A new strategy for development of hepatitis E vaccine: Epitope-based vaccines

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Vaccination is commonly known as the most desirable strategy for controlling infections. However, the traditional vaccine approaches are incapable of preventing emerging infectious diseases as well as antigenically hypervariable pathogens that are still a main threat for world health. Recent advances in computational approaches developed for epitope identification and in silico analysis have paved the way for the development of epitope-based vaccines against these kinds of infectious agents. The aim of these novel vaccines is to induce immune responses in a more effective way than the conventional vaccines. In this review, we describe the principles of epitope-based vaccine design, along with the application of these approaches in design and development of high density multiepitope construct as the first epitope-based vaccine candidate against hepatitis E virus.

Keywords: Epitope-based vaccine; Multiepitope construct; High epitope density; In silico vaccine design; Epitope selection; Optimization; Hepatitis E virus; Immunity


Hepatitis E virus (HEV) infection is usually a self-limiting disease but may progress to fulminant hepatitis and even lethal liver failure, causing considerable mortality especially when Hepatitis E is accompany with pregnancy and underlying chronic liver problems [1,2]. The mortality rate of hepatitis E is about 0.5-4% in epidemics but may reach 10-25% in pregnant women and up to 75% in individuals with underlying liver problems [1]. Hepatitis E is distributed all around the world, with a higher prevalence in East and South Asia [3]. Large outbreaks usually occur in developing countries with limited access to health services and essential water, while sporadic form of the infection is found in developed countries [4].

HEV is a small, non-enveloped positive-stranded RNA virus, belongs to Hepevirus genus and Hepeviridae family [5]. This virus has been classified into four genotypes with different epidemiology [6]. Genotypes 1 and 2 infect humans, while genotypes 3 and 4 are usually seen in animals [2,5]. However in some developed countries, sporadic infection with genotypes 3 and 4 are also reported in humans [5,7,8]. HEV is transmitted through ingestion of fecal contaminated water and food supplies derived from infected animals [1]. Blood transfusion and congenital transmission are some other possible ways to spread HEV infection [4].

In the era of effective vaccination, many infectious diseases have been eradicated or eliminated. However, HEV infection still affects an increasing number of the world’s
population. This infection is more prevalent in less developed societies, where 30 to 80% of the adult population are seropositive [9]. According to WHO report, 20 million new cases of HEV infection emerge each year globally. Also, 3.3 million acute cases and approximately 56600 deaths due to HEV-related acute liver failure occur worldwide annually [3]. Therefore, HEV infection is a real problem to public health and its prevention through vaccination is highly desirable. Meanwhile, the development of inactive or live attenuated vaccines against HEV has not been possible, largely because the production of sufficient amounts of HEV is difficult in culture system [5, 10]. Therefore, the development of vaccines has relied upon production of recombinant HEV proteins in various prokaryotic and eukaryotic expression systems [5, 8].

Among three open reading frames (ORFs) of HEV genome, only ORF2 has been proven to be effective for HEV vaccine development. ORF2 produces capsid protein, which is immunogenic in nature and develops production of neutralizing antibodies [6, 11]. Immunological analysis and sequencing showed that residues 271–286, 199–214, 463–478, 583–598, and 631–646 in HEV capsid protein are highly immunogenic [12]. The amino acids 112 to 660 assembles into VLPs (virus-like particles) and initiates strong immune responses [13]. The amino acids 455-606 exposed on the surface of virion forms a spike and contains the main B cell epitopes [5]. However, when full-length capsid protein (660 amino acids) is expressed, its immunogenic properties are masked due to insolubility. Therefore, most efforts to design HEV vaccine have focused on the truncated or short forms of the capsid protein [6, 11].

The currently developed vaccine candidates against HEV have been consisted of the various truncated forms of ORF2 protein, including trpE-C2 (aa 221-660) [14], pE2 (aa 394-604) [15], HEV 239 (aa 368-606) [16], 53 kDa (aa 112-578) [17], 56 kDa (aa 112-607) [17], 62 kDa (aa 112-660) [18], rHEV VLP (aa 112-608) [13] and T1-ORF2 (aa 126-621) [19] vaccines. Among all of these vaccine candidates, only HEV 239 and 56 kDa vaccines have been assessed in human clinical trials [5], and HEV 239 vaccine has succeed to reach phase III trial, and then was licensed by SFDA (China’s State Food and Drug Administration) in 2011 [1, 20-22].

HEV 239, designated Hecolin, is the first prophylactic human vaccine against HEV infection [1, 5]. This vaccine has been derived from genotype 1 Chinese HEV strain and following expression in E. coli, assembles into VLPs with high neutralization capacity for cross-protection against different HEV genotypes [1, 5, 16]. The reason is that the four genotypes are considered as a single serotype due to sharing common neutralizing epitopes [5, 8]. Despite the success of this vaccine in china, more evaluations are required to verify the safety and efficacy of this vaccine for children, pregnant women and patients with underlying liver problems, and to make this vaccine available globally [1].

