International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Identification of T Cell and B Cell Specific Peptide Vaccine for the Treatment of Ross River Virus

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Abstract:

Introduction:

Ross River virus, also known as Ross River fever and epidemic polyarthritis, is an infection that is spread to humans through mosquitoes.Ross River virus infection can cause fever, rash, joint inflammation and pain, fatigue and muscle aches. Most people recover completely within 3 to 6 months, although some people have intermittent symptoms for a year or more. Ross River virus infection is caused by an alphavirus(RNA Virus).Ross River virus is named after the Ross River in Townsville, which is the place where it was first identified. Ross River fever is the most common mosquito-borne disease in Australia, and nearly 5000 people are reported to be infected with the virus each year.The T and B lymphocytes (T and B Cells) are involved in the acquired or antigen-specific immune response given that they are the only cells in the organism able to recognize and respond specifically to each antigenic epitope.Developing a B-cell and T-cell specific peptide vaccine for Ross River Virus (RRV) would involve identifying specific antigenic targets that can stimulate the immune response.

Materials and methods: Sequence was retrieved, B cell epitope and T cell epitope prediction was carried out and the results were plotted in an excel sheet and graphical representation of immunogenicity regions of Ross Virus was plotted

Results: In the B cell epitope prediction ;Peptide RNQPYLFKTNPNYKGNDIKCTSTSRDK was selected as it was homologous (100%) to the C. burnetiid htpB and the length was appropriate for a candidate peptide vaccine. In the T cell epitope prediction ;

Conclusion: The discovery and development of B cell and T cell specific peptide vaccines has the potential to provide a personalised and successful strategy to combating Ross River Virus infection, hence providing hope for future prevention and treatment techniques against this viral disease.

Keywords: B cell specific peptide vaccine - T cell specific peptide vaccine - Ross River Virus. - peptide score prediction - HLA allele binding - MHC I binding - Lesser side effects - More efficacy

Introduction:

Viruses cause a great deal of harm to people and are the primary global cause of morbidity and mortality from infectious diseases. Since they were discovered in antiquity, viral illnesses in humans have influenced

human history. The 19th century saw the emergence of scientific methods for the research of virus and infectious disease, which eventually led to the discovery of certain viral disease entities. Many viral infections have been identified and numerous viral diseases have been distinguished (e.g., smallpox vs. chickenpox and measles vs. rubella) thanks to meticulous clinical observations. The pathophysiology of multiple viral diseases has been identified thanks to advancements in our comprehension of disease at the level of cells and tissues, best demonstrated by the groundbreaking research of Virchow.

At the close of the 19th century, the initial viruses were discovered. The tobacco mosaic virus was detected by Ivanovsky and Beijerinck, whereas the foot-and-mouth disease virus was found by Loeffler and Frosch. The U.S. Army Yellow Fever Commission (Reed W et al. 1902)(1)and Walter Reed conducted revolutionary studies on the etiology of yellow fever shortly after these discoveries, leading to the identification of the yellow fever virus. Tumor viruses, bacteriophages, influenza viruses, mumps viruses, and other viruses carried by arthropods were detected by the end of the 1930s. Up until now, the procedure of discovery has continued with increasing momentum. The most recent discoveries include the recently discovered Merkel cell polyomavirus, which is linked to skin cancer; two new Old World arenaviruses that cause fatal disease; three bat-related respiratory coronaviruses, five and reoviruses; seven; and eight novel influenza viruses with avian and swine origins.(Zaki AM et al 2012) (2)(Zhang X et al 2008).(3)

