Guts & Glory *H. pylori:* Cause of Peptic Ulcer

Ashley Johnson*, Bryan Kratz*, Lorraine Scanlon*, and Alina Spivak*
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Summary

Due to the 1983 discovery of *H. pylori bacteria as* the leading cause of peptic ulcers, the understanding of the disease dramatically changed. We now know that stress and spicy foods are not the leading causes of peptic ulcers. Symptoms including acute abdominal pain, vomiting of blood, and weight loss are characteristic of peptic ulcers. Ulcers form because of the inflammation caused by *H. pylori* leading to sensitivity of gastric cells to the acid secreted by the infected patient's stomach. Although more than half of the world's population is infected with *H. pylori*, most people remain asymptomatic. Current research suggests that several bacterial virulence genes such as *CagA* and *VacA,* as well as the individual host's genetic predisposition, are factors that influence progression of disease. The mechanism of *H. pylori* infection has been recently examined in detail clarifying the morphological changes of the host cell and how this promotes the formation of a peptic ulcer. Present studies to explain the persistence of *H. pylori and* propose how this bacterium evolved key mechanisms to evade the host's immune response. Due to the advances in the understanding of peptic ulcers, effective treatments have been proposed to treat and eliminate this disease.

History

In 1983, Australian scientists Robin Warren and Barry Marshall showed that the leading cause of peptic ulcers is the infection of the stomach lining with a helical (spiral) shaped gram negative bacterium *Helicobacter pylori (H. pylori)*. It was previously believed that peptic ulcers were caused by stress and consumption of spicy foods (1).

Characteristics

H. pylori infection leads to inflammation of the gastric mucosa in 80% of peptic ulcer cases. *H. pylori* cause elevated acid secretion in people who develop duodenal ulcers, and decreased acid secretion in those who develop gastric ulcers and gastric cancer (3, 5). Duodenal ulcers form due to acid hypersecretion in response to antral inflammation. In patients with gastric ulcers, *H. pylori* cause corpus inflammation which leads to decreased acid secretion and gastric atrophy (Figure 1). Peptic ulcers are 0.3-0.4 cm in diameter in the affected area of the stomach. The remaining 20% of peptic ulcer cases are caused by nonsteroidal anti-inflammatory drugs (NSAIDS) like aspirin, which irritate the stomach lining (3). NSAIDS hinder the protective mechanisms of the stomach including mucus and Bicarbonate secretions, and reduce blood circulation which aids in cell renewal and repair. With the host's defenses down, stomach acid can irritate the sensitive lining, thus causing ulcers (5, 6).

Gut Wrenching Diseases

Peptic Ulcers
Peptic ulcers form on the epithelial cells of the stomach lining. An ulcer consists of two major structures: a distinct ulcer margin and granulation tissue at the ulcer base. A distinct ulcer margin is formed by the adjacent non-necrotic mucosa - the epithelial component. The granulation tissue consists of fibroblasts, macrophages, and proliferating endothelial cells, which form microvessels. On the molecular level, the pathogenesis of ulcer disease is believed to reflect an imbalance between increased corrosive stomach byproducts and decreased protective factors. As a result of stimulation arising from the sight, smell, taste, or thought of food, acetylcholine, a neurotransmitter, and gastrin, a hormone, are released and act on the parietal cells to produce acid. The mast cells in turn release histamine, which also stimulates gastric acid secretion. In patients infected with *H. pylori*, the parietal cells have increased sensitivity to gastrin and possibly to histamine. The increased sensitivity causes corrosion of the stomach lining, leading to the formation of an ulcer (2).

Gastric Cancer
H. pylori trigger the host's immune system to release immune response mediators. These molecules, such as reactive oxygen species and nitrogen made by neutrophils, are released in the stomach and undergo lysis due to low pH levels. These molecules can often damage DNA. Patients with gastric cancer often have constantly activated oncogenes, such as *c-met, c-erbB-2, K-sam*, or inactivated tumor-suppressor genes, such as *p53, p16, and APC*. Those affected also show abnormal alterations of genes implicated in cell proliferation and apoptosis, such as *cyclin D1, bcl-2, E2F-1,* and *SC-1*(7).

Not-So-Glorious Symptoms

One of the major symptoms of gastric ulcers is abdominal pain, which usually occurs during mealtimes as more acid is secreted into the stomach. Hematemesis (vomiting of blood) is often seen in patients with gastric ulcers, which leads to a noticeable reduction in the patient's weight. Melena (i.e. foul smelling feces) is another symptom of gastric ulcers that is often caused by the presence of oxidized iron from hemoglobin (2).

Environmental Pitfalls
A diet high in salt and lacking antioxidant vitamins might promote low acid secretion and cause gastritis, which leads to gastric ulcers and gastric cancer. Salt may change acid secretion by suppressing parietal cells, causing gastric atrophy. Also, the antioxidant vitamins in fresh fruit might protect specialized gastric cells from reactive oxygen species released by inflammatory cells. Diet confirms why there is a high prevalence of ulcers in China and Japan. These countries not only have a high prevalence of *H. pylori* but also a traditionally salty diet.

