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Abstract

There are no specific drugs or vaccines for Nipah virus (NiV), which is a new *Paramyxovirus* that infects swine and humans. This study was conducted to investigate B-cell epitope mapping of the NiV attachment glycoprotein and to construct peptide-based vaccine candidates using the reverse vaccinology approach. To generate the linear B-cell epitope, the NiV isolates were extractad from GenBank, NCBI, using the IEDB web server; peptide modeling was conducted using PEP-FOLD3; docking was conducted using PatchDock and FireDock; and *in silico* cloning was designed using SnapGene. Various peptides were successfully identified from the NiV attachment glycoprotein based on B-cell epitope prediction, allergenicity prediction, similarity prediction, and toxicity prediction. An *in silico* cloning design of the pET plasmic was also developed. The peptide "RFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLD" (1G peptide) is predicted to be a potential candidate for the NiV vaccine as it has several good vaccine characteristics. It increases the immune response of B cells through activation, differentiation into plasma cells, the formation of memory cells, and it may increase IgM/IgG antibody titres for viral neutralization. However, the results of this study should be further verified through *in vivo* and *in vitro* analyses.

Keywords: attachment protein, B-cell epitope, nipah virus, peptide-based vaccine, reverse vaccinology approach

Introduction

NiV occurs in Southeast Asia and is a highly pathogenic zoonotic paramyxovirus [1] with a high mortality rate [2]. The first outbreak in humans occurred in Malaysia in approximately 1998 to 1999, where 250 people were infected and 100 died. Subsequent outbreaks have occurred in Bangladesh (2001–2015), India (2001, 2007, 2018, and 2019), and other countries [2–4].

A bat-like animal belonging to the genus *Pteropus* is known to be a natural reservoir for NiV [5], but swine (*Sus scrofa*) is also strongly suspected to be an intermediate host prior to human infection [6, 7]. Infected swine in Malaysia were found to be asymptomatic, and close contact with swine was determined as the main NiV

transmission route [7, 8]. The practice of consuming fresh date palm sap in various Asian countries is zoonosis transmission mode from bats to humans [9, 10]. Furthermore, person-to-person transmission of NiV is known to occur via bodily fluids or through close contact between an infected person and household members [11, 12].

NiV is a negative-stranded RNA virus with a genome size of 18 kb (NCBI accession number NC_002728.1); taxonomically, it belongs to the genus *Henipavirus* from the family *Paramyxoviridae* [1, 9]. This virus is closely related to other viruses such as *Mojiang virus*, *Cedar virus*, and *Hendra virus*. NiV has an envelope and its genome encodes six structural proteins (3'-N-P-M-F-G-L-5') [9]. Genome analyzes have reported that NiV has two genotypes: NiV-Bangladesh (NiV-BD), which is recognized in India and Bangladesh, and NiV-Malaysia (NiV-MY), which is recognized in Cambodia and Malaysia [2].

The NiV attachment glycoprotein (G protein) is known to be one of the virus' receptors, and it can be attached to Ephrin-B2 and Ephrin-B3 in humans or other mammals [13–16]. The G protein also has the capability of inducing an immune response in a host cell, and it has thus been used in constructing a vaccine against NiV [13, 17–19]. The NiV genome has been characterized and sequenced based on the disease in bat species (*Pteropus vapmyrus*, *Pteropus medius*, *Pteropus lylei*, and *hypomelanus*) across Asia and also that originating in human patients [20, 21]. It is anticipated that the data obtained will enable the discovery therapeutical treatments and the identification of virus mutations, and it will also assist in diagnostic accuracy and the development of a vaccine against NiV [22–24]. Many research institutes globally are endeavoring to develop an NiV vaccine [19].

