CHAPTER 1

INTRODUCTION

In the 19 years, since the discovery of \textit{H. pylori}, there has been remarkable scientific progress, especially from developed countries providing increased knowledge about its role in important human diseases like peptic ulcers, chronic gastritis and gastric cancers, methods of diagnosis and effective treatments. An improved understanding of the pathogenesis of infection will facilitate further medical progress for better diagnostic treatments. In India the infection of \textit{H. pylori} is high and recent reports indicated 80-95\% as the infection rate, particularly in specific regions of the country (Graham et al., 1991; Gill and Desai, 1993). Hence this study pertaining to the nature of \textit{H. pylori} pathogenesis particularly in the Indian clinical isolates is important in understanding variations of its known pathogenic profile. Therefore, the work presented in this thesis has been an attempt to understand the molecular pathogenesis of this organism especially with respect to the Indian clinical isolates.

1.1 NATURAL HISTORY OF \textit{H. PYLORI} INFECTION

From studies conducted by hundreds of investigators all over the world, it has become clear that \textit{H. pylori} infection has a natural history (Fig. 1). Within weeks after the organism is acquired, chronic superficial gastritis develops (Morris \textit{et al.}, 1991). In asymptomatic persons the lesion persists without any consequences for the duration of infection (Dooley \textit{et al.}, 1989).
The disease progresses into a chain of events in different groups such as peptic ulcers, variety of lymphoproliferative disorders, and chronic superficial gastritis proceeding to atrophic gastritis. The latter lesion is important because it is the major recognised risk factor for the development of gastric cancer (Dixon et al., 1995). The manifestation of cancer takes 3-6 decades of infection.

1.1.1 Discovery of *H. pylori* and Historical Development of its role in Human Disease

Peptic ulcer disease is a chronic inflammatory condition of the stomach and duodenum. The disease has relatively low mortality, but it results in substantial human sufferings and high economic loss. In the early 20th century, the pathogenesis of the disorder was believed to be related to stress and dietary factors. Thus, treatment focused on hospitalisation with bed rest and prescription of specially made bland foods. Later it was thought to be due to the injurious effects of digestive secretions such as gastric acid. Hence, antacids became the standard therapy. In 1971, Sir James Black identified a subtype of the histamine receptor (H$_{2}$ receptor) that appeared to be the principal mediator of gastric acid secretion. Antagonists to this receptor proved to be safe and effective therapy for peptic ulcer disease. More recently, inhibitors of the proton pump (H+, K+-ATPase) in gastric parietal cells have proved to be rapidly effective and extremely potent antiulcer drugs (Nagata et al., 1993). Other drugs that appear to enhance mucosal defence such as bismuth compounds, sucralfate (aluminum sucrose sulphate, basic) and prostaglandin have also been applied to the treatment of peptic ulcers. Despite these sophisticated therapeutic agents, the disturbing problem of high recurrence rate of peptic ulcer, even after complete healing, remained.
The first isolation of *Helicobacter pylori* in 1982 by Marshall and Warren (1984) ushered in a new era in gastric microbiology. Spiral organisms nestled in the narrow interface between the gastric epithelial cell surface and the overlying mucous gel had been observed many times in the preceding century. However, the isolation of *H. pylori*, in conjunction with the interest in the pathogenesis of gastroduodenal diseases, as well as the availability of clinical specimens via endoscopic biopsy, has led to important breakthroughs in medical care. In 1979 Robin Warren, a pathologist in Perth, Western Australia began to notice that curved bacteria were often present in gastric biopsy specimens submitted for histological examination (Warren and Marshall, 1983). These organisms were not present within the gastric mucosa, but were present in the mucous layer overlying the tissue. Since the organisms had the appearance of curved, gram-negative rods, Campylobacter isolation method was used by plating the biopsy on to the selective media and growing under microaerobic conditions. Initially when colonies were not visible within 2 days plates as in the case of campylobacters, the plates were discarded, but it was observed by chance that after 5 days colonies were seen. Subsequently organisms were isolated and characterised as *Campylobacter pyloridis* (Marshall and Warren, 1984). By 1984, it had become clear that *H. pylori* infection was strongly associated with the presence of inflammation in the gastric mucosa, and especially with polymorphonuclear filtration. It was several years that sufficient evidence was gathered to prove its etiologic role (Blaser, 1990). A wide body of evidence now indicates that once acquired, *H. pylori* persists, usually, for life, unless eradicated by antimicrobial therapy (Blaser, 1996; Blaser, 1997a). Marshall and Warren noted that *H. pylori* infection was associated with the duodenal ulceration. By 1994, consensus conference convened by the National Institutes of Health concluded that *H. pylori* was a major cause of peptic ulcer disease (NIH consensus report, 1994). Evidences showed that chronic gastritis was linked to adenocarcinoma of the
stomach (Correa, 1992), the most important stomach malignancy of the world, but the causation of the gastritis was then unknown. In 1991, 4 reports first showed association between *H. pylori* infection and the presence (Talley *et al.*, 1991) and development (Parsonnet *et al.*, 1991) of gastric cancer. In 1994, the International Agency for Cancer Research, an arm of the World Health Organization, reviewed the available evidence and declared that *H. pylori* was a carcinogen of humans. *H. pylori* infection also has been associated with the development of gastric non-Hodgkin's lymphomas (Parsonnet *et al.*, 1994) and with another lymphoproliferative disorder, gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Wotherspoon *et al.*, 1991). Thus, in total, *H. pylori*, a previously obscure organism, has now been associated with many of the most important diseases involving gastroduodenal tissue. *H. pylori* infection is usually asymptomatic, but some individuals develop disease. The disease establishment and progression is associated with impaired immune function and host response that results in chronic infection. Therefore damage in *H. pylori* infection is probably the result of both pathogen and host mediated processes.

### 1.1.2 Epidemiology of *H. pylori* infection

*H. pylori* has been found in the stomachs of humans in all parts of the world and is commonly isolated from nonhuman primates as well. In developing countries, 70 to 90% of the population carries *H. pylori* acquired before the age of 10 years. In developed countries, the prevalence of infection is lower ranging from 25 to 50% (Taylor and Parsonnet. 1995)(Fig.1). Most studies suggest that males and females are infected at approximately same rates, although reports show male sex as a significant risk factor for infection (Reddy *et al.*, 1996). Several studies show that prevalence of *H. pylori* changes over time and it has been decreasing in prevalence with a 50% reduction in the past 25 years.
There are two distinct patterns of *H. pylori* prevalence with respect to age depending on the geographical region studied. The first pattern is widespread infection early in childhood with elevated prevalence rates of close to 80% throughout adulthood, and the second is increasing prevalence with age. This variability in pattern suggests a difference in infectivity or transmissibility of *H. pylori* infection. Potential determinants of these differences including environmental, bacterial and host factors have also been reported. The most important determinant is socioeconomic class, which affects living conditions and sanitation, thus altering exposure to the bacterium. Host factors also play a role, perhaps via host receptors for *H. pylori*. Bacterial factors may also contribute, although compelling evidence is lacking.

In developed countries, persons of higher socioeconomic status have lower infection rates, although among certain minorities, high rates persist in spite of economic advancement (Graham, 1991; Mendall *et al.*, 1992) DNA motifs at several informative loci like Cag, Vac etc., in more than 500 isolates of *Helicobacter pylori* from five continents were studied by PCR and sequencing to gain insights into the evolution of this gastric pathogen. Five types of deletion, insertion, and substitution motifs were found at the right end of the *H. pylori cag* pathogenicity island. Of the three most common motifs, type I predominated in Spaniards, native Peruvians, and Guatemalan Ladinos (mixed Amerindian-European ancestry) and also in native Africans and U.S. residents; type II predominated among Japanese and Chinese; and type III predominated in Indians from Calcutta. Sequences in the *cagA* gene and in *vacA* type alleles of the vacuolating cytotoxin gene (*vacA*) of strains from native Peruvians were also more like those from Spaniards than those from Asians. These indications of relatedness of Latin American and Spanish strains, despite the closer genetic relatedness of Amerindian and Asian people
themselves, lead us to suggest that *H. pylori* may have been brought to the New World by European conquerors and colonists about 500 years ago. This thinking, in turn, suggests that *H. pylori* infection might have become widespread in people quite recently in human evolution (Kersulyte et al., 2000). Available evidence also strongly suggests an inverse correlation between socio-economic status and prevalence of *Helicobacter pylori*.

The prevalence of duodenal ulcer in South India is high but there are very few reports regarding the prevalence of *H. pylori* infection in various upper gastrointestinal disorders in south Indians. It is more closely associated with duodenal ulcers (Kate et al., 1993). In this population, the prevalence of infection in asymptomatic individuals was nearly as high as that in duodenal ulcer, underlining the need for further study to identify the differences in host response or bacterial pathogenicity that lead to the development of ulcer in only some individuals (Prasad et al., 1994). In developing countries, however, *H. pylori*, like other enteric infections are common in infants also. In a study from Bangladesh, *H. pylori* infection has been shown in 41 of 90 (46%) infants aged 1-12 months (Sarker et al., 1995) and has been recognised that *H. pylori*, like most enteric infections, is mainly acquired in childhood. In India, like other developing countries, due to poor socio-economic status, poor hygiene and overcrowding the prevalence of *H. pylori* infection is very high. A study from Delhi in children (Bansal et al., 1998) has shown that there is no correlation of these above-mentioned factors and *H. pylori* infection. In 1991, a study from Hyderabad (Graham et al., 1991) has shown that *H. pylori* infection increases with age and the prevalence reaches 84% by 20 years of age. In a similar study from Bombay in 1994 (Gill et al., 1994), it has been shown that the prevalence of IgG antibody was 22%, 56% and 87% in 0-4, 5-9 and 10-19 years age group, respectively in 340 subjects. Another study from Bangalore (Dore et al., 1997)
Fig. 1.1 Map showing percentages of population infected with *H. pylori* as determined by epidemiological studies
(Adapted from www.helico.com)
has detected \textit{H. pylori} infection in 82\% of 50 children (6-18 years) by $^{13}$C urea breath test. There are two studies in adults, one from Karnataka (Katelaris et al., 1991) and Allahabad (Misra et al., 1997), which have shown a 77.2\% and 78\% prevalence of \textit{H. pylori} in adults. The overall seroprevalence of \textit{H. pylori} in Chennai is high; the age-specific prevalence is different from what has been reported earlier in India, the pattern resembling that of a developed country (Alaganatham et al., 1999). All these studies have shown that \textit{H. pylori} infection is very common in India and most of the Indian pediatric population is infected.

1.1.3 Transmission

A determination of its prevalence has been performed around the world, and regardless of how exotic a location, \textit{H. pylori} is found in a substantial proportion of a population. \textit{H. pylori} remains among the most universal of infections. It is realised that infection can be gained and lost at higher frequency than previously believed. Oral-Oral and feco-oral transmission account for most cases of infection. \textit{H. pylori} infection has declined rapidly in developed countries, which probably has contributed to declines in duodenal ulcer disease and gastric cancer. The reason for this potential elimination of infection is still unknown.

There appears to be no substantial reservoir of \textit{H. pylori} aside from the human stomach. Three routes have been described for its transmission. The first and least common is iatrogenic, in which tubes, endoscopes, or specimens in contact with the gastric mucosa from one person are introduced to another person (Akamatsu et al., 1996). Occupationally acquired infections also have been reported, especially the endoscopists (Mitchell et al., 1989). Fecal-oral
transmission is the second mode indicating that shedding is intermittent. Klein et al., (1991) reported isolation of \textit{H. pylori} from faeces in children and therefore fecally contaminated water may be a source of infection. Oral-oral transmission, the third mode, has been identified (Megraud, 1995), but there is no identified association of infection with sexual transmission. Recent studies among patients with gastrointestinal disorders suggest a potential role of conjugal transmission. These results support the hypothesis of a major role of spouse-to-spouse transmission of \textit{H. pylori} infection. \textit{Helicobacter pylori} have also been found in some domestic cats, but at present, the risk of infection from these animals appears rare. The pattern of acquisition and loss of \textit{Helicobacter pylori} infection in a cohort of 212 children from a biracial community with a homogeneous socioeconomic class were studied. The children were followed over 12 years (1973-1974 to 1985-1986) from childhood to young adulthood. \textit{H. pylori} status was assessed by the presence of serum IgG antibodies to \textit{H. pylori}. (Malaty et al., 1999) The prevalence of infection with \textit{H. pylori} in developed countries was about 20\%, on the other hand in developing countries it reached over 80\%. In developing countries 40\% of infants already had anti-\textit{H. pylori} antibody and the prevalence of infection rapidly increased and then reached the peak (80\%) in teenager. In contrast, in developed countries the rate of infection with \textit{H. pylori} was below 20\% in teenager and gradually increased by age (1\% per 1 year). These results suggested \textit{H. pylori} infection would be closely associated with childhood living conditions than current living (including socioeconomic) status. Exposure to \textit{H. pylori} occurs early in India and is widespread, even in control subjects. The high prevalence of the organism in young Indian control subjects and the comparable prevalence of antibodies to \textit{H. pylori} may suggest a feco-oral mode of transmission (Gill et al., 1993). From this point of view, the prevention of infection with \textit{H. pylori} in childhood will be most important to prevent the gastroduodenal disease
related with *H. pylori* in the future. In summary, although *H. pylori* is present in the stomachs of half of the world's population, its transmission is not yet clearly understood and the mode of its spread is a subject of ongoing debate.