* This paper was written in BIO221 Cellular and Molecular Biology, taught by Dr. Shubhick DebBurman

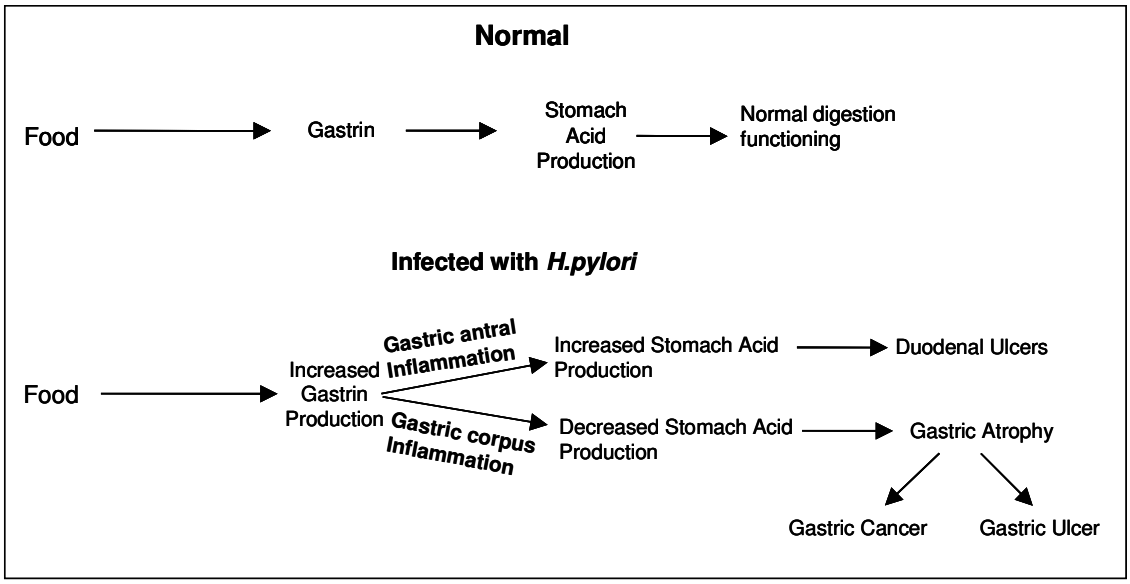

Figure 1. *H. pylori* induced changes in stomach functioning lead to several gastrointestinal diseases Infection with *H. pylori* leads to hypergastrinemia which may lead to inflammation depending on bacterial virulence, environmental factors, and host genetic differences. Patients who develop inflammation of the gastric corpus exhibit decreased acid production which may lead to gastric cancer or gastric ulcer. Those who maintain a healthy gastric corpus but have gastric antral inflammation exhibit increased acid production, which may lead to duodenal ulcers.

Cigarette smoking also strongly predisposes to both duodenal ulcer and gastric cancer (3).

You've "Gut" to Take These

The most effective treatment for peptic ulcers is a three drug regimen consisting of a proton pump inhibitor (PPI) and two antibiotics. Proton pump inhibitors work to expose *H. pylori* to the drug treatment. The most common antibiotic used is amoxicillin and the most prevalent PPI is Omeprazole. Once the bacteria are eradicated by the drug regimen, the normal immune response has the full potential to regenerate the stomach lining and heal the ulcer.

What's Left to Stomach

Although a lot is already known about H. pylori and the diseases it causes, there are four major areas that are currently being expanded on. The fact that the bacterium resides in so many people yet symptoms of disease only appear in a few people is intriguing. This question led researchers to investigate whether bacterial virulence factors and differences in the host attribute to this discrepancy. In addition, the persistence of the bacterium in the infected individual suggests the possibility, addressed by current research, that H. pylori evolved key mechanisms to evade the host's immune responses. Studies of the molecular mechanism of the invasion of the gastric cells with H. pylori have been elaborated upon in recent years. Also, new therapeutic agents and methods of treatment of gastrointestinal diseases have been proposed in response to new findings.

Disease or No Disease…That is the question

Current research suggests that several bacterial virulence factors such as CagA and VacA genes, as well as the individual host's genetic predisposition, influence progression of H. pylori-related diseases. The World Health Organization recently has classified H. pylori as a class I carcinogen because of the risk factor of developing gastric carcinoma as a result of chronic infection (4, 5, 6).

VacA: Evacuate my body

The H. pylori vacuolating cytotoxin gene, vacA, is naturally polymorphic. The two most diverse regions being are the signal region (which can be type s1 or s2) and the mid region (m1 or m2). The type s1/m1 and s1/m2 strains of vacA are associated with peptic ulcer and gastric cancer, whereas while the type s2/m2 strains are non-toxic and associated with lower risk of peptic ulcer and gastric cancer. The features of vacA that determine the nontoxicity of these strains were determined by Letley et. al. (2003). They did this by deleting parts of vacA and constructing isogenic hybrid strains in which regions of vacA were exchanged between toxigenic and non-toxigenic strains. They showed that a naturally-occurring 12-amino acid hydrophilic N-terminal extension found on
s2 VacA blocks vacuolating activity as while its removal (making the strain s1-like) confers activity. They did chromosomal replacement of vaca in a nontoxigenic strain with vacA from a toxigenic strain and found full activating activity, proving that the vacoulation is controlled entirely by elements within vacA. This research defined why determined that H. pylori strains with different vacA allelic structures have differing toxicity (10).

Virulent Strain Carries CagA Gene

Another bacterial virulence factor is the polymorphism of the CagA protein. All H. pylori strains have the cagPAI DNA segment, but only some strains have the cagA gene that encodes the 145 -kDA CagA protein. These strains are called cagA+ strains, and while the strains lacking the cagA gene are called cagA-. The cagA+ strains are more virulent than the cagA- strains and are associated with gastric carcinoma. The CagA is injected by the bacterium and subsequently

undergoes tyrosine phosphorylation. The phosphorylated CagA specifically binds SHP-2 phophatase, activates the phophatase activity, and thereby induces morphological transformation of cells. SHP-2 plays an important role in both cell growth and cell motility. This morphological change is referred to as the hummingbird phenotype because the cell undergoes dramatic elongation by means of the attachment of cagA+. Higashi et. al. (2002) found that Western and East Asian CagA both contain tyrosine phosphorylation sites but they differ in structure. Western strains can have repeating tyrosine phosphorylation sites. The larger the number of binding sites, the greater the amount of tyrosine phosphorylation, which leads to increased SHP-2 binding and greater morphological changes. In contrast, the East Asian strains have a different tyrosine phosphorylation sequence at the region corresponding to the Western sequence that binds SHP-2 stronger and induces greater morphological changes to the cell than the Western sequence, causing East Asian CagA proteins to be more potent and leading to high gastric cancer incidence rates (9).

