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Review

Chemokine expression in *Helicobacter pylori*-infected gastric mucosa

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Abstract: Inflammatory response to *Helicobacter pylori* is characterized by infiltration of neutrophils, monocytes, and lymphocytes into the gastric mucosa. Interleukin-8 (IL-8), a prototype of the CXC-chemokine subfamily, may be a key modulator in inducing neutrophil migration and activation in *H. pylori*-infected gastric mucosa. IL-8 is produced by gastric epithelial cells in response to *H. pylori* infection, and IL-8 expression is induced by local production of proinflammatory cytokines and attachment of *H. pylori* organisms to the gastric epithelial cell surface. Multiple genes in the *H. pylori cag* pathogenicity island seem to be involved in inducing the epithelial IL-8 response to *H. pylori* attachment. Activation of the transcription factor, nuclear factor κB (NF-κB), is associated with this IL-8 response. Reactive oxygen intermediates whose production is increased in *H. pylori*-infected gastric mucosa may also modulate IL-8 expression in the gastric mucosa. Recent reports also suggest that the local production of CC-chemokines, another chemokine subfamily, is important in *H. pylori*-associated gastritis.

Key words: chemokine, interleukin-8, *Helicobacter pylori*

Introduction

Helicobacter pylori has been implicated in the pathogenesis of most clinically important gastroduodenal diseases, including peptic ulcer, gastric carcinoma, and gastric MALT (mucosa-associated lymphoid tissue) lymphoma.[1–3] *H. pylori* infection is associated primarily with chronic active gastritis characterized by neutrophil infiltration in the gastric epithelium and underlying lamina propria,[4,5] as well as the infiltration of other inflammatory cells such as monocytes and lymphocytes. *H. pylori* organisms are not invasive, but they are able to induce significant inflammatory responses by interacting with epithelial cell surfaces.

The local production of chemokines, a group of cytokines with chemoattractant activity and other biological functions, is an important factor in the recruitment and activation of inflammatory cells.[6] More than 30 chemokines have been reported thus far, and the numbers are still increasing. Chemokines can be divided into four subfamilies, CXC-, CC-, C, and CX_3C-chemokines, based on overall homology, the disposition of the first two cysteine residues, and the location of the genes (Table 1).[6,7] Neutrophils express CXCR1 (interleukin [IL]-8RA) and CXCR2 (IL-8RB) chemokine receptors[6] and are recruited and activated by CXC-chemokines such as IL-8, growth related protein (GRO)α, and epithelial-derived neutrophil attractant (ENA)78. This chemokine response appears to be a primary factor in the induction of inflammatory responses.

IL-8 expression in *H. pylori*-infected gastric mucosa

Crabtree et al.[8] first reported that IL-8 content was increased in *H. pylori*-infected gastric mucosa. Subsequently, they reported an immunohistochemical IL-8 expression study that localized IL-8 to the epithelium in histologically normal mucosa and that showed increased IL-8 expression in *H. pylori*-infected mucosa.[9] They also showed that gastric epithelial cell lines, such as KATOIII and MKN45, constitutively expressed IL-8. The increased IL-8 production in *H. pylori*-infected gastric mucosa was confirmed by other investigators.[10–14] Therefore, locally produced IL-8 appears to be a key cytokine in *H. pylori*-associated gastritis, and epithelial

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Table 1. Chemokine superfamily

CXC-subfamily (4q12–21)
IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, GCP-2, PF-4, IP-10, Mig, PBSF/SDF-1(10q11)
CC-subfamily (17q11–21)
MCAF/MCP-1, MCP-2, MCP-3, MCP-4, eotaxin, RANTES, MIP-1α, MIP-1β, HCC-1, MIP-3α/LARK(2q33–37), MIP-3β/ELC(3q13), I-309, TARC(16), MIPF-1, MIPF-2/eotaxin-2, MDC, DC-CK1/PARC, SLC(9q13)
C-subfamily (1q23–25)
lymphotactin
CX_3C-subfamily (16q)
fractalkine

cells are likely to be a major source of IL-8 production in the gastric mucosa.