### 1.2 CHARACTERISTICS OF THE PATHOGEN

#### 1.2.1 Microbiological characteristics

**Morphology**

*H. pylori* organisms are spiral, microaerophilic, and gram-negative bacteria showing bluntly rounded ends in gastric biopsy specimens (Goodwin et al., 1987). However culturing on solid media, the bacteria assumes a rod-like shape (Goodwin and Armstrong, 1990). After prolonged culture on solid or liquid medium, coccoid forms predominate, which are metabolically active but cannot be cultured *in vitro* (Chan et al., 1994). Coccoid forms appear as U shaped bacilli with the ends of the two arms joined by a membranous structure. The protein components of the coccoid forms are similar to that of the spiral forms and they are also reported to be the manifestation of cell death, contributing to the pathogenesis during *H. pylori* infection. In gastric biopsy specimens, organisms are 2.5 to 5.0μm long and 0.5 to 1.0μm wide and there are 4-6 unipolar-sheathed flagella for motility. Flagella exhibit a characteristic terminal bulb, which is an extension of the flagellar sheath (Geis et al., 1993). Ultrastructurally the outer membrane of *H. pylori* is coated with glycocalyx like structure. In gastric biopsy specimens, the surface of individual bacteria may be linked to gastric epithelial microvilli by thread like extensions of the glycocalyx (Goodwin et al., 1987). The surface of viable *H. pylori* cells grown on agar lates is coated with 12-15 nm ring-shaped aggregates of urease and *HspB*, a homolog of the groEL heat shock protein (Austin et al., 1992).
1.2.1.2 Nutritional Requirements

Defined media are not available for culture of *H. pylori*; the organism requires complex basal medium with supplements such as whole blood, heme, serum, charcoal, cornstarch, or egg yolk emulsion (Hachem, *et al.*, 1995). Supplementation of media with cyclodextrins supports excellent growth of *H. pylori* (Marchini *et al.*, 1995). Fresh isolates of *H. pylori* grow best under microaerobic conditions. However, after laboratory passage, some strains become sufficiently aerotolerant that they can grow in 10% CO₂ (Goodwin and Armstrong, 1990).

1.3 PATHOGENESIS OF INFECTION

1.3.1 Animal Model studies

Ever since the realisation that *H. pylori* was intimately associated with the development of gastritis and peptic ulcer disease in humans, there has been a need for a simple animal model in which modes of pathogenicity, transmission, immunisation and chemotherapeutic intervention can be evaluated. *H. pylori* has a very narrow host range and will not colonise many of the usual laboratory animal species, including conventionally reared rats, mice, rabbits, guinea pigs, specific pathogen-free pigs and gnotobiotic rats and mice (Cantorna *et al.*, 1990; Radin *et al.*, 1990). An apparent exception was colonisation of *H. pylori* by Mongolian gerbils, after induction of gastric lesions with indomethacin (Yokota *et al.*, 1991). Owing to narrow host range of colonisation, naturally occurring *H. mustelae* infection of ferrets (Fox *et al.*, 1988) *H. pylori* in gnotobiotic piglets (Krakowka *et al.*, 1987) and *H. felis* model in mice and rats (Lee *et al.*, 1990; Fox *et al.*, 1991) were considered for understanding the disease process. But all these animal models are still only
substitutes for the "target" organism in \textit{H. pylori}. Gastric colonisation of \textit{H. pylori} in mice was first reported by Karita et al., (1991) who showed that Balb/c nude mice could support long-term colonisation associated with gastritis and their euthymic counterpart demonstrated only transient infection for two weeks. Gastric pathology resembling human disease has also been shown in specific pathogen free (SPF) CD1 mice or conventional (CV) Balb/C mice (Marchetti et al., 1995). This animal model reproduces many of the characteristics of human \textit{H. pylori} infections and offers considerable potential for comparative treatment studies. Marchetti et al., (1995) evaluated whether oral vaccination with bacterial lysate or purified antigens (Urease/VacA) in the presence of heat-labile enterotoxigenic toxin of \textit{E.coli} could prevent colonisation. This model reported that purified \textit{VacA} from Type I bacteria only protected against Type I challenge but not Type II. The facts that fresh clinical isolates or mouse-adapted bacteria were necessary for colonisation in previously reported studies (Karita et al., 1991) suggest that quality of the challenge inoculum was vital for successful analysis of pathogenesis. Important determinants of successful colonisation have also been elucidated using simple mouse model referred as Glaxo model (Mc Colm et al., 1995a). Using this model the marked predilection for the cardiac antrum area was observed during \textit{H. pylori} colonisation. This model also served as a rapid screening strategy for identification and initial selection of novel anti-\textit{Helicobacter} agents (Mc colm et al., 1995b).

The first reports of experimental \textit{H. pylori} infection of pigs used piglets delivered by caesarian section in to sterile isolation units (Krakowa et al., 1987). Gnotobiotic piglets challenged with \textit{H. pylori} can be maintained up to two months in germ free conditions. Histopathological chronic active gastritis occurred in all piglets and increased in intensity with time.
Neutrophilic aggregates and some monocytes are present mostly in the nonglandular regions of the cardia initially and later predominated by mononuclear cells, forming large lymphoid follicles in the submucosa and lamina propria. Gnotobiotic pigs reproduce many of the features of diseases associated with *H. pylori* in humans. A characteristic of the pig model is the patchy distribution of *H. pylori* over gastric mucosa (Engstrand et al., 1992), which may result in false negative results for cultured endoscopic biopsies. Precise topographic mapping of urease distribution on the gastric mucosa is a more accurate method in a pig model but necessitates the killing of the animal during evaluation.

Gnotobiotic beagle pups have been successfully colonised with *H. pylori* grown in brucella broth and maintained in sterile isolation units up to 1 month (Radin et al., 1990). In spite of showing lower *H. pylori* densities and different pattern of bacterial distribution compared to humans, dogs can be maintained in gnotobiotic conditions for several years thus allowing prolonged studies. Domestic cats also have been identified as a natural host for *H. pylori* infections (Handt et al., 1994). The animals develop multifocal gastritis, comprising lymphoid aggregates and nodules mostly in the antral gastric mucosa with *H. pylori* visible within the glandular crypts (Fox et al., 1995). Cats are the only other species except for certain other species of nonhuman primates to have been positively shown to acquire *H. pylori* infections naturally. Consequently, this model is an important advance offering considerable scope for examination of mechanisms of transmission, pathogenesis, and perhaps factors important in the long-term progression to atrophic gastritis and gastric carcinoma. In addition it is also a suitable secondary stage therapy model for further evaluation of compounds selected in a primary mouse test.
1.3.2 Evidences of the role of *H. pylori* in Gastric inflammation

Extensive evidences implicate *H. pylori* in the pathogenesis of chronic superficial gastritis. Voluntary ingestion of the bacterium by two human volunteers resulted in acute or chronic gastritis (Morris et al., 1991) and is cleared by antimicrobial therapy (Valle et al., 1991). Also a persistent immunological response is seen in untreated infected individual and the titer of *H. pylori* specific antibodies decreases after eradication. *H. pylori* exhibits specificity of tissue involvement by associating exclusively to gastric mucosal cells and seen in the duodenal bulb only in association with gastric metaplasia. *H. pylori* is associated with specific gastroduodenal pathology specifically in chronic superficial and type B atrophic gastritis (Morris, 1989). Infection with *H. pylori* induces more severe gastric mucosal inflammation, resulting in enhanced DNA damage in patients with gastric cancer. In response to DNA damage, apoptosis of gastric epithelial cells occurs with insufficient cell proliferation, which might be inhibited by the presence of VacA. It follows that atrophic gastritis is more extensive in patients with gastric cancer than in subjects with chronic gastritis. Differences in the extent of mucosal inflammation, which might be associated with increased gastric epithelial DNA damage, and the extent of atrophic gastritis possibly contribute to the development of gastric cancer (Yoshimura et al., 2000).

1.3.3 Histopathology of infection

*H. pylori* is most commonly associated with chronic superficial gastritis characterised by mononuclear inflammatory cell infiltration associated with neutrophilic infiltration of the epithelium and is not specifically associated with metaplastic change, granuloma formation, or fundus gland atrophy (Dixon.
1995) with highly variable rate of inflammation. The inflammatory response to \textit{H. pylori} in children differs from that of adults. Endoscopy reveals a finely granular or nodular mucosal surface, which microscopically corresponds to lymphonodular hyperplasia, especially in the antrum (Genta et al., 1993).

1.4 MOLECULAR PATHOGENESIS: MAJOR VIRULENCE FACTORS

1.4.1 Motility

The motility of \textit{H. pylori} is essential for colonisation facilitating the bacteria to spread through the viscous mucous covering the epithelial cells of the gastric mucosa (Hazell et al., 1986). Studies show the inability of an aflagellate, non-motile variants of \textit{H. pylori} to infect gnotobiotic piglets (Eaton et al., 1992). \textit{H. pylori} possesses two to six polar sheathed flagella encoded by \textit{flaA} and \textit{flaB} genes (Josenhans et al., 1995). The flagellar sheath is composed of a double layer of phospholipids probably protecting the flagella from gastric acidity (Geis et al., 1993). Jones et al. (1997) showed the existence of a flagellar sheath protein identical to the HpaA protein, which has been reported to be an N-acetyl-neuraminylactose binding a hemagglutinin (Evans et al., 1993). The movement of \textit{H. pylori} in the viscous mucin layer is enhanced by chemotactic activity toward urea and bicarbonate (Nakamura et al., 1998).

1.4.2 Bacterial adhesins and cellular receptors

Disease caused by bacterial pathogens is the result of the outcome of host-pathogen interactions. The first step in an infectious disease requires specialised protein factors which allow its binding of the cells. Such adhesins determine the severity of the disease and the outcome of the host-pathogen
interaction and is important for virulence expression of many bacterial pathogens in the human host (Beachey, 1981). The adhesins of pathogenic bacteria are involved in the sequential recognition of epithelial cell receptors and adherence promotes the delivery of bacterial toxins (Zaffiri et al., 1987). Adhesins are proteins, which usually recognize carbohydrates on eukaryotic cells. Most bacteria encodes for more than one adhesin eg. Bordetella pertussis. 

*H. pylori* also has developed efficient strategies to bind to cell surfaces for the survival in the harsh acidic environment. Following binding, the brush border architecture is disrupted, with an intimate contact between the bacteria and the plasma membrane (Hessey et al., 1990). Electron micrographs of antral biopsies showed *H. pylori* adhered to the apical membrane of mucous-secreting gastric epithelial cells (Tricottet et al., 1986). Adhesion is characterised by intimate contact between the bacterial cell and the host cell membrane in the form of adhesion pedestals and rearrangement of microskeletal cell proteins (Bode et al., 1988). Bacterial attachment to adherence pedestals was also seen in ferret model of *H. pylori* associated disease (Fox et al., 1990). Depletion of microvilli and disruption of cytoskeletal filaments were observed at points of bacterial attachment. Attachment of *H. pylori* to epithelial cells of gastric origin *in vitro* was first demonstrated by Hemalatha et al. (1991). Adhesion of *H. pylori* to epithelial cells was described as similar to the binding of "attaching and effacing" (A/E) *E. coli* (Hemalatha et al., 1991; Neman-Simha and Megraud, 1988). Adherence was significantly reduced at extreme acidity probably related to the reduced viability.

The intimate association of *H. pylori* with primary human gastric epithelial cells and with AGS cells appeared very similar by transmission electron microscopy. Actin polymerisation, demonstrated by phalloidin staining, occurred in AGS cells exposed to *H. pylori*, which correlated with the
cellular projections observed on electron micrographs (Smoot et al., 1993). The accumulation of actin filaments below adherent bacteria were less dense than those observed in EPEC adherence. Although *H. pylori* closely adhered to microvillus-denuded areas of the plasma membrane of cultured HEp-2 cells, Kato-III, HEL, CHO cells, F-actin pedestals were absent both *in vitro* and *in vivo* when a human gastric mucosal biopsy was used (Dytoc et al., 1993).