Interleukin-1 β̃ What alleles do you have?

Host genetic factors that affect interleukin-1-beta may determine why some individuals affected with H. pylori develop gastric cancer while others do not. Polymorphisms in human cytokine genes affect the level of cytokine production by cells after contact with H. pylori. Specific polymorphisms in the IL-1b gene and the IL-1 receptor-antagonist gene (IR-1RN) lead to increased gastric mucosal levels of IL-1b in individuals infected with H. pylori. IL-1b (interleukin-1-beta) is an important pro-inflammatory cytokine and a powerful inhibitor of gastric acid secretion (5). The three reported diallelic polymorphisms in IL1B which have been reported all represent C-to-T base transitions at positions –511, -31, and +3954 basepairs from the transcriptional start site. El-Omar et al., demonstrated that individuals who were carriers for of the interleukin-1 beta- 31T allele had low acid secretion. The polymorphisms also increase the risk of gastric atrophy, hypochlorhydria, intestinal metaplasia, and gastric cancer (5, 8). Using electrophoretic mobility shift analysis to assess DNA binding in vitro, the interleukin-1 beta -31T allele was associated with a five-fold increase in DNA-binding after lipopolsaccharide stimulation. Individuals carrying the interleukin-1 beta -31 T allele are more susceptible to developing hypochlorhydria, and subsequently gastric cancer, in the case of an infection by the bacterium H. pylori. Thus, the interleukin-1 beta gene is a crucial factor in determining if a person will develop gastric cancer (8).

Gastric Cancer

Infection with the human microbial pathogen *Helicobacter pylori* is assumed to lead to invasive gastric cancer. *H. pylori* activate the hepatocyte growth factor/scatter factor receptor c-Met (oncogene), which is involved in invasive growth of tumor cells. The *H. pylori* effector protein CagA intracellularly targets the c-Met receptor and acts as an oncoprotein, promoting cellular processes that lead to changes in cell polarity, motility and differentiation. These changes may be related to the development of gastric cancer. CagA could represent a bacterial adaptor protein that associates with phospholipase Cγ (PCγ), but not with Grb2-associated binder 1 or growth factor receptor–bound protein 2. The *H. pylori*–induced motogenic response is suppressed and blocked by the inhibition of PLCγ and of MAPK, respectively. Thus, upon translocation, CagA modulates cellular functions by deregulating c-Met receptor signaling. The activation of the motogenic response in *H. pylori*–infected epithelial cells suggests that CagA could be involved in tumor progression (8).

H. pylori Persistence

The primary response of the body to infection of *H. pylori* is inflammation. This is caused by the infiltration of the gastric mucosa with neutrophils, macrophages, B cells, and T cells following release of interleukins. T lymphocyte responses in acute *H. pylori* infection are predominantly of the CD4+ Th1 (mainly cell-mediated) cell phenotype (3, 5). Although a seemingly large immune response is initiated, it is mostly ineffective, because *H. pylori* bacteria are rarely completely eradicated from an infected individual. The persistence of *H. pylori* and the high reinfection rate suggest that the host has significant anergy and is unable to build protective immunity.

VacA Does What?

Previous studies showed that VacA inhibits release of IL-2 in Jurkat cells (human T-cell leukemia cells). This inhibition is linked to the ability of VacA to inactivate the Nuclear Factor of Activated T-cells (NFAT). This transcription factor is critical to the transcription of IL-2; therefore, if VacA inactivates NFAT, IL-2 secretion is inhibited, and Jurkat T cell proliferation is therefore decreased (11).

However, Sundrud et al. (2004) propose that VacA has a different effect on primary human T_h cells. Similar testing with the human T_h cells suggested that VacA has only a modest effect on IL-2 secretion. VacA did not cause a reduction in IL-2 levels in either naïve or memory Th cells. Therefore, it is now predicted that VacA inhibits IL-2-driven proliferation of primary human T_h cells by a non-NFAT mechanism. Further studies suggested that VacA suppresses cell cycle progression in T_h cells, similar to drugs such as rapamycin which induce G1 arrest. Therefore, instead of blocking IL-2 secretion, and by that inhibiting T_h cell proliferation in human T_h cells, VacA might be blocking normal cell cycle progression of these cells. Sundrud et al. found that VacA must have an intact hydrophobic domain within its N-terminal region. This component of VacA structure is necessary for both inhibition of IL-2 secretion in Jurkat cells and inhibition of IL-2-driven proliferation of human primary T_h cells. This region is attributed to making VacA anion-selective channels, which may cause depolarization of the T_h cell plasma membrane and lead to inhibition of IL-2- dependent T-cell proliferation. Interestingly, a mutant VacA (VacA-(6-27) that completely lacks this entire hydrophobic region actually has a dominant negative effect and fully blocks the wildtype VacA mediated inhibition of T cell proliferation both in Jurkat and primary human T_h cells. Thus, these scientists concluded that VacA has immunosuppressive properties that help *H.pylori* evade the host's immune response (11).

