Molecular and Cellular Biochemistry **253:** 207–215, 2003.

Molecular mechanism of action of major *Helicobacter pylori* virulence factors

Suman Kumar Dhar,[1] Rajesh K. Soni,[1] Bimal K. Das[2] and Gauranga Mukhopadhyay[1]

[1]*Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi;* [2]*Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India*

Abstract

Although *Helicobacter pylori* infects 50% of the total human population, only a small fraction of the infected people suffer from severe diseases like peptic ulcers and gastric adenocarcinoma. *H. pylori* strains, host genotypes and environmental factors play important role in deciding the extent and severity of the gastroduodenal diseases. The bacteria has developed a unique set of virulence factors to survive in the extreme ecological niche of human stomach. Together these virulence factors make *H. pylori* one of the most successful human pathogenic bacteria colonizing more than half of the human population. Understanding the mechanism of action of the major *H. pylori* virulence factors will shed light into the molecular basis of its pathogenicity. (Mol Cell Biochem **253**: 207–215, 2003)

Key words: *Helicobacter pylori*, virulence factors, type IV secretion system, VacA, PA

Introduction

Helicobacter pylori are Gram-negative spiral shaped bacteria that infect more than 50% of humans globally [1]. The infection normally takes place through faecal-oral pathways. It is now generally accepted that *H. pylori* causes gastric and duodenal ulcer diseases. Additionally, *H. pylori* is associated with the development of gastric adenocarcinoma and lymphoma which is linked with the chronic gastritis found in infected individuals [2]. World Health Organization has classified *H. pylori* as number one carcinogen causing gastric cancer. Gastric cancer is the second most common cancer in the world [3]. A large number of patients suffering from this disease die since it is mostly diagnosed at the advance stage.

Though *H. pylori* infects 50% of the population, most infected people remain asymptomatic. Only a small fraction (15–20%) of the infected people shows the signs of gastroduodenal abnormalities including stomach and duodenal ulcers, gastric adenocarcinoma and lymphoma. The extent and severity of the infection depends on the degree of the host response against the bacteria, bacterial polymorphisms and environmental factors [4].

Several aspects of *H. pylori* infection and of the host reactions have been uncovered so far. Current research is undergoing to clarify the molecular mechanisms of action of *H. pylori* virulence factors. A number of factors contribute to the development of severe gastroduodenal diseases. Among them, the most important ones are the virulence factors produced by the pathogenic strains. Pathogenic strains contain a cluster of genes forming a pathogenicity island, vacuolating cytotoxin (VacA), a neutrophil activating protein (HP-NAP) and the urease [5]. Other *H. pylori* virulence factors include bacterial flagella [6], lipopolysaccharides (LPS) [7] , Lewis^{x,y} antigen [8], IceA [9], PicB [10] and several other outer membrane proteins.

In this review, primarily we will discuss about the recent advances in molecular basis of pathogenesis caused by major *H. pylori* virulence factors. We will also try to explore how the host factors modulate the host-bacteria equilibrium leading to the onset of gastroduodenal diseases. Finally, the implications of these virulence factors in human immune response will also be discussed.

Address for offprints: S.K. Dhar, Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, India (E-mail: skdhar2002@yahoo.co.in)

Urease: A key component for buffering stomach pH

Before the bacterium swims into the mucus layer to make contact with the intestinal epithelial cells, it has to survive the extreme pH of the stomach lumen. A gene cluster containing seven genes is responsible for the biosynthesis of the enzyme urease [11, 12]. These genes encode for UreA (26.5 kDa) and UreB (60.3 kDa) and five other proteins required for the uptake of Ni^{+2}, the cofactor required for its activity [12, 13]. Crystal structure of urease reveal that the two subunits (α and β) first form a $\alpha\beta$ heterodimer. Three $\alpha\beta$ heterodimer form a trimeric assembly $(\alpha\beta)_3$ with 3 fold symmetry. $(\alpha\beta)_3$ units of *H. pylori* urease further assemble to form a unique supramolecular dodecameric assembly, $((\alpha\beta)_3)_4$, which is organized as a double ring of 13 nm in diameter [14].

Urease hydrolyses urea and generates ammonia that buffers the cytosol and periplasm and helps forming a neutral layer around the bacterial surface. The uptake of urea by *H. pylori* takes place through a proton-gated channel which is open only at lower pH [15]. This is an excellent way of maintaining intracellular and extracellular pH. At neutral pH, the urea channels are blocked in order to avoid over-alkalinization.

Stingl *et al*. recently proposed a model in favour of the high cytoplasmic urease activity of *H. pylori* [16]. This activity is essential for maintaining the cytoplasmic pH of this organism at a value close to neutral under extreme low pH conditions. This mechanism involves the formation of ammonium ions (combining NH_3 released by urea and H^+ ions present in the cytoplasm) followed by the electrogenic export of ammonium ions from the cytoplasm via a yet to be discovered transport system (Fig. 1). The authors also propose that the extracytoplasmic urease activity creates a cloud of ammonia around the cells leading to the formation of a microenvironment of neutral pH.

Urease is found both in the cytosol and surface of the bacteria. It is synthesized and accumulated mainly in the cytosol together with a heat shock protein HspB. These proteins are released from the cytosol following bacterial autolysis and are adsorbed on the surface of the live bacteria. So far this is the only acceptable mechanism of surface expression of urease since no leader sequence has been found in urease which can export it from the cytoplasm to the bacterial surface. This mechanism may ensure survival of the population at the ex-