Australia and the nearby Pacific Islands are home to the endemic viral disease known as Ross River fever, which is spread by mosquitoes. It belongs to the arthritogenic group of alphaviruses, it is a single stranded RNA virus primarily responsible for the disease's crippling polyarthritis, rash, and fever. There is no approved medication or vaccination for this condition, and our understanding of the mechanisms behind human protective humoral immunity is lacking(Powell et al 2020)(4).RRV is transmitted through the bite of Aedes and Culex mosquitoes.Typical infection symptoms include fever, rash, and—most importantly—disabling pain in the muscles and joints that lasts for three to six months(Harley D et al 2001).(5)Since the disease's national alert program was established in 1993, 4,600 cases on average have been recorded in Australia per year for RRV(Australian government department of health).In the past, it was believed that kangaroos and wallabies, which are native to Australia, were the reservoirs of RRV (Claflin et al 2015).(6)Recent data, however, suggests that additional mammalian species, including flying foxes, rabbits, and rodents, may serve as the virus's reservoirs and aid in its dissemination (Lau C et al 2017)(7). This discovery raises questions about the possibility of RRV spreading outside the borders of Australia and the Pacific Territories and raises concerns about the transmission of the virus to humans in the future. Vaccines are undoubtedly the most successful biological achievement in illness prevention. Over 100 million children worldwide receive vaccines each year to avert diseases that were once common and connected to significant medical issues or even death. Childhood immunizations that are globally provided comprise those for ,mumps , measles,influenza virus which is seasonal ,rubella, hepatitis B ,tetanus, polio,pertussis , diphtheria and others. Vaccines for illnesses that are endemic to specific locations, such as Yellow fever virus, whose mosquito vectors spread year-round in tropical and subtropical climates, are also given to the broader population. Vaccination is predicted to prevent between 2-3 million lives each year (WHO).

Because many vaccines against viral infections are predicated on generating antibody responses, they are often poor stimulants of T cell responses (Plotkin SA et al 2013)(8). T cell stimulating vaccines are needed

because T cells are vital in protecting against numerous viral diseases. Specific T cell responses can be elicited by inserting tiny protein fragments (peptides) in a vaccination that can be delivered by MHC-molecules to CD4+ and CD8+ T cells.The primary benefit of peptide vaccines versus traditional immunizations is the ability to specifically trigger T cell responses and the ease with which these vaccines may be produced. Aichele et al. published the initial synthetic peptide vaccination capable of inducing a T cell response in mice. A 15-mer peptide isolated from the NP protein of LCMV was suspended in incomplete Freund's adjuvant (IFA) in this vaccination (Aichele P et al 1990)(9). Further research revealed that such peptide vaccines could provide some protection against virus challenge (Kast WM ate al 1991)(10). These promising results constituted the primary motivation for the current study.

Therefore the current study employed the identification of specific T cell and b cell peptide vaccines against Ross River Virus.

Materials and methods:

Sequence retrieval:

All the available Protein databases were evaluated for potential targets for the discovery of vaccination candidates using the server https://www.uniprot.org/proteomes.The NCBI Reference Sequence: (GenBank: NC_075016) structural polyprotein of Ross river virus was chosen for the investigation.The amino acid sequence for the preceding entry was obtained from the NCBI database.Fasta sequences were utilised to predict B and T cell epitopes.

Linear B cell epitope prediction:

Using the BepiPred 2 software application, the protein sequences were utilised to anticipate probable linear B-cell epitopes . As a default, the epitope threshold was set at 0.5. The program's default scoring for epitope (E) is 0.5, and adjustments to this would modify the degree of sensitivity and specificity of the epitope's immunogenic efficacy [13]. Positions over the threshold and longer than 20 mer were deemed possible Bcell epitopes.The BepiPred program took into account each epitope's structural predictions (sheet, helix, or coil), epitope locations, and surface accessibility (hidden or exposed).

T cell epitope prediction:

T cell epitope prediction is to find the shortest peptides present in an antigen that can activate T cells or elicit immunogenicity [39]. These antigens can activate either CD4+ or CD8+ T lymphocytes. The NetCTL 1.2 server can forecast CTL epitopes. The chosen protein was submitted, and MHC I - binding T cell epitope prediction was performed using the internet server program http://tools.iedb.org/mhci/.The Immune Epitope Database (IEDB), which contains an archive of scientifically identified T cell epitopes, contains information regarding MHC ligand elution and MHC binding studies.Using the HLA allele reference set, the interacting alleles (MHC I-binder) with these epitopes were then identified.

Results:

Linear B cell epitope prediction:

The anticipated Linear B cell epitope for Ross River virus varies in length from 3 to 27 mer. The peptide RNQPYLFKTNPNYKGNDIKCTSTSRDK was chosen because it was 100% identical to the C. burnetiid htpB and the length was adequate for a potential peptide vaccine.

