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Immune Response to *Helicobacter pylori*

Batool Mutar Mahdi

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1. Introduction

Helicobacter pylori (H. pylori) is a Gram-negative microaerophilic spiral bacterium previously known Campylobacterpylori. It has a significant attention since its isolation and characterisation in 1983 [1]. It colonizes the gastrointestinal mucosa of its host in spite of a strong persistent humoral and cellular immune response to *H. pylori* at the local and systemic level, the organism persists for the lifetime of its host.

It is a pathogen, playing an important role in the aetiology of gastritis (especially active antral gastritis). Gastritis is a histopathologic term characterized by chronic inflammation of the stomach mucosa. It is classification based on the underlying cause (eg, *H. pylori,* that cause atrophic gastritis, bile reflux, nonsteroidal anti-inflammatory drugs [NSAIDs], autoimmunity, or allergic response) [2]. Gastritis is also classified as erosive or nonerosive based on the severity of mucosal injury by endoscopy. Other classification is according to the site of involvement (cardia, body, antrum). Histological classification is acute or chronic based on the inflammatory cell type. Acute gastritis is characterized by PMN infiltration of the mucosa of the antrum and body. Chronic gastritis implies some degree of atrophy or metaplasia. It predominantly involves the antrum with subsequent loss of G cells and decreased gastrin secretion or the corpus with loss of oxyntic glands, leading to reduced acid, pepsin, and intrinsic factor [3, 4, 5].

H. pylori causes more than half of peptic ulcers worldwide. The bacterium causes peptic ulcers by damaging the mucous membrane of the stomach and duodenum. Damage to the mucous membrane causing stomach acid to get through to the sensitive lining layer. Together, the stomach acid and *H. pylori* irritate the lining of the stomach or duodenum and cause an ulcer [6].Ulcers in the stomach and duodenum result when the consequences of inflammation allow stomach acid and the digestive enzyme pepsin to overwhelm the mechanisms that protect the stomach and duodenal mucous membranes. The location of colonization of *H. pylori*, which

affects the location of the ulcer, depends on the acidity of the stomach [7].The majority of patients with duodenal ulcer (DU) are infected with *H. pylori* [8].

H. pylori infection is a major cause of gastric adenocarcinoma, specifically non-cardia gastric cancer. H. pylori infection also causes gastric mucosa-associated lymphoid tissue (MALT) lymphoma [9]. Some *H. pylori* bacteria had a toxin produced by a gene called cytotoxin-associated gene A (*cagA*) injected into the junctions where cells of the stomach lining. This toxin (known as CagA) alters the structure of stomach cells and allows the bacteria to attach to them more easily. Long-term exposure to the toxin causes chronic inflammation [10]. Other type of cancer is gastric MALT lymphoma is a rare type of non-Hodgkin lymphoma that is charac-terized by the slow multiplication of B lymphocytes. Normally, the lining of the stomach lacks lymphoid tissue, but growth of this tissue is often stimulated in response to colonization of *H. pylori* [11]. Nearly all patients with gastric MALT lymphoma show signs of *H. pylori* infection, and the risk of developing this tumor is more than six times higher in infected people than in uninfected people [12]

The prevalence of gastric cancers among *H. pylori*-infected patients varies between individuals, countries, and geographic areas, *H. pylori* disease-related outcomes are believed to be deter-mined by interaction between host factors, bacterial factors, and their *interaction* with the *environment* [13] produces different diseases in different persons. High and low acid secretion rates probably contribute to duodenal ulceration and gastric carcinogenesis, respectively. *H. pylori*products and certain cytokines released in gastritis release gastrin from G-cells but inhibit parietal cells. Also tumour necrosis *factor* alpha inhibits somatostatin-cells and interleukin 1 beta inhibits enterochromaffin-like cells. The net result is that antral gastritis tends to increase, whilst corpus gastritis tends to decrease acid secretion. Corpus atrophy further lowers acid through loss of parietal cells. Factors postulated to increase corpus gastritis include: *host* genetics, early acquisition of bacteria, more aggressive strains, poor general health and diets high in salt or lacking in antioxidant vitamins [14]. *Other factor is environmental* factors that play an important role in the manifestation, course, and prognosis of diseases of *H. pylori*-induced gastritis. This disease is the outcome of allelic group of a *host* and the microbial (microflora) and physical environments. *Host* alleles predisposing to a disease in one genomic and/or *environmental* surroundings may not be deleterious in other group, in addition to that microbes can have different effects in different hosts and under different *environmental* conditions [15]. *H. pylori* eradication may not eliminate the risk of gastric cancer. Therapy of this bacteria may be used in high-risk populations to reduce gastric cancer incidence. It can reverse many biochemical, genetic, and epigenetic changes that *H. pylori* infection induces in the stomach [16]. The major factors playing a pathogenic role in *H. pylori*-related non-cancer diseases are host polymorphisms in genes involved in inflammation and protection against oxidative damage, host exposure to dietary genotoxic agents, and bacterial genetic polymorphisms [17]. The study of *host*-bacterial *interaction* is key to detection the molecular and cellular pathways involved and will lead to developing preventive and therapeutic modules against this pathogen [18].

