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Full Length Article

Designing a Multiepitope Vaccine (MEV) using *In Silico* Approach against Newcastle Disease (ND) Virus

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Abstract

Virulent strains of the Newcastle disease virus (NDV) cause a highly contagious disease in domestic poultry that has resulted in major economic losses worldwide. Recurrent mutations in the viral genome and emersion of new strains are attributed to disease constant spread. These repeated episodes of infection have been questioning the efficacy of vaccines in use. However, recent advancement in molecular biology has enabled scientists to design recombinant vaccines which will be safer and provide long-lasting immunity. Thus, this study aims to analyze the Fusion glycoprotein (F0) of the NDV using a computational approach to identify highly antigenic and conserved epitopes that can be used for the development of a therapeutic peptide vaccine. The sequence of F0 glycoprotein of a virulent strain was subjected to software for the prediction of T cell and B cell epitopes. Epitopes were scrutinized based on their antigenicity, toxicity, conservancy, and topology. Cytotoxic T-lymphocytes (CTL) epitopes were checked for their ability to interact with chicken BF alleles through molecular docking. A 308 amino acid long MEV was constructed by joining cytotoxic T cell, helper T cell and interferon-gamma (IFN-γ) epitopes along with an adjuvant. The construct was found highly antigenic, stable, non-allergenic and stable. The ability of MEV to elicit host immune response is demonstrated by its strong binding affinity with chicken toll-like receptor (TLR-4). Our findings suggest that the designed MEV can act as a potent vaccine candidate against variant virulent NDV strains and can help in reducing the number of outbreaks. © 2022 Friends Science Publishers

Keywords: Newcastle disease virus; *In silico*; Fusion glycoprotein; Epitopes; Multiepitope vaccine; Docking

Introduction

The agricultural sector plays a pivotal role in Pakistan's economy with a major contribution of almost 21% to the Gross domestic production (GDP) (Abdisa and Tagesu 2017). Its subsector includes crops, livestock, fishery and forestry, where only the first two are considered crucial. Livestock is the most productive subsector of agriculture with a fair share of 11.9% of GDP and is a source of income for thousands of rural families (Shahid *et al.* 2020). Of all livestock products, poultry meat is considered the most important as it offers low-cost animal protein. The poultry industry in Pakistan has been playing an important role in narrowing down the gap between the supply and demand of protein. However, the main risk to this industry is low biosecurity measures and persistent occurrence of infectious diseases which ultimately lead to low production (Absalón *et al.* 2019).

Among all poultry diseases, Newcastle disease (ND) exhibits devastating economic effects on commercial as well as domestic produce (Mayers *et al.* 2017). Not only its outbreaks are expensive but the preventive measures taken against the disease are also very costly. The disease was reported as early as 1926 when two main outbreaks occurred at two different poles of the world, *i.e.*, New-Castle upon Tyne, England and the island of Java, Indonesia (Alexander 2009). Pakistan witnessed a devastating outbreak in 2012, which killed almost 45 million chickens in Punjab alone (Rehan *et al.* 2019).

ND, also known as a pseudo-fowl pest, is a zoonotic disease that occurs after infection with lethal virus *avian paramyxovirus I* or also known as NDV (Alexander 2000). The virus contains a single-stranded and negative sense ribonucleic acid which is almost 15,200 nucleotides in length. Its genome encodes for six important proteins *i.e.*, phosphoprotein (P), nucleoprotein (NP), hemagglutinin-neuraminidase (HN), matrix (M), fusion (F) and RNA polymerase (L). HN and F proteins are located outside the viral envelop and are mainly responsible for host invasion and infectivity (Al-Garib *et al.* 2003).

Different strains of virus display varying clinical pictures and based on mean death time these are divided into three pathotypes, *i.e.*, lentogenic, mesogenic, and

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velogenic (Songhua *et al.* 2003). This difference in the pathogenicity index is credited to a peptide motif located in the F protein. Cleavage of this motif is carried out by host proteases which in turn convert inactive precursor F0 (65 kD) to active F1 (55 kD) and F2 (10 kD) proteins. This step is crucial for the initiation of infection and is linked to virulence as the cleavage site is different among all NDV strains (Panda *et al.* 2004).

Different vaccines have been used so far to protect chickens and other exotic birds against ND, however, none of the live or killed vaccines has been found effective in reducing the virus transmission rate. To cope with this presenting problem, scientists have been working on developing recombinant vaccines that can generate a better immune response with limited safety concerns (Jorge and Dellagostin 2017). The advent of knowledge of antigen recognition and the role of host major histocompatibility complex (MHC) I and II receptors in immunity generation has resulted in the development of specific motif or epitope based vaccines (Fleri *et al.* 2017).

To develop an effective subunit vaccine, accurate antigenic determinants are selected and an adjuvant is added to enhance its immune response in the host. The concept of modern vaccines has enabled scientists to produce recombinant vaccines against various infectious viruses such as fowlpox (Swayne *et al.* 2000), vaccinia virus (Sebastian and Gilbert 2016), turkey herpesvirus (Liu *et al.* 2019) and pigeon pox virus (Skinner *et al.* 2005). *In silico* analysis can help in the development of safe, suitable and effective vaccines (Soria-Guerra *et al.* 2015). Given the current prevailing outbreaks of ND in Pakistan, it is a basic necessity to develop a recombinant vaccine against NDV to prevent any further loss to poultry birds. This study aims to investigate NDV Fusion protein using the computerized approach to identify all possible immunogenic epitopes which can be used as a possible vaccine target.

