Helicobacter

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Inflammation, Immunity, and Vaccines for *Helicobacter*

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Abstract

Helicobacter pylori represents the major etiologic agent of gastritis, gastric, and duodenal ulcer disease and can cause gastric cancer and mucosa-associated lymphoid tissue B-cell lymphoma. It is clear that the consequences of infection reflect diverse outcomes of the interaction of bacteria and host immune system. The hope is that by deciphering the deterministic rules – if any – of this interplay, we will eventually be able to predict, treat, and ultimately prevent disease. Over the past year, research on the immunology of this infection started to probe the role of small noncoding RNAs, a novel class of immune response regulators. Furthermore, we learned new details on how infection is detected by innate pattern recognition receptors. Induction of effective cell-mediated immunity will be key for the development of a vaccine, and new work published analyzed the relevance and contribution of CD4 T helper cell subsets to the immune reaction. Th17 cells, which are also induced during natural infection, were shown to be particularly important for vaccination. Cost-efficiency of vaccination was re-assessed and confirmed. Thus, induction and shaping of the effector roles of such protective Th populations will be a target of the newly described vaccine antigens, formulations, and modes of application that we also review here.

Helicobacter pylori remains one of the most prevalent pathogens worldwide, infecting every second human being. Infection causes gastritis that in most infected people remains clinically asymptomatic for decades. However, *H. pylori* is the etiologic agent of a majority of gastric and duodenal ulcer diseases and can lead to gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) B-cell lymphoma [1]. The factors that determine these diverse clinical outcomes are subject to continuous investigations, but it has become clear that variant pathogen virulence factors, host genetics [2] and environmental variables, such as co-infections [3], contribute to the course of the disease triggered or promoted by the infection. Here, we review selected literature that has advanced our understanding of the innate and adaptive immune responses to infection as well as advancing efforts to develop a vaccine against this medically important pathogen.

Inflammatory and Innate Immune Response to Infection

Over the last two decades, the concept of recognition of patterns associated with microbes as envisaged by the late Charley Janeway has led to the discovery of a multitude of so-called pattern recognition receptors (PRR) [4,5]. Depending on their subcellular localization, they sense their cognate class of ligands at the cell surface or in intracellular vesicles – such as members of the Toll-like receptor family (TLR) – or in the cytoplasm – e.g. the retinoic acid-inducible gene I (RIG-I)-like or the nucleotide-binding domain and leucine-rich repeat-containing receptors (RLR and NLR, respectively). The latter are multi-domain proteins with an N-terminal effector, a central nucleotide oligomerization (NOD), and the C-terminal leucine-rich repeat domain. These PRR families recognize diverse classes of abundant microbial structures like lipoproteins, LPS; peptidoglycan derivatives (by TLR-2, -4, and NOD-1, respectively) or particular structures and forms of RNA and DNA (e.g. TLR-3, TLR-7 to -9, RIG-1, MDA-5). Functioning as sentinels their role upon ligand recognition is to trigger signaling cascades that start an alarm and immediate defense program that mostly relies on de novo gene expression and has a critical impact on both innate and adaptive immunity. Four families of transcription factors, nuclear factor kappaB (NF-κB), interferon regulatory factor (IRF), AP1, and nuclear factor of activated T

cells (NF-AT) orchestrate this program [6,7]. Given that combinatorial signaling is the rule, it is difficult to appreciate which cascade contributes what to the overall response.