The mechanisms of binding of *H. pylori* has been demonstrated *in vitro* with several cell lines including HEp-2, HEL, CHO, KatoIII, HeLa, human buccal, and human intestinal undifferentiated 407 cells (Dytoc et al., 1993; Fauchere and Blaser, 1990; Figueroa et al., 1992; Neman and Megraud, 1988). All these cell lines, however, lack a brush border at their apical membrane, in contrast to gastric epithelial cells. Theulaz et al., (1996) demonstrated adhesion of *H. pylori* to a polarized epithelial cell line T84. Confocal laser scanning microscopy and electron microscopy showed a focal loss of typical brush border microvilli at the site of adherence. Rearrangement of F-actin network was reflected by the decrease of villin labeling (An actin-associated protein restricted to the brush border cytoskeleton). Labelled *H. pylori* cells were found associated with the apex of the intestinal cells, in vicinity to villin labeling. This study also concluded that acidic pH favors *H. pylori* adhesion and that binding is followed by survival of the bacteria in pockets in apical membrane (Theulaz et al., 1996). The haemagglutinins contribute to adherence *in vivo* and the ability of strains to adhere to different cell lines correlate with their ability to produce agglutination of erythrocytes. Variable expression of specific adhesins on bacterial surface may explain the variation in binding by different strains of *H. pylori*. Most bacterial adhesins are haemagglutinins, which is supported by the fact that bacterial adhesins share properties of moderate to strong hydrophobicity and the ability to recognize
specific receptors (Beachey et al., 1981). The chemical composition of the membrane of RBCs from various species differs and may give the clue to the receptor and adhesin necessary for bacterial adherence. Studies on haemagglutination profiles have suggested that adherence may be important in the colonisation and pathogenesis of \textit{H. pylori} strains (Evans \textit{et al.}, 1988). Potential receptors for \textit{H. pylori} have been purified and partially characterized (Lingwood \textit{et al.}, 1989). Both \textit{H. mustelae} and \textit{H. pylori} agglutinated RBCs of various species. (Morgan \textit{et al.}, 1991). Other potential receptors for \textit{H. pylori} binding include extracellular matrix components, which may be exposed after injury to the gastric epithelium. In vitro, \textit{H. pylori} strains bind laminin, fibronectin, various collagens, and heparan sulphate (Valkonen \textit{et al.}, 1993; Ascencio \textit{et al.}, 1993).

1.4.3 Urease

The enzyme urease is abundantly produced by all gastric \textit{Helicobacter} species. \textit{H. pylori} urease has unusual characteristics compared to those of other bacterial species. First, the enzyme is found in the cytoplasm as well as on the bacterial surface (Phadnis \textit{et al.}, 1996). Second it has two optimal pHs, one of which is acidic (Marais \textit{et al.}, 1999). Third it is composed of only two subunits, UreA and UreB (Hu \textit{et al.}, 1992), whereas other bacterial ureases contain three subunits (Mobley \textit{et al.}, 1995). The native urease of \textit{H. pylori} has a molecular mass of approximately 540kDa and is a nickel containing hexameric molecule consisting of two subunits, Ure A, 30-kDa and Ure B 62 kDa in a 1:1 molar ratio. \textit{H. pylori} urease cluster contains nine genes, including the \textit{ureA} and \textit{ureB} structural genes, as well as regulatory genes involved in the synthesis and assembly of the holoenzyme. Mutant with \textit{ureB} disruption does not cause gastritis in nude mice due to difficulty in colonisation.
In addition, an ureG mutant does not colonize the stomach in normochlorhydric or achlorhydric piglets. These results suggest that the role of urease in bacterial colonisation is not limited to the neutralization of gastric acid (Fig.2). Urease does not play a direct role in adhesion, since adherence of the bacteria to gastric cells is not affected by ureB. Urease is an essential colonisation factor of *Helicobacter* species. Isogenic urease-negative mutants were unable to colonize gnotobiotic piglets, and stomachs of nude mice. Urease activity is required for the production of a neutral microenvironment for the organism within the gastric lumen. There is considerable evidence that urease is associated with the outer membrane of *H. pylori*, but urease activity is also observed within the cytoplasm, suggesting a role in the assimilation of organic nitrogen. Urease encoding region of *H. pylori* genome is composed of two gene clusters, common to all strains (Cussac et al., 1992), one comprising the ureAB genes encoding the structural urease subunits and the other containing the ureEFGH genes encoding the accessory proteins required for nickel incorporation in to the urease active site. ureI gene of unknown function immediately upstream from this cluster and the close distance between these genes suggest that ureI-ureE-ureF constitutes an operon, and cotranscription of ureI and ureE has been demonstrated by northern blot analysis (Akada et al., 2000). *H. pylori* also has such an aliphatic amidase hydrolyzing acetamide and propioinamide in vitro (Skouloubris et al., 1997) Urease cluster of *H. pylori* is unique among the many urease operons of Gram negative bacteria that have been sequenced (Mobley et al., 1995) in that it has an extra gene, ureI. UreI is not required for full urease activity during in vitro growth, but essential for the survival of *H. pylori* (Skouloubris et al., 1998). Urease is an important factor involved in colonisation and survival in the gastric environment (Labigne et al., 1991; Eaton and Krakowa, 1994). Urease induces vacuolation in the presence of added ureA through the generation of ammonia from ureA. (Smoot
Fig. 1.2 Localization of *Helicobacter pylori* in the host

The bacterium is acquired via the oral route and survives the low pH of the stomach lumen because of the protective action of the urease, which buffers the pH around the bacterial surface. It penetrates into the mucus layer covering the superficial stomach cells. This layer is poorly permeable to bicarbonate anions, while it is very permeable to protons in the direction of the stomach lumen. As a consequence, the pH above the cells is much less acidic than in the lumen. *H. pylori* adheres strongly to the apical cell surface via adhesins and VacA and induces a rearrangement of the underlying cytosol (Montecucco et al., 1999).
et al., 1990). Cell vacuolation by urease indirectly by metabolizing ureA to ammonia was reported by a simple, quantitative neutral red uptake assay. This assay is on the basis of rapid uptake of neutral red into intracellular vacuoles. (Cover et al., 1991). The synthesis of active urease in H. pylori requires expression not only of the ureA and ureB structural genes but also of the accessory genes ureE, ureF, ureG, and ureH.

1.4.4 Vacuolating Cytotoxin

H. pylori cytotoxin activity was first described by Leunk et al., (1988) who showed that approximately 50% of H. pylori strains cause vacuolation of eukaryotic cell lines. Pathogenic strains of H. pylori produce a potent cytotoxin, VacA, which causes massive vacuolar degeneration in target cells in vitro. (Leunk et al., 1988). Vacuolating cytotoxin is a member of the A-B family of bacterial toxins, consisting of distinct moieties A and B involved in cytotoxic enzyme activity and membrane translocation respectively (Lupetti et al., 1996). A-B toxins consist of B domain involved in cell surface binding and in the entry in to the cell of the catalytically active A promoter (Cover et al., 1997). VacA purified from the supernatant of H. pylori cultures is seen as high molecular weight oligomeric structures with either 6 or 7- fold radial symmetry. Each oligomer is formed from monomers of approximately 90 kDa, with two distinct subunits. After release from the bacteria, the 90 kDa monomer can undergo specific proteolytic cleavage at a hydrophilic loop to generate 37 and 58 kDa fragments, which remain, associated in the oligomeric structure. Vacuolation induced by H. pylori culture filtrates in primary human mucosal epithelial cells from jejunum is also reported (Harris et al., 1996). To facilitate further study of the cell vacuolation, induced by H. pylori supernatant, a quantitative assay for cell vacuolation on the basis of the rapid uptake of neutral red in to intracellular
Vacuoles induced by VacA are acidic because their limiting membrane contains the vacuolar ATPase proton-pump (V-ATPase), whose activity is essential for vacuole formation and enlargement (Papini et al., 1993). Vacuolating activity was associated with the presence of 128 and 82 kDa bands, as revealed by the comparison of proteins present in the culture supernatants from Tox+ and Tox- strains. Vacuoles induced by the vacuolating cytotoxin originate from late stages of the endocytic route and contain protein markers of late endosomes and lysosomes (Papini et al., 1994; Molinari et al., 1997). VacA is expressed in the cytosol of HeLa cells, where it causes the formation of vacuoles indistinguishable from those induced by VacA added to the medium and endocytosed by cultured cells (de Bernard et al., 1997). Cytosolic activity of VacA resides in the aminoterminal region of the toxin and that it's entire N-terminus is required for the induction of vacuoles by acting in the cell cytosol. Large portion of the 58-kDa fragment is not necessary for the cytosolic activity of VacA. This finding is in agreement with the fact that p58 is membrane active and increases the permeability of liposomes to K⁺ (Moll et al., 1995), a property shared by the B promoters of several A-B toxins.

In the host cells RPTPβ, a Vac binding protein is postulated to be VacA receptor (Yahiro et al., 1999). This was confirmed by transfection studies of BHK21 cells, which are insensitive to VacA acquire VacA sensitivity when transfected with RPTPβ. PMA induces differentiation of human leukemic HL-60 cells in to cells with macrophage like characteristics and enhances the susceptibility of HL-60 cells to H. pylori VacA toxin (de Bernard et al., 1998). This shows that acquisition of VacA sensitivity by PMA treated HL-60 cells results from the induction of RPTPβ, which functions as a VacA receptor (Padilla et al., 2000).
VacA induces a reorganization of late endosomes (LE) and lysosomes (LY) compartments resulting in the marked decrease of the cell proteolytic activity and extensive alteration of the protein trafficking to LE which are likely to be directly relevant to infection and disease pathogenesis. It is also involved in the inhibition of antigen processing and presentation (Molinari et al., 1998), which may be a part of a strategy of survival of \textit{H. pylori} since a depression of antigen processing within the mucosa would significantly contribute to the long lasting infection that \textit{H. pylori} establishes in the stomach. which due to the. As a consequence of the VacA-induced alteration of LE/LY, compartments, the lysosomal acid hydrolases are released and thus cause the degradation of the protective mucous layer. VacA induces increase of epithelial conductivity in the absence of cell vacuolation and this is not inhibited by V-ATPase inhibitors. This shows that epithelial permeabilisation induced by VacA is distinct from vacuolation (Montecucco et al., 1999).

1.4.5 CagA

The \textit{cagA} gene is part of the cagA-pathogenicity island encoding a highly immunogenic outer membrane protein of variable molecular mass, 120-140 kDa and of unknown function. It consists of an open reading frame encoding 1,147 to 1,181 amino acids. The \textit{cagA} gene has a highly conserved 5' region, while variation in the size of the cagA protein has been correlated with the presence of a variable no. of repeat sequences encoded by the 3' region of the \textit{cagA} gene (Covacci et al., 1993; Tummuru et al., 1993). CagA was initially found in the culture supernatant of many cytotoxic strains of \textit{H. pylori} suggesting that it plays a role in pathogenesis. Earlier reports indicated that this is present only in cytotoxic-producing strains and hence the name cytotoxin-associated-antigen (Crabtree et al., 1992). Specific antibodies detected in sera of virtually
all infected individuals, suggested this protein to be very immunogenic (Gerstnecker et al., 1992). The presence of repeating sequences in antigenic molecules is a finding common to many pathogens such as *Streptococcus, Clostridium difficile, Trichomonas vaginalis, Plasmodium falciparum* and *Trypanosoma cruzi* (Michel et al., 1992; Pereira et al., 1991). Such mechanism generates antigenic diversity or immunodominant nonprotective epitopes.

The CagA antigen is mostly present in culture supernatant, as it is surface associated and is released due to action of proteases in the serum containing growth medium. The absence of a typical leader peptide sequence suggests the presence of sec-independent export system that is involved in the export of this protein. Analysis of the 3' sequences of the 155 cagA⁺ isolates identified 4 types of cagA structure, designated A, B, C and D based on their primary sequences and structural organization (Yamaoka et al., 1998a). Type C strains were associated with more severe degrees of gastric atrophy, higher levels of anti-cagA antibody and gastric cancer. This finding suggested that the cagA genotype may provide useful marker for difference in virulence among cagA⁺ isolates (Yamaoka et al., 1998b). Six of the cag genes are homologous to well-known genes present in a collinear arrangement in operons of *Bordetella pertussis, Agrobacterium tumafaciens, E. coli, Legionella pneumophila, Rickettsia prowazekii, and Brucella suis*. These operons code for type IV export machineries specialized in transfer of a variety of multimolecular complexes across the bacterial membrane to the extracellular space (Covacci and Rappouli, 1998). The translocation of cagA to gastric epithelial cells is through type IV secretion encoded by the cag PAI (Odenbreit et al., 2000). CagA was reported to be the first bacterial virulence protein translocated by a type IV secretion system. This finding suggested that cagA, so far considered as an immunodominant antigen without apparent
function, represents a module inserted by bacteria into eukaryotic signal transduction pathways, which is likely to play a role in *H. pylori*-host cell interactions and pathogenesis (Stein et al., 2000).