The expression of other proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ), and interleukin-6 (IL-6), is also increased in *H. pylori*-associated gastritis.[10–13,15,16] *H. pylori* lipopolysaccharide (LPS) and released surface proteins stimulate lamina propria mononuclear cells to produce IL-1β and TNF-α,[17] although *H. pylori* LPS has relatively weak biological activity compared to that of *Eschericia coli*.[18] TNF-α and IL-1β are potent inducers of IL-8 expression in many cell types, and in vitro studies using gastric epithelial cell lines have demonstrated that IL-8 expression in these cells is up-regulated by TNF-α or IL-1β.[19–23] The increased IL-8 production by gastric epithelial cells in *H. pylori* gastritis is probably mediated, at least in part, by these locally produced proinflammatory cytokines.

Evidence also suggests the direct induction of IL-8 expression in gastric epithelial cells by *H. pylori* organisms.[19–21,24] Direct induction of IL-8 expression by bacteria is not unique to gastric epithelial cells. Similar observations were reported in a variety of other epithelial cells, including respiratory, intestinal, and cervical epithelial cells.[25–27] Attachment of *H. pylori* organisms to gastric epithelial cell surfaces appears to be necessary to induce a significant IL-8 response.[19,20,24] *H. pylori* culture supernatant and dead *H. pylori* do not yield a significant response.[20,24]

CagA and IL-8 expression

H. pylori strains differ markedly in their ability to induce IL-8 expression.[24] Crabtree et al.[24] demonstrated that this ability is related to the strain's CagA status. They showed that gastric epithelial cell lines secrete larger amounts of IL-8 in response to CagA-positive *H. pylori* strains than in response to CagA-negative strains. CagA is an outer membrane protein of *H. pylori*,[28] and infection with a CagA-positive strain is associated with severe gastric mucosal inflammation.[29] Approximately 60% of *H. pylori* strains have CagA protein, although there are geographical differences in the prevalence of CagA. Peek et al.[30] also showed that gastric mucosal IL-8 production is increased in patients infected with CagA-positive *H. pylori* strains compared to findings with CagA-negative strains. A subsequent study, however, unexpectedly has shown that disruption of the *H. pylori cagA* gene does not affect the degree of IL-8 induction in gastric epithelial cells,[31] suggesting that the CagA protein itself is not involved directly in the IL-8 response. Tummuru et al.[32] reported that, instead of *cagA*, disruption of the *picB* gene located upstream of *cagA* resulted in marked reduction in the gastric epithelial cell IL-8 response to *H. pylori*. More recently, Censini et al.[33] have identified the presence of a *cag* pathogenicity island (*cag* PAI) that contains more than 30 genes, including *cagA*. Multiple genes in the *cag* PAI are necessary to induce the IL-8 response.[33] The *cag* PAI gene products are likely to play a role in exporting molecules that induce gastric epithelial cell IL-8 expression. However, further studies are needed to identify the molecules that are directly responsible for inducing epithelial IL-8 expression. Figure 1 summarizes the gastric mucosal IL-8 response to *H. pylori.*

Role of nuclear factor-κB (NF-κB) in IL-8 expression

The transcription factors, nuclear factor-κB (NF-κB), nuclear factor-IL6 (NF-IL6), and activator protein (AP)-1 are involved in the transcriptional regulation of the IL-8 gene,[34–36] with the role of NF-κB appearing to be the most important. NF-κB regulates the expression of a wide variety of inducible genes.[37] In gastric epithelial cells, induction of IL-8 expression by proinflammatory cytokines, such as TNF-α or IL-1β, is associated with NF-κB activation.[35] Recently, Aihara et al.[38] demonstrated that co-culturing *H. pylori* with MKN45, a gastric epithelial cell line, activated NF-κB and increased the transcription of IL-8. In their study, mutating the NF-κB site completely abrogated *H. pylori*-simulated epithelial IL-8 expression, suggesting that NF-κB plays a pivotal role. Keates et al.[39] also reported similar NF-κB activation by *H. pylori* infection, using the gastric epithelial cell lines AGS and KATOIII.

