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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 induced 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 finding suggests 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 was shown in a urinary tract infection model in which mice deficient in the receptor were unable to clear an *Escherichia coli* infection (6). Because epithelial expression of IL-8R can be an effective means by which to augment the inflammatory response required for bacterial clearance, we examined whether epithelial cells express IL-8R in response to *H. pylori* infections.

MATERIALS AND METHODS

Collection of gastric biopsy samples. Clinical samples were obtained from 20 volunteers (18- to 50-year-old healthy males and females) enrolled in a clinical research trial (June 1999 to June 2000) in the Hepato-Gastroenterology Department at the Centre Hospitalier Universitaire Edouard Herriot (Lyon, France). Patients who had taken nonsteroidal anti-inflammatory drugs, proton pump inhibitors, or antibiotics during the preceding 3 months were excluded from the study. The *H. pylori* status of the volunteers was determined by serological Pyloriset Dry tests (Orion Diagnostica, Espoo, Finland) and enzyme-linked immunosorbent assays (ELISAs) as pre-endoscopy screening. Endoscopies were performed under local anesthesia, and two biopsy samples were obtained from adjacent areas of the gastric antrum. RNA and DNA were extracted with TRIZOL (Gibco BRL, Cergy Pontoise, France). By using a PCR-based method (1), we found that the biopsy samples were infected to the same degree independently of the *cag* PAI genotype of the infecting strain.

Cells, bacterial strains, and growth conditions. The human epithelial cell line AGS (ATCC CRL 1739, human gastric carcinoma) was cultured in 24-well cell culture plates in RPMI 1640 medium supplemented with 25 mM HEPES, 2 mM L-glutamine (Life Technologies, Stockholm, Sweden), and 10% fetal calf serum (Integro b.v., Zaandam, Holland) at 37°C in 5% CO_2. *H. pylori* strains 67:20 (*cag* PAI negative) and 67:21 (*cag* PAI positive) are two different subclones isolated from the same patient; in strain 67:20, the *cag* PAI has been excised (3). Both strains were grown on brucella agar plates containing 5% bovine blood under microaerophilic conditions at 37°C for 48 to 72 h. Bacteria were subcultured in brucella broth supplemented with 5% fetal calf serum and 1% IsoVitox enrichment (Dalynn Biologicals, Calgary, Alberta, Canada) for 16 h at 37°C under microaerophilic conditions. Bacteria were thoroughly washed in phosphate-buffered saline before they were added to the tissue culture medium at a density of 2×10^6 CFU/ml, which corresponds to a multiplicity of infection of 10. The supernatants were collected, and total RNA was isolated with TRIZOL at 2 and 6 h postinfection (Gibco BRL). The supernatants were analyzed for IL-8 by ELISA (Diaclone, Besançon, France), and the statistical significance of differences was determined by Student's *t* test.

Determination of IL-8 and IL-8R expression. First-strand cDNA synthesis was performed on 5 μg of DNase I-treated total RNA with random hexamers and a ProSTAR First Strand RT (reverse transcription)-PCR kit (Stratagene, Amsterdam, The Netherlands). All expression quantification was performed by real-time RT-PCR with the double-stranded DNA-specific dye SYBR Green I and a LightCycler apparatus (Roche Diagnostic, Mannheim, Germany). Human glyceraldehyde-3-phosphate dehydrogenase-specific primers were obtained with Prime software from a 1,272-bp mRNA sequence (EMBL accession number X01677) as previously described (15). To detect human IL-8 expression, we used a ready-to-use kit in accordance with the manufacturer's (Search-LC GmbH, Heidelberg, Germany) instructions. PCR primers for IL-8RA (GenBank accession number L19592) (forward, 5′-TGTCGTCCTCATCTTCCTGC-3′ [located on exon 1]; reverse, 5′-CCGATGAAGGCGTAGATGATGG-3′ [located on exon 2]) and for IL-RB (GenBank accession number M99412) (forward, 5-TC CAAGGTGAATGGCTGG-3′ [located on exon 1]; reverse, 5′-ATGAGTAGA CGGTCCTTCGG-3′ [located on exon 2]) were obtained with Prime software. Quantification of each gene's expression was performed with 70 ng of total RNA from each preparation. Quantitative analysis was performed with the LightCycler software (Roche Diagnostic). Background fluorescence was removed manually by setting a noise band, and the log-linear portion of the standard curve was identified. The identity and specificity of the PCR products were confirmed by melting-curve analysis, which is part of the LightCycler analysis program. Cor-

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responding RNA samples not previously subjected to RT were also amplified to measure the amount of contaminating chromosomal DNA. All results were standardized to glyceraldehyde-3-phosphate dehydrogenase expression. Gene expression was normally distributed according to the Shapiro-Wilks test, and the statistical significance of differences in the mean number of copies of transcripts was performed with Student's *t* test. Expression of IL-8R protein was determined by using Fluorokine on 10^4 AGS cells with a fluorescence-activated cell sorter in accordance with the manufacturer's (R&D Systems, Oxon, United Kingdom) instructions.