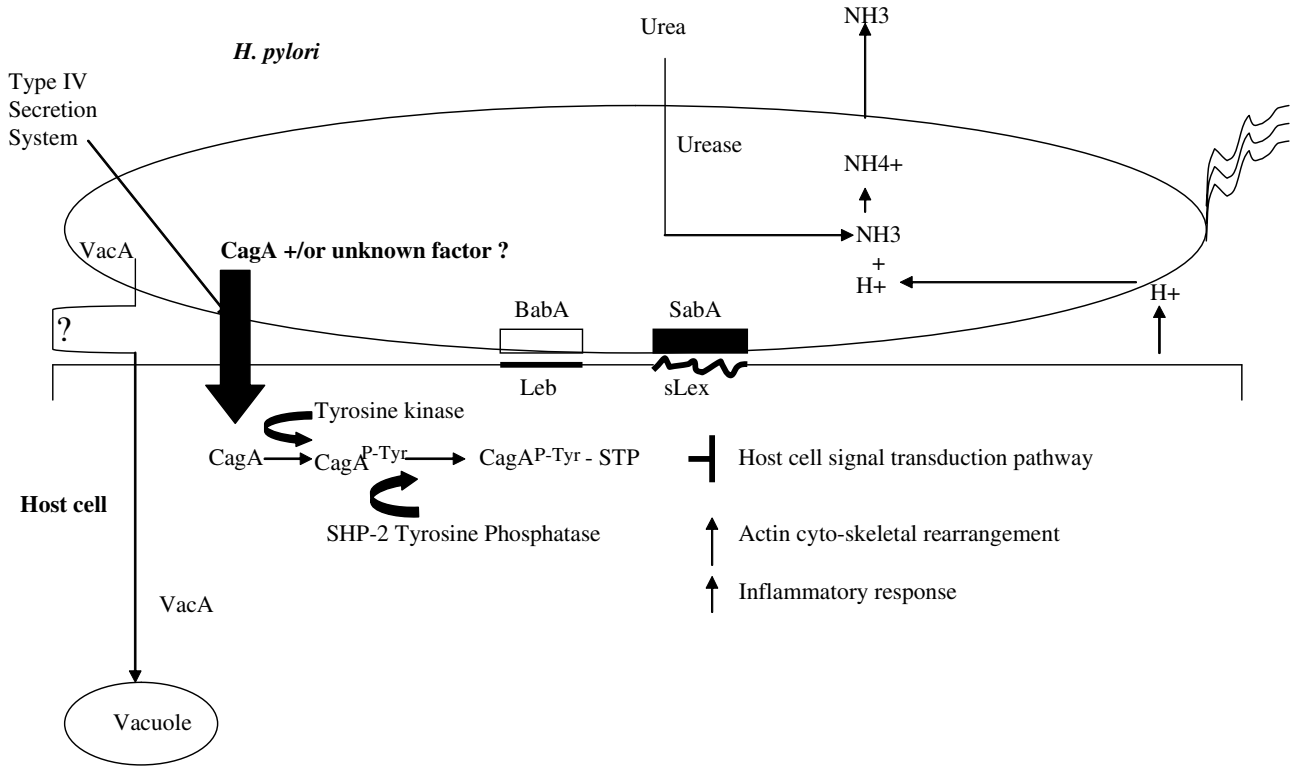

Fig. 1. Overview on *H. pylori*-host cell interactions. Major adhesins, BabA, SabA and corresponding host cell surface antigens are indicated. Translocation of CagA through the type IV secretion system into host cell and its fate has been marked. Perturbation of host cell signaling pathway and up regulation of actin cytoskeletal rearrangement and inflammatory response by phosphorylated CagA are shown. Type IV secretion system is shown by thick arrow. Neutralization of cytoplasmic acid by ammonia generated by the action of *H. pylori* enzyme urease on urea is indicated.

pense of individual cells. Urease is also found on the surface of the *H. pylori* present in human biopsies [17, 18].

Urease defective *H. pylori* mutants cannot colonize the stomach of gnotobiotic piglets [19]. This result strongly suggests that the urease activity is essential for colonization by *H. pylori*. However, urease activity may also contribute to toxicity by the production of ammonia which is toxic to the cells. Ammonia may also react with the reactive intermediates generated by the neutrophil myeloperoxidases to form carcinogenic agents responsible for *H. pylori*-associated stomach adenocarcinoma [20–22]. In *in vitro* system, in the presence of ammonium chloride, VacA rapidly induces formation of larger vacuoles in HeLa cells compared to the vacuoles generated by VacA or ammonia alone suggesting a synergistic effect of urease and VacA in damaging cells [23, 24]. Urease is also capable of activating monocytes for proinflammatory cytokines production. The local production of cytokines by urease-stimulated mononuclear phagocytes may play a central role in the development of *H. pylori* gastroduodenal inflammation [25]. It is one of the major antigens recognized by the human sera. However, the mode of host immune response towards this virulence factor is still not known.

Flagella and adhesins required for the bacterial motility and attachment to the host cells

To avoid prolonged exposure to acid and getting discharged in the intestine, *H. pylori* must reach the stomach epithelial cell line under the thick mucous layer. The bacteria contain flagella which acts as a propeller to travel the viscous mucus layer like a screw into a cork. Different factors like urea, bicarbonate ions are required for the chemotactic movement of the bacteria [26]. Non-motile mutant bacteria cannot colonize the stomach suggesting that flagellar movement is essential for the bacteria to cross the mucous barrier [27].

Once *H. pylori* reach the stomach epithelial cells under the mucous layer, it adheres strongly to gastric cells. This binding involves several proteins and glycolipids. Electron microscopy shows extensive areas of adhesion to the host cells. Several surface molecules have been shown to be responsible for binding. Out of them BabA, the Leb blood group antigen binding adhesin, an outer membrane protein of *H. pylori* binds to Lewis B type antigen of human cells [28, 29]. Recently, Mahdavi *et al*. [30] identified a sialic acid-binding adhesin (SabA; JHP662/HPO725) which binds to sialylated Lewis x antigens expressed during chronic inflammation, might also contribute to virulence (Fig. 1). *H. pylori* protein (HpaA, HP0410) also binds to sialylated glycoconjugates [31, 32]. Two closely related outer membrane proteins AlpA and AlpB, required for *H. pylori* adherence to gastric epithelial cells were identified recently. The pattern of AlpAB-dependent adherence of *H. pylori* to the gastric epithelial surface showed a clear difference to the BabA2-mediated adherence to Lewis, suggesting the involvement of a different receptor [33]. An *H. pylori* lectin binds to the sialic residues of laminin [34]. Additional bacterial proteins also help establishing contact with the host cells [35, 36]. It is unlikely that mutation in a single gene can create an adhesion defective mutant bacteria incapable of making contacts with the host cells.

Binding of the bacteria to the host cells is followed by the rearrangement of the plasma membrane below the contact area. The plasma membrane changes its shape and extends in order to increase the bacterial surface area of contact [37]. *H. pylori* does not seem to invade the epithelial cells.

CAG pathogenicity island (PAI)

More severe form of the gastroduodenal diseases is associated with the presence of a 40 kb pathogenicity island containing more than thirty genes [38]. Depending on the presence or absence of this region, *H. pylori* strains are termed as cag^+ or cag^-. The origin of pathogenicity island is not understood clearly so far. It is assumed that it has been acquired from another species or even genus since the GC content of this region is different from the rest of the genome [39].

