytotoxin activity and increased gastric inflammation.[5 6] The s1/m1 strains produce higher toxin activity in vitro than s1/m2 strains,[5] m1 strains are associated with increased gastric epithelial damage,[6] and s1a strains are associated with increased mucosal neutrophil and lymphocyte infiltration in vivo.[6] These results suggest s1a/m1 strains are the most virulent allelic type. In fact in US populations, infection with *vacA* s1 strain is more frequent in patients with ulcer disease than in those with chronic gastritis only (table 1).[6] However, the association between *vacA* diversity and clinical outcome is not as evident in Asian populations as it is in Western populations. Recent studies show that most *H pylori* strains in Japan have a s1a/m1 genotype even in patients with chronic gastritis only (table 1).[7 8]

cag PATHOGENICITY ISLAND

The cytotoxin associated gene A (*cagA*) is the most studied non-conserved gene of *H pylori*. *cagA*, which encodes a high (120–140 kDa) molecular weight immunodominant protein, is present in approximately 60% of *H pylori* strains.[9] In vitro studies have shown that the ability of *H pylori* to induce chemokines in gastric epithelial cell lines varies, the response being restricted to strains with the CagA phenotype.[10–12] In vivo, infection with CagA positive strains results in increased mucosal immune responses and more intense gastritis.[13–15] Several studies have shown that infection with CagA positive strains is highly associated with peptic ulcer disease,[15 16] atrophic gastritis,[17 18] and gastric cancer.[19–21]

Recent studies show that *cagA* is part of a 40 kilobase pathogenicity island (*cag* PAI) which contains over 30 genes.[22 23] The importance of gene products from *cag* PAI in the stimulation of epithelial chemokine responses has been assessed using isogenic mutant strains. These studies have shown that the deletion of *cagA* has no effect on epithelial secretion of interleukin (IL) 8,[12 24] but deletion of many other genes in the *cag* PAI abolishes the ability of the bacterium to stimulate IL-8 production.[22 23 25 26] These studies show that the CagA is a phenotypic marker for virulent strains and the epithelial chemokine responses depends on multiple genes in the *cag* PAI. The proteins encoded by *cag* PAI genes are thought to function as a secretion system for the export of bacterial factors involved in host epithelial activation.[22 23]

LIPOPOLYSACCHARIDE

H pylori LPS has also been implicated in the pathogenesis of *H pylori* infection.[27] The immunological activity of *H pylori* LPS has been considered to be low.[27] Early studies showed the lethal toxicity in mice of *H pylori* LPS was 500-fold lower compared with that of

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Table 1 H pylori vacA gene signal sequence type in patients with peptic ulcer disease and chronic gastritis from the USA and Japan

	USA[6]		Japan[8]
Signal sequence	*Peptic ulcer disease*	*Chronic gastritis*	*Both conditions*
sla	17	1	84
slb	8	6	1
s2	2	8	0

Values presented as number of patients.

salmonella LPS.[28] Several studies also showed that secretion of the proinflammatory cytokines tumour necrosis factor (TNF) α, interleukin (IL)1 and IL-6 from human mononuclear cells and IL-8 secretion from neutrophils following stimulation with *H pylori* LPS was significantly lower than that induced by *Escherichia coli* and salmonella LPS.[29 30] This low biological activity of *H pylori* LPS is a result of the phosphorylation pattern of its lipid A component and may prolong *H pylori* infection and contribute to chronic inflammation in the gastric mucosa.[27]

H pylori LPS may have an important role in autoimmune responses in the gastric mucosa. The structure of LPS O-specific antigen in different *H pylori* strains is similar to that of host Lewis^X or Lewis^Y blood group antigens[31 32] which are expressed in normal human gastric mucosa.[33] This molecular mimicry may account for some of the gastric autoantibodies induced by *H pylori*. Appelmelk and colleagues[34] showed that the β chain of the H^+, K^+ proton pump has Lewis^Y epitopes and anti-Lewis^Y antibodies induced by *H pylori* may contribute to the development of atrophic gastritis.[35] Recent studies suggest that peptide epitopes on gastric H^+,K^+ ATPase may also be the target of autoimmune responses in chronic gastritis.[36] Lewis antigens are expressed more frequently on *cagA* positive than on *cagA* negative strains.[37] This suggests that *cagA* positive strains could potentially be more likely to stimulate autoimmune responses and contribute to the association between *cagA* positivity and atrophic gastritis.[17 18] However, *H pylori* infected subjects without serum antibodies to Lewis^X are at increased risk of atrophic gastritis.[38] This may reflect the immunodominant nature of CagA rather than the absence of expression of Lewis epitopes.

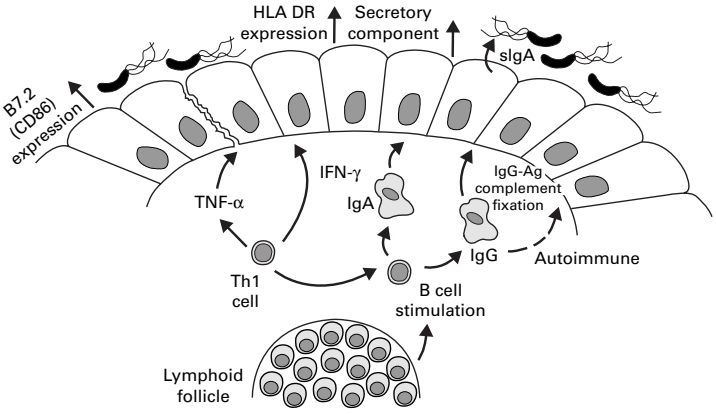

Figure 1 Induction of H pylori specific immune responses in the gastric mucosa.