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 2×10^6 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.

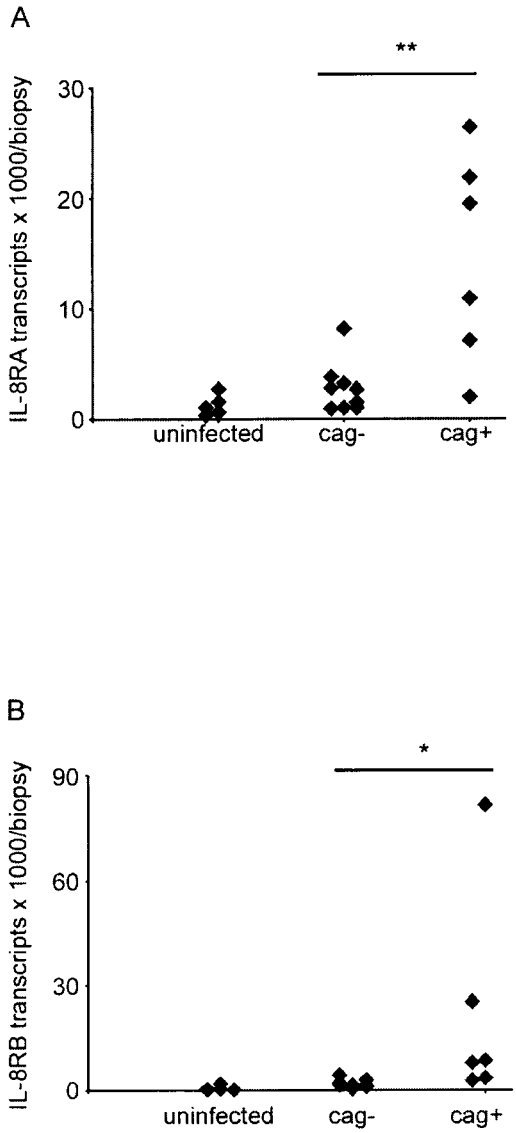

FIG. 1. *H. pylori* infection induces IL-8R in human biopsy samples. IL-8RA (A) and IL-8RB (B) expression was determined by quantitative real-time RT-PCR for uninfected patients ($n = 5$), *cag* PAI-negative strain-infected patients ($n = 9$), and *cag* PAI-positive strain-infected patients ($n = 6$). *, $P < 0.05$; **, $P < 0.01$.

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.

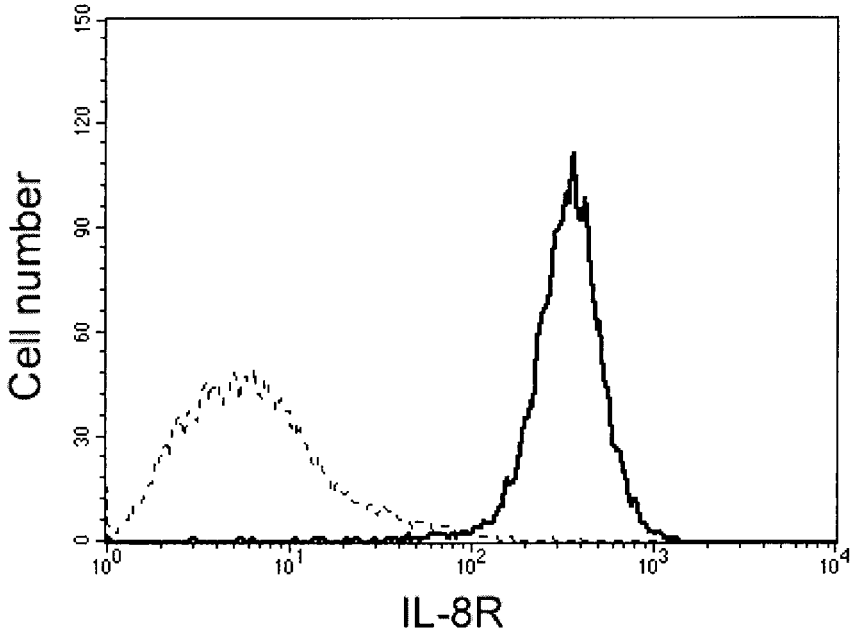

FIG. 2. AGS cells express IL-8R. Binding of IL-8 to AGS cells was determined by flow cytometry (solid line). The dashed line shows the background fluorescence of the streptavidin control. These data are representative of three independent experiments.

