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In silico identification and characterization of common epitope-based peptide vaccine for Nipah and Hendra viruses

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ABSTRACT

Objective: To explore a common B- and T-cell epitope-based vaccine that can elicit an immune response against encephalitis causing genus *Henipaviruses*, Hendra virus (HeV) and Nipah virus (NiV).

Methods: Membrane proteins F, G and M of HeV and NiV were retrieved from the protein database and subjected to different bioinformatics tools to predict antigenic B-cell epitopes. Best B-cell epitopes were then analyzed to predict their T-cell antigenic potentiality. Antigenic B- and T-cell epitopes that shared maximum identity with HeV and NiV were selected. Stability of the selected epitopes was predicted. Finally, the selected epitopes were subjected to molecular docking simulation with HLA-DR to confirm their antigenic potentiality *in silico*.

Results: One epitope from G proteins, one from M proteins and none from F proteins were selected based on their antigenic potentiality. The epitope from the G proteins was stable whereas that from M was unstable. The M-epitope was made stable by adding flanking dipeptides. The 15-mer G-epitope (VDPLRVQWRNNSVIS) showed at least 66% identity with all NiV and HeV G protein sequences, while the 15-mer M-epitope (GKLEFRRNNAIAFKG) with the dipeptide flanking residues showed 73% identity with all NiV and HeV M protein sequences available in the database. Molecular docking simulation with most frequent MHC class-II (MHC II) and class-I (MHC I) molecules showed that these epitopes could bind within HLA binding grooves to elicit an immune response.

Conclusions: Data in our present study revealed the notion that the epitopes from G and M proteins might be the target for peptide-based subunit vaccine design against HeV and NiV. However, the biochemical analysis is necessary to experimentally validate the interaction of epitopes individually with the MHC molecules through elucidation of immunity induction.

1. Introduction

A consequence of Nipah virus (NiV) infection in humans is encephalitis which causes headache, stiff neck, fever and altered mental status due to inflammation of parts of the brain, spinal cord and meninges [1]. Since 1994, there have been 31 outbreaks of Hendra virus (HeV); 17 of these outbreaks occurred in 2011. In 1998–1999, the first outbreak of NiV was documented in Malaysia and Singapore, where 283 cases of NiV encephalitis were observed, with 109 fatalities [2]. To date, there have been a total of 600 reported cases of NiV infection in people with fatality rates up to 100% for some outbreaks [3]. Most importantly, in Bangladesh, outbreaks occurred almost every year between 2001 and 2015, which have had higher fatality rate averaging about 75% [4]. In January, 2012, NiV outbreak occurred in the Joypurhat district of Bangladesh, resulting in the death of six individuals, with case fatality rate 100% indicating the continuous role of this virus in disease outbreaks in Bangladesh [5]. In the past, NiV showed

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transmission from bats to pigs and then to humans suggesting that the pig was required as amplifying host. Recent outbreaks in Bangladesh from 2001 to 2005 reported transmission of NiV directly from bats to humans and even human-to-human [6]. NiV belongs to the order *Mononegavirales* and is a member of the *Paramyxoviridae* family. Because of unique biological and genetic characteristics, NiV and the closely related HeV form a new genus *Henipavirus* within the subfamily *Paramyxoviridae* [7]. HeV, like NiV, is a zoonotic pathogen containing non-segmented, negative-stranded RNA genome [5]. To date, four of the seven human infections with HeV recorded so far have been fatal [5]. In nature, both NiV and HeV are found in several pteropous fruit bat species (flying foxes) and possess a broad host tropism that includes pigs, horses, cats, dogs, guinea pigs, hamsters, ferrets, monkeys and humans. Unlike NiV, HeV is transmitted from bats-to-horses and then to humans [3]. Both viruses can cause relapsed encephalitis in infected individuals. The case fatality rate of human infection varies between 50% and 100%, made both NiV and HeV, the most deadly viruses known to infect humans [5].