Recognition of *Helicobacter pylori* by TLR

H. pylori has already been shown to be detected by the receptors TLR-2, -4, -5, -7, -8, -9, and signal in a MyD88-dependent manner in antigen-presenting cells [8]. TLR-5 can putatively be ruled out as a sensor of *H. pylori* flagellin [9]; however, deciphering *H. pylori* effectors and the single receptors involved remains a major goal. Rad et al. [10] addressed this problem by exploiting PRR gene-deficient mice as a proxi to establish which PRR may be relevant in *H. pylori*-detection by professional antigen-presenting cells (APC). Comparing *H. pylori* strains that differed with respect to their status of the functional type 4 secretion system (T4SS) encoded by the *cag* pathogenicity island (*cag*PAI), they reported that bone marrow-derived dendritic cells (DC) detect the bacteria by the surface PRR TLR-2 and -4 and sense bacterial DNA after phagocytosis of the pathogen by TLR-9 probably in late, acidified endosomes. In addition, their data suggest that *H. pylori* RNA (not *Escherichia coli* RNA) may be sensed by RIG-1 (but not MDA59) activating IRFs and inducing type 1 interferons. They also proposed a dominant role of TLR-2 resulting in increased transcription of the immunosuppressive IL-10. Increased IL-10 may be responsible for blunting a protective adaptive immune response [11]. The *cag*PAI status of *H. pylori* seemed not to matter for the response triggered by these PRR in professional APC. Whether this is also the case for RNA recognition by RIG-1 is an interesting issue. Functional heterogeneity in TLR genes can impact the course of disease. To analyze putative correlations with disease outcome, Ng et al. [12] investigated a polymorphism in the TLR-9 promoter region. Mutations within this region created a novel functional NF-κB-binding site in HeLa cells, suggesting this alteration could increase the sensitivity of cells to TLR-9 ligands. Indeed, certain *Tlr-9* mutations correlated with low gastric acid production and more pronounced atrophy within a cohort of *H. pylori*-infected patients.

Helicobacter pylori and NOD Family Receptors

Several groups have focused on the role of the NLR member NOD-1 in *H. pylori* detection, thereby complementing the above analyses. NOD-1 was initially described by Viala et al. [13] to recognize *H. pylori* peptidoglycan in a *cag*PAI T4SS-dependent manner. Recent studies by Ferrero's group now suggest that a functional T4SS may not be necessary, because outer membrane vesicles (OMV), commonly shed by Gram-negative bacteria including *H. pylori,* were taken up by epithelial cells in a cholesterol-dependent manner, thereby triggering the NOD1-dependent transcription of NF-kB reporters and IL-8 release [14]. In accordance with this, gastric gavage of *H. pylori*-derived OMV to mice induced NOD1-dependent chemokine responses in the stomach and OMV-specific serum IgG production; a finding that makes OMV interesting for vaccine development [15]. This group also reported that *H. pylori* induced the antimicrobial peptide human β-defensin 2 in epithelial AGS cells, and this appeared to be mediated by NOD1-dependent activation of NF-kB [16]. This host cell response was reported to be *cag*PAI-dependent but OMV-mediated triggering of NOD1, which seemed to be less efficient than the *cag*PAI-dependent pathway, was not tested. Because whole bacteria, *H. pylori* lysates and OMVs, contain multiple possible PRR ligands, Watanabe et al. [17] used a more specifically defined NOD1 ligand, namely γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP), for determining the ability of NOD1 ligands to trigger NF-κB activation. In epithelial cells, iE-DAP-activated NOD1 was not linked to NF-κB activation but to IRF7 stimulation resulting from RICK-binding to TNF receptor-associated factor TRAF-3. IRF7-induced type I interferon-β (IFN-β). The latter triggered an autocrine/paracrine loop that led to the synthesis of chemokines such as CXCL10, IL-8, and i-TAC [17]. Interruption of this loop in a mouse model increased bacterial load early after infection, indicating that such NOD1 emanating signals trigger anti-bacterial effector functions in mice. To reconcile the divergent findings with regard to NOD1-mediated NF-κB activation in *H. pylori* infection, it will be necessary to refine analyses whether (and how) NOD1 activation and NF-κB signaling are linked. This may extend to the newly reported *H. pylori* NOD1 association with the MAPK and AP-1 signaling cascades [18].

Cell Biology Correlates of Recognition of *Helicobacter pylori* by PRR

Obviously, *H. pylori* can be sensed by members of all PRR families. Furthermore, Gringhuis et al. [19] showed that certain *H. pylori* strains can, dependent on carbohydrate expression on their surfaces, negatively influence IL-6 and IL-12 secretion, but positively influence IL-10 secretion via binding to the dendritic cell-specific C-type lectin DC-SIGN. Despite the obvious challenges of weighing the relative contribution of these recognition processes, understanding the cell biology basis, e.g. of intracytoplasmic recognition of

bacterial ligands, may hold interesting surprises. Necchi et al. mapped the intracellular distribution of NOD-1 in histologic sections of infected patients and also in cells exposed to *H. pylori* in vitro, revealing so-called Particle-rich Cytoplasmic Structures (PaCS) [20]. These structures may be a kind of ''aggresome'' structures that are known to be linked to intracellular protein degradation [21]. Indeed they found NOD1 to be co-localized with *H. pylori* remnants (defined antigens such as VacA and outer membrane proteins) and with ubiquinated proteins and proteasomes. Furthermore, this study highlights another level of complexity in host–*H. pylori* interplay, i.e. the impact of the pathogen's subcellular distribution. Our current knowledge of the subcellular localization of *H. pylori* and the existence of differential localization between strains remains minimal. Nevertheless, interpretation of existing data regarding PRR-*H. pylori* interactions within a cellular biology context will undoubtedly be rewarding.