Western blot and *in vitro* kinase assays show a *cagA*+ induced, dose-dependent activation of extracellular signal-regulated kinases (ERK), p38 and c-jun N-terminal kinase (JNK) MAP kinases upon coculture of AGS cells with *H. pylori in vitro*. MAP kinases regulate cell proliferation, differentiation, programmed death, stress and inflammatory responses. Activation of gastric epithelial cell MAP kinases by *H. pylori cagA*+ strains may be instrumental in inducing gastroduodenal inflammation, ulceration and neoplasia (Keates et al., 1999). In *H. pylori*, no specific function has been identified for *cagA*, there is increasing evidence that *cagA*+ strains are associated with increased intensity of gastric inflammation and increased mucosal concentration of cytokines, particularly IL-8. *cagA*+ strains, unlike *cagA*− strains, provoke potentially damaging inflammatory responses in infected host tissue and induces synthesis of the proinflammatory cytokine IL-8 in gastric biopsies and cultured cells (Crabtree et al., 1994a; Sharma et al., 1995). But *cagA* itself is not needed for the induction process. Chromosome walking and mutational experiments identified several genes upstream of the *cagA* that are absent from *cagA*− strains and that are needed for IL-8 induction (Tummuru et al., 1995). One of these genes is *picB*, which is homologue of *ptlF* and *virB4* genes of *Bordetella pertussis* and *Agrobacterium tumafaciens* respectively. Further analysis indicated that *cagA-picB* gene cluster is part of the cag PAI unique to *cagA*+ strains (Censini et al., 1996). Much remains to be learned about the role of the cag-PAI in colonisation, persistence and disease, the evolutionary forces that determine the differences in prevalence of the cag-PAI among *H. pylori* isolates from different human populations.
1.4.6  Cag-pathogenicity Island

In an attempts to thoroughly understand the CagA protein and virulence, the DNA regions flanking the CagA gene was studied by Censini et al., (1996), which led to the discovery of extensive pathogenicity island (Akopyants et al., 1998). The cag-PAI has many of the features of classic pathogenicity islands namely, (i) a G+C content of 35 mol% that differs from the mean of 39 mol% for the rest of the H. pylori genome, (ii) flanking direct repeats involved in the integration of the 40 kb locus into the genome, (iii) putative virulence genes and genes that encode a type IV secretion system and (iv) insertion sequences. The nucleotide sequencing of the 40 kb cag-PAI revealed the presence of a novel insertion sequence, IS605, which consists of two genes, tnpA and tnpB, which are flanked by two nucleotide sequences of 33 bp and 41 bp, respectively. The maximal number of copies of IS605 was associated with cag-PAI+ strains, while cag-PAI -strains did not appear to possess the IS605 element (Censini et al., 1996). However, Akopyants et al. (1998) found that 9 out of 16 cag-PAI +strains lacked IS605 and Hook-Nikanne et al (1998) reported that the prevalence of IS605 was similar in 37 cag-positive (27%) and in 13 cag-negative (23%) strains. The presence of IS605 did not correlate with production of the VacA toxin, with the s-type and m-type of vacA alleles, or with clinical manifestations of H. pylori infection. From one to nine copies of IS605 have been detected per genome (Hook-Nikanne et al., 1998). Interestingly, five full-length copies of IS605 occur in the genome sequence of strain 26695, but none of them is associated with the cag-PAI (Tomb et al., 1997). Significant geographic differences among strains exist in the Cag pathogenicity island. One half and two thirds of US and European strains are Cag+ carrying the Cag pathogenicity island. All East Asian strains carry Cag- PAI independent of the disease status. In India reports show the existence of
CagPAI vacS1 alleles more abundant in Calcutta than in the west. 10-20% of Calcutta strains lacked Cag PAI and these strains were of VacS2 genotype (Mukhopadhyay et al., 2000). The structure of the cag-PAI varies among strains (Fig.3): (i) it can consist of a single uninterrupted unit; (ii) it can be split into two regions, cagI and cagII, by IS605;(iii) it can be split into cagI and cagII by a large piece of chromosomal DNA that is flanked by IS605; and (iv)-(vi) it can consist of partially deleted segments of cagI, cagII or both. This variation in the structure of the cag-PAI is most likely due to the insertion of IS605 followed by homologous recombination, which may explain the existence of cag-PAI⁺ and cag-PAI⁻ strains and their intermediates (Censini et al., 1996).

1.4.7 Outer membrane proteins

Approximately 4% of the coding capacity of both strains is devoted to outer membrane proteins. This amount is significantly larger than that of any other bacterial genome sequenced to date. The majority of these proteins belong to three paralogous families, the largest having 20 and 21 members in J99 and 26695 respectively (Alm et al., 2000). Several members of the largest paralogous family are porins (Exner et al., 1995) or adhesins specific for Lewis B carbohydrate moiety found on host cells (Ilver et al., 1998). Three adhesins described in H. pylori belong to this large OMP family (Boren et al., 1993; Odenbreit et al., 1999). They are AlpA and AlpB, which may act as adhesins and recognize different receptors on the cell surface, and the BabA adhesin, which mediates attachment to the Lewisb antigen (Ilver et al., 1998). The outer membrane profile of H. pylori on sodium dodecyl sulfate-polyacrylamide gels differs from that of other gram-negative bacteria, as the highly abundant nonselective porins (Escherichia coli OmpF and OmpC-like) are absent and a number of less abundant species of proteins are observed
(Doig and Trust, 1994). The two complete genomic sequences of *Helicobacter pylori* J99 and 26695 were used to compare the paralogous families (related genes within one genome, likely to have related function) of genes predicted to encode outer membrane proteins, which were present in each strain. The large family of Outer membrane proteins of *H. pylori* consists of four protein family; 1) *Helicobacter* outer membrane proteins (Hop), Those with Hop-like motifs are named as *hop* genes. 2) Proteins related to the Hop family, but lacking the N-terminal is named as *hor* (hop related) genes. 3) The family of 50-kDa outer membrane protein genes has been named as *hof* (*Helicobacter* outer membrane family) genes. 4) The smaller family of outer membrane genes is called *hom* (*Helicobacter* outer membrane) genes. A family of five outer membrane proteins from *H. pylori*, termed HopA to HopE, possess N-terminal sequence homology and have been shown to function as porins (Exner et al., 1995). The sequence similarity between these characterized outer membrane proteins has been used to define a much larger paralogous family with extensive C-terminal sequence homology (Tomb et al., 1997; Alm et al., 1999). Analysis of the *H. pylori* 26695 sequence identified 21 proteins with this characteristic N terminus, 20 of which had orthologous members encoded by the genome of *H. pylori* J99. Most of the Hop proteins are predicted to contain amphipathic antiparallel β sheets (Alm et al., 2000).

The regions of outer membrane proteins which are exposed on the surface of a bacterium display a much higher rate of sequence divergence than regions located within the membrane or exposed to the periplasm, and surface-exposed proteins overall vary more than non-surface-exposed proteins (Whittam, 1995). This sequence diversity may be driven by the immune system but may also reflect different functional capabilities. When the sequence diversity of orthologous pairs of the outer membrane proteins of strains J99 and
26695 was examined, which showed that of the 20 orthologous pairs of Hop proteins, 7 share 95% identity, with 6 having 90 to 95% and 7 having between 80 and 90% identity. There was a similar distribution of nucleotide and protein similarity between the Hop orthologs which was not reflected when all of the orthologs between J99 and 26695 are compared, as the higher drift in the third (wobble) position of the coding triplet results in a higher amino acid identity than nucleotide identity (Alm et al., 1999; Doig et al., 1999). The hor gene family, which is made of 11 members and previously grouped with hop family, are even more highly conserved. Most orthologous protein pairs (equivalent genes between two genomes, same function) shared considerable identity between the two strains. The unusual set of outer membrane proteins and the specialized outer membrane may be a reflection of the adaptation of *H. pylori* to the unique gastric environment where it is found. One subfamily of proteins, which contains both channel forming and adhesin molecules, is extremely highly related at the sequence level and has likely arisen due to ancestral gene duplication. In addition, the largest paralogous family contained two essentially identical pairs of genes in both strains. The presence and genomic organization of these two pairs of duplicated genes were also analyzed in a panel of independent *H. pylori* isolates. The results showed that while one pair was present in every strain examined, one allele of the other pair appeared partially deleted in several isolates (Alm et al., 2000).

The organizational differences and shuffling of the outer membrane protein genes observed between *H. pylori* J99 and 26695 have also been detected in other *H. pylori* isolates, which suggests that a subtle mechanism of regulation may be occurring. The reason(s) for such possible regulatory mechanisms in *H. pylori* is not known. However, this ability to possibly perform phase variation may play a role in evading the host’s immune system.
and could be especially important in light of the limited sequence variation between orthologs.

1.4.8 *iceA* gene

Recently a new candidate gene designated *iceA* (for induced by contact with epithelium) was suggested to be associated with peptic ulcer. This gene was identified in *H. pylori* following transcriptional up regulation on contact with gastric epithelial cells. The *iceA* gene has 2 main allelic variants, *iceA1* and *iceA2*. Studies show only *iceA1* RNA is induced following adherence *in vitro* (Peek et al., 1998). The deduced *iceA1* products demonstrates a strong homology to a restriction endonuclease NlaIII in *Neisseria lactamica*; however mutations and deletions found in majority of *iceA1* sequences preclude translation of a full-length homolog. The *iceA2* has no homology to known proteins and its structure reveals patterns of repeated peptide cassettes. *iceA2* can encode a protein of 59 aminoacids (aa) with two conserved outer domains of 14 and 10aa, respectively, that flank three internal peptide domains of 13, 16 and 6 aa, respectively (Figueredo et al., 2000). *H. pylori iceA1* strains have been associated with duodenal ulcer disease and enhanced neutrophilic infiltration of the gastric mucosa, yet considerable heterogeneity exists in the levels of inflammation among persons harbouring iceA1 strains (Nishiya et al., 2000; Van doorn et al., 1998). Geographic differences were also observed in the *iceA* genotypes. The *iceA1* allele was predominant in Japan and Korea, and the *iceA2* allele was predominant in the United States and Colombia. But neither *iceA* nor the combinations of *iceA*, *vacA*, and *cagA* were seemed to be helpful in predicting the clinical presentation of an *H. pylori* infection. RT-PCR results show that *iceA1* expression is significantly related to the host mucosal response while *iceA2* expression may be more highly influenced by gene structure (Peek et al., 2000).
There was also reports showing the association of iceAl significantly related to the severity of the acute mucosal inflammation and also iceAl was predominant in Europe and East Asia (Ito et al. 2000). The report of the absence of iceA gene in H. pylori J99 suggests that it may not represent an informative epidemiological marker of pathogenicity and virulence (Doig et al., 1999; van Doorn et al., 1998). iceA sequence variations may be and useful tool for analysis of the population genetics of H. pylori. Data concerning the geographic distribution of iceA alleles are scarce and information on the association of the gene with a disease is rare and still controversial.

1.4.9 Catalase and superoxide dismutase

There appear to be three principal mechanisms, which enable H. pylori to resist oxidative damage, and they are catalysed by the enzymes superoxide dismutases, catalase, and alkylhydroperoxide reductase (Ahp) (Hazell et al., 1991; Pesci et al., 1994). There is significant homology in the genes encoding the superoxide dismutase (Spiegelhalder et al., 1993) and catalase (Odenbriet et al., 1996) enzymes of H. pylori to those of intracellular pathogenic microorganisms suggesting a role in the resistance to killing by polymorphonuclear leukocytes. These enzymes are surface associated thereby essential for protection against oxygen-dependent killing of H. pylori by neutrophils (Phadnis et al., 1996). Acute inflammatory response to H. pylori infection consists chiefly of polymorphonuclear leukocytes, which through their oxidative burst are cytotoxic for bacteria. To combat this H. pylori and many other bacteria produce enzymes such as catalase and superoxide dismutase to catalyze the elimination of toxic oxygen species (Beaman and Beaman, 1990). The importance of catalase to the survival of H. pylori is clearly demonstrated by catalase negative mutants of H. pylori (Hazell et al., 1991). Superoxide
dismutase is similar to facultative intracellular pathogenic microorganisms and that is either surface-associated or actively secreted (Speigelhalder et al., 1993), in protection against oxidative damage from processes external to the cell. This proves to be an essential function in the pathogenesis of *H. pylori*, allowing the organism to resist attack from the host’s armory of inflammatory cells. Characterisation of *H. pylori* catalase has indicated that this enzyme is a highly expressed enzyme with four subunits and approximately 50 kDa located throughout the cytosol and in periplasmic space (Hazell et al., 1991). It is also proposed as a vaccine antigen because they are highly conserved and fundamental to the survival of the organism (Radcliff et al., 1997).

