Vagus Nerve Versus Helicobacter pylori: New View on Old Secrets of Gastroduodenal Pathology

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Author’s contribution

This whole work was carried out by author OS.

ABSTRACT

Despite the long history of research on etiology and pathogenesis of gastroduodenal pathology, there are still a lot of unclear points in this field. Identification of Helicobacter pylori has been widely considered to be a major breakthrough in pathophysiology of gastroduodenal diseases. However, Helicobacter pylori infection, as a host-pathogen interaction, involves bidirectional cross-talk and requires assessment not only in terms of Helicobacter pylori virulence, but the immune reactivity of the host too. One of the major tasks of mucosal immunity is to discriminate between dangerous and harmless antigens, and it depends on neuroimmune cross-talk, which is orchestrated in gastroduodenal area by vagus nerve. In this review, we propose to recall and assess the role of the vagus nerve in the gastroduodenal homeostasis maintenance and anti-Helicobacter pylori fight, focusing on Helicobacter pylori-gastric mucosa-vagus nerve interaction. The following issues are discussed in this review: 1) Vagus nerve regulation of mucins production as a key element of gastrointestinal barrier against Helicobacter pylori colonization; 2) Virulence factors of Helicobacter pylori and cytoprotective effects of vagus nerve; 3) Peculiarities of Helicobacter pylori-induced innate immunity response, involving host cytokine network activation, and vagus nerve effects, realized through cholinergic anti-inflammatory pathway; 4) Modulation of maturation and activity of dendritic cells by Helicobacter pylori and acetylcholine; and 5) Alteration of adaptive immunity during Helicobacter pylori infection and role of acetylcholine. The structural and immune homeostasis of gastroduodenal area can be affected not only by Helicobacter pylori virulence factors, but

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theoretically by any shifting in vagus nerve reactivity and/or interaction with enteroendocrine and immune cells in gastroduodenal mucosa.

Keywords: Vagus nerve, Helicobacter pylori, acetylcholine, mucosal immunity.

ABBREVIATIONS

Ach – Acetylcholine; Alpha7nachr – Alpha7 Nicotinic Acetylcholine Receptors; Cag A – cytotoxin-associated gene A; CAMP – Cyclic adenosine monophosphate; DAG – diacylglycerol; DC – Dendritic Cells; GDP – Gastroduodenal Pathology; HMGB1 – High Mobility Group Box 1; HP – Helicobacter pylori; I3F – inositol-1,4,5-triphosphate; IL – Interleukin; INOS – Inducible Nitric Oxide Synthase; LPS – lipopolysaccharide; MR - Muscarinic Receptor; MUC – Mucin; NF - kB – Nuclear Factor Kappa Beta; NO – nitric oxide; PAMP – Pathogen -Associated Molecular Patterns; PGE2 – Prostaglandin E2; PRRs – pattern recognition receptors; TCR – T cell receptor; TGFβ – transforming growth factor β; TLR – Toll-like receptor; TNFα – Tumor necrotic factor α; Treg – Regulatory T cells; VacA – vacuolating cytotoxin A; VIP – Vasointestinal Peptide; VN – Vagus Nerve.

1. INTRODUCTION

Nowadays, gastroduodenal pathology (GDP) occupies one of the leading positions in the structure of human diseases. Despite the long history of GDP etiology and pathogenesis research, there are still a lot of unclear points in this field. To understand the problem and develop new therapeutic approaches for treating of GDP it is necessary to look back to the history. The story of GDP pathogenesis began in 17th century from the early ideas on gastric secretion (Spallanzani and de Reaumur) and with first descriptions of food digestion (Dupuytren and Bichat, Beaumont, early 18th century) [1]. Modern gastroenterology has started at the beginning of 19th century by Prout who confirmed the active secretion of hydrochloric acid by the stomach and related it to the symptoms of dyspepsia [2]. The research continued with the first descriptions of gastric glands as the source of gastric acid and their changes upon digestive stimulus (Purkinje and Golgi, mid and late 19th century). Thus, gastric acid and pepsin (Schwann, early 19th century) were found to be essential for food digestion, subsequent studies pointed to histamine, being the most potent final common chemostimulator of oxyntic cells [1]. The role of the vagus nerve in the control of gastric acid secretion was identified in the early and mid-19th century by Brodie, and further explored by I. P. Pavlov, who discovered the neuro-reflex stimulation of gastric secretion [1,3]. He proposed the concept of nervism or entire neural control of gastric secretion that was widely recognized at that time, and in 1904, for the first time in gastroenterology, the proponent of this concept was awarded the Nobel Prize [1]. When concept of nervism or complete neural control of all digestive functions reached its apogee in Eastern Europe, on the other side of it, in United Kingdom in 1906, E. Edkins discovered that a hormone, gastrin, may serve a chemical messenger in stimulation of gastric acid secretion, while in 1916 L. Popielski revealed that histamine is the most potent gastric secretagogue [1]. K. Schwartz, without considering neural or hormonal nature of gastric secretory stimulation, enunciated in 1910 the famous dictum: "no acid no ulcer", that later induced the term of “mucosal defense” and the notion that the breaking of “gastric mucosal barrier” represents the initial step in the process of mucosal injury (Davenport, Code and Scholer, mid-20th century) [2,3].
Recent milestones in the understanding of gastric acid secretion and treatment of acid-peptic disorders include the discovery of histamine H$_2$-receptors and development of histamine H$_2$-receptor antagonists by J. W. Black, who was awarded in 1972 the second Nobel Prize in gastroenterology; identification of H$^+$, K$^+$-ATPase as a proton pump of oxyntic cells and development of proton pump inhibitors; identification of *Helicobacter pylori* (*HP*) as the major cause of gastric and duodenal ulcer, and development of effective eradication regimens [1,4]. The discovery of *HP* in 1980 by B. J. Marshall and R. J. Warren, Australian clinical researches, was awarded in 2005 the Nobel Prize for the third time in the history of gastroenterology, this has been widely considered to be a major breakthrough in pathophysiology of gastritis and peptic ulcer, which for the first time can be definitively cured by merely eradication of a germ infecting stomach [4]. Nowadays eradication therapy is considered to be a key of gold standard treatment of GDP [5,6]. Thus, solving the problem of GDP treatment was turned from nerves and hormones to bacteria!