Materials and Methods

Sequence retrieval and analyses

To evaluate the practicability of our approach for a subunit vaccine against NDV, a sequence of target antigen F0 encompassing 1665 nucleotides was obtained in FASTA format from the NCBI database (https://www.ncbi.nlm.nih.gov/). Few mutations were induced at the cleavage site to convert the protein into a less virulent form.

Bioinformatic analysis of fusion protein

F gene was translated using the JustBio tool and its physicochemical properties were determined through Expasy's Protparam (https://web.expasy.org/protparam/). Secondary structure was analyzed using Chou and Fasman tool was used (http://www.biogem.org/tool/chou-fasman/).

The antigenicity of synthetic F0 protein was evaluated via the VAXIJEN-v.20 online server which predicts immunogenicity based on its physicochemical properties.

Epitope prediction and evaluation

B-cell epitope prediction: B cell epitopes were predicted using ABCPred and then further evaluated using the Bipepred linear epitope prediction tool of Immune epitope database analysis (IEDB). Surface accessible epitopes were obtained through the Emini surface accessibility prediction tool, whereas protein antigenic sites were determined using Kolaskar and Tongaonkar antigenicity method. Ellipro from IEDB is used for the identification of continuous B-cell epitopes with a threshold value set at 0.5 (http://tools.iedb.org/ellipro/).

T-cell epitope prediction: Cytotoxic T-cell (CTL) epitopes that are presented by MHC-I alleles were obtained by NetMHC 4.0 server (http://www.cbs.dtu.dk/services/NetMHC/) and Helper T-cell (HTL) epitopes were retrieved through NetMHCII 3.2 server (http://www.cbs.dtu.dk/services/NetMHCII/).

IFN-γ epitope prediction: To find out regions in F0 protein that tend to induce IFN-γ, a web-based server IFNepitope was used (http://crdd.osdd.net/raghava/ifnepitope/).

Evaluation of Epitopes

Antigenicity of B cell and T cell epitopes was evaluated using Vaxijen v. 2.0 where a threshold value of 0.5 was used and only highly immunogenic epitopes were selected. Epitope toxicity was found using ToxinPred (http://crdd.osdd.net/raghava/toxinpred/), this online software predicts toxicity based on physicochemical properties. Allergenicity was determined using AllerTOP and Topology was predicted through TMHMM. To identify the degree of the conservancy of epitopes within a set of protein sequences, the IEDB conservancy analysis tool was used (http://tools.iedb.org/conservancy/).

Homology modeling

For the construction of the tertiary structure of ND F0 protein, RaptorX (http://raptorx.uchicago.edu/ContactMap/) was used which is a distance-based protein structure prediction server. The protein sequence of HLA alleles and chicken alleles (BF2 21:01 & BF2 04:01) were obtained from NCBI (accession no. NP_001026509.1 and CAK54660.1) and submitted to PHYRE2 for homology modeling (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index). Whereas, 3D structures of MHC epitopes were obtained through PEP-FOLD at RPBS MOBYLE portal for docking analysis.

Molecular docking

The molecular interaction of proposed MHC peptides was

analyzed with HLA alleles and chicken BF alleles, respectively. Docking was conducted based on peptide-binding groove affinity using Molecular Operating Environment (MOE) software which is an integrated computer-aided molecular design platform.

Construction of MEV

For the construction of a highly immunogenic and stable MEV, an adjuvant was linked with the first CTL epitope through an EAAAK linker. The remaining epitopes were linked together using AAY and GPGPG spacers to preserve their activity.

Evaluation of vaccine construct

Physicochemical properties of MEV were obtained using Protparam and its sequence was evaluated for its antigenicity and allergenicity using online software. The secondary structure of the chimeric vaccine was predicted using Psipred (http://bioinf.cs.ucl.ac.uk/psipred/) and the 3D tertiary structure was retrieved using Robetta (https://robetta.bakerlab.org/). The stereochemical properties of the predicted 3D structure were done using Procheck, ERRAT and Verify3D (https://saves.mbi.ucla.edu/). To refine MEV 3D structure Galaxy Refine (http://galaxy.seoklab.org/cgi-bin/submit_REFINE.cgi) was used.

Docking of vaccine construct with chicken immune receptors

Molecular docking was conducted to analyze the binding affinity of our final vaccine construct with a chicken toll-like receptor. For this purpose, an automated protein docking server ClusPro 2.0 was used, and results were visualized using Chimera.

Results

Target protein sequence and structural analysis

The nucleotide sequence of NDV F0 gene was retrieved from Genbank and sequence of its cleavage motif was altered to revert velogenic strain into a less virulent form *i.e.*, lentogenic. Cleavage motif is located at 336 to 354 bp where sequence for velogenic strain (AGGAGACAGAAACGCTTT) was converted into a less virulent lentogenic strain (GGAAGACAAGGTAGACTC).

Physicochemical properties were obtained through Protparam, an Expasy-based online tool. F0 protein is found to be alkaline and the isoelectric point is 8.46. The molecular weight of synthetic F0 protein is estimated to be 58864 D. The instability index for fusion protein is computed as 36.99 which indicates its stability in *E. coli* cells.