1.4.10 Heat shock protein analogs

A protein associated with the urease holoenzyme was identified to be a homolog of the class of heat shock proteins (Hsps), to which *E. coli* GroEL belongs (Austin et al., 1992). The highly conserved *HspB* gene is a part of a bicistronic operon (*hspA-hspB*) and has been cloned and sequenced. The *hspA* gene located upstream of the *HspB* gene, codes for the *H. pylori* homolog of the GroES heat shock protein homolog. Expression of the *hspA* and *HspB* heat shock proteins together with *H. pylori* urease increases the activity of urease in functional complementation experiments. Analysis of the DNA region upstream of *HspB* revealed a second open reading frame designated *hspA* that encoded a protein with a deduced molecular mass of 13kDa. (Suerbam et al., 1994).

A remarkable feature of *H. pylori* *hspA* is the presence of series of histidine and cysteine residues at the C-terminal domain of the polypeptide and contains a nickel-binding site. Vaccination with GroEL proteins induces protective immunity against intracellular pathogens such as *Mycobacterium tuberculosis*.
Certain bacterial GroEL proteins are able to induce protective immune responses. In case of *H. pylori* it has been speculated that its GroEL homolog HspB is an important proinflammatory factor and is believed to mediate the chronic inflammation seen in *H. pylori* infected individuals. (Sharma et al., 1994). Induction of protective immunity in mice after immunization with *H. pylori* HspA is an example where GroES class proteins act as an antigen (Ferrero et al., 1995). In addition purified Hsp60 induced IL-8 secretion from primary human gastric epithelial cells (Yamaguchi et al., 1999).

### 1.5 HOST-PATHOGEN INTERACTION AND MECHANISMS OF TISSUE INJURY

*H. pylori* is able to colonize and persist in a unique biological niche within the gastric lumen. The putative pathogenic determinants of *H. pylori* can be divided into two major groups: 1) Virulence factors, which contribute to the pathogenic effects of the bacterium, and 2) Maintenance factors, which allow the bacterium to colonize and remain within the host. Virulence factors contribute to the three major pathogenic effects of *H. pylori*, Gastric inflammation, disruption of the gastric mucosal barrier, and alteration of gastric physiology (Fig.1.3). Many *H. pylori* factors function as both virulence and maintenance factors *in vivo*.

#### 1.5.1 Induction of gastric inflammation: Cytokine expression of *H. pylori*

Inflammation may be important for the survival of *H. pylori* *in vivo* caused by infiltration of the gastric mucosa by polymorphonuclear leukocytes and/or mononuclear cells resulting in superficial gastritis. Cytokines play an important role in protection against bacterial and viral infections and also in the
Fig. 1.3 Model of *H. pylori* regulation of host physiology to maintain homeostasis

*H. pylori* regulate gene expression in response to particular environmental signals. Bacterial secretion of proinflammatory molecules results in tissue injury. Secretion of Nε-methyl histamine stimulates the host to increase acid production, when pH is high for optimum microbial function. In this model, the equilibrium established by the colonizing population in each host is dynamic and unique. A consequence of this interaction is that the nature of the equilibrium determines characteristics such as epithelial cell proliferation and apoptosis, gastric secretion and antigenic stimulation. These factors in turn would determine the likelihood under differing environmental conditions (adapted from Blaser et al., 1997a).
pathogenesis of infectious diseases. The defense mechanism and pathogenesis vary depending on such factors as the kind of pathogens, the stages of infections (acute or chronic), and the site of infections (systemic or topical). Chemokines are possible candidates to act as second signals following the contact of bacteria with the epithelium, thereby contributing to the pathological changes leading to inflammation. In the gastrointestinal tract, not only inflammatory cells, but also epithelial and mesenchymal cells secrete cytokines in response to various stimuli. Inflammatory cytokines play critical roles in inflammation and with gastrointestinal infections.

Cytokines are soluble glycoproteins produced by a wide variety of cells and are involved in host defense and inflammatory response. *H. pylori* resides in the mucous layer overlaying the epithelium and does not invade epithelial cells. Epithelial cells colonized by *H. pylori* produce immune response mediators, eg. proinflammatory cytokines/chemokines, that leads to rapid mobilization of phagocytic cells to the sites colonized by the bacteria. Because gastric epithelial cells are the first sites of contact with the bacteria, activation of cytokine genes would act as an early warning system in the host organism. Cytokines in the gastric milieu provide bi-directional communication between inflammatory cells such as mucosal macrophages and epithelial cells and thus may regulate the degree of inflammation and the extent of epithelial cell degeneration in *H. pylori* infection. Parasitism by *H. pylori* may lead to altered homeostasis in the gastric mucosa resulting in an imbalance between proinflammatory and anti-inflammatory cytokines leading to inflammation and disease (Blaser and Parsonnet 1994).

Proinflammatory cytokines also have activities that are damaging to the integrity of the epithelium, and this can result from an accentuation of
that an extract from *H. pylori* itself exhibited *in vitro* chemotactic activity for monocytes and neutrophils. In contrast it is also known that the *H. pylori* components such as porin and lipopolysaccharide stimulate the production of cytokines and chemokines by polymorphonuclear leukocytes (Tufano et al., 1994). Several studies show the production of IL-8 *in vitro* upon coculture with *H. pylori* in human gastric cancer cell lines (Sharma et al., 1995; Huang et al., 1995). Increased IL-8 contents in gastric epithelial cells *in vivo* (Crabtree et al., 1994b) and in tissue homogenates of mucosal biopsy specimens has also been reported (Moss et al., 1994). *cag A*+ strains can enhance IL-8 production by gastric epithelial cells compared with *cagA*− strains (Crabtree et al., 1994b). *picA* and *picB* gene products, a part of *cagA* pathogenicity island were strongly associated with the induction of IL-8 in gastric epithelial cells. (Tummuru et al., 1995). Disruption of the *cagE, cagF, cagG, cagH, cagI, cagL* and *cagM* but not *cagA* reduced the ability to induce IL-8 secretion from gastric epithelial cells. Several human gastric cancer-derived cell lines were examined for the production of IL-8 *in vitro* upon coculture with *H. pylori*. Contradictory results were shown for MKN 45 cell lines in the expression of IL-8 at marginal level and constitutive. MKN45 cells produced IL-8 only after direct contact with an *H. pylori*-derived cell component(s), which is lost by heat or glutaraldehyde treatment (Aihara et al., 1997). The significance of direct contact to epithelial cells as a possible prerequisite to cytokine stimulation was demonstrated by Reider et al. (1997). An increase in IL-8 concentration was observed when the bacterial-epithelial cell ratio was 10/100:1. Cytokine stimulation aided with direct contact or adhesion has been observed in Uropathogenic *E. coli* for the stimulation of IL-6 *in vitro* and *in vivo*.
1.5.1.2 Interleukin-6

IL-6 is a multifunctional cytokine with immunoregulatory and proinflammatory effects. IL-6 may be involved in chronic inflammatory changes through its broad effects on growth and differentiation of mononuclear cells, including T and B-lymphocytes and macrophages (Akira et al., 1990). These biological properties may be relevant to the pathogenesis of gastroduodenal inflammation. It was previously shown that there were some factors other than the cag pathogenicity island, which induced IL-6 production in *H. pylori* infection (Yamaoka et al., 1996). In situ hybridisation studies and the immunogold silver staining of the biopsy specimens suggested that IL-6 is over expressed at the margin of gastric ulcer in *H. pylori*-positive gastritis (Furukawa et al., 1998). Recently it was showed that IL-6 production was induced in response to urease in human gastric epithelial cells (Tanahashi et al., 2000). Immunohistochemistry also revealed positive staining for IL-6 in the epithelial cells localized to the superficial and the neck regions of the stomach glands in *H. pylori*-infected patients (Lindholm et al., 1998). These studies indicated that *H. pylori* urease contributes to IL-6 production, at least in part, in human gastric epithelial cells, whereas ELISA did not detect IL-6 production in low-dose urease stimulation that had been judged positive by RT-PCR (Tanahashi et al., 2000).

1.5.1.3 Role of IFNγ in *H. pylori* infection

IFNγ is thought to be important in immune responses, because it induces the expression of classII major histocompatibility complex of antigen presenting cells and activates macrophages and Natural Killer cells. Dalton et al. (1993) showed that IFNγ enhanced the expression of class II MHC during
Mycobacterium bovis infection. Thus IFNγ appear to be involved during bacterial infection and in induction of inflammation. The finding that the expression of IFNγ mRNA always took place in gastric mucosa indicates its role in the induction of immune responses in gastroduodenal mucosa (Yamaoka et al., 1995). Reduction in the gastric inflammation score accompanied by significant reduction in IFNγ in vitro in mice infected with H. felis also substantiates the role of IFNγ in H. pylori infection. (Mohammadi et al., 1996). The role of IFNγ in protection from H. pylori infection and in the development of gastric inflammation is implicated in H. pylori infected IFNγ−/− mouse model (Sawai et al., 1999).

1.5.2 Neutrophil adherence

Evans et al. have characterized a 150-kDa protein which increases the expression of neutrophil CD 11B/CD18 and increases neutrophil adherence to endothelial cells. The protein, designated HP-NAP, is a polymer of 10 identical subunits. The gene (napA) shows homology to the gene encoding the bacterioferritin family of proteins (Evans et al., 1995).

1.5.3 Lipopolysaccharide

The mammalian host has learnt to recognize the presence of unwanted gram-negative bacteria within tissues by responding to lipopolysaccharide (LPS) that comprise the outer surface molecules of the bacteria. However, LPS from H. pylori has very low biological activity or immunostimulatory activity compared to pathogens such as E. coli and Salmonella spp. (Mai et al., 1991) The hypothesis is that this was a consequence of evolution to long-term survival on the gastric surface. This
essential feature of low proinflammatory activity is a phenomenon mediated by its unique lipid A structure. The O polysaccharide region of *H. pylori* LPS is a major antigenic determinant (Mills et al., 1992). The O polysaccharide contains epitopes that mimic the structures of the Lewis antigens, as shown by chemical and immunological studies (Aspinall and Monteiro, 1996). *H. pylori* LPS disrupts the gastric mucous coat by interfering with the interaction between mucin and its mucosal receptor. LPS mediates binding of the bacteria to laminin and interferes with gastric cell receptor-laminin interaction thereby potentially contributing to the loss of mucosal integrity. *In vitro* observations of inhibition of sulphated mucin synthesis and stimulation of pepsinogen secretion by LPS suggests new mechanisms for *H. pylori* induced mucosal damage.

1.5.4 **Lewis antigens**

Microbial expression of host antigens is another mechanism to escape elimination by the host immune response. For example, *Neisseria gonorrhoeae* LPS resembling host glycolipids (Mandrell et al., 1988), *Campylobacter jejuni* similarity with glycosphingolipids (Aspinall et al., 1993). Several gram-negative bacteria can express human ABO-blood group antigens (Springer et al., 1961) and *streptococcus bovis* expresses sialyl-lewis\(^x\). Aspinall et al. (1996) first reported the presence of Lewis antigens on the O-antigen regions of the LPS. The lewis antigens are polymorphic fucosylated glycoconjugates, best recognised on erythrocyte surfaces but also present in a wide variety of epithelial cells, including gastric mucosa. Aspinall et al. (1996) found that LPS of several *H. pylori* strains contained Le\(^x\) and/or Le\(^y\) as part of their polysaccharide chains. *H. pylori* strains have been shown to express Le\(^a\), Le\(^b\), Le\(^x\), Le\(^y\) or sialo-Le\(^x\) antigens. Virtually all strains express these antigens, and they often express more than one (Wirth et al., 1996). Expression of Le\(^x\) and Le\(^y\) was designated as type 2
determinants, and Le\(^a\) and Le\(^b\) as type1. cagA\(^+\) strains show higher level Le expression than do strains lacking cagA. Immuno electron microscopy and enzyme-linked immunosorbent assay confirmed the presence of Le\(^a\) in \textit{H. pylori} strains. (Sherburne and Taylor, 1995). Appelmelk \textit{et al.} (1996) demonstrated the possible pathogenic role of Anti-Le antibodies in \textit{H. pylori} induced gastritis. Thus the molecular mimicry that exists between \textit{H. pylori} and host glycoconjugates may give rise to autoimmune phenomena. Le\(^a\), Le\(^y\) which are common epitopes no \textit{H. pylori} LPS, induce autoantibodies in animals and humans. This may induce antibodies and T-cells to bacterial cell components that can recognize itself and immune mediated damage follow.

