T-cell-mediated mucosal immunity in the absence of antibody: lessons from *Helicobacter pylori* infection

Peter B. Ernst and Jacques Pappo

Departments of Pediatrics, Microbiology & Immunology and the Sealy Center for Molecular Sciences, University of Texas Medical Branch, Galveston, Texas, and Department of Immunology, Infection Discovery, AstraZeneca R&D Boston, Waltham, Massachusetts, USA

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Approximately 50% of humanity is infected with *Helicobacter pylori*. This lifelong infection elicits a marked host response, including a robust gastric IgA response. However, natural infection fails to yield protective immunity. Rather than providing protection, the chronic inflammatory response associated with natural infection can contribute to tissue damage and the pathogenesis of gastroduodenal disease, including atrophic gastritis, peptic ulcer, and gastric cancer. These immune responses are attributed to a subset of helper T cells, so-called Th1 cells, that enhance cell-mediated immunity and induce damage to the gastric epithelium. Thus, it is desirable to have effective vaccines that could prevent and cure infection and that may modify the host response in a manner that prevents immune-mediated disease. Using animal models as a tool to understand the immunobiology of *Helicobacter* infections, several investigators have shown that effective vaccines can be developed. Thus, prophylactic and even therapeutic vaccines have been described in various animal models. The basis for the effectiveness of these vaccines appears related to their ability to alter the gastric immune response, from a homogeneous Th1 response to a mixed Th1 and Th2 response. Interestingly, immunity can occur in the absence of B cells, suggesting that novel IgA-independent mechanisms exist that confer protection against a luminal infection. Thus, *H. pylori* infection provides a model with which new mechanisms of immunological protection can be identified and applied to other mucosal infections. □ *Cytokines; immunity; immunopathogenesis; T cells; vaccines*

Peter B. Ernst, D.V.M., Ph.D., Children's Hospital, Room 2300, UTMB, 301 University Blvd., Galveston, TX 77555-0366, USA. Tel: +1 409 772-1750, fax: +1 409 772-1761, e-mail: Pernst@utmb.edu

Overview of diseases associated with *Helicobacter pylori*

Helicobacter pylori infects more than 50% of humanity and is responsible for several gastroduodenal diseases (1–8). Approximately 90% of recurrent duodenal ulcers will disappear after successful eradication of the organism. *H. pylori* is also responsible for approximately 50% of gastric ulcers and appears to be a factor contributing to autoimmune gastritis. It is the only bacterial species that has been designated by the World Health Organization as a carcinogen because infection is a factor contributing to gastric adenocarcinoma and gastric MALT (mucosa-associated lymphoid tissue) lymphoma. Although *H. pylori* infection has the potential to induce very serious disease, it should be considered that only 10%–15% of infected individuals actually develop one of the more severe outcomes of infection.

Whereas most of the clinical manifestations of *H. pylori* infection are limited to the gastroduodenal regions of the digestive tract, there are more far-reaching elements. For example, the search for an understanding of disease transmission has pointed to both fecal–oral transmission and dental plaque as potential sources of the bacteria (9–14). It is also interesting to compare the immunobiology of *H. pylori* as a model for other types of chronic inflammatory diseases caused by infections of persistent stimulation with environmental or dietary antigens. As discussed below, the ability to study the host response to a known pathogen such as *H. pylori* may well have implications for our understanding of mucosal immunity throughout the digestive tract, including the oral cavity.

The immunopathogenesis of disease

The interaction between the host and the bacteria represents 'two solitudes' from which to consider the pathogenesis of disease. Obviously, the microbial trigger itself is essential, and in the case of *H. pylori* several interesting virulence factors have been suggested to play a key role in the pathogenesis of gastroduodenal disease (5–7, 15). However, with severe disease occurring in a small minority of infected subjects, other environmental or host factors must be important. Indeed, recent genetic studies have associated diversity in the structure of genes encoding proteins that regulate the immune response in the pathogenesis of gastroduodenal disease, including interleukin (IL)-1 (16) and IL-10 (17). This discussion will focus on the immunological aspects of the pathogenesis.