In Mongolian gerbil models, cag^- bacteria cause mild inflammation of the stomach whereas the cag^+ strains cause severe inflammation, gastric ulcers and tumors. This result strongly support the data obtained from the epidemiological studies showing that severe gastric diseases are always associated with cag^+ strains [40]. Although the reports from East Asian and Indian populations do not show strong correlation between disease status and the presence of cag^+ strains [41–43]. It is possible that East Asian strains may contain some virulence factor(s) other than CagA. Though the role of CagA in pathogenicity is shown by Mongolian gerbil model, the exact molecular mechanism behind the severe inflammation and the occurrence of gastric cancer is still not clear.

Genes in the pathogenicity island are involved in different pathogenic process including the induction of host cells to release pro-inflammatory chemokines and modification of intracellular signaling [44].

One of the genes of Cag-PAI, the cagA gene encodes a 128 kDa protein. CagA is always present in *H. pylori* strains associated with the more severe form of the disease. The exact function of CagA is not clearly understood so far. CagA protein is injected from the attached *H. pylori* into host cells using the type IV secretion system [10]. Upon transfer into the host cells, CagA is tyrosine phosphorylated [45, 46].

Introduction of $cagA^+$ in the host cells also triggers morphological changes (humming bird phenomenon) similar to those induced by growth factors [47]. Recently, SHP-2 tyro-

sine phosphatase has been identified as an intracellular target of *H. pylori* CagA protein (Fig. 1). CagA formed a physical complex with the SH2-containing tyrosine phosphatase SHP-2 in a phosphorylation dependent manner and stimulated the phosphatase activity leading to the dephosphorylation of some cellular proteins. Wild-type but not phosphorylatrion-resistant, CagA induced a growth factor like response in gastric epithelial cells. Deregulation of SHP-2 by CagA may induce abnormal proliferation and movement of gastric epithelial cells, promoting the acquisition of a cellular transformed phenotype [48].

Two members of the src kinases family, c-Src and Lyn, account for most of the CagA-specific kinase activity in host cell lysates [49, 50]. Tyrosine phosphorylation of CagA is restricted to a repeated sequence called D1 located in the C-terminal half of the protein. It contains the five-amino-acid motif EPIYA, which is amplified by duplications in a large fraction of clinical isolates. Tyrosine phosphorylation at the EPIYA motifs may lead to the cytoskeletal rearrangements, cell elongation and increased cellular motility. CagA shows sequence diversity among different *H. pylori* strains. The phosphorylation domain 'EPIYA' is located within the repeat region of CagA. Therefore, the number and sequence polymorphism of the CagA phosphorylation sites may be critical for the binding affinity of CagA to SHP-2, which in turn may be found useful to elucidate the clinical outcome of infection by different cagA$^+$ *H. pylori* strains [48].

The vacuolating cytotoxin A (VacA)

In cultured cells, *H. pylori* vacuolating cytotoxin (VacA) forms large cytoplasmic vacuoles, which eventually fill up the entire cytosol [51]. The molecular mass of VacA is 95 kDa and it is present in half of the *H. pylori* isolates. It is one of the major virulence factor of *H. pylori* which is a strong immunogen in humans [52].

VacA gene encodes a protoxin approximately 140 kDa in mass. An amino terminal signal sequence and a carboxy-terminal fragment are proteolytically cleaved to produce a ~ 88 kDa mature toxin [51]. Mature toxin molecules are either secreted in the extracellular space or it may be retained in bacterial surface [53]. Mature VacA may further undergo cleavage leading to the formation of N-terminal 34 kDa (p37) and C-terminal 54 kDa (p58) fragments which remain non-covalently associated. Secondary structure prediction programs indicate that p37 is rich in β-pleated sheets. This region contains a 32-residue hydrophobic segment with a propensity to insert into membrane [54]. p58 is predicted to contain two domains separated by a flexible segment of variable length. The first domain is highly conserved whereas the second domain is genetically diverse. VacA is purified as a large oligomeric complex (> 900 kDa), which under electron microscope is appeared as 'flower' like structure with 30 nm in diameter [55, 56]. These structures are thought to be composed of one or two rings each comprised of 6–7 VacA monomer. The oligomeric complex is disassembled in monomers when exposed to acidic or basic pH. Monomeric forms show increased cytotoxocity than oligomers [57].

VacA is released and placed in between the mucus layer and the apical domain of the stomach epithelial cells. Using radioactive VacA, a number of non-saturable low-affinity receptors were found on the surface of HeLa cells suggesting that VacA may not need specific receptors on the cell surface. Upon insertion into the plasma membrane, VacA forms anion specific channels of low conductance [58]. These channels release bicarbonate and organic anions from the cell cytosol to support bacterial growth. The toxin channels are slowly endocytosed and finally reach late endosomal compartments. The presence of vacuolar ATPase proton pump on the membrane of the endosome increases hydrogen ion concentration inside the lumen of the endosome. These proton pumps are essential for the formation of the vacuoles. In the presence of weak bases like ammonia generated by the *H. pylori* urease, osmotically active acidotropic $NH4^+$ ions are accumulated inside the endosome. This leads to water influx and vesicle swelling leading to the vacuole formation. By an unknown mechanism, VacA alters the tight junctions and increases the permeability of iron and nickel ions through the paracellular route. These nutrients are essential for *H. pylori* growth. VacA may mediate this activity by specific interaction with recently identified cytosolic protein VIP54 [59].

Apart from vacuolar ATPase, several cellular enzymes are required for vacuole development and maintenance. These include small GTP binding proteins Rab7 [60] and Rac1 [61]. Rab7 may be needed to support membrane deposition and homotypic fusion between late endosomes, and Rac1 may control cytoskeletal elements affecting membrane trafficking.

Purified and activated VacA applied externally to cells induce cytochrome c release into the cytosol. In order to find out specific role of p37 and p58 in VacA mediated cytosolic cytochrome c release, HEp-2 cells were transfected with DNAs encoding either the N-terminal (p37) or the C-terminal (p58) fragment of VacA. p37 was found to be localized specifically to mitochondria, whereas p58 was cytosolic. Incubated in vitro with purified mitochondria, VacA and p37 but not p58 translocated into the mitochondria. p37-GFP or VacA-GFP transfected HeLa cells induced the release of cytochrome c from mitochondria and activated the caspase 3, a key enzyme for apoptosis. This was determined by the cleavage of poly(ADP-ribose) polymerase (PARP) and interestingly, PARP cleavage was antagonized specifically by co-transfection of DNA encoding Bcl-2, known to block mitochondria-dependent apoptotic signals. This clearly suggests that p37 is specifically targeted to mitochondria followed by the induction of apoptosis in cultured cells [62]. However,

it is interesting to note that externally applied toxin failed to induce a significant apoptosis.

