

The Immune Response to *Helicobacter pylori*

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Summary

The immune response to *Helicobacter pylori* involves different mechanisms that are both protective and damaging to the host. The innate and the adaptive immune responses lead to inflammatory as well as anti-inflammatory responses, allowing for persistence of many infections. Thus, developing new therapeutics and effective vaccines against *H. pylori* has proven to be arduous. Despite many immunisation experiments, using various routes of immunisation with classical as well as recombinant *H. pylori* vaccines (urease, CagA, HP-NAP, HspA, DNA, chimeric molecules, live vectors, microspheres), no effective vaccine is currently available for humans. New directions for successful vaccine construction should follow a profound knowledge of immunopathological events during natural *H. pylori* infection and factors leading to resolution of infection: mandatory is a new knowledge about the interplay of the innate response to *H. pylori*, mucosal inflammation, *H. pylori* virulence factors inducing immune responses, regulation of the adaptive responses to *H. pylori* as well as construction of novel vaccine platforms for achieving a broad immune response, leading to a sterilizing immunity.

Key words: *Helicobacter pylori*, immune response, lymphocyte activation, vaccine construction

Introduction

Helicobacter pylori is well established as the causative agent of chronic gastritis. Other diseases associated with *H. pylori* infection include duodenal ulcer, gastric ulcer and gastric carcinoma. An etiological role of this organism in the development of these diseases is still unclear, although evidence strongly supports its major role. *H. pylori* infects an estimated 50 % of the world's population, but only a small proportion of individuals develop clinical symptoms of gastritis, peptic ulceration or gastric cancer. The variations in disease presentation may be due to factors that influence the bacterial virulence or the host response to infection with *H. pylori*. The local cellular and humoral immunity as well as non-specific mucosal defence mechanisms have been suggested to be involved in the pathogenesis of *H. pylori*-related diseases as well (1).

Infection with *Helicobacter pylori* induces strong local immune responses in the gastric mucosa of *H. pylori*-in-

fectured hosts. It is characterized by the recruitment of neutrophils, T and B lymphocytes, plasma cells, macrophages and dendritic cells (DCs), together with epithelial cell damage. Gastric mucosa of *H. pylori*-infected individuals contains increased concentrations of several pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-2, IL-6, IL-8, IL-12 and tumour necrosis factor (TNF)- α and anti-inflammatory cytokines IL-4 and IL-10 (2). The increased production of inflammatory cytokines in response to *H. pylori* infection results in the development and recruitment of specific T lymphocytes sensitized to *H. pylori* antigens and B cells producing immunoglobulin (Ig)A and IgG. Still, the immune response cannot eliminate the bacteria, and unless antibiotic treatment is used, the infection is usually lifelong (2,3).

When considering the immunity against such a successful and adapted pathogen like *H. pylori*, we must first reconsider some of the main protective forces of the host that could damage the microorganism and prevent

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the infection. Since *H. pylori* colonises gastric mucosa, the gastric protective mechanisms play a central role in the parasitic-host events in *H. pylori* infections (1). The human stomach, as part of gastrointestinal tract, is exposed to a broad spectrum of dietary and bacterial antigens. The stomach epithelium is the primary cellular barrier between the outside world and the host. The immune system must effectively protect the stomach against pathogens without damaging the epithelium or perturbing its functions. Furthermore, the immunity must distinguish microbial from dietary antigens to prevent an immune response against dietary antigens (2,4).

Adaptation Mechanisms of *H. pylori* to the Host

During infection *H. pylori* colonises the surface of the gastric epithelium especially in the antrum. *H. pylori* does not reside within the acidic environment in the lumen of the stomach. Instead, the microorganism colonises more neutral microenvironment of the mucus layer lining and the surfaces of the epithelial cells. In addition, the *H. pylori* produces an urease. An ammonia, produced by the enzyme, forms a cloud around the bacterium, thus protecting it from the acid. Because the turnover of the gastric mucus layer and gastric epithelial cells is very rapid, *H. pylori* has developed several adaptations that avoid its rapid removal from the stomach. The specialised motility of *H. pylori* allows it to move from the lumen down through the mucus onto the gastric cell surface. *H. pylori* developed a vast number of adhesion molecules, interacting with various structures in the mucus and stomach epithelial surfaces (5).

