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Immunity and Vaccine Development Against *Helicobacter pylori*

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Abstract

Helicobacter pylori is a highly-adapted gastrointestinal pathogen of humans and the immunology of this chronic infection is extremely complex. Despite the availability of antibiotic therapy, the global incidence of *H. pylori* infection remains high, particularly in low to middle-income nations. Failure of therapy and the spread of antibiotic resistance among the bacteria are significant problems and provide impetus for the development of new therapies and vaccines to treat or prevent gastric ulcer, and gastric carcinoma. The expansion of knowledge on gastric conventional and regulatory T cell responses, and the role of T_H17 in chronic gastritis from studies in mouse models and patients have provided valuable insights into how gastritis is initiated and maintained. The development of human challenge models for testing candidate vaccines has meant a unique opportunity to study acute infection, but the field of vaccine development has not progressed as rapidly as anticipated. One clear lesson learned from previous studies is that we need a better understanding of the immune suppressive mechanisms *in vivo* to be able to design vaccine strategies. There is still an urgent need to identify practical surrogate markers of protection that could be deployed in future field vaccine trials. Important developments in our understanding of the chronic inflammatory response, progress and problems arising from human studies, and an outlook for the future of clinical vaccine trials will be discussed.

Keywords

Helicobacter pylori · Gastritis · Immunity · Vaccine · Regulatory T cell

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1 Introduction

A recent meta-analysis of publications from 73 countries reported that *Helicobacter pylori* infects 44.3% of the global population (Zamani et al. 2018). It is believed that after acquiring infection in childhood, most patients are infected for many decades. What is fascinating about *H. pylori* infection, is that only around 15% of these develop symptomatic gastric disease in the form of chronic gastritis, peptic ulcer, mucosa associated lymphoma, and 1–3% develop carcinoma (Parsonnet et al. 1991; Posselt et al. 2013). Given that *H. pylori* establishes a chronic infection in such a high percentage of the

population, it is perhaps more pertinent to ask the question of why such a low proportion of infected persons develop symptoms? It would appear that this long- term relationship between the bacterium and host is finely balanced.

One overarching explanation for this tight relationship lies in the long period of co- evolution of *H. pylori* with the human host (Moodley et al. 2012). The studies that document the migrations of human populations with their *H. pylori* and have elegantly illustrated this (Falush et al. 2003). Consistent with the success in the wide distribution of this pathogen, and hand in hand with idea of co-evolution, is the proposal that *H. pylori* was a member of the gastric microbiota until relatively recently (Otero et al. 2014). It has been reasoned that improvements in hygiene, and widespread use of antibiotics in the decades since they became widely available in the 1940s mean that *H. pylori* is now carried by less people. The status of a flora organism may go some way to explain the minimal response in many *H. pylori* infected individuals. Indeed, some workers have described *H. pylori* as a pathobiont (Arnold et al. 2017).

The human host also shows evidence of co-evolution insofar that although an immune response is generated after infection, the bacterium is not cleared. Indeed, the nature of the natural immune response to *H. pylori* infection appears to be aimed not at clearance, but rather at the suppression of overt inflammation, and therefore symptomatic disease. The current status of our understanding of this suppressive milieu will be discussed here, with a focus on the knowledge gained from analysis of clinical material.

The recommendations of the 2017 consensus report on clinical management of *H. pylori* infections include what has been called a paradigm shift in the clinical view- that *H. pylori* disease is an infectious disease that should be treated in all patients were infection is detected, not just in symptomatic patients (Malfertheiner et al. 2017). This shift provides additional impetus for development of better therapies, and for prevention and vaccine development.

Given the extent of *H. pylori* disease worldwide, and problems with antibiotic resistance and treatment failure, an efficacious vaccine is a desirable aim. Vaccine development although initially promising, has struggled to progress in recent years and lacks investment. The current status of vaccine clinical trials will be discussed in the context of our understanding of the natural immune response and mechanistic studies in animals. Finally, we discuss a view to the way forward for vaccine development against this challenging pathogen.

2 The Natural Immune Response to *H. pylori* – A Tight Balance of Chronic Inflammation and Suppression That Ultimately Fails to Control Infection

From an immunological standpoint, chronic infection with *H. pylori* is a complex equilibrium between the inflammatory and suppressive aspects of immunity. A combination of bacterial and host factors are now known to orchestrate this.

Histologically, the chronic-active gastritis seen in the "snap-shot" from biopsies from symptomatic adults infected with *H. pylori* is characterized by strong infiltrates of monocytes, polymorphs, and lymphocytes. Lymphoid follicles containing primed B cells form, and infection persists despite the production of *H. pylori* specific IgA, and IgG (Dixon 2001). More detailed analysis of gastric infiltrates has revealed that in addition to macrophages and neutrophils, $CD4^+$ and $CD8^+$ and B lymphocytes, are present (Bamford et al. 1998a; Muñoz et al. 2007).

Early studies established that *H. pylori* gastritis is T_H1 biased with significant amounts of IL-8 and interferon-γ (IFN-γ) detected in infected humans (Bamford et al. 1998b; Lindholm et al. 1998) and mice (Eaton et al. 2001). Studies in $CD4^+$T cell depleted mice revealed that $CD4^+$ T cells were required for the development of *H. pylori* gastritis (Eaton et al. 2001). Seemingly at odds with these pro-inflammatory responses, is the presence of a $CD4^+$ subset of regulatory T cells (Treg). Treg are MHCII restricted and have the ability to reduce the effector functions of activated T cells, B cells, dendritic cells (DC) and natural killer cells (NK) (Sakaguchi et al. 1995).