As it was proved, *HP* is a Gram-negative, microaerophilic bacterium which selectively colonizes the gastric mucosa of more than 50% of the human population, causing chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer [7]. The pathogenicity of *HP* depends on expression of several unique virulence factors, including vacuolating cytotoxin A (VacA), which induces apoptosis of epithelial cells, and cytotoxin-associated gene A (CagA) and CagL, that initiates signal transduction events in infected cells [8]. Another important factor required for in vivo colonization of stomach by *HP* is urease, which neutralizes gastric acid by generating ammonium from urea. In addition, *HP* cell division-related gene A (cdaA) and *HP* lipopolysaccharides (LPS) are closely studied in recent years regarding their impact on gastric epithelial cells kinetics and immune response alteration respectively [9,10]. The release of *HP* proteins facilitates survival of the bacterium in the gastric mucosa, but also results in the potent innate and adaptive immune response. These facts had grounded the methods of diagnostics, prognostic biomarkers and strategy of *HP*-associated pathology treatment [5].

For today, we have more than a 30 years’ experience of this fight. Let’s answer the question: What did we expect and what do we really have? The frequency of GDP is still high, eradication of *HP* does not stop the gastritis and even less so gastro-esophageal reflux disease and duodenitis [6,11], “gold standard” treatment does not prevent the complications of ulcers, for instance bleeding that is a life-threatening medical emergency, and gastric cancer development [12]. Despite the therapy patients with GDP have periodic exacerbation and relapses [5].

As a matter of fact, the *HP*-infection, as a host-pathogen interaction, involves bidirectional cross-talk and requires assessment not only in terms of *HP* virulence, but the immune reactivity of the host too [13]. The latter primarily includes the local mucosa-associated immune system that is tightly associated with gastric endocrine cells and nerves. The central nervous system interacts dynamically with the immune system to modulate inflammation through neuro-endocrine pathways [14,15]. One of the main participant of this cross-talk is VN and its neurotransmitter acetylcholine (ACh). Without neglecting the role of *HP* as a risk factor of GDP, we suggest to turn back from *HP* eradication to understanding the systemic mechanisms of gastroduodenal disorders. That is why in this review, we propose to recall and assess the role of the VN in *HP* associated GDP.

As historically VN was recognized to be the key causer of gastroduodenal diseases, at the beginning of discussion is quite important to clarify, what is more dangerous to alter gastroduodenal homeostasis: VN hyperstimulation or *HP* infection?
2. WHO IS A FOE: *H. pylori* OR HYPERACTIVE VAGUS NERVE?

An overactive vagus is considered to be one of the main “causers” of the increased acid secretion and progressive damage of gastroduodenal mucosa. Is this really so? To answer this question, it is necessary to analyze the mechanisms of influence of ACh, as the main vagal neurotransmitter, on the parietal cells [15,16]. The stimulating effect of ACh on the parietal cells is carried through M3-muscarinic cholinoreceptors (MR). Via a Gq-protein ACh activates phospholipase C, with the formation of inositol-1,4,5-triphosphate (I3F) and diacylglycerol (DAG) [1,3]. I3F links with glycosylated receptor protein P400, which is a Ca2+ pump, that increases the level of intracellular Ca2+ that through protein kinase C stimulates expression of membrane transporters and pumps. This cascade leads to the main effect of ACh – activation of Na+-K+-ATPase on the parietal cells and increases production of HCL that is supposed to be the main aggressive peptic factor [17]. However, initiation of acid secretion mechanisms is accompanied with simultaneous activation of feedback system. This phenomenon is associated with the DAG hydrolysis that results in the release of arachidonic acid, which is metabolized under physiological conditions via the cyclooxygenase pathway to prostaglandin E2 (PGE2) [18]. The latter is a direct antagonist of histamine H2-receptors on parietal cells and activates adenyl cyclase, inducing the antisecretory effect [1]. An increased PGE2 production not only limits excessive acid production, but also provides protection of gastroduodenal mucosa [3]. This effect of PGE2 is connected with an increased microcirculation and mucus secretion, as well as bicarbonate transport in epithelial cells [19]. In addition, prostaglandins reduce secretory activity of ECL- and G-cells, decreasing histamine and gastrin secretion [18].