A special adaptation may be the feature that *H. pylori* expresses adhesion molecules only in certain phases of bacterial growth. This means that bacteria are bound to the gastric epithelium in certain growth phases and in other phases they are not. This interchangeable binding prevents *H. pylori* from being removed completely from the stomach together with the superficial epithelial cells that are shed into the lumen. In this way, attached and unattached bacteria may exist in the stomach all the time. While the attached bacteria are shed together with the stomach epithelia, the unattached bacteria are able to infect new yet uninfected epithelial lining. This is one of the proposed mechanisms that enable *H. pylori* to persist in the stomach (6).

Inflammatory Response of the Host

Microbial invasion is, in part, prevented by the epithelial tight junctions and is complemented by mucus production and various antibacterial proteins such as lysozyme, lactoferrin, interferon and defensin/cryptidin. In response to epithelial stress (chemical, microbial, toxic), inflammatory changes are initiated by the release of cytokines and other mediators, which recruit and activate polymorphonuclear cells, monocytes and mast cells. The host innate immune system plays a key role in the initiation and the subsequent progression of the *H. pylori*-associated pathogenesis (7). Gastric epithelial cells (GECs) are primary target for *H. pylori* infection, and actively contribute to the innate immune responses by signalling through pattern recognition receptors, such as Toll-like

receptors (TLRs). GECs are the first point of contact for *H. pylori* and express TLRs that may activate an innate immune response. Although lipopolysaccharide (LPS) is the classical bacterial ligand for TLR4, *H. pylori*-derived LPS has been reported to signal through TLR-2 and has low binding affinity for the TLR4. *H. pylori* was shown to inhibit macrophage release of nitric oxide in response to *H. pylori* LPS in a mouse model of infection. *H. pylori* LPS was also shown to suppress TLR-4 signalling, but enhance IL-12 and IL-18 production, which was suggested to be linked to the chronic inflammation commonly seen during infection (8).

Inflammation is a crucial player in the *H. pylori* immune response. During inflammation resulting from the infection, bone marrow-derived mesenchymal stem cells are recruited to the gastric mucosa. These cells are thought to be underlying promoters of gastric cancer. A recent study shows that *H. pylori* infection of gastric epithelial cells induces migration of mesenchymal stem cells, which was dependent upon NF- κ B activation and TNF- α production in an *in vitro* model (9).

Specific Immune Responses

Apart from the non-specific inflammatory response, the immune responses are relatively sparse in uninfected stomachs. The intra-epithelial lymphocytes (IEL) are all T cells and the majority have the suppressor/cytotoxic (CD8) phenotype. The lamina propria contains both T and B lymphocytes, and the majority of the T cells are of helper (CD4) phenotype. The plasma cells in the lamina propria secrete predominantly IgA. IgA is selectively transported across the epithelial surfaces by the polymeric immunoglobulin receptor (secretory component) and can provide immunity without inducing much inflammation (10).

D'Elios *et al.* (11) found high mRNA expression of interferon (IFN)- γ , TNF- α , and IL-12, but not IL-4 in antral biopsies from the *H. pylori*-infected patients, but not from uninfected controls. Furthermore, upon *in vitro* lysate stimulation, T lymphocytes from the *H. pylori*-infected patients derived CD4⁺ T cell clones mostly (over 80 %) producing IFN- γ , but not IL-4 or IL-5. Results provide evidence for *H. pylori*-specific Th1 effectors in the gastric antrum of *H. pylori*-infected patients.

Besides Th1 response, *H. pylori* is able to drive gastric Th17 response. Amedei *et al.* (12) demonstrated that *in vitro* produced T cells from *H. pylori*-infected patients with distal gastric adenocarcinoma produced IL-17 and IL-21 in response to the secreted peptidyl prolyl *cis-trans*-isomerase of *H. pylori*.

Innate immune sensing and signalling is the starting point of specific immunity. Central to this process, dendritic cells (DCs) integrate innate information and convey it to the lymphocytes. The DCs activation by *H. pylori* is mediated largely by the TLRs (13).