Treg Cells don't regulate but promote disease

Recent studies show that the host's immune response often leads to immunopathology in an infected person (3, 5, 7). This conclusion stems from the fact that T_h

cells have a poor responsiveness to *H. pylori* antigens. CD4+ T cells proliferate more during *H. pylori* infection in comparison to CD8+ T cells. Also, Lundgren et al. (2003) suggest that memory cells in infected individuals proliferate a lot less in comparison to the memory cells of healthy individuals, and naïve cells barely proliferate in either case. In fact, this difference in proliferation rates of memory cells was nonexistent when both individuals were treated with another toxin (Tetanus-toxin). This implies that the reduced responsiveness of memory T cell proliferation in infected individuals was limited to *H. pylori* specific cells. This finding led to the assumption that regulatory $CD4^{+}CD25^{high}$ T cells (Treg cells) suppress proliferation of memory T-cells. Treg cells are vital for controlling the immune response to foreign antigens and preventing autoimmune responses. Therefore, it is currently suggested that repetitive stimulation of T cells with *H. pylori* antigen may lead to activation of Treg cells that actively suppress the response of memory cells. Therefore, these authors showed that with prolonged infection, the host's own immunity activates *H. pylori* specific Treg cells, which suppress memory cell proliferation promoting pathogenesis (12).

(COX) ^{4} Lowers Immune Response

Meyer et al. (2003) found that *H. pylori* induce production of cyclooxygenase (COX) ^{4}-2. COX is an enzyme that is attributed to inhibition of epithelial apoptosis, increased cell proliferation, and angiogenesis. Studies have shown that a byproduct of *H. pylori*, urease, allows the bacteria to survive the acidic pH of the stomach and also induces (COX) ^{4}-2 expression (5, 13). (COX) ^{4}-2 then produces prostaglandins such as prostaglandin E_2 (PGE_2) which mediate inflammation. Therefore, the induction of (COX) ^{4}-2 by the host is a defense strategy that works by making PGE_2 that reduces inflammation. Also, Meyer et al. (2003) found that a decrease of inflammation has been attributed to increased bacterial colonization. Therefore, *H. pylori* inhibit the effectiveness of the host's immune response leading to increased pathology (13).

Le+ *H. pylori* have an advantage

Horizontal gene transfer and translational frame shifts contribute to the large genetic diversity of this bacterium (5). Bergman et al (2005) showed that *H. pylori* express Lewis blood group Antigen (Le) in their lipopolysaccharide (LPS) that is phase variable, resulting in Le+ and Le- population of *H. pylori* within a single strain. Similar to HIV, Le+ antigen of *H. pylori* variants can bind to the C-type lectin DC-SIGN and present on gastric dendritic cells (DCs). This interaction induces inhibition of Th1 cell differentiation as compared to nonbinding variants. Le+ antigen alter the host's T cell ability to differentiate by reducing the amount of IL-6 produced and blocks Th 1 cell polarization. Similar to the Treg suppression of the immune response, the binding of Le+ antigen to DC-SIGN reduces IL-6 levels which may lead to increased T cell sensitivity to suppression. Therefore, *H. pylori* targets DC-SIGN to block a polarized Th1 cell response by phase-variable expression of Le antigens. Once again, decreased proliferation of Th1 cells lead to a decrease in the host's immune response (14).

***H. pylori* Learned to Avoid TLR**

It is widely known that eukaryotic organisms have evolved many mechanisms to recognize bacterial agents so that a proper immune response can be activated to eradicate the bacterium. One such immunity are Toll-like receptors (TLRs), which recognize components of bacterial membrane LPS and a bacterial protein flagellin that are released by many gram-negative bacteria. Gewirtz et al. (2004) suggested that although *H. pylori* contain both LPS and flagella, they are still able to evade this immune response. The scientists found that *H. pylori* releases much smaller amounts of flagellin than other gram-negative bacteria and the flagellin that they do release is barely potent. The flagellin that is released does not play a large role in mediating proinflammatory gene expression in the host. The usual effect of gram-negative bacteria is the activation of TLR, which induces IL-8 secretion of a proinflammatory cytokine. However, *H. pylori* are able to evade TLR mediated immunity by producing impotent flagellin and preventing the release of this potentially immunogenic, proinflammatory protein (15).

CagA vs. Mucus

CagA plays a major role in morphological changes induced by the *Helicobacter pylori* bacterium upon entry of the gastric epithelial cells. Al-Mahroon et al. (2004) preformed an experiment to test the effect of CagA (+) or CagA (-) strains of *H. pylori* on the mean gastric mucus thickness in humans when compared to an uninfected individual. Biopsies taken from each of the patients were submitted to PCR to determine the presence of CagA (+). After staining and treating the biopsies, the mucus layer thickness was determined using an integration of light microscopy, CCD camera, and specific computer software. The results showed that, on average, the mucus layer thickness was not affected in a manner that was statistically significant (20).

I SAID Drop Your Apical Junctions Now!

Another side effect of *H. pylori* infection is faulty apical junctions and loss of cell-to-cell adhesion. Scientists wondered if CagA plays a role in the mediation of this effect and how it causes this abnormal morphological change. Bagnoli (2005) preformed an experiment in which CagA and ZO-1(a known tight junction scaffolding protein) were tagged with antibodies so that they could be easily seen under the microscope. The results showed that in CagA expressing cells, the ZO-1 protein was mislocated to the basolateral membrane (Figure 2). It was also found that the apical junction perimeter and the surface area of the apical membrane had become substantially reduced. As a result and consistent with their hypothesis, CagA expressing cells acquired an elongated, spindle-shaped morphology, and lost their connections with the apical junctions of neighboring cells (19).

Hey SHP-2 Wanna Bind Tonight?