The role of Tregs in suppressing inflammation and promoting chronic infection has been investigated in depth, and *Helicobacter*-specific Tregs were demonstrated in both humans (Lundgren et al. 2003) and mice, (Raghavan et al. 2004). In functional studies, Tregs were depleted from infected C57BL/6 mice using anti-CD25 antibody, resulting in increased levels of gastritis, increased antibody titres, and bacterial numbers (Rad et al. 2006). A study published in the same year reported however that depletion of Tregs from infected BALB/c mice did not affect either gastritis or colonization, but antibody IgG1 titres were increased (Kaparakis et al. 2006). Further, the effect was the same when the mice were infected with *H. pylori*, or the close relative *H. felis* (Kaparakis et al. 2006). The T_H2 bias of the BALB/c background probably explains the difference seen in the effects of depleting $CD25^+$ Tregs on *H. pylori* induced inflammation, and this highlights the fact that the cytokine milieu also has an overriding effect on Treg function, an observation that has also been made for other "non- responsive" mouse strains (Nedrud et al. 2012). Another aspect that Treg depletion studies have brought to light is the potential for autoimmune reactions, the use of anti-CD25 antibody to deplete Tregs did not increase the incidence of autoimmune gastritis in wild-type C57BL/6 or BALB/c *H. pylori* infected mice, but did in 1E4-TCR mice that are predisposed to auto-immune gastritis (Kaparakis et al. 2006). As discussed, there is potential for bias in results due to the genetic background of the mice used, however if Tregs are to be investigated as therapeutic targets for ulcer and tumours, the development of autoreactivity should be included in study design.

2.1 First Contact- the Intensity of the Initial Inflammatory Response Is Limited by Host Factors

The gastric mucous layer has a complex structure and is composed predominantly of gel-forming mucins MUC5AC and MUC6 which forms a protective layer above the epithelium (reviewed in (McGuckin et al. 2011)). This barrier is not only physical, and a signalling role for Muc1 has been demonstrated. Studies using *H. pylori* infected $Muc1^{-/-}$ knockout mice show reduced inflammation compared to wild-type littermates (McGuckin et al. 2007; Guang et al. 2012) and inhibition of the NLRP3 inflammasome (Ng and Sutton 2016). Studies in AGS cells with Muc1 knock-down or overexpression, have resulted in the conclusion that Muc1 acts to prevent activation of the pro-inflammatory transcription factor NF-κB (Guang et al. 2010), and that it also interacts with both CagA and β-catenin, resulting in attenuation of IL-8 production (Guang et al. 2012). A number of antibacterial epithelial factors also inhibit *H. pylori* in the inner gastric gel layer including MUC6, defensins (HBD2, LL37), galectin 3, and Trefoil factors (TFFs) also play roles in preventing access (reviewed in (Dhar et al. 2016)). The protective effects of the mucous layer, in combination with lipopolysaccharide (LPS) that is less pyrogenic and immunoreactive than that of other Gram-negative pathogens (Moran 2007), in part explain the comparatively mild inflammation triggered even in acute infections. Recently developed *in vitro* models employing organoid cultures of polarized, mucin secreting, human gastric epithelia have also provided new potential for investigations on the interactions between *H. pylori* and the epithelium (Schlaermann et al. 2016; Sigal et al. 2017). These models also present exciting possibilities to investigate the interactions between immune cell populations and the gastric epithelium.

2.2 *H. pylori* Drives Dendritic Cells (DC) to a Tolerogenic Phenotype

Activation of the gastric epithelium brings DCs into play. Dendritic cells patrol mucosal surfaces to provide early recognition of pathogens. Intra vital microscopy has shown DCs in the upper area of the stomach mucosa in mice (Kao et al. 2010), and cells with DC-like morphology were also detected by electron microscopy in biopsies from heavily infected humans (Necchi et al.

2009). In the latter study, projections were seen to contact *H. pylori,* consistent with earlier reports of intraluminal sampling of other pathogens by DCs in the intestine (Rescigno et al. 2001). This observation indicates that despite the establishment of long- term Treg populations, DCs continue to patrol and present antigen, potentially contributing to the chronic-active nature of *H. pylori* gastritis.

Another feature that may prevent excessive inflammation overall is the focal nature of *H. pylori* infection. Local damage to the mucous or epithelial barrier facilitates focal colonisation. Morey and co-workers have described a possible specific mechanism, in that the actions of *H. pylori* cholesterol-α-glucosyltransferase effectively deplete cholesterol in the epithelium, effectively interfering with inflammatory signalling and thus permitting inflammation only in adjacent areas (Morey et al. 2018). Foci of inflammation are reported in human biopsies (Cherdantseva et al. 2014) and colonisation is not distributed uniformly throughout the stomach. This is also one reason for the consensus recommendation of a minimum of two biopsies from the antrum and two from the middle of the gastric body for clinical diagnosis (Malfertheiner et al. 2017).

Despite the actions of a number of host factors which appear to dampen responses, an *H. pylori*-specific immune response does develop. Expression of epithelial Toll-like receptors (TLRs) is increased (Lagunes-Servin et al. 2013, Pachathundikandi et al. 2015), and cytokines and chemokines are secreted which attract DCs and neutrophils. Dendritic cells co-localized with lymphocytes secreting IL-23 and IL-17 were reported in antral biopsies from *H. pylori* infected patients (Khamri et al. 2010), and CagA-positive *H. pylori* were more effective in stimulating DC to produce IL-23, promoting IL-17 secretion by autologous $CD4^+$ T cells (Fig. 1). Other studies have implicated bacterial factors in driving a DC response that is skewed towards Treg responses. This response was originally reported to be independent of CagA or Vac A in murine DC *in vitro* (Kao et al. 2010). In a later study however, vacA and γ-glutamyl transpeptidase (GGT) both independently promoted generation of tolerizing DCs in a mouse model (Oertli et al. 2013). In the case of CagA, the mechanism of this effect appears to be that it impairs maturation of human DCs, and therefore promotes development of tolerogenic DCs (Kaebisch et al. 2013). In addition, a recent study using peripheral blood mononuclear cells (PBMC) suggested that *H. pylori* heat shock protein 60 (HpHSP60) acts on macrophages to trigger secretion of IL-10 and TGF-β to promote production of Tregs (Hsu et al. 2018) (Fig. 1).

