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Citation Classic

Detecting a Bacterial Protein to Understand Cancer Risk

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Featured Article: Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111–5.[4]

In 1982, *Helicobacter pylori* was discovered in the human stomach in association with inflammatory cells infiltrating the gastric mucosa, a condition known as chronic gastritis. Barry Marshall and Robin Warren received the 2005 Nobel Prize in medicine for the discovery of *H. pylori* and determining its role in peptic ulcer disease. Within several years, it became clear that persons carrying *H. pylori* were also at increased risk for the most prevalent types of gastric cancer *(1)*. Yet, *H. pylori* colonization is highly prevalent, and only a fraction of colonized individuals become ill. Work in my laboratory since 1985 has sought to define the antigens of *H. pylori*, to identify virulence factors, and to develop diagnostic tests to ascertain carriage and genotype. Studies initiated in 1988 by postdoctoral fellow Dr. Timothy Cover focused on an activity in culture supernatants from some but not all *H. pylori* strains that induced vacuole formation in epithelial cells. Cover ultimately purified a protein, which we called VacA, that specifically signals host cells.

In the 1980s, to characterize the vacuolating activity Cover prepared culture supernatants from toxin-positive and toxin-negative strains, which we probed in Western blot analyses with serum from persons carrying such strains. The strategy worked! We identified a band migrating at 128 kDa that was recognized by serum IgG from persons with toxin-positive strains but not from those with toxin-negative strains *(2)*. Importantly, only approximately 60% of persons with gastritis showed antibodies to this 128-kDa protein, in contrast to 100% of patients with duodenal ulceration. The following year, when Crabtree et al. reported that gastric IgA antibodies recognized a 120-kDa band in nearly identical proportions of patients with gastritis and ulcer disease *(3)*, we knew that we were on the right track!

We also screened libraries of *H. pylori* genes in bacteriophage λ gt-11 by using serum from a person (me) who had strong antibody responses to *H. pylori*. This approach yielded *Escherichia coli* clones that produced *H. pylori* antigens recognized by my serum IgG. In July 1989, we purified a clone with a high molecular weight antigen. Dr. Murali Tummuru, another postdoctoral fellow in the lab, completed the cloning and found a 128-kDa protein product *(4)*, which we discovered to be the same protein that Tim Cover studied. We initially called the gene *tagA* (toxin-associated gene A); however, we learned that Italian colleagues (Antonello Covacci, Rino Rappuoli, and others) had discovered the same protein *(5)*, which they had intended to call *caiA* (cytotoxin-associated immunodominant gene A). We compromised and coined a single gene name (*cagA*) that has persisted into the present *(4, 5)*.

Our work and that of Crabtree et al. *(2, 3)* provided evidence that carrying *cagA*-positive strains increased the risk of peptic ulcer disease. We then focused on gastric cancer to determine whether *cagA* positivity was associated with an increased risk for that disease. We used a recombinant fragment (orv660) as an antigen to detect specific serum anti-CagA IgG *(6)*. We validated the assay with serum from persons who were known to be *H. pylori* negative or who were *H. pylori* positive but from whom a *cagA*-negative strain was isolated. These studies showed a strong specificity for the assay, and nearly all those who had a *cagA*-positive strain were seropositive (indicating the high sensitivity of the assay).

With Dr. Abraham Nomura, we had previously found an association between *H. pylori* infection and intestinal-type distal gastric cancer in Japanese American men in Hawaii *(1)*. Using the now-validated recombinant CagA assay, we assessed CagA associations with such cancers. Our studies showed that *H. pylori*–positive men who carried a *cagA*-positive strain had a risk of developing intestinal-type distal gastric adenocarcinoma in the subsequent 21 years that was increased by 130% (odds ratio, 2.3; 95% CI, 1.0–5.2), compared with men carrying a *cagA*-negative strain *(6)*. Thus, the polymorphisms of *H. pylori* permitted the development of an assay that detects serum responses to a specific bacterial protein and is thereby able to predict the risk for developing the most common form of gastric cancer worldwide.

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Much subsequent work has confirmed our observations, and we now know that *H. pylori* injects the CagA protein into its host's epithelial cells! The injected CagA interacts with host proteins to determine cell properties and fate. Our work that used host antibody responses detected in clinical samples to probe bacterial antigens has advanced our understanding of gastric carcinogenesis and ultimately opened the door to exploring whether other important clinical conditions are influenced by CagA status.

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