Gastric inflammation nearly always precedes the development of peptic ulceration, and is a critical component in initiating the multi-step progression towards gastric carcinogenesis due to this interaction between these three factors [19].

H. pylori is spread from person to person by two routes, oral-oral and faecal-oral route [20].Food prepared under less ideal conditions or exposed to contaminated water or soil may increase the risk. Inadequate sanitation practices, low social class, and crowded or high-density living conditions seem to be related to a higher prevalence of *H. pylori* infection. Therefore poor hygiene and crowded conditions may facilitate transmission of infection among family members Understanding the route of *H. pylori* transmission is important if public health measures to prevent its spread. Iatrogenic transmission of *H. pylori* following endoscopy is the only proven mode. The person-to-person mode of transmission is supported by the higher incidence of infection among institutionalized children and adults and detection of *H. pylori* DNA in vomitus, saliva, dental plaque, gastric juice, and feces. Waterborne transmission, probably due to fecal contamination or contaminated well water, may be an important source of infection, especially in parts of the world in which untreated water is common. *H. pylori* has been isolated in domestic cats, housefly. However, evidence is lacking that H. *pylori* can be transmitted to humans from flies that have been in contact with *H. pylori*-infected feces. Mode of transmission of *H. pylori* is important to prevent its spread and identifying high-risk populations [21, 22].

The pathogenesity of this bacterium depend on the production of several virulence factors. The most important ones are CagA (cytotoxic associated gene A) and VacA (vacuolizating cytotoxin A). The immunodominant CagA protein is encoded by genes called the cagA-PAI (pathogenicity island), a 40 kb genomic fragment containing ORFs (open reading frames) encoding approximately 31 genes that forms a type IV secretion system, which is found in 60–70% of *H pylori* strains and can be divided into two regions, cag I and cag II, according to a novel insertion sequence [23] This secretion system (T4SS) forms a pilus that delivers CagA, an oncoprotein, into the cytosol of gastric epithelial cells through a rigid needle structure covered by CagY, a VirB10-homologous protein and CagT, a Virb7-homologous protein, at the base [24]. This is accomplished by a specialized adhesin of the pilus surface, the CagL protein, which binds to and activates host cell integrins for subsequent delivery of CagA across the host cell membrane. Injected CagA becomes tyrosine-phosphorylated by Src and Abl family kinases and mimics a host cell protein in binding and activation of multiple signalling factors. After the induction of membrane dynamics, actin cytoskeletal rearrangements and the disruption of cell-to-cell junctions as well as proliferative, pro-inflammatory and anti-apoptotic nuclear responses. All these signalling cascades contribute in *H. pylori* pathogenesis [25]. Therefore, CagA remains the only identified effector mechanism that is translocated by the T4SS of *H. pylori* into gastric epithelial cells where it induces host cell kinases that phosphorylate tyrosine residues in CagA adjacent to the site of bacterial adhesion on the host gastric epithelial cells, which in turn activate eukaryotic signal transduction pathways and cytoskeletal plasticity [26]. Wild-type *H pylori* (not phosphorylation-resistant CagA)induced a growth factor-like response in gastric epithelial cells. CagA formed a physical complex with the SRC homology 2 domain (SH2)-containing tyrosine phosphatase SHP-2 in a phosphorylation-

dependent manner and stimulated the phosphatase activity. On the other hand, the CagA effect on cells was reproduced by constitutively active SHP-2. Thus, upon translocation, CagA perturbs cellular functions by deregulating SHP-2 [27]. Injected CagA associates with the epithelial tight-junction scaffolding protein ZO-1 and the transmembrane protein junctional adhesion molecule, causing an ectopic assembly of tight-junction components at sites of bacterial attachment, and altering the composition and function of the apical-junctional complex. Long-term CagA delivery to polarized epithelia caused a disruption of the epithelial barrier function and dysplastic alterations in epithelial cell morphology [28].