Additionally to direct control of oxyntic cells, ACh provides stimulating effect on the G-cells [20]. Gastrin stimulates histamine release from endocrine and mast cells by histidine decarboxylase activation. Schematically, the sequence of events in the gastric mucosa can be represented as follows: Vagus nerve stimulation – ACh release – G-cells stimulation – increased gastrin production – ECL-cells stimulation – increasing histamine production [21]. In its turn, histamine through H2-receptors increases acid production by activation of adenyl cyclase, increasing cAMP, stimulation of cAMP-dependent protein kinase A, that induces the translocation and insertion of H+-K+-ATPase into the apical plasmalemma of oxyntic cells [22]. However, it should be mentioned that histamine is also able to activate H1-receptors of ECL-cells, and, in this way, inhibit their activity in autocrine manner [21]. The autocrine regulative loop restricts the hydrochloric acid production and limits the effects of vagal stimulation, as well as ACh release.

In contrast, *HP* infection is associated with decrease in gastric acidity, and can persist without any clinical symptoms. What is the price for such a “positive” gift, though? As it is well known, *HP* releases numerous proteins, some of which, for instance, VacA, are associated with the host damage by pores formation in the membranes of host cell, disrupting membrane trafficking and inducing apoptosis [9]. It was shown that VacA decreases level of some cellular survival proteins, such as Stat3 and Bcl-2 family protein [7,8]. Similarly, other scientists showed that expression of a pro-apoptotic member of the Bcl-2 family, Bax, was induced through VacA activation [10,23]. A recent study further expanded the knowledge of the role of VacA in the host cell damage by a detailed examination of the death mechanisms, showing a caspase independent process that included the histone-binding protein high mobility group box 1, which is consistent with known necrosis pathways [24].
The next important * Hp* virulence factor is CagA protein, which can be injected into the host cells [25]. Recently, CagA has been termed an oncoprotein due to its' intracellular activities that lead to dysregulation of cell division. Once inside cells, CagA is phosphorylated by src tyrosine kinases, stimulating gastric epithelial cells proliferation and motility, suggesting its potential role in metastasis. In addition, CagA was shown to induce overexpression of microRNAs, leading to increased NF-kB and Erk1/2 signaling, targeting, and inducing epithelial-mesenchymal transition and intestinal metaplasia of gastric epithelial cells [26].

Additionally, it should be noted that * Hp* is a potent modulator of the host inflammatory reaction. * Hp* cell division-related gene a (cdrA) was shown to induce NF-kB activation and IL-8 production by gastric epithelial cells. * Hp* lipopolysaccharide (LPS) has been shown to modulate both innate and adaptive immunity mechanisms [9]. Despite the fact that * Hp* LPS induces weaker immune response than LPS from other bacteria, it is one of the main determinants of host specific immunosuppression and long bacteria persistence in human organism [10,26].

Thus, * H. pylori* alters gastric epithelium kinetics and repair, causes inflammatory reaction and modulates specific immunity of human organism. During its long coexistence with humans, * Hp* has developed complex strategies to limit the degree of gastric mucosal damage and inflammation, as well as immune activity [8,10]. The next part of the paper is focused on * Hp*–gastric mucosa–VN interaction describing reciprocal relations between the pathogen and regulatory host systems during such processes as:

1) Bacteria colonization of gastric mucosa  
2) Induction of innate immunity  
3) Adaptive immunity reaction

### 3. * H. pylori* AGAINST MUCINS: VAGUS NERVE DETERMINES THE WINNER

It is well known that mucous-bicarbonate barrier plays a pivotal role in gastroduodenal mucosa against peptic factors [27]. The main components of mucous layer covering the surface of gastroduodenal area are mucins (MUC). Mucins are heavily glycosylated glycoproteins with high molecular weight, whose family comprises 21 members (MUC1 to MUC21). Mucins can be produced either as the membrane-bound or secreted products [24]. All mucins have similar features: a tandem repeat domain including sequences of amino acids repeated in tandem, rich in proline, threonine and serine residues, constituting the Pro/Thr/Ser domains. These domains are extensively glycosylated at the serine and threonine residues [28]. The healthy gastric mucosa is covered with mucus gel layer, including MUC5AC and MUC6 [1]. In addition, the membrane-associated MUC1 is expressed in epithelial cells of gastric pits. The secreted MUC5AC is considered to be a major constituent of the surface mucous gel layer, whereas the expression of the secreted MUC6 is limited to the glands [29].

Regarding * Hp*-induced GDP, maintenance of mucus bicarbonate barrier is quite important as mucus layer builds a physical barrier preventing * Hp* adhesion to epithelial cells. At low pH, gastric mucins form a gel that effectively traps bacteria. However, * Hp* has a remarkable ability to colonize gastric mucosa [27]. Since production of gastric acid, lowering pH to 1-2, limits luminal colonization of the stomach, adhesion of * Hp* to the gastric epithelial cells is a crucial step in * Hp* survival and subsequent development of infection. * Hp* uses diverse strategies to survive in such challenging conditions [10]. The highly important mechanism for acid resistance is bacterial urease, catalyzing production of ammonium ions that raise pH to
near neutral [8]. This leads to mucus gel transitions to a viscoelastic solution through which \textit{HP} can swim [9]. Furthermore, urease and other bacterial enzymes (lipase and proteases) facilitate flagellar motility through the mucus layer by changing the viscoelasticity properties of gastric mucins [28] due to the loss of the polymeric structure of mucins. In addition, \textit{HP} induces impairment of mucins expression in the gastric mucosa. It was shown that MUC6, normally associated with mucous cells of the gastric glands, is expressed in covering epithelial cells of \textit{HP} infected patients while the MUC5 component was decreased [30]. Navabi et al. have reported that MUC1 turnover and level are also decreased upon \textit{HP} infection in mice [31].

