Induction of interleukin-8 secretion from gastric epithelial cells by a cagA negative isogenic mutant of *Helicobacter pylori*

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Abstract
The ability of *Helicobacter pylori* strains to induce interleukin-8 (IL-8) gene expression and protein secretion from gastric epithelial cell lines in vitro is variable. This cellular response is associated with bacterial expression of the CagA protein present in type I H *pylori* strains. To determine the role of CagA in this host cell response, an isogenic cagA negative mutant, N6.XA3, was constructed. The cagA negative isogenic mutant and the wild-type parental cagA positive strain, N6, were co-cultured with AGS, ST-42 and KATO-3 gastric epithelial cell lines and secreted interleukin-8 assayed by enzyme linked immunosorbent assay. In all three cell lines there was no significant difference in the IL-8 secretion induced by the cagA negative isogenic mutant, N6.XA3, and the wild-type parent strain, N6. These studies show that CagA is not the inducer of IL-8 secretion from gastric epithelial cells. As all wild-type CagA positive strains studied to date induce IL-8, the bacterial factor(s) inducing this inflammatory response is closely associated with the expression of CagA.

Keywords: Interleukin-8, *Helicobacter pylori*, CagA, epithelial cells, gastritis.

The CagA surface protein of *Helicobacter pylori* is highly immunogenic and is expressed in about 60 to 70% of *H pylori* strains. Mucosal IgA antibody recognition of this protein has been linked with peptic ulcer disease and the activity of gastritis and systemic IgG responses to CagA are also elevated in ulceration. Strains of *H pylori* which have the gene coding for CagA and express this immunogenic protein usually coexpress the vacuolating cytotoxin (VacA). Strains with this genotype/phenotype have recently been classified as type I bacteria. Type II strains lack the cagA gene and express neither the CagA protein nor the VacA protein. While the VacA protein is thought to be an important mediator of gastric mucosal damage, this protein does not elicit gastric inflammatory cell infiltration in animal models.
Our previous studies have shown that gastric mucosal production of the neutrophil chemotactic and activating peptide interleukin-8 (IL-8) is increased in patients with *H. pylori* infection and epithelial polymorph infiltration (active gastritis). 7 In vivo an important source of IL-8 is the gastric epithelium. 8 We have recently demonstrated that type I strains expressing the cytotoxin and CagA protein directly induce IL-8 mRNA expression and IL-8 protein secretion in gastric epithelial cell lines. 9 Using natural phenotypic variant strains of *H. pylori* with disparate CagA and VacA expression, 9 we have also shown that the up-regulation of epithelial IL-8 is associated with the CagA phenotype and not with expression of VacA. 10

To determine whether the CagA protein is the bacterial mediator inducing gastric epithelial IL-8, we have constructed an isogenic cagA negative mutant strain of *H. pylori* and examined the ability of the mutant strain and parental strain to induce IL-8 secretion.

**Methods**

**CONSTRUCTION OF ISOGENIC cagA NEGATIVE MUTANT N6.XA3**

The cagA gene, cloned in the Bluescript vector SK+ (pA), was disrupted by the insertion of a kanamycin resistance gene at nucleotide position 2370 (pA::Km). DNA from plasmid pA::Km was transferred into the recipient cagA/ CagA positive N6 strain of *H. pylori* (kindly provided by Dr A Labigne) by electroporation. Briefly, *H. pylori*, incubated in a microaerophilic atmosphere, were collected, resuspended and washed three times in WEB solution (10% glycerol in deionised water) at 4°C. Bacteria were centrifuged and resuspended at a final concentration of 250 OD/ml. Aliquots of 25 μl were frozen in liquid nitrogen and stored at −80°C. For electroporation, 25 μl of frozen bacteria were mixed with 1 μl of DNA (10 μg/ml in deionised water) in pre-cooled 0-1 cm cuvettes (BioRad) and exposed to an electric field of 2-0 Kv, 25 microFD, 200 Ohms. Bacteria were recovered and plated onto non-selective plates for two days. Bacteria were then isolated and plated onto selective media for three days. 11 Kanamycin resistant *H. pylori* colonies were screened for allelic exchange by Southern hybridisation and polymerase chain reaction. The expression of the CagA protein was assessed by western blot assay using a rabbit anti-CagA specific antiserum. The isogenic cagA negative mutant N6.XA3 showed no expression of CagA in western blot assays.

**BACTERIAL STIMULATION OF IL-8 SECRETION IN GASTRIC EPITHELIAL CELL LINES**

The isogenic cagA negative mutant N6.XA3, the wild-type parental strain N6 and the type strain NCTC 11637 (CagA positive control) were grown on blood agar base Number 2 (Oxoid, Basingstoke, UK) including 7% fresh horse blood. Bacteria were harvested on day 4 and after centrifugation, resuspended at 2-5 × 107/ml in RPMI 1640 (ICN-Flow Laboratories, High Wycombe, UK) containing 10% fetal calf serum (FCS) (Sera Lab, Crawley, Surrey, UK) and used immediately.

