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Research paper

A novel design of a multi-antigenic, multistage and multi-epitope vaccine against *Helicobacter pylori*: An *in silico* approach

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ABSTRACT

Helicobacter pylori have colonized the gastric mucosa of half of the population worldwide. This bacterium is classified as a definitive type I carcinogen by the World Health Organization and no effective vaccine has been found against it yet. Thus, a logical and rational vaccine design against *H. pylori* is necessary. Because of its tremendous complexity and elicited immune responses, the vaccine design should considered multiple antigens to enhance immune-protection, involved in the different stages of pathogenesis besides inducing a specific immune response by B- and T-cell multi-epitopes. In this study, emphasis was placed on the design of a new unique vaccine named CTB-multiHp. *In silico* techniques were used to design a chimeric construct consisting of cholera toxin B subunit fused to multi-epitope of urease B (residue 148–158, 188–198), cytotoxin-associated gene A (residue 584–602), neutrophil activating protein (residue 4–28), vacuolating cytotoxin gene A (residue 63–81), *H. pylori* adhesine A (residue77–99), heat shock protein A (residue 32–54) and gamma glutamyl transpeptidase (residue 271–293). The tertiary structure and features of the vaccine were analyzed. The chimeric protein was expressed in *Escherichia coli* BL21 and the serology analyses indicated that the CTB-multiHp protein produced exhibit immune-reactivity. The results showed that CTB-multiHp could be a good vaccine candidate against *H. pylori*. Ongoing studies will evaluate the effects of CTB-multiHp against *H. pylori* infection.

1. Introduction

Helicobacter pylori is a Gram-negative bacterium that colonizes the human stomach mucosa of more than half of the population worldwide, causing gastric diseases ranging from gastritis to cancer (Testerman and Morris, 2014). Although over 80% of infected people show no symptoms, the bacterium has been categorized as a class I human carcinogen by the World Health Organization (Ferlay et al., 2015). There are still no effective treatments 100% for your eradication (Testerman and Morris, 2014). Thus, the need for an effective vaccine against *H. pylori* is urgent and of prime importance to global public health (Del Giudice et al., 2009). To achieve this objective, a logical and rational design for a vaccine against *H. pylori* is essential.

The design of an effective vaccine against *H. pylori* must consider the interference with the mechanisms of pathogenic bacteria. Therefore, vaccine design should contain molecules derived from different stages (multistage) of *H. pylori* pathogenesis. Recently, significant progress has been made in understanding *H. pylori* pathogenesis and the role of its virulence factors in gastric diseases. Several studies have revealed that at least three distinct and sequential stages are required for *H. pylori* to exert its virulence on the colonized stomach: adhering to and colonizing the surface of gastric epithelial cells, evading and attenuating the host defense, and invading and damaging gastric mucosa (He et al., 2014). Some individual virulence factors, such as Urease B, CagA, VacA, and others, in their native or recombinant forms can confer certain level of protection against *H. pylori* (Del Giudice et al., 2009). However, a single recombinant antigen has shown to induce insufficient immunity with limited protective effect (Czinn and Blanchard, 2011). Hence, many formulation strategies have been tested, resulting in a significant reduction of bacterial load. Strong immune responses have been reported with the use of a multiantigenic vaccine (as VacA, CagA, NAP), which is currently under development for human use; another multiantigenic vaccine incorporates UreB, HspA, HpaA with adjuvant

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by oral administration in mice (Wang et al., 2010). Also attenuated *Salmonella* expressing VacA, CagA and UreB has shown a reduction of *H. pylori* colonization (Liu et al., 2011), among others.

A focus on vaccine design and development has moved to the generation of recombinant multi-epitope vaccines as a new strategy. The potential benefits of vaccines based on epitopes include a specific immune response avoiding the side effects of other unfavorable epitopes in the complete antigen and increased safety (Sbai et al., 2001). An epitope-based vaccine could also include single antigenic molecules combined from different epitopes for increased potency and it could lead to an effective strategy for the control of *H. pylori* (Sette and Fikes, 2003). Immunological studies have shown that *H. pylori* infection induces an activation of adaptive B- and T-cell responses (Backert and Yamaoka, 2016). As we already know, protein epitopes recognized by both T and B cells are the best candidates for vaccines because of their high specificity properties (Olsen et al., 2011). In this context, bioinformatics tools nowadays have a significant role to identify appropriate epitopes, in addition to saving time and being cost effective (Li et al., 2005). Recent studies have reported epitopes prediction of *H. pylori* using bioinformatics. Moise et al. (2008) predicted conserved T-cell epitopes among two *H. pylori* genomes; Ardito et al. (2011) identified a core genome from seven *H. pylori* strains and predicted consensus T cell epitopes; Naz et al. (2015) predicted vaccine candidates, and then, their epitopes were predicted for conservation using 39 genomes of *H. pylori*, suggesting the possibility of development of a multi-epitope or multi-component vaccine. Similarly, several research groups have focused on the design of chimeric genes containing different epitopes for the development of vaccines against *H. pylori*. Moss et al. (2011) designed a multi-epitope construct containing 25 T cell epitopes (previously identified) and optimized the epitope order. After, Haghighi et al. (2013) designed a chimeric construct consisting of three fragments from CagA, NaPA and OipA with a high density of B- and T-cell epitopes. The construct was fused with the D3 domain of *Pseudomonas* flagellin as adjuvant. Recently, Mohammad et al. (2016) designed a chimeric construct consisting of four fragments from FliD, Urease B, VacA and CagA with a high density of B- and T-cell epitopes.