Several online software were used to predict the secondary structure and the generated files were compared for the identification of protein structure. In the predicted secondary structure, helices lie in the peptide region of 75 to 108, 121 to 170 and 511 to 540, respectively.

The antigenicity of the F0 protein was evaluated using the VAXIJEN_v2.0 tool at a threshold level of 0.5. Overall prediction of antigenic protein was found to be 0.5306 which declared F0 as a potent immunogen.

A protein can only function properly in its final folded form and any change in its shape will alter its function. Protein tertiary structure was determined using RaptorX which is a template-based tertiary structure modeller and provides high-quality structure models. The predicted model for F0 has an RMSD value of 2.564 and an overall GDT value of 66, where a model having a GDT value of above 50 is considered a good and stable protein. Ramachandran plot analyses of the model show that 94% of amino acids fall under the favorable region.

***B*-cell epitope prediction**

These epitopes are a portion of an antigenic protein that interacts and activates B lymphocytes and is responsible for triggering an immune response against virus-infected cells. Fusion glycoprotein linear B-cell epitopes were retrieved using ABCPred which were further analyzed using Bipepred, Kolaskar and Tongaonkar antigenicity, and Emini surface accessibility of the IEDB online tool.

From all IEDB predicted epitopes, fifteen were selected after screening according to their allergenicity, antigenicity, topology, and conservancy. All B cell epitopes were found to be highly conserved after testing them against aligned F0 sequences. However, epitopes that satisfy and overlap all prediction tools were $_{8}$KIPAPMMLTIRVALVL$_{23}$, $_{29}$ANSIDGRPLAAAGIVV$_{44}$, $_{221}$FGPQITSPALNKLTIQ$_{236}$, $_{243}$GGNMDYLLTKLGIGNN$_{258}$, $_{289}$LPSVGNLNNMRATYLE$_{304,}$ and $_{327}$GSVIEELDTSYCIETD$_{342}$. Selected epitopes are presented in Table 1 and graphical presentations of peptide regions that are likely to be recognized as epitopes by a B cell response are represented in Fig. 1.

Ellipro was used to find out discontinuous B cell epitopes that allows the prediction of epitopes based on a protein 3D structure. A total of 136 residues from 93 to 181 and 390 to 438 with a score of 0.82 and 0.76 respectively, were declared conformational B cell epitopes.

T-cells epitope analysis

CTL epitopes were retrieved through NetMHC 4.0 which were generated to show interaction with different HLA alleles. A higher interaction score between epitope and HLA indicates higher chances of the epitope being presented to HTL. In this study human MHC-I alleles were selected instead of chicken B-F alleles because of their unavailability

Table 1: Selected B cell epitopes based on their immunogenicity, allergenicity, topology and conservancy

Peptide sequence	ABCPred Score	Antigenicity score	Allergenicity	Toxicity	Topology	Conservancy	Minimum Identity %
GSVIEELDTSYCIETD	0.85	0.5014	Non-allergen	Non-Toxic	Outside	97%	93.75
LPSVGNLNNMRATYLE	0.77	0.8710	Non-allergen	Non-Toxic	Outside	98%	93.75
ANSIDGRPLAAAGIVV	0.76	0.6780	Non-allergen	Non-Toxic	Outside	96%	93.75
FGPQITSPALNKLTIQ	0.72	0.5006	Non-allergen	Non-Toxic	Outside	97%	93.75
GGNMDYLLTKLGIGNN	0.65	0.8280	Non-allergen	Non-Toxic	Outside	96%	87.5
KIPAPMMLTIRVALVL	0.64	0.6073	Non-allergen	Non-Toxic	Outside	92%	81.25