Increased levels of apoptosis have been well documented in the gastric epithelium of patients colonized by *H. pylori* [63]. Therefore, the increased occurrence of cell death probably plays a role in the appearance of atrophic gastritis, an established pre-neoplastic condition [63].

VacA-induced vacuolization results in decreased proteolytic activity in the endocytic pathway and proteolysis of antigens in the antigen-processing compartment of antigen presenting cells [64]. As a result, it inhibits the stimulation of T-cell clones specific for epitopes generated in the antigen-processing compartment. *H. pylori* inhibits the local immune response as evidenced by the low frequency of VacA specific $CD4^+$ T cells found in the stomach mucosa. Persistent *H. pylori* infection also down regulates specific $CD8^+$ cytotoxic T cell response suggesting that *H. pylori* may take this strategy for prolonged survival leading to the chronic infection of the human stomach.

In conclusion, although many aspects of VacA function have been elucidated so far, several important aspects are still missing. Presently two models have been postulated for VacA function. In the first model, VacA is an A/B toxin and its toxic activity requires either p37 or the entire protein which is translocated in the cytoplasm and then acts on an intracellular target(s). The other model proposes that active VacA binds to targets on the cell plasmamembrane followed by formation of transmembrane anion selective channels. Membrane associated VacA molecules are endocytosed and accumulate in endo-lysosomal compartments leading to the vacuole formation. These two models may be alternative or complementary to each other.

Helicobacter pylori neutrophil activating protein

Infection with *H. pylori* induces a state of chronic inflammation which often leads to gastric or duodenal ulcers or more rarely to gastric adenocarcinoma or mucosa-associated lymphoid tissue (MALT) lymphoma. *H. pylori* induced inflammation is associated with the infiltration of phagocytes (mainly neutrophils) to the gastric mucosa. The tissue damage may be attributed to the combined effects of bacterial factors and host inflammatory mediators. Recently, a protein capable of promoting neutrophil infiltration and adhesion to endothelial cells was identified. The purified protein was termed as HP-NAP. It is a 150-kDa dodecameric (composed of 15 kDa subunits) iron-binding protein that promotes adhesion of PMNs to endothelial cells [65, 66]. Purified recombinant HP-NAP stimulates phagocyte chemotaxis, NADPH oxidase assembly, and production of reactive oxygen species (ROS) via a cascade of intracellular activation events including an increase in cytosolic calcium ion concentration and phosphorylation of proteins. HP-NAP is less powerful than PMA with respect to neutrophil activation. It has been suggested that HP-NAP induces moderate inflammation, which alters the epithelial tight junction leading to the release of nutrients from the mucosa [67].

The interaction of the HP-NAP with target cell is mediated through the glycoconjugate moieties present on the membrane of human granulocytes [68]. In solid phase assay, HP-NAP bound to acid glycosphingolipid fraction from human neutrophils, whereas no binding to the non-acid glycosphingolipids or polyglycosyl ceramides from these cells was obtained.

HP-NAP plays an important role in immunity. Vaccination of mice with HP-NAP induced protection against *H. pylori* challenge. This is consistent with the finding of HP-NAP specific antibodies present in the majority of *H. pylori* infected patients [69]. Therefore, HP-NAP is a virulence factor important for the *H. pylori* pathogenic effects at the site of infection and a candidate antigen for vaccine development.

Type IV secretion system

H. pylori type I strains are characterized by the presence of cag pathogenicity island (PAI) which contains 31 open reading frames. Some of these ORFS share significant homology with the virulence (*vir*) genes *virB4*, *virB7*, *virB8*, *virB9*, *virB10*, *virB11*, and *virD4* of the so-called VirB/D complex of type IV secretion systems known from *Agrobacterium tumefaciens* and *Bordetella pertussis* [46, 70]. Generally type IV secretion systems are involved in conjugative DNA transfer of prokaryotes and in the delivery of bacterial virulence factors into the eukaryotic cells (Fig. 1).

CagA is the only protein known so far to be injected by *H. pylori* into host cells (Fig. 1). This is the first evidence of a functional secretion apparatus in *H. pylori*. In recent studies, each gene in the cag-PAI was deleted without causing polar effect on the expression of the downstream genes. Seventeen out of 27 genes including the VirD homologue were found to be absolutely essential for translocation of CagA into host cells [71]. This clearly suggests that an intact type IV secretion system is required for the translocation of CagA. Attachment of the type I *H. pylori* strains to host cells induces the production and secretion of chemokines such as interleukin-8 (IL-8) [44]. The mechanism of this chemokine induction is not clearly understood. Several genes of the cag-PAI or components of the type IV secretion apparatus seem to be involved in this process by activation of AP-1 [72] and NFκB [10] leading to the induction of chemokines. Inactivation of VirD4 homologue which affected CagA translocation had no effect on IL-8 secretion. This clearly suggests that CagA translocation and IL-8 secretion may take place independent

to each other. VirD4 is thought to be an adapter protein guiding CagA into the transport channel. Both CagA translocation and IL-8 secretion require intact type IV secretion system since inactivation of VirB4 which does not allow functional assembly of transporter affect both the activities. IL-8 secretion may take place either due to the transportation of an yet another unidentified molecule through the type IV secretion system or by binding of the transporter itself to relevant cell surface receptors (receptor hypothesis) followed by activation of cellular signaling mechanism.

Overall biological effects

We have discussed the cellular effects of major *H. pylori* virulence factors in details (Fig. 1). Multiple virulence factors modulate host in different ways giving rise to *H. pylori* pathogenicity. It is important to note that host factors also play important roles in determining the extent and severity of the damage of the host tissues. Biological effects of *H. pylori* on the host cells include cellular proliferation, inflammation and apoptosis.

