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Immunoinformatics approaches to design a novel multi-epitope subunit vaccine against HIV infection

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ABSTRACT

The end goal of HIV vaccine designing requires novel strategies to elicit a strong humoral and cell-mediated immune response. The emergence of drug resistance and the requirement of next line treatment necessitate the finding of the potential and immunogenic vaccine candidate. This study employed a novel immunoinformatics approach to design multi-epitope subunit vaccine against HIV infection. Here, we designed the subunit vaccine by the combination of CTL, HTL and BCL epitopes along with suitable adjuvant and linkers. Physiochemical characterization of subunit vaccine was assessed to ensure its thermostability, theoretical PI, and amphipathic behavior. In further assessment, subunit vaccine was found to be immunogenic with the capability to generate humoral and cell-mediated immune response. Further, homology modeling and refinement was performed and the refined modeled structure was used for molecular docking with the immune receptor (TLR-3) present on lymphocyte cells. Consequently, molecular dynamics simulation ensured the molecular interaction between TLR-3 and subunit vaccine candidate. Disulfide engineering was performed by placing the cysteine residues in the region of high mobility to enhance the vaccine stability. At last, *in silico* cloning was performed to warrant the translational efficiency and microbial expression of the designed vaccine.

1. Introduction

The human immunodeficiency virus (HIV) has become a major public health concern for the developing world. Around 36.7 million people throughout the world are currently living with this severe complication, and almost 40% patients are being unaware of their diseased condition. The worst-hit areas are regions of sub-Saharan Africa where one in every 25 individuals is affected by this retroviral syndrome [1]. In spite of the steps taken by the National AIDS Control Organization (NACO) to spread awareness about this syndrome, in the year 2015, around 2.1 million new individuals were enthralled to this infection. Although there are no specific symptoms of HIV infection, some common flu-like symptoms may be experienced [2]. Over the time, HIV infection leads to acquired immunodeficiency syndrome (AIDS) which was common in intravenous drug users, male homosexuals and hemophiliacs in the United States [3]. Broadly, HIV is classified into two categories namely HIV-1 and HIV-2. Among these two strains, formerly is responsible for the 95% of the global spread of AIDS, while the influence of HIV-2 is mainly restricted to Central and Western Africa. The strain of HIV-1 can be additionally ordered into four groups to be specific M, N, O, and P [4]. Of these, M (95% cases) is the major group further classified into nine genetically distinct subtypes namely A, B, C, D, F, G, H, J and K. Of these, B-subtype is dominant in the Australia, America and Western Europe while C-subtype has shown its prevalence in the southern Africa and India. Both these subtype contributes to the major HIV infectious cases, globally.

HIV genome consists of gag, pol, and env genes flanked by long terminal repeats. Apart from this, HIV also has six regulatory genes that are rev, tat, nef, vif, vpr, and vpu [5,6]. Rev is an RNA binding protein that performs a nuclear export of intron-containing HIV-1 RNA [7]. Tat protein binds to trans-activation response RNA, downstream of the transcription start site, hence is crucial for activating transcription. [8]. Nef protein is crucial for viral survival by aiding in evasion of the immune system and anti-apoptosis of the HIV infected cells [9]. Vif protein hinders two human enzymes namely APOBEC3F and APOBEC3G, the cellular cytosine deaminases by forming a complex with these two enzymes. It can also interact

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and inhibit translation of APOBEC3G [10]. Vir protein causes cell cycle arrest in G2 phase which gives ample amount of time to the virus for the expression of the viral genome [11]. HIV protein Vpu works along with Nef to escape host immune surveillance. Vpu depletes virion tethering bone marrow stromal antigen-2 (BST2). The viral RNA is reverse transcribed into proviral DNA by reverse transcriptase encoded by the viral RNA. Integrase performs the task of integrating proviral DNA into the host genome by performing various cleaving reactions followed by ligation [12]. Newly formed HIV particles are released with a thick layer of radially arranged Gag and Gag-pol precursors. It is the protease that performs the cleaving of these precursors into their mature counterparts in an organized series of steps. These proteases are aspartyl proteases in nature and perform cleaving during or shortly after budding [5].

Currently, available HIV/AIDS treatment consists of antiretroviral therapy (ART). ART has evolved from days of high toxicity and pill burden to a more effective option that has less toxic side effects. Although with current treatment, complete eradication of the disease is not possible, ART helps to increase the life expectancy of AIDS patient. A lot of efforts are still needed to find some drug alternatives like a vaccine. Vaccines symbolize the most cost-effective life-saving device in history that prepares our body beforehand to fight the deadly and contagious disease. Many approaches can be taken for designing of a vaccine which includes its mode of infections and how immune system of the host responds. Types of vaccine that currently exist are live attenuated, inactivated vaccine, subunit vaccine, toxoid vaccine, conjugate vaccine, DNA vaccine and recombinant vector vaccine. All of these have their respective advantages and disadvantages and hence are used according to in different disease conditions. Regardless of many years of efforts, the failure to develop a vaccine against the worldwide pandemics of HIV needs to search for the new vaccine candidate that can confer protective immunity. Subunit vaccines do not consist of any live pathogenic components, therefore, nullify the chances of pathogenicity reversal as in case of live attenuated vaccine [13,14]. It consists of only the antigenic part of the pathogens which may have the capability to elicit a protective immune response within the human body. The major advantage of using subunit vaccine includes its application to a person with the weak immune system, long-lived immunity and low risk of reaction. Therefore, this study made an effort to design a novel subunit vaccine against HIV infection. This study utilizes the immunogenic epitopes viral proteome to be specific tat, rev, vpu, vpr, vif, nef, protease, integrase and reverse transcriptase. Our designed vaccine consisting of humoral as well as cell-mediated immune response specific immunogenic epitopes. These epitopes were further checked for their conservancy among other HIV subtypes and worldwide population coverage. Subunit vaccine was further evaluated on the antigenicity, allergenicity, and physiochemical parameters. Nextly, the 3D model of subunit vaccine was docked against the TLR-3 receptor and the complex stability was determined by molecular dynamics simulation studies. Vaccine stability was enhanced by disulfide engineering, and *in silico* cloning was performed to ensure the high expression in *E. coli* K12 expression system. Overall, this study applied a combinatorial approach to design an immunogenic, thermostable and non-allergic vaccine candidate to tackle the HIV infection.

