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Review Article

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IgY Antibodies for the Prevention and Treatment of *Helicobacter pylori* Infections

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Abstract

Major progress has been made both in the field of vaccination and also in therapies against *H. pylori* infections in recent years. But, the increasing number of *H. pylori* infections and its resistance to current antibiotics has become a worldwide problem, due to the direct correlation between *H. pylori* infections and gastritis, gastric ulcers as well as cancer. In this regard, new alternative therapies or prevention methods turned into a global need. Therefore, an oral administration of chicken antibodies IgY specific to *H. pylori* can be very advantageous. Antibodies like anti-UreB IgYs or anti-VacA IgYs, produced by adult avian species immunized previously with UreB and VacA antigens respectively, are highly effective as a prophylaxis against *H. pylori* but as well as a treatment of already existing infection.

Keywords: IgY; *H. pylori*; Prevention; Oral vaccination; Antibodies

Introduction

H. pylori is a gastritis, gastric ulcer and cancer causing bacterium (specifically non-cardia gastric cancer and gastric mucosa-associated lymphoid tissue lymphomas) [1,2]. Although the infection rate in the human population fluctuates between 15% to 58% [3,4], the number of patients with cancer attributable to *H. pylori* has increased from 5.2% to 6.2% of all cancers [5], hence, it has become a serious global problem. Supported by statistical data, the infection rate in several regions in the world has been shown to depend on the economic level of a country, hygienic education, social status of a family, culture (diet), age and sex [3-5]. The prevalence of *H. pylori* infections is higher in developing countries e.g. the number of cases in Eastern European countries is substantially more than those in the Western countries [4]. Statistics show that the upper limit for infection rate in South America, Asia and Africa, is around 70% to 85% [3,4,6,7].

But Western countries also have a continuous growing problem with *H. pylori* infections. Baker et al. researched the association with *H. pylori* and drinking water from untreated well water in the USA and found an increase in the *H. pylori* infection rate [8]. Furthermore, the simple transmission of *H. pylori* via an oral-oral and/or a fecal-oral route increases the risks of interfamilial infections which could quickly expand the outbreak and consequently, presents a major international problem.

The common therapy against *H. pylori* is based on a proton pump inhibitor, clarithromycin and amoxicillin, which is known as the standard triple therapy. Unfortunately, due to the worldwide antibiotic abuse, bacterial resistance for this treatment has developed [9]. Therefore, the current priority for health institutions and scientists is to find out a new strategy for eradication of *H. pylori* [10-12]. With the consideration of the scale of infection, universality and relatively low costs should be the main priorities in the development of a new effective therapy.

H. pylori Infection and Detection

H. pylori is a gram-negative bacterium which colonizes in human and nonhuman primates' gastric mucosa. It clings to a gastric epithelium due to Adhesins and Lewis antigens, and moves by using Flagellae [13]. Characteristically *H. pylori* is able to survive in acidic conditions of gastric lumen and gastric mucosa because of its ability to synthesize AmiF, Ami F, RocF and urease enzymes. It hydrolyzes urea in the stomach of a host organism, and as a result ammonia and CO_2 is produced, which increases the pH value. Moreover, it was discovered, that even though these enzymes have similar roles, the crucial factor for this process is a Nickel containing hexadimer-Urease, with two subunits Ure A and Ure B (UreC) [13,14]. The ammonia-producing cycle is regulated by the ArsSR system based on histidine kinase ArsS which is essential for the resistance of *H. pylori* to acidic conditions, by sensing low p^H [14]. *H. pylori* inflammation results in continuous inflammation called chronic gastritis, but it can also turn into atrophic gastritis, intestinal metaplasia, dysplasia and finally gastric adenocarcinoma [13,15,16]. There are several possible explanations why it causes cancer [17]. One of the reasons can be a chronic response placed in the gastritis mucosa: in this process macrophages and granulocytes are recruited. They contain nitric oxide synthase generating NO in significant amounts. At the same time, ROS are produced (reactive oxygen species) causing oxidative stress. The combination of both of these processes can lead to DNA damage, DNA repair system deregulation, increased cell proliferation and inactivated apoptotic mechanisms, which support carcinogenesis. *H. pylori* infections also result in an increased rate of epidermal growth factor (EGF) and its receptor (EGFR) [17]. Current research also supports the concept of viral agents of *H. pylori* such as: CagA, VacA, DupA, IceA, OipA, BabA, HopQ, which initiate and promote routes leading to tumor changes [18-21].