2.3 Reduced Inflammatory Responses in *H. pylori* Positive Children

H. pylori infection is thought to be acquired in early childhood in the majority of cases, however symptoms frequently do not manifest until decades later. An understanding of the progression of inflammatory responses in young children will enhance our understanding of how the gastritis is largely suppressed, and perhaps how best to target a prophylactic vaccine. Studies in a cohort of Chilean children showed that in an area of high prevalence approx. 60% of children had positive stool antigen detected by ELISA by 2 years of age (O'Ryan et al. 2015). Of these children, 22% were persistently infected (several sequential positive stool antigen tests) by the age of 5, and 11% were only intermittently infected. Significantly, only 2% of children in the study had clinical and pathological findings after follow-up by a gastroenterologist. The latter observation is consistent with the findings of a number of studies that have all reported that low levels of gastritis in children (Muñoz et al. 2007; Harris et al. 2008). These reports suggest that the Treg response is established soon after initial infection, and indeed increased proportions of Tregs were reported in children compared to (symptomatic) adults in both Chile and Brazil (Harris et al. 2008; Freire de Melo et al. 2012). Results from studies with neonatal mice are consistent with this, and neonatal infection resulted in reduced inflammation that was dependent on Tregs (Arnold et al. 2011). While reduced gastric inflammatory response minimizes symptoms it does not however

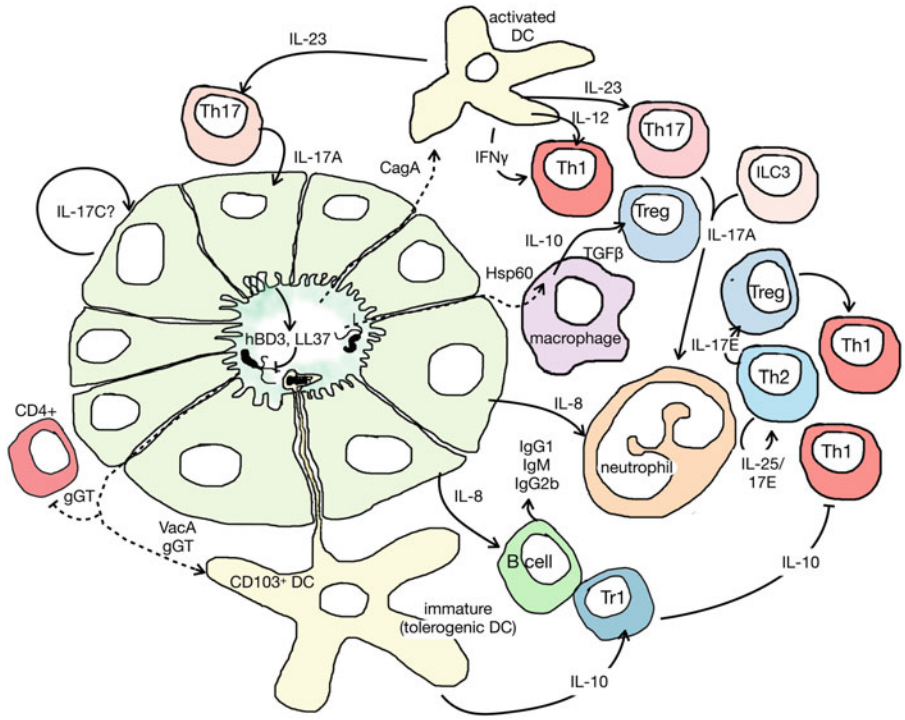

Fig. 1 Complex control of chronic inflammation in *H. pylori* infection is mediated by a balance of T_H1/T_H2/T_H17 and Treg responses. Bacterial factors (dotted lines) VacA and GGT prevent maturation in dendritic cells (DC), promoting Treg responses, GGT also interferes with cell- cycle progression in CD4 + T cells. HpHsp60 drives macrophages to secreted TGF-β and IL-10, promoting induction of Treg. CagA positive strains stimulate IL-8 production by epithelial cells which attracts neutrophils and B cells (solid arrows), CagA can also activate DC to secreted IL-23 and IL-12 promoting pro-inflammatory T_H1 and T_H17 T cell populations (red shades). T_H17 (and possibly ILC3), secrete IL-17A which induces antimicrobial peptide (eg. HBD3, LL-37) production by epithelial cells, contributing to mucosal defence. Anti-inflammatory T cell populations (blue shades) Treg suppress the actions of T_H1 and T_H17 cells by contact dependent means, Tr1 secrete IL-10 which also suppresses T_H1

suppress the growth of *H. pylori,* and children have similar levels of colonization as adults (Freire de Melo et al. 2012), whereas neonatally infected mice had significantly higher levels of colonization (Arnold et al. 2011).

2.4 Chronic- Active Gastritis Is Driven by the Actions of $T_H1/17$ and Treg

The central role of $CD4^+$ T cells in *H. pylori* gastritis is still not completely understood and a strong focus has been the study of the role of T_H1/T_H2/T_H17 cells and the cytokines that regulate these populations. Early studies showed that *H. pylori* specific $CD4^+$T cell clones can be isolated from the stomach of chronically infected subjects, and that they secreted IFN-γ and tumour necrosis factor-α (TNF-α) but not IL-4 or IL-5 (D'Elios et al. 1997). Since then, levels of inflammatory cytokines IFN-γ, TNF-α, IL-1, IL-6, IL-7, IL-8, IL-17-A, IL-18, and IL-23, as well as anti-inflammatory cytokines- transforming growth factor (TGF-β) and IL-10, have all been reported to be present at increased levels in the mucosa of *H. pylori* infected compared to healthy patients (Di Tommaso et al. 1995; Luzza et al. 2000; Mizuno et al. 2005; Caruso et al. 2008; Bagheri et al. 2017).