Regarding VacA, there is a significant polymorphism in this gene (vacA s, vacA i and vacA m). There are two types of the s (s1 and s2), i (i1 and i2), and m (m1 and m2) regions of *vacA* [29]. Three polymorphic subtypes, namely m1T, m1Tm2 and m2. The s1a/i1/m1Tm2 genotype had a higher frequency of lymphoid follicle formation in the corpus than the s1a/i1/m1T and s1a/i1/m2 strains while *vacA* s1/m1 strains are most closely associated with gastric carcinoma [30]. The method PCR- genotyping from gastric biopsy had advantages over routine diagnostic tools such as histological *H. pylori* detection, which has high interobserver variation, very much depending on the experience of the pathologist, and over *H. pylori* culture, which is more time-consuming, requires expertise, and is not always successful. In general, PCR-based techniques show high sensitivity for *H. pylori* detection and permit further characterization of bacteria virulence-associated genotypes. In archive materials, the genotyping of the *vacA* i region allows the analysis of the *H. pylori* strains in the same biopsy specimen that can also be used for histopathological evaluation, conferring a more reliable measurement of the effects of local infecting strains. Another advantage of the use of archive materials is that it permits retro-spective studies to be performed without the need of *H. pylori* isolation from fresh biopsy specimens [31].

In most cases, the infection is asymptomatic and clinical manifestation appears in only 10-15% of infected individuals. This is due to virulence of *H. pylori* strains and host immune response to this bacterium [32]. The different clinical outcomes may be explained by bacterial factors and the host immune responses. *H. pylori* induce a strong immune response with infiltration of neutrophils, B- and T-cells into the gastroduodenal mucosa that fails to clear the infection. Several virulence factors have been associated with the development of gastroduodenal disease, e.g. the cytotoxin-associated gene A (CagA) and a vacuolating toxin [33] individuals infected with strains lacking these genetic markers may still develop peptic ulcers, and that many individuals infected with bacterial strains bearing these genotypes do not develop any symptoms [34]. On other hand, the lower epithelial cytokine responses may be of importance for the pathogenesis of *H. pylori*-induced ulcers, most likely can be explained by host factors, i.e. mainly a decreased ability of the epithelium to produce cytokines, but possibly partly also down-regulation by regulatory T cells [35]. *H. pylori*infection induces strong antibody responses in the human gastric mucosa, both in asymptomatic carriers and in ulcer patients [36].

This will shed a light on the immune response to *H. pylori* colonization and its effect on clinical outcomes.

2. Host immune response to *H. pylori*

The immune response towards bacterial pathogens can be divided into an innate and an adaptive response.

