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REVIEW: INFLAMMATION, IMMUNOLOGY, VACCINES

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Abstract – The immune response to *Helicobacter pylori* is important in persistence of the infection and development of peptic ulcer disease and gastric cancer. *H. pylori* influences the host gastric and systemic immune response via a complex network of mechanisms. Understanding these could lead to the development of new therapeutics. Research on vaccines against *H. pylori* has been ongoing for several decades, but unfortunately with little success. This review summarizes some important findings in the areas of *H. pylori*-mediated inflammation, immunity and vaccines during the period from April 2021 to March 2022.

Keywords: *Helicobacter pylori*, Inflammation, Innate immunity, Cytokines, Adaptive immunity, Vaccines.

INFLAMMATION

Epithelial Interactions

Several articles[1-7] characterised canonical and non-canonical nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) signalling. Binding of tumour necrosis factor (TNF) receptor-associated factor (TRAF)-interacting protein to forkhead-associated domain (TIFA) with TRAF6 led to transforming growth factor beta (TGFβ)-activated kinase 1 (TAK1) binding and canonical NF-κB signalling. TIFA interaction with TRAF2 activated the alternative NF-κB pathway[1]. Taxauer et al[2], found that *H. pylori* adhesin HopQ, binding to carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) was important for non-canonical NF-κB activation. Tegtmeyer et al[3] demonstrated that cortactin, the actin-binding protein, is involved in canonical NF-κB activation in response to *H. pylori*. Cortactin is needed for efficient activation of Src kinase, which then stimulates NF-κB activation. This is important, as cortactin regulation of the actin cytoskeleton is disrupted by cytotoxin-associated gene A (CagA) signalling during infection with *cag*A positive *H. pylori*[4].

Groups studied other inflammatory mechanisms, including the role of Regulated in Development and DNA Damage Responses-1 (REDD1) in *H. pylori*-mediated gastritis[5]. REDD1 expression was increased in gastric tissues from infected patients and mice, and *H. pylori* induced REDD1 expression in human gastric epithelial cell lines and primary cells in a *cag*A-dependent manner. REDD1 in gastric epithelial cells controlled expression of the chemokine CXCL1, which then attracted migration of MHC class II$^+$ (MHCII$^+$) monocytes. When infected, *Redd1*$^{-/-}$ mice had reduced MHCII$^+$ monocyte infiltration than wild-type mice, confirming regulation by REDD1 and CXCL1. The role of the *H. pylori* virulence factor TNFα inducing protein (Tipα), in inducing gastric inflammation in mice was investigated[6]. Mice infected with wild-type *H. pylori* had more severe gastritis and a stronger TNFα response, than those infected with a *tip*A-deficient mutant.

Microarray analysis of the gastric epithelial cell response to Tipα, indicated its participation in the gastric inflammatory response to *H. pylori, via* NF-κB. A single nucleotide polymorphism in the autophagy gene *ATG16L1* rs2241880 was investigated in inflammation and gastric cancer[7]. Using gastric organoids and gastric epithelial cell lines, increased interleukin-8 (IL-8) and decreased TNF-α expression was found in cells that were homozygous for the *ATG16L1* rs2241880 G-allele, which may explain its association with gastric carcinogenesis.

IMMUNOLOGY

Lymphocytes

In investigations of *H. pylori* effects on B-cells, Tamrakar & Kodgire[8] stimulated cells with recombinant Helicobacter outer membrane (Hom) proteins HomA and HomB. They found reduced expression of cytidine deaminase (AID), leading to impairment of immunoglobulin class switch recombination, and reduced IgG and IgA production. The HomA/B treatment also resulted in upregulated expression of the immunosuppressive cytokines IL-10 and IL-35, and anti-programmed death ligand 1 (PDL1). A new role for CagY in stimulating B- and T-cell proliferation in gastric MALT lymphoma was also reported[9].