On the other hand, however, maintaining the normal pattern of mucins secretion in host limits \textit{HP} infection [29]. Recently, it has been demonstrated that the membrane-associated MUC1 can limit \textit{HP} adhesion to gastric epithelial cells both by steric inhibition of binding to other cell surface ligands and by acting as a releasable decoy [32]. These data are in agreement with previous studies showing that mice deficient in MUC1 were more susceptible to \textit{HP} infection [9]. It is an interesting fact that mucins can modulate proliferation and gene expression of \textit{HP}. These properties can range significantly in different individuals from stimulatory to inhibitory, depending on the type of mucins and their ability to bind to \textit{HP} [33]. For instance, tumor-derived mucins as well as mucins from the surface mucosa have potential to stimulate \textit{HP} proliferation, while gland-derived mucins tend to inhibit it [31]. However mucins from healthy uninfected individuals showed little effect. Additionally to this, mucins and trefoil peptides protect the viability of the cells and control the cytokine production modulating \textit{HP}-induced host signaling [29]. Hence, direct and indirect VN impact on mucus-bicarbonate barrier can be involved in host-specific effects, determining self-protection and \textit{HP} proliferation, gene expression and virulence.

Concerning the role of mucins in the prevention of \textit{HP} colonization, it is quite important to point out the key regulators of mucous gel production. As it was shown in several studies, there is a hierarchy of mucins regulation, comprising local and systemic factors. The most important local factors are PGE$_2$ and nitric oxide (NO), which are considered to be the key VN-dependent mediators of gastric cytoprotection, stimulating mucin exocytosis in antral mucous cells [28,34]. \textit{HP}, as a nitric-utilizing bacterium, is in concurrent interaction with host L-arginin metabolism. It was shown that \textit{HP} arginase inhibits nitric oxide production by eukaryotic cells, including gastric mucosa cells and monocytes, therefore results in limitation of cytoprotective system activity in gastroduodenal mucosa [35]. In addition, such hormones as histamine, serotonin, melatonin and VIP are also known to be potent stimulators of mucins secretion, as well as HCO$_3$ transport. Interestingly, both regulatory links are controlled by VN that is the main orchestrating regulator maintaining the gastrointestinal barrier [35]. VN stimulation with subsequent PG E$_2$, serotonin, VIP, histamine and NO release provides activation of mucins expression in epithelial cells of stomach and duodenum [36]. This improves the gastrointestinal barrier, which limits both acute and chronic colonization by \textit{HP}, and also reduces the inflammation induced by \textit{HP} infection [14].

Additionally to mucins secretion, VN stimulation is associated with protective effect on gastrointestinal epithelium, stimulating bicarbonate transport and expression of occludin and ZO-1 via alpha7 nicotinic acetylcholine receptors (alpha7nAChR) [19]. This effect is extremely important bearing in mind that \textit{HP} colonization is potentially associated with alteration of cell to cell and cell to matrix interaction of mucosa through its CagA-mediated disruption of tight-junctions via inhibition of Par-1b, adherence junctions via E-cadherin, and focal adhesions via activation of SHP-2 [7].
Thus, balance between bacterial pathogenicity and VN tone determines the success of the first defense barrier against *HP* formed by mucus-producing epithelial cells lining, and efficacy of *HP* colonization.

### 4. *H. pylori* ALTERS HOST INNATE IMMUNITY

The host innate immune system plays the key role in the initiation and subsequent progression of the *HP*-associated GDP [10]. Invasion of *HP* generates the cytokine response in gastric mucosa that is considered to be the main factor in GDP development. The first targets for *HP* are gastric epithelial cells and mucosal leukocytes, which actively contribute to the innate immune responses [38]. Activation of these cells is realized through several mechanisms including the following: *HP* LPS and flagelline, CagL RGD-dependent activation of α5β1 integrin activates pro-inflammatory cytokine interleukin-8 (IL-8) and urease that exhibits chemotactic activity for human monocytes [9, 37] and strongly stimulates production of IL-1β, IL-6, and TNF-α by neutrophils [38]. In addition, *HP* infection induces activation of macrophages, whose response includes increased expression of inducible NO synthase (iNOS) and cyclooxygenase-2, and production of inflammatory mediators such as reactive oxygen intermediates, TNF-α, and IL-1 [7].