2. Methodology

2.1. HIV genome analysis and selection of crucial proteins

A thorough literature survey was done to identify the crucial proteins of the HIV metabolic pathways. Total nine proteins encoded by HIV-1 group M subtype B genome were selected consisting of two regulatory proteins namely Tat (UniProt ID: P04608) and Rev (UniProt ID: P04618); four accessories proteins Vpu (UniProt ID: P05919), Vpr (UniProt ID: P69726), Vif (UniProt ID: P69723), Nef (UniProt ID: P04601), and three enzymes encoded by pol (UniProt ID: P04585) namely Protease (UniProt ID: P04585), Integrase (UniProt ID: P04585) and Reverse transcriptase (UniProt ID: P04585), were selected for the purpose of subunit vaccine development. Sequences of all these proteins were retrieved from UniProt database (http://www.uniprot.org/) in FASTA format.

2.2. Helper T-lymphocytes epitope prediction

Helper T-lymphocyte cell (HTLs) receptor specific epitopes were screened among the above-selected nine HIV-1 group M subtype B proteins by utilizing Immune Epitope Database (IEDB) (http://tools.iedb.org/mhcii/) MHC-II epitope prediction tool [15,16]. In this server, different prediction methods were available for the epitope prediction purpose but we selected the IEDB recommended option to use the best-suited prediction method for the epitope selection. During species selection, the human was selected as target species. In the MHC allele selection panel, IEDB recommended allele option was selected, consisting of a reference panel of 27 alleles with a coverage of >99% population. The selected MHC-II alleles were HLA-DRB1∗01:01, HLA-DRB1∗03:01, HLA-DRB1∗04:01, HLA-DRB1∗04:05, HLA-DRB1∗07:01, HLA-DRB1∗08:02, HLA-DRB1∗09:01, HLA-DRB1∗11:01, HLA-DRB1∗12:01, HLA-DRB1∗13:02, HLA-DRB1∗15:01, HLA-DRB3∗01:01, HLA-DRB3∗02:02, HLA-DRB4∗01:01, HLA-DRB5∗01:01, HLA-DQA1∗05:01/DQB1∗02:01, HLA-DQA1∗05:01/DQB1∗03:01, HLA-DQA1∗03:01/DQB1∗03:02, HLA-DQA1∗04:01/DQB1∗04:02, HLA-DQA1∗01:01/DQB1∗05:01, HLA-DQA1∗01:02/DQB1∗06:02, HLA-DPA1∗02:01/DPB1∗01:01, HLA-DPA1∗01:03/DPB1∗02:01, HLA-DPA1∗01/DPB1∗04:01, HLA-DPA1∗03:01/DPB1∗04:02, HLA-DPA1∗02:01/DPB1∗05:01, and HLA-DPA1∗02:01/DPB1∗14:01. As the majorly HIV-1 affected countries are Sub-Saharan Africa followed by Eastern Europe and Central Asia, North America, Latin America, south and south-East Asia, and Oceania. For the HTL epitope shorting, allelic population coverage was taken into consideration and only one epitope per protein was selected that belongs to the alleles of the aforementioned region which is severely affected by the HIV-1 infection.

2.3. Cytotoxic T lymphocyte epitope prediction

Cytotoxic T lymphocyte cells (CTL) are the key player in MHC-I mediated cellular immune response. They perform their function by killing the cancerous cells, virus-infected cells or other damaged cells by recognizing the epitope presented by MHC-I molecule on the cell surface. CTL epitopes were predicted for the same HIV proteins by utilizing the NetCTL 1.2 server [17] at the sensitivity of 0.80. Among the different parameters of prediction, A2, A3, and B7 were selected as a supertype to cover 83–88.8% person in different ethnic groups [18], weight on C terminal cleavage and tap transport efficiency was kept default at 0.15 and 0.05, respectively. While the threshold score set for the prediction was 0.75.

2.4. T-cell (HTL & CTL) epitope conservancy analysis

The only objective of this study of subunit vaccine development was to provide broader protection against multiple HIV-1 strains using conserved epitopes. Therefore, it was necessary to check the conservancy of CTL and HTL epitopes among different strains of HIV-1 group-M strains. Aforementioned, T-cell epitopes were predicted for the HIV-1 group M subtype B, therefore IEDB Epitope Conservancy Analysis tool [19] was used to calculate the degree of

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