Helicobacter pylori adherence to gastric epithelial cells: a role for non-adhesin virulence genes

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Helicobacter pylori is a major aetiological agent in gastroduodenal disorders and adherence of the bacteria to the gastric mucosa is one of the initial stages of infection. Although a number of specific adhesins has been identified, other H. pylori virulence factors may play a role in adherence to gastric epithelial cells directly or through interaction with other adhesins. This study assessed the effect of 16 H. pylori virulence factors on the adherence of the bacteria to gastric AGS cells and on gastric epithelial cell cycle distribution. Defined isogenic H. pylori SS1 mutants were used. After co-incubation of gastric AGS cells and bacteria, adherence of H. pylori to AGS cells was visualised by immunofluorescence microscopy and quantified by flow cytometry. Cell cycle phase distribution was analysed by flow cytometry with propidium iodide staining. Mutants were tested for their ability to adhere to AGS cells and compared with the wild-type SS1 strain. Mutations in genes in the cag pathogenicity island showed that cagP and cagE mutants adhered less than the wild-type strain to AGS cells, whereas a cagF mutant showed no reduction in adherence. Mutations in genes involved in flagellar biosynthesis showed that the adherence ability of fliQ, fliM and fliS mutants was reduced, but a flhB mutant possessed wild-type levels of adherence. Mutations in genes coding for the urease (ureB) and phospholipase (pldA) enzymes did not affect adherence, but mutation of the tlyA gene encoding an H. pylori haemolysin resulted in a reduced adherence. A fliQ mutant, with reduced adherence to AGS cells, was less able to induce AGS cell apoptosis than SS1. The ability to induce G0G1 cell cycle arrest was also abolished in the fliQ mutant. However, an increased cell number in S phase was observed when AGS cells were exposed to the fliQ mutant compared with SS1, suggesting that unattached bacteria may still be able to stimulate cell proliferation. In addition to known adhesins, other bacterial virulence factors such as CagE, CagP, FliQ, FliM, FliS and TlyA appear to play a role in H. pylori adherence to gastric epithelial cells. Mutations in these genes may affect H. pylori pathogenicity by reducing either the ability of the bacteria to attach to gastric epithelial cells or the intensity of bacteria–host cell interactions.

Introduction

Chronic infection with Helicobacter pylori, the main cause of chronic gastritis, results in various disease outcomes including peptic ulceration, gastric adenocarcinoma and gastric lymphoma [1]. The bacterium colonises the gastric mucosa by adhering to and penetrating the mucus layer lining the gastric epithelium [2]. Colonisation of the gastric mucous layer protects the bacteria from the extreme acidity of the gastric lumen and displacement from the stomach by forces such as those generated by peristalsis and gastric emptying. The majority of the colonising bacteria remain in the gastric mucous layer, but some adhere to the gastric epithelial cells. The clinical significance of the host–pathogen interactions that follow attachment of H. pylori to human gastric cells remains to be fully elucidated. It is considered unlikely that chronic infection with H. pylori could occur in the absence of adhesin–host cell interactions [3]. Therefore, adherence of the bacteria to the gastric mucosa is
one of the initial steps of \textit{H. pylori} infection and is an important virulence factor.

Bacterial surface structures play an important role in determining the mode of interaction of individual bacteria with other bacteria and with other factors in their immediate surroundings. \textit{H. pylori} has evolved a repertoire of interactive surface molecules that enable the bacteria to adhere to human gastric epithelial cells and phagocytic cells via adhesins interacting with host-cell receptors. Many different \textit{H. pylori} adhesins have been identified [4], implying that adherence is a multifactorial process. However, there is no consensus as to which adhesins are most important in vivo, or whether adhesin(s) and receptor(s) for the \textit{H. pylori} epithelial cell interactions are similar to the \textit{H. pylori}–phagocytic cell interactions. Furthermore, although adhesins are a group of bacterial surface molecules, their adhesive functions may be inhibited or enhanced by other bacterial virulence factors during the processes of adhesin synthesis, transportation, secretion and activation. The real challenge in this post-genomic era is not only to study known adhesins to determine which are important in vivo, but also to identify other bacterial factors which may have an effect on \textit{H. pylori} adherence.