Thus, acute inflammation is a crucial component of the *HP*-associated GDP. Imbalance in the highly regulated local inflammatory response to infection and injury can result in the excessive production of TNFα, IL-1β, high mobility group box1 (HMGB1) and other inflammatory molecules by immune cells and their subsequent release into the circulation [8, 38]. These systemic cytokine responses are associated with unrestrained inflammation, secondary tissue injury and clinical signs of GDP [37]. However, the next studies have shown that, in addition to varied *HP* virulence, immune reactivity of the host is extremely important to the outcome of *HP*-associated GDP. Recent studies indicated that polymorphisms of pro-inflammatory cytokines or related genes involved in *HP*-initiated innate immune response in the host may strongly influence pathological outcome [39]. Furthermore, *HP* has several strategies to escape from host innate immune response [8].

Detection of pathogen-associated molecular patterns (PAMPs) by epithelial cells and leukocytes occurs via distinct classes of pattern recognition receptors (PRRs), one of which is Toll-like receptors (TLRs). The most important of them are TLR4 (binding LPS), TLR2 (receptor for lipoteichoic acid and lipoproteins), TLR3 (recognizing dsRNA and polynucleotides: polycytidyl acid), TLR5 (links to flagellin) and TLR9 (unmethylated CpG) [9]. *HP*s able to avoid detection by several types of PRRs that are crucial for the recognition of other Gram-negative pathogens [40].

Evolutionally developed strategies allow *HP* to avoid classical recognition by TLR-4. LPS of *HP*s 1000-fold less bioactive than E. coli LPS, and can not induce strong IL-1β, IL-6 or IL-8 responses [37]. It is explained by low affinity of *HP* LPS to TLR4, through which only limited NF-κB activation occurs. Moreover, *HP* LPS was also shown to suppress TLR-4 signaling, but enhance IL-12 and IL-18 production [41], which was suggested to be linked to the chronic inflammation commonly seen during infection [40].

In contrast, *HP*-derived LPS reported to signal through TLR-2. TLR-2 mediates NF-κB signaling that activates proinflammatory pathway in epithelial and innate immune cells [9, 38]. A further role TLR-2 was shown to be resulted in a shift from cagPAI-dependent to cagPAI-independent signaling leading to the secretion of IL-8 and TNF-α [8]. In NK cells, TLR-2 was shown to be activated by *HP* lipoprotein HpaA, leading to IFN-γ production in an IL-12
dependent manner [9]. Detection of HP non-LPS ligands by TLR2 represents yet another example of how HP exploits the immune system for the induction of anti-inflammatory responses. Activation of TLR2 triggers the MyD88-dependent expression of a number of anti-inflammatory genes, most notably IL-10 [37]. Recent studies have shown that different TLRs are expressed on different T cell subsets. Expression of these TLRs on T cells suggested the potential of the respective TLR ligands to directly influence T cell, thus, modulating not only innate but also adaptive immune responses [42]. It has been shown that CD4+ effector memory T cells are the main population responding to TLR7, TLR8, TLR2, TLR5 and TLR6 stimulation [43]. Toll-like receptor 2 (TLR2) serves as a co-stimulatory receptor for human T cells by enhancing T cell receptor (TCR)-induced cytokine production and proliferation through promotion of NFκB, Erk1/Erk2 and Akt activation [44]. The same was shown during stimulation of TLR7/8 of CD4+ T cells. However, this effect depended on microenvironment: contact of T cells with monocytes completely changed the effect of TLRs 7/8 stimulation. It was shown that monocytes transmit a negative signal to T cells inhibiting their proliferation [43].

Thus, HP was shown to be able to either stimulate or inhibit acute inflammatory reaction. As it is supposed nowadays this choice depends not only on HP strains diversity, but on host immunity regulation, too. It is well known that the nervous system and the immune system communicate bidirectionally, and the central nervous system modulates inflammation through neuroendocrine pathways. HP infection initiates activation of gastric mucosal sensors by HP-released cytotoxins and inflammatory products, as well as the reactive oxygen species generation in gastric mucosa during inflammation that results in the long vago-vagal reflexes with ACh release.

## 5. VAGUS NERVE AND ACETYLCHOLINE REDUCE INNATE IMMUNITY RESPONSE TO H. pylori

Nowadays it is widely recognized that ACh modulates cytokine production in immune cells. This effect involves cells of innate and adaptive immunity and includes direct and indirect mechanisms [45]. Hence, what is the role of vagus nerve and ACh in the modulation of the immune response to HP? Recently, in animal models of sepsis, the vagus nerve has been put forward in the regulation of the “inflammatory reflex”, a prototypical reflex circuit that maintains immunological homeostasis [46]. In details, the chain of events includes the following steps. First, molecular products of infection or injury activate sensory neurons transmitting signal to the brain stem in vagal centers [14,47]. Next, these incoming signals generate action potentials that travel from the brain stem to the spleen and other organs. All above-mentioned culminates in release of acetylcholine, which can attenuate innate immune responses, modulating activation of the nuclear factor kappa beta (NF-kB) with subsequent pro-inflammatory cytokines production and activation of leukocytes recruitment mechanisms [48,49]. These effects occur not only via alpha7nAChR subunit-dependent activation of monocytes and neutrophils, but also through T-lymphocyte-dependent signaling to spleen via vagus nerve. This efferent arm of the inflammatory reflex is referred to as the “cholinergic anti-inflammatory pathway” [50,51].