A study conducted by Shiho Yamazaki et al. (2003) suggests that the CagA protein then may bind, undergo tyrosine phosphorylation, and form an activated-complex with SRC homology 2 Domain (SHP-2). The phosphorylation of CagA and activation of SHP-2 are thought to induce the hummingbird phenotype: a

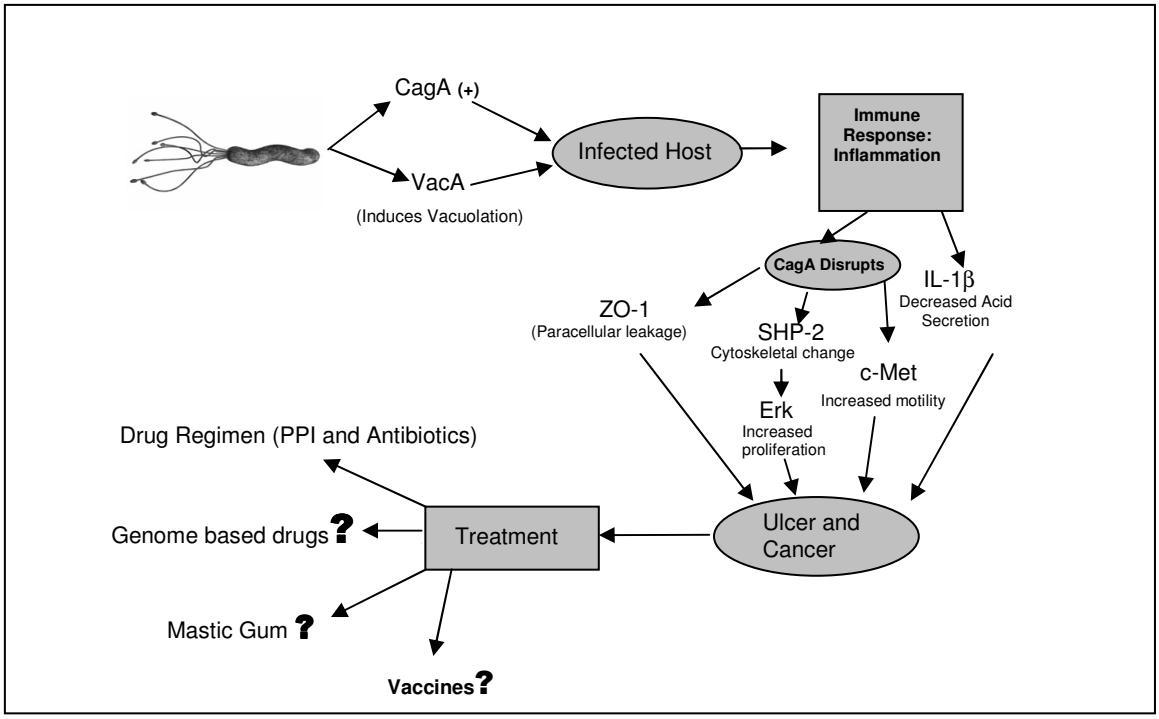

Figure 2. Pathogen-Host Interactions in the Pathogenesis of *H. pylori* Infection
Bacterial virulence factors CagA and VacA cause damage to the host cell. In response to bacterial colonization, a host mounts an immune response which often leads to disease. Several host proteins: ZO-1, SHP-2, c-Met, IL-1b, and Erk have been noted to be affected by CagA leading to formation of duodenal and gastric ulcers as well as gastric cancer. However, there are other effects of CagA that remain unknown and must be addressed in future research. Several treatments are used as a means to eradicate *H. pylori* infection such as antibiotics. Recent research proposes several alternative methods of treatment such as: mastic gum, genome-based drugs, and vaccines. Further research is still needed to clearly understand the pathology caused by *H. pylori* and optimal treatments.

morphological change characterized by elongation and contraction of the cell and increased cell motility. Normal SHP-2 is actively involved in regulation adhesion, spreading, and migration of cells. The scientists took biopsies at eight different parts of the stomach lining from fifteen patients who had either gastritis or early gastric cancer. The biopsies were submitted to immunoblotting and immunoprecipitation in conjunction with antibodies to detect CagA, phosphotyrosine, and SHP-2. The results detected the presence of tyrosine phosphorylated CagA protein and CagA-coimmunoprecipitated endogenous SHP-2. This suggests that deregulation of SHP-2 by translocated CagA can cause abnormal morphology and movement of gastric epithelial cells (16).

Please Don't Phosphorylate When Erk is Home

Hideaki Higashi et. al (2004) investigated cellular proteins that bind to phosphorylated tyrosine but not non-phosphorylated CagA and form complexes SHP-2 and subsequently with extracellular signal-regulated kinase (Erk), a MAP kinase signaling molecule that is thought to effect cell proliferation and motility. To test the effect on the humming bird phenotype, they created a knock-out SHP-2 and transfected it into AGS cells. They found that only phosphorylated CagA complex with SHP-2 binds to and abnormally prolongs the activation of Erk (17).

CagA Sticks like C-Met

C-Met is a hepatocyte growth factor/scatter factor receptor that is involved in invasive growth of tumor cells. In a study conducted by Yuri Churin et al. (2003), the interaction of CagA with this receptor was tested.

Small interference RNA was used to block the expression of c-met. The blocking of c-Met expression inhibited scattering in AGS cells infected with CagA (+) *H. pylori* (18).

What's a Gut to do?

Current treatment of Helicobacter pylori infection, which ultimately leads to the development of peptic ulcers, is based on multiple drug therapies (22). Currently, the most effective therapy consists of a proton-pump inhibitor and a series of three antibiotics chosen from macrolide antibiotics, ß-Lactam antibiotics, or metronidazole antibiotics (22). Other therapies, including two drugs (proton pump inhibitor and an antibiotic) and four drugs (proton pump inhibitor, three antibiotics), have also proven to eradicate *H. pylori* infection in humans (21).

