

Vaccine development against *Helicobacter pylori*

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Abstract. *Helicobacter pylori* (Hp) colonizes and persists in the gastric mucosa. It was the Class I carcinogenic factor, and the International Agency for Research on Cancer attributed 92% of stomach cancers to Hp, and according to data, nearly 50% of people in China are infected with *Helicobacter pylori*. At present, antibiotics are mainly used in clinical treatment, but with the increase of Hp resistance, antibiotics will lose their effect. The development of Hp vaccine has become a new way to prevent and control Hp infection. Components such as flagella, urease, virulence factors and outer membrane proteins involved in the process of Hp infection, colonization and reproduction, and have also become candidate antigens in the development of Hp vaccine. Specifically, study have suggested that flagellin vaccine can decrease infection rate. In the meantime, recombinant Hp antigens and dmLT were efficient for protection from the Hp infection. This article summarizes the progress of Hp vaccine research and development, hoping to provide reference for related research.

Keywords: *Helicobacter Pylori*, Vaccine, MV-Hspa, Bacterial Outer Membrane Vesicles (OMV).

1. Introduction

The spiral-shaped *Helicobacter pylori* (Hp) was classed into gram-negative bacterium, a kind of extracellular parasite that can specifically infect gastric mucosa and damage gastric epithelial cells, leading to chronic gastritis, ulcers, and even gastric cancer. WHO has designated Hp as a primary carcinogen. As the White Paper on "Prevention and Control of Hp Infection in China" pointed out, the infection rate of Hp among residents in China is nearly 50%, with infection rates ranging from 35.4% to 66.4% among different populations. Gastric cancer is a typical type of disease that is caused mostly by the infection of Hp. The International Agency for Research on Cancer attributed 92% of stomach cancers to Hp. The cancers are the important aspects scientists want to work out; they are trying to stop cancer by preventing infection with pathogens that help the development of cancer.

People used to Hp with antibiotics, however, with the increasingly antibiotic resistance of bacteria, it became an approaching major issue to the countries. The people of developing nations and those who

are severely affected by Hp must benefit from a vaccine production and delivery plan that is both efficient and affordable. Since the inflection of Hp was mainly cured with antibiotics, the drug resistance of Hp accelerated demand for vaccines. The Hp vaccines deal with microorganisms and can beneficially prevent cross-reactions and carcinogenesis.

This paper introduces the information of possible vaccine, which may become an approaching major issue to the countries. The people of developing nations and those who are severely affected by Hp can benefit from vaccine production and delivery plan that is both efficient and affordable.

2. Flagellin vaccine

A pathogen-associated molecular pattern (PAMP) flagellin is able to stimulate innate and adaptive immunity, is a versatile adjuvant that can be used with a variety of vaccines and immunotherapies because it is a protein-based Toll-like receptor agonist [1]. Its unique structural features allow it flexible and efficient adjuvant function [2].

2.1. Flagellin vaccine for Helicobacter pylori

Despite the fact that Hp flagellin can induce anti-flagellin antibodies in body, TLR5 is unable to detect it. Most bacteria's flagellin activate TLR5, which is included in the Toll-like receptor family. Four domains can be distinguished in the flagellin structure. During the research, area necessary for TLR5 recognition was discovered to be present at the D0 and D1 domain, which were the conserved N- and C-terminal ends, but the D2 and D3 domains were exposed and immunodominant for the antibodies generation. Some recombinant chimeric flagellins make the ability of activating TLR5 recreated in Campylobacter flagellins. These researchers created a chimeric flagellin (CF), making the Escherichia coli to have the N- and C-terminal segments of the Hp flagellin, which would still be antigenic to Hp while gaining the capacity to activate the TLR5 -where activate the TLR5 receptor. In order to cause mice to produce a significant antibody response. CF's efficacy as an adjuvant was demonstrated when they compared aluminum adjuvants with it. Mice given the CF vaccine developed antibodies, which can be against the H. pylori variable domain and recognized the native FlaA flagellin from the pathogen [3].

2.2. Clinical effect

The flagellin's carboxyl and amino domain bind to TLR5 can increase the flagellin's immune response toward the antigen. In the help of the attracting of antigen, they can better meet the immunity of the humanity. The flagellin vaccine get a great success for immunity. Based on the data analysis, infection rate reduced 40%-60% minimum rate among the vaccinated population [4]. Since flagellin vaccine have some hidden danger in this flagellin vaccine, so there was a high data on unvaccinated population. Thereby, the flagellin vaccine reach a high data on unvaccinated population [5]. The previously existing immunity to flagellin did not significantly affect its adjuvant effect, and effective adjuvant effects can be induced at relatively low doses before inducing maximum innate immunity. And the safety of flagellin vaccine should be stated, since researchers cannot make sure the 100 percent safety of the vaccine with the bacterium. So, it may be the better way to extract different harmful VLP and antigens.

3. Urease vaccine

Urease plays a crucial part in the gastric colonization of Hp, which can hydrolyze urea into ammonia to neutralize gastric acid and construct a neutral pH microenvironment around the bacteria. In a previous study, a vaccine made of UreA, UreB, and neutrophil-activating protein (NAP) was created by Zhong and coworkers to immunize mice with double mutant heat-labile toxin (dmLT) [6].