1.5.5 \textit{babA}

The BabA adhesin of \textit{H. pylori}, which binds to the fucosylated Lewis b (Le\(^b\)) histo-blood group antigen, is a putative virulence factor and is strongly associated with the presence of the \textit{cag}-pathogenicity island. The gene encoding BabA was recently cloned by Ilver \textit{et al.} (1998). Three \textit{bab} alleles have been identified, but it is not yet known whether \textit{bab} alleles are present in all \textit{H. pylori} strains. Two of these alleles, namely \textit{babA2} and \textit{babB}, encode functional proteins. However, the third allele \textit{babA1} is identical to the \textit{babA2} allele with the exception of a 10 bp deletion of a repeat motif in the signal peptide sequence, which results in ablation of the translational initiation codon. This suggests that hot spots for phenotypic (phase) variation exist within the \textit{bab} gene family. The \textit{bab} genes belong to a family of over 30 \textit{H. pylori} genes which have been proposed to encode outer-membrane proteins showing extensive amino acid sequence identity in the N-terminal and C-terminal domains, suggesting the potential for recombinational events leading to mosaicism (Tomb \textit{et al.}, 1997; Ilver \textit{et al.}, 1998).
1.6 DISRUPTION OF THE GASTRIC MUCOSAL BARRIER

H. pylori can inhibit the secretory response of mucous cells in vitro, indicating a potential deleterious effect on this primary defense mechanisms of the gastric mucosa (Micots et al., 1993). The protective phospholipid-rich layer at the apical membrane of the mucous cells is disrupted by phospholipases A2 and C expressed by H. pylori (Weitkamp et al., 1993). It is proposed that Mucinase activity of H. pylori also would probably contribute to the disruption of mucosal barrier. H. pylori possess a gene not yet characterized, which has identity to a mucinase gene of Vibrio cholerae (Smith et al., 1994).

H. pylori also induces the synthesis of reactive oxygen species (ROS) in gastric mucosa in vivo. The extent of gastric mucosal injury is directly associated with the amount of ROS and the infective load of H. pylori. There is no evidence for ROS participation in gastric mucosal injury in cases not related to H. pylori infection (Davies et al., 1994). Levels of 9 dehydroxydeoxyguanosine, a marker for oxygen free radical-induced DNA damage, are increased in H. pylori infected individuals compared to controls (Baik et al., 1996). Many anti-ulcer drugs function as scavengers of ROS helping to explain how the gastric mucosal injury induced by H. pylori. The gastric mucosal production of ROS both in vitro (Bagchi et al., 1996) and in vivo stimulated by H. pylori is very likely to be of pathogenic significance (Davies et al., 1994). High-output nitric oxide production by inducible nitric oxide synthase (iNOS) is associated with immune activation and tissue injury. Eradication of H. pylori reduces iNOS in human gastric epithelial cells, suggesting its role in inducing activity of this enzyme (Mannick et al., 1996).
1.6.1 Apoptosis

Apoptosis is a genetically programmed form of cell death characterized by distinct morphologic and molecular features (Raff, 1998). Gastric epithelial apoptosis is a programmed physiological event in the superficial aspect of the mucosa and is important for the healthy cell turnover. Programmed cell death plays an important role in the regulation of epithelial cell numbers in the gastrointestinal tract (Jones and Gores, 1997). In addition, deregulation of the apoptotic pathway is implicated in a number of disease processes in the intestine including carcinogenesis (Merritt et al., 1995). Microbes have developed mechanisms to stimulate the apoptotic signal transduction cascade, which likely plays a role in pathogenesis (Zychlinsky and Sansonetti, 1997). Microbial pathogenesis, or their products, can directly activate the cell death-signaling cascade (Kim et al., 1998).

The intestinal mucosa is an important route of entry for microbial pathogens. Following bacterial entry, intestinal epithelial cells rapidly (within 60-90 minutes) upregulate expression of an inflammatory gene program (Eckmann et al., 1993; Jung et al., 1995). First few hours of bacterial invasion, human colon epithelial cells produce mediators that have the potential to orchestrate the onset of the mucosal inflammatory response (Kagnoff and Eckmann, 1997). Some of these mediators also have the potential to induce apoptosis of human colon epithelial cells (Abreu-Martin et al., 1995). Epithelial cells that line the human intestinal mucosa are the initial sites of host invasion by bacterial pathogens. Human colon epithelial cells are shown to undergo apoptosis following infection with invasive enteric pathogens, such as Salmonella or Shigella (Eckmann et al., 1993). Ensuing phenotypic expression of apoptosis is delayed for 12-18 hour after bacterial
entry, TNF-α and NO are produced as components of the intestinal epithelial cell proinflammatory program in the early period after bacterial invasion, play an important role in the later induction and regulation of epithelial cell apoptotic program (Witthoft et al., 1997).

Apoptosis in response to bacterial infection may function to delete infected and damaged epithelial cells and restore epithelial cell growth regulation and epithelial integrity that are altered during the course of entire infection. Following bacterial infection, TNF-α production is upregulated (Eckmann et al., 1993) and blocking TNF-α activity blocked apoptosis. Induction of proinflammatory cytokine TNF-α mediates apoptosis during Salmonella infection in vitro. Cytotoxic lymphocytes can induce apoptosis of hepatitisC virus-infected cells (Ando et al., 1997). H. pylori infection reportedly promotes such a cell death response. Alterations in the gastric epithelial cell cycle, including both enhanced proliferation and increased apoptosis of gastric cells are identified during H. pylori infection (Moss et al., 1996). Because apoptosis regulates the cycle of cell turnover in balance with proliferation, dysregulation of apoptosis or proliferation evoked by H. pylori colonisation would be linked to gastric carcinogenesis (Shirin et al., 1999). Investigations of the molecular determinants mediating apoptosis have identified both enhanced expression of the tumour suppressor p53 (Jones et al., 1997). Increased expression of proapoptotic protein Bak in response to H. pylori infection has also been reported (Chen et al., 1997). However it is not clear whether immune factors or bacterial factors contribute to cell death. One study emphasizes the direct role of H. pylori in the induction of gastric epithelial cells in vitro. Also the mechanisms of cell death differed between cell lines, the KatoIII cells undergoing necrosis and AGS cells undergoing apoptosis mimicking the in vivo setting. H. pylori infection results in Fas-
triggered apoptosis, but the factors mediating enhanced Fas receptor expression are not known (Jones et al., 1999). To support this result a recent study showed an increase in Fas ligand mRNA expression lymphocytes within the lamina propria of H. pylori-infected gastric biopsy tissue (Rudi et al., 1998). Incubation of gastric epithelial cells with H. pylori led to a time- and concentration-dependent reduction of epithelial cell growth and a concomitant induction of DNA fragmentation. Treatment of gastric cells with tumor necrosis factor alpha, a receptor-activating CD95/APO-1/Fas antibody, and interferon gamma markedly potentiated H. pylori-induced DNA fragmentation.

Thus H. pylori affects gastric epithelial cell growth by direct induction of apoptosis and inhibition of DNA synthesis and indirectly by sensitization of epithelial cells for apoptosis induced by proinflammatory stimuli (Wagner et al., 1997).

1.7 ALTERED GASTRIC HOMEOSTASIS

There is changes in the expression of the acid-stimulating peptide gastrin and acid-inhibitory hormone somatostatin caused by H. pylori, the effects related to the degree of gastric inflammation (Calam et al., 1995). Acid secretory studies show that H. pylori increase and also decrease the duodenal acid load under different (Sumii et al., 1994) circumstances. These effects may not be due to H. pylori per se but may be related to the degree of inflammation present.
1.7.1 Gastrin and Acid secretory pathophysiology

Physiologists have speculated for many years that disturbances in gastric acid secretion are the key factors in ulcerogenesis. Peptic ulcer patients were typically characterized with higher concentrations of serum gastrin, more acid production in the duodenal ulcer and less acid production than normal in the case of gastric ulcer (Isenberg and Thompson, 1997). No acid-No ulcer was the basis of the treatment of peptic ulcer disease. However there are mounting evidence that gastrin and acid secretory function are altered through a variety of mechanisms during *H. pylori* infection. Reports showed a higher basal serum gastrin levels in *H. pylori* infected duodenal ulcers compared to uninfected controls (Peterson et al., 1993). In addition meal-and gastrin-releasing-peptide-stimulated gastrin levels are elevated in *H. pylori* infection (El-Omar et al., 1995a). Such alterations in gastric levels were also observed in children with *H. pylori* infected gastric cell metaplasia (Oderda et al., 1993).

Hypergastrinemia in *H. pylori*-infected individuals is a result of larger amounts of G-17, the biologically active hormone species. The major source of G-17 is the G cells of the gastric antrum. Somatostatin, a hormone produced by the antral D cell, is the major inhibitory influence on production of gastrin by the antral G cells (Mulholland et al., 1993). Antral somastatin levels, as well as D cell numbers and somastatin numbers are reduced in infected cases (Sumii et al., 1994). This findings revealed that hypergastrinemia is involved in the disruption of the inhibitory effect of somastatin on the G cell. The local inflammatory response and the resultant release of cytokines that accompanies bacterial infection are thought to be responsible for these changes (Graham et al., 1993). Elimination of infection and associated inflammation results in normalization of basal and stimulated gastrin levels (Witteman et al., 1994). The complexity of *H. pylori* infection on acid secretion has been shown by the
bacterial products interfering directly with acid secretion (Jablonowski et al., 1994). The diffuse gastritis in some patients over time lead to atrophic gastritis with loss of parietal cells resulting in reduced acid output. Following bacterial eradication in gastric ulcer patients, the disturbances in the acid secretion become normalized except for maximal acid output, which remains high (El-Omar et al., 1995b).

1.8 NF-κB ACTIVATION DURING H. PYLORI INFECTION

NF-κB is a human transcription factor targeting the genes encoding IL-8, IL-1β, IL-6 and TNF-α (Baeuerle and Henkel. 1994). Activation of the NF-κB/Rel transcription family, by nuclear translocation of cytoplasmic complexes, plays a central role in inflammation through its ability to induce transcription of proinflammatory genes (Baldwin Jr. 1996). This pathway is activated upon appropriate cellular stimulation, most often by signals related to pathogens or stress. The NF-κB/Rel family includes NF-κB1 (p50 / p105), NF-κB2 (p52/p100), p65 (RelA), RelB, and c-Rel (Chen et al., 1999). NF-κB is a heterodimer consisting of a p50 or p52 sub-unit and p65, which contain transactivation domains necessary for gene induction. NF-κB exists in the cytoplasm in an inactive form associated with regulatory proteins called inhibitors of κB (IκB), of which the most important may be IκBα, IκBβ, and IκBε. Activation occurs via phosphorylation, ubiquitinilation, and proteolytic degradation of IκB, the inhibitory subunit (Brown et al., 1995). Phosphorylation of IκB, an important step in NF-κB activation, is mediated by IκB kinases (IKK). Phosphorylated IκBα is then ubiquitinilated, which targets it for degradation by the 26S proteasome, thereby releasing NF-κB dimers from the cytoplasmic NF-κB–IκB complex and allowing them to translocate to the nucleus. NF-κB then binds to κB enhancer elements of target genes, inducing
transcription of proinflammatory genes. (Tak and Firestein. 2001). Thus Various NF-κB proteins play a pivotal role in the defense of host against certain pathogens.

Earlier it was reported by Munzenmaier et al., (1997) that exposure of gastric epithelial cells to *H. pylori* potentially activates NF-κB. Unlike other bacteria, where NF-κB induction is through lipopolysaccharide, in *H. pylori* infection the genes in the cag pathogenicity island is involved. In a study by Glocker et al. (1998) it was shown that cagE, cagG, cagH, cagJ, cagL, and cagM are absolutely necessary, since isogenic mutants of these loci no longer induce NF-κB activity. The induction of IL-8 in AGS gastric cells by *H. pylori* is upregulated via NF-κB-dependent transcriptional process and *pic* determinants expressed by cag+ strains play a critical role in NF-κB–mediated up-regulation of IL-8 expression (Sharma et al., 1998). *H. pylori* directly induces the expression of ICAM-1 on gastric epithelial cells in an NF-κB-dependent manner (Mori et al., 2000). Helicobacter pylori–associated gastritis is also marked by increased NF-κB activity in gastric epithelial cells (van den Brink et al., 2000). The number of NF-κB positive cells correlates with the degree of gastritis. The understanding of the host cell response mechanism may reveal potential targets for drug intervention in the case of pathological activation of this transcription factor in inflammatory diseases.

