Gastric mucosal response to *Helicobacter pylori*

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Abstract. Since Marshall’s discovery before 20 years, *Helicobacter pylori* (*H. pylori*) infection is reportedly to be associated with a variety of clinical outcomes including peptic ulcer disease and gastric cancer. The first step of the *H. pylori* colonization might be its adhesion to the surface epithelial cells, which evokes gastric inflammatory events initiated by neutrophil recruitment from the microcirculation. Mongolian gerbil is one of the suitable animal models for *H. pylori* infection, which exerts gastric ulcer and cancer with its bacterial infection. In *H. pylori*-colonized gerbils, extensive levels of microvascular leukocyte adhesion and migration into the parenchymal side and significant levels of inflammatory cell infiltration are encountered. Bacterial urease not only neutralizes gastric luminal acid, but also plays as an adhesion factor to the surface epithelium. Recently, such an adhesion to the epithelium is reported to be important for bacterial type IV secretory system, which intermediates Cag A injection into the epithelial cells. Then, multiple chemokine and cytokine networks are activated and mucosal inflammatory lesion formation would be completed. In the long-term colonization of *H. pylori*, gastric mucosal cell turnover would be modified due to persistent inflammation and then such deregulation of cell turnover might link to the precancerous lesion formation.

Key words: leukocyte, free radical, antioxidant, microcirculation, IgY

Introduction

In 1982, Marshall and Warren in Australia became the first in the world to succeed in culturing *Helicobacter pylori* (*H. pylori*) isolated from the gastric mucosa of patients with gastritis.¹ Since the report by Palmer,² who denied the existence of micro-organisms in human stomach, it had been thought almost impossible for any micro-organism to survive in the acidic environment within the stomach. However, such spiral micro-organisms had been already discovered in 1890s.³

Rokuzo Kobayashi (1887–1969) was a Professor Emeritus of Keio University School of Medicine. He and his co-worker Katsuya Kasai when they were at Kitasato Institute found that a spirochete-like organism, that might have been *Helicobacter felis*, was colonizing in the stomach of dogs and cats, but not of laboratory animals, and demonstrated that when the rabbits infected with the spirochetes isolated from dogs or cats were inoculated in combination with the *virus fixe*, remarkable hemorrhagic inflammation in the gastric mucosa was encountered.⁴ They also demonstrated that spirochetes inoculated into the gastric mucosa of mouse were eradicated by the administration of arsaminol⁴ (A modern equivalent of arsaminol is thought to be a bismuth, a close relative of arsenic). Then, Kobayashi was appointed Professor of Bacteriology, Keio University School of Medicine in 1924, and made valuable achievement in bacteriology, especially in the field of infection and host immunity. It took 70 years until scientists again attempted to treat gastritis associated *Helicobacter*. Kobayashi’s work about the intragastric bacteria in 1919 has been revisited by Anthony T.R. Axon⁵ at the symposium “*Helicobacter pylori* – a revolution in understanding” held in Japan in 1994. To commemorate Kobayashi’s achievements in the field of *Helicobacter* research, “Rokuzo Kobayashi Memorial Symposium on *Helicobacter pylori*” was held on May 11th, 2002 at the Kita-kan Hall of the Keio University Mita Campus. At the present time, the important role played by *H. pylori* infection in the onset of gastritis and of peptic ulcer diseases is becoming clearer.⁶ *H. pylori* eradication therapy has been officially recognized also in Japan since November 1st, 2000.⁷

*H. pylori* Urease

*H. pylori* is a spiral-shaped gram-negative rod, a few microns in length. At the end of the organism, there are
a number of long flagella. These are flailed vigorously in a screw-like motions, developing a driving force that causes the organism to advance by spinning like the bit of a drill. How can *H. pylori* survive in the strong acid of the stomach? *H. pylori* has a large amount of urease, an enzyme that breaks down urea. When it releases urease, the urea in the mucus layer is converted to ammonia, which quickly neutralizes the surrounding acid and enables *H. pylori* to protect itself against its acidic environment. Urease, an acid neutralizer, together with vigorous movements of bacterium enables *H. pylori* to infect and colonize the gastric mucosa.