It appears that individual patient differences in the expression levels of these cytokines determine the severity of inflammation and disease outcomes. Indeed, polymorphisms in the

promotor regions of many cytokines, are associated with increased cancer risk (reviewed in (Rivas-Ortiz et al. 2017)). In a recent comparison of paired antral and corpus biopsies, the frequencies of IL-17A and IFN-γ^+ cells were increased in *H. pylori* infected patients, particularly in those with gastric ulcers (Adamsson et al. 2017). Of note, was that although *H. pylori* were detected in both antrum and corpus, increased cytokine expression and the inflammation were only seen in the antrum. Studies in the mouse model have shown that IL1-7A secreted by T_H17 cells drives IL-8 production by epithelial cells, recruits neutrophils, and B cells (Algood et al. 2009), thus contributing to mucosal defence, but also to chronic active gastritis (reviewed by (Chamoun et al. 2018)) (Fig. 1).

The link between proinflammatory and regulatory responses is intrinsically linked by T_H17 plasticity (Morrison et al. 2013), and the decision of antigen stimulated cells to differentiate in to T_H17 or Tregs is dependent on the cytokine-driven activation of the transcription factors RORγt (T_H17) or FOXP3$^+$ (Treg) (Omenetti and Pizarro 2015). The actions of IL-17 are mediated by binding to multimeric receptors (IL-17RA-E) that are expressed on a variety of cell types including epithelial cells, lymphocytes, and myeloid cells, as such IL-17R signalling acts as a link between innate and adaptive immunity (Gaffen 2009).

Most studies on the role of IL-17 in *H. pylori* pathogenesis to date have focussed on IL-17A, and the related IL-17F, and studies using IL17 and IL-17R deficient mice, and depletion of IL-17 with antibodies have revealed an import role in the control on gastritis (Delyria et al. 2009) and on vaccine-induced protection (Velin et al. 2009; Flach et al. 2012). Mice that are deficient in IL-17RA $^{-/-}$ do not control *H. pylori* numbers as well as wild-type mice, and this is linked to reduced recruitment of neutrophils, but enhanced B cell recruitment (Algood et al. 2009). In contrast to IL-17A which is secreted chiefly by T cells, other members of the IL-17 family (IL-17B-E) are secreted by a wide range of cell types including the gastric epithelia, and investigation of these in the future is likely to bring new understanding to the control of inflammation in the gastric mucosa. A recent study showed that levels of IL-17C were increased in biopsies of *H. pylori* infected patients, and that expression was localized to epithelial, and chromogranin A positive endocrine cells (Tanaka et al. 2017). The mechanistic role of IL-17C remains unresolved but its receptor IL-17RE is expressed in stomach glandular tissue, suggesting a role in epithelial responses to infection.

In a model of experimental colitis, IL-17E (also known as IL-25) was reported to inhibit secretion of both IL-23 and IL-12 (Caruso et al. 2009), and is considered to promote T_H2 responses, and to enhance the function of Treg (Tang et al. 2015). IL-17E signals through a multimeric IL-17RA/RB receptor and Horvath and co-workers reported that despite some reduction in T_H2 responses, colonization and inflammation was similar in IL-17RB$^{-/-}$ and wild-type mice, indicating that neither IL-17B or E are critical for control of natural infection (Horvath et al. 2013). It would be of interest to test whether this also applies in a vaccination model.

2.5 Treg Sub-populations and the Control of Gastritis

Almost all studies on Tregs and *H. pylori* infection have focussed on FoxP3$^+$ Tregs (Lundgren et al. 2004; Enarsson et al. 2006; Harris et al. 2008; Aebischer et al. 2008). This population is CD25^{hi}, CD4$^+$, FoxP3$^+$, and are antigen-specific. A few studies have investigated the suppressive capacity of Tregs isolated from the gastric mucosa of patients (Rad et al. 2006; Enarsson et al. 2006) and mice (Raghavan et al. 2004), demonstrating the specificity for *H. pylori* antigens. Depletion of Tregs was also shown to increase severity of gastritis in mice (Raghavan et al. 2003; Rad et al. 2006).

The presence of Tregs is not sufficient to prevent disease, however, and two studies have reported that Tregs are found in both symptomatic and asymptomatic *H. pylori* infected individuals (Lundgren et al. 2004; Robinson et al. 2008). Further, a study in patients with

gastric ulcers revealed that these patients had lower frequencies of $CD4^+/CD25^+$, and lower expression of FOXP3, compared to infected donors with no ulcer (Robinson et al. 2008).

$FoxP3^-$negative Tregs (Tr1) have been detected in *H. pylori* infected mice (Blanchard et al. 2004; Nedrud et al. 2012). Tr1 cells are not antigen-specific and mediate suppressive activity via IL-10 secretion and induction of cytotoxic killing (Maynard et al. 2007) (Fig. 1). An interesting additional aspect has been raised by a recent study that reported that frequencies of Tr1 cells were increased in asymptomatic *H. pylori* infected patients, and further that the Tr1 cells from patients with GC also had reduced suppressive function (Song et al. 2018). This also raises the question of whether the combined actions of antigen specific $FOXP3^+$ Treg and $FoxP3^-$Tr1 act in concert in *H. pylori* disease. The study by Song and co-workers unfortunately did not report on the relative frequencies of $FoxP3^+$ Treg in the same patients (Song et al. 2018). More detailed studies of Treg populations isolated from non-ulcer dyspepsia, ulcer and carcinoma lesions, using approaches such as single cell expression profiling are required to provide clarify the roles of Treg subpopulations in the pathogenesis of *H. pylori* disease.

2.6 Unconventional Lymphocytes in *H. pylori* Infections

Mucosal-associated invariant T cells (MAIT) are a class of innate-like T cells that have been found in many organs and are involved in anti-microbial defences. Investigations in the mucosa and blood of *H. pylori* infected patients revealed that significant numbers of MAIT cells were present in the gastric mucosa (Booth et al. 2015; D'Souza et al. 2018), there was however no difference in the frequency of MAIT cells in the gastric mucosa of infected and healthy patients, the numbers in the blood were significantly decreased in *H. pylori* negative patients (Booth et al. 2015). These unconventional T cell populations may have as yet unknown roles in regulating inflammation.