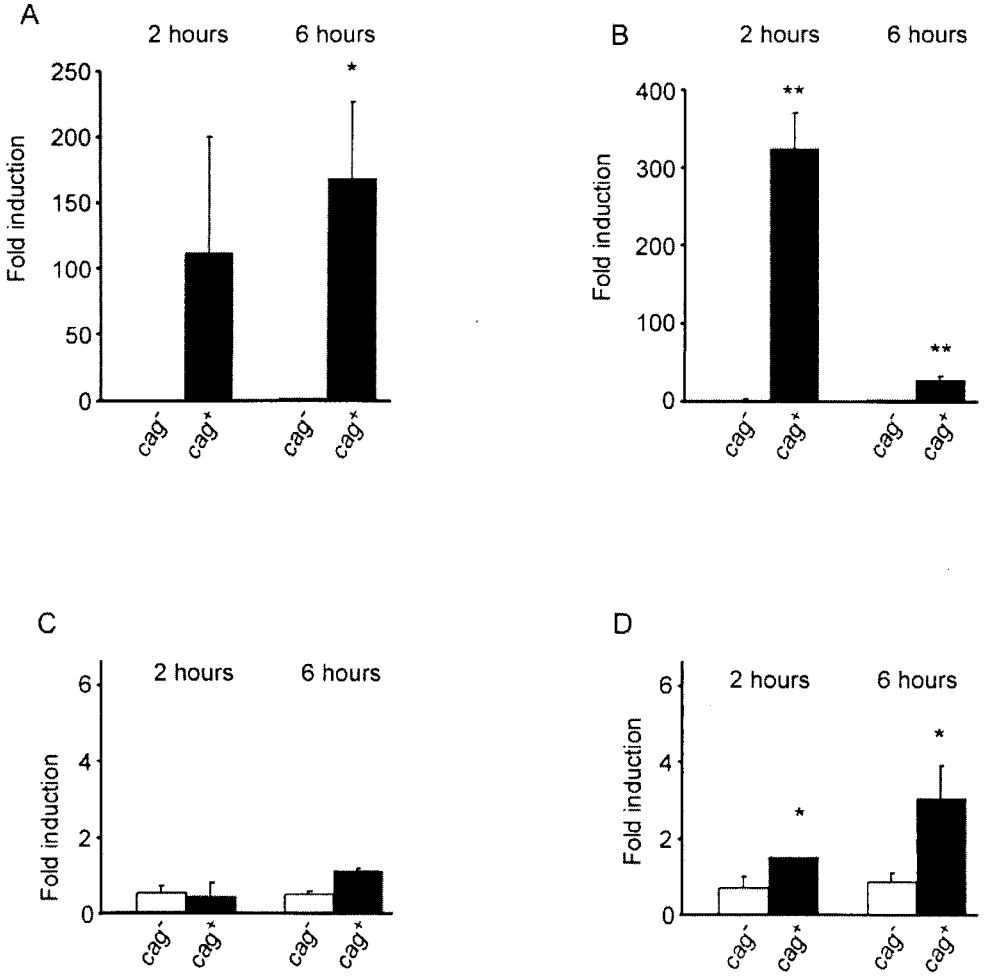

FIG. 3. Gastric epithelial cells respond to *H. pylori* infection by expression of IL-8 and IL-8R. AGS cells were infected with *H. pylori* strain 67:20 (*cag* PAI negative, white bars) or 67:21 (*cag* PAI positive, black bars) for 2 or 6 h. IL-8 expression was determined by ELISA (A) and quantitative 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 fine tune the IL-8 response in AGS cells, we pretreated the cells with blocking antibodies directed to IL-8RB. However, we could not obtain any evidence that supports this hypothesis (data not shown). The function of IL-8R expression in epithelial cells in response to bacterial infections could be to bind endogenously produced IL-8 and thereby locally increase the IL-8 concentration, which maintains the chemotactic gradient for neutrophil chemotaxis and transmigration.

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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