No	Start	End	Peptide	Length
1	9	11	DQT	3
2	13	15	NNQ	3
3	17	66	NRSLTALQVLPTAANTEASSHRLGTGVVPALQAAETGASSNASDKNLIET	50
4	68	77	CVLNHHSTQE	10
5	98	106	TGTQNNTDGY	9
6	144	147	GELV	4
7	161	172	PKPTSRDSFAWQ	12
8	208	222	YPTFGEHLQANDLDY	15
9	241	244	EKSP	4
10	267	293	RNQPYLFKTNPNYKGNDICTSTSRDK	27

Table 1. Prediction of B cell epitope

T cell epitope prediction :

The IEDB tool peptide_binding_to_MHC_class_I_molecules and the HLA class I set (Weiskopf et al., 2013) were used to determine T-cell epitopes for the protein. Percentile rank with the least threshold was set as the best peptide score(threshold ranges from 0 to 1%) resulting in a high antigenic score.A peptide with a high antigenicity score as well as the ability to bind to a greater number of alleles is thought to have high potential to elicit a powerful defence response. The highest potential peptides were selected as ATNPSVFV with a score of 0.989845 and percentile rank or antigenicity score of 0.01% and the second highest being ETAIGNFF with score of 0.973605 and percentile rank of 0.01.Protein-peptide interactions play an important role in biological signaling networks. The protein's peptides 'ATNPSVF' and 'ETAIGNFF' were predicted to bind to HLA-A*11:01 and HLA-A*68:01.There were 31 reported epitope-protein interactions, and the best prediction was chosen.

allele	sequence number	length	peptide	score	percentile rank
HLA-A*11:01	3	9	ATNPSVFVK	0.989845	0.01
HLA-A*68:01	2	10	ETAIGNFFSR	0.973605	0.01

HLA-B*57:01	3	9	MSPASAYQW	0.967831	0.04
HLA-A*68:01	1	9	QTVNNQVNR	0.967226	0.02
HLA-B*58:01	3	9	MSPASAYQW	0.966579	0.02
HLA-A*26:01	3	9	ELVPQLLQY	0.965979	0.01
HLA-A*03:01	3	9	ATNPSVFVK	0.965599	0.01
HLA-A*02:03	3	9	KMTDPPAQV	0.961658	0.01
HLA-A*24:02	2	9	TYMRFDAEF	0.95932	0.01
HLA-A*68:01	2	9	TAIGNFFSR	0.959123	0.03
HLA-B*57:01	3	10	KPTSRDSFAW	0.957813	0.05
HLA-B*07:02	3	10	APKPTSRDSH	0.944011	0.04
HLA-B*40:01	4	10	TEKSPHSITL	0.941925	0.04
HLA-B*44:03	2	9	QETAIGNFF	0.9379	0.03
HLA-B*35:01	3	10	VPFMSPASAY	0.936474	0.03
HLA-A*02:01	3	9	KMTDPPAQV	0.934079	0.03
HLA-A*23:01	2	9	TYMRFDAEF	0.930885	0.01
HLA-A*31:01	4	9	RAWIPRPLR	0.916803	0.02
HLA-B*58:01	3	10	KPTSRDSFAW	0.914624	0.05
HLA-B*40:01	4	9	GEHLQANDL	0.911691	0.06
HLA-B*15:01	4	9	YLFKTNPNY	0.908292	0.02
HLA-B*44:03	3	10	GELVPQLLQY	0.901071	0.04
HLA-B*35:01	3	9	VPQLLQYMY	0.899017	0.04
HLA-A*68:01	1	10	DQTVNNQVNR	0.96949	0.07
HLA-B*44:02	2	9	QETAIGNFF	0.885828	0.03
HLA-A*30:01	3	9	ATNPSVFVK	0.882022	0.01
HLA-B*35:01	4	10	SPHSITLRVY	0.868311	0.05

HLA-B*07:02	4	9	RPLRNQPYL	0.866564	0.06
HLA-B*51:01	4	9	SPHSITLRV	0.859877	0.02
HLA-B*57:01	3	10	FMSPASAYQW	0.857175	0.16
HLA-B*08:01	5	9	TSRDKITL	0.854896	0.03

Table. 2 Prediction of T cell epitope

Immunogenicity regions of Ross River Virus:

Immunogenicity is the measure of the efficacy of the synthesised vaccine and measures the immune response regions created over a magnitude of time.