Protective effect of VN stimulation is connected with modulation of innate immune responses to infection, injury and ischemia through stimulation of alpha7nAChR signaling [48]. Both, ACh and nicotine, can effectively attenuate macrophage activation by decreasing the production of diverse proinflammatory mediators, including HMGB1, TNFα, IL-1β, and IL-6, activated by HP trough TLR2-pathway. Li et al. [52] have shown that deficiency of
alpha7nAChR in bone marrow-derived cells significantly impaired vagus nerve-mediated regulation of TNF-alpha, whereas alpha7nAChR deficiency in neurons and other cells had no significant effect. Matteoli G et al. have shown that VN stimulation reduced intestinal inflammation [53]. This effect was associated with inhibition of leukocytes recruitment at the sites of injury by suppressing the expression of VCAM1. Such effect was associated with fall of C5aR- and Fcgamma R-triggered generation of reactive oxygen species, decreased release of TNF-alpha and other proinflammatory cytokines under stimulation of alpha7nAChR on human neutrophils [49]. The selective 7nAChR agonist GTS21 decreased neutrophils accumulation and release of cytokines and chemokines at sites of injury as well as inhibited leukotriene B4 and IL-8 production [48]. In addition, stimulation of macrophage alpha7nAChR inhibited nuclear translocation of NF-κB, likely by blocking degradation of the NF-κB inhibitor or via activation of signal transducer and activator of transcription (STAT3) [54]. This effect resulted in decreased production of IL-1 and TNFα, but increased the IL-10 secretion [50,52,55] and was realized via micro RNA-124, activated under alpha7nAChR stimulation. Anterograde labeling fails to detect vagal efferents contacting resident macrophages, but shows close contacts between cholinergic myenteric neurons and resident macrophages expressing alpha7nAChR [44]. Instead, the vagus nerve interacts with cholinergic myenteric neurons, which are in close contact with macrophages. This direct neuro-immune interaction is an alternative for humoral regulation of immunity by the cytokine network [55].

Although it is unclear whether an elevated inflammatory response induced under HP infection is due to the low vagal activity, it is possible that activation of the cholinergic outflow by the vagus nerve may be beneficial for improvement of the autonomic dysfunction and may have clinical implications in the treatment of inflammatory disorders in gastroduodenal area.

6. H. pylori AND VAGUS NERVE EFFECTS ON DENDRITIC CELLS

Another issue for discussion is HP and VN impact on dendritic cells (DC) maturation and phenotype, as they play the crucial role as antigen-presenting cells that switch on the adaptive immune response. Several studies have demonstrated that the immunological outcome of HP infection depends on the DCs activation state and subtype [56]. Immature DCs sample and process antigens, and efficiently sense a large variety of signals from the surrounding environment. During maturation process induced by exogenous and endogenous stimulators, DCs display high levels of surface molecules, including CD40, CD80, CD86 and MHC [56]. DCs maturation also promotes such functional features as high cytokine production, low antigen-uptake and high migratory capacity. After activation, DCs become capable of activating naïve T cells and directing differentiation and polarization of effector T lymphocytes [57].

In the past few years many advances in research have made it clear that different signals are able to determine distinct programs of DCs’ differentiation and different kinds of immunity and tolerance [56]. Concerning DCs activity in the HP-infected stomach, there are some controversial notions. Mucosal CD11c+ DCs are located near the surface of normal gastric epithelium, and their numbers increase after HP infection [56], although it was shown, that HP-activated DCs are less responsive compared with other gastrointestinal bacteria [9, 40]. On the one hand, the assessment of bone marrow-derived DCs respond after stimulation with HP have demonstrated proinflammatory potential based on secretion of IL-12 or IL-23 or activation of Th1 and Th17 cells [57]. Really, wild-type HP bacteria promoted the
maturation of DCs, in which DC maturation was independent of the cag PAI and VacA statuses of \textit{HP} [7]. In contrast to the presence of whole bacteria, CagA protein of \textit{HP} negatively regulated the maturation of DCs. The similar effect was described by Kim JM et al. who indicated a possible mechanism of immune inhibition by VacA-mediated prevention of DCs maturation [58].

Moreover, in vivo \textit{HP}-stimulated DCs fail to induce effector T-cell responses of the Th1 and Th17 types [9]. The same effect was demonstrated by Kao JY et al. who have shown that \textit{HP} alters the DC-polarized Th17/Treg balance toward a Treg-biased response, which suppresses the effective induction of \textit{HP}-specific Th17 immunity [59]. Instead, such DCs preferentially induce the expression of the Treg-specific transcription factor FoxP3, the surface marker CD25 and anti-inflammatory cytokine IL-10 in naïve T-cells [8]. This is consistent with a growing body of literature documenting the prevalence and function of Treg cells in the host response to \textit{HP}. Research is now focused on characterizing how \textit{HP} induces such activity in DCs and identifying the mechanisms by which \textit{HP}-exposed DCs activate Treg cells. Some authors suggested that gastric DCs recognize \textit{HP} much like DCs in the gut that recognize commensal organisms to promote immune tolerance through Treg response [10,40].