Urease is present both in the cytoplasm and bound to the outside surface of the bacteria. The origin of the surface urease continues to be controversial. Since the amount of externalized urease increases with time in culture, urease is thought to be released into the extracellular milieu by autolysis. Scott et al. have shown that intrabacterial, not surface, urease is responsible for acid resistance in *H. pylori* when the external pH is less than 4.0. In particular since surface urease is not only inactive but irreversibly inactivated at this pH or below. The mechanism whereby intrabacterial urease protects the organism from high acidity requires expression of a proton gated urea channel, which results in an influx of urea into the cytoplasm. Urea is rapidly hydrolyzed by cytoplasmic urease and the ammonia produced diffuses into the periplasm, resulting in an increase of periplasmic pH to levels consistent with cell survival and growth. External urease can play only a minor role, if any, in acid resistance since it is irreversibly inhibited at pH 4.5 and below, whereas the 24-hour median pH of the human stomach is 1.4. Even though lysis is responsible for the presence of surface urease, this urease component does not contribute to survival or growth of *H. pylori* in gastric acid.

Such a surface urease might play as an adhesion factor to the surrounding mucin or surface epithelium. Acid-driven high affinity adherence of *H. pylori* surface urease to mucin and lipopolysaccharides contributes to gastric mucosal colonization by the bacterium. Icatlo et al. examined the effect of a urease-binding polysaccharide in combination with a histamine H2 receptor antagonist (famotidine) on *H. pylori* colonization in vivo and demonstrated that the use of urease-targeted polysaccharides concurrently with a gastric acid inhibitor could warrant consideration as an additional component of the standard multidrug chemotherapy of *H. pylori* infection. Anti-*H. pylori* immunoglobulin is another candidate for targeting the bacteria-mucin or bacteria-epithelial adhesion. Recently, anti-surface urease of *H. pylori* IgY antibody produced in chicken egg is applied for the biotherapy of *H. pylori* infection (Fig. 1).

**Mongolian Gerbil Models for *H. pylori* Infection**

A recently established animal model for *H. pylori*-associated gastric disease, produced by inoculating this bacterium to Mongolian gerbils, is useful for *in vivo* observation of *H. pylori*-infected gastric mucosa. The Mongolian gerbil was first established as an experimental animal by Tatsuji Nomura and his mother Masuko Nomura in the Central Laboratory of Experimental Animals, Tokyo, Japan. In this model, we previously reported the significant levels of gastric mucosal neutrophil accumulation, and the increase in the contents of thiobarbituric acid (TBA) reactive substances, a marker of lipid peroxides. In the same model, Japanese researchers revealed the formation of gastric cancer by inoculating only *H. pylori*. The exact mechanisms by which *H. pylori* infection results in gastric mucosal injury are still unclear.

**The Onset of Gastric Mucosal Injury due to *H. pylori***

Six week-old Mongolian gerbils (MGS/Sea, body weight: 50–60 g) inoculated with *H. pylori* (ATCC 43504: 1.5 × 10⁸ CFUs/0.5 ml/animal) has been generally used for the infection model of *H. pylori*. Gastric microcirculation was visualized through an intravital...
microscope using a digital color 3CCD camera. The spiral shaped bacterial body was detected in the mucus layer by high-power lens. The velocity of rolling leukocytes on the venular endothelium and the number of adherent leukocytes was calculated by the playback image analysis of recorded VCR tape. In the normal uninfected gastric mucosa of gerbils, the mesh of a capillary network is seen between the collecting venules. The epithelial cell layer that covers the surface of the gastric mucosa is observed as a paved stone-like pattern. On the initial phase of H. pylori infection, the bacterial body moves in the mucus layer by the screw-like force of flagella and adheres on the surface of epithelial cells. As a result of the energetic movement of the H. pylori and the activity of urease, the bacteria broke through the mucus layer and colonized to the gastric mucosa.

**Microvascular Leukocyte Activation after H. pylori Colonization**

A month after the bacterial inoculation, no conspicuous changes in the gastric mucosa was seen macroscopically. However, when we observed by the intravital microscope, some microcirculatory events occurred in the venules of gastric mucosa. In particular, we documented a phenomenon in which leukocytes, especially neutrophils, were rolling along the luminal surface of the venular endothelium, or were adhering to it.