H. pylori infection is associated with enhanced cellular proliferation of host cells. However, the mechanism behind the proliferation is not clearly understood. Expression of various cell cycle markers associated with the proliferation was checked in the host cells following co-culture of gastric epithelial cells with bacteria. In a recent report, Cyclin D1 transcription in gastric cancer (AGS) cells was enhanced by co-culture with *H. pylori* [73]. This activation of cyclin D1 was partly dependent on the *cag* pathogenicity island but not on *vacA*. Among various cyclins, cyclin D1 regulates passage through the restriction point and entry into the S phase. Furthermore, cyclin D1 overexpression shortens the G_1 phase and increases the rate of cellular proliferation. Co-culture of *H. pylori* with epithelial cells was reported to reduce expression of the cell-cycle regulatory protein p27, which leads to epithelial-cell G_1 arrest [74]. Cell proliferation may also be induced by *H. pylori* as a result of host response to bacteria. Production of gastrin by mucosal G cells increases in the presence of *H. pylori*. *In vitro*, gastrin enhances the proliferation of gastric epithelial cells. Transgenic mice overexpressing gastrin yielded gastric adenocarcinoma more frequently and in less time in the presence of *H. pylori* than in the absence of bacteria suggesting that high gastrin level and *H. pylori* colonization may cooperatively enhance the incidence of gastric cancer [75].

H. pylori also causes inflammation. Pro-inflammatory cyclooxygenase (COX) enzymes (Cox1 and 2) catalyze inflammatory prostaglandin formation. COX-1 is expressed constitutively whereas COX-2 is induced by cytokines and its expression in the gastric epithelial cells is increased when co-cultured with *H. pylori* [76]. Phospholipase A_2, a key enzyme catalysing the formation of the prostaglandin precursor arachadonic acid, also gets activated in the presence of *H. pylori* both *in vitro* and *in vivo* [77, 78]. In the presence of *H. pylori*, activated neutrophils may also induce inflammation by releasing superoxide radicals causing DNA damage.

H. pylori has been associated with increased and decreased level of apoptosis depending on the host. *In vitro*, *H. pylori* by itself and purified urease and VacA induces apoptosis in gastric epithelial cells. T_H1 cytokines induced by *H. pylori*, such as IFN-γ, might induce epithelial-cell apoptosis through a Fas-mediated pathway. Expression of Fas receptors on the surface of epithelial cells increase in the presence of *H. pylori*. *In vitro*, *H. pylori* may also induce apoptosis by activating NF-κB [79]. There should be a delicate balance between apoptosis and cell proliferation. Increased rates of apoptosis could result in formation of atrophic gastritis, with a concomitant increased risk of distal gastric adenocarcinoma. Whereas, reduced rates of apoptosis, accompanied by hyperproliferation, could lead to the development of gastric cancer.

Host factors and onset of gastric cancer

We have discussed the effects of *H. pylori* virulence factors. Several host factors are also associated with the onset of gastric cancer. Th1 type of immune response is induced in humans by *H. pylori* whereas *Helicobacter felis* can induce same type of immune response in mice [80]. Gastric atrophy initiated by *H. felis* can be reduced by Th2 immune response [81].

H. pylori infected individuals show differential level of IL-1β secretion within the gastric mucosa [82]. IL-1β gene promoter contains several polymorphic regions affecting protein expression. The chances of getting gastric atrophy and gastric adenocarcinoma are higher in *H. pylori* infected individuals with higher IL-1β secretion than average [83]. In Mongolian gerbil models, gastric acid production is reduced with increasing IL-1β secretion after 6–12 weeks following infection. IL-1β, the proinflammatory cytokine could play a major role in the formation of gastric adenocarcinoma [84].

Polymorphism of the TNF-α promoter is associated with increased risk of gastric cancer [85]. TNF-α expression is increased in *H. pylori* infected mucosa as a result of Th1 immune response. It has been reported recently that mutation in RAS together with increased level of TNF-α may cause carcinogenesis in genetically susceptible hosts [86].

Some major histocompatibility complex (MHC) genotype may also play some role in *H. pylori* induced gastric cancer. Class II MHC molecules expressed on gastric epithelial cells are upregulated in the presence of *H. pylori* suggesting that MHC haplotype might partially influence epithelial cell response to pathogens [87].

Host cells and *H. pylori* constantly exchange signals with each other leading to a formation of dynamic equilibrium. In most cases the equilibrium is unchanged whereas in some cases the equilibrium is shifted causing disease depending on the host and bacterial characteristics. This might explain why some people get disease and majority don't get affected by *H. pylori*. Individuals infected with Cag^+ *H. pylori* strain and with high level of IL-1β and TNF-α expression due to genetic polymorphism in host are at more risk than individuals infected with either Cag^- strain or low expression of IL-1β and TNF-α in the host.

Therefore, the onset of gastric cancer may be the net result of bacterial strain variation, host genotype and environmental factors.

Development of vaccines

To date, *H. pylori* is one of the most common infections of mankind. Infection usually occurs during childhood, and when left untreated results in lifelong colonization of the stomach. Antimicrobial therapy is currently the method of choice for the treatment. However, complex dosing, inconsistent efficiency, development of antibiotic resistance, costs and side effects compromise widespread use. As a result, it is important to explore new strategies for the prevention and eradication of *H. pylori*. Development of an effective vaccine may be useful since it will be both effective and economic in use. Natural infection with *H. pylori* usually results in a strong inflammatory Th1-type CD4(+)T-cell response that does not seem to have any protective effects. A Th2-type response is required for protection. Unfortunately, the exact mechanisms involved in protective immunization are still poorly understood.

H. pylori virulence factors like Urease, CagA, VacA, HP-NAP are major antigens in the human immune response to *H. pylori* infection [52]. Introduction of these antigens in animal models, result in induction of immune response which may either protect the animals from infection (prophylactic vaccines) or eradicate an already existing infection (therapeutic vaccine). Both oral and systemic immunization should be considered in this regard. Although commercial development of vaccines for clinical trial is underway, many important issues, such as lack of a suitable mucosal adjuvant, and prevention of potential side effects, such as postimmunization gastritis, need to be resolved. If a suitable vaccine against *H. pylori* is found, elimination of a common pathogen which has been living in human for such a long time will be made possible.

Acknowledgement

SKD acknowledges the University Grant Commission, India for support.