At least five different \textit{H. pylori} adhesins have been described [4]. One group of putative adhesins is the \textit{H. pylori} outer-membrane protein (Hop) family, represented in the strain 26695 genome sequence by 32 members [5]. Three previously identified \textit{H. pylori} adhesins belong to the Hop family [6–8]: AlpA and AlpB, which may act as adhesins and recognise different receptors on the gastric epithelial cell surface, and the BabA adhesin, which mediates attachment to the blood group antigen Lewis$^b$ (Le$^b$) [9]. It is suggested that other members of the Hop family may also act as adhesins. Recently, Namavar et al. [10] identified a 16-kDa surface protein that adhered to oligosaccharide ligands such as sulphated Lewis$^b$ (Le$^b$) antigens present on mucin glycoproteins. Tomb et al. [5] showed that the Hop genes have highly homologous domains at the 5' and 3' ends. This feature suggests the possibility of recombination and hence an increase in antigenic variation. One of these genes, hopZ, has been characterised recently. A hopZ mutant showed significantly reduced binding to human gastric epithelial cells compared with the wild-type strain ATCC 43504 [11].

This study assessed the effect of mutations in 16 \textit{H. pylori} virulence genes not previously associated as adhesins on bacterial adherence to gastric AGS cells. All the genes studied can be defined as virulence determinants, either through previous \textit{H. pylori} studies or by their similarity to virulence genes in other bacteria. To further assess the importance of bacterial adherence on \textit{H. pylori} pathogenicity, the effect of some of these mutants on AGS cell cycle progression was also investigated.

### Materials and methods

#### Construction of defined isogenic \textit{H. pylori} mutants

\textit{H. pylori} wild-type strain SS1 was used in this study [12]. Defined isogenic \textit{H. pylori} SS1 mutants were constructed as described previously [13]. Briefly, specific amplifying primers for each gene were designed from the \textit{H. pylori} 26695 genome sequence. The resulting PCR products were amplified from \textit{H. pylori} 26695 chromosomal DNA and cloned into pUC19. A defined deletion and unique \textbf{BgII} site were introduced into each of the cloned genes by inverse PCR mutagenesis [14, 15]. A kanamycin resistance gene was cloned into the unique \textbf{BgII} site and these constructs were introduced into the SS1 wild-type strain by electroporation [16]. Double-crossover mutants were selected and screened as described previously [13]. The \textit{H. pylori} wild-type strain and the mutant strains used are listed in Table 1.

#### Cell culture and growth of \textit{H. pylori} strains

Gastric AGS cells, derived from human gastric carcinomas, were grown in culture medium consisting

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**Table 1. \textit{H. pylori} SS1 and its isogenic mutants**

<table>
<thead>
<tr>
<th>Code no.</th>
<th>Gene</th>
<th>HP no.</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS1</td>
<td>Wild-type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ND1</td>
<td>cagP</td>
<td>0536</td>
<td>Cag pathogenicity island; function unknown</td>
</tr>
<tr>
<td>ND4</td>
<td>cagE</td>
<td>0544</td>
<td>Cag pathogenicity island; IL-8 induction</td>
</tr>
<tr>
<td>ND8</td>
<td>cagF</td>
<td>0543</td>
<td>Cag pathogenicity island; function unknown</td>
</tr>
<tr>
<td>ND3</td>
<td>ureB</td>
<td>0072</td>
<td>Urease β-subunit (urea amidohydrolase)</td>
</tr>
<tr>
<td>ND5</td>
<td>pldA</td>
<td>0499</td>
<td>Phospholipase [13]</td>
</tr>
<tr>
<td>ND6</td>
<td>fliI</td>
<td>1420</td>
<td>Flagellar export protein (ATP synthase) [17, 18]</td>
</tr>
<tr>
<td>SF1</td>
<td>fliG</td>
<td>0352</td>
<td>Flagellar motor switch protein [19]</td>
</tr>
<tr>
<td>SF3</td>
<td>fliQ</td>
<td>1419</td>
<td>Flagellar biosynthetic protein [19, 20]</td>
</tr>
<tr>
<td>SF4</td>
<td>fliM</td>
<td>1031</td>
<td>Flagellar motor switch protein</td>
</tr>
<tr>
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<td>fliB</td>
<td>1575</td>
<td>Flagellar biosynthetic protein [19, 20]</td>
</tr>
<tr>
<td>MW1</td>
<td>fliS</td>
<td>0753</td>
<td>Flagellar biosynthetic protein [19]</td>
</tr>
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<td>MW2</td>
<td>fliF</td>
<td>0351</td>
<td>Flagellar basal-body M-ring protein [19]</td>
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<tr>
<td>EA2</td>
<td>cheY1</td>
<td>0867</td>
<td>Iron (II) transport protein, ferrous binding protein [21]</td>
</tr>
<tr>
<td>RS1</td>
<td>cheY2</td>
<td>1067</td>
<td>Chemotaxis response regulator [22]</td>
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<tr>
<td>SF6</td>
<td>cheA2</td>
<td>0392</td>
<td>Chemotaxis histidine kinase [22]</td>
</tr>
<tr>
<td>RS7</td>
<td>tlyA</td>
<td>1086</td>
<td>Haemolysin [23]</td>
</tr>
</tbody>
</table>
of RPMI 1640 medium, fetal calf serum (FCS; Sigma-Aldrich, Poole) 10%, penicillin 100 IU/ml, streptomycin 100 μg/ml and 2 mM L-glutamine. The cells were maintained in a humidified atmosphere of CO2 5% and air 95% at 37°C. All the reagents and medium were purchased from GibcoBRL (Paisley).