The Basic PPIs

Proton pump inhibitors (PPIs) play an essential role in the eradication of *H. pylori*. PPIs act within the parietal cells of the stomach to inhibit H^+, K^+-ATPase activity. This enzyme maintains the balance of H^+ and K^+ ions within the cell so that pH is maintained inside and outside of the cell. PPIs bind to the H^+, K^+-ATPase on the outer luminal membrane and inhibit phosphorylation of ATP molecules. This in turn prevents the exchange of H^+ and K^+ ions. With the enzyme blocked, the acidic pH of the stomach is made more basic so that

antibiotics, which are taken along with PPIs, may reach the *H. pylori* living within the epithelial cells of the stomach (23).

All PPIs are NOT Created Equal

There are several PPIs that may be used in combination with antibiotics to eradicate *H. pylori*. The most common PPIs used are Omeprazole, Pantoprazole, Lansoprazole, and Rabeprazole. PPI differences depend on the H^+, K^+-ATPase binding location and their pharmacokinetic properties.

In comparison, Hellstrom and Sigurd (2004) found that Rabeprazole was very quick to inhibit acid production compared to the others; however, Omeprazole offered the most potent acid inhibition. Pantoprazole and Lansoprazole are not far behind Omeprazole and Rabeprazole in speed and potency, indicating that all four of these PPIs are effective ways to inhibit the function of the H^+, K^+-ATPase enzyme (23).

Dealing the Drugs

Current therapies used to eradicate *H. pylori* in the stomach all include at least one antibiotic in combination with a PPI. The main categories of antibiotics used are: macrolide antibiotics, ß-Lactam antibiotics, and Metronidazole antibiotics (21).

Holy Macrolide

Macrolide antibiotics accumulate in the epithelial tissues of the stomach. Here, they are able to inhibit RNA- dependent protein synthesis by binding to the 23S ribosomal RNA in the 50S subunit of prokaryotic ribosomes.When the macrolides bind to the ribosomes, they inhibit peptidyl transferase reactions and cause incomplete peptide chains to be detatched from the ribosome. Proteins are essential for a cell to funciton, so without properly formed proteins, the bacteria die quickly (24).

And The Walls Came Tumbling Down

ß-Lactam antibiotics are analogues of D-alanyl-D-alanine, which is an amino acid that makes up peptidoglycan. This close relationship allows ß-Lactam antibiotics to bind to the active site of penicillin binding protiens (PBPs) within bacteria. PBPs facilitate the transpeptidation of the cell walls of bacteria. When ß-Lactam
antibiotics bind to the active site of PBPs and inhibit transpeptidation of peptidoglycan, they prevent cell wall synthesis within the bacteria (25). Without cell walls, parent cells are not able to undergo mitosis to generate a new generation of bacterial cells. Therefore, the bacteria are soon eradicated (26).

Where's the Air

Metronidazole antibiotics only work on anaerobic bacteria like *H. pylori* (27). When a metronadizole antibiotic enocunters an anaerobic bacterium, the nitro group of the metronidazole is reduced, thus interfering with DNA synthesis and making it possible for the antibiotic to interact with intracellular macromolecules and ultimately kill the bacterium (28).

Just Say No to Drugs

The reason that there are so many choices in antibiotic combinations when considering treatment of *H. pylori* is antibiotic resistance. A patient's level of resistance to an antibiotic can cause a drug regimen to fail in erradicating infection. Ecclissato et. al. (2002) studied the effects of antibiotic resistance in two common regimens used to treat infection by *H. pylori.* In both a three drug regimen and a two drug regimen, it was shown that when a patient was resistant to just one antibiotic, the overall eradication rate of the regimen was decreased by half (28). This has serious implications for the treatment of patients for *H. pylori* infection.

Currently, doctors do not test patients for antibacterial resistance before they are prescribed a regimen to treat *H. pylori* infection (28). If these patients are resistant to the bacteria, the regimen is likely to fail. In countries such as the United States, where drugs are readily available ,regimen failure is not as serious as in countries where drugs are not easily obtained (28).

Bacteria form resistance to antibiotics in ways unique to each antibiotic. *H. pylori* resistance to ß-Lactam antibiotics is due to alteration in the Penicillin Binding Protein (PBP) (26). Studies have shown that the replacement of the the wild-type HP0597 (PBP1A) gene by the Hardenberg PBP1A resulted in a huge increase in the minumum inhibitory concentration (MIC) of amoxicillin (a ß-Lactam antibiotic) (26). Antibacterial resistance is usually due to the bacteria evolving ways to produce ß-Lactamase even in the presence of anti β-lactam antibiotics. Structural alterations in a PBP or changes in other proteins that are involved in cell wall synthesis are also involved in antibacterial resistance.

Macrolide antibiotics face two main modes of resistance. There is target site modification, during which the bacterium makes an enzyme that methylates the rRNA, thus inhibiting the binding of erythromycin (or other macrolides) (24). The second mode of resistance is alteration in transport of the antibiotic. This mode of resistance involves two macrolide efflux pumps: A and E. The pumps pump macrolides out of the cell; however, this mode of resistance only works on fourteen or fifteen membered macrolides (24). Metronidazole resistance has been accredited to mutations in the *rdxA* gene that make the gene inoperative (29). This gene coded for an oxygen-insensitive NADPH nitroreductase (29). Without the expression of this gene, the Metronidazole cannot energize its anabolic functions.

Glimpses of Future Glory

Hit Them Where it Hurts
Currently, Genome-based drugs and vaccines are being worked on. Genome-based drugs are drugs that attack a specific target, which is essential to cell function (21). Researchers are trying to find proteins involved in cell envelope synthesis and integrity, cell division, protein synthesis, nucleic acid biosynthesis, gene expression and regulation, cell metabolism and other protein essential to *H. pylori* function that may be easily and safely targeted (21).

Just Give it a Shot
An important topic of research that many scientists are

very interested in is the possibility of a vaccination for *H. pylori* infection. It is believed that a vaccination is possible due to the immune response generated by the host at the onset of *H. pylori* infection (29). It has been found that *H. pylori* actually benefit from this response when first colonizing a new host. The antigens formed in this process may be used to treat established infections (30).