References

1. Parsonnet J: The incidence of *H. pylori* infection. Ailment Pharmacol Ther 9:(suppl 2) 45–51, 1995
2. Peek RM, Blaser MJ: *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nature Rev Cancer 2: 28–37, 2002
3. Correa P: *Helicobacter pylori* and gastric cancer: State of the art. Cancer Epidemiol Biomarkers Prev 5: 477–481, 1996
4. Go MF: What are the host factors that place an individual at risk for *Helicobacter pylori*-associated diseases? Gastroenterology 113: S15–S20, 1997
5. Montecucco C, Papini E, de Bernard M, Zoratti M: Molecular and cellular activities of *Helicobacter pylori* pathogenic factors. FEBS Lett 452: 16–21 1999
6. Suerbaum S: The complex flagella of gastric *Helicobacter* species. Trends Microbiol 3: 168–170, 1995
7. Moran AP, Linder E, Walsh EJ: Structural characterization of the lipid A component of *Helicobacter pylori* rough- and smooth-form lipopolysaccharides. J Bacteriol 179: 6453–6463, 1997
8. Appelmelk BJ, Negrini R, Moran AP, Kuipers EJ: Molecular mimicry between *Helicobacter pylori* and the host. Trends Microbiol 5: 70–73, 1997
9. Peek RM Jr, Thompson SA, Donahue JP, Tham KT, Atherton JC, Blaser MJ, Miller GG: Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, iceA, that is associated with clinical outcome. Proc Assoc Am Physicians 110: 531–544, 1998
10. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A: Cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci USA 93: 14648–14653, 1996
11. Hu LT, Mobley HLT: Purification and N-terminal analysis of urease from *Helicobacter pylori*. Infect Immun 58: 992–998, 1990
12. Labigne A, Cussac V, Courcoux P: Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. J Bacteriol 173: 1920–1931, 1991
13. Achtman M, Suerbaum S (eds): In: *Helicobacter pylori*: Molecular and Cellular Biology. Horizon Scientific, Norfolk, 2001
14. Ha NC, Oh ST, Sung JY, Cha KA, Lee MH, Oh BH: Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. Nat Struct Biol 8: 505–509, 2001
15. Weeks DL, Eskandari S, Scott DR, Sachs G: A H^+-gated urea channel: The link between *Helicobacter pylori* urease and gastric colonization. Science 287: 482–485, 2000
16. Stingl K, Altendorf K, Bakker EP: Acid survival of *Helicobacter pylori*: How does urease activity trigger cytoplasmic pH homeostasis? Trends Microbiol 10: 70–74, 2002
17. Phadnis SH, Parlow MH, Levy M, Ilver D, Caulkins CM, Connors JB, Dunn BE: Surface localization of *Helicobacter pylori* urease and a heat shock protein homolog requires bacterial autolysis. Infect Immun 64: 905–912, 1996
18. Dunn BE, Vakil NB, Schneider BG, Miller MM, Zitzer JB, Peutz T, Phadnis SH: Localization of *Helicobacter pylori* urease and heat shock protein in human gastric biopsies. Infect Immun 65: 1181–1188, 1997
19. Eaton KA, Brooks CL, Morgan DR, Krakowka S: Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylo*ri in gnotobiotic piglets. Infect Immun 59: 2470–2475, 1991
20. Megraud F, Neman-Simha V, Brugmann D: Further evidence of the toxic effect of ammonia produced by *Helicobacter pylori* urease on human epithelial cells. Infect Immun 60: 1858–1863, 1992
21. Suzuki M, Miura S, Suematsu M, Fukumura D, Kurose I, Suzuki H, Kai A, Kudoh Y, Ohashi M, Tsuchiya M: *Helicobacter pylori*-associated ammonia production enhances neutrophil-dependent gastric mucosal cell injury. Am J Physiol 263: G719–G725, 1992