*Helicobacter pylori* strains were stored at −80°C in Brain Heart Infusion (BHI) broth (Oxoid) containing glycerol 15% v/v and FCS 10% v/v. Strains were grown in BHI broth supplemented with FCS 10% v/v or on *Helicobacter* selective agar (DENT), consisting of Blood Agar Base No. 2 (Oxoid) supplemented with lysed defibrinated horse blood (TCS Microbiology, Botolph Claydon) 7% v/v and DENT selective supplement (Oxoid), in a micro-aerobic atmosphere at 37°C. For the adherence assay, overnight broth cultures were resuspended in phosphate-buffered saline (PBS) to a final concentration of $5 \times 10^8$ cfu/ml (OD$_{450}$ ≈ 1.0). For the cell cycle analysis, the bacteria were grown on Columbia Blood Agar containing horse blood 5% (Oxoid) for 48 h under micro-aerobic conditions.

### *H. pylori* adherence assay

The adherence assay was performed as described previously [24]. Gastric AGS cells (5 x 10⁶ cells) and *H. pylori* ($5 \times 10^8$ cfu) were incubated at 37°C for 1 h with agitation (150 rpm). Non-adherent bacteria were removed by centrifugation with 10 ml of sucrose 15% w/v solution. Cells were washed once with PBS and then incubated with a 1 in 5 dilution of polyclonal anti- *H. pylori* antibody (SkyTek Laboratories, Logan, USA) on ice for 30 min. After washing with 15 ml of PBS, the cells were incubated for an additional 30 min on ice in a 1 in 20 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Sigma-Aldrich). The cells were washed and resuspended in 1 ml of formaldehyde 1%. Before flow cytometric analysis, the adherence of the bacteria to AGS cells was visualised by fluorescence microscopy. A FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) was used to measure bacteria adhering to AGS cells. By using a dot-plot display of forward light scatter and side (90°) light scatter, the machine was gated to include single cells and to exclude cell debris and unbound bacteria. Fluorescence data were acquired in log-model on a 256-channel scale by an analysis of 10,000 individual cells. The results were expressed as the percentage of fluorescent cells calculated from fluorescence frequency distribution histograms. The threshold of cells without adherent bacteria was established for each experiment, the cells being stained with the two-step FITC-conjugated *H. pylori* antibody method described above. All experiments were repeated at least three times.

### Flow cytometric analysis for gastric epithelial apoptosis and cell cycle phase distribution

Subconfluent AGS cells were incubated with or without *H. pylori* (bacteria:cell ratio 100:1) for 48 h. The effect of *H. pylori* on gastric epithelial cell cycle phase distribution was assessed by flow cytometry as described previously [25]. Briefly, a pool of detached and adherent cells was washed in PBS, then the cells were fixed in ice-cold ethanol 70% overnight. The cell pellets were resuspended in 1 ml of a solution containing ribonuclease (Sigma-Aldrich) 200 μg/ml and propidium iodide (Sigma-Aldrich) 50 μg/ml for 60 min at 37°C. Cell cycle analysis was performed on a FACScan flow cytometer equipped with FACStation™ and CellQuest software (Becton Dickinson Immunocytometry Systems, Oxford). Debris was eliminated from the analysis with a forward angle light scatter threshold. Cell doublets and clumps were gated out of the analysis with a dot-plot display of area and width; 10,000 cells were analysed for each sample and the apoptotic cells were considered to constitute the sub-G₁ cell population. All experiments were repeated at least three times.