2.1. Innate immune response to *H. pylori*

Recognition of bacterial antigenic molecules is mediated by TLRs (Toll-like receptors) that are expressed by distinct cell types throughout the gastrointestinal tract, and play an important role in regulation of the innate immune response especially TLR4 It is expressed on antigen-presenting cells such as monocytes and dendritic cells. Bacterial contact with monocytes and other APCs leads to the secretion of proinflammatory cytokines such as TNF-α (tumour necrosis factor-α), IL (interleukin)-1β and IL-8. *H. pylori* infection has been shown to be associated with increased levels of these cytokines which, in turn, act as local chemoattractants, inducing granulocytic infiltration [37]. The gastric epithelia of children respond to *H. pylori* infection by increasing the expression of TLR2, TLR4, TLR5, TLR9 and the cytokines IL-8, IL-10 and TNF-α that participate actively in innate immune responses [38]. Their role in the progression of gastric lesions leading to cancer is associated with decreasing levels of TLRs inhibitors and elevated TLRs levels throughout all the spectrum of lesions [39].
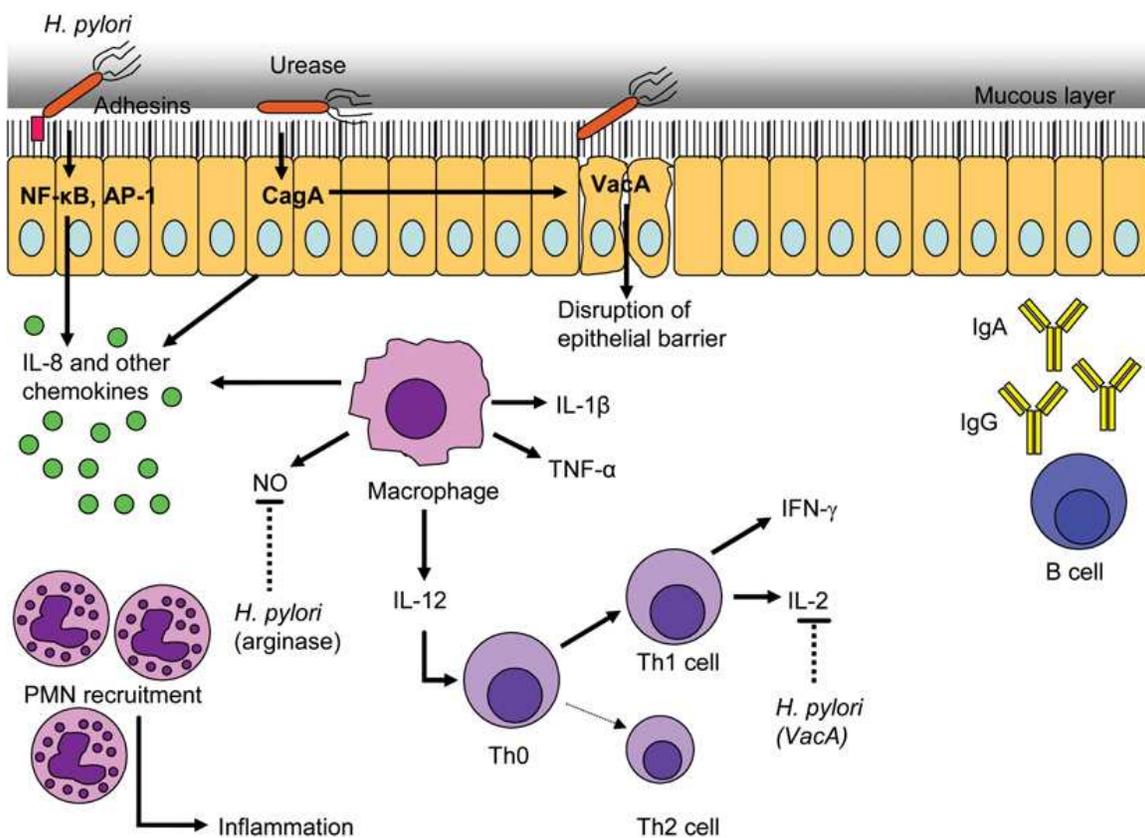

Figure 1. *H. pylori* pathogenesis, the inflammatory immune response and some of escape mechanisms [54].

2.2. Adaptive immunity: cellular immune response and humoral response

2.2.1. Cellular immune response

Adaptive immune responses towards *H. pylori* infection have been developed after failure of innate immune response to eliminate the pathogen. The immune response to *Helicobacter pylori* is a versatile group of mechanisms involving responses that are both protective and damaging to the host. The innate and the adaptive immune responses lead to damaging inflammatory responses allowing for persistence of many infections [40]. *H. pylori* associated inflammatory reaction is characterized by a mucosal infiltration of different cells like poly-morphonuclear leukocytes (PMN), T cells, macrophages, and plasma cells [41] in addition to that *H. pylori* adheres to the cells of gastric mucosa and secretes different molecules that can change gastric epithelial cell function [42]. Chronic active gastritis is associated with an increased CD4/CD8 T-cell ratio within the gastric mucosa and accumulation of CD4+ T-helper lymphocytes in the lamina propia of the gastric mucosa. *H. pylori* infection results in a Th1-predominant host immune response in the gastric mucosa and induction of IFN-γ (interferon-γ) and IFN-γ -related genes. A Th1-predominant immune response is associated with elevated levels of the pro-inflammatory cytokines IL-12, IL-18 and TNF-α([43]. Other cell that infiltrates the gastric mucosa was Th17 which are CD4+ T cells associated with infections and inflamma-tion. Th17 are induced during both *H. pylori* infection and gastric cancer in the inflammatory process of gastric stroma and may be an important link between inflammation and carcino-genesis [44]. The host genetic milieu contributes to the inflammatory response to *H. pylori* infection is IL-1B. The *IL-1B* gene encodes the expression of IL-1β, a potent pro-inflammatory cytokine and powerful inhibitor of gastric acid secretion that had a most important responsi-bility in initiating and amplifying the inflammatory response to *H. pylori* infection [45]. It had been that IL-1 polymorphism had an effect on IL-1 production in gastric mucosa infected with *H. pylori* [46]. This indicate that individual genetic polymorphism had an effect on disease expression.