Interpreting these data, it is quite important to stress the role of microenvironment for DC modulating their maturation and the final response to stimuli. As it is known, the activity of DCs can be affected with PAMPs, cytokines, chemokines and some hormones including serotonin, ghrelin, VIP, histamine and prostaglandins [56]. Under physiological and pathological conditions, response of DCs, expressing a wide spectrum of receptors, depends on the content of regulatory factors cocktail. There are plenty evidences to prove that VN may also be involved as a key component of such cocktails. VN can modulate DCs activity by direct and indirect influence through the enteric nervous system and/or enteroendocrine cells [60]. Direct effect of VN via alpha7nAChR activation, the same to \textit{HP}, drives immature DCs towards an anti-inflammatory phenotype, resulting in TGF-β secretion and FoxP3+ Treg cells activation [39]. Interestingly, the same effect was described for VIP secreted by myenteric neurons and endocrine cells [14,61]. However it was found that DCs express not only nicotinic, but muscarinic M3, M4 and M5 receptors too. Maturation of dendritic cells in the presence of the cholinergic agonistcarbachol resulted in the stimulation of TNF-alpha and IL-8 production [62]. All these effects were prevented by atropine, a MR antagonist. Incubation of DCs with carbachol after the differentiation increased the expression of HLA-DR, improved the T cell priming ability of DCs, and stimulated the production of TNF-α, but not IL-12 or IL-10 [38,57].

Thus, the final immunomodulating effect of VN and ACh depends at least on DCs receptors expression prevalence.

7. \textit{H. pylori} ENTR APS THE HOST ADAPTIVE IMMUNITY: WHAT IS THE ROLE OF VAGUS NERVE?

Recently, some advances were made in evaluation of the contribution of bacterial virulence factors and host response in the outcome of \textit{HP} infection, that have somewhat filled the gap in the puzzle of GDP. To maintain coloniztion in the gastric mucosa in spite of the robust immune response, \textit{HP} activates escaping mechanisms and exerts immunomodulatory effect on the host immune system, securing long-term coexistence through various factors [9,40].
Over the last decades, it has become evident that chronic infection by HP is due to two main mechanisms: hiding from host immunity; and alteration of adaptive immune defense mechanisms. With regard to adaptive immunity, the recognition and presentation of HP-derived antigens by gastric epithelial cells, DCs and macrophages leads to the creation of a complex cytokine milieu, promoting the differentiation of various T-cell lineages, including the Th1 cells (characterized by IL-12, 18, TNF-α, IL-1β), Th2 cells (IL-4, IL-5), Th17 cells (IL17A, F, IL-23) and Treg cells (TGF-β1, IL-10) [7,63,64]. The functional activity of these lineages contributes to the pathological nature of the underlying gastritis. Numerous studies have been published on Th1-dominated cytokine milieus (TNF-α, IL-1β, IFN-γ) in HP-induced gastritis [8,65]. Th1 activation drives inflammation that, if prolonged, results in pathological outcome. On the other hand, experimental data showed that polarized Th2 response with B-cell activation and antibodies production alone does not guarantee protection, suggesting that specific Th1 response appropriately tuned by Th2 cells would lead to a balanced, protective response [38].

Nowadays it is thought that HP inhibits cell-mediated adaptive immune response. As minimum, two virulent factors have been specifically involved in the inhibition of human T-cells. VacA inhibits T-cell proliferation by interfering with the T-cell receptor/interleukin-2 signaling pathway at the level of the Ca²⁺/calmodulin-dependent phosphatase calcineurin [66]. Another HP virulence determinant involved in T-cell inhibition is γ-glutamyl-transpeptidase [67]. Similar to VacA, γ-glutamyl-transpeptidase is a secreted factor that blocks proliferation of T-cells through a mechanism that involves the inhibition of cyclin-dependent kinase activity in the G1 phase of the cell cycle disrupting the Ras signaling pathway [9]. In addition, HP employs a variety of tools to inhibit the T cells response by activation of host tolerance mechanisms, including activation of Treg cells [68]. Several human-based studies demonstrated that the degree of active HP-induced inflammation negatively correlates with the numbers of Treg cells in gastric mucosa [65]. HP-induced TGF-β was shown to inhibit CD4⁺ T cell proliferation and lead to Treg development, suggesting a mechanism that is used to subvert the host response and colonize gastric mucosa. Treg cells were found to be associated with an increasing bacterial colonization, chronic inflammatory changes and the expression of immunosuppressive cytokines [6]. Eradication therapy led to a significant reduction of Treg cells and corresponding cytokine levels in gastroduodenal mucosa [63].

Notably, Treg cells are capable of suppressing Th1-derived effector cells by producing IL-10 and TGF-β. Tregs are induced when TGF-β is present, along with PDL-1 expression on antigen presenting cells [69]. Another novel mechanism of Treg development during HP infection was established in the mouse model where IL-18 was shown to be required for Treg development and was produced by DCs during infection [9].

It was surprising to find out that HP impact on Treg cells formation is the same as VN impact on gastrointestinal mucosa, maintaining immune homeostasis by interference into immunity and tolerance with Treg-mediated mechanism [59]. As it was mentioned above, VN stimulation initiates Treg activation through DCs modulation by Ach. Moreover, Rosas-Ballina M. et al. identified an acetylcholine-producing memory phenotype T cell population in mice that is integral to the inflammatory reflex [70]. Pena G. et al. described cholinergic CD4 (+)CD25(-) lymphocytes [71]. Ach synthesized and released from T-lymphocytes acts as an autocrine and/or paracrine factor regulating immune function. It is considered that acetylcholine-producing T cells are required for inhibition of cytokine production by VN stimulation. Does it mean that VN and Ach cooperate with HP for coexistence? Not at all, as Ach effects are realized via numerous types of nicotinic and muscarinic Ach-receptors,
which are widely expressed in endothelial cells, enterocytes, T lymphocytes, B lymphocytes, DCs, monocytes, macrophages, neutrophils etc. [9]. It was shown that ACh-producing T and B lymphocytes regulate local innate immunity controlling the recruitment of neutrophils and activity of macrophages [72].