Several review articles have described the morphological changes associated with *H. pylori* infection (2, 18, 19). The histopathology can be best described as chronic, active inflammation. During infection, neutrophils are present in the gastric lamina propria, adjacent to the

Table 1. Host responses in different mucosal sites within the digestive tract

Factors	Normal	Inflamed
Chemokines	Low to undetectable	Increased IL-8, ENA-78
Neutrophils	Few	Marked increase
T-cell responses	Few	Increased, often marked Th1 responses
Antibody	Predominantly IgA	Increase in IgG, IgM, and activated complement
Epithelial cell responses		Increased expression of chemokines, class-II MHC, and PIgR on epithelial cells Adenocarcinoma in some forms of chronic inflammation

Normal mucosal immune responses in the digestive tract are often characterized as having a 'low-grade' inflammatory response. In response to normal flora, these responses include a mixed cellular infiltrate comprising B cells, plasma cells, T cells, and occasional polymorphonuclear cells. Most of the antibody response in these sites is IgA. These 'common' mucosal responses are believed to be important in protecting the host from damage that could occur in the course of an overly exuberant response. One would predict that the failure of normal responses would lead to less desirable responses and inflammation. Indeed, in periodontitis, gastritis, celiac disease, and inflammatory bowel disease, the host responses have several common elements. The most dramatic is the increase in phlogistic antibodies including IgG and IgM in periodontitis (67), gastritis (68), celiac disease (69), and inflammatory bowel disease (70), usually in association with activated complement (27, 71–73). In the presence of chronic bacterial stimulation, an increase in chemokine expression is associated with an accumulation of neutrophils. Often, Thl-like responses are found. Several changes also occur in epithelial cell gene expression, including an increase in chemokines, class-II major histocompatibility complex (MHC), and the polymeric immunoglobulin receptor (PIgR), whereas subjects with *Helicobacter pylori*-induced gastritis, celiac disease, and ulcerative colitis have an increased risk of adenocarcinomas, presumably due to persistent oxidative stress.

epithelium, and can even be seen migrating across the epithelium into the lumen. There is also an increase in mononuclear cells in the gastric lamina propria including T cells, B cells, plasma cells, macrophages, and mast cells. Lymphoid aggregates are particularly characteristic of the infection. The normal tissue architecture can be disrupted, and changes include increased epithelial cell proliferation, offset by a compensatory increase in apoptosis, mucosal atrophy, and, in some cases, epithelial cell metaplasia.

Neutrophils and macrophages

The innate response is triggered by bacteria that stimulate gastric epithelial cells to produce chemokines, including IL-8 (20, 21) and epithelial neutrophil-activating peptide 78 (ENA-78) (22, 23), which recruit and activate neutrophils. This chemokine production is exacerbated by cytokines produced by local T cells and macrophages, including tumor necrosis factor-α (TNF-α) and interferon-gamma (IFN-γ) (24). These two cytokines can collaborate with the bacteria to stimulate additional production of chemokines by the epithelium.

There are many potential roles in the pathogenesis of gastroduodenal disease for the activated neutrophil and macrophage. Both cell types are excellent sources of reactive oxygen and reactive nitrogen species that can modify cell signaling and impart significant damage, particularly to host cell DNA. Given the protracted course of the infection, chronic inflammatory responses may result in epithelial stem cell injury and be a factor contributing to the development of gastric adenocarcinoma (2, 25).

B cells

Other cells, present during the host response, contribute to the inflammation, epithelial cell damage, and possibly to gastroduodenal disease. B cells are present throughout the mucosa, and it has been shown that *H. pylori* can stimulate a B-cell response that recognizes gastric epithelial cells including the parietal cell (26). Thus, by molecular mimicry and/or nonspecific activation of autoreactive B cells, a local autoimmune-like reaction can evolve. This notion is supported by the observation that activated complement is present in the infected gastric mucosa (27) and, thus, may contribute to immune complex-mediated damage.