A more recent approach for HEV vaccines development is DNA vaccine. In this realm, two different vaccines, including pHEVORF2 and Lipo-NE-DP vaccines have been developed using plasmid DNA containing the complete sequence or the truncated sequence of ORF2 (encoding aa 458-607), respectively. Despite being fully protective in animal models, no further development of these two vaccines has been reported [23-25].

The currently developed vaccines against HEV, though exhibit promising outcomes, suffer from the limited extent and duration of immunity as well as high cost of vaccine preparation. Therefore, new approaches for the development of novel vaccines against HEV infection are needed. One of the most promising approaches is epitope-based strategy. This strategy was introduced for the first time by Jackob in 1985 to develop vaccine against cholera [26, 27]. The main idea behind the development of epitope-based vaccines is the identification of immunodominant epitopes in a given protein sequence that is expected to induce the immune responses against a target pathogen [27, 28].

An epitope is defined as an immunogenic region of a protein that recognizes by T cell receptors (T cell epitopes), or antibodies (B cell epitopes), thereby inducing a cellular or humoral immune responses, respectively [29]. B cell epitopes are conformational, while T cell epitopes are groups of 8-11 amino acids or 12-25 amino acids that present to cytotoxic T lymphocytes (CTLs) or helper T lymphocytes (HTLs) by MHC class I or MHC class II, respectively [27]. B cells neutralize pathogens through antibody production, CTLs initiate lysis of the epitope presenting cells, and HTLs secret cytokines that in turn initiate activation of B cells and CTLs [30, 31].

Experimentally, epitopes are identified by sequencing epitopes eluted from HLA-epitope complexes or screening overlapping peptides derived from the antigen [32]. Although these two conventional methods have been quite successful, but suffer from a few technical limitations. In the overlapping peptide method, the presence of possible epitopes in the overlapping regions may be overlooked [32]. In the sequencing method, the identified epitopes may be from multiple processed proteins but not a single target protein [32]. Moreover, only a few of pathogen-derived epitopes that are displayed in the HLA complexes can be immunodominant, which is expected to induce effective immune responses [33]. Overall, during the expression and purification processes...
exists the possibility that the transiently produced antigens may be missed, while the abundantly expressed proteins may not necessarily induce strong immune responses [34]. On the other hand, the difficulties and high cost of the experimental procedures address the need of new methods to reduce time and expenses [27, 35]. Therefore, computational approaches to assist the identification of the immunogenic epitopes were created and introduced to the scientific community [28, 36, 37]. These approaches provide computational tools based on epitope mapping and HLA binding prediction algorithms that facilitate the identification of epitopes [27, 30, 33].

Despite the considerable advances in in silico prediction of epitopes, such computational methods have yet to be improved [34]. It has been observed that in silico approaches may fail to predict some of the potentially immunoreactive epitopes. This might be due to some limitations in the predictive algorithms [34]. On the other hand, some differences might exist between the algorithm predicted epitopes and the cellular processed epitope, therefore in silico methods might not reliably predict naturally presented epitopes by the infected cells [30].

However computational procedures and in silico analysis facilitate the prediction of epitopes due to reducing the number of candidate epitopes to be experimentally tested, still cannot replace experimental approaches and only can be a starting point [38]. Finally, the laboratory experimentation should be performed to validate the accuracy of the in silico prediction [30, 34, 39]. The combination of computational and experimental procedures is a desirable method for efficient selection of epitopes [38, 40].

Following identification of epitopes, some critical challenges in epitope selection are the genetic diversity of pathogenic strains and the extensive variability of HLA alleles among human population [28, 37, 41]. Computational approaches through in silico analysis have overcome these problems by selecting epitopes from the conserved regions among the various strains of a pathogen, and epitopes capable of binding to various HLA alleles [29, 34, 35, 41]. However an emphasis is placed on the broad coverage of allelic diversity in an attempt to cover the most of world population, but a vaccine can economically contain a limited number of epitopes [28]. One reasonable way to minimize the number of epitopes is the use of epitopes restricted by the most common HLA alleles in the global population or epitopes recognized by HLA supertypes [28, 30, 42]. A HLA supertype is known as a set of HLA alleles with overlapping peptide binding properties [43, 44]. The supertype-based strategies might lead to a lower response compared to the multiple allele-based strategies. Since HLA supertypes are not prevalent in populations of all parts of the world. Therefore, these kinds of vaccines can only be useful in populations with high prevalence of HLA supertypes [37].

The conserved regions in multiple protein sequences are determined by alignment tools such as ClustalW and BLAST [29]. However, the central problem is that sometimes the highly conserved epitopes might be similar to host self-protein and less efficiently recognized by T cells, therefore induce self-tolerance; or might be conserved between the host and the pathogen, therefore induce responses against host self-protein, a phenomenon that is known as autoimmunity [29]. Therefore, selection of epitopes should be with care to avoid these undesired responses.

Another consideration is low immunogenicity of single epitope or small peptide compared to the whole protein or entire pathogen [34, 45-47]. Therefore, the idea of multiepitope-based strategy was proposed, in which multiple epitopes are included in a single construct [39]. Furthermore, multiepitope construct containing high epitope density enhances immunogenicity as well as antigenicity [8, 48, 49]. Such high density multiepitope construct have been developed for influenza A virus and HIV and, HEV, with promising results [8, 49-53].