### Statistical analysis

Any significance in differences between two data sets was determined by the Student’s t test and by single-factor ANOVA for more than two data sets. p values <0.05 were considered significant in all analyses.

### Results

#### Effect of *H. pylori* strains on bacterial adherence to gastric AGS cells

The morphology and growth rate of the 16 isogenic SS1 mutants were assessed and showed no significant differences when compared to the wild-type strain. The ability of these mutants to adhere to cultured human AGS cells was analysed (Table 2). The results are presented as a percentage figure calculated from the total number of AGS cells adhered to by *H. pylori* strains and the total number of AGS cells analysed by flow cytometry. Statistical analysis was performed on each data set and a reduction in the ability of an individual mutant to adhere to AGS cells was deemed significant if p <0.05. Reduced adherence was observed with mutations of the *cagP, cagE, fliQ, fliM, fliS* and *tlyA* genes. The reduction in adherence to AGS cells is obviously not a result of the introduction of the kanamycin resistance cassette during mutagenesis, as many *H. pylori* SS1 mutants showed wild-type levels of adherence.

#### Effect of *H. pylori* strains on gastric AGS cell apoptosis and cell cycle progression

To examine the effect of *H. pylori* adherence on the pathogenicity of the bacterium, gastric AGS cell cycle
distribution was further analysed after exposure to the H. pylori SS1 wild-type strain and ureB, pldA and fliQ isogenic mutants. A significant induction of AGS cell apoptosis was observed by incubating either with the wild-type strain or its mutants (p = 0.002, 0.0003, 0.0007 and 0.00001, respectively), but the rates induced by the mutants were markedly lower than their parent strain SS1 (p = 0.007, 0.01 and 0.01, respectively) (Fig. 1). There were no significant differences in the induction of AGS cell apoptosis among the three mutants. Analysis of cell cycle phase distribution of surviving cells indicated that cells exposed to strain SS1 showed significant inhibition of cell cycle progression with 62.8 ± 1% of live cells arrested in G0/G1 phases (Fig. 2). However, the ability to induce G0/G1 cell cycle arrest was abolished in all three mutants (p = 0.0008, 0.0004 and 0.001, respectively). Interestingly, the fliQ mutant, which has a reduced adherence to gastric cells, showed a marked increase in cell numbers in S phase compared with untreated control cells (p < 0.0001) and the wild-type strain (p < 0.001). The ureB and pldA mutants showed no obvious effect on the cell cycle phase distribution of surviving AGS cells when compared to untreated control cells (p = 0.2 and 0.1, respectively) and to the wild-type strain (p = 0.2 and 0.1, respectively).

Discussion

Clinical isolates of H. pylori are known to differ in virulence and those from individuals with peptic ulcers are mostly type I strains which express both VacA and CagA [26]. The cagA gene is a marker for a large 40-kb locus containing >40 genes, termed the cag pathogenicity island (cag PAI), and the majority of these genes encode membrane-associated proteins with features similar to other bacterial secretion systems, particularly the type IV system epitomised by Bordetella pertussis toxin secretion [27–29]. A cagE mutant induced only mild inflammation in Mongolian gerbils, whereas the wild-type strain and vacA mutants induced more severe gastritis [30]. Mutation of cagE abolishes the ability of H. pylori to induce the cytokine interleukin-8 (IL-8), a neutrophil chemotactic factor, in Kato-3 cells [27, 29, 31, 32]. Adherence has been shown to play a role in the induction of H. pylori-associated IL-8 secretion [33]. The present study has clearly shown that mutation of cagE markedly reduced the adherence of H. pylori to gastric AGS cells. This finding suggests that the inability of a cagE mutant to stimulate IL-8 induction may be partly due to the loss of adherence capacity to gastric epithelial cells. A recent study has also found that a cagE mutant failed to colonise both C57BL/6j and BALB/c mice [34]. The present study also found that a cagP mutant had reduced adherence to gastric AGS cells, but not a cagF mutant. The function of CagP and CagF has yet to be defined. Although the reasons leading to the reduced adherence capacity in these mutants remain unclear, data from the present study suggest that some genes in the cag PAI play a role in H. pylori adherence to gastric epithelial cells.