Yuceyar etal 2002 [47] found that there is no alteration in total T and B lymphocytes and CD4+ T, CD8+ T lymphocytes and natural killer cells of both duodenal ulcer and chronic antral gastritis patients compared to normal persons. Although there was a slight increase in the proportion of active T lymphocytes in duodenal ulcer and chronic antral gastritis groups comparing to healthy subjects the difference was not statistically significant. This indicate that there is no systemic alteration in the specific immune system in response to *H. pylori* in patients with duodenal ulcer and chronic antral gastritis. Other study confirmed the systemic immune response to helicobacters at the cellular level in patients with *Helicobacter pylori* infection by leukocyte migration inhibition test was performed and a highly significant inhibitory effect on leukocyte migration was found in patients with *Helicobacter pylori* infection [48].

2.2.2. Humoral immune response

H. pylori induce a strong specific systemic and local antibody response and infected individual had antibodies against whole bacteria or part of it [49]and increase in plasma cells in gastric mucosa which produce IgA [50]. Other important antibody was IgG that binds to *H. pylori* and

enhance phagocytosis [51].This antibodies lead to complement activation by either classical or alternative pathways [52]. In addition to that, the role of the secretory IgA is important in neutralizing urease and VacA as well as inhibiting adherence of *H. pylori* to gastric mucosa [53].The immune response to *H. pylori* can be summarized by figure -1- [54]. There is high seroprevalence of *H. pylori* antibodies undervalue of past infection, the relation of the *H. pylori* cytotoxin to gastric precancerous lesions is warranted. There is a strong mucosal IgA response to *H. pylori* in non-neoplastic antral mucosa of gastric cancer patients irrespective of the biopsy urease results [55].

2.2.2.1. Evasion of immune response by H. pylori

Helicobacter pylori is a gastric bacterial pathogen that evades host immune responses in vivo by different mechanisms like inducing apoptosis of macrophages in association with altera-tions in the mitochondrial pathway [56].

Elimination of this key immunomodulatory cell may represent a *mechanism* employed by the bacterium to evade host immune responses. It can survive intracellularly within macrophages by interfering with lysosomal proteins, similar to *Mycobacterium tuberculosis* [57]. *H. pylori* like many other pathogen escape the immune system by production an enzyme arginase that prevent nitrous oxide production [58].

Vacuolating cytotoxin secreted by *H. pylori* has turned out to be a potent immunomodulatory toxin. VacA-deficient *H. pylori* induced significantly higher expression of integrin-linked kinase (ILK) and endothelial nitric oxygen synthase (eNOS), and significantly more production of reactive oxygen species (ROS) in monocyte/macrophage-like U937 cells, as compared with isogenic vacA+ *H. pylori*. Thus, vacA-deficient *H. pylori* appears to upregulate ILK expression, which modulates the expression of eNOS and as a result, stimulates the production of ROS. It is VacA that prevents such a process by inhibiting ILK expression, helping *H. pylori escape* host immunoreaction and persist in gastric mucosa [59].

In addition to that, *H. pylori* LPS exhibits a reduced endotoxic potency in terms of pyrogenicity, lethality, toxicity, mitogenicity and the lower immune response elicited by *H. pylori* LPS in comparison with other enterobacterial LPS may represent an *escape mechanism* from the host [60]. *H. pylori*, possess flagellin molecules that cannot be recognized by TLR5 that is important for its survival [61]. *H. pylori* infection could alter cellular gene expression processes that evade host immune mechanism by activating NF-κB and Wnt/β-catenin signaling pathway, disturb metal ion homeostasis, and induce carcinogenesis [62].