Most research concerning vaccines has been carried out in animal models with promising results. It was found by Ghiara et. al. (1997) that mice that had chronic *H. pylori* infection were able to receive therapeutic vaccinations of recombinant VacA and CagA together with a genetically detoxified mutant of the heat-liable enterotoxin LTK63, intragastrically, to eradicate *H. pylori* infection (31). Furthermore, the vaccination protected the mice from re-infection for 12 weeks after eradication (31).

Using animal models, scientists are currently testing different possible vaccines for efficacy and safety, as well as considering the best mode of delivery (32). A big challenge for scientists to overcome in eradicating *H .pylori* is antibiotic resistance.

Gutsy Alternative Treatments:

In order to lessen the possibility of antibacterial resistance and subsequent ineffectiveness of drugs in treating *H. pylori*, alternative treatments should be studied. Some of the alternative methods to antibiotics include: Vitamin C supplements, Lactobacilli,
Mastic gum, and garlic, among others (33). Mastic gum is a resinous substance obtained from the stem and leaves of the mastic tree (*Pistacia lentiscus*). The direct mechanism of action of mastic gum in healing ulcers and eradicating *H. pylori* should be studied to determine its therapeutic properties. Lactobacilli have been shown to reduce the incidence of antibiotic-induced gastrointestinal side effects such as diarrhea, bloating, and taste disturbance. In addition, clinical evidence suggests that Lactobacilli such as *L. salivarius* enhance the effects of antibiotic treatment; however, research needs to be conducted to confirm these findings (33).

H. pylori May Be Good

Some scientists worry that eradication of *H. pylori* may be more harmful than its presence. Scientists like Martin Blaser of Vanderbilt University in Nashville suggest that the bacterium's presumed long acquaintance with mankind may offer benefits (34). It is possible that the benefits of having *H. pylori* infection may balance the costs, or else we would have evolved a better immune response. *H. pylori* have been around for at least 100,000 years. This preliminary evidence suggests that people who are not infected with *H. pylori* are more likely to develop reflux—a painful disease in which acid from the stomach backs through a leaky valve and inflames the esophagus. In addition, it is possible that the bacterium may also reduce the risk of the cancer of the esophagus. While gastroesophageal reflux disease is now a growing problem in some developed countries, Graham notes that excess acid secretion can be easily managed by current medications. The immune response stimulated by *H. pylori* could help the human immune system fight other, more harmful, invaders. Putsep et al. reported that *H. pylori* makes a compound that kills other bacteria. With these intriguing findings, Putsep et al. suggest that physicians should wait for more studies on possible benefits of *H. pylori* before aggressively pursuing any program to eradicate the bacterium (34).

Conclusion

Since its discovery in 1983, research has shown *Helicobacter pylori* to be the cause of peptic ulcers and a contributor to gastric cancer. Further studies on the bacterium have given scientists insight into how the bacterium functions in the human body and how it may be eradicated. Advances in the knowledge of *H. pylori* will help scientists and physicians effectively treat gastric and duodenal ulcers as well as gastric cancer.

Acknowledgements

We would also like to thank Michael Zorniak, Jenny Riddle, Katie Hampton and Michael Wollar for their guidance and expertise. We would like to thank Dr. DebBurman for his time and patience.

Note: Eukaryon is published by students at Lake Forest College, who are solely responsible for its content. The views expressed in Eukaryon do not necessarily reflect those of the College.

References

1. *Pathophysiology of Peptic Ulcer Disease*. (n.d.). Retrieved April, 2006, from First Principals of Gastroenterology Database: http://gastroresource.com/GITextbook/en/chapter16/Default.htm
2. *Helicobacter pylori*. (2006, April 14). Retrieved April 14, 2006, from Wikipedia database: http://en.wikipedia.org/wiki/Helicobacter_pylori
3. Calam, John., Baron J.H. ABC of The Gastrointestinal Tract: Pathophysiology of Duodenal and Gastric Ulcer and Gastric Cancer. *BMJ*. **323**. 980-982. (2001)
4. Suerbaum, Sebastian, Michetti, Pierre. *Helicobacter pylori* Infection. New England Journal of Medicine.**347**. 1175-85. (2002)
5. Atherton, John C. The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal disease. *Annual Review of Pathology: Mechanisms of Disease*. **1**. 63-96. (2006).
6. Sachs George, Weeks, David, Melcher, Klaus, Scott, David R. The Gastric Biology of *Helicobacter pylori*. Annu. Rev. Physiol.. **65**:349–69 (2003)
7. Chen, Jie. Rocken, Cristoph, Malfertheiner, Peter, Elbert, Mattias P.A. Recent Advances in Molecular Diagnoses and Treatment of Gastric Cancer. Dig Dis. **22**:380–385 (2004)
8. El-Omar, Emad M. et. al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*. **404**. 398-402. (2000).
9. Higashi, Hideaki., et. al. Biological activity of the *Helicobacter pylori* virulence factor CagA is Determined by variation in the tyrosine phosphorylation sites. *Proc. Natl. Acad. Sci.* **99**. 14428-33. (2002).
10. Letley, Darren. P., Rhead, Joanne L., Twells, Rachel J., Dove, Brian. Atherton, John. C. Determinants of non-toxicity in the gastric pathogen *Helicobacter pylori*. *The Journal of Biological Chemistry*. **278**. 26734-41. (2003).
11. Sundrud, Mark S., Torres, Victor J., Unutmaz, Derya, Cover, Timothy L. Inhibition of primary T cell proliferation by *Helicobacter pylori* vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. PNAS.**101**.no. 20. 7727-32. (2004)
12. Lundgren, Anna, Suri-Payer, Elisabeth, Enarsson, Karin, Svennerholm, Ann-Mari, Lundin, B. Samuel. *Helicobacter pylor*-Specific $CD4^+$ $CD25^{high}$ Regulatory T Cell Suppress Memory T-Cell Responses to *H.pylori* in Infected Individuals.Infection and Immunity. **71**, No.4. 1755-62 (2003)
13. Meyer, Frank, Ramanujam, Kalathur S., Gobert, Alain P., James, Stephen P., Wilson, Keith T.