Innate Inflammatory Mediators

Infection with *H. pylori* is known to lead to the release of many chemo- and cytokines; however, more comprehensive characterization of their individual roles is still required. Wong et al. [22] recent characterization of macrophage migration inhibitory factor (MIF) expression in mice infected with *H. pylori* revealed that a negligible inflammatory response in *H. pylori*-infected MIF-deficient mice correlated with a substantially reduced inflammatory T-cell response, characterized by lower IFN-γ and TNF-α production. Inflammation in response to *H. pylori* infection may not only be induced by recruitment of leukocytes, but, alternatively, the induction of IL-1β by *H. pylori* neutrophil-activating protein (HP-NAP) may increase survival of inflammatory monocytes, and in turn neutrophils extending the local life time of these cells, as shown by Cappon et al. [23]. Several studies have shed more light to the many facets of IL-1β in this infection, such as the loosening of tight junctions by disrupting claudin-4 [24], and the involvement of sonic hedge hog signaling in IL-1-dependent reduction in gastric acid output [25]. Thus, step-by-step, we are gaining an increased understanding of why the genetic background of IL-1/IL-1R impacts the course of *H. pylori*-triggered disease [26].

Small Noncoding RNAs and *Helicobacter pylori*

In recent years, the study of a novel class of regulators, small RNAs, has gained momentum [27]. Small or micro RNAs (miR) are noncoding RNAs mostly transcribed by RNA polymerase II. They are processed by ribonucleases in the nucleus and further in the cytoplasm by the machinery that also generates small interfering RNAs and by other enzymes. The mature miRs (classified using a nomenclature of the kind miR followed by a number, e.g. miR-155) preferentially bind to complementary sequences in the 3′ UTRs of target mRNAs leading to degradation or inhibition of translation. Depending on the target gene, this can affect multiple host cell processes, including cell development, differentiation, and even malignant transformation, possibly also gastric cancer [28]. Over 700 miR species are predicted from the human genome, and for a number of them a role in regulating expression of genes in cells of the immune system has been demonstrated (for recent review see [27]). Specific microarrays have been produced to detect miR sequences in samples of small RNAs to allow parallel assessment of miR expression. Matsushima et al. [29] used this technology to investigate signatures of 470 miRs in biopsies from Japanese *H. pylori* infected patients in comparison with non-infected controls. From a total of 242 miRs detected, 55 miRs showed differential abundance in these samples. Validation with another patient cohort revealed that the levels of 30 miRs were consistently decreased in infected patients. Only one miR, miR-223, expressed in myeloid cells [27], correlated with the activity score of gastritis, while most of the other differentially detected miRs lacked correlation with histologic features including pre-neoplastic changes. Using a similar approach, Xiao et al. [30] analyzed miR patterns in a gastric epithelial cell line, GES1, after exposure to *H. pylori*. They focused on miR-155 which is expressed in many hematopoietic cell lineages and showed that miR-155 was induced by *H. pylori* and present in increased amounts in the stomach mucosa of *H. pylori*-infected patients. The degree of induction was dependent on the bacterial strain and, as expected from other work, was mediated by NF-κB and AP-1 transcription factors. In phagocytes, miR-155 is thought to function as a negative regulator of pro-inflammatory gene expression [27]. Accordingly, miR-155 overexpression reduced *H. pylori*-triggered synthesis of IL-8 and Gro-α in vitro. Tang et al. [31] revealed another aspect of miR-155-mediated negative feedback on inflammatory responses, showing that mir-155 reduced MyD88 translation in AGS epithelial cells. In their system, low MyD88 concentrations led to a roughly twofold reduced IL-8 secretion. The role of miR-155 was also investigated by Fassi et al. [32] with a focus on T cells. They confirmed miR-155 expression by *H. pylori* infection in vivo by analyzing RNA from biopsies of experimentally challenged volunteers previously reported [33]. In addition, they showed that upregulation of miR-155 in Jurkat cells exposed to