VacA of *H pylori* act as an immunomodulator by interfering with the IL-2 signalling pathway in T-cells by blocking Ca2+ mobilization and the activity of the Ca2+/calmodulin-dependent phosphatase calcineurin [63]. It also interferes with antigen presentation mediated by MHC class II [64]. VacA by itself act as immunosuppressive by a direct action on T-cells rather than APCs [65]. *VacA* is a crucial element for *H. pylori* to *escape* from host immune defense by means of differentially regulating the expression of some related genes by altering their mRNA expression at different times [66].

2.2.2.2. Vaccination against H. pylori infection

Helicobacter pylori can persist in the gastric mucosa of infected individuals for life, in the face of chronic inflammation and low pH. Efforts to develop vaccines have largely failed and, in the wake of emerging antibiotic resistance, novel therapeutic approaches have developed [67].

There is a consent that vaccines are essential to limit the severity of this infection. Great development has been made since its detection 25 years ago the virulence factors and several aspects of the pathogenesis of the *H. pylori* gastric diseases. Several key bacterial factors have been identified: urease, vacuolating cytotoxin, cytotoxin-associated antigen, the pathogenicity island, neutrophil-activating protein, and among others. These proteins, in their native or recombinant forms, have been shown to confer protection against infectious challenge with *H. pylori* in animal models. However, a quantity of clinical trials in healthy volunteers have been conducted using urease given orally as a soluble protein or expressed in bacterial vectors with limited results or a mixture of *H. pylori* antigens was reported to be highly immunogenic in *H. pylori*-negative volunteers following intramuscular administration of the vaccine with aluminium hydroxide as an adjuvant [69].

H. pylori is a mucosa-associated organism, it was initially thought that an IgA type anti-*Helicobacter* antibody response would be essential for protective immunity [69]. An adjuvant is an important component of any oral/mucosal vaccine as it is responsible for stimulating immune system, but due to toxicities associated with these agents, there are currently no suitable and safe adjuvants available for use in humans. Another important limitation to effective oral immunization is that it requires multiple doses and a large amount of antigen administration. These studies showed that mucosal immunity can be induced by oral or intranasal routes of immunization. The intranasal route of immunization is similar to the oral route in that it also requires the administration of bacterial antigen in conjunction with an adjuvant multiple times [70].

Considering the side effects of oral adjuvants, researches have focused attention on making oral immunization safe and effective for human usage. Thus, *E. coli LT* heat-labile toxin that was used as an oral adjuvant in humans did show a significant decrease in gastric *H. pylori* density but was associated with cramping and diarrhea [71]. Another approach is to use other mucosal routes such as the nasal mucosa and the rectal mucosa for effective immunization. When mice were immunized orally, rectally and intranasally in combination with *E. coli* heat-labile toxin (LT) and subsequently challenged with purified *H. pylori* antigen, mice immunized by either of the routes were found to be protected against this challenge compared with controls. These studies showed that mucosal immunity can be induced by oral or intranasal routes of immunization. The intranasal route of immunization is similar to the oral route in that it also requires the administration of bacterial antigen in conjunction with an adjuvant multiple times [72].

Although the intransal route of immunization seems the most efficient and effective route of mucosal immunization, it still has some disadvantages histologic inflammation in the olfactory bulb and cause paralysis of facial nerves [72]. Intranasal route could also result in oral ingestion thus exposing the subject to various side effects and toxicities. Eriksson et al. [73] found that

CTA1-DD adjuvant was to be safe for intranasal administration without any accumulation in the nervous tissue.

Other modalities of administering vaccines such as the intraperitoneal (i.p.) and subcutaneous (s.c.) routes are also being done. Mice were vaccinated i.p. using *H. pylori* antigen in combination with aluminum hydroxide and upon rechallenge with *H. pylori* were found to confer protection that was shown by absence of bacteria, both histologically and in culture of gastric biopsy tissues. This immunity was noted to be antibody independent and achieved by IL-5-secreting T cells [74]. The results of these experiments suggest that until a safe, effective, and inexpensive mucosal (oral/nasal) vaccine becomes available, systemic immunizations should be a consideration.

Urease is important as a vaccine antigen has been confirmed by numerous studies in mice, ferrets, and non-human primates [75]. Urease conferred protection against helicobacter infection when delivered either as a native protein or as an enzymatically inactive recombinant protein [76]. Therapeutic immunisation with urease has recently been reported in ferrets naturally infected with *H. mustelae*. [77]. Infection with *H. mustelae* occurs in ferrets soon after weaning, persists for life, induces active chronic gastritis, and is associated with the development of ulceration in a subgroup of infected animals [78]. Thus, therapeutic immunisation is possible in a natural setting of helicobacter infection, confirming that the inability of the natural immune response to clear helicobacter infection can be overcome.