Diagnostic methods for the determination of *H. pylori* infections can be divided into two groups: invasive and non-invasive tests. The first one involves: endoscopy, histology, rapid urease test, *H. pylori* culture, antibiotic susceptibility testing and molecular methods (PCR and rtPCR) requiring biopsy specimens. In the second group are included: 13C Urea Breath Test, stool antigen tests and serology (antibody tests e.g. ELISA for IgG and pepsinogens detection) [4,22,23]. Even though, there are already several diagnostic approaches, problems still arise with early gastric cancer detection because of its almost imperceptible symptoms and its non-specificity [22].

Prevention and Current Therapies

The ultimate prevention and eradication of *H. pylori* infections and minimalizing its possible consequences, includes a proper lifestyle, early and correct diagnosis, as well as if needed an effective therapy. Due to *in vivo* experiments in Mongolian gerbils infected with *H. pylori*, research showed that higher doses of salt in the diet have a considerable impact for the increase of stomach carcinogenesis [24]. Moreover, a diet rich in fruit and vegetables is highly recommended [22]. There are also currently ongoing studies on preventable antioxidants (e.g. ascorbic acid, carotenoid, beta-carotene, polyphenols) and chemicals (non-steroidal anti-inflammatory drugs e.g. aspirin, rofecoxib, etodolac and celecoxib, which are inhibitors of COX-2 overexpressed in stomach cancers) [22]. Furthermore, it was discovered that organic acids produced by lactic acid bacteria can affect the viability of *H. pylori* in *in vitro* experiments [25]. Additional investigations are necessary before these factors can be implemented so that the ideal routine medical prevention method can be established.

Due to the failure of the standard triple therapy against *H. pylori* (proton pump inhibitor PPI with antibiotics clarithromycin and amoxicillin), caused by antibiotic-resistance strains, scientists are working on alternative treatment methods. As it was summarized in the Maastricht IV/Florence Consensus Report on the management of *H. pylori* infection [26], only in the regions with low clarithromycin resistance (less than 20%) is still recommended to use as a first line treatment based on PPI, clarithromycin and amoxicillin/ metronidazole, alternatively bismuth quadruple therapy. And as a second line the same composition treatment, but instead of clarithromycin, it is advised to use levofloxacin, and also as an alternative the bismuth-containing quadruple therapy. In areas with the high resistance to clarithromycin, the bismuth-containing quadruple therapy is used as a first line, alternatively non-bismuth quadruple therapy (sequential or concomitant). Second line therapy is based on PPI, amoxicillin and levofloxacin. In all cases the further treatment depends on antimicrobial susceptibility testing [9-11]. However, it was discovered that there is a risk of gastric neoplasm even after successful *H. pylori* eradication. According to the research of Kitamura et al. it was observed that after elimination of *H. pylori*, an epithelium with low-grade atypia can appear which continues with the gastric tumor [27].

Nevertheless, there are also several clinical trials on gastric cancer targeted therapies, parallel to surgical methods and chemo-radiotherapy. They are mainly based on antibodies and molecular inhibitors targeting the ErbB family of proteins (EGFR, HER2), MET (hepatocyte growth factor receptor), mTOR (mammalian target of rapamycin complex), VEGF (Vascular endothelial growth factor), claudin 18.2 and T cell regulatory molecules (CTLA4, PD-1, PD-L1) [12]. Moreover, there are ongoing studies on anti-Helicobacter pylori vaccination, bringing very optimistic results [28].