H. pylori has developed a variety of mechanisms to persist in the gastric mucosa. Regulatory T cells (Tregs) are induced during infection. They express the FoxP3 transcription factor and inhibit inflammatory T cell responses by secretion of inhibitory cytokines IL-10 and TGF- β . Tregs are induced when TGF- β is present, along

with PDL-1 expression on antigen-presenting cells (13, 14). A unique feature of the gastric epithelium is the ability to act as antigen-presenting cells in expressing class II MHC and co-stimulatory and co-inhibitory molecules. Gastric epithelial cells were shown to produce TGF- β after exposure to *H. pylori* (12). *H. pylori*-induced TGF- β was shown to inhibit CD4⁺ T cell proliferation and lead to Treg development, suggesting a mechanism that it uses to subvert the host response and persist in the gastric mucosa (15).

***H. pylori* Virulence Factors and Immune Responses**

In the past decade extensive studies confirmed the pathogenic role of the cytotoxin-associated gene-pathogenicity island (cagPAI) as a virulence factor linked to carcinogenesis. Recently, CagA has been termed an oncoprotein due to its intracellular activities that lead to dysregulation of cell division (16,17). Once inside cells, CagA is phosphorylated by Src tyrosine kinases. CagA increases the motility of gastric epithelial cells (18), hence its potential for a metastatic role. CagA also induces over-expression of microRNAs, leading to increased NF- κ B and Erk1/2 signalling, targeting, and inducing epithelial-mesenchymal transition and intestinal metaplasia of gastric epithelial cells (19).

The vacuolating cytotoxin A (VacA) virulence factor has long been associated with host damage by forming pores in host cell membranes, disrupting membrane trafficking and inducing apoptosis. Mechanisms associated with apoptosis include VacA-induced inhibition of Stat3 and Bcl-2 cell survival proteins (20). In addition, a caspase-independent process is activated, which involves the histone-binding high mobility group box 1 protein, consistent with known necrosis pathways. The end result is epithelial cell necrosis and release of inflammatory proteins that contribute to pathogenesis (20).

H. pylori cell division-related gene A (*cdrA*) was shown to induce NF- κ B activation and IL-8 production. This expression of *cdrA* is accompanied by higher levels of mucosal IL-8 in the *cdrA*-positive samples compared to the *cdrA*-negative samples (21).

Urease enables *H. pylori* to colonise the acidic environment of the stomach. A protective mechanism of the host, CD46, C3b/C4b-binding complement regulator bound to *H. pylori*, inhibits urease and thus enables the bacteria to survive in an acidic environment (22). The urease B subunit leads to Th17 responses in the mouse model of *H. pylori* infection (23). When recombinant urease B was incubated directly with mouse splenic lymphocytes, the number of IL-17-producing cells increased and when macrophages were incubated with recombinant urease B, IL-6 and IL-23 were produced to support Th17 development.

H. pylori lipopolysaccharide (LPS) has been shown to induce weaker immune responses than LPS from other bacteria. Particularly, LPS from *H. pylori* did not induce strong IL-1 β , IL-6, or IL-8 responses (24), as other bacterial LPSs do. *H. pylori* LPS was also shown to induce little NF- κ B activation through TLR4, but was shown

to induce IL-12 and IL-18 responses, which are thought to be pro-inflammatory.

Progress in Vaccine Development against *H. pylori*

Numerous studies of possible vaccines against *H. pylori* demonstrate that it is possible to achieve an immune response that is effective enough for its therapeutic eradication (2). Efforts to develop a vaccine for the prevention and treatment of *H. pylori* infection began in earnest in the early 1990s, with the recognition that *H. pylori* is the most important cause of peptic ulcer disease and gastric cancer. Although the prevalence of *H. pylori* is declining in developed countries, the current understanding is that vaccination of infants to prevent *H. pylori* infection would be cost effective. However, one can hardly escape the impression that results to date have been disappointing. Sterilizing immunity has rarely been achieved in animal models, there is no consensus on the choice of antigens, adjuvants, or delivery route, and the few clinical trials have generally been unsuccessful. Nevertheless, some progress is being made in the identification of novel antigens and in our understanding of mechanisms of protection, which increasingly focuses on the role of Th1- and Th17-biased cellular immune responses, as well as Tregs (25).