After three months of the inoculation, closer examination revealed considerable exfoliation of the epithelial cells and stagnation of the microvascular blood flow. There were great numbers of neutrophils adherent to the venular walls, and many neutrophils had infiltrated through the narrow passage in the venular walls, showing the enhanced level of leukocyte-endothelial adhesion in the mucosal as well as in the submucosal venules (Fig. 2). The velocity of rolling leukocytes in the mucosal and submucosal venules decreased in the H. pylori-colonized group.

Histological findings showed a large number of leukocytes mainly in the submucosa in the stomach with H. pylori infection. Myeloperoxidase (MPO) activity, an index of tissue-associated neutrophil accumulation, was also determined by a modification of the method of Grisham et al. MPO activity of the stomach started to increase significantly in the gerbils of H. pylori-colonized group at 3 months after the inoculation to the level eight fold of the control group.

It is known that, as a result of leukocyte activation, reactive oxygen metabolites are produced through the NADPH oxidase on the membrane of neutrophils. Of these substances, the monochloramine (NH2Cl) which is produced by the H. pylori-derived NH3 and neutrophil-derived HOCl, plays an important role on the gastric mucosal injury (chromatin condensation and DNA fragmentation) in H. pylori-infected stomach. Although it is technically difficult to measure the tissue level of NH2Cl, it might be enhanced in the gastric mucosa of H. pylori-inoculated gerbils. Such a nitrous compound might become more potent when the level of gastric mucosal pH would elevate as a result of the extension of corpus gastritis or long-term antisecretory therapy.

**Mast Cell and H. pylori-associated Gastritis**

In the gastrointestinal mucosa, cells located outside microvessels, such as mast cells, could affect the
dynamics of microvascular events. Mast cell in the gastric mucosa is strategically located at the optimal site and interacts with invading bacteria. It is reported that mast cell increases in *H. pylori* gastritis. Bamba et al. investigated the kinetics of mast cells and mast cell growth factor (stem cell factor; SCF) in *H. pylori*-positive and -negative gastric mucosa and found that densities of mast cells, proliferating cell nuclear antigen (PCNA)-positive mast cells, and SCF-positive cells were significantly greater in *H. pylori*-positive than *H. pylori*-negative subjects. They also reported that SCF was expressed in mast cells and fibroblasts and that the density of SCF-positive fibroblasts increased in *H. pylori*-positive gastritis and decreased after bacterial eradication, suggesting the importance of SCF for mast cell increment.

Montemurro et al. showed that a neutrophil-activating protein in *H. pylori* (HP-NAP) induces β-hexosaminidase release and interleukin-6 (IL-6) production in peritoneal mast cells, two actions which were completely inhibited by pertussis toxin. They also showed that in polarized epithelial cell monolayers, HP-NAP translocates from the apical to the basolateral domain, where mast cells were located.

On the other hand, presence of VacA, the virulent *H. pylori* cytotoxin, is correlated with the severity of *H. pylori*-induced gastritis. Inoculation of VacA resulted in epithelium vacuolization and marked infiltrations of mast cells and mononuclear cells into the mucosal epithelium. In an in vitro study using bone marrow-derived mast cells, VacA directly bound and showed a chemotactic activity to the mast cell. In addition, VacA induced bone marrow-derived mast cells to produce proinflammatory cytokines, TNF-α, macrophage-inflammatory protein-1 α, IL-1 β, IL-6, IL-10, and IL-13 in a dose-dependent manner without causing degranulation, suggesting that early activation of mast cells by VacA might be the host early response to clear the bacteria and also may contribute to the pathogenesis of *H. pylori*-induced gastritis (Fig. 3).

The WBB6F1 W/Wv mice, which show a lack of tissue mast cells with a lack of hair pigmentation, are resistant to gastric lesion formation induced by ethanol administration. There is also a possibility that the stomach of mast cell-deficient, WBB6F1 W/Wv mice, are resistant to *H. pylori* infection. Such a study should be completed in near future.

Only a little aspect of gastric mucosal response to *H. pylori* has been summarized in this review. More and more efforts to investigate the host response to *H. pylori* and to elucidate the mechanism of *H. pylori*-associated gastric mucosal injury should be performed.

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References