Cutting Edge: Cyclooxygenase-2 Activation Suppresses Th1 Polarization in Response to *Helicobacter pylori*. The Journal of Immunology.**171**:3913-17(2003)

14. Bergman, Mathijs P. Engering, Anneke, Smits, Hermelijn H., van Vliet, Sandra J., van Bodegraven Ad A., Wirth, Hans-Peter, Kapsenberg, Martien L., Vandenbroucke-Grauls, Christina M.J.E., van Kooyk, Yvette, Appelmelk, Ben J. *Heliciobacter pylori* Modulates the T Helper 1/T Helper Cell 2 Balance through Phase-variable Interaction between Lipopolysaccharide and DC-SIGN. J. Exp. Med.**200**. 979-90.(2005)
15. Gewirtz, Andrew T., Yu, Yimin, Krishna, Uma S. Israel, Dawn A., Lyons, Sean L., Peek, Richard M. Jr. *Helicobacter pylori* Flagellin Evades Toll-Like Receptor 5-Mediated Innate Immunity. The Journal of Infection Diseases.**189**. 1914-20 (2004)
16. Yamazaki, S. The CagA Protein of Helicobacter pylori is .Translocated into Epithelial Cells and Binds to SHP-2 in Human Gastric Mucosa. *The Journal of Infectious Diseases*, **187**, 334-337. (2003).
17. Higashi, H. Helicobacter pylori CagA Induces Ras-independent. Morphogenetic Responce through SHP-2 Recruitment and Activation. *The Journal of Biological Chemistry, **279***, 17205-17216. (2004).
18. Churin, Y. Helicobacter pylori CagA protein targets the c-Met receptor and enhances the motogenic responce. *The Journal of Cell Biology, **161***(2), 249-255. (2003).
19. Bagnoli, F. *Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells*. The National Academy of Sciences of the USA. (2005).
20. Al-Marhoon, M. S. Effects of cagA+ and cagA- strains of Helicobacter pylori on the human gastric mucus layer thickness. *Journal of Gastroenterology and Hepatology*, (20), 1246-1252. (2004).
21. Trust, T. J., Alm, R. A., Pappo, J. *Helicobacter pylori*: Today's Treatment, and Possible Future Treatment. *The Infection Discovery*. **586**, 82-88. (2001).
22. Okada, M., et al. A New Quadruple Therapy for *Helicobacter pylori*: Influence of Resistance Strains on Treatment Outcome. *Aliment Pharmacol Ther*. **13**, 769-774. (1999).
23. Hellstrom, P. M., Vitols, S. The Choice of Proton Pump Inhibitor: Does it Matter? *Basic and Clinical Pharmacology & Toxicology*. **94**, 106-111. (2004).
24. Jain, R., Danziger L. H. The Macrolide Antibiotics: A Pharmacokinetic and Pharmacodynamic Overview. *Current Pharmaceutical Design*. **10**, 3045-3053. (2004).
25. Wikipedia contributors (2006). Beta-lactam antibiotic. *Wikipedia, The Free Encyclopedia*. Retrieved 06:05, April 18, 2006 from http://en.wikipedia.org/w/index.php?title=Beta-lactam_antibiotic&oldid=48122422.
26. Gerrits, M. M. et al. Alterations in Penicillin-Binding Protein 1A Confer Resistance to ß-Lactam Antibiotics in *Helicobacter pylori*. *Antimicrobial Agents and Chemotherapy*. **46**, 2229-2233. (2002).
27. Wikipedia contributors (2006). Metronidazole. *Wikipedia, The Free Encyclopedia*. Retrieved 01:02, April 19, 2006 from http://en.wikipedia.org/w/index.php?title=Metronidazole&oldid=48384810.
28. Ecclissato, C., et al. Increased Primary Resistance to Recommended Antibiotics Negatively Affects *Helicobacter pylori* Eradication. *Helicobacter*. **7** (1), 53-59. (2002).
29. Goodwin, A. et al. Metronidazole Resistance in *Helicobacter pylori* is Due to Null Mutations in a Gene (rdxA) the Encodes an Oxygen-Insensitive NADPH Nitroreductase. *Molecular Microbiology*. **28**(2), 383-393. (1998).
30. Blaster, M. J., Kirschnet, D. Dynamics of *Helicobacter pylori* Colonization in Relation to the Host Response. *Mathematics, Microbiology*. **96**, 8359-8364. (1999).
31. Ghiara, P. et al. Therapeutic Intragastric Vaccination against *Helicobacter pylori* in Mice Eradicates an Otherwise Chronic Infection and Confers Protection against Reinfection. Infection and Immunity. **65** (12), 4996-5002. (1997).
32. Kleanthous, H. et al. Rectal and Intranasal Immunizations with Recombinant Urease Induce Distinct Local and Serum Immune Responses in Mice and Protect against *Helicobacter pylori* Infection. *Infection and Immunity*. **66** (6), 2879-2886. (1998).
33. Gaby, Alan R. Alternative Medicine Review. 6. no 4. 358-66. (2001).
34. Pütsep, Katrin , Brändén, Carl-Ivar, Boman, Hans G., Normark, Staffan. Antibacterial Peptide from H. pylori. Nature 398. 671-72 (1999).