3.1. Mechanism

In a proprietary mouse enterotoxicity experiment that gauges the rise in intestinal weight brought on by toxin-induced fluid secretion, DmLT is essentially nontoxic when compared to native LT. A powerful adjuvant for mucosal vaccination with Hp antigens, dmLT has also been reported to effectively boost

immune responses to other delivered vaccines [7]. In conclusion, preventative protection against Hp infection is provided by recombinant H. pylori antigens and dmLT administered orally to mice [6].

3.2. Clinical effect

Zhong and others studies the effects of this vaccine on Hp challenged in mice. They found that compared to unimmunized mice, the gastric colonization of Hp were obviously reduced by oral immunization with candidate antigens and dmLT 6 weeks after challenge. Furthermore, the immunization strengthened antigen-specific lymphocyte proliferation and serum IgG and mucosal IgA responses. In conclusion, in comparison to whole bacterial lysates, oral administration of recombinant Hp antigens (UreA/UreB/NAP) and dmLT provides more effective prophylactic protection against Hp infection in BALB/c mice [6]. Most vaccine candidates are still in the early stage of R&D and have not been successfully licensed. The choice of which antigen and urease to choose to form a multivalent vaccine can optimize the immune efficacy still needs further research.

4. Virulence Factor vaccine - heat shock proteins (Hsp)

Heat shock proteins (Hsp), a heat-stress protein widely found in bacteria and mammals, have a core regulatory function in cells. It is essential for maintaining the normal structure of the protein, while at the same time they can repair misfolded conformations. When an organism is exposed to high temperatures, it stimulates the synthesis of this protein, which is used to protect the organism itself. Hsp is a conserved molecule located on the surface of Hp, which can initiate innate immunity as a molecular chaperone protein with high immunogenicity. Therefore, Hsp is one of the potential vaccine candidates [8].

4.1. MV-HspA vaccine

Hp may cause stomach cancer, which can lead to tumors. Oncolytic virus therapy is a new anti-tumor method. Now studies have shown that measles virus (MV) can target and infect tumor cells and replicate in tumor tissues, continuously expanding and killing virus-infected tumor cells through a specific independent apoptotic mechanism [9]. Strains generated from MV Edmonston are desirable vector platforms for oncolytic viral therapy and vaccine development.

Hp heat shock protein A (HspA) is a bacterial heat shock chaperone protein that has an important function of clearing nickel ions. The HspA gene encodes 118 amino acid residues [10]. Tomb et al. [11] believe that HspA plays a role in nickel ion transport and delivery to apoenzyme proteins. HspA, like UreB and VacA, is an effective antigenic component and can be used as a candidate molecule for vaccines. A recombinant MV-HspA vaccine created by Iankov et al [12]. could be utilized to treat cancer and prevent infection with H. pylori by immunoprophylaxis. By cloning the HspA gene onto the attenuated MV Edmonston backbone on extra transfection before N gene, the study team was able to successfully rescue the MV-HSPA strain.

4.2. Vaccine mechanism of MV-HspA

H.pylori strain 26695 HspA fragment PCR product was cloned into a vector. The MluI/AatII enzyme cut fragment was then added to the full-length p (+) MV EGFP infective clone plasmid of attenuated MV Edmonston strain. By using restriction enzymes and DNA sequencing, it was determined that the HspA transgene and recombinant MV.2 full-length p (+) MV-HspA were both intact. By co-transfecting MV plasmid with plasmids expressing N and L proteins in a 293-3-4 cell culture, Mv- HspA was produced. Vero cell lines were used to grow MV-HspA to its second and third generations, and titers were assessed on Vero cells. The equivalent 2 and 3 generations were used to amplify the control MV strain in the same way. Growth kinetics of MV-infected Vero cells revealed that the replication pattern of MV-HSPA differed from other MV strains when the infection multiplicity was 1. In the first 24 to 48 hours after infection, Mv-HspA multiplies more quickly in Vero cells than MV strains expressing green fluorescent protein (MV-GFP), and it quickly accumulates in the supernatant released by cell-associated and infected viral particles [12].

4.3. Vaccine test results

Iankov et al. prepared and identified the immunogenicity of MV attenuated strains encoding HspA transgenes (MV-HSPA). The replication rate of MV-HSPA was accelerated, the titer was increased by more than 10 times, and the accumulation rate of MV protein was accelerated within 48 h after infection. Additionally, it has been demonstrated to have outstanding tumor-removal effectiveness, such as sarcomas, ovarian malignancies, and breast cancers. Only one of the nine mice tested did not develop a long-term anti-HSPA antibody response and had no negative effect on the formation of protective anti-MV immune memory. The result demonstrated that MV-HSPA may be an effective treatment against *H. pylori* infection. In the meantime, it is a candidate vaccine for cancer treatment and [12]. According to the above studies, the recombinant MV-HspA vaccine has good immunogenicity, but it has not yet entered the clinical trial stage, and it is the first platform for a live, attenuated viral vaccination that expresses the HSP antigen of *H. pylori* or other novel bacteria.