1.9  **HELCIOBACTER PYLORI-A CONUNDRUM OF GENETIC DIVERSITY**

1.9.1  *H. pylori* genome and plasmids

A complete genome sequence of *H. pylori* strain 26695 was published by Tomb et al. (1997), which was originally isolated from a patient
with gastritis. This strain colonizes piglets, elicits an immune and inflammatory response and is also known to be toxigenic, plasmid-free and naturally competent. It contains a single circular chromosome of approximately 1.73 Mb with 1590 predicted ORFs, one third of which are of unknown function (Berg et al., 1997). The G+C composition averages 35.2 mol %. The \textit{H. pylori} genome possesses at least two copies each of the 16S and 23S rRNA genes (Taylor et al., 1992). The variable location of multiple genes in genomic maps suggests extensive rearrangement of the genome occurs (Jiang et al., 1996). The \textit{H. pylori} genome possess at least two copies each of the 16S and 23S rRNA genes (Taylor et al., 1992). The variable location of multiple genes in genomic maps suggests that extensive rearrangement of the \textit{H. pylori} genome occurs (Jiang et al., 1996). Allelic variation in six genes by multilocus enzyme electrophoresis has confirmed the genetic diversity of \textit{H. pylori} strains (Go et al., 1996). The biological significance of such diversity is not known. Approximately 40% of \textit{H. pylori} isolates contain plasmids ranging in size from 1.5 to 23.3 kb, but the plasmids do not contain recognised virulence factors (Kleanthous et al., 1991).

In the past 15 years the recognition of \textit{Helicobacter pylori} infection as a cause of gastroduodenal morbidity has probably had more impact on health care worldwide than any other emerging infection barring HIV. \textit{H. pylori} is one of the commonest human gastrointestinal pathogens, infecting almost 50% of the human population (Taylor and Blaser, 1991). One of the most intriguing aspects is its genetic diversity, which is at a level as yet unseen in other bacterial species, the biological significance of which is enigmatic (Logan and Berg, 1996; Covacci and Rappouli, 1998).
The diverse pathologies associated with *H. pylori* can be attributed to the host genetic factors together with the environmental factors depending on the specific properties of the pathogen (Mobley, 1997). Such genomic variation can be analyzed at two different levels, between strains originating from different individuals and variations in population within an individual host. Numerous studies based on plasmid profiles, restriction fragment length polymorphism (RFLP) analysis of chromosomal DNA (Hirschl et al., 1994; Taylor et al., 1995) or specific loci (Atherton et al., 1995; Kansau et al., 1996 and repetitive extragenic palindromic PCR (Go et al., 1995) have shown that there are substantial levels of variation among natural isolates of *H. pylori*. The comparison of genome sequences from strains J99 and 26695 (Alm et al., 1999) conclude that the overall organization, gene order, and predicted proteomes of both strains are quite similar and this diversity is an overestimation of the extent of genetic variation in *H. pylori*, due to the results through usage of lower resolution techniques such as PFGEs and RFLP. Different mechanisms, such as a point mutations or mosaicism within the conserved gene, non-conservation of some genes, chromosomal rearrangements, variation in a number of intragenic cassettes and extragenic elements could contribute to genetic diversity. Heterogeneity in the gene observed through RFLP analysis indicates the existence of point mutation (i.e., only a single base pair mutation being silent, due to the changes in the neutral point of codon, third position). These point mutations were shown as silent nucleotide variations within genes, therefore no polymorphism was detected at the genomic level. In contrast, cagA gene which is not conserved in all strains, the ratio between the mutations accumulated in the first and second positions and those in the third position of the codon is approximately 1.1:0.9 which suggests that the selective pressure on *H. pylori* genome is not uniform.
1.9.2 Genotypic and Phenotypic Variation in the vacA gene

The mechanisms involved in phenotypic and antigenic variation of *H. pylori* can be divided into three types: gene mosaic organization, intragenic recombination, and on-off switching owing to DNA slippage or repeat motif insertion within a coding sequence (Marais et al., 1999).

The vacuolating cytotoxin is produced by approximately 50% of *H. pylori* strains (Cover and Blaser, 1992) and this lack of expression of the vacuolating cytotoxin was thought to be due to the sequence divergence of *vacA* genes of the vac' strains (Cover et al., 1994). But Forsyth et al. (1998) showed that there exists a heterogeneity in the levels of *vacA* transcription which may contribute to the phenotypic differences of the vacuolating activity, apart from this inter-strain heterogeneity, VacA also exhibit a type of genotyping variation known as mosaicism with conserved regions and highly divergent regions occurring within these alleles (Atherton et al., 1995). The gene segment encoding the C-terminus of the protoxin, and the segment encoding the region near the amino terminus appear to be conserved in all isolates. However there is sufficient diversity in the mid-region of the gene to define at least four allelic types, designated m1, m2, m1-like (m1*), and m1*-m2, and at least three different families of *vacA* signal sequences, designated s1a, s1b and s2 can be defined, indicating the sequence diversity between strains of the *H. pylori* isolates (Fig. 1.4). The existence of different s genotypes and m genotypes of *vacA* yields the possibility of multiple combinations (Atherton et al., 1995; Pan et al., 1998). The variance in the s and m genotypes appears to be correlated with different levels of production of VacA cytotoxin and hence may be a marker for differences in the virulence potential of strains (Atherton et al., 1997). Additional variants have been identified in Germany, designated m1a.
(Strobel et al., 1998), in Taiwan, designated m1T and m1Tm2 (a chimaeric type) (Wang et al., 1998), and in China, designated m1b and m1b-m2 (a hybrid type) (Pan et al., 1998), suggesting that vacA typing schemes need to be revised. There is much evidence for a strong correlation between the vacA genotype of a strain and disease outcome. Strains of the signal sequence /mid-region combination type s1/m1 were identified by Atherton et al. (1995) as producing high levels of the vacuolating cytotoxin in vitro and were associated with increased gastric epithelial damage, enhanced gastric inflammation and duodenal ulceration (Atherton et al., 1997). Furthermore, strains of signal sequence type s1 are associated with the presence of the cagA gene and numerous studies have highlighted the prevalence of type s1 strains in individuals with peptic ulcer disease (Rudi et al., 1998; Strobel et al., 1998; van Doorn et al., 1998a). Interestingly, strains of signal sequence type s1a and mid-region type m1 were isolated with remarkable prevalence (almost 100%) in Japan (Ito et al., 1997), with no correlation with clinical consequences. The same high prevalence of type s1a strains was also found in Taiwan and China, but in contrast to Japan, the dominant mid-region type was m2 (Pan et al., 1998; Wang et al., 1998). However, strains with the type m1b and m1b-m2 alleles produced significantly higher levels of the VacA cytotoxin than did those with type m2 alleles (Pan et al., 1998) and strains with the type m1T allele occurred statistically more often in peptic ulcer disease patients than did strains with type m2 alleles (Wang et al., 1998). In India, the strains carrying toxigenic vacAs1 allele with cag PAI was found to be abundant (Mukhopadhyay et al., 2000).

Such an enormous genotyping variation in vacA raises questions about the mechanism of acquisition. The genome sequencing of 26695 revealed three vacA-related genes (Tomb et al., 1997), but the encoded putative proteins
(Strobel et al., 1998), in Taiwan, designated m1T and m1Tm2 (a chimaeric type) (Wang et al., 1998), and in China, designated m1b and m1b-m2 (a hybrid type) (Pan et al., 1998), suggesting that vacA typing schemes need to be revised. There is much evidence for a strong correlation between the vacA genotype of a strain and disease outcome. Strains of the signal sequence/mid-region combination type s1/m1 were identified by Atherton et al. (1995) as producing high levels of the vacuolating cytotoxin in vitro and were associated with increased gastric epithelial damage, enhanced gastric inflammation and duodenal ulceration (Atherton et al., 1997). Furthermore, strains of signal sequence type s1 are associated with the presence of the cagA gene and numerous studies have highlighted the prevalence of type s1 strains in individuals with peptic ulcer disease (Rudi et al., 1998; Strobel et al., 1998; van Doorn et al., 1998a). Interestingly, strains of signal sequence type sla and mid-region type m1 were isolated with remarkable prevalence (almost 100%) in Japan (Ito et al., 1997), with no correlation with clinical consequences. The same high prevalence of type sla strains was also found in Taiwan and China, but in contrast to Japan, the dominant mid-region type was m2 (Pan et al., 1998; Wang et al., 1998). However, strains with the type m1b and m1b-m2 alleles produced significantly higher levels of the VacA cytotoxin than did those with type m2 alleles (Pan et al., 1998) and strains with the type m1T allele occurred statistically more often in peptic ulcer disease patients than did strains with type m2 alleles (Wang et al., 1998). In India, the strains carrying toxigenic vacAs1 allele with cag PAI was found to be abundant (Mukhopadhyay et al., 2000).

Such an enormous genotyping variation in vacA raises questions about the mechanism of acquisition. The genome sequencing of 26695 revealed three vacA-related genes (Tomb et al., 1997), but the encoded putative proteins
(a) VacA protein family

![Diagram of VacA protein family]

(b) 3' regions of the cagA genes

![Diagram of cagA gene types]

Fig. 1.4 Schematic diagram of vacA and cagA gene structure

(a) Schematic representation of the diversity in the vacA family. The s2/m2 combination of vacA alleles produces no detectable cytotoxin (adapted from Blaser, 1997).

(b) Schematic presentation of the primary structure of the 3' regions of the cagA gene types A, B, C and D. R1 repeats-15bp, R2 repeats-42bp, and R3-147bp (adapted from Yamaoka et al., 1998).
were predicted not to be secreted toxins (Atherton, 1997). The genome sequence comparison studies also encountered with the same observation (Alm et al., 1999). The presence of such genes may be due to the intragenic recombination occurring between these genes to generate new VacA variants (Tomb et al., 1997).

1.9.3 Generation of genomic diversity of *H. pylori*

The generation of genetic diversity can result from exchange of DNA within a particular bacterial cell or lineage (intra-strain rearrangements) or exchange of DNA between different strains or in some rare cases even different species (horizontal transfer). The genome sequence of *H. pylori* (Tomb et al., 1997) reveals that *H. pylori* possesses a large number of repetitive DNA sequences. As well as several full-length and partial copies of the IS605 insertion sequence, there are four copies (two full-length and two partial) of a novel insertion element, IS606. Due to the number of copies and their presence throughout the chromosome, these elements may lead to recombinational events resulting in chromosomal rearrangements.

The short tandem repeats identified in *H. pylori* may also be associated with genomic rearrangements. Such repeats, like simple repeat DNA of eukaryotes, may be hot spots for intra-genomic rearrangements, such as gene conversion and illegitimate recombination. The phenomenon of horizontal transfer is likely to have played a significant role in the genetic make-up of *H. pylori*. The entire genome sequence of *H. pylori* strongly suggests that inter-species horizontal transfer of genes has occurred during evolutionary development of *H. pylori*. Many of the proteins described in this bacterium show sequence similarity to proteins from different major taxonomic groups,
incidence of adenocarcinoma. Several other factors include genetic predilection and dietary intake of antioxidants and fats which influence the infection process. It is time to stop doing serologic studies to confirm the association of gastric cancer with \textit{H. pylori} and instead expend our efforts on eliminating the infection and investigating the mechanism(s) and interactions.

1.11 DIAGNOSIS

A variety of tests is now available to diagnose \textit{H. pylori} infection. These are histological examination of gastric tissue, bacterial culture, rapid urease testing, use of DNA probes and PCR analysis. Tests involving gastric tissue require endoscopy, therefore they are expensive and involve slight complication due to the procedure. In contrast breath tests, serology, gastric juice PCR, and urinary excretion of $\text{N}^{15}$ ammonia are noninvasive tests that do not require endoscopy. The choice of test used for diagnosis of \textit{H. pylori} infection will depend on the clinical information sought and the local availability and cost of individual tests.

1.11.1 Methods Requiring Endoscopy

The distribution of \textit{H. pylori} and the associated inflammation occur in patches. This patchy nature of infection can lead to endoscopic sampling error resulting in false negative biopsy culture and rapid urease test results. A minimum of two biopsy specimens taken from within 5 cm of the pylorus should be obtained at endoscopy, with multiple sections being examined histologically (Sobala et al., 1991). Genta and Graham (1994) however reported a sensitivity of 100% with biopsy specimens taken from the angularis of stomach.
1.11.1.1 Culture

Culture of *H. pylori* has two major advantages. First it allows antimicrobial susceptibility testing and second, isolates obtained by culture can be characterised in detail. Although the sensitivity of culture in experienced laboratories is greater than 95% other methods for the diagnosis of *H. pylori* infection are simpler and prone to less variability and not time consuming. Culture of gastric biopsy specimens typically provides the greatest yield of *H. pylori*. Culture of gastric juice has been successful occasionally and culture of faeces is also reported (Kelly et al., 1994).

1.11.1.2 Histologic assessment

*H. pylori* can be visualised at high magnification with conventional hematoxylin and eosin (H&E) stained sections. Bacteria are located in the mucous adherent to the surface epithelium and are often found deep within the crypts. Using special stains such as the Warthin Starry and modified Giemsa stains facilitates histological identification of bacteria (el-Zimaity et al., 1996). The distribution of *H. pylori* in the stomach is not uniform nor is organism usually found in areas of intestinal metaplasia (Genta and Graham, 1996). Factors that influence the ability to correctly identify *H. pylori* include bacterial density, type of stain used and the enthusiasm and experience of the laboratory technician (Kolts et al., 1993). A sensitive staining technique consisting of a combination of H&E Steiner silver stain and alcian blue has been developed by Genta et al. (Genta et al., 1994) This stain reportedly allows ready detection of *H. pylori* while simultaneously allowing evaluation of gastric histology. Immunohistochemical staining techniques also have been developed to detect *H. pylori* (Ashton-key et al., 1996). Such techniques are usually not necessary
but may prove worthwhile in cases where tinctorial stains are difficult to interpret. Histologic assessment combined with molecular markers that correlate with cancers can be important in identifying individual at high risk of cancer. The tracing of infection relapse with histologic features in gastric biopsies is possible with histological assessment.