22. Sommi P, Ricci V, Fiocca R, Romano M, Ivey KJ, Cova E, Solcia E, Ventura U: Significance of ammonia in the genesis of gastric epithelial lesions induced by *Helicobacter pylori*: An *in vitro* study with different bacterial strains and urea concentrations. Digestion 57: 299–304, 1996
23. Cover TL, Halter SA, Blaser MJ: Characterization of HeLa cell vacuoles induced by *Helicobacter pylori* broth culture supernatant. Hum Pathol 23: 1004–1010, 1992
24. Ricci V, Sommi P, Fiocca R, Romano M, Solcia E, Ventura U: *Helicobacter pylori* vacuolating toxin accumulates within the endosomal-vacuolar compartment of cultured gastric cells and potentiates the vacuolating activity of ammonia. J Pathol 183: 453–459, 1997
25. Harris PR, Mobley HL, Perez-Perez GI, Blaser MJ, Smith PD: *Helicobacter pylor*i urease is a potent stimulus of mononuclear phagocyte activation and inflammatory cytokine production. Gastroenterology 111: 419–425, 1996
26. Yoshiyama H, Nakamura H, Kimoto M, Okita K, Nakazawa T: Chemotaxis and mobility of *Helicobacter pylori* in a viscous environment. J. Gastroenterol 34(supl 11): 18–23, 1993
27. Josenhans C, Suerbaum S: In: M. Achtman, S. Saeubaum (eds). *Helicobacter pylori*: Molecular and Cellular Biology. Horizon Scientific, Norfolk, 2001, pp 171–184
28. Boren T, Falk P, Roth KA, Larson G, Normark S: Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science 262: 1892–1895, 1993
29. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T: *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 279: 373–377, 1998
30. Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, Angstrom J, Larson T, Teneberg S, Karlsson KA, Altraja S, Wadstrom T, Kersulyte D, Berg DE, Dubois A, Petersson C, Magnusson KE, Norberg T, Lindh F, Lundskog B, Arnquist A, Hammarstrom L, Boren T: *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science 297: 573–578, 2002
31. Evans DG, Karjalainen TK, Evans DJ, Graham DY, Lee CH: Cloning, nucleotide sequence and expression of a gene encoding an adhesion subunit protein of *Helicobacter pylori*. J Bacteriol 175: 674–683, 1993
32. Jones AC, Logan RP, Foynes S, Cockayne A, Wren BW, Penn CW: A flagellar sheath protein of *Helicobacter pylori* is identical to HpaA, a putative N-acetylneuraminyllactose-binding hemagglutinin, but is not an adhesin for AGS cells. J Bacteriol 179: 5643–5647, 1997
33. Odenbreit S, Till M, Hofreuter D, Faller G, Haas R: Genetic and functional characterization of the alpAB gene locus essential for the adhesion of *Helicobacter pylori* to human gastric tissue. Mol Microbiol 31: 1537–1548, 1999
34. Valkonen K, Wadstrom T, Moran AP: Identification of the N-acetylneurainyllactose-specific laminin binding protein. Infect Immun 65: 916–923, 1993
35. Lingwood CA, Wasfy G, Han H, Huesca M: Receptor affinity purification of a lipid-binding adhesin from *Helicobacter pylori*. Infect Immun 61: 2474–2478, 1993
36. Namavar F, Sparrius M, Meerman ECI, Applemelk BJ, Van Vandenbroucke-Grauls CM: Neutrophil activating protein mediates adhesion of *Helicobacter pylori* to sulphated carbohydrates of high molecular weight salivary mucin. Infect Immun 66: 444–447, 1997
37. Segal ED, Falkow S, Tompkins LS: *Helicobacter pylori* attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. Proc Natl Acad Sci USA 93: 1259–1264, 1996
38. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A: Cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci USA 93: 14648–14653, 1996
39. Hacker J, Blum-Oehler G, Muhldorfer I, Tschape H: Pathogenicity islands of virulent bacteria: Structure, function and impact on microbial evolution. Mol Microbiol 23: 1089–1097, 1997
40. Ogura K, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M: Virulence factors of *Helicobacter pylori* responsible for gastric diseases in Mongolian gerbil. J Exp Med 192: 1601–1610, 2000
41. Mukhopadhyay AK, Kersulyte D, Jeong JY, Datta S, Ito Y, Chowdhury A, Chowdhury S, Santra A, Bhattacharya SK, Azuma T, Nair GB, Berg DE: Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, India. J Bacteriol 182: 3219–3227, 2000
42. Pan Z J, van der Hulst RW, Feller M, Xiao S D, Tytgat GN, Dankert J, van der Ende A: Equally high prevalences of infection with *cagA*-positive *Helicobacter pylori* in Chinese patients with peptic ulcer disease and those with chronic gastritis-associated dyspepsia. J Clin Microbiol 35: 1344–1347, 1997
43. Chattopadhyay S, Datta S, Chowdhury A, Chowdhury S, Mukhopadhyay AK, Rajendran K, Bhattacharya SK, Berg DE, Nair GB: Virulence genes in *Helicobacter pylori* strains from west Bengal residents with overt *H. pylori*-associated disease and healthy volunteers. J Clin Microbiol 40: 2622–2625, 2002
44. Crabtree JE, Covacci A, Farmery SM, Xiang Z, Tompkins DS, Perry S, Lindley IJ, Rappuoli R: *Helicobacter pylori* induced interleukin-8 expression in gastric epithelial cells is associated with CagA positive phenotype. J Clin Pathol 48: 41–45, 1995
45. Asahi M, Azuma T, Ito S, Ito Y, Suto H, Nagai Y, Tsubokawa M, Tohyama Y, Maeda S, Omata M, Suzuki T, Sasakawa C: *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med 191: 593–602, 2000
46. Odenbreit S, Puls J, Sedlmaier B, Gerland E, Fischer W, Haas R: Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science 287: 1497–1500, 2000
47. Segal ED, Cha J, Lo J, Falkow S, Tompkins LS: Altered states: Involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc Natl Acad Sci USA 96: 14559–14564, 1999
48. Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M: SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 295: 683–686, 2002
49. Stein M, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ, Covacci A: c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol 43: 971–980, 2002
50. Selbach M, Moese S, Hauck CR, Meyer TF, Backert S: Src is the kinase of the *Helicobacter pylori* CagA protein *in vitro* and *in vivo*. J Biol Chem 277: 6775–6778, 2002
51. Cover TL, Blaser MJ: Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J Biol Chem 267: 10570–10575, 1992
52. Del Giudice G, Covacci A, Telford JL, Montecucco C, Rappuoli R: The design of vaccines against *Helicobacter pylori* and their development. Annu Rev Immunol 19: 523–563, 2001
53. Telford JL, Ghiara P, Dell'Orco M, Comanducci M, Burroni D, Bugnoli M, Tecce MF, Censini S, Covacci A, Xiang Z *et al.*: Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. J Exp Med 179: 1653–1658, 1994
54. McClain MS, Cao P, Cover TL: Amino-terminal hydrophobic region of *Helicobacter pylori* vacuolating cytotoxin (VacA) mediates transmembrane protein dimerization. Infect Immun 69: 1181–1184, 2001
55. Lupetti P, Heuser JE, Manetti R, Massari P, Lanzavecchia S, Bellon PL, Dallai R, Rappuoli R, Telford JL: Oligomeric and subunit structure of the *Helicobacter pylori* vacuolating cytotoxin. J Cell Biol 133: 801–807, 1996