Vaccination as Prevention Against *H. pylori* Infections

The main argument for supporting research for an anti *H. pylori* vaccination is the prevention of the *H. pylori* infection before its onset in contrast to the therapies used today which can only be initiated after gastritis detection has taken place. Immunization should be performed as a prophylactic. There are different strategies for anti-*H. pylori* vaccines, ranging from oral administration of whole cell vaccines or those consisting of subunits, combined with labile toxin or cholera toxin, through attenuated genetically modified bacteria expressing desired antigens, next encapsulated antigens and intramuscular vaccination containing *H. pylori* subunits and aluminium hydroxide, and ending with naked DNA immunization [29-31]. There is a big genetic diversity among *H. pylori* strains, however, there is a core of 1281 genes similar for all strains [32]. Due to proteomic analysis, it was possible to find out a few antigens with vaccine potential common to most of the strains: UreB, CagA, VacA, neutrophil-activating protein NapA, antigen for the colonization factor HpaA, antigens of flagellar FlaA and FlaB, adhesin antigens BabA, SabA, AlpAB and outer membrane inflammatory protein oipA [31,33]. Additionally, it was shown that the oral delivery of *H. pylori* antigens (defined native or recombinant) can successfully immunize animals for protection against their infection, hence, leading mostly the Urease antigen trials to the clinical stage [28,29,34]. Unfortunately, the oral administration requires significant amounts of antibodies which increases the costs of potential vaccine production, thus the egg yolk immunoglobulin Y (IgY) became an attractive alternative.

IgYs for the Treatment of *H. pylori* Infections

Immunoglobulin Y (IgY), functionally comparable to mammalian IgG, is an antibody class produced in the response to immunization of the mature avian species. It is enriched within the egg yolk (parental immunization) in high doses (1 ml contains >9.4 mg of IgY) [35,36]. In previous studies, IgY has been called IgG, due to its function and serum concentration in comparison with the mammalian IgG. Nowadays, it has become clear that this is not a correct term, due to clear differences in molecular structure. Even though both types of Immunoglobulins have two heavy and two light chains, IgY's heavy chain consists of four constant domains and one variable, where IgG has three constant domains and one variable. Additionally, IgY does not possess a hinge region, which makes the Immunoglobulin Y less flexible in antigen binding to a broad range of epitopes. Moreover, IgY is more hydrophobic than IgG, and has an isoelectric point in the range 5.7-7.6 [36,37].

One method for immunization and production of IgY is to use whole *H. pylori* cell lysate, but since it can bring the risk of cross reactivity with other bacteria, preferably and the more effective way is the immunization with selected antigens [38-40].

There are many advantages of using chickens for the production of polyclonal antibodies: easy purification (only one class of antibodies IgY in egg yolk and high IgY concentration), production of antibodies against conserved mammalian proteins, recognition of different epitopes than mammalian and a less invasive method of production [41-45]. The most important advantages of IgY is its ability to be used in passive immunization and as a ready and specific antibody that can be delivered directly into an organism. The idea of oral administration of immunoglobulins specific to host pathogens is not new, but using IgYs for this purpose is definitely an attractive approach especially against pathogens infecting the gastrointestinal track. Eggs as an everyday food product do not cause the risk of toxic side effects. Even concerns about allergy are not necessary because a final IgYs batch does not contain allergenic albumin, and IgY itself does not activate the mammalian complement system, does not interact with rheumatoid factors, proteins A and G, nor with mammalian Fc receptors [37]. IgY generated against *H. pylori* antigens can suppress the growth and colonization of this pathogen. IgY designed specifically to the adhesion, virulence or motility factors can provide host protection by blocking cell-to-cell spread and microbial attachment to epithelial cells. Another option are IgYs specific to toxins/enzymes produced by pathogen-they bind to the active site and inhibit or neutralize their activity [36]. On the basis of these facts, IgYs can be implemented in a therapy through oral administration or as prevention against intestinal infections of *H. pylori* by using IgY enriched food products [35,38,46,47].