Innate lymphoid cells (ILC) also represent an understudied area with regard to *H. pylori* infection. ILCs are of particular interest because they are distributed at epithelial surfaces and appear to act as intermediates between innate and acquired immune cells (Moro and Koyasu 2015). The cytokine secretion patterns of ILCs allow them to be classified into types that correspond generally to T_H1 (ILC1), T_H2 (ILC2) and T_H17 (ILC3). One study implicated ILC2s to be increased in *H. pylori* infected patients, and mice (Li et al. 2017). Similarly, ILC3 are a potential source of IL-17 which may drive gastritis (Fig. 1). Because there are to date no animal models that are deficient in ILC, the specifics of their roles remain to be investigated.

2.7 Acute Infection, Spontaneous Clearance and Re-Infection in Adults

The development of *H. pylori* challenge models in adult volunteers has permitted study of acute infection in adults and provided new insight in to the early infection process. In the first study using this model, volunteers were infected with a single dose of the Baylor strain (CagA-negative) (Graham et al. 2004). While some developed mild/moderate dyspepsia, all resolved by the end of the study developed inflammation with the histological appearance of "acute chronic" gastritis by 2 weeks post infection. Immunological studies on these patients were unfortunately limited, and no data on induction of Tregs were reported. Only a small number of studies have been performed with the infection model, but the data that are available resulted in a number of findings, some of which are unexpected, including apparent clearance of the infection in a proportion of those challenged (discussed later).

The participants in the challenge volunteer trials described above were all previously *H. pylori*-negative. A report from a small study on re-infection in two *H. pylori* positive individuals provides evidence for a lack of protection induced by natural infection, and both volunteers could be repeatedly re-infected after eradication (Stenström et al. 2016).

Interestingly an epidemiology study with 10-year follow-up in Sorbo San Basile in a rural area in Italy with high infection rates for *H. pylori* reported reversion to sero-negativity in only 3% of participants (Luzza et al. 2014). It seems that clearance is unusual in adults with infections that were presumably established in childhood. *H. pylori* negative adults may however possess immune mechanisms that are at least partially effective at preventing the bacterium from establishing.

These studies in acutely and chronically infected adults have revealed two interesting points: that acutely infected adults develop chronic gastritis and Treg responses are established within a short period, and further that spontaneous clearance in adults acquiring infection for the first time is relatively frequent. Further investigation of this phenomenon may reveal useful information to guide vaccine development. The successful reinfection after eradication in the study by Stenström and co-workers was cited as a potential ground for the lack of viability of a therapeutic vaccine (Stenström et al. 2016). Analysis of the mucosae of vaccinated mice has however shown that vaccination induces a different "program" in the mucosa from infection (Mueller et al. 2003; Walduck et al. 2004). Vaccinated human volunteers, also had distinct gene expression signatures (Aebischer et al. 2008).

3 Progress Towards a Vaccine Against *H. pylori*

3.1 The Need for a Vaccine and Its Potential

The current *H. pylori* eradication treatment regime using antibiotics combined with proton pump inhibitors have been improved in recent years and is discussed in detail elsewhere in this book (Chapter 13: Treatment of *Helicobacter pylori* Infection). Indeed, currently the most cost-effective method of preventing *H. pylori* induced peptic ulcers (gastric and duodenal) is antibiotic treatment. However, since peptic ulcers and cancers can be regarded as two mutually exclusive diseases, vaccination ideally should target those individuals that do not have a previous history of peptic ulcers. Thus, it would be useful to screen *H. pylori* infected individuals particularly in regions of the world where both *H. pylori* infection and gastric cancer are highly prevalent, and to select subjects for vaccination that show pre-malignant changes in their gastric mucosa. The concept of a vaccine to prevent inflammation and cancer is well established for the prevention of cervical cancer caused by the human papilloma virus (HPV). The HPV vaccine, has been successful in dramatically reducing the prevalence of infections (Machalek et al. 2018), and are projected to reduce the number of cases of cervical cancer and mortality by over 30% by 2035 (Hall et al. 2018). Gastric cancer (GC) is responsible for 9% of cancer deaths world-wide (around 80% of which are *H. pylori* associated), and a vaccine that would prevent *H. pylori*-induced GC could be expected to have a significant impact. Ideally, a highly efficacious vaccine should be based on our understanding of the *H. pylori* bacterium and its interplay with the human host in order to eradicate the infection and protect against reinfection.

Mathematical modelling studies by Rupnow and co-workers (Rupnow et al. 2000, 2001) have been helpful in understanding the potential impact of introducing a prophylactic *H. pylori* vaccine for children in developed versus low- and middle-income countries. They report that vaccines against *H. pylori* infection could be cost-effective from a long term perspective in reducing both the prevalence of the infection, and incidence of GC in the US and Japan (Rupnow et al. 2001, 2009). Since the prevalence of the infection in developed countries is decreasing naturally without intervention, a vaccination scheme running for 10 years was predicted to almost eradicate the infection at the population level (Rupnow et al. 2009). In low and middle-income countries however the scenario is different; the high prevalence rate of *H. pylori* infection means that it would require a longer time (>10 years of continuous vaccination) and also a wider vaccine reach for it to effectively reduce the

prevalence of *H. pylori* infection in the population (Rupnow et al. 2001). These are some aspects that should be considered when discussing mass vaccination schemes, which have been effective in reducing the incidence of other enteric infectious diseases.

Vaccination using a number of different strategies, and using both mucosal and parenteral route have been tested in mouse models, and in general significant reductions in colonization, but not sterilizing immunity have been observed in most models. These pre-clinical studies are too numerous to be discussed in detail here (for recent reviews see Raghavan and Quiding-Järbrink 2016; Sutton and Boag 2018).