56. Lanzavecchia S, Bellon PL, Lupetti P, Dallai R, Rappuoli R, Telford JL: Three-dimensional reconstruction of metal replicas of the *Helicobacter pylori* vacuolating cytotoxin. J Struct Biol 121: 9–18, 1998
57. Yahiro K, Niidome T, Kimura M, Hatakeyama T, Aoyagi H, Kurazono H, Imagawa K, Wada A, Moss J, Hirayama T: Activation of *Helicobacter pylori* VacA toxin by alkaline or acid conditions increases its binding to a 250-kDa receptor protein-tyrosine phosphatase beta. J Biol Chem 274: 36693–36699, 1999
58. Szabo I, Brutsche S, Tombola F, Moschioni M, Satin B, Telford JL, Rappuoli R, Montecucco C, Papini E, Zoratti M: Formation of anion-selective channels in the cell plasma membrane by the toxin VacA of *Helicobacter pylori* is required for its biological activity. EMBO J 18: 5517–5527, 1999
59. de Bernard M, Papini E, de Filippis V, Gottardi E, Telford J, Manetti R, Fontana A, Rappuoli R, Montecucco C: Low pH activates the vacuolating toxin of *Helicobacter pylori*, which becomes acid and pepsin resistant. J Biol Chem 270: 23937–23940, 1995
60. Papini E, Satin B, Bucci C, de Bernard M, Telford JL, Manetti R, Rappuoli R, Zerial M, Montecucco C: The small GTP binding protein rab7 is essential for cellular vacuolation induced by *Helicobacter pylori* cytotoxin. EMBO J 16: 15–24, 1997
61. Hotchin NA, Cover TL, Akhtar N: Cell vacuolation induced by the VacA cytotoxin of *Helicobacter pylori* is regulated by the Rac1 GTPase. J Biol Chem 275: 14009–14012, 2000
62. Galmiche A, Rassow J, Doye A, Cagnol S, Chambard JC, Contamin S, de Thillot V, Just I, Ricci V, Solcia E, Van Obberghen E, Boquet P: The N-terminal 34 kDa fragment of *Helicobacter pylori* vacuolating cytotoxin targets mitochondria and induces cytochrome c release. EMBO J 19: 6361–6370, 2000
63. Shirin H, Moss SF: *Helicobacter pylori* induced apoptosis. Gut 43: 592–594, 1998
64. Molinari M, Salio M, Galli C, Norais N, Rappuoli R, Lanzavecchia A, Montecucco C: Selective inhibition of Ii-dependent antigen presentation by *Helicobacter pylori* toxin VacA. J Exp Med 187: 135–140, 1998
65. Yoshida N, Granger DN, Evans DJ Jr, Evans DG, Graham DY, Anderson DC, Wolf RE, Kvietys PR: Mechanisms involved in *Helicobacter pylori*-induced inflammation. Gastroenterology 105: 1431–1440, 1993
66. Evans DJ Jr, Evans DG, Takemura T, Nakano H, Lampert HC, Graham DY, Granger DN, Kvietys PR: Characterization of a *Helicobacter pylori* neutrophil-activating protein. Infect Immun 63: 2213–2220, 1995
67. Blaser MJ: *Helicobacter pylori*: Microbiology of a 'slow' bacterial infection. Trends Microbiol 1: 255–260, 1993
68. Teneberg S, Miller-Podraza H, Lampert HC, Evans DJ Jr, Evans DG, Danielsson D, Karlsson KA: Carbohydrate binding specificity of the neutrophil-activating protein of *Helicobacter pylori*. J Biol Chem 272: 19067–19071, 1997
69. Satin B, Del Giudice G, Della Bianca V, Dusi S, Laudanna C, Tonello F, Kelleher D, Rappuoli R, Montecucco C, Rossi F: The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. J Exp Med 191: 1467–1476, 2000
70. Christie PJ, Vogel JP: Bacterial type IV secretion: Conjugation systems adapted to deliver effector molecules to host cells. Trends Microbiol 8: 354–360, 2000
71. Fischer W, Puls J, Buhrdorf R, Gebert B, Odenbreit S, Haas R: Systematic mutagenesis of the *Helicobacter pylori* cag pathogenicity island: Essential genes for CagA translocation in host cells and induction of interleukin-8. Mol Microbiol 42: 1337–1348, 2001
72. Naumann M, Wessler S, Bartsch C, Wieland B, Covacci A, Haas R, Meyer TF: Activation of activator protein 1 and stress response kinases in epithelial cells colonized by *Helicobacter pylori* encoding the cag pathogenicity island. J Biol Chem 274: 31655–31662, 1999
73. Hirata Y, Maeda S, Mitsuno Y, Akanuma M, Yamaji Y, Ogura K, Yoshida H, Shiratori Y, Omata M: *Helicobacter pylori* activates the cyclin D1 gene through mitogen-activated protein kinase pathway in gastric cancer cells. Infect Immun 69: 3965–3971, 2001
74. Shirin H, Sordillo EM, Oh SH, Yamamoto H, Delohery T, Weinstein IB, Moss SF: *Helicobacter pylori* inhibits the G1 to S transition in AGS gastric epithelial cells. Cancer Res 59: 2277–2281, 1999
75. Wang TC, Dangler CA, Chen D, Goldenring JR, Koh T, Raychowdhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, Fox JG: Synergistic interaction between hypergastrinemia and *Helicobacter* infection in a mouse model of gastric cancer. Gastroenterology 118: 36–47, 2000
76. Romano M, Ricci V, Memoli A, Tuccillo C, Di Popolo A, Sommi P, Acquaviva AM, Del Vecchio Blanco C, Bruni CB, Zarrilli R: *Helicobacter pylori* up-regulates cyclooxygenase-2 mRNA expression and prostaglandin E2 synthesis in MKN 28 gastric mucosal cells *in vitro*. J Biol Chem 273: 28560–28563, 1998
77. Pomorski T, Meyer TF, Naumann M: *Helicobacter pylori*-induced prostaglandin E(2) synthesis involves activation of cytosolic phospholipase A(2) in epithelial cells. J Biol Chem 276: 804–810, 2001
78. Nardone G, Holicky EL, Uhl JR, Sabatino L, Staibano S, Rocco A, Colantuoni V, Manzo BA, Romano M, Budillon G, Cockerill FR III, Miller LJ: *In vivo* and *in vitro* studies of cytosolic phospholipase A2 expression in *Helicobacter pylori* infection. Infect Immun 69: 5857–5863, 2001
79. Gupta RA, Polk DB, Krishna U, Israel DA, Yan F, DuBois RN, Peek RM Jr: Activation of peroxisome proliferator-activated receptor gamma suppresses nuclear factor kappa B-mediated apoptosis induced by *Helicobacter pylori* in gastric epithelial cells. J Biol Chem 276: 31059–31066, 2001
80. Mohammadi M, Czinn S, Redline R, Nedrud, J: Helicobacter-specific cell-mediated immune responses display a predominant T_H1 phenotype and promote a delayed-type hypersensitivity response in the stomachs of mice. J Immunol 156: 4729–4738, 1996
81. Fox JG, Beck P, Dangler CA, Whary MT, Wang TC, Shi HN, Nagler-Anderson C: Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. Nat Med 6: 536–542, 2000
82. Noach LA, Bosma NB, Jansen J, Hoek FJ, van Deventer SJ, Tytgat GN: Mucosal tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8 production in patients with *Helicobacter pylori* infection. Scand J Gastroenterol 29: 425–429, 1994
83. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS: Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404: 398–402, 2000
84. Takashima M, Furuta T, Hanai H, Sugimura H, Kaneko, E: Effects of *Helicobacter pylori* infection on gastric acid secretion and serum gastrin levels in Mongolian gerbils. Gut 48: 765–773, 2001
85. El-Omar EM: The importance of interleukin-1β in *Helicobacter pylori* associated disease. Gut 48: 743–747, 2001
86. Suganuma M, Kurusu M, Okabe S, Sueoka N, Yoshida M, Wakatsuki Y, Fujiki H: *Helicobacter pylori* membrane protein 1: A new carcinogenic factor of *Helicobacter pylori*. Cancer Res 61: 6356–6359, 2001
87. Engstrand L, Scheynius A, Pahlson C, Grimelius L, Schwan A, Gustavsson S: Association of *Campylobacter pylori* with induced expression of class II transplantation antigens on gastric epithelial cells. Infect Immun 57: 827–832, 1989