Even though the infection of *H. pylori* can be caused by different strains, UreB was detected in all serums of examined patients with *H. pylori*, which brings the IgY-urease B as the strongest candidate for a universal vaccine [48,49]. UreB is a Urease subunit containing an active site responsible for the enzyme activity. By its inactivation, H. pylori is not able to survive in acidic p^H of gastric lumen and gastric mucosa. It was observed in animal models that anti-ureaseB-IgY was able to reduce the *H. pylori* population by 65% to 80%, to decrease bacterial adhesion and growth, and attenuate gastritis and mucosal injuries, as a result of neutralization of urease activity after its interaction with administered antibodies [35,50].

Additionally, it was discovered, that lactic acid bacteria and spent culture supernatants from fermented milk products showed a strong suppressing activity against *H. pylori* [25]. Further studies reported that a yoghurt enriched with 1% urease specific IgY, and containing *Lactobacillus* spp and *Bifidobacterium* spp reduced *H. pylori* infection in treated volunteers [51]. Another clinical study was performed to analyze the influence of anti-urease IgY on *H. pylori* infections. Volunteers were tested 4, 8 and 12 weeks after daily 2 cups consumption of specific IgY enriched yogurt. Measurements of *H. pylori* infection were done using C13-urea breath tests. After the 8^{th} and 12^{th} week 55.1% and 57.2% decrease in urea breath tests values were observed, respectively [52,53]. Similar results regarding anti-*H. pylori* urease containing drinking yogurt were also obtained in research done by Chen et al. [54].

Another promising result brings the research on anti-VacA toxin IgY against *H. pylori*. Vac A is a toxin secreted by *H. pylori* and is considered as one of the significant virulence factors. It allows bacteria expansion by binding to the host cell and causing vacuolation. Experiments done on *H. pylori* infected gerbils, showed that after specific IgY introduction, decreased VacA activity, host cell apoptosis, and as a consequence increased animal viability was observed [55].

Conclusions

Although research on *H. pylori* produced conflicting results, the International Agency for Research on Cancer declared this bacterium as a carcinogen (cancer-causing agent) in humans in 1994. Continued research has confirmed that the risk of gastric cancer and of gastric mucosa-associated lymphoid tissue (MALT) lymphoma is increased with the colonization of *H. pylori* in the stomach. Now *H. pylori* is known to be the major cause for gastric cancer. But other factors contribute to the risk of developing gastric cancer. Studies supported that the onset of gastric cancer is influenced by the economic level of a country, hygienic education and social status of the family, diet, age and sex. In detail, older age, male sex, a diet high in salted, smoked, or poorly preserved foods and low in fruits and vegetables and tobacco smoking contributes to the disease. But also, chronic gastritis, pernicious anaemia and a family history correlates with the development of gastric cancer [56,57].

In the times of increasing resistance of *H. pylori* to antibiotic therapies, it is highly important to find an alternative way to eradicate its infections. The most reasonable solution seems to be a therapeutic approach based on anti-VacA- or anti-UreB-IgY, which not only protects against *H. pylori* infections but also decreases the effects of current inflammation. As both are common markers for all of *H. pylori* strains, the use of IgYs specifically generated against them would be beneficial in the prevention and treatment of *H. pylori* infections. Passive oral immunization with IgYs would consequently result in a major impact against gastric cancer.

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Not applicable

Competing Interests

TD is the President and CEO of Igy Immune Technologies and Life Sciences Inc. CO is a consultant to IgY Immune Technologies and Life Sciences Inc.

Authors' Contributions

JZ, AS, TD and CO: Contributed to the original plan for the paper, wrote sections of the paper and contributed to draft revisions. All authors read and approved the final manuscript.

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