B cells can directly recognize epitopes on antigens without the help of T cells; maturation then occurs and antibodies are produced that can bind to pathogens for the neutralization process. An advantage of B-cell-based vaccines is that IgM antibodies can be produced and undergo isotype switching to neutralize the virus [47–48]. Preliminary NiV vaccine candidate studies for humans can therefore be designed based on the B-cell pathway. This study was conducted with the aim of investigating B-cell epitope mapping of the NiV attachment glycoprotein, and the reverse vaccinology approach was used to construct a peptide-based vaccine candidate.

Methods

Isolate retrieval. The NiV attachment glycoprotein (G) sequence with the accession number NC_002728.1 was obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). The sample was downloaded in FASTA format. The NiV genome sequence consists of a complete genome (18,246 bp); however, this study used only the G protein as the target vaccine design candidate.

Protein modeling and validation. The 3D structure of the NiV G protein in this study was obtained from the results of homology modeling conducted on the SWISS-MODEL server (https://swissmodel.expasy.org/). This method works by aligning query sequences and templates to determine the 3D structure of the target protein [22, 25]. The 3D model was validated using the Ramachandran plot with a score threshold of 90% [26–28].

B-Cell epitope mapping, antigenicity, similarity, allergenicity, and toxicity prediction. A probability prediction of the B-cell epitope on the henipavirus G protein was conducted using the immunoinformatics web server (https://services.healthtech.dtu.dk/) with the BepiPred 2 tool (https://services.healthtech.dtu.dk/service.php?BepiPred-2.0). This prediction has a threshold score of 0.5 for determining the epitope region of the target protein, and candidate peptides can be identified based on the specific epitope position [29]. The VaxiJen v2.0 web server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) was used to identify the antigenicity of peptides in triggering an immune response; to be classified as an antigen, the predicted results required a score greater than the 0.4 threshold [30]. A similarity test with the BLASTp web server (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) was conducted to identify the similarity between peptide constituent sequences and receptors on the *Homo sapiens* cell, with the aim of avoiding autoimmunity following vaccination [31]. Allergenicity was conducted via the AllerTOP v2.0 web server (https://www.ddg-pharmfac.net/AllerTOP/); the prediction aims to determine the potential level of allergens in the peptides used as vaccine candidates and to ensure that the peptides are non-allergens [32]. The ToxinPred web server (https://www.crdd.osdd.net/raghava/toxinpred/) was employed to identify the toxicity level of the vaccine candidate peptide, where a threshold score of 0.1 is given for non-toxins [33].

Physicochemical prediction. The predicted physico-chemical properties of candidate peptides were screened using the ProtParam web server (https://www.web.expasy.org/cgi-bin/protparam/protparam) to identify the grand average of the hydrophobicity (GRAVY) score, Aliphatic index, instability, molecular weight, and theoretical pI [34].

Peptide 3D construction. The peptide sequence of the NiV vaccine candidate is classified as having a primary protein structure. In this study, the PEP-FOLD 3.5 server (https://www.bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) was used to construct secondary protein

3D structures from peptides through the *de novo* method based on predicting the amino acid conformation [22, 35].

Peptide-protein docking. Molecular docking simulations were used to determine the pattern of the binding interactions between proteins and ligands [35-37]. This study used the ID 5IFH B-cell receptor (BCR), which was downloaded from RCSB PDB (https://www.rcsb.org/). Molecular docking in this study was conducted using the PatchDock web server (https://www.bioinfo3d.cs.tau.ac.il/PatchDock) and refinement was performed via the FireDock web server (https://www.bioinfo3d.cs.tau.ac.il/FireDock) to obtain a global score for energy, attractive, repulsive Van der Waals, atomic contact energy (ACE), and hydrogen bonds based on rank [38, 39].

Molecular dynamic and 3D visualization. Molecular dynamics (MDs) are used in research to determine the fluctuation in the stability and flexibility of complex docking results, and these were employed here via the CABS-flex 2.0 server (https://www.biocomp.chem.uw.edu.pl/CABSflex2/index) (Kuriata *et al*., 2018; Kharisma *et al*., 2022). Visualization of 3D structures and staining selection were conducted through PyMol software v.2.5.2 via an academic license (Schrödinger, Inc., USA) [28, 40, 41].