H. pylori motility is essential for colonisation [35]. Motility enables the bacterium to spread into the viscus mucous layer covering the gastric epithelium. At least 40 proteins in the H. pylori 26695 genome appear to be involved in the regulation, secretion and assembly of the flagellar architecture [5]. In Salmonella enterica serovar Typhimurium, the first structure in flagellar assembly is the MS ring (FliF). The next structure assembled is the C ring which contains the switch proteins, FliG, FliM and FliN. This is followed by rod assembly for which several proteins, including FliI, FliQ and FliB, are required in addition to the rod structural proteins [36, 37]. These proteins, believed to be located at the cytoplasmic side of the basal body.
near to the switch [37], are thought to be components of the flagellum-specific export apparatus, exhibiting pronounced amino acid similarities with proteins involved in export of virulence factors [36]. FliS, required for efficient elongation of the filament in S. Typhimurium, is thought to be a cytoplasmic chaperone for flagellin export [37]. Factors responsible for colonisation may not affect the direct adherence to the cells, as adhesion to and colonisation of the gastric mucosa are separate stages of H. pylori infection [19, 20]. This is supported by data from the present study which show that some of the non-motile mutants are still able to adhere to AGS cells. However, the fliQ, flIM and fliS mutants all showed a reduced adherence to gastric epithelial cells. This may be due to the fact that these mutants are defective in some adherence pathway. For example, FliQ is probably involved in a type III export pathway in H. pylori [19, 20]. Therefore, the reduced level of adherence may underline the inability of the fliQ mutant to export a protein with a role in adherence. In contrast, mutants such as flhB or flil, which do not appear to be involved in the type III export pathway, showed wild-type levels of adherence. Mutations in genes involved in the regulation of chemotaxis (cheAY2 and cheY1) did not appear to affect adherence to gastric epithelial cells.

Like other pathogens, H. pylori possesses iron-scavenging systems for survival under the low iron conditions encountered in the human host [4]. Genomic analysis suggests that H. pylori has several systems for iron uptake [5]. One system for iron uptake present in H. pylori appears to be similar to the ferrous (feo) iron uptake system present in Escherichia coli. The feoAB operon encodes proteins which contribute significantly to the iron supply of E. coli cells under anaerobic conditions [38]. An feoB-like gene (HP0687), coding for a putative cytoplasmic protein with homology to ATPase, was identified in H. pylori, whereas no feoA orthologue seems to be present. Although FeoB has been shown to be important in both Fe^{2+} and Fe^{3+} transport [21, 39], the data from the present study did not support a role for this protein in H. pylori adherence.

One of the notable features of H. pylori grown on unlysed blood agar plates is haemolytic activity and it has been suggested that haemolysins may affect the biological activity of bacterial adhesins [40, 41]. It is known that E. coli haemolysins, by interacting with bacterial adhesins, can accelerate and enhance the rate of phagocytosis of bacteria by neutrophils and also increase the release of histamine and leukotriene from inflammatory cells [42]. Mutation of the gene encoding the H. pylori haemolysin TlyA significantly reduced the adherence of the organism to AGS cells. This suggests that there may be some interaction between TlyA and H. pylori adhesins, although the underlying mechanisms remain unclear. In contrast, mutation of the gene encoding the H. pylori phospholipase PldA, which also results in reduced haemolytic activity [13], showed a similar level of adherence to the wild-type strain. This suggests that PldA, whilst possessing haemolytic activity, has no role in adherence.

Bacterial adherence to host cells has been shown to be an important factor in the induction of cell cycle changes [43]. To examine whether the changes observed in H. pylori adherence to gastric epithelial cells have any effect on bacteria-associated cell damage, the
previously studied H. pylori-induced gastric epithelial cell cycle changes after exposure to SS1 and three mutants: ureB, pldA and fliQ. Both UreB and PldA are important virulence factors. Mutations in these genes produced ureB and pldA mutants with normal levels of adherence to AGS cells, whereas a fliQ mutant showed a markedly decreased adherence ability to AGS cells. Apoptosis is a prominent feature of H. pylori-induced epithelial cell damage. Many factors are responsible for the induction of H. pylori-associated gastric epithelial cell apoptosis [44]. The data from the present study further confirm that phospholipase and urease activities plus bacterial adherence to host cells are important factors in the induction of apoptosis.

