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Intracellular *Helicobacter pylori* and Gastric Carcinogenesis: An “Old” Frontier Worth Revisiting

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See “Intracellular, Interstitial, and stromal invasion of gastric mucosa, preneoplastic lesions, and cancer by *Helicobacter pylori*” by Necchi V, Candusso ME, Tava F, Luinetti O, Ventura U, Fiocca R, Ricci V, and Solcia E on page 1009–1023.

It has been more than 20 years since Warren and Marshall demonstrated that the human stomach can be colonized by a spiral bacterium, now called *Helicobacter pylori*, and that there is a strong association between the presence of *H pylori* and gastric and duodenal ulcer.¹ This observation was initially received with skepticism, but the impetus was given for many laboratories to investigate the pathogenic role of *H pylori* in gastric diseases. The results of these studies progressively convinced the medical community that the infection was present in a majority of patients with peptic ulcer disease and that the ulcer disease condition was cured if *H pylori* was eradicated by antibiotics.² Soon after the discovery of *H pylori*, several groups demonstrated an association between *H pylori* and gastric adenocarcinoma,^{3,4} confirming an earlier prediction that one or several environmental factors may play a role in gastric carcinogenesis.⁵ The association between gastric adenocarcinoma and *H pylori* was confirmed by many subsequent investigations, leading to the consensus that the bacterium is a class I carcinogen.⁶

However, even today, we do not know why about 80% of *H pylori*-infected subjects do not have peptic ulcer disease or gastric cancer and, as a corollary, how *H pylori* colonization of the antrum produces gastroduodenal ulceration or gastric adenocarcinoma in the remaining subjects. In an attempt to explain this conundrum, the role of bacterial virulence and of host predisposition to the disease was explored. First and foremost, peptic ulcer disease and gastric adenocarcinoma appear to be present preferentially in subjects infected by virulent strains carrying genes such as the vacuolating cytotoxin *vacA* and the cytotoxin-associated pathogenicity island (*cag* PAI).⁷ In addition, persons with cytokine gene polymorphisms and/or low pepsinogen I levels may be at a higher risk of developing gastric adenocarcinoma.^{8,9} Unexpectedly, early studies showed that *H pylori* was not detected in the gastric lumen of patients with precancerous and cancerous lesions, indicating that persistence of the infection was not necessary to promote the late stages of cancerization. However, as discussed below, more recent studies¹⁰ have demonstrated that this hypothesis needs to be revised. In addition, *H pylori* is generally present in patients with early gastric carcinoma, and eradication of *H pylori* infection may prevent relapse following mucosal resection in these patients.¹¹

To understand *H pylori* pathogenicity, it is useful to detail the process of gastric colonization by the bacterium. The majority of *H pylori* are located in the gastric lumen and mucus where

they congregate in dense colonies. In addition, individual bacteria adhere to epithelial cells through the binding of multiple adhesins to the corresponding host receptors.^{12–14} These intimate interactions facilitate the entry of bacterial proteins into the host cells through a type IV secretion system with resulting cytoskeletal rearrangements and tyrosine phosphorylation of host proteins.¹⁵ In addition, infection increases the expression of genes implicated in modulation of angiogenesis and proinflammatory and mucosal defenses in host cells, and decreases expression of tumor suppressor genes in those cells.¹⁶ Finally, an intense humoral and cellular inflammatory response is mounted by the innate and adaptive immune systems,¹⁷ which are unable to clear the infection. The initial trigger for the immune response is believed to be the interaction of the bacterium with toll-like receptors¹⁸ of gastric epithelial cells, which function as antigen-presenting cells and, in turn, stimulate lamina propria immunocytes.¹⁹ The ensuing influx of neutrophils, mononuclear cells, and T-helper 1 (Th1) cells is a response typically directed against intracellular bacteria.¹⁷ However, *H pylori* is generally considered to be noninvasive, perhaps because it was not seen inside the gastric mucosa by some early investigators.²⁰

During the past 20 years, however, the penetration of *H pylori* into the gastric mucosa was documented by many light and transmission electron microscopy (TEM) studies of cell lines and biopsy specimens, as reviewed in 2003.²¹ In brief, the steps of *H pylori* internalization by epithelial and immune cells were documented, including their total engulfment and enclosure within cytoplasmic vacuoles. In one of the studies, many intracellular spiral *H pylori* were usually observed within defined membrane-bound vacuoles, but they did not appear to replicate and seemed to degenerate.²² Other authors found that although many *H pylori* indeed degenerated, some of them maintained a normal ultrastructural morphology even after phagocytosis by immunocytes²³ and were viable by the acridine orange method.²⁴ In addition, immunocyto-chemistry (including immunogold staining) and in situ hybridization studies demonstrated that intracellular *H pylori* can express virulence genes within gastric epithelial cells, as well as immune cells, both in vitro and in vivo.^{10,25,26} *H pylori* was also detected within progenitor cells, which may be critical for its life-long persistence in the human stomach.²⁷ Finally, *H pylori* were observed within gastric wall capillaries²⁸ and were detected by hemoculture,²⁹ demonstrating that *H pylori* can be an invasive organism. Importantly, these intracellular *H pylori* do express specific mRNA and proteins corresponding to virulence factors within preneoplastic and neoplastic cells of North American patients (Figure 1) and the number of intracellular and interstitial *H pylori*- and *cagA*-positive clusters are increased in patients with gastric intestinal metaplasia and cancer compared to control subjects with gastritis.¹⁰