***In silico* cloning.** The pET30a(+) expression vector was selected for cloning, and its nucleotide sequences were collected from the Addgene vector database (https://www.addgene.org/vector-database/) [42]. SnapGene software v.6.1 (GSL Biotech LLC, USA) was then used to pursue the *in silico* cloning of the peptide-based vaccine component against NiV [43].

Immune simulation. The C-ImmSim web server (https://www.kraken.iac.rm.cnr.it/C-IMMSIM/) was employed to simulate the B-cell humoral response generated when following vaccine administration. Several simulation parameters were used as default settings. A step simulation setting of 1000, adjuvant 100, volume 10, and random seed 1234 was then selected [44, 45].

Results

Protein modeling and validation. This study used the NiV G protein as the vaccine design target, and the 3D structure of the target was modeled using the homology modeling method via the SWISS-MODEL [25].

The 3D structure model of the NiV G protein (modeling template ID 3d12.1A) is composed of β-sheet, α-helix, and coil structures; the Ramachandran plot score shows no bad bonds, 92.22% favored bonds, 0.47% outliers, and GMEAN and QMEAN values of 0.55 and -1.43, respectively. The model score is 100%, and the cyrillic structure of the NiV G protein, which consists of an outer and an inner region, is shown in Figure 1. The local quality estimate relates to the estimate of each model residue (x-axis) and its expected similarity value to the original structure (y-axis). The fluctuating graph (Figure 1B) shows a low similarity score lower than 0.6 for some of the residues that make up the model with the template. The bad bonds in the Ramachandran plot relate to weak chemical interactions, such as van der Waals bonds, that affect the protein structure, and the presence of these interactions causes the formation of weak structures compared to the results of hydrogen bond interactions. However, the NiV G protein model constructed in this study is considered to be valid because it has a similarity score of 100% and the Ramachandran plot favors 92.22% of bonds.

B-Cell epitope mapping, antigenicity, similarity, allergenicity, toxicity, and physicochemical prediction. The B cells of the NiV G protein were directly recognized through BCR, and the prediction of epitopes in this study was conducted using the BepiPred 2 method on the immunoinformatics server with peptide naming given the suffix "G" [29]. We obtained 7 epitopes; the longest 79-mer was found in position 136–214 and the shortest 11-mer was found in position 75–85 (Table 1). B-cell epitopes producing vaccine candidate peptides initiated direct recognition in the formation of humoral immune responses via the B cells. To determine a good vaccine candidate, it is necessary to predict the antigenicity, similarity, allergenicity, and toxicity of the seven peptides on the G NiV protein, and these were predicted by eptiop through the VaxiJen, BLASTp, AllerTOP, and ToxinPred servers [45]. The 1G peptide was used in further analysis because it was predicted to initiate the formation of a B-cell immune response (Table 2). The physicochemical properties of the 1G peptide were predicted through ProtParam (Table 3). The vaccine candidate requires a molecular weight lower than 36 kDa, and a GRAVY score between -0.14 to -0.45 is required to allow natural hydrophilic interactions to form in the vaccine. However, neither parameter was met by the 1G peptide. Nevertheless, the aliphatic index score for the 1G peptide was high (64.13 to 80.42), and this indicates stability at several temperatures. The instability index score of the 1G peptide was also low (<40), and this enables its stability when constructed and triggers the initiation of immunogenic reactions [34].