Nevertheless, VN and ACh (neuronal and non-neuronal) are supposed to be important regulators of adaptive immunity, too. This suggestion is based on the following recognized facts. First of all, lymphocytes express both muscarinic and nicotinic ACh receptors, and their selective stimulation produces various biochemical and functional changes, modulating both cell-mediated and humoral immunity reactions [73]. Immunological activation of T cells enhances synthesis of ACh and transcription of choline acetyltransferase, M5 cholinergic receptor and acetylcholinesterase. Activation of muscarinic and nicotinic ACh receptors on lymphocytes increases intracellular Ca\(^{2+}\) concentration and stimulates c-fos gene expression and NO synthesis [74]. However, long-term exposure of lymphocytes to nicotine reduces intracellular Ca\(^{2+}\) signaling via alpha7nAChR-mediated pathways [75].

Furthermore, acetylcholine is thought to be an additional mediator to modulate activation of interacting T and B lymphocytes. Alpha 7nAChRs were found recruited into immune synapses between human T and B lymphocytes, both of which produced ACh [76]. Moreover, cholinergic system of lymphocytes demonstrates high plasticity as different immune cells activation is associated with diversity of Ach receptor subtypes expression. In vitro activation of CD4 T cells through TCR/CD3 cross-linking was associated with the appearance of \(\alpha_4\) and \(\alpha_7\), up-regulation of \(\alpha_5\), \(\beta_4\), M1 and M5 and down-regulation of \(\alpha_9\) and \(\beta_2\). In vitro activation of CD8 T cells, in comparison, also featured appearance of \(\alpha_4\) and \(\alpha_7\), as well as up-regulation of \(\alpha_2\), \(\alpha_5\), \(\beta_4\), M1 and M4, and down-regulation of \(\alpha_10\), \(\beta_1\), \(\beta_2\) and M3. Polarization toward Th1 cells lineage was associated with a reduction of \(\beta_2\), \(\beta_4\) and M3 expression; while activation of Th2 cells was accompanied with down-regulation of \(\alpha_9\) and \(\beta_3\), and stimulation of M2 and M5. Induction of Th17 phenotype formation led to down-regulation of \(\alpha_9\), \(\alpha_10\), \(\beta_2\) and M3R [77].

Finally, interesting data were obtained in experimental research with modeling of autoimmune myasthenia gravis. Increase of anti-acetylcholine receptor serum IgG level under this condition was accompanied with shifting in Th1/Th17/Tfh and Th2/Treg/Tfr cell types balance. These changes were associated with increase in follicular helper T cells (Tfh, defined as CD4\(^{(+)}\)CXCR5\(^{(+)}\)ICOS\(^{(\text{high})}\)) and a decrease in follicular regulatory T cells (Tfr, defined as CD4\(^{(-)}\)Foxp3\(^{(+)}\)CXCR5\(^{(-)}\)ICOS\(^{(\text{median})}\)) [78]. From these data, it is possible to come up with a suggestion that cholinoreceptors are involved in determination of immune response polarity. Partly it is related with discrimination between MRs and nicotinic cholinoreceptors stimulation effects. Stimulation of muscarinic receptors on T and B cells with ACh or another agonists elicits intracellular Ca\(^{2+}\) signaling, up-regulation of c-fos expression, increased NO synthesis and IL-2-induced signal transduction via M3 and M5 mediated pathways [76]. M1 cholinoreceptors pathway activation is associated with enhancement of IL-6 production and IgG secretion, whereas there was no significant change in gamma interferon secretion [74]. Acute stimulation of nicotinic receptors with ACh or nicotine causes rapid and transient Ca\(^{2+}\) signaling in T and B cells, probably via alpha7nAChR-mediated pathways. Chronic nicotine stimulation, by contrast, down-regulates alpha7nAChR expression and suppresses T cell activity [76]. Some interesting findings were described in Qian J, et al. research. The authors have shown that nicotinergic stimulation up-regulated interferon-\(\gamma\) and down-regulated IL-17 secretion, whereas the muscarinic stimulation enhanced IL-10 and IL-17 and inhibited interferon-\(\gamma\) secretion. In addition, Fujii YX et al. showed that M1 receptors play a crucial role in the differentiation of CD8\(^{(+)}\) T cells.
into cytolytic T lymphocytes [75]. In any case, mulling over these data, it is possible to assume that prevalence of muscarinic or nicotinic cholinoreceptors signaling may determine the landscape of adaptive immunity, as well as final response of immune system under HP-pathology.

8. CONCLUSION

The structural and immune homeostasis of gastroduodenal area can be affected not only by HP virulence factors, but theoretically by any shifting in vagus nerve reactivity and/or interaction with enteroendocrine and immune cells in gastroduodenal mucosa carrying cholinergic receptors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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