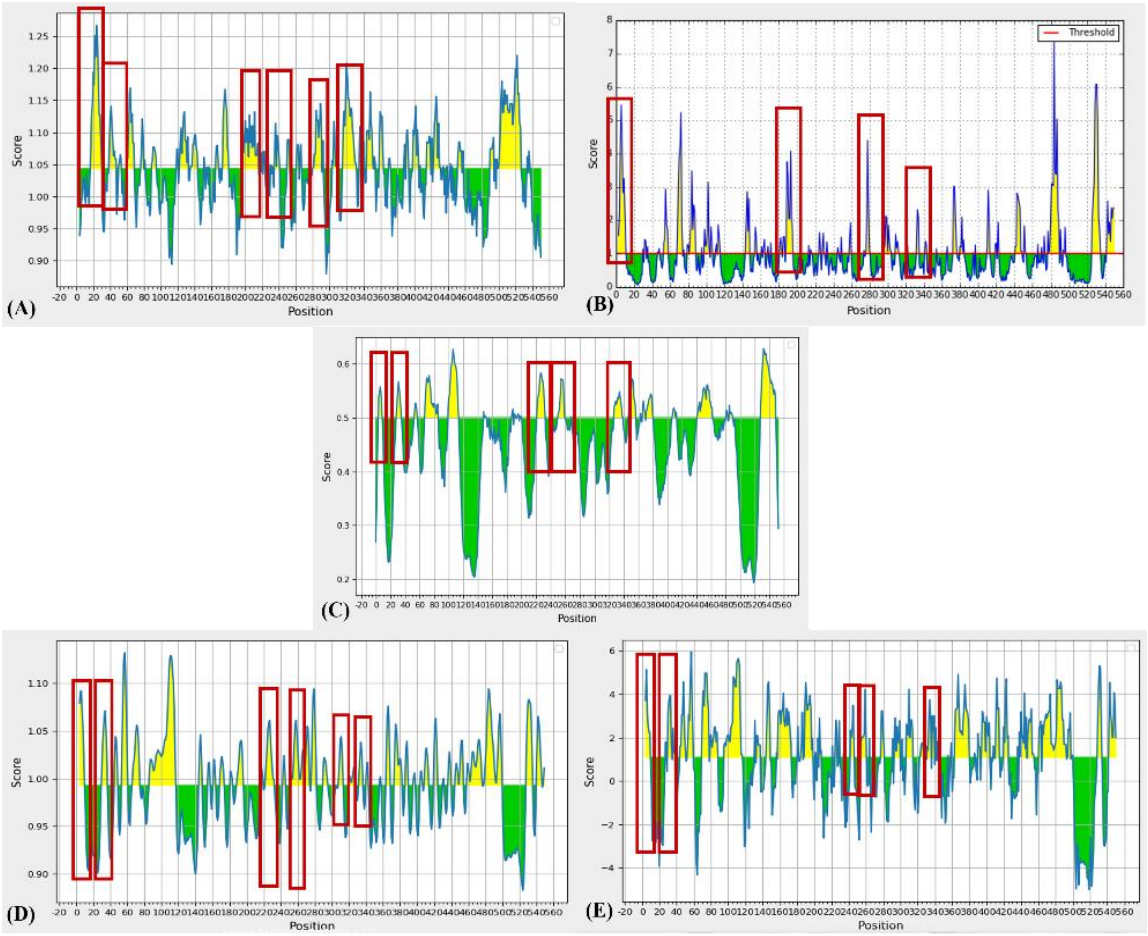

Fig. 1: Prediction of B cell epitopes using (**A**) Kolaskar and Tongaonkar antigenicity prediction (**B**) Emini surface accessibility prediction (**C**) Bipepred linear epitope prediction (**D**) Karplus and Schulz flexibility prediction (E) Parker hydrophilicity prediction. The yellow area above the threshold level is supposed to be potent epitopes where peaks highlighted in a maroon box are the final B cell epitopes selected

in epitope prediction software; however, they both show biochemical and functional similarity.

A total of 31 MHC-I epitopes were selected that showed the ability to interact with multiple alleles and have strong defense capabilities. However, only five MHC I epitopes were found to be antigenic, conserved and located on the protein's outer surface.

CTL epitope showing high affinity with their respective alleles are; $_{42}$VALVLSCICPANSI$_{55}$ interact with five alleles HLA-E*01:01 and HLA-B (51:01, 48:01, 07:02) alleles, $_{231}$VELNLYLTELTTVF$_{244}$ interact with seven alleles HLA-B (35:03, 18:01, 46:01, 44:03) and HLA-A (11:01, 30:02, 68:02), $_{258}$IQALYNLAGGNMDY$_{271}$ interact with six alleles HLA-A (29:02, 30:02, 11:01, 01:01) and HLA-B (15:02, 07:02), $_{312}$LPSVGNLNNMRATY$_{325}$ show interaction with four alleles HLA-A*30:02 and HLA-B (15:01, 53:01, 35:01) and $_{524}$LITYIVLTIISLVF$_{537}$ interact with five alleles HLA-A (23:01, 24:02, 02:01, 68:02) and HLA-B*46:01.

On the other hand, HTL is involved in provoking adaptive immune response as they help in the activation of B cells and CTL to kill infected cells. As avian BL alleles are difficult to determine, we selected human MHC class II alleles, HLA and DR, however, only two epitopes $_{201}$ALITYIVLTIISLVF$_{225}$ and $_{210}$ELNLYLTELTTVFGP$_{224}$ were selected after scrutiny.