Several studies[10-12] examined the role of IL-17 and IL-17-secreting T-helper (Th17) cells. He et al[10] examined the impact of IL-9 on *H. pylori* immunity in mice. IL-9 is secreted mainly by Th9 cells, but also Th17, and may have pro- or anti-inflammatory activity. IL-9 was upregulated in response to the infection and was correlated with increased expression of IL-17A, and decreased expression of interferon-gamma (IFNγ). IL-9 deficient mice had increased colonisation and gastritis severity, and their effector T-cells secreted higher IFNγ and lower IL-17A levels than wild-type mice, indicating IL-9 control with a mechanism involving IL-17A. Dixon et al[11] hypothesized that IL-17 receptor (IL-17RA) signaling in lymphocytes downregulates cytokine production and controls *H. pylori* colonisation and gastritis. AGS gastric epithelial cells (GECs) responded strongly to incubation with IL-17A or the heterodimeric form of IL-17 (IL-17A/F), with expression of inflammatory genes such as IL-8. IL-17A and IL-17A/F synergized with TNFα and IL-22 to induce further inflammatory gene expression. In mice, deficiencies of IL-17A or IL-17F alone did not significantly change the severity of *H. pylori*-induced gastritis, but deficiency of both cytokines resulted in a hyperinflammatory lymphocytic response. *H. pylori*-infected IL-17RA deficient mice had more severe gastritis than wild-type mice, but this was not observed in mice with T-cell-targeted knockdown of IL-17RA. The impact of IL-17RA knockdown could therefore not be attributed to T-cells. The role of IL-17A in *Helicobacter hepaticus* infection and colitis in mice was also described[12]. IL-17A deficient mice had increased colonization and more severe colitis compared to wild-type mice, confirming that IL-17A is important in regulating mucosal responses to infection.

A novel IL-21-secreting $CD4^+$ T-cell subset in the infected gastric mucosa was identified, which expressed markers of T follicular helper (TFH) cells[13]. TFH cells regulate antigen-specific B-cell responses. The authors found links with glucocorticoid-induced tumour necrosis factor receptor (GITR), which was upregulated in gastric $CD4^+$ cells from *H. pylori*-infected patients, compared to healthy controls. The GITR ligand (GITRL) was also upregulated in macrophages in the infected gastric mucosa, and this signal induced polarization of mucosal $CD4^+$ cells towards the IL-21 TFH-like subset. IL-21 induced B-cell proliferation, and expression of inflammatory cytokines and chemokines by gastric epithelial cell lines, demonstrating its role in the mucosal response to *H. pylori.*

Müller's group contributed new information on the Treg response to *H. pylori.* Artola-Borán et al[14] used a co-infection model of *H. pylori* and *Mycobacterium bovis* BCG, as a model for *Mycobacterium tuberculosis*, a co-infection which commonly occurs in many parts of the world. Immune responses to each infection reciprocally influenced colonisation and the host response to the other. In the presence of *H. pylori*, immune regulation of the response to *M. bovis* was impaired, and there was an augmented Th1 response in the gastric mucosa with more severe gastritis. The effects were shown to be due to the redirected homing of Tregs to *M. bovis* infection sites and could be reversed by CXCR3 blockade. Fallegger et al[15] showed that expression of TGFβ by tissue-resident eosinophils, in response to Helicobacter infection

or allergen exposure in mice, caused the expansion of an unusual population of extrathymic forkhead box P3 (Foxp3)-positive Tregs that express markers including retinoic acid-related orphan receptor gamma t (RORγt). TGFβ expression by eosinophils required contact with the bacteria. When *tgfb* expression was targeted in eosinophils, defects in the Treg response occurred and exacerbated the T-cell response. These studies[14-15] provide novel mechanisms to understand how the Treg response to infection might be induced, and the potential for other immune stimuli to interrupt this.

Increased numbers of $CD4^+CD8\alpha\alpha^+$ double positive intraepithelial lymphocytes (DP T-cells), reportedly having immunosuppressive activity, were found in the gastric mucosa of *Helicobacter felis*-infected mice by Ruan et al[16]. These were located in the mucosal epithelial layer and expressed the activation markers CD80 and CD86, and α4β7 integrin and CD103 which mediate homing to mucosal tissues. Some Treg markers were expressed, but not Foxp3. *In vitro*, the cells had inhibitory activity on dendritic cells (DCs) and effector T-cells. The frequency of gastric DP T-cells in *H. felis*-infected mice decreased significantly after injection of neutralizing antibodies against CD8α, accompanied by more severe gastritis and reduced colonisation. The findings indicated that gastric DP T-cells may modulate the gastric inflammatory response to *H. pylori* and protect against disease.