There is currently no vaccine or antiviral therapy licensed for use in humans or animals. During the initial NiV outbreak in Malaysia, ribavirin used against NiV, did not show much success [8]. In Australia, ribavirin failed to show a good therapeutic effect in several patients infected with HeV [9]. Additionally, treatment with chloroquine and ribavirin proved ineffective for one HeV-infected individual in 2009 as no clinical benefit was observed [10]. Therefore, a vaccine against both NiV and HeV should be designed that will be safe and effective for using in humans. *In silico* design of epitope based vaccines has become the standard approach to vaccine discovery in the current post-genomic era as these offers inexpensive, more specific, potential and easy means of vaccine development for treating infectious diseases [11]. Suitable antigenic determinants of fusion protein (F), attachment glycoprotein (G) and matrix protein (M) might be potential target to design epitope-based vaccine against encephalitis caused by NiV and HeV infection. In the present study, we analyzed F, G and M proteins of HeV and NiV by using different bioinformatics tools to identify the best antigen that might be an effective vaccine target against both HeV and NiV. We conducted investigations to find the best B- and T-cell epitopes from F, G and M proteins of NiV and HeV following antigenicity analysis and showed the binding ability of the epitopes with MHC class-I (MHC I) and class-II (MHC II) by *in silico* molecular docking approach.

2. Methodology

2.1. Prediction of antigenicity of different Henipavirus membrane proteins

All the complete amino acid sequences of each F, G and M proteins of NiV and HeV were retrieved from protein databases (http://www.us.expasy.org/sprot; http://www.ncbi.nlm.nih.gov/protein/) and non-identical sequences were analyzed with VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) antigen prediction server [12]. For highest accuracy, a threshold value of 0.5 was used to check the antigenicity of each full length protein. Amino acid sequences from F and G Proteins that have antigenic score >0.5 were chosen. Only for the M proteins that have antigenic score >0.4 were selected as described previously [13]. Each chosen full length amino acid sequence was then subjected to transmembrane topology analysis using TMHMM v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/) prediction server to identify exo-membrane (surface exposed) amino acid sequences of each protein [14].

2.2. Prediction of antigenic B-cell epitopes

For the prediction of B-cell epitopes, each selected full length protein sequence was subjected to BCPreds (http://ailab.cs.iastate.edu/bcpreds/predict.html) analysis where both BCpred and AAP prediction methods were used [15]. All predicted B-cell epitopes (16-mer) having a BCPreds cutoff score >0.8 were selected and subsequently checked for membrane topology by comparing with TMHMM results for exo-membrane amino acid sequences. Surface exposed B-cell epitope sequences having the cutoff value >0.8 for BCPreds were then analyzed using VaxiJen at threshold 0.5 to check the antigenicity. Finally, two-three epitopes with the top VaxiJen scores were selected to use in prediction of T-cell epitopes.

2.3. Prediction of T-cell epitopes from selected B-cell epitopes

T-cell epitopes were predicted from the selected B-cell epitopes and two screening steps were followed. In the first screening, the selection criteria were: i) the T-cell epitope sequence should bind to both the MHC I and MHC II molecules and the least number of total interacting MHC molecules should be >15, ii) the T-cell epitope sequence must interact with HLA-DRB1*0101 of MHC-II, and iii) T-cell epitope sequence should be antigenic based on VaxiJen score. Propred-1 (http://www.imtech.res.in/raghava/propred1/) [16] and Propred (http://www.imtech.res.in/raghava/propred/) [17] servers that utilize amino acid position coefficients inferred from literature employing linear prediction model [18], were used to identify common epitopes that bind to both MHC I and MHC II molecules. Total numbers of interacting MHC alleles were counted. For quantitative structure–activity relationship (QSAR) simulation approach, the half maximal (50%) inhibitory concentration (IC_{50}) and antigenicity of common epitopes (predicted by Propred-1 and Propred) were calculated using MHCPred v.2 (http://www.ddg-pharmfac.net/mhcpred/MHCPred/) server [19] and VaxiJen v2.0, respectively. Epitopes with highest antigenicity and those bound more than 15 molecules of both MHC I and MHC II alleles and had less than 100 nmol/L IC_{50} scores for DRB1*0101 were selected. The second screening was based on structure and QSAR simulation methods using T-Epitope Designer (http://www.bioinformation.net/ted/) [20] and MHCPred, respectively. T-epitope Designer can screen peptides for >1000 HLA alleles. In the second screening, the criteria were as follows. The peptide should bind >75% of total HLA molecules; the peptide must bind with high scores to (i) HLA-A*0201, HLA-A*0204, and HLA-B*2705, and (ii) DRB1*0101 and DRB1*0401. T-epitope Designer was used for first two criteria and MHCPred was used for the final criteria. The final list of T-cell epitopes was made with peptide sequences that pass these above mentioned criteria and VaxiJen

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