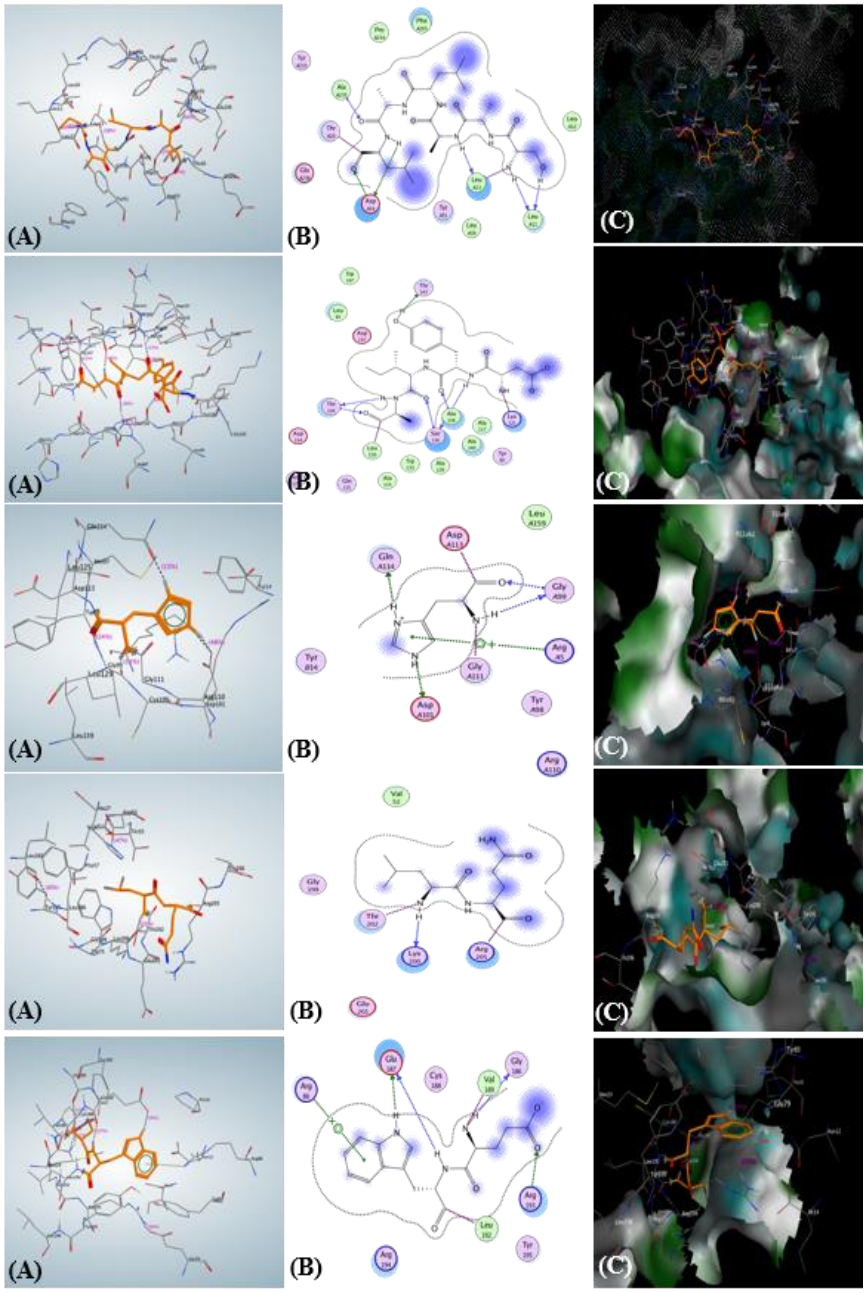

Fig. 2: Docking interaction. (**A**) Interaction of CTL epitopes with their respective HLA alleles. (**B**) Interacting residues. (**C**) Binding of peptide and allele within the pocket

IFN-γ epitope analysis

IFN-γ plays an active role in provoking both innate and adaptive immune responses. Nineteen epitopes were predicted using IFNepitope, out of which, only three $_{29}$GVALGVATAAQITAA$_{44}$, $_{194}$LPSVGNLNNMRATYL$_{209}$, and $_{398}$VKLTSTSALITYIVL$_{413}$ showed positive results.

Interaction analysis of epitopes with respective alleles

Interaction between selected epitopes and their respective alleles was evaluated by performing molecular docking using Molecular environment operating (MOE) software. This software is designed to support molecular modeling and structure-based designs. Interactions are classified according to their S-score and RMSD value and the energy of HLA alleles was minimized before docking. London DG tool was used for scoring and refinement was done using the force field. Out of 10 predicted conformations, only those were selected that show high scores and RMSD values between 2 to 3.

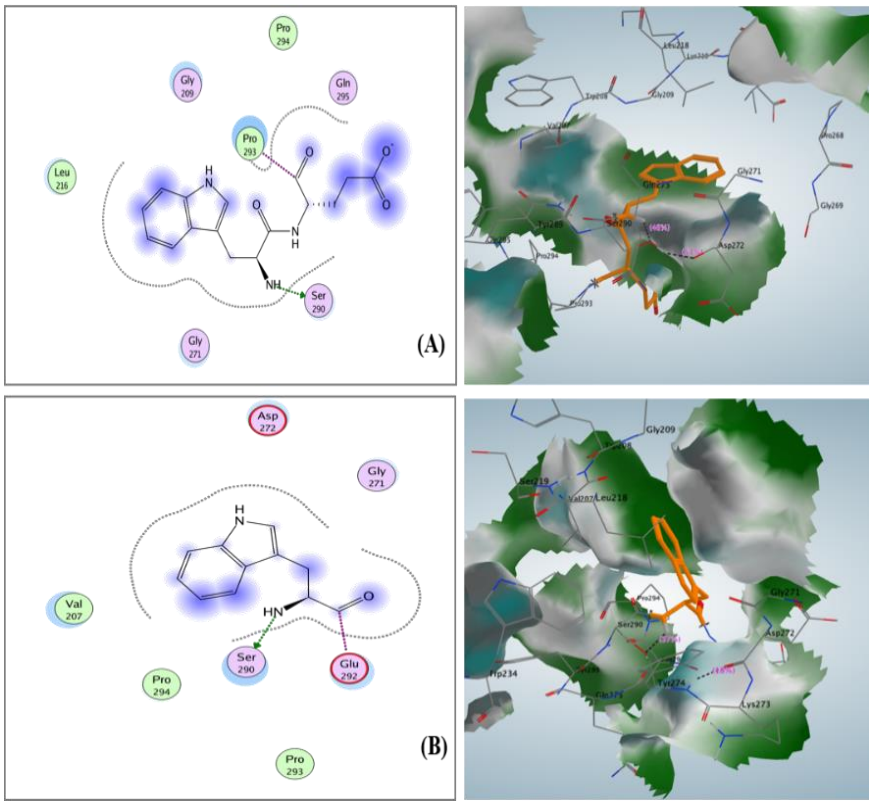

Fig. 3: Molecular docking of proposed CTL epitope and chicken MHC class I alleles. (**A**) Interaction of IQALYNLAGGNMDY with BF2 21:01. (**B**) Interaction of IQALYNLAGGNMDY with BF2 04:01