The study by Borody *et al.* (26) tested the hypothesis that an impaired immune response may contribute to failed eradication after standard antibiotic therapy. The ability of lymphocytes to produce IFN- γ and IL-4 was measured. They found a significant reduction in the secretion of IL-4 from blood T cells in subjects failing to eradicate *H. pylori*, compared with those who successfully eradicated the infection in lymphocyte cultures. A significant difference in IL-4 secretion was also detected in antigen-stimulated cultures, compared with that in *H. pylori*-positive subjects. Low levels of IL-4 secretion were detected irrespective of the number of courses of antibiotic therapy. In our experiments, we did not find differences in cytokine production in non-stimulated lymphocyte cultures.

Moss *et al.* (27) developed a vaccine platform using a computational method to predict novel T cell epitopes. The multi-epitope vaccine was administered intranasally or intramuscularly to *H. pylori*-infected mice, followed by a boost with the peptides formulated in liposomes with CpG oligonucleotides and heat-labile enterotoxin. The vaccine induced a broad immune response, which was determined by IFN- γ production, and led to a sterilizing immunity 32 weeks after challenge in five out of 19 mice.

Another promising vector platform for expression of *H. pylori* antigens was developed by Iankov *et al.* (28). They produced a measles virus (MV) vaccine strain encoding the *H. pylori* neutrophil-activating protein (NAP). Nine months post vaccination all animals immunized with MV strains, which expressed the secretory NAP antigen, developed a strong humoral immunity against NAP. IFN- γ ELISpot assay confirmed the effective NAP-specific cell-mediated immunity. Their experiments demonstrated that immunization with a live replication-

-competent vaccine expressing *H. pylori* molecules (NAP or potentially CagA, VacA, etc.) induced not only robust antibody production but also distinctive cell-mediated response against *H. pylori* antigens.

Improved efficacy of vaccines may be achieved with new vaccine formulations that include multiple antigens and use different methods to optimize cellular immunity. Chen *et al.* (29) used a *H. pylori oipA* gene-encoded construct co-delivered by IL-2 and B subunit heat-labile toxin of *Escherichia coli* gene-encoded construct. Intradermal co-delivery of adjuvant(s) enabled the shift of the immune response from being Th2- to being Th1-biased, which resulted in a greater reduction in bacterial load after *H. pylori* infection. A similar approach used *Salmonella* vector construct that expressed fusion proteins complexed with *H. pylori* CagA, VacA and UreB in different arrangements (30). Oral therapeutic immunization significantly decreased *H. pylori* colonization in the stomach; protection was related to the combination of Th1, serum IgG and mucosal IgA responses.

In our attempt to construct effective vaccine formulation, we constructed a chimeric flagellin, in which both terminal segments of *H. pylori* flagellin (that is TLR5-non-activating flagellin) were replaced by the corresponding segments from TLR5-activating *E. coli* flagellin (25). Recombinant chimeric flagellin folded correctly and was able to activate TLR5. Significantly increased serum IgG and IgA antibody responses were determined in mice vaccinated with chimeric flagellin in comparison with mice vaccinated with a control protein (FlaA) or negative control. Antibody titers remained high even 8 months after the last immunization. Antibodies were able to bind native flagellin from *H. pylori* lysate. Vaccination with chimeric flagellin provided mice with significant protection against *H. pylori*. The approach of chimeric flagellin can therefore generate effective immunogens that enable activation of innate and adaptive immune response and can be used to construct efficient vaccines against *H. pylori* or other flagellated bacteria that evade TLR5 recognition.

Guo *et al.* (30) used a fusion protein construct of cholera toxin B subunit expressed in *E. coli* and a UreA epitope of *H. pylori* urease to develop a vaccine with good immunogenicity and immunoreactivity that could induce specific neutralizing antibodies. However, the efficiency of the vaccine should still be confirmed by a sterilizing immunity trial, since urease vaccine targets have a long history of rather disappointing results.

Our Experiences – Mucosal Immunity in *H. pylori* Infection

The local cellular and humoral immunity in gastric mucosa was suggested to be involved in the pathogenesis of *H. pylori*-related diseases as well. It is well documented that a simple chronic gastritis is associated with an excessive local IgG production, T cell infiltration and increased epithelial HLA class II expression (1,31).