Experiments were undertaken with AGS, ST42 and KATO-3 gastric epithelial cell lines described in previous studies. 9,10 Cells were routinely maintained in RPMI 1640 supplemented with 10% FCS and 40 μg/ml gentamicin. Confluent monolayers of AGS and ST42 (approximately 5 × 105/ml) in 24 well plates (ICN-Flow Laboratories) and suspensions of non-adherent KATO-3 (5 × 105/ml) were cultured for 24 hours in quadruplicate with N6.XA3, the wild-type parental strain N6 and the type strain NCTC 11637 at a concentration of 2-5 × 105/ml as previously described. 9,10 Cultures were undertaken in gentamicin free media. After 24 hours, supernatants were aspirated and stored at −70°C until assayed in duplicate for IL-8 by enzyme linked immunosorbent assay (ELISA). The ELISA was carried out as previously described 9,10 and uses a mouse monoclonal antibody to IL-8 (Sandoz, Vienna, Austria) and a phosphatase conjugated goat anti-IL-8 polyclonal antibody (Sandoz).

**Results**

The 24 hour secretion of IL-8 from AGS and ST42 cells after stimulation with *H. pylori* is shown in the figure. The cagA positive N6 wild-type strain and the cagA negative isogenic mutant N6.XA3 both induced IL-8 secretion relative to cell only control cultures. IL-8 secretion with the isogenic cagA negative mutant strain was 78% (AGS) and 84% (ST42) that of the wild-type parent strain. There was no significant difference between IL-8 secretion induced by the cagA positive wild-type strain and the cagA negative isogenic mutant (p = 0.66). Similarly, there was no significant difference in IL-8 secretion by KATO-3 cells following culture with N6 (mean (SEM), 11-7 (0-20) ng/ml and N6.XA3 (9-11 (0.5), n = 3); cell only controls (1-77 (0-13)); positive control NCTC 11637 (11-2 (0-45)).
Discussion
These studies with the isogenic cagA negative mutant strain of H pylori demonstrate that deletion of the CagA protein does not effect the ability of H pylori to induce IL-8 secretion from gastric epithelial cells. The CagA protein is, therefore, not functionally involved in this response but is a marker for strains with pro-inflammatory properties. As all of the wild-type CagA positive strains we have studied to date induce epithelial IL-8 secretion, the bacterial factor(s) stimulating this inflammatory response is clearly closely associated with the expression of cagA. The function of the CagA protein remains to be determined.

The CagA protein shows considerable size variation in different strains of H pylori, ranging from 128 to 150 kD.3 Intragenic repeat sequences are the basis of this heterogeneity.1 Studies with native4 and recombinant CagA protein5–7 have clearly demonstrated the high immunogenicity of this protein both locally and systemically. It is important to consider the role of this antigen specific response in the immunopathogenesis of chronic gastritis.

If the inflammatory epithelial response generated by type I strains is important for bacterial nutrient supply, one bacterial survival strategy may be to avoid immunological recognition of bacterial inflammatory factors and tissue damaging agents such as the cytotoxin. The highly immunogenic CagA protein may therefore be an "immunological decoy" diverting host responses away from these functionally important bacterial factors.

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A practical single sample dry latex agglutination test for Helicobacter pylori antibody detection

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Abstract
Assessment of a single serum sample for Helicobacter pylori antibodies is frequently requested in routine diagnostic laboratories. Current enzyme linked immunosorbent assay (ELISA) kits are not ideal for testing small numbers of serum samples and some have low sensitivities, specificities or large grey zones. A panel of 90 serum samples from patients who had presented for routine upper endoscopy was used to compare three kits for the detection of H pylori antibodies: (1) Pyloriset Dry, total antibody latex agglutination, Orion Diagnostica, Espoo, Finland; (2) Pyloriset enzyme immunoassay (EIA), IgG ELISA, Orton; and (3) Hel-p, IgG ELISA, Amrad, Kew, Victoria, Australia. Diagnosis of H pylori positivity was made if culture results and either rapid urease test or histopathology were positive. The sensitivity, specificity, positive and negative predictive value for each test was as follows: Orton: latex 93.3%, 95.6%, 95.5%, 93.3%, respectively; Orton: EIA-G 84.4%, 97.8%, 97.4%, 84.4%, respectively; and Amrad: EIA-G 100%, 88.9%, 90%, 100%, respectively. The latex test performed better than the ELAs with respect to sensitivity and specificity.


Keywords: Helicobacter pylori, serology, diagnostic test.