H. pylori was likely in response to VacA and γ-glutamyl transpeptidase. Furthermore, miR upregulation depended on FoxP3 expression and cAMP, which were both increased in Jurkat T cells during *H. pylori* infection. These findings are largely consistent with data from mouse models (c.f. references in [27]). However, based on miR-155-deficient mice, it appears that repression of other miRs (e.g. miR-142–3) by FoxP3 may be functionally more important. For *H. pylori*'s effect on T cells, this awaits testing. Future work is likely to reveal more about miRs and their role in the acute and chronic inflammatory response to *H. pylori*. As *H. pylori* produces its own small RNAs [34] which are likely to reach the host cell cytoplasm (see [10] above), it is tempting to speculate that bacterial small RNAs can interfere with the host miR regulatory system.

Adaptive Immune Response

Detection of *H. pylori* by PRR generates signals that ultimately impact on the adaptive immune response. Accumulating evidence suggests that cell-mediated immunity was a driving selective force in the evolution of *H. pylori's* immune evasion mechanisms [35]. The $CD4^+$ T helper cells (Th) are of particular interest as indicated by the study of Ermak et al. in 1998 [36] that showed that these cells are responsible for the antigen-specific control of *H. pylori* burden. Stimulation, expansion, and differentiation of CD4 T cells into so-called effector cells are instructed primarily by professional APC, mainly DCs. There are many kinds of DCs [37] and CD4 T cells can differentiate into a multitude of effector and memory cells [38]. T-cell differentiation leads to cells that can be distinguished by functional assays and their production of characteristic cytokines. This cellular differentiation is commonly viewed as a kind of lineage commitment associated with the expression of master regulators within this process, i.e. the transcription factors T-bet, Gata-3, RorγT, Bcl6, and FoxP3 that drive differentiation of Th1, Th2, Th17, follicular T helper (Tfh), and regulatory Treg cells, respectively. However, there is growing evidence for plasticity in this process, and in the context of Th cell function in chronic infections such as *H. pylori*, one should keep in mind that subtype populations may not be as committed as generally believed [38].

DC in the Healthy and *Helicobacter pylori*-Infected Mucosa

The generation and maintenance of a T-cell response depends on DC, and it has been a longstanding challenge to decipher whether DCs are present in noninflamed gastric mucosa. Studies in mice had shown that *H. pylori*-specific responses might be induced more distally in the gastrointestinal system, in Peyer's patches, by passing *H. pylori*, implying that this was a functional consequence of the gastric mucosa lacking DC [39,40]. Several groups have now re-addressed this issue. Bimczok et al. [41] showed that cells with typical DC markers such as MHC II, CD11c, DC-SIGN, and CD206 can be isolated from biopsies taken from both normal and *H. pylori*-infected patients. The frequency of these cells increased around fivefold in biopsies from infected patients in which DCs also exhibited an activated phenotype and molecules which act as co-stimulatory ligands to T cells, such as CD86, were upregulated. In vitro, these gastric DCs phagocytose and process *H. pylori.* DCs secreted IL-6, -8, and -10 as well as triggering expansion of Th1 cells. Presence of DCs in normal human gastric mucosa and their increase in *H. pylori* colonized mucosa were confirmed by Necchi et al. [42]. It is interesting in this context that Khamri et al. also detected monocyte-derived DCs in inflamed mucosa, and using DCs derived by IL-4 and GM-CSF from blood monocytes, they found that these cells express IL-23 and IL-12, but the former was produced earlier [43]. IL-23 is a positive regulator of Th17 cells, and Kamzi et al. could visualize IL-23 expressing DCs as well as Th17 cell in inflamed gastric mucosa. They also showed that in vitro stimulated $CD4^+$ T cells produce more IL-17 in response to *H. pylori* exposed DCs, a process mediated by IL-23 and IL-1. Kao et al. reported essentially similar findings when analyzing mice [44]. They showed that bone marrow-derived DCs (which are more accessible) preferentially induced Treg and Th17 cells. Differentiation of these $CD4^+$ subsets requires TGF-β, but Th17 cells also need IL-6 and are further promoted by IL-23 and IL-1. The bias toward Treg cells in the murine system was probably because *H. pylori* was not a strong activator of bone marrow-derived DCs and TFG-β is also produced by nonstimulated DCs. Overall, these and past studies dealing with the examination of *H. pylori*-derived effects on DCs suggest that local and monocyte-derived DC populations in the gastric mucosa may differ functionally and support conditions for a diverse population of T cells. It will require further studies, but by exploiting the murine model these intricate relations may be dissectible.