In our studies, we defined changes in the local immune response between the chronic gastritis, associated with *H. pylori* (urease-positive gastritis) and the chronic urease-negative gastritis. In order to define the type of local immune response that may predict the outcome of

H. pylori infection, we analysed lymphocytes infiltrating inflamed gastric mucosa. After isolation of gastric mucosa lymphocytes, we evaluated the expression of lymphocyte molecules that determine differences in lymphocyte activation between the gastritis caused by *H. pylori* and other types of gastritis (32).

We demonstrated a significant increase in IL-2 receptor expression in gastric mucosa T cells in ulcer patients compared with non-ulcer dyspeptic patients (33). In the following study we analysed the expression of IL-2 receptor and intracellular lymphokine (IFN- γ , IL-4) production in gastric mucosa infiltrating T lymphocytes in duodenal ulcer patients before and after *H. pylori* eradication. We detected a prevalence of Th1 T cells compared to Th2 T cells in gastric mucosal tissue. A proportion of Th2 T cells did not change significantly in patients one or twelve months after *H. pylori* eradication. On the other hand, the percentage of IFN- γ -producing cells diminished significantly after *H. pylori* eradication (34).

Our results suggest that long-term lymphocyte gastric mucosa infiltration after *H. pylori* eradication may reflect homing of memory lymphocytes induced by mucosal antigen-presenting cells. Dendritic cells (DCs) and other antigen-presenting cells constitute the initial immune response towards *H. pylori*. Immature DCs in the mucosa-associated lymphoid tissue are capable of migrating through epithelial tight junctions to gain access to the gastrointestinal lumen (35). Upon exposure to bacterial components, immature DCs differentiate into mature, antigen-presenting cells with upregulated expression of major histocompatibility complex (MHC) class I and II, adhesion/costimulatory molecules and induce production of inflammatory cytokines (36).

As DCs play a pivotal role in shaping the immune response, we investigated whether the strain-dependent differences in *H. pylori*-derived LPS influence the activation of the DCs and cytokine secretion. To examine the modulatory role of *H. pylori* strains in antigen presentation, we use cathepsin X inhibitor, a monoclonal antibody 2F12. Cathepsin X activity promotes adhesion of DCs as well as their phagocytic function (37). We demonstrated that cathepsin X mediates the activation of b2 integrin receptor Mac-1 (CD11b/CD18), and that it may also regulate the maturation of DCs, a process which is crucial for the initiation of adaptive immunity (38).

We also examined dendritic cells stimulated by *H. pylori* isolated from patients with repeated antibiotic eradication failure and found that in them human leukocyte antigen (HLA-DR), CD86, TLR-2, and interleukin-8 (IL-8) were less expressed compared to *H. pylori* strains susceptible to antibiotic therapy; the latter expressed lower production of IL-10 (39). Polymyxin B inhibition of LPS reduces IL-8 secretion in the group of *H. pylori* strains susceptible to antibiotic therapy. The differences in IL-8 secretion between both groups are LPS dependent, while the differences in secretion of IL-10 remain unchanged after LPS inhibition. Inhibitor of cathepsin X Mab 2F12 reduced the secretion of IL-6, and the secretion was significantly lower in the group of *H. pylori* strains isolated from patients with repeated antibiotic eradication failure. Our results suggest that different *H. pylori* strains, susceptible/resistant to antibiotic eradication therapy,

differ in their LPS capability to induce DC maturation and antigen-presenting function.

Conclusions

The immune response to *Helicobacter pylori* involves innate and adaptive immune changes, which lead to damaging inflammatory reactions, but may also allow persistence of infection. Despite many immunisation experiments, using various routes of immunisation with classically as well as recombinantly prepared *H. pylori* vaccines (urease, CagA, HP-NAP, HspA, DNA, chimeric molecules, live vectors, microspheres), no effective vaccine is currently available for humans. New directions for successful vaccine construction should follow a profound knowledge of immunopathological events during natural *H. pylori* infection and factors leading to resolution of infection: mandatory is the new knowledge about the interplay of the innate response to *H. pylori*, mucosal inflammation, *H. pylori* virulence factors inducing immune responses, regulation of the adaptive responses to *H. pylori* as well as construction of novel vaccine platforms for achieving a broad immune response, leading to sterilizing immunity.

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