T cells

The gastric T cells have also been implicated in contributing to inflammation and tissue damage. Chemokines, including RANTES (23, 28), are increased in response to infection and can recruit and activate T cells to the site of infection. Reports have now described the presence of IL-12 (29, 30) and IL-18 (31) in the gastric mucosa. Both of these cytokines can direct the differentiation of gastric T cells to a subset referred to as T helper (Th) 1 cells. Helper T cell subsets differ in the production of cytokines and the effects these cytokines have on selecting for a particular effector cell response. Th1 cells are best known for the production of IL-2, TNF-α and IFN-γ . In contrast, Th2 cells do not produce significant levels of these cytokines but do produce IL-4, IL-5, IL-10, and IL-13. Other subsets are being described, including Th3 cells, which resemble Th2 but also produce TGF-β, and so-called Tr1 cells—yet another subset that will be discussed below. Whereas these functional distinctions may seem complex, the T cells generated in response to *H. pylori* in natural infection of humans are almost all Th1 cells (29, 32–37). This type of homogeneity is not protective, as infection persists for life. Moreover, gastric Th1 cells can damage the epithelium directly or indirectly by producing cytokines that stimulate other inflammatory responses (38–40). Although we may not understand what governs the expression of genes in Th1 and Th2 cells at a

particular point in time, it is important to realize that they generally correlate to at least two very different responses. Th1 cells are generally proinflammatory and, when they persist, can contribute to autoimmune diseases, whereas the other subsets can favor controlled responses and immunity.

Several similarities exist in the local mucosal response in healthy and diseased tissues of the oral cavity, stomach, small intestine, and colon (Table 1). An interesting aspect of understanding the immunopathogenesis of *H. pylori* is therefore what this knowledge can teach us about other chronic inflammatory diseases. In contrast to the oral cavity or colon, where hundreds of species of bacteria reside, the gastric flora is very limited. Having a specific pathogen in hand is an outstanding resource, and thus, insight into the pathogenesis of *H. pylori* or effective approaches in vaccine development may well predict directions for advancement in our understanding of host/pathogen interactions in other mucosal sites.

Mechanisms of immunity

Even though research related to *H. pylori* has only emerged since 1983, investigators in this field have enjoyed several advantages over their colleagues studying other chronic inflammatory diseases in the digestive tract. First of all, a single etiologic agent that triggers gastroduodenal disease has been identified. Second, there are excellent animal models that have facilitated the research.

The role of antibodies in protection against Helicobacter

One of the first interesting advances was the development of a vaccine to prevent infection with *H. pylori*. Czinn et al. (41) showed that the passive administration of high concentrations of IgA were effective at preventing infection in mice. At the same time, others showed that human breast milk was capable of neutralizing the bacteria and prevent infection in an animal model (42). These observations were consistent with the general view on protective effector mechanisms in the mucosa. However, it should be pointed out that the level of specific or nonspecific antibody has seldom represented a consistent correlate of immunity.

Several groups have examined the emergence of mucosal immunity in the *Helicobacter spp.* model in laboratory animals (43–46). Given that infection persists in humans and laboratory animals, it was easy to conclude that the host response was ineffective in preventing infection. Since the major T-cell response was Th1-like, and antigen-specific IgA responses are believed to be largely Th2-dependent, it followed that a vaccination protocol that induced Th2 responses might be effective. Using the effective mucosal adjuvant, cholera toxin, several groups have now shown that many different *H. pylori* antigens administered with cholera toxin can induce protective immunity (47–51). In addition, *Escherichia coli* toxins can be similarly effective as adjuvant (52, 53). It was further shown that vaccination not only prevents, but also can treat, a pre-existing infection (43, 54–56).

These observations were consistent with many paradigms for mucosal immunity that were developed over the past 30 years. However, the advent of genetically engineered animals has provided a new resource that has exposed the gaps in our knowledge and hinted about exciting new effector mechanisms of immunity. Perhaps the most surprising observation that challenged the traditional view of mucosal immunology was that mice that were rendered deficient in IgA still were capable of mounting protection against challenge to *Helicobacter* (57). This observation is consistent with other studies in which it was reported that effective immunity could be established in mice that were completely deficient in B cells (57, 58). Moreover, there is no apparent increase in infection rate in humans lacking IgA (59). This does not prove that B cells cannot provide protection, but they do point to the fact that other effector mechanisms are also important in providing immunity to luminal pathogens.