CD4 T Helper Cell Subsets in *Helicobacter pylori* Infection

Th17 and Treg $CD4^+$ cell subsets have been the focus of many recent immunologic studies on the course of Helicobacter infection. Regulatory T cells are thought to

expand and eventually dominate in chronic infection hindering the function of protective T cells. Recent work is substantiating this scenario; for instance, Kindlund et al. [45] showed that eradication of *H. pylori* reduced Treg numbers, and Jang et al. [46] reported increased numbers of Tregs in the stomachs of *H. pylori*-positive gastric cancer patients. Treg differentiation depends on TGF-β but, in the presence of IL-6, TGF-β rather promotes Th17. Th17 cells have become a new focus in this field because of their role in neutrophil recruitment and activation. Th17 thrive in particular when IL-1 and IL-23 are also present [47]. Shi et al. [48] confirmed the latter scenario after *H. pylori* infection of mice and found that Th17 and Th1 cells contribute to the overall pro-inflammatory T-cell response. Similar to other infection and autoimmune disease models, Th17 and Th1 cells modulate each other. However, in the study by Shi et al., Th17 cells promoted an inflammatory component and Th1 response that correlated with higher *H. pylori* colonization when wild-type mice were compared with IL-17-deficient or normal mice treated with an anti-IL-17 antibody just before infection. Similarly, IL-17, when delivered by recombinant adenovirus just before *H. pylori* infection, increased inflammation and bacterial load 4 weeks later. These findings are at odds with work by Otani et al. [49], who observed an increase in gastritis and Th1 cytokines in mice treated with anti-IL-17 antibodies 6 months after infection. It also contradicts work by Kao et al. [44] who showed a negative correlation of IL-17 production and *H. pylori* burden. Complicating the issue further, Algood et al. [50] reported that mice deficient in the IL-17A receptor developed increased inflammation over a 6 -month time scale but also suffered tenfold increased bacterial burdens. Consistent with the model that IL-17 amplifies recruitment of neutrophils, the inflammatory infiltrate contained more lymphocytes, in particular B cells at the expense of granulocytes. In humans, serum levels of IL-17 seem to correlate with severity of disease; for instance, Jafarzadeh et al. [51] found increased levels of IL-17 in duodenal ulcer patients when compared to asymptomatic *H. pylori*-positive patients. Moreover, genetic typing for IL-17A alleles in over 800 individuals, 300 of which were gastric cancer patients, by Shibata et al. [52] associated the hypermorphic G-197A promoter allele with increased risk of gastric cancer. Cytokines often display pleiotropic effects, and further studies will be necessary to appreciate fully the role of IL-17, which is not a single cytokine but represents a system of several subforms and receptors. In addition, the role of this cytokine family may change over the course of infection, and $CD4^+$ T cells may alternate between Treg and Th17 function [38].

Inflammatory Th Cell Subsets: Friends or Foes

The discrepant findings with IL-17 highlight that different types of inflammatory responses exist, and context has to be carefully assessed. Work by Sayi et al. [53] corroborated the correlation between long-term increased inflammation and reduced bacterial colonization, in this case by *Helicobacter felis*. Given that *H. felis* promotes long-term inflammatory reactions and pre-cancerous changes that are not conducive to eliminating the pathogen, the authors stress the view that vaccine-mediated protection may rely on pro-inflammatory Th cells such as Th1 cells that seem also involved in promoting cancerous changes if bacteria cannot be eliminated. This idea is seemingly enforced by findings of Stoicov et al. [54] which show that mice deficient in T-bet, the transcription factor required for Th1 differentiation, do not develop cancerous lesions. Yet one cannot rule out the possibility that this is largely because of effects of T-bet in cells other than Th1 cells. To date, however, histologic analyses of gastric inflammation in human volunteers immunized and challenged with *H. pylori* did not reproduce evidence for increased gastritis, at least during the first 2–3 months postinfection [33].

Th1, Th2, Th17, and Treg cells have all been detected in *H. pylori* infection. But our comprehension of the network of pro-inflammatory Th1 and Th17 and regulatory Treg cells remains incomplete. We should be encouraged to improve our understanding of these networks as they seem to be critical for clinical outcomes. We will also be curious to see whether IL-9 or IL-22 producing Th cells and, in particular the newly discovered follicular helper $CD4^+$ T cells (Tfh, [55]) also play a role in *H. pylori* infection, the latter in particular when infection leads to MALT lymphoma.