3.2 Current Status of Clinical Trials of Candidate *H. pylori* Vaccines

After extensive pre-clinical studies that have explored a range of approaches to the design of a *H. pylori* vaccine, three have progressed to further clinical trials. These are:

1. Mucosal vaccination with *H. pylori* whole cell vaccine antigens and active *Escherichia coli* heat-labile toxin (LT) or with an LT mutant, LT_{R192G} as adjuvant (Michetti et al. 1999; Kotloff et al. 2001).
2. Oral vaccination with live attenuated *Salmonella* carriers expressing *H. pylori* urease (DiPetrillo et al. 1999; Angelakopoulos and Hohmann 2000; Bumann et al. 2001; Metzger et al. 2004; Aebischer et al. 2008) and finally;
3. Parenteral immunization with recombinantly produced *H. pylori* antigens and alum adjuvant (Malfertheiner et al. 2008, 2018).

Unfortunately, all three approaches have failed to provide a strong candidate for further clinical development. This has been in part due to setbacks experienced during the trials that were not expected, despite the designs being based on extensive pre-clinical data as discussed below.

The *H. pylori* vaccine containing the enterotoxin LT as an adjuvant, as might be anticipated resulted in side-effects such as diarrhoea in a large proportion of the volunteers (Michetti et al. 1999). Furthermore, even though both adjuvants, LT or LT_{R192G} were able to induce strong specific serum IgG or mucosal IgA antibody against the co-administered antigens, *H. pylori* colonisation was either not studied (Kotloff et al. 2001), or not affected by vaccination (Michetti et al. 1999). Thus, the long-term efficacy of the vaccine on *H. pylori* colonization will not be known due to the difficulties in studying colonization levels in the *H. pylori* infected vaccinated subjects.

Vaccination with *Salmonella* carrier expressing urease, or another candidate antigen (Hp0231), presented other challenges than the enterotoxicity of the LT or LT_{R192G} adjuvant. The *S.* Typhimurium Ty21a-based vaccine, although safe and successful in inducing strong immune responses to the *Salmonella* carrier indicating uptake and presentation to the immune system, unexpectedly resulted in only weak immune responses to the urease antigen and decreased the bacterial load only in a few volunteers (Metzger et al. 2004; Aebischer et al. 2008). The mechanisms leading to the weak immune responses to urease, particularly antibody response has not been fully explored and could possibly relate to low *in vivo* expression of urease by the *Salmonella* carrying the plasmid. In addition, another aspect to consider regarding the *Salmonella* carrier vaccination approach is that pre-existing immunity to *Salmonella* might prevent uptake during booster immunizations (Metzger et al. 2004). The two aforementioned approaches discussed above have focused on vaccination via the mucosal route.

Malfertheiner and colleagues have instead investigated the potential of a systemic route of immunization since there are known adjuvants that are registered for human use. Thus, parenteral administration of a mixture of putative protective antigens, CagA, VacA and NapA, together with an alum adjuvant (Malfertheiner et al. 2008) led to strong circulating humoral and cellular

immune responses in *H. pylori* negative healthy volunteers, which was very encouraging. A follow up clinical study with the same intramuscular immunizations with CagA, VacA and NapA, together with an alum adjuvant was carried out combined with a challenge with live *H. pylori* $CagA^+$ Baylor strain 300. The results of the study were recently reported and, compared with placebo, intramuscular immunization with alum and *H. pylori* antigens, CagA, VacA and NapA did not confer additional protection against *H. pylori* infection, in spite of the strong antigen-specific antibody and T cell responses induced (Malfertheiner et al. 2018). The study also reports that a spontaneous clearance of *H. pylori* infection in the healthy adult population occurs, a phenomenon that was also reported in a proportion of the subjects in the previous challenge study. (Aebischer et al. 2008). Since at least some adults seem to spontaneously eradicate the *H. pylori*, it has to be concluded that adults are not ideal subjects for testing vaccine-efficacy as colonization cannot be used as a primary endpoint of the trial. In the discussion of their findings, Malfertheiner and colleagues suggest that the next vaccine trial with a candidate *H. pylori* vaccine should be carried out in children that become naturally infected (Malfertheiner et al. 2018).

Indeed, vaccination of *H. pylori*-negative children and follow up of acquisition of infection was explored in a phase III trial performed between 2005 and 2007 in Nanjing, Jiangsu Province, China (Zeng et al. 2015). No prior data pre-clinical studies or phase I/II trials had been published before the phase III trial was reported. The vaccine consisted of a fusion protein of *H. pylori* urease and the non-toxic *E. coli* heat labile toxin B subunit (LTB). The LTB subunit presumably functioned as an adjuvant in the vaccine, since strong enhancement of *H. pylori* specific IgG and IgA responses against the urease antigen was reported in the subjects. The immune responses were also associated with a 72% efficacy at follow up year 1, and 65% at year three. Indicating that (1) the urease-LTB fusion protein is safe and immunogenic and (2) it could provide protection against acquisition against *H. pylori*. However, since the study was performed more than 10 years ago with no follow up, the future of this vaccine remains unclear.

3.3 Ongoing Clinical Trials Listed on Public Clinical Trials Database

At the time of writing this book chapter there was one trial recruiting for a vaccine against *H. pylori* infection (www.clinicaltrials.gov). This vaccine consists of GGT and a non-toxic derivative of cholera toxin as an adjuvant. The enzyme GGT secreted by *H. pylori* has a unique function in that it can inhibit T cell proliferation by inducing cell cycle arrest in the G1 phase of T cells (Gerhard et al. 2005) (Fig. 1). Thus, the premise of the vaccine is different from those previously tested, and is based on the principle that by inducing neutralizing antibodies through vaccination blocking GGT activity, T cell inhibition can be overcome leading to a productive T cell response to *H. pylori* antigens and protection against infection. Pre-clinical work has shown that therapeutic vaccination with GGT and adjuvant, induces neutralizing antibodies to GGT and reduction in the number of the bacteria in the stomach of mice (Anderl et al. 2012). In the ongoing phase 1a/b trial (Clinical Trials Identifier: NCT03270800), safety is the primary end point and the secondary end point is the analysis of neutralizing antibodies to GGT in both in *H. pylori* positive and *H. pylori* negative subjects. Although protection against *H. pylori* infection will not be evaluated in this trial, it still holds promise for an effective vaccine against *H. pylori* infection due to the unique mechanism of targeting GGT.