Immune mediated damage

Histologically, the host's response to *H pylori* infection is characterised by infiltration of plasma cells, lymphocytes, neutrophils, and monocytes into the gastric mucosa.[39] The inflammatory host immune response may play a major role in the induction of gastric mucosal damage by *H pylori* infection. This review focuses on cellular responses as the role of B cells and autoantibodies is discussed elsewhere in this supplement.

NEUTROPHIL ACTIVATION

In acute gastritis caused by infection with *H pylori*, neutrophils comprise the initial inflammatory component of the response to the pathogen. Activated neutrophils have many properties which may contribute to tissue damage. Neutrophil chemotaxis and activation can be induced directly by products of *H pylori* and also indirectly through the inflammatory cytokine cascade.[39] A water soluble neutrophil activating 150 kDa protein, HP-NAP, has been purified and extensively studied.[40 41] The *napA* gene which encodes this protein is present in all *H pylori* strains but expression of HP-NAP varies in vitro.[40] Recent studies suggest HP-NAP may have multiple functions, being induced in the bacterium by acid stress.[42] *H pylori* LPS also affects neutrophils; it does not stimulate neutrophils directly to release superoxide but it primes neutrophils to oxidative metabolism.[43]

Neutrophil infiltration and activation will also be stimulated indirectly by the cytokine cascade induced by *H pylori* infection.[10–12] The gastric epithelium secretes chemokines which have neutrophil attractant properties, such as IL-8 and GROα in response to bacterial infection. In vivo, increased IL-8 immunoreactivity and increased IL-8 mRNA expression in *H pylori* infected mucosa have been demonstrated.[13 14 44 45] In vitro the expression of IL-8 in gastric epithelial cells is up-regulated by the inflammatory cytokines such as TNF-α and IL-1,[46] which are produced in the gastric mucosa in *H pylori* infection.[47 48] Inflammatory cytokines are increased in patients infected with strains of the CagA phenotype.[13 14 39] As described earlier CagA is not the direct inducer of IL-8 but CagA positive strains are associated with increased gastric IL-8 mRNA expression[13] and IL-8 protein in vivo.[14] Although proteins which stimulate mononuclear cell cytokine production have not been studied as extensively, previous investigations showed the potential role of urease and porins of *H pylori* in inducing inflammatory cytokine secretion in mononuclear cells.[49 50] Additionally, water soluble extracts of *H pylori* up-regulate neutrophil adhesion to endothelial cells which occurs via CD11b/CD18 dependent interactions with ICAM-1.[51] Hatz and colleagues[52] recently showed increased expression of ICAM-1 and VCAM-1 in *H pylori* positive gastric mucosa which will facilitate neutrophil endothelial adhesion and extravasation. Interestingly, other adhesion molecules such as E-selectin are not increased in *H pylori* infected mucosa.[52]

T CELL ACTIVATION

Following initial acute inflammation and associated changes in gastric permeability,[53] continuous exposure to antigen results in the generation of *H pylori* specific T cell and B cell responses (fig 1).[39] Evidence from murine studies suggests that T cell responses to *H pylori* play a role in the induction of gastric mucosal damage.[54] For the activation of T cells, the interactions of B7–1 or B7–2, also known as CD80 and CD86, on antigen presenting cells with CD28 on T cells is required. Recent studies show that expression of B7–2, as well as class II molecules, is increased on gastric epithelial cells in *H pylori* positive mucosa, suggesting gastric epithelial cells could potentially act as antigen presenting cells in *H pylori* infection.[55]

The infiltrating T cells in *H pylori* positive gastric mucosa are predominantly of the CD45RO+ phenotype, a marker for antigen committed cells, and correlate with enhanced HLA-DR expression on the gastric epithelium in *H pylori* gastritis.[56 57] Isolated gastric mononuclear cells from patients with *H pylori* chronic gastritis frequently secrete interferon (IFN) γ but not IL-4.[58] The majority of *H pylori* specific CD4+ T cell clones secrete IFN-γ in response to antigen stimulation indicative of a Th1 phenotype.[59] A recent study has also shown that live *H pylori* selectively stimulate IL-12 production from peripherial blood leucocytes, which is likely to be important in inducing Th1 responses.[60] A gastric Th1 response is more frequent in patients with peptic ulcer disease.[59] Perturbations in acid secretion may be greater in the presence of a gastric Th1 response and thus contribute to increase risk of duodenal ulceration. Mohammadi and colleagues[54] showed that a significant reduction in gastric mucosal inflammation occurs in mice after neutralisation of IFN-γ. Conversely, IL-10, which suppresses Th 1 responses, is also increased in *H pylori* positive gastritis but a protective role in mucosal damage is unproved.[61]

COMPLEMENT ACTIVATION

Complement activation may also be another factor which contributes to neutrophilic response in the gastric mucosa. Early studies suggested that *H pylori* can activate the classic pathway of complement, resulting in neutrophil activation in vitro.[62] Recently, Berstad and colleagues[63] showed that activation of both complement pathways occurs in *H pylori* gastritis. The presence of activated C3b was significantly related to neutrophil infiltration in vivo. These results suggest that activated complement may have a role in neutrophil chemotaxis and epithelial damage.

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