In the present issue of GASTROENTEROLOGY, Necchi et al³⁰ use TEM and immunogold staining to present the first independent confirmation of the presence of *H pylori* within gastric preneoplastic and neoplastic cells as well as blood capillaries, and extend the previous observation to a European population. They first verified the sensitivity and specificity of their antibodies by using molecular and histologic methods and cultures of wild-type *H pylori* compared with mutants lacking the *cagA* and *vacA* genes. They then examined gastric biopsies from 20 dyspeptic patients and specimens from 20 gastric cancers using a combination of light microscopy, immunocytochemistry, and TEM with immunogold staining. Importantly, invasive *H pylori* were present in a majority of the *H pylori*-positive gastric mucosae examined. In addition, the intracellular “*H pylori*-like bodies” observed by Necchi et al³⁰ were alive by their normal ultrastructural appearance and also were *H pylori* because the immunogold staining showed that they were capable of expressing virulence factors specific to this bacterium. The authors’ detailed observations also suggest that the bacteria may enter the gastric mucosa by trespassing disrupted tight junctions that subsequently reconstitute after *H pylori*-passage, using the same route as neutrophils passing from mucosa to lumen and from lumen to mucosa in the presence of gastrointestinal inflammation.³¹

Necchi et al³⁰ extend earlier in situ hybridization and immunohistochemistry demonstrations that intracellular and interstitial *H pylori* can express virulence factors by localizing VacA and CagA antigens at the ultrastructural level to discrete bacterial body spots by gold particles. In particular, CagA was detected at sites corresponding to bacterial/cell adhesion sites. In addition, their immunogold procedure demonstrates that the VacA cytotoxin can be secreted into intracellular vacuoles and mucus (eg, Figure 7A). Interestingly, their Figures 7A and 9A illustrate that VacA antigens are detectable not only in different parts of *H pylori*, but also in the membrane of the host vacuole, indicating that *H pylori* can produce native vacuolating cytotoxin within infected cells; that is, in close proximity to vital host proteins and genes. Such localization may represent the in vivo illustration of the entry of virulence products into host cells that was observed in vitro.³² Also of great interest is their observation that the immunogold reactivities to CagA as detected in intracellular *H pylori* were different in distribution and intensity compared to those detected in intraluminal or cultured bacteria. The potential implications of these differences are unclear, but these alterations may represent one of the *H pylori* adaptations that ensure its intracellular or interstitial survival.

The extraordinary precision of the present ultrastructural observations has several tradeoffs. First, the studies are limited to a small number of patients because few centers have the resources to perform TEM procedures, and archived specimens adequately fixed in glutaraldehyde are not generally available. Second, in contrast to in situ hybridization and immunocytochemistry, quantification of differences between groups is difficult in TEM pictures, as illustrated by the fact that the authors did not attempt to verify whether distribution and intensity of immunogold reactivity was different in gastric cancer versus gastritis subjects as previously reported.¹⁰

Importantly, however, these novel observations provide convincing ultrastructural evidence that *H pylori* can enter and survive within normal and neoplastic epithelial cells. Because relatively few *H pylori* are present within the wall of the gastric mucosa, the invasiveness of the bacterium has often been considered irrelevant for carcinogenesis. However, it may be worthwhile to consider that direct intracellular expression of virulence genes such as *vacA* and *cagA* by *H pylori* may be more likely to modify critical molecular processes involved in gastric carcinogenesis than having these products injected into the host cells through a type IV secretion system. The carcinogenic effect may be magnified if differentiated cells are dividing rapidly and/or are epithelial progenitor cells.²⁷ In addition, *H pylori* invasiveness may play an important role in the generation of an intense local and humoral immune response. Finally, it may also help to understand why antimicrobial therapy often fails to eradicate *H pylori* infection, and reinforces the importance of using antibiotics that achieve high intracellular concentrations. New studies specifically addressing these questions will be necessary to demonstrate that *H pylori* invasiveness plays a principal or accessory role in *H pylori* pathogenicity.

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Figure 1.

Detection of intracellular *Helicobacter pylori* in patients with gastric adenocarcinoma. Sections of formaldehyde-fixed and paraffin-embedded biopsies were prepared using in situ hybridization and a probe recognizing either *cagA* (A, B) or *babA* (C, D), and were counterstained with nuclear fast red as described. The blue stain of *H. pylori* mRNA expression demonstrates the presence of the bacteria between and within the pink host cells. Neoplastic cells are characterized by large elongated nuclei and increased nuclear-to-cytoplasmic ratio and are associated to inflammatory cells (A, B). In other patients, neoplastic cells have marked hyperchromatism of nuclei and increased nuclear-to-cytoplasm ratio (C) or are poorly differentiated with pleomorphism, patchy hyperchromatism of nuclei, variable nuclear-to-cytoplasm ratio, and cytoplasmic vacuoles (D; arrows). Both in situ hybridization (this figure) and TEM with immunogold (see pages 1009–1023) specifically identify *H. pylori* and they can complement each other. The latter allows precise ultrastructural analysis of the relation between *H. pylori* and host cells and the former permits screening of a large number of host cells and their spatial relation with the bacteria. Bars: 10 μ M. Biopsy specimens provided by Dr V. Simko (Brooklyn VA Medical Center, Veterans Administration New York Harbor Healthcare System, Brooklyn, New York). Figure prepared by C. Semino-Mora, Uniformed Services University, Bethesda, MD.