1.11.1.3 Biopsy Urease Tests

The early observation that *H. pylori* produces large amounts of urease activity led to the development of methods for the indirect detection of the organism in gastric biopsy tissue. The sensitivity of all urease-based tests for detection of *H. pylori* is dependent upon the bacterial load in the stomach (Laine et al., 1996a).

The first commercially available biopsy urease tests, designed specifically for *H. pylori* detection was the CLOtest (Delta West Ltd Bently Australia) developed by Marshall (Cutler et al., 1995). It consists of an agar gel containing phenol red and urea in the presence of urease; the urea is hydrolyzed, leading to a pH (and hence a color) change of the indicator. The test is interpreted up to 24 hr after placement of the gastric biopsy sample onto the agar gel. Of the available biopsy urease tests (also known as rapid urease tests) the CLOtest has been the most widely studied (Cutler et al., 1995; Laine et al., 1996b). Two other biopsy urease tests are available commercially: Hpfast, a gel test similar to CLO tests, but with a different pH indicator at a lower pH and PyloriTek, a strip test. In the latter test, in the presence of urease, ammonia is produced from urea impregnated into a pad. An overlying pH indicator detects the diffusion of ammonia through a membrane. A potential advantage is that interpretation requires no more than 1 hr. Comparative studies
of the sensitivity and specificity of the CLOtest, Hpfast, and PyloriTek tests have been performed. The overall sensitivities were equivalent (88 to 93%) and the specificities were excellent (99 to 100%). At 1 hr (the end point for reading pyloriTek) the sensitivities of the gel tests were significantly lower (66 to 71%). The PyloriTek test seems to be the test of choice, if a rapid result (1 hr or less) is desired. However, if rapid results are not needed, all three tests provide equivalent accuracies and the choice may be made based on cost, regulatory issues, availability, physician preference, or other factors. An important point to keep in mind is that increased incubation times may lead to improved sensitivity of biopsy urease tests but to decreased specificity of detection of *H. pylori*.

1.11.1.4 Polymerase Chain Reaction (PCR)

PCR offers great promise as a highly sensitive and specific technique for the detection of *H. pylori*. PCR techniques for the detection of *H. pylori* in gastric biopsy specimens have been described by a number of laboratories, although the accuracy of such techniques varies widely (El-Zaatari et al., 1995). Factors affecting test accuracy include choice of primers, target DNA, specimen preparation bacterium density and technical issues related to PCR procedure. Li et al. (1996) have developed a PCR assay, which is reportedly 100% sensitive and specific for detection of *H. pylori* infection with gastric mucosal biopsy specimens. A potential advantage of PCR is that it may enable the diagnosis of *H. pylori* to be made non-invasively by detecting *H. pylori* DNA in nongastric fluids such as saliva. In one study, the sensitivity of PCR for detecting *H. pylori* in saliva was 84% (Li et al., 1996). PCR techniques for the detection of *H. pylori* are still in its infancy. It is unlikely that such techniques will have widespread use in the initial detection of *H. pylori* except in the
research environment. There are also studies indicating that PCR was less accuracy than immunohistologic staining of bacteria. The potential for obtaining false positive results by PCR should also not be underestimated. However, PCR methods hold great promise in the detection of genetic differences between \textit{H. pylori} strains for research and epidemiologic studies.

1.11.2 Nonendoscopic Methods

1.11.2.1 Antibody detection

Infection of the gastric mucosa with \textit{H. pylori} results in systemic as well as local immune responses, thus allowing the development of serologic tests for detection of the bacteria. Serologic methods have proven, especially valuable in screening large number of individuals in epidemiologic studies. Such tests are non-invasive, relatively rapid and simple to perform and much less expensive than tests requiring endoscopic biopsy. Further serologic tests are less likely to be confounded by suppression of \textit{H. pylori} infection by bismuth compounds, proton pump inhibitors, or antibiotics taken for unrelated conditions than are urease based tests, which as dependent upon and reflect the current bacterial load. Although a wide variety of serologic methods for detection of \textit{H. pylori} have been described in the literature, most tests available commercially are enzyme linked immunosorbent assay methods.

The utility of any serologic test for the detection of \textit{H. pylori} specific antibodies is dependent on the antigen preparation used. In general, three types of antigen have been used. These include crude antigens such as whole cells or whole cell sonicates, cell fractions such as glycine extracts or heat stable antigens and enriched antigens such as urease or a 120-kDa antigen (Andersen \textit{et al.}, 1995). The sensitivities and specificities of tests involving all three types
of antigen preparation typically approach 95%. However, a recent Meta analysis of studies of 11 commercial enzyme linked immunosorbent assay kits and one latex agglutination kit found an average sensitivity of 85% and specificity of 79% (Loy et al 1996).

In the absence of therapeutic intervention antibody levels remain elevated, perhaps for a lifetime reflecting the duration of infection. After eradication of *H. pylori* specific immunoglobulin G (IgG) and IgA levels tend to decrease, typically to approximately half of the pretreatment value within 6 months (Kosunen et al., 1992; Hirschl et al., 1996). Low levels of specific IgG tend to persist for months even after eradication of *H. pylori*. Therefore, using serologic tests to assess the effects of treatment may be problematic unless the pre and post treatment sera can be directly compared.

1.11.2.2 Urea breath tests

The principle of urea breath test is similar to that of the urease tests for the detection of *H. pylori*. Urea is provided as a substrate, and is ingested as either $[^{13}\text{C}]$ or $[^{14}\text{C}]$ urea. *H. pylori* urease hydrolyses the ingested urea into labeled bicarbonate. The exhaled CO$_2$, which is labeled, is collected and detected by scintillation counter or mass spectrometry. The accuracy of the test depends on various factors such as timing of breath collection, the form of delivery of urea, the gastric emptying time etc., UBTs can also be used to confirm eradication, but the test must be performed at least 4 weeks after the completion of treatment (Slomianski et al., 1995) Therapy with antibacterial, bismuth or proton pump inhibitors will reduce production causing false-negative test results (Bazzoli et al., 1997). The accuracy of urea breath test depends on the timing of breath collection the form of delivery of urea and the gastric emptying time.
14C urea breath test is not suitable for children or during pregnancy, while 13C urea breath test is preferred. Measurement of 14C in the urine and 13C in the serum has been reported to accurately reflect the H. pylori status of patients.

1.12 TREATMENT

In vitro, H. pylori strains are susceptible to penicillins, some (cefuroxime) but not all (cephalexin) of cephalosporins, macrolides, tetracyclines, nitroimidazoles, nitrofurans, quinolones, bismuth salts, and proton pump inhibitors (PPIs) (Ansorg et. al., 1996). They are intrinsically resistant to H2-receptor blockers (e.g., cimetidine and ranitidine), polymyxin, and trimethoprim (Piotrowski et al., 1995). Many inconsistencies exist between in vitro susceptibility testing for H. pylori and patient response to therapy. For example, methods for determining antimicrobial susceptibility and the breakpoints for defining resistance of H. pylori are not standardized, there may be an apparent reversibility of metronidazole resistance in vitro (Weel et al., 1996). The effects of antimicrobial resistance on the efficacy of treatment may be variable and unpredictable; the role of susceptibility testing in guiding therapy is not established. In addition extrinsic factors such as smoking may adversely influence the results of metronidazole treatment.

There are a number of reports on the outcome of treatment regimens based on retrospective analysis of antibiotic resistance. In regimens involving metronidazole, in vitro resistance of the H. pylori strain to metronidazole has not consistently influenced efficacy. This variability may relate, in part, to the choice of accompanying antimicrobial and other agents. Macrolide antibiotics, particularly clarithromycin, are increasingly used in regimens against H. pylori and, when used in combination with metronidazole and other agents, may help
to mitigate the effect of metronidazole resistance. \textit{In vitro} resistance to clarithromycin, however, appears to adversely affect treatment outcome more directly (Graham et al., 1996).

The prevalence rate of metronidazole resistance among \textit{H. pylori} strains is highly variable. In developed countries, the prevalence of resistance ranges from 11 to 70%. Resistance to metronidazole is even more prevalent in developing countries (Vasquez et al., 1996); where up to 95% of isolates may be resistant. In contrast, the prevalence of clarithromycin resistance generally is not more than 10% (Olson and Edwards, 1995) resistance tends to be lower in countries where clarithromycin only recently has become available and where other macrolides are not widely used. An exception has been noted in Peru, however, where 50% of \textit{H. pylori} isolates were found to be clarithromycin resistant. Resistance to both metronidazole and clarithromycin has been reported. Resistance to tetracycline has been noted in up to 6% of isolates in the United Kingdom (Karim et al., 1996). There are reports on tolerance to amoxicillin, an antibiotic also frequently used in anti-\textit{H. pylori} regimens have been reported (Dore et al., 1997). The combination of a PPI, bismuth, tetracycline, and metronidazole has resulted in excellent cure rates despite the presence of metronidazole-resistant strains (Borody et al., 1995). The current treatment regimens include traditional triple therapy consisting of bismuth salicylate, metronidazole and tetracycline all taken 4 times daily for 14 days. An addition of acid-suppressive therapy is reported to be highly desirable. This combination of triple therapy with proton pump inhibitor (PPI) has been shown to be more efficacious (de Boer et al., 1995). Vaccination has been successful in a variety of animal models (Ferrero et al., 1995). Although target human populations for prevention of \textit{H. pylori} infection have not been defined, the development of vaccines is still under way.
1.13 OBJECTIVES OF THE PRESENT STUDY

The study of bacterial pathogenesis has undergone a dramatic resurgence in interest, because of the emergence of new diseases. The recent focus has been on the molecular cross talk between pathogens and their host cells towards the elucidation of interactions that helps to understand the molecular basis of disease. Towards this direction, several studies using the live bacteria, or bacterial products and mutants lacking virulence factors with the availability of \textit{in vitro} conditions have contributed in understanding the role of these virulent factors in the pathogenesis or the disease process.

\textit{H. pylori} is considered as one of the most common pathogenic infections of mankind. Despite its worldwide distribution, the pathogenesis of \textit{H. pylori} associated gastroduodenal diseases remains poorly understood. There are several uncertainties about the significance of their occurrence in healthy people and characters of the organism that are contributory for pathogenic potentials. Additional knowledge in terms of the nature of the clinical isolates, a reproducible serological assay for diagnosis based on Indian strains and rapid molecular method for typing the toxigenic strains are urgently required in our country. The work presented thesis is focused on understanding the pathogenic mechanism of the local strains in Chennai, its ability to induce host-signaling changes by analysing the bacterial products and therefore identify the putative virulence factors involved in the pathogenesis. This work involves the evaluation of the potential role of outer membrane proteins as a putative virulence factor in the pathogenesis of \textit{H. pylori}.

The variability of the disease outcome is determined by differences in the virulence of the infecting bacteria and the effectiveness of the host response.
The pathogens are well equipped to induce alteration in the host cell such as the membrane trafficking events, immune modulation, activation of transcriptional factors as well as the cytoskeleton machinery that significantly contribute to the development of the disease. Another important aspect of *H. pylori* is its highly diverse nature at specific loci of virulence genes and also at the genomic level. The study of the Chennai population of *H. pylori* strains will aid in understanding the toxigenic or nontoxigenic strain in this geographical location. This will further facilitate to analyse host-pathogenic interactions with clinical isolates from different clinical presentations of *H. pylori* associated diseases. The objectives of this study can be summarised as follows:

1) Preliminary characterisation of the clinical isolates as virulent or less virulent.

2) Identification of putative virulence factor by analysing the cellular fractions of the bacteria.

3) To evaluate the immunogenicity of such virulence associated factor as an ideal candidate antigen in diagnosis by immunoblot assay.

4) To understand the host cell response during infection with different clinical isolates in terms of transcription factor activation, cytokine expression and cytoskeletal rearrangements.

5) Genotyping of the clinical isolates in this geographic location based on specific primers for the specific loci of virulence.

Definition of the signal transduction and regulatory mechanisms that govern interactions between *Helicobacter pylori*, epithelial cells of the gastric mucosa, and inflammatory and immune cells in the adjacent mucosa may lead to new therapeutic approaches for manipulating and regulating inflammatory and immune responses at the mucosal surfaces of the gastrointestinal tract.