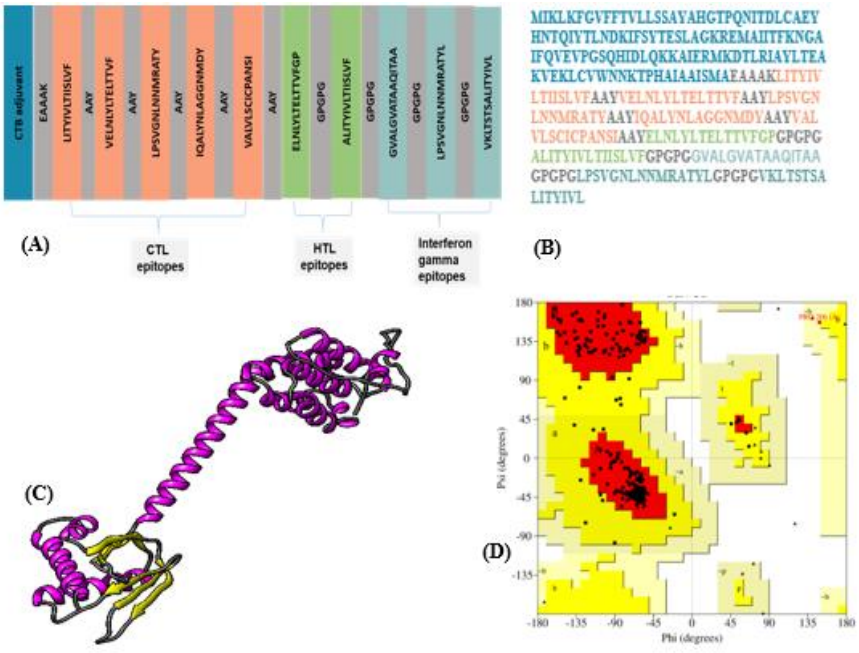

Fig. 4: Designing and structure validation of MEV construct. (**A**) An adjuvant linked final construct of MEV where CTL, HTL, and IFN-γ epitopes along with their linkers are shown. (**B**) A 308 amino acid long sequence of MEV which consists of CTB adjuvant (blue) and epitopes are linked together using AAY and GPGPG linkers (grey). (**C**) 3D structure of MEV vaccine. (**D**) Ramachandran plot analysis

Table 2: Selected MHC class I, II and Interferon-gamma epitopes based on their interaction with MHC-I alleles, antigenicity, allergenicity, topology and conservancy

Epitope sequence	MHC I alleles	Antigenicity score	Toxicity	Allergenicity	Topology	Conservancy	Minimum identity
			MHC class I				
LITYIVLTIISLVF	HLA-A*23:01, HLA-A*24:02, HLA-B*46:01, HLA-A*02:01, HLA-A*68:02	0.7306	Non-toxic	Non-Allergen	Outside	93.00% (93/100)	92.86
VELNLYLTELTTVF	HLA-B*35:03, HLA-B*18:01, HLA-A*11:01, HLA-A*30:02, HLA-A*68:02, HLA-B*46:01, HLA-B*44:03	0.6869	Non-toxic	Non-Allergen	Outside	98.00% (98/100)	92.86
LPSVGNLNNMRATY	HLA-B*35:01, HLA-A*30:02, HLA-B*15:01, HLA-B*53:01	0.8204	Non-toxic	Non-Allergen	Outside	98.00% (98/100)	92.86
IQALYNLAGGNMDY	HLA-A*29:02, HLA-B*15:02, HLA-A*30:02, HLA-B*07:02, HLA-A*11:01, HLA-A*01:01	0.7712	Non-toxic	Non-Allergen	Outside	96.00% (96/100)	92.86
VALVLSCICPANSI	HLA-E*01:01, HLA-B*51:01, HLA-B*48:01, HLA-B*07:02, HLA-B*07:02	0.8500	Non-toxic	Non-Allergen	Outside	94.00% (94/100)	92.86
			MHC class II				
ELNLYLTELTTVFGP	HLA-DRB1*04:21, HLA-DRB1*08:01	0.574	Non-Toxic	Non-Allergen	Outside	98.00% (98/100)	93.33
ALITYIVLTIISLVF	HLA-DRB1*15:06, HLA-DRB1*08:13	0.75	Non-Toxic	Non-Allergen	Outside	93.00% (93/100)	93.33
			Interferon-gamma				
GVALGVATAAQITAA		0.899	Non-Toxic	Non-Allergen	Outside	97.00% (97/100)	86.67%
LPSVGNLNNMRATYL		0.8743	Non-Toxic	Non-Allergen	Outside	98.00% (97/100)	93.33%
VKLTSTSALITYIVL		0.737	Non-Toxic	Non-Allergen	Outside	95.00% (97/100)	93.33%

Table 3: Docking results retrieved from MOE by using three-dimensional structures of CTL epitopes against human and avian alleles

