Helicobacter pylori infection induces interleukin-8 receptor expression in the human gastric epithelium

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The gastric pathogen Helicobacter pylori is known to activate multiple proinflammatory signaling pathways in epithelial cells. In this study, we addressed the question of whether expression of the interleukin-8 receptors IL-8RA (CXCR1) and IL-8RB (CXCR2) is upregulated in H. pylori-infected human gastric biopsy samples. Biopsy samples from patients infected with H. pylori strains harboring the cag pathogenicity island (PAI) expressed larger amounts of both receptors. In addition, IL-8RB expression was observed in the gastric epithelial cell line AGS upon infection with a clinical isolate containing the cag PAI, while a strain lacking the cag PAI did not. Our findings suggest that gastric epithelial cells express IL-8R in response to H. pylori infection.

Helicobacter pylori is a gram-negative bacterium that is specialized in colonizing the human gastric mucosa, where it causes a variety of clinical outcomes ranging from asymptomatic carriage to gastritis, peptic ulcers, and cancer (12). One of the most important virulence determinants of H. pylori is the 40-kb pathogenicity island (PAI) that harbors the cag genes, which encode effector molecules and a type IV secretion system (4). The type IV secretion system is required for translocation of effector molecules, e.g., CagA, into host cells (14).

Epithelial cells form tight mucosal barriers and are the first cells that encounter a microbial challenge and have evolved numerous ways to detect and respond to infectious agents (2). One of the most important effector molecules produced by the epithelium is the chemokine interleukin-8 (IL-8) (9, 11). Binding of IL-8 to two high-affinity receptors, IL-8RA (CXCR1) and IL-8RB (CXCR2), induces chemotaxis and transepithelial migration of neutrophils (8, 10, 13). The importance of IL-8R cation of effector molecules, e.g., CagA, into host cells (14).

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Infection Induces Interleukin-8 Receptor Expression in the Human Gastric Epithelium

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RESULTS AND DISCUSSION

It was recently reported that, in addition to producing IL-8 and other chemokines upon bacterial infection, renal epithelial cells also express IL-8RA and IL-8RB in response to E. coli (7). To investigate whether H. pylori induces IL-8R expression in the gastric epithelium, we analyzed biopsy samples obtained from uninfected (n = 5) and H. pylori-infected (n = 15) patients. We found that infected biopsy samples showed increased levels of IL-8RA (4.1-fold) and IL-8RB (8.1-fold) expression. When the biopsy samples were separated in accordance with the cag PAI genotype of the infecting strain, we found that biopsy samples infected with cag PAI-positive strains (n = 6) induced IL-8RA 11-fold while biopsy samples infected with cag PAI-negative strains (n = 9) only induced IL-8RA 2-fold (Fig. 1A). The corresponding induction of IL-8RB expression was 26-fold versus 4-fold (Fig. 1B). Accordingly, in addition to IL-8 production (1), the cag PAI status of the infecting H. pylori strain affects cellular IL-8R expression in gastric biopsy samples. Because we found slightly more neutrophils in biopsy samples infected with cag PAI-positive strains, we could not exclude the possibility that neutrophils contributed to the observed increase in IL-8R expression. We found, however, that biopsy samples infected with cag PAI-negative H. pylori strains had transcript levels of the leukocyte marker CD18 (fivefold higher than those of uninfected samples) similar to those of cag PAI-positive H. pylori strain-infected biopsy samples (fourfold higher than those of uninfected samples) and that the degrees of inflammation, as scored by the updated Sydney system (5), were similar (data not shown).

To examine whether epithelial cells express IL-8R, we used the gastric epithelial cell line AGS as a model system. We harvested the cells and incubated them with biotinylated IL-8 and found that most of the cells bound this chemokine on the cell surface (Fig. 2). This finding suggests that cell-surface-localized IL-8Rs are expressed by gastric epithelial cells. To investigate whether gastric epithelial cells increase IL-8R expression upon H. pylori infection, we infected AGS cells with 2 × 10⁶ CFU of H. pylori strain 67:20 (cag PAI negative) or 67:21 (cag PAI positive) per ml. To ensure that the cells were properly infected, we determined the bacterium-induced IL-8 production by ELISA (Fig. 3A), as well as by quantitative real-time RT-PCR (Fig. 3B). We found that a rapid increase in IL-8 mRNA was accompanied by IL-8 protein synthesis when the cells were infected with strain 67:21 but not when they were infected with strain 67:20. H. pylori infection had no effect on IL-8RA expression (Fig. 3C), while IL-8RB expression was increased threefold 6 h after the addition of cag PAI-positive strain 67:21 (Fig. 3D). The induction of IL-8RB was strictly cag PAI dependent, as infection with strain 67:20 did not induce any IL-8RB expression. However, the cag gene(s) responsible for IL-8RB expression remains to be identified.

In order to investigate whether IL-8 is responsible for the induction of IL-8RB, we infected AGS cells with H. pylori strain 67:21 in the presence of IL-8-neutralizing antibodies and found that this addition did not affect IL-8RB expression. To further confirm this finding, we stimulated the cells with 10 ng of recombinant human IL-8 per ml without observing any effect on IL-8RB expression (data not shown). This concentration is fivefold greater than what is produced by AGS cells at 6 h postinfection. Collectively, this demonstrates that induction of IL-8R occurs independently of the secreted IL-8 and that cag PAI-positive H. pylori causes inflammation by a synergistic mechanism that directly activates IL-8 and its receptors by independent pathways.
FIG. 2. AGS cells express IL-8R. Binding of IL-8 to AGS cells was determined by flow cytometry (solid line). The dashed line shows the background fluorescence of the streptavidin control. These data are representative of three independent experiments.

FIG. 3. Gastric epithelial cells respond to *H. pylori* infection by expression of IL-8 and IL-8R. AGS cells were infected with *H. pylori* strain 67:20 (cag PAI negative, white bars) or 67:21 (cag PAI positive, black bars) for 2 or 6 h. IL-8 expression was determined by ELISA (A) and quantitative real-time RT-PCR (B). IL-8RA (C) and IL-8RB (D) expression was determined by quantitative real-time RT-PCR. The values shown are the means ± the standard errors of three independent experiments. *, *P* < 0.05; **, *P* < 0.01 (versus cells infected with strain 67:20).
IL-8R-deficient mice are unable to regulate their production of macrophage inflammatory protein 2, an IL-8 homologue in mice, during urinary tract infections (6). Prolonged macrophage inflammatory protein 2 production leads to accumulation of neutrophils in the submucosa that are unable to transmigrate through the epithelium. Accordingly, these mice cannot clear the infection and develop symptoms of systemic disease. To investigate whether the increase in IL-8RB was involved in a negative feedback loop, acting to disease. To investigate whether the increase in IL-8RB was inflammatory protein 2 production leads to accumulation of neutrophils in the submucosa that are unable to transmigrate through the epithelium. Accordingly, these mice cannot clear the infection and develop symptoms of systemic disease. To investigate whether the increase in IL-8RB was involved in a negative feedback loop, acting to disease.

In conclusion, we demonstrate here that biopsy samples obtained from H. pylori-infected patients expressed higher levels of both IL-8RA and IL-8RB than did uninfected biopsy samples. The level of expression of both receptors was higher in biopsy samples infected with cag PAI-positive strains than in biopsy samples infected with cag PAI-negative strains. Our results suggest that the gastric epithelium can be one cellular source of these receptors and thus imply that the mucosal response to bacterial infections is broader than previously appreciated.

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F.B. and E.T. contributed equally to this work.

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