The link between *H. pylori* adherence to gastric epithelial cell surfaces and NF-κB activation is poorly understood, although some reports do address this issue. Beales and Calam[40] showed that tyrosine kinase inhibitors abolished *H. pylori*-induced and proinflammatory cytokine-induced IL-8 expression in gastric epithelial cells, suggesting that tyrosine phosphorylation is involved in the IL-8 response. Segal et al.[41] recently

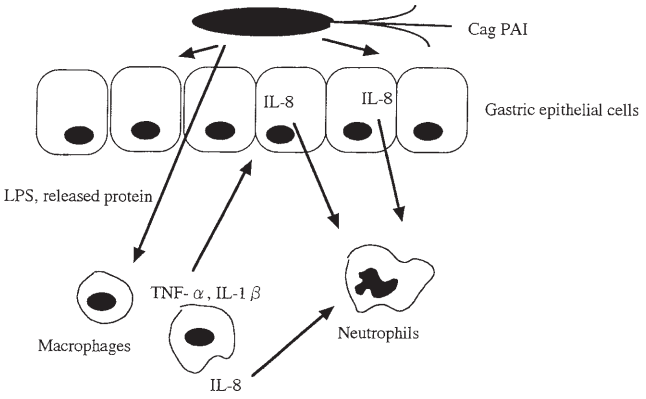

Fig. 1. Gastric mucosal interleukin (*IL*)-8 response to *Helicobacter pylori*. *PAI*, Pathogenicity island; *LPS*, lipopolysaccharide; *TNF*, tumor necrosis factor

demonstrated that mutations in a number of *cag* PAI genes abolished both tyrosine phosphorylation and IL-8 expression. Collectively, these findings may suggest that attachment of *H. pylori*-derived molecules whose export is dependent on the presence of *cag* PAI to the gastric epithelial cell surface will induce intracellular tyrosine phosphorylation and trigger a signaling cascade. The molecular mechanisms governing the interaction between *H. pylori* and gastric epithelial cells will be an interesting field of *H. pylori* research.

Modulation of IL-8 expression by reactive oxygen intermediates (ROI)

There seem to be additional factors affecting IL-8 expression in gastric epithelial cells. Local production of reactive oxygen intermediates (ROI) is increased in *H. pylori*-infected gastric mucosa,[42] and ROI are likely to be responsible for the mucosal damage associated with *H. pylori* infection. Evidence is also accumulating that ROI can affect intracellular signal transduction pathways, and the expression of certain genes is redox (reduction-oxidation)-sensitive.[43] We recently showed that IL-8 expression in gastric epithelial cells was up-regulated by oxidative stress and down-regulated by antioxidants.[44] It is interesting that IL-8 recruits and activates neutrophils and that activated neutrophils produce large amounts of ROI that may further stimulate IL-8 secretion. The use of antioxidants may be of therapeutic significance in *H. pylori*-associated gastritis.

CC-Chemokines in *H. pylori*-associated gastritis

Although little attention has been focused on the CC-chemokine subfamily, locally produced CC-chemokines that target monocytes and lymphocytes may also play important roles in chronic inflammation associated with *H. pylori* infection. Consistent with this notion, the in vivo expression of CC-chemokines, such as monocyte chemotactic protein (MCP)-1 or regulated upon activation, normal T-cell expressed and presumably secreted (RANTES), is as high in the gastric mucosa as that of IL-8.[23] Although inflammatory cells seem to be the major source of these CC-chemokines, we have shown that gastric epithelial cells also secrete MCP-1 in response to proinflammatory cytokines and *H. pylori* organisms.[23] The balance between local production of CXC- and CC-chemokines may determine the pattern of inflammatory responses in *H. pylori*-infected gastric mucosa.

Anti-inflammatory cytokines

H. pylori infection induces a significant inflammatory and immune response in the gastric mucosa. However, spontaneous clearance of *H. pylori* is extremely rare, and infection continues for decades. In chronic *H. pylori* infection, the presence of persistent inflammation may cause mucosal damage. Generally, anti-inflammatory cytokines, such as IL-4 and IL-10, play a role in down-regulating inflammatory responses.[45] As for chemokine response, neutrophil chemokine production is reported to be down-regulated by IL-4 and IL-10.[45] Since expression of IL-4 and IL-10 is shown in *H. pylori*-infected gastric mucosa,[46–48] these anti-inflammatory mechanisms may also be important in *H. pylori*-associated chronic gastritis. However, as far as we could determine, gastric epithelial IL-8 expression was not affected by these anti-inflammatory cytokines (unpublished observation). Gastric epithelial cells may lack receptors for these anti-inflammatory cytokines. It appears that the only way to down-regulate the gastric epithelial chemokine response is to eliminate *H. pylori* organisms from the gastric mucosa.

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