Epitope	MHC I Allele	Docking score	RMSD value	Interacting Residues
LITYIVLTIISLVF	HLA-B*46:01	-19.36	2.5	Leu11, Leu13, Thr20, Asp54, Ala235
VELNLYLTELTTVF	HLA-B*18:01	-15.32	2.2	Lys 121, Ala 136, Ser 116, Leu 126, Thr 134, Thr 143
LPSVGNLNNMRATY	HLA-B*35:01	-28.45	2.68	Arg 5, Gly 99, Asp 101, Gly 111, Asp 113, Gln 114
IQALYNLAGGNMDY	HLA-A*29:02	-33.85	2.5	Lys 200, Thr 202, Arg 205
VALVLSCICPANSI	HLA-B*48:01	-23.36	2.4	Arg 86, Gly 186, Glu 187, Val 189, Leu 192, Arg 193

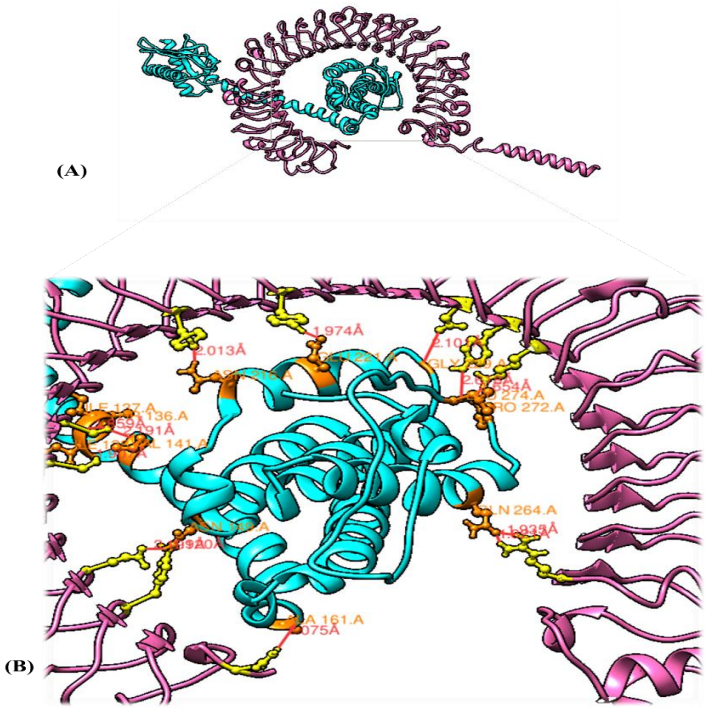

Fig. 5: Molecular docking. (**A**) Docking complex of MEV in cyan and TLR4 in brown. (**B**) 3X magnification of docked complex, where interacting residues of MEV are highlighted in purple and chicken immune receptors in red

Docking scores and interacting residues are listed in Table 3. Epitope IQALYNLAGGNMDY showed the highest binding score of -33.85 and made strong H bonds with the HLA allele using Lys_{200}, Thr_{202} and Arg_{205} residues. The interaction of all selected CTL epitopes is shown in Fig. 2. All epitopes showed efficient binding with their respective HLA alleles indicating that predicted peptides are capable to bind and generate immunity in the host body.

CTL epitope IQALYNLAGGNMDY was then tested for its ability to bind with chicken BF alleles. Docking results based on RMSD value and binding energy scores showed that CTL epitope achieved stronger binding affinity with BF2 04:01 with two hydrogen bonds at residues Ser_{290} and $Glu_{292.}$ On the other hand, interaction with BF2 21:01 showed only one hydrogen bond at Ser_{290}. Interacting residues and binding of epitope at BF2 allele binding site are shown in Fig. 3.

Construction of MEV

The selected MHC-I, II, and IFN-γ epitopes were used to construct an MEV. To increase vaccine immunogenicity, a 123 amino acid long cholera toxin B adjuvant was linked at the N-terminal of MEV using an EAAAK linker. Epitopes were then merged sequentially using AAY and GPGPG spacers, respectively. These linkers help in increasing stability and avoiding junctional epitopes. The final vaccine construct was 308 amino acids long. The final vaccine construct is shown in Fig. 4.

Evaluation of MEV

Physicochemical properties evaluated using Protparam indicate that the molecular weight of MEV protein is 32.8 kD which is slightly alkaline with an isoelectric point of 7.62. GRAVY index of 0.488 shows that it is hydrophobic, whereas, the instability index of 23.82 indicates protein as stable. Antigenicity evaluated through Vaxijen shows the value of 0.6143 (threshold: 0.5) which confirms the immunogenic nature of the vaccine, and AllerTOP confirms that it is non-allergen in nature.

Secondary structure predicted through Psipred shows that MEV comprises 45% α-helices, 17% *β*-strands, and 38% coils. The 3D tertiary structure was generated using Robetta which shows a confidence level of 0.63 over the predicted structure, where a confidence level of 0.5–1.0 is considered good. The tertiary structure was further evaluated using ERRAT and the overall quality factor was achieved as 96.92, as a value of 80–100% shows that constructed model is reliable.

Ramachandran plot built using PROCHECK indicates that 93% of residues fall under the most favorable region and 6.7% under the allowed region. The 3D structure was then refined using Galaxy Refine and the quality analysis of the improved model evaluated using ERRAT, reached up to 99.29% and almost 96% of residues fall under the favorable region.