3.4 What Have We Learned from the Clinical Trials That Can Help Us to Make a Better Vaccine?

There are important factors that need to be taken into consideration when designing a vaccine against *H. pylori*, namely, the choice of antigen, the route of immunization, and the safety of an adjuvant. An optimal combination of these three

factors will be crucial for the success of a *H. pylori* vaccine in the future.

Antigens Vaccines based on *Salmonella* carriers expressing urease are not currently being pursued, due to the weak antibody and T cell responses generated to the heterologous antigen. In this regard, whole-cell vaccines might be more consistent in enhancing immune responses to *H. pylori* antigens, as long they can be combined with a non-toxic mucosal adjuvant since the *H. pylori* pathogen associated molecular patterns (PAMPs) are very poor at activating the immune system. Currently two independent studies have reported growth of *H. pylori* in fermenter scale and inactivation using formalin to prepare the whole-cell vaccine (Summerton et al. 2010; Holmgren et al. 2018). *H. pylori* whole cell vaccines seem to be effective in enhancing immune responses (Kotloff et al. 2001), but there is uncertainty as to whether the immune responses are strain specific. In this regard, a selection of highly immunogenic antigens produced recombinantly has the advantage of a streamlined production with minimal batch-to-batch variation (Satin et al. 2000; Malfertheiner et al. 2008).

Route The mucosal route of vaccination should be considered since the infection is acquired via the mucosal route and would need mucosal homing $CD4^+$ T cells expressing $\alpha4\beta7$ to be induced for protection (Michetti et al. 2000). In addition, since *H. pylori* is prevalent in low- and middle-income countries which are also target population for the vaccine, a drinkable needle-free preparation will be much safer to introduce for mass-vaccinations.

Adjuvants Safe and non-toxic mucosal adjuvants are available such as the non-toxic double-mutant *E. coli* heat-labile toxin (dmLT) which has shown a good safety profile in human volunteers in a bacterial vaccine directed against enterotoxigenic *E. coli* (ETEC) (Lundgren et al. 2014). It is also possible that multiple rounds of immunizations might be necessary as reported in the trial by Zeng and co-workers (Zeng et al. 2015).

The implementation of a prophylactic vaccine in children is going to require further careful work before it will have wide application. There is however potential for application of a prophylactic vaccine to benefit other patient groups. Administration of prophylactic vaccine to *H. pylori*-positive individuals at the same time as, or after treatment with antibiotics would be an excellent strategy to promote cure, and to prevent re-infection. Clinical studies in *H. pylori*-positive subjects have shown that eradication therapy frequently has an unacceptably low success rate (Graham et al. 2007), so a prophylactic vaccine could have impact as an adjunct therapy. Further, as discussed above studies in the human challenge model have shown that some volunteers spontaneously eradicate the infection, making it difficult to assess protection in vaccinated subjects. Thus, the next trial of a candidate *H. pylori* vaccine should recruit uninfected individuals (or those treated with antibiotics) and then follow the rate of infection or reinfection over a 3–5 year period, much in the same way as the previous field trial (Zeng et al. 2015). The *H. pylori* challenge model trials have unfortunately raised more questions than they have been able to answer regarding the protective efficacy of vaccine candidates, and so in our opinion field trials are going to be the only possible approach. As mentioned previously, the target for such a vaccine would initially be principally children, and possibly adults identified to be at the risk for developing GC, and possibly also patients with recurrent *H. pylori*-induced peptic ulcers.

3.5 Is a Vaccine Actually an Achievable Goal?

Given the challenges discussed above, a number of aspects will have to be addressed for a successful vaccine.

3.5.1 Vaccines Should Not Induce or Exacerbate Inflammation

Because there is no inflammation in the stomach in the absence of infection, a prophylactic vaccine

can be expected to be quite safe to administer. However, when the subjects eventually acquire *H. pylori* naturally through contaminated food or water, there is a risk that in the vaccinated individuals, chronic inflammation may be activated. In the pre-clinical models, vaccinated mice receive a very high dose of bacteria to achieve consistent colonization in the sham-treated mice (usually in excess of 10^8 colony forming units (CFU)). This often leads to what is termed "post-immunization gastritis" reported in several studies (eg. (Sutton et al. 2001; Raghavan et al. 2002a, b; Velin et al. 2005; Becher et al. 2010). In reality, the dose of *H. pylori* bacteria that humans will be exposed to might well be considerably lower, and might therefore not induce significant inflammation and permit eradication by the vaccine-induced memory responses with minimal inflammation. Evidence from the studies in volunteers suggests that vaccinees do not develop post-immunization gastritis since even with the high dose of *H. pylori* bacteria administered in the challenge studies (dose of 10^5-10^6 CFU), the subjects did not have a higher pathology score of inflammation compared unvaccinated subjects (Aebischer et al. 2008; Malfertheiner et al. 2018).

The major challenge for the development of a prophylactic vaccine against *H. pylori* is the induction of long-lasting immunity. This is particularly important in the context of protection against GC since it develops much later in life. A vaccine should also be able to prevent infection/reinfections with *H. pylori* in the elderly as this group are thought to have increased susceptibility to acquire *H. pylori* infection in elderly care facilities (Regev et al. 1999) as they become immunocompromised with age. There are indications from pre-clinical studies in other infections that mucosal vaccination can generate long-lasting tissue resident memory $CD4^+$ T cells that act as a first-line of defence upon second encounter with the pathogen (Schenkel and Masopust 2014). Thus, $CD4^+$ tissue resident memory cells could potentially be induced by a *H. pylori* vaccine and even in the absence of the antigen and could provide long-term protection against infection.