Other method of delivery of the antigen used in mice by immunisation with genetically engineered bacteria expressing *H. pylori* antigens elicits a protective immune response against gastric helicobacter infections. Immunisation with live bacterial vaccines usually requires only one or two doses, does not depend on the addition of extra mucosal adjuvant, and the vaccine can be produced at very low cost. Additional improvements to the vaccine should result from a better understanding of the natural immune responses to helicobacter infection and of the mechanisms by which vaccination restores protection [79].

Zhang etal, 2013 [80] used recombinant technology, Lactococcus lactis (L. lactis) could serve as an antigen-delivering vehicle for the development of edible *vaccine*. They produced edible UreB (urease B) *vaccine* derived from L. lactis against H. *pylori*. The UreB subunit is the most effective and common immunogen of all strains of H. *pylori*. The UreB was produced as a chimeric protein fused with IL-2 (human interleukin 2) as the mucosal adjuvant. Mucosal immunization of mice with recombinant L. lactis NZ9000 containing the UreB-IL-2 protein elicited more anti-UreB antibody that specifically bounded to the purified bacterial UreB protein and more cytokines such as IFN-γ, IL-4, and IL-17, and had a lower H. *pylori* burden and urease activity than control mice. These results suggest that the recombinant L. lactis expressing UreB-IL-2 can be potentially used as an edible *vaccine* for controlling H. *pylori* infection.

Altman etal, 2013 [81] demonstrated that synthetic glycoconjugates based on delipidated lipopolysaccharide (LPS) of *Helicobacter pylori* and containing an α(1-6)-glucan chain induced broadly cross-reactive functional antibodies in immunized animals. Thus, they prepared glycoconjugates based on dextrans produced by lactic acid bacteria Leuconostoc mesenter-

oides B512F and consisting of linear α(1-6)-glucan chains with limited branching. Three dextrans with averaged molecular masses of 5, 000 Da, 3, 500 Da and 1, 500 Da, respectively, were modified with a diamino group-containing linker and conjugated to a carrier protein, tetanus toxoid (TT) or diphtheria toxoid (DT. The conjugates were immunogenic in both rabbits and mice and induced specific IgG responses against α(1-6)- glucan -expressing H. *pylori* LPS. Studies performed with post-immune sera of mice and rabbits immunized with dextran-based conjugates demonstrated cross-reactivity with LPS from typeable and non-typeable strains of H. *pylori* and selected mutants. The post-immune sera from rabbits that received the conjugates exhibited functional activity against α(1-6)-glucan-positive strains of *H. pylori*. These data provide evidence that dextran-based conjugates may offer a simplified approach to the development of carbohydrate-based *vaccines* against *H. pylori*.

Helicobacter pylori neutrophil-activating protein (NAP) is a toll-like receptor 2 (TLR2) agonist and potent immunomodulator inducing Th1-type immune response. Iankov etal 2013 [82]studied the humoral immune response against NAP-tagged antigens, encoded by attenuated measles virus (MV) vector platform, in MV infection susceptible type I interferon receptor knockout and human CD46 transgenic (Ifnarko-CD46Ge) mice. Immunogenicity of MV expressing a full-length human immunoglobulin lambda light chain (MV-lambda) was compared to that of MV expressing lambda-NAP chimeric protein (MV-lambda-NAP). MV-lambda-NAP immunized Ifnarko-CD46Ge mice developed significantly higher (6-20-fold) anti-lambda ELISA titers as compared to the MV-lambda-immunized control animal group, indicating that covalently-linked NAP co-expression significantly enhanced lambda immunogenicity. In contrast, ELISA titers against MV antigens were not significantly different between the animals vaccinated with MV-lambda or MV-lambda-NAP. NAP-tagged antigen expression did not affect development of protective anti-measles immunity. Both MV-lambda and MV-lambda-NAP-immunized groups showed strong virus neutralization serum titers in plaque reduction microneutralization test. They concluded that MV-encoded lambda-NAP is highly immunogenic as compared to the unmodified full-length lambda chain. Boost of immune response to poor immunogens using live vectors expressing NAP-tagged chimeric antigens is an attractive approach with potential application in immunoprophylaxis of infectious diseases and cancer immunotherapy.