Vaccine Development

Since the first proof-of-principle nearly 20 years ago [56,57], the development of a vaccine against *H. pylori* (for review, see [58]) remains a desirable option, because of cost-efficiency, to control *H. pylori* infection [59]. Although originally thought to be antibody-dependent, it was later shown that protection by active immunization required Th cells [36], and antibodies are not helpful or even counter-protective [60,61]. Clinical studies that combined vaccination with experimental infection with *cag*PAI-negative *H. pylori* indicated that T-cell immunity exists but that the vaccines tested required much improvement [33]. Improvement strategies would clearly benefit from a molecular understanding of the events that link Th cells with the effector mechanisms for protection.

Protective Mechanism

Over the past year, researchers have probed the role of Th17 cells in vaccine-mediated protection. Blanchard's team demonstrated that Th17 seem to mediate protection in a mouse model of *H. pylori* infection [62], as did Velin et al. [63] using *H. pylori* and *H. felis*. In the latter study, neutralizing IL-17 significantly reduced vaccine efficacy although bacterial burdens were not back to levels of nonvaccinated controls, indicating that additional factors may also be important. The role of IL-17 in protection is further supported by the therapeutic effect of recombinant IL-17 given to infected mice which reduced bacterial burden, thus agreeing well with the opposite effect noted by Otani et al. [49] when they treated nonimmunized but infected mice with anti-IL-17. Since Th17 cell expansion depends on IL-23, an IL-12 family heterodimer between IL-12p40 and a p19 subunit, these findings corroborated previous data on reduced vaccine efficacy in IL-12p40-deficient but not IL-12p35-deficient mice. Mast cells had been implicated in Th cell vaccine effects [64] while others showed that neutrophils were relevant [62]. A study by Ding et al. [65] now suggests that these findings can be reconciled as mast cells contributed to protection but did so by amplifying neutrophil recruitment and effects of IL-17.

Novel Antigens and Vaccine Formulations

The above mechanistic studies will eventually provide the basis for rationale vaccine improvement. Meanwhile, others have concentrated on the identification of additional vaccine antigens, modes of production, and routes of administering them [66–70]. Meinke et al. applied a novel strategy to identify and screen for antigens recognized during natural infection: the ANTIGENome approach [71]. Short sequences of 50–300 bp generated by shearing genomic DNA from *H. pylori* isolates were cloned such as to get them expressed on the surface of *E. coli*. Based on their surface exposition, the fragments were detected using reactive sera, allowing isolation of the respective *E. coli* clone and subsequent determination of antigenic peptide encoding sequences. In comparison with immunoproteomics, the ANTIGENome approach is less prone to technical bias and a novel, highly dominant antigen, HP1341, was identified. HP1341 is a predicted surface membrane-located transporter which had, like many other membrane proteins, escaped detection in previous proteomic analyses. Since surface topology or secretion generally increases immunogenicity [72], antigens detected by the ANTIGENome method will expand the choice of vaccine candidates.

Needle-independent routes of vaccine administration are clearly preferred. Oral vaccination has therefore been an option, but mucosal adjuvants pose a significant problem because of inefficacy or intolerable reactogenicity. Summerton et al. [73] further improved a vaccine based on whole killed *H. pylori* by admixing them with a novel mutant form of *E. coli* heat labile toxin (LT). This adjuvant had similar potency in mice compared to a less attenuated LT form that was found too reactogenic in previous clinical studies. Together with improvements of the killed bacteria formulation, this vaccine may show superior characteristics in future clinical trials. Hickey et al. [74] followed a different approach and tested transcutaneous vaccine delivery of a bacterial lysate formulated with a lipid mixture and CpG oligonucleotides as a further immune stimulants. This vaccine reduced *H. pylori* burdens in mice roughly by one to two orders of magnitude. It induced high levels of specific secretory IgA but comparatively little serum IgG, an interesting aspect given that Ig may be counter-protective.

In summary, vaccine development against *H. pylori* remains a focus of research. Progress is made but is incremental. There is need for a still better understanding of the protective mechanism and for improving efficacy. It will also be necessary to evaluate gain by protection versus the alleged danger of the same immune mechanism contributing to disease. Further clinical studies may help to avoid blurring this important issue by incongruent animal models.

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Conflict of Interest

The authors declare no conflict of interest.

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