3.5.2 Therapeutic Vaccines Must Overcome Suppression

We know from pre-clinical studies in mouse models that therapeutic vaccination is able to reduce colonisation, but is unable to eradicate an ongoing infection (Koesling et al. 2001; Raghavan et al. 2002a, b; Sjökvist Ottsjö et al. 2015). This is possibly due to the suppressive effect of an ongoing chronic infection on the vaccine-induced response. Indeed, we have shown that the T cell response and cytokine secretion after vaccination is lower after infection of the mice compared to the response in naïve mice (Sjökvist Ottsjö et al. 2013; Holmgren et al. 2018). A lower immune response after vaccination was also observed in *H. pylori*-positive compared to *H. pylori*-negative subjects in a clinical trial of a *H. pylori* whole cell vaccine and LT_{R192G} adjuvant (Kotloff et al. 2001). Together this suggests that an ongoing *H. pylori* infection can suppress the immune responses to a *H. pylori* vaccine. To avoid the complication of still carrying the *H. pylori* bacteria for an extended period of time and a suppressed antigen-specific immune response, the vaccination might be combined with antibiotic therapy for protection against reinfection. Follow-up of acquisition of infection in vaccinees could perhaps be combined with screening for gastric cancer (Ohata et al. 2004).

3.6 Mechanisms of Protection- What Are Desirable Responses?

3.6.1 Surrogates of Protection for Human Trials

Confirming eradication or colonisation levels with biopsies in vaccine trials is invasive and expensive. Identifying surrogates of protection remains a problem for screening of candidate prophylactic vaccines. Irrespective of the type of vaccine, $CD4^+$T cell responses are clearly essential for protection as elucidated in pre-clinical models and recently in clinical studies (Kotloff et al. 2001; Aebischer et al. 2008). Since $CD4^+$T cells cannot directly access or target the bacteria, the secretion of cytokines by the $CD4^+$T cells

contributes to the anti-bacterial response seen after vaccination. Several studies in both mice and humans have suggested that IFN-γ and/or IL-17A are important effector cytokines but not T_H2 related cytokines. The IFN-γ and IL-17A secreting cells have distinct functions. Secretion of IFN-γ by the $CD4^+$T cells in the stomach leads to macrophage activation, making the environment unfavourable for colonization (Fig. 1). While the secretion of IL-17A by $CD4^+$T cells activated the epithelial cells to continue to secrete IL-8 which sustains the chronic recruitment of neutrophils to the stomach (Luzza et al. 2000) (Fig. 1). Very few clinical studies have systematically measured T cell responses in subjects after vaccination and thus we have only limited knowledge regarding the activation of cellular immune responses after vaccination. Since it is technically challenging to measure the immune cells in gastric biopsies, blood is probably the samples of choice to measure the frequency of circulating and mucosal homing $\alpha4\beta7^+CD4^+$ T cells secreting IFN-γ and IL-17A and possibly also TNF-α in response to *H. pylori* antigens (Fig. 1). These advances will greatly improve our understanding of vaccine-induced responses to *H. pylori* in subjects which is greatly needed.

The desirable biomarkers for protection will however depend on the vaccine and the type of response that it can induce. For instance, because the vaccine trials based on the vaccine GGT are based on a different strategy, analysing the neutralizing IgG antibody responses to GGT will actually be the marker for an effective vaccination schedule optimizing doses and frequency (Anderl et al. 2012).

3.7 Strategies for Design of the Next Generation of Candidate Vaccines.

3.7.1 Multi Epitope Vaccines

Single antigen vaccine strategies are less likely to induce broadly effective protection, and the rationale for testing multi-antigen or multi- epitope subunit vaccines is clear. There are two major challenges facing the design of a universal *H. pylori* vaccine for global application: firstly, the variation in strains of *H. pylori* in different regions means that vaccines containing the well-studied virulence factor antigens such as CagA and VacA would have to include a broad range of antigens to cover all types; and secondly subunit vaccines will have to be carefully designed to account the spectrum of HLA types. The second problem might be overcome by the whole cell vaccine approach, but it is not clear whether even whole cell vaccines could induce protection against all strains.

One approach to address this problem is described by Ali and co-workers, who have used a comparative genomics and pathogenomics approach to compare the genome sequences and proteomes of 39 global representative strains (Ali et al. 2015). The study selected conserved regions of the genome and used computational approaches to attempt to identify universal proteins that might be selected as targets for vaccines of therapeutics. A total of 28 proteins were identified, none of which have been previously tested in pre-clinical models (Ali et al. 2015). While the rationale for this approach is sound, and given the challenges that have arisen from the currently tested antigens to date, perhaps a completely new approach is required. To our knowledge no follow-up studies have demonstrated proof-of-principle of this approach.

4 Conclusions

Is an H. pylori vaccine an achievable aim? Decades of research by several groups worldwide on effects of different approaches to vaccination on immune responses to *H. pylori* in mice and human subjects has led to several important findings regarding the host response and correlates to protection. *H. pylori* bacteria have evolved to adapt to the hostile stomach environment and can express specific antigens that can dampen the host mucosal immune response. This could explain why in spite of a vigorous systemic immune response generated in all infected individuals, the infection is rarely spontaneously eradicated and can persist for decades in the

human host. Several candidate vaccines have been evaluated in phase I clinical trials, and one study has advanced to Phase II/III clinical trials and has given us valuable insights into vaccine design and delivery. Yet, we need to consider for future clinical trials that: (1) the adult challenge model is not optimal for evaluating the efficacy of a *H. pylori* vaccine, (2) as a primary endpoint, it is difficult to accurately assess level of colonization after vaccination in *H. pylori* infected individuals and (3) evaluation of long term re-infection rates is probably the most effective method to assess the protective efficacy of a *H. pylori* vaccine.

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