Peptide 3D construction, peptide-protein docking, and molecular dynamic analysis. The 3D peptide structure of 1G was obtained through modeling on PEP-FOLD using the fold recognition method [22, 31]. The 1G vaccine candidate peptide was then allowed to bind by recognition to B cells, and molecular docking was

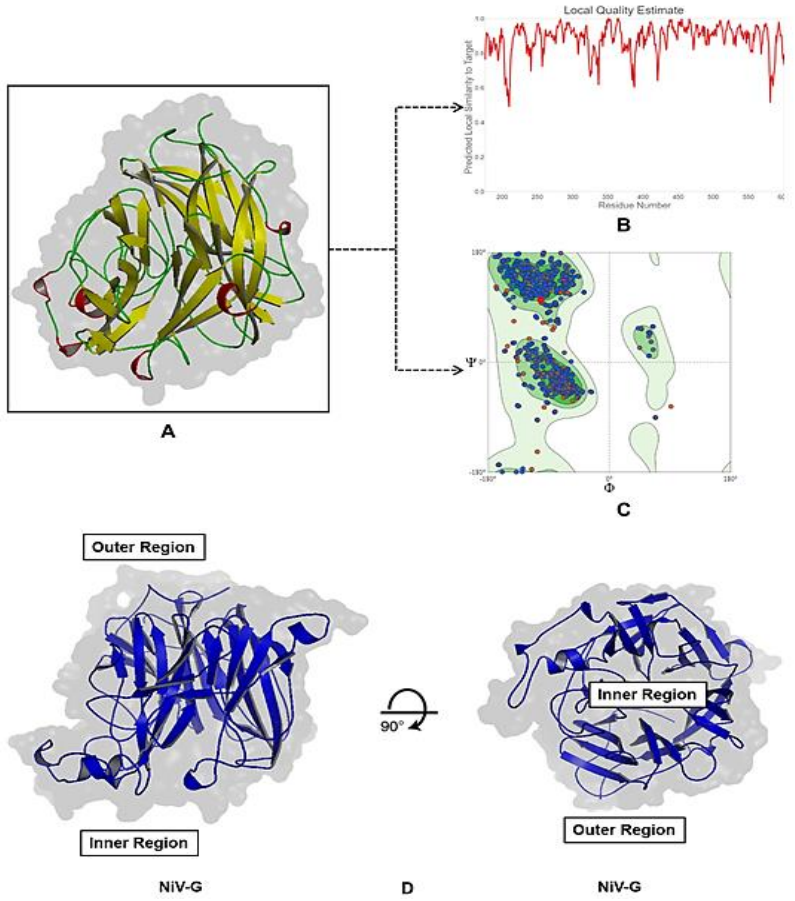

Figure 1. Visualization of NiV G Protein. (A) 3D NIV Structure of G Protein with Structural Staining, Yellow (β-sheet), Red (α-helix), and Green (Coil); (B) Local Quality Estimate Graph of NiV G Protein; (C) Ramachandran Plot Results, and (D) NiV G Protein

Table 1. BepiPred 2.0 Prediction Result

Peptide	Length	Position	Sequence
1G	35	8–44	RFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLD
2G	11	75–85	RSTDNQAVIKD
3G	79	136–214	ASINENVNEKCKFTLPPLKIHECNISCPNPLPFREYRPQTEGVSNLVGLPNNI-CLQKTSNQILKPKLISYTLPVVGQSG
4G	13	300–313	VGDPILNSTYWSG
5G	19	324–342	KSNGGGYNQHQLALRSIEK
6G	35	371–405	VRTEFKYNDSNCPITKCQYSKPENCRLSMGIRPNS
7G	17	483–499	TVISRPGQSQCPRFNTC

Table 2. Antigenicity, Similarity, Allergenicity, and Toxicity Prediction Results

Peptide	Antigenicity	Similarity	Allergenicity	Toxicity
1G	Antigen	Non-similar	Non-allergen	Non-toxin
2G	Non-antigen	-	-	-
3G	Antigen	Non-similar	Non-allergen	Toxin
4G	Non-antigen	-	-	-
5G	Antigen	Non-similar	Allergen	-
6G	Antigen	Non-similar	Allergen	-
7G	Non-antigen	-	-	-

conducted on PatchDock and FireDock using the 1G peptide as the ligand and BCR (PDB ID 5IFH) as the target. Fast rigid body and refinement docking methods were used on both servers, and the binding energy output consisted of global energy, van der Waals, ACE, and hydrogen bonds [38, 39]. The best docking results were attributed to the molecular complex at solution 10, because it provided the lowest binding energy and allowed the peptide to trigger BCR activity (Table 4). The 3D complex structure sequence of the docking results in solution 10 was displayed through PyMol software, and staining selection was conducted to differentiate the regions and structures and display secondary proteins [40]. As shown in Figure 2A, the 1G