Molecular docking of MEV with chicken TLR4

For an efficient immune response in the host body, a strong association between antigen and immune receptors is required. Chicken TLR4 is well studied and broadly expressed in all body tissues. To check its proper engagement with MEV, molecular docking was conducted using ClusPro, which presented the 10 best models for interaction. These models were then investigated using Chimera and only that model was selected which showed efficient binding, least binding score and maximum clustering members. The docked complex had binding energy of -1286.3 and 48 clustering members and a total of 9 hydrogen bonds were found between MEV and TLR4 complex. Interacting residues of MEV and TLR4 complex are shown in Fig. 5.

Discussion

Vaccination is considered the most effective method for the prevention of an infectious disease that acts by presenting a foreign antigen to the host immune system. Many attenuated and inactive forms of NDV have been in use for eliciting antigen-antibody response but repetitive outbreaks of ND are raising questions regarding their potential for protection against infection and reduction of viral transmission (Milić *et al.* 2017). Pakistan has been exporting Mukteshwar R2B, a mesogenic vaccine, which is reported to have adverse reactions and is even lethal for immune-compromised chicks (Shahid 2017). Desired immunity against NDV is achieved when the vaccine protects against variant viral strains with minimum side effects. The recent knowledge on immune receptors and their interaction with different viral antigens has led to the concept of epitope based vaccines. These vaccines are the safest option as they are highly antigenic but least virulent in nature. Raza *et al.* (2022) and Mozafari *et al.* (2022) have previously designed multiepitope vaccine against HN protein of NDV. However, there was a need to target fusion glycoprotein as it is highly antigenic in nature, exhibits 16 conserved immune epitopes on its outer surface, and is a major determinant of viral virulence.

So this study aimed to design an MEV for NDV in particular for F0 protein with the help of computational methods. The purpose was to identify new highly immunogenic T and B cell epitopes that are supposed to provoke a long-lasting immunity against infection. Another study by (Arora *et al.* 2010) demonstrates the comparison between the live vaccine and viral protein fractions (F alone and HN-F), where the author stated that immunity generated by F0 protein is similar to a whole vaccine or both proteins together. F0 was found highly antigenic, also it is a membrane-bound protein and thus it is involved in signaling and acts as a receptor.

Several immunoinformatic tools were used for the prediction of B and T cell peptides at primary, secondary, and tertiary structural levels of protein. To determine an effective antigenic peptide against B cells, the epitope should show a score above the threshold level when analyzed using Emini surface, Bipepred linear and Tongaonkar prediction methods (Zhang *et al.* 2008). IEDB server shows that a large portion of the F0 sequence is

immunogenic and acts as an epitopic region that can be identified by B cells for the development of antibodies in the host body. Ellipro confirms that selected epitopic regions are located at the outer surface of the protein.

Vaccines are mostly designed to elicit B cell immunity but it is now studied that immunity generated through T cells is stronger and long-lasting (Broere and Eden 2019). This modern strategy has been successfully used to design a vaccine against malaria and cancer (Oyarzún and Kobe 2016). MHC class I and II epitopes were finalized based on their conservancy, antigenicity, topology, and their ability to interact with multiple alleles. Unfortunately, no software can predict the binding interaction of a protein with chicken class I and II MHC alleles (Milona *et al*. 2007), however, some studies show similarities between chicken and human MHC alleles. Table 2 presents epitopes that show interaction with different class MHC-I and II alleles. These docked epitopes were then interacted with BF2 21:01 and BF2 04:01 alleles to confirm the presence of real CTL epitopes. These alleles were selected based on a study by (Koch *et al.* 2007) which suggests that these alleles have novel peptide binding affinity and accommodate a variety of peptides presented as epitopes to CTL.

Lately, MEV constructs are displaying promising results for the control of different viral infections. It requires accurate identification of epitopes and efficiently combining those using linkers and adjuvant without disturbing their actual structure and function. MEV is usually poorly antigenic and requires the addition of an adjuvant which can overall improve the quality and quantity of immune response (Khan *et al.* 2019).

Various bacterial endotoxins are known to have adjuvant properties but CTB can efficiently bind mucosal epithelial cells and elicit long-term immunological memory. Designing an MEV without linkers can result in the formation of either an entirely new protein with different features or a functionless abnormal peptide. Thus, vaccine design can be improved by adding tandem repeats such as EAAAK, KK, AAY and GPGPG (Meza *et al.* 2017). MEV designed in our study was found to be highly immunogenic, non-toxic to cells and non-allergenic to the host body, thus, portraying its potential to elicit a strong immune response.

While designing a subunit vaccine, molecular docking is very important to check whether the designed antigen is binding to specific host immune receptors or not. Docking analysis was conducted to figure out the immune response of TLR4 against vaccine construct. Results of this study suggest that MEV can be a potential candidate that can be further analyzed both *in vitro* and *in vivo* to develop an effective vaccine against the Newcastle disease virus.

Conclusion

Recent studies show that highly antigenic epitopes of some proteins can act as vaccine targets as they can successfully elicit an immune response and protect the host organism from pathogen attack. Thus, in this current study, *in silico* approach was used to develop a vaccine that is based on stable epitopes which are highly antigenic and conserved and show the capability to interact and make bonds with host immune receptors. A combination of analyses was used for the construction of an immunogenic MEV, however, it requires experimental validation to ensure the efficacy of the vaccine. We hope that the presented MEV construct will help design a safe and long-lasting vaccine against NDV.

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Author Contributions

The project was conceived and supervised by MSK, however, SS being student carried out the work and prepared the manuscript. The manuscript was critically reviewed by MSK, FAJ and MAZ.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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