As we can see a variety of studies have proved that each individual design has contributed to vaccines development. According to Pahl and Beitz (2013), a novel design incorporates new solution principles, performed by combining known principles. Thus, the holistic application of a combined approach could result in an effective vaccine design. This study, we propose a novel strategy for the logical and rational design of a multi-antigenic, multistage and multi-epitope vaccine against *H. pylori*, using bioinformatics tools.

2. Materials and methods

The flow chart representing the overall procedures of vaccine design is illustrated in Fig. 1.

2.1. Selection of antigen targets

The selection of antigen targets was based on two main criteria: (1) antigens involved in the stages of pathogenesis according to He et al. (2014) and (2) the potential to be vaccine candidate (essential *H. pylori*-derived antigen, proposed function, immunogenicity and protective in murine model) through literature search.

2.2. Sequences and alignments

The entire antigenic protein sequences were retrieved from NCBI protein database (http://www.ncbi.nlm.nih.gov/protein/) in FASTA format. For antigens with variable sequences, a consensus sequence was generated, using sequences of *H. pylori* representative strains in the world are shown in the Supplementary material (Table S1). Multiple sequence alignment was performed with EMBOSS CONS (http://emboss.bioinformatics.nl/cgi-bin/emboss/cons), T-COFFEE (http://www.ch.embnet.org/software/TCoffee.html) and CLUSTALW – JALVIEW (http://www.hongyu.org/software/clustal.html) software.

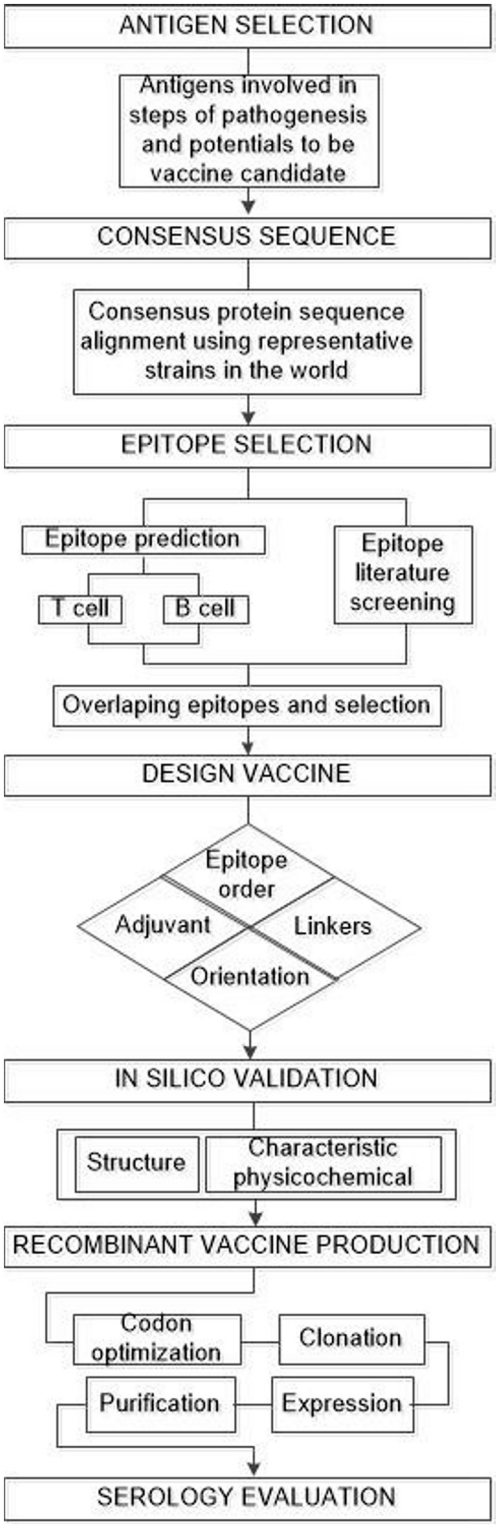

Fig. 1. Flowchart summarizing the steps of a *Helicobacter pylori* vaccine design.

2.3. Bioinformatics analyses

All antigenic sequences were analyzed separately to identify the best segments containing both B and T-cell epitopes, using bioinformatics