5. HP outer membrane vesicles (OMV) vaccine

5.1. Basic information of OMV

Bacterial outer membrane vesicles (OMV) are nanosphere-shaped double-layer (20-250 nm) vesicles that are naturally released from Gram-negative bacteria into the extracellular environment. They are a double-layer membrane structure that is secreted by bacteria during the growth process and does not have the ability to replicate. OMV can enter the cell interior or other specific sites through micropinocytosis and clathrin-mediated endocytosis. Proteomic and biochemical analysis showed that OMV contained a variety of components derived from parent bacteria. OMV is composed mainly of outer membrane proteins, but also includes other substances such as DNA, RNA, lipopolysaccharide (LPS), proteins, enzymes and peptidoglycans. Its surface is composed of phospholipid bilayer with lipopolysaccharide outer layer (LPS), outer membrane protein and receptor. Internally, OMV has a thin peptidoglycan layer, periplasmic protein, and nucleic acid. Most bacterial release vesicles contain specific cargo molecules and thus have different functions, including transmission of virulence factors, DNA transfer, phage interception, induction of pathogenesis, signal transduction, mediating communication between bacteria and host cells, and between bacteria and each other. In addition, bacterial outer membrane vesicles also have the function of regulating immune activity, and therefore have great potential for applications as vaccines, anti-cancer drugs and nanotechnology.

5.2. The application of OMV vaccine

A conventional vaccination for human is a pharmaceutical drug which activates the body's immune system to thwart microorganism infections in their earliest stages of development. In order to promote a potent and permanent immune response, a vaccine product must be appropriate in size and possess both disease-specific antigens alongside PAMPs.

OMVs are of the appropriate dimension (20-200 nm) for entering lymph vessels while be taken up by antigen-presenting cells. As a result of their resemblance to the pathogen's bacterial antigenic surface, they are inherently comprised of elements which elicit both humoral and cellular immune responses. Hp have been shown to produce OMV in vitro and in vivo. OMVs, which are results of bacterial fermentation, will inherently include a range of chemicals that can engage the innate immune system. LPS will initially be present as the vital ingredient that comprises the outer leaflet of the exterior membrane. By specifically recognizing the TLR4/MD2 receptor, inducing the activation of NF- κ B and IRF3 driven gene transcription, it is a particularly strong stimulator of immune cells including monocytes and macrophages. The ensuing activation involved in subsequent adaptive immune response, which is extremely beneficial for OMV usage as vaccines.

Within a C57BL/6 mouse experimental model, Qiong Liu and colleagues found that mice were given 200 g of OMVs or the *H. pylori* whole cell vaccine orally, either along with or without the adjuvant cholera toxin. The OMVs immunization groups had been demonstrated to produce significantly more IL-4, IL-13, and IL-10 as well as sIgA and IgG1 antibodies than the WCT immunization groups. The

results showed that compared to the group receiving the WCV vaccine, OMVs might provoke the high humoral immune response and increase mucosal immune response. The outcomes further supported the hypothesis that OMVs mostly produced Th2-biased immune responses, which can considerably lower bacterial loads after infection. At present, there are few studies on Hp OMV vaccine, and the mechanism of OMV in the diseases caused by fine diseases has not been fully clarified. There are still many problems to be solved in the successful development of Hp OMV vaccine.

The following issues may need to be addressed in order to develop an effective OMV vaccine: (1) the high reactivity of PAMPs like LPS; (2) the low expression degrees of related protective antigens; (3) strain variation, which results in multiple subtypes of a specific antigen and low coverage; (4) immunodominant antigens that mislead the immune response; (5) immunosuppressive substances or particles that might otherwise conflict with the protective immune response. Consequently, by deleting, adding codon, or changing OM as well as other substances, the genetic manipulation of OMV-producing strains was used to optimize their vaccine application, resulting in a trustworthy and effective OMV product that is ready for usage.

6. Conclusion

We address the most used antigens and their respective immunological responses against Hp expected or registered up to now. They are divided into mainly 4 parts: flagellin vaccine, urease vaccine, virulence factor vaccine and Hp OMV vaccine. For flagellin vaccine, infection rate reduced among the vaccinated population. Recombinant Hp antigens and dmLT were efficient for protection from the Hp infection. According to evidence for the virulence factor vaccine, MV-HspA is hopeful for the treatment of cancer and immunoprophylaxis against Hp. OMVs for the Hp OMV vaccine generate potent humoral and mucosal reactions and mostly elicit Th-2 type immune responses. Although Hp vaccine research has made breakthrough progress, there are still many problems, including (1) the mechanism of Hp is not clear; (2) The effective use of vaccines is affected by many factors; (3) Immune response triggered may cause damage to human stomach tissue. Therefore, there is still some way to improve before Hp vaccine can be used in clinic. We hope that HP vaccine development will focus on solving the above problems and develop vaccines with high efficiency and little or no side effects, so as to reduce HP infection and gastric cancer coverage.

Author contribution

All the authors contributed equally, and their names were listed in alphabetical order.

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