An important consideration in the development of subunit *vaccines* is the loss of activity caused by physical instability of the protein. CagL is a protein present in strains of *H. pylori*. It contributes to bacterial adherence via $\alpha5\beta1$ integrin, thereby making it an attractive subunit *vaccine* candidate. Choudhari etal, 2013 [83] used CagL in different pH and temperature conditions using a variety of spectroscopic techniques. Stability was assessed in terms of transition temperature with the accumulated data, and then incorporated into an empirical phase diagram (EPD) that provided an overview of CagL physical stability. These analyses indicated maximum CagL stability at pH 4-6 up to 40°C in the absence of excipient. Using this EPD analysis, aggregation assays were developed to screen a panel of excipients with some found to inhibit CagL aggregation. These analyses will help in the formulation of a stable *vaccine* against H.*pylori*.

Helicobacter pylori is a major human pathogen that colonizes the stomach and is the lead etiological agent for several pathologies. Sutton P and Chionh YT 2013 [84] demonstrated that effective *vaccine* against these bacteria would be invaluable for protecting against gastric adenocarcinoma. However, the development of such a *vaccine* has stalled and the field has progressed little in the last decade. The key problems that are preventing the development of a *H. pylori vaccine*. Primarily, this involves the inability to produce a completely protective immune response. The knock-on effects of this include a loss of industry investment. Over-coming these problems will likely involve defeating the immune-evasion defenses of *H. pylori*, in particular the mechanism(s) by which it evades antibody-mediated attack.

Chronic *H. pylori* infection can be successfully eradicated by intragastric vaccination with *H. pylori* antigens such as recombinant VacA and CagA, which were administered together with a genetically detoxified mutant of the heat-labile enterotoxin of Escherichia coli (referred to as LTK63), in which the serine in position 63 was replaced by a lysine. The therapeutic vaccination confers efficacious protection against reinfection [85]. Vaccines against *Helicobacter pylori* could circumvent the problem of increasing antibiotic resistance. They would be particularly useful in developing countries, where re-infection rates are high following standard eradication regimes. The vaccine could be given orally with an *H. pylori* whole cell sonicate preparation and cholera toxin adjuvant. Protection was associated with increased serum *H. pylori* IgG antibodies. In this type of vaccine the levels of gastric IL-12p40 and IFN-gamma transcripts were significantly decreased. Gastric IL-10 and TGF-beta transcripts were found only at relatively low levels [86]. Other type of vaccines were used a recombinant antigen ureB138 (a segment of the beta-subunit of urease) against *Helicobacter pylori* infection [87]. Using mucosal vaccination with *H. pylori* antigens when given together with cholera toxin (CT) adjuvant, but not without adjuvant, can induce protective immune responses against *H. pylori* infection. However, the toxicity of CT prevents its use as a mucosal adjuvant in humans. The using a nontoxic double mutant Escherichia coli heat-labile toxin, LT(R192G/L211A) (dmLT), as a mucosal adjuvant in an experimental H. pylori vaccine and compared it to CT in promoting immune responses and protection against *H. pylori* infection. Immunization via the sublingual or intragastric route with *H. pylori* lysate antigens and dmLT resulted in a significant decrease in bacterial load after challenge. Cellular immune responses in the sublingually route resulting in enhanced *in vitro* proliferative and cytokine responses from spleen and mesenteric lymph node cells to *H. pylori* antigens. Thus, dmLT is an attractive adjuvant for inclusion in a mucosal vaccine against *H. pylori* infection [88].

3. Conclusions

Helicobacter infection is an important cause of cancer development. Immune response to this bacterial infection is playing an important role in gastric inflammation. The infection may be innocent or subclinical. Therefore, frequent patients screening for this bacteria and eradication of *Helicobacter* spp. is very crucial. Researches about these bacteria are very essential starting from laboratory animal to application of the researches from lab bench to clinic. Innate and adaptive immune responses of the host are seriously significant for the development of new

plans to prevent the development of *H. pylori*-induced gastroduodenal disease and production a vaccine to eradicate this infection. It will be necessary to determine the feasibility of using different epitopes extracted from *H. pylori* in a vaccine design.

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