Table 3. Physicochemical Properties Prediction Result

Peptide	Theoretical pI	Molecular Weight (Daltons)	GRAVY	Index	
				Aliphatic	Instability
1G	Antigen	Non-similar	Non-allergen	75.14	-8.76 (stable)

Table 4. Peptide-protein Docking Simulation Result

Peptide	Solution	Global Energy (kcal/mol)	Van der Waals		ACE	Hydrogen
			Attractive	Repulsive		
1G_BCR	10	-28.66	-25.83	17.28	-1.04	-2.45
1G_BCR	3	-22.57	-19.82	15.58	-0.88	-0.29
1G_BCR	1	-12.37	-27.42	15.37	10.78	-2.05
1G_BCR	5	3.21	-28.55	10.16	6.89	-1.54
1G_BCR	4	4.79	-3.50	0.37	4.07	-1.22
1G_BCR	6	5.67	-28.34	25.99	3.73	-3.00

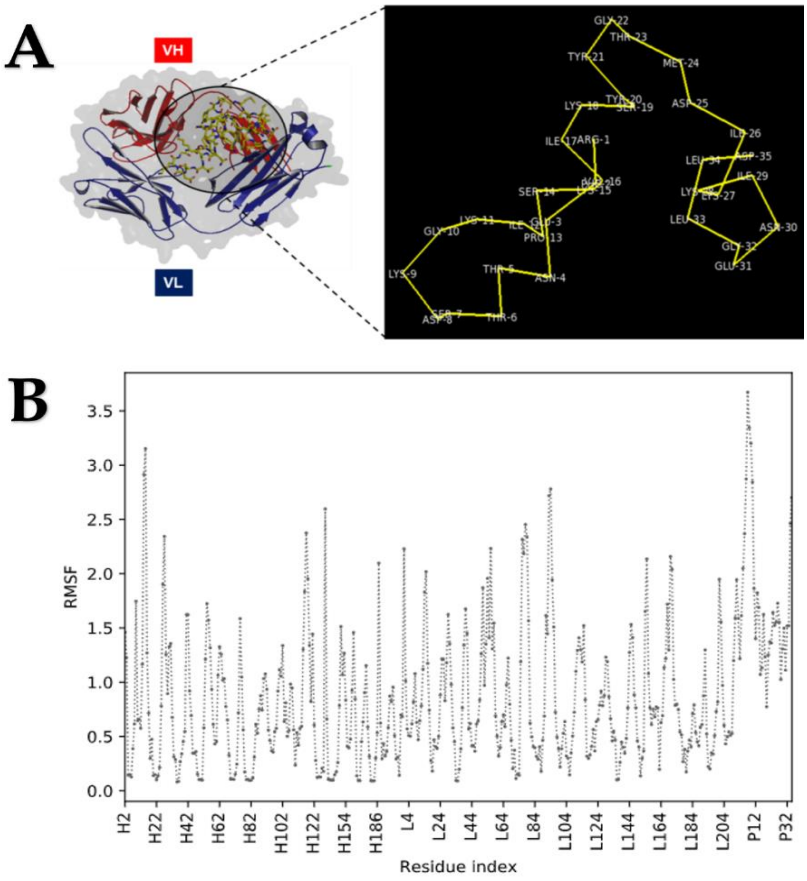

Figure 2. (A) Molecular Docking 1G Peptides using BCR. The Yellow Color Indicates 1G Peptide, Light Blue Indicates Light Chains (VL), and Red Indicates Heavy Chains (VH). (B) RMSF Heavy (H) and Light (L) in BCR When Bounded with 1G Peptide (P) Vaccine Candidate for NiV