Dendritic Cells, Macrophages and Neutrophils

An important study by Oster et al[17] showed that presence of a *H. pylori* infection reduced the efficacy of cancer immunotherapy drugs in patients and in mice. The mechanism involved effects of *H. pylori* on the cross-presentation activities of dendritic cells (DCs). In mice engrafted with colon adenocarcinoma or melanoma cells and receiving cancer treatments, the tumour volumes in *H. felis*-infected mice were significantly larger than those of uninfected controls. The total numbers and activation states of T-cells, Tregs, DCs, macrophages and monocytes in the spleens and lymph nodes of infected and uninfected animals were not significantly different. There was a decrease in numbers and activation status of specific $CD8^+$ T-cells in the tumours of infected mice treated with cancer immunotherapies. DCs from infected mice inhibited tumour-specific $CD8^+$ T-cell proliferation, and thereby reduced the effectiveness of the immunotherapy drugs.

When investigating the impact of *H. pylori* urease B subunit (UreB) on macrophage activation, stimulation of mouse bone marrow-derived macrophages with UreB increased expression and accumulation of PDL1[18]. This had a subsequent effect on $CD8^+$ T-cells from infected mice, decreasing their proliferation and cytotoxic activity. UreB bound to myosin-9 (Myh9) on macrophages, leading to activation of mammalian target of rapamycin complex 1 (mTORC1) signalling. The data indicate a novel mechanism for UreB suppression of the immune response to *H. pylori.* Codolo et al[19] also explored the impact of a *H. pylori* factor on macrophages, but in a zebrafis, model transplanted with human melanoma cells. Exposure to neutrophil-activating protein (HP-NAP) caused the recruitment of pro-inflammatory macrophages into the tumour site, and tumour growth was inhibited. Depletion of macrophages dramatically inhibited these anti-tumour effects, indicating the potential of HP-NAP in cancer therapeutics.

Wen et al[20] investigated the role of Notch signalling in the macrophage response to *H. pylori,* and revealed its importance in inducing proinflammatory and bactericidal activity. Expression of the Notch ligand, Jagged1, was increased in macrophages cultured with *H. pylori,* and increased frequencies of Jagged1-expressing macrophages were present in the gastric mucosa of infected patients compared to healthy controls. Overexpression of Jagged1 in macrophages augmented their bactericidal activity against *H. pylori,* which might be exploited therapeutically.

Two interesting studies[21,22] reported on the effects of metabolites in the *H. pylori* lipopolysaccharide (LPS) biosynthesis pathway on macrophages. Faass et al[21] found that ADP-heptose, together with stimulation from the *cag* type IV secretion system, could strongly activate human macrophages, and bias the differentiation of monocytes into bactericidal M1 macrophages. In contrast, Coletta et al[22], reported that ADP-heptose upregulates the expression of miR146b in macrophages, leading to suppressive effects on a regulator of human leukocyte antigen (HLA) class II genes. This caused a reduction in HLA-II expression, and the reduced ability to present antigen may contribute to an inability of the immune response to clear the infection.

Wen et al[23] investigated the role of Toll-like receptor 2 (TLR2) in *H. pylori* neutrophil-activating protein (HP-NAP)-induced cytokine secretion in neutrophils. Their results showed that TLR2 participated in HP-NAP-induced IL-8 secretion, and PTX-sensitive G proteins also played a role in this. Competitive binding assays indicated that direct interactions between HP-NAP and TLR2 in neutrophils are involved in cytokine expression.

VACCINES

Immunogenic Antigens

Altman et al[24] aimed to produce a novel synthetic carbohydrate-based vaccine, using a recombinant α-1,6-glucosyltransferase enzyme from *H. pylori* to synthesize a α-1,6-glucan structure present in the outer core region of *H. pylori* LPS. When conjugated to bovine serum albumin or tetanus toxoid, the glucan formulations produced strong LPS-specific IgG responses in mice and rabbits. Recombinant flagellin FliD was also evaluated as a vaccine candidate[25], since serum from infected patients contained higher levels of rFliD-specific antibodies compared to healthy controls. FliD stimulated more IFNγ than IL-4 from patients' peripheral blood mononuclear cells and purified $CD4^+$ cells *in vitro*, indicating that rFliD could be a useful vaccine candidate.