T-cell-mediated immunity in the absence of B cells

Although immunity against *H. pylori* infection appeared not to be dependent on B cells, the role of T cells still remained to be clarified. Michetti et al. (60) have shown that T cells expressing a receptor that directs homing to mucosal tissues are important in immunity to *H. pylori*. Studies in class-I-deficient mice have shown that protective immunity could be induced but not in animals lacking class-II major histocompatibility complex (MHC) (58, 61). These findings suggest that T cells bearing CD8 are not essential. This hypothesis is in agreement with the fact that *H. pylori* exist almost exclusively in the lumen, and it would therefore have been surprising if cytotoxic T cells were required for immunity. CD4 helper T cells that recognize class-II MHC are, however, evidently important for vaccine immunity.

This returns the discussion to the role of the cytokine profile in Th subsets. Clearly, the phenotype of the Th cells in the infected mucosa cannot provide sterile immunity; thus, vaccines must induce a different, or complementary, change in cytokine gene expression. In fact, several publications have shown that Th cells resembling the Th2 phenotype seem to be more effective at conferring protection against *H. pylori* infection than Th 1 cells (62, 63). Complementation of the host response by mixing both Th1 and Th2 may be even more important, given that the absence of the Th1-derived cytokine, IFN-γ, is associated with increased bacterial load (64). While these studies implicate the importance of T-cell phenotypes, the effector mechanisms driven by any of these T-cell subsets remain to be defined.

The role of the salivary glands in immunity

Another interesting manipulation has shown that

removal of the salivary glands in mice makes the animals incapable of responding effectively to vaccines. Shirai et al. (65) performed sialoadenectomy before and after intragastric immunization, using whole-cell sonicates of *H. pylori* and cholera toxin as adjuvant. Subsequently, infection was assessed. The procedure decreased the levels of both total gastric IgA and post-immunization *H. pylori*-specific IgA by approximately 50%. Interestingly, the surgical treatment affected the induction of immunity preferentially, as animals that had their salivary glands removed after immunization still had protective immunity that decreased bacterial density 1 month after surgery. Five months later, however, immunity was impaired, and bacterial densities were increased. These observations suggested that immunity was not sterile and that the recrudescence of the infection was attributable to the removal of the salivary glands. Nonetheless, this paper raises the point that remote sites may contribute to mucosal immunity in unknown ways.

Other novel mechanisms contributing to mucosal immunity

The complexity of understanding effector mechanisms in immunity can be appreciated best when one considers the millennia during which the host response has evolved. Innate responses have developed in the most simple species. Remnants are found in humans, but the impact of these innate mechanisms is unclear. For example, molecules such as Toll-like receptors, complement proteins, defensins, various proteases, lactoferrin, lyzozyme, pH, and so forth can all contribute significantly to immunity. Specifically, somatostatin was recently shown to inhibit *H. pylori* bacterial cell proliferation (66). Whereas this report was limited to in vitro observations, it indicates that many different molecules may contribute to the interaction with the bacteria and limit their ability to inflict damage. However, to which extent T cells can regulate expression and secretion of innate host factors, and how such factors may contribute to immunity post-immunization, is now just beginning to be investigated.

Implications for mucosal immunity

It appears likely that inappropriate immune responses to a given pathogen or cadre of microbial flora may lead to disease in genetically susceptible hosts. The fact that polymorphisms in genes encoding pro- or anti-inflammatory cytokines are associated with gastroduodenal disease supports the notion that dysregulation of the immune response is important. What is equally interesting is the fact that the host response can be manipulated in a manner to prevent or treat infection. The mechanisms for immunity to *H. pylori* appear to be novel and may provide a completely innovative approach to the prevention of diseases in the digestive tract. Future studies will undoubtedly unmask additional aspects of the role of the host response in the pathogenesis and prevention of gastroduodenal disease associated with *H. pylori* infection. This can be expected to contribute significantly to our understanding of mucosal immunity in other sites.

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