peptide consists of a sticks and ribbon structure, where the colors relate to its constituent atoms and amino acid residues. The illustrated target protein structure has transparent surfaces on which staining selection was conducted (Figure 2A). The G1_BCR complex was analyzed by MD through CABS-flex to identify the root mean square fluctuation (RMSF) level when the G1_BCR complex was formed [46]. Fluctuating residues were found in heavy chain (H) 15, 27, 117, 129, 187, 218, light chain (L) 15, 56, 76, 77, 93, 27, 155, 170, and peptide G1 vaccine candidate (P) 5, 6, 7, 8, 9, 10, 34, 35; all of which had a similar RMSF of 2-3 Å (Figure 2B). The MD simulation results show that the 1G peptide is stable when it binds to BCR, and it is able to initiate target receptor activity because it has the lowest binding energy.

***In silico* cloning and immune simulation.** In this study, we successfully inserted the G protein gene into the pET30a(+) expression vector (considering *NdeI* and *HindIII* restriction sites). The ability of the vaccine candidate peptides to stimulate the immune response of the B cells and form specific antibody isotypes, memory, and population were determined through C-ImmSim [45]. It was predicted that peptide G1 could trigger the formation of memory B-cell activity >500 per mm^3 after five days of injection, and this was increased up to 35 days. The IgM+IgG isotype antibody titter produced by plasma B cells (PLB) increased for five days after injection until the peak on day 13, with the number of cells reaching 20 mm^3. These results show that peptide G1 triggers the activity of B-cell differentiation into plasma and memory cells and produces IgM and IgG antibody type titres for the process of neutralizing NiV.

Discussion

The mapping and surveillance of emerging and reemerging diseases is required to enable the early detection of a potential pandemic-causing agent, such as that associated with SARS-CoV-2 (COVID-19 pandemic) [22]. NiV has patterns of spread that are similar to those of SARS-CoV-2 [2], and further immediate research on NiV is required to assist in preventing such a pandemic.

Bioinformatics have enabled the rapid development of vaccinology, and vaccines developed using the latest technology (including computational based research) are designed to be safer, more effective, and cheaper than traditional vaccines [45]. However, a deep understanding of the disease agent is required to induce the precise immunological reaction, and this mainly requires genomic analysis and epitope prediction [31]. The significant potential of epitopes in vaccine design, disease prevention, diagnosis, and therapy has prompted significant research interest [19]. Through using the latest technology, specific epitopes that could replace all pathogens in vaccines have been isolated. However, not all epitopes have the same antibody-producing capabilities [24].

In silico research on vaccine design based on epitopes has been conducted for various kinds of viruses, such as SARS-CoV-2 [24,31], Zika virus (ZIKV) [35], and Dengue virus (DENV) [36]. In this study, we aimed to construct a vaccine based on the G protein from NiV based on NiV data from the NCBI reference sequence with accession number NC_002728, which has a genome size of 18,246 bp. The G protein is believed to be one of the virus receptors that attaches to Ephrin-B2 and Ephrin-B3 in humans or other mammals [15], and it also has the ability to induce a host immune response [14]. The presented vaccine candidate showed promising potential through an *in silico* analysis, but further *in vitro* and *in vivo* investigations are required to confirm its efficacy

Conclusions

In summary, the results of this study show that the "RFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLD" peptide (1G peptide) is a good candidate for the NiV vaccine because it is immunogenic, has low toxicity, and it does not trigger an autoimmune response. In addition, it can increase the immune response of B cells through activation, differentiation into plasma cells, the formation of memory cells, and by increasing in IgM/IgG antibody titres for viral neutralization. However, it is crucial to verify the results of this study through further *in vivo* and *in vitro* analyses.

Conflicts of Interest

The authors declare that there are no conflicts of interest with the data contained in the manuscript.

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