In Silico Vaccine Design

Xie et al[26] used the Immune Epitope Database and Analysis Resource (IEDB) to predict B- and T-cell epitopes in HpaA, UreB, and catalase, and combined these with LTB adjuvant into a multivalent vaccine named LHUC. In mice, oral immunisation induced specific IgG responses which inhibited activities of urease and catalase enzymes *in vitro.* Splenocytes from the immunised mice secreted IFN-γ, IL-4, and IL-17 when stimulated with *H. pylori.* When tested in prophylactic immunisation, oral administration of LHUC resulted in a reduced colonisation density and less severe gastritis than the control groups. A similar protective effect was found when LHUC was tested as a therapeutic vaccine. Calcado[27] used algorithms to predict well-conserved linear B-cell epitope sequences in vacuolating cytotoxin A (VacA), CagA, blood group antigen binding adhesin (BabA), UreB and flagellin A (FlaA). The peptides were tested for reaction with serum from *H. pylori*-immunised rabbits. All were recognised by the serum, indicating that this approach could be useful in future vaccine design. Gohsh et al[28] and Ma et al[29] also employed in-silico approaches to design multi-valent epitope vaccines based on outer membrane proteins (OMP), with further bioinformatics tools to test molecular docking with toll-like receptors. It was hypothesized that these constructs would stimulate a strong immune response.

Vaccine Adjuvants and Delivery Modes

Wang et al[30] used an attenuated *Listeria monocytogenes* strain as a live vaccine vector for *H. pylori. L. monocytogenes* was engineered to express a multi-epitope chimeric antigen, containing UreB with B-cell epitopes from FlaA, OMP AlpB, sialic acid binding adhesin (SabA), and *H. pylori adhesin A* (HpaA) (EGDeAB-MECU). Using a therapeutic vaccine approach, *H. pylori*-infected mice were given oral doses and intravenous injections of EGDeAB-MECU, EGDeAB control, or PBS. Lower *H. pylori* colonisation and gastritis, higher specific serum IgG levels and gastric IgA responses were observed in both groups receiving *L. monocytogenes,* compared to the PBS control group*. H. pylori*-stimulation of splenic lymphocytes from mice immunised with EGDeAB-MECU or EGDeAB induced IFNγ, IL-4, and IL-17. The authors concluded that cross-reactions with *L. monocytogenes* could control *H. pylori* infection in the stomach, and that attenuated *L. monocytogenes* has potential as a *H. pylori* vaccine vector.

Soudi et al[31] evaluated outer inflammatory protein A (OipA) as a vaccine candidate in mice, delivered subcutaneously or orally, with propolis adjuvant. Propolis is a compound gathered by bees, that has immune stimulating properties. IFNγ mRNA expression in the spleen was

elevated following OipA administration with propolis via both routes, and most marked following oral gavage. The response was similar to that from OipA in Freund's adjuvant, indicating the potential for propolis as an oral vaccine adjuvant. Melittin, a component of bee venom which enhances Th1 responses, was suggested as adjuvant for a vaccine containing multiple predicted B-cell epitopes from UreB, HpaA and neutrophil activating protein subunit A (NapA)[32]. Efficacy of this formulation is untested.

Using a DNA vaccine approach with the *fla*A gene[33], the response in mice was monitored following four intramuscular injections at weekly intervals. Total serum concentrations of IgG, IgM, IL-2, IL-4, IL-12, and IFNγ were significantly increased. IL-4 was the most increased cytokine, and reached the highest concentrations, indicating a Th2-predominant response.

Immune Responses and Vaccine Efficacy

Vaillant et al[34] investigated the role of gastric eosinophils in prophylactic vaccination. When mice were immunised intranasally with urease and cholera toxin prior to infection, colonisation was reduced, with enhanced recruitment and activation of eosinophils in the gastric mucosa. When eosinophil-deficient mice were immunised and infected, there was reduced colonisation compared to eosinophil-sufficient mice, indicating that eosinophils inhibited the protective immune response. For successful immunisation, adjuvant-induced responses should therefore not mobilize eosinophils. The authors also investigated the role of granulocyte macrophage colony-stimulating factor (GM-CSF) in vaccine-induced reduction of *H. pylori* colonisation[35]. Immunised and infected mice had increased gastric IL-17 and GM-CSF mRNA expression compared to an infection-only group. The gastric mucosa of immunised infected mice contained GM-CSF$^+$ Th1/Th17 cells, which were absent in the infection-only group. Vaccine-induced effects on colonisation were less marked in GM-CSF-deficient compared to wild-type mice, demonstrating the importance of this factor.

Ruan et al[16] compared adjuvants and immunisation routes in the induction of suppressive CD4$^+$CD8$\alpha\alpha^+$ T-cells in the murine stomach. Using silk fibroin as the adjuvant induced fewer DP T-cells and reduced *H. felis* colonisation more effectively.

Conflict of Interest

None.

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Informed Consent

None.

Authors' Contribution

None.

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