H. pylori possesses several different phospholipase (PL) activities, such as PLA1, PLA2 and PLC [45]. The main activities of these PLs are thought to be the degradation of the phospholipid components of the mucosal barrier and normal phospholipid bilayer of the epithelial cell membrane [46]; but it may also influence the synthesis of prostaglandin [47]. Phospholipids constitute c. 20% of the dry weight of the human gastric mucus and are believed to be important in generating a hydrophobic barrier and maintaining normal cell functions. It has been reported that mucosal hydrophobicity is reduced in H. pylori-positive patients with duodenal ulcer compared with those having gastritis only [48]. The significantly diminished induction of apoptosis and cell cycle arrest by the pldA mutant suggests that H. pylori phospholipase activity may play an important role in H. pylori-associated gastric epithelial cell damage.

H. pylori produces high levels of urease, which makes up about 6% of the total bacterial protein [49]. This enzyme breaks down urea into ammonia and carbon dioxide, providing an acid-neutralising cloud of ammonia that could protect the bacterium from gastric acidity [50]. It has become increasingly clear that this is not the only function of urease in the physiology of H. pylori [51]. Although previous studies have suggested that the urease activity of H. pylori may be important in bacterial colonisation [4, 13, 52, 53], this study did not find a role for UreB in adherence to gastric epithelial cells. This may be because factors responsible for colonisation may not affect the direct adherence to the cells as adherence to and colonisation of the gastric mucosa are separate stages of H. pylori infection [37]. However, a ureB mutant showed a reduced ability in inducing AGS cell apoptosis and an inhibited induction of G0/G1 cell cycle arrest. These data further characterise the role of urease activity in H. pylori pathogenesis. Significant reductions in the levels of apoptosis and AGS cell cycle arrest were observed after exposure to the fliQ mutant, which has a significantly decreased adherence ability to AGS cells compared with the wild-type strain. Because the fliQ mutant still possesses intact H. pylori virulence factors, such as UreB and PldA, the reduced abilities to induce apoptosis and cell cycle arrest further demonstrate the importance of H. pylori adherence. Similarly, the ability of H. pylori to induce G0/G1 cell cycle arrest was abolished in all three mutants. This further suggests that bacterial virulence factors and bacterial adherence are both important determinants in H. pylori-induced cell cycle changes. Also, cells co-incubated with the fliQ mutant showed a significantly increased cell number in the S phase of cell cycle compared with cells exposed to the wild-type strain and untreated control cells, suggesting that exposure to the fliQ mutant may stimulate AGS cell proliferation. This may represent the effect of unattached bacteria on gastric epithelial cells. Although a proportion of H. pylori adhere to the gastric epithelial cells of gastric tissue, the majority of the organisms in infected patients remain in the mucus layer [54]. Therefore, investigation of the behaviour of unattached bacteria in the stomach may be of clinical importance. The enhanced AGS cell number in S phase following exposure to the fliQ mutant suggests that H. pylori may stimulate gastric epithelial cell proliferation, even without adherence to the epithelial cells. Further work is required to substantiate this hypothesis.

In summary, this study assessed the adherence of 16 SS1 isogenic mutants to gastric epithelial cells by fluorescence microscopy and flow cytometry. Mutations in genes in the cag PAI showed that cagP and cagE had significantly reduced adhesion, whereas a cagF mutant showed no difference compared to SS1. Mutation of fliQ, fliM or fliS genes significantly reduced the adherence of H. pylori to AGS cells compared with the wild-type strain. Mutations of the pldA, ureB and feoB genes did not affect adherence, but a tlyA mutant showed significantly reduced adhesion to AGS cells. Induction of AGS cell apoptosis was markedly reduced in cells exposed to the pldA, ureB and fliQ mutants. Analysis of cell cycle phase distribution of surviving cells showed that mutations in fliQ, ureB or pldA reduced the ability of the bacterium to induce AGS cell cycle arrest. Apart from adhesins, many other bacterial virulence factors such as CagE, CagP, FliQ, FliM, FliS and TlyA appear to play a role in H. pylori adherence to gastric epithelial cells. Mutation of the encoding genes may not only influence bacterial adherence, but may also have an effect on the pathogenicity of the organism.

References

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