In regard to the design of multiepitope construct, two strategies including sequential arrangement of epitopes and multiple Ag peptides (MAP) have been investigated [40, 54]. Since the production of MAP construct in large quantities is challenging, the other strategy is preferred. However, the design of multiepitope construct based on sequential arrangement may lead to the creation of junctional epitopes [54].

A junctional epitope is defined as an irrelevant epitope, composed of C-terminal and N-terminal linkage of two distinct epitopes in a linear arrangement [45, 54, 55]. Formation of such epitope is a serious problem in the multiepitope vaccine design, which can either suppress the immune responses to the target epitopes or create undesired immune responses [45, 54]. While the use of glycine/proline sequences (GPGPG spacer) between neighboring epitopes can minimize the creation of junctional epitopes and also optimize epitope processing, thereby improve the potency and breadth of the immune responses [54, 55]. On the other hand, the presence of GPGPG spacers in multiepitope construct might facilitate the expression and purification of the protein due to formation of secondary and tertiary structures [54, 56].

Another issue is the different combinations in which the epitopes can be arranged in a multiepitope construct. Different arrangements of epitopes in the construct have direct effect on the potency and extent of responses. Thus,
the epitope arrangement should be optimized for the design of multiepitope constructs \[40, 55\].

Multiepitope vaccines offer several advantages over conventional vaccines, including the ability to induce immune responses against multiple strains of a pathogen or different pathogens and protect the majority of the human population \[34, 37, 46\], the possibility to exclude antigenic sequences activating unwanted reactions such as suppressor responses and reversion to virulence similar to those observed with live attenuated vaccines \[35, 37, 47\]. They are also safe, thermostable, cost-effective and easy to produce in large amounts \[30, 35\]. The most outstanding advantage of these kinds of vaccines is the opportunity to develop vaccines for hypervariable pathogens and those pathogens that are impossible or difficult to culture in vitro \[34, 35, 57\].

We investigated the feasibility of the epitope-based strategy to design a high density multiepitope construct as vaccine candidate for HEV. We focused on T cell epitopes conserved among the four standard strains of HEV genotype 1 as well as those recognized by the most frequent HLA alleles in the human population in an attempt to design a vaccine with high population and pathogen coverage \[8\]. This high density multiepitope was used to stimulate PBMCs of patients recovered from acute HEV infection. This recombinant protein activated specific cellular immune responses, as confirmed by cytokines secretion, Elispot assay and lymphocyte proliferation. This is the first time that a high density multiepitope construct containing T cell epitope is designed as a vaccine candidate against HEV, while the results encourage the further development of this vaccine candidate \[8\].

Epitope-based vaccines can be optimized to improve both MHC binding capacity and processing efficiency of epitopes \[55, 58\]. Some epitopes in the context of multiepitope construct are processed ineffectively, while by introducing a positively charged residue immediately after the C-terminus of the epitope, the efficacy of processing can be increased \[55\]. In addition, some epitopes have suboptimal anchors, thereby bind HLA with low or intermediate affinity. One strategy to overcome this shortcoming is to construct epitopes analogues in which suboptimal anchors are replaced by residues with high affinity \[55\]. Furthermore, an ideal vaccine should be able to induce both humoral and cellular immune responses in an effort to provide broad protective immunity against pathogens \[30, 35, 47, 59\]. To achieve this goal, a multiepitope construct should consist of combinations of both B cell and T cell epitopes capable of producing neutralizing antibodies to eliminate free pathogens and activating cytotoxic T cell to destroy the pathogen-infected cells, respectively \[30\]. Therefore, the epitope-based vaccines are applicable for the development of prophylactic vaccines as well as therapeutic vaccines especially against chronic infections \[30\].

Recently, several epitope-based vaccines against infectious diseases have been produced and evaluated in preclinical and clinical trials. Some of these vaccines against HCV, HIV, HPV, HSV2 and influenza A viruses have shown promising outcomes in human clinical trials \[30, 34\]. In addition, the efficacy of these kinds of vaccines in tumor immunotherapy has gained considerable attention \[27, 28\]. Therefore, there is a possibility that epitope-based vaccines will be on the market in near future \[30\].

**Conclusion**

This review has provided important information for epitope-based vaccine studies. Recent progresses in the field of computational techniques have led to the development of novel vaccines against antigenically variable pathogens such as influenza A viruses, HIV and HCV. However, these kinds of vaccines are still in development, but the promising results of human clinical trials encourage the use of this in silico strategy for a variety of pathogens towards which no effective conventional vaccine is available. Accordingly, we used this novel strategy based on in silico prediction to design a high density multiepitope construct as the first epitope-based vaccine candidate for HEV. However, the preliminary analysis and in vitro experiments showed a promising outlook, but still in vivo evaluation are required to validate the protective efficacy of this novel vaccine candidate against HEV.

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**Conflict-of-interest**

The authors declare there are no conflicts of interest in the content of this review.

**Author contributions**

Reza Taherkhani and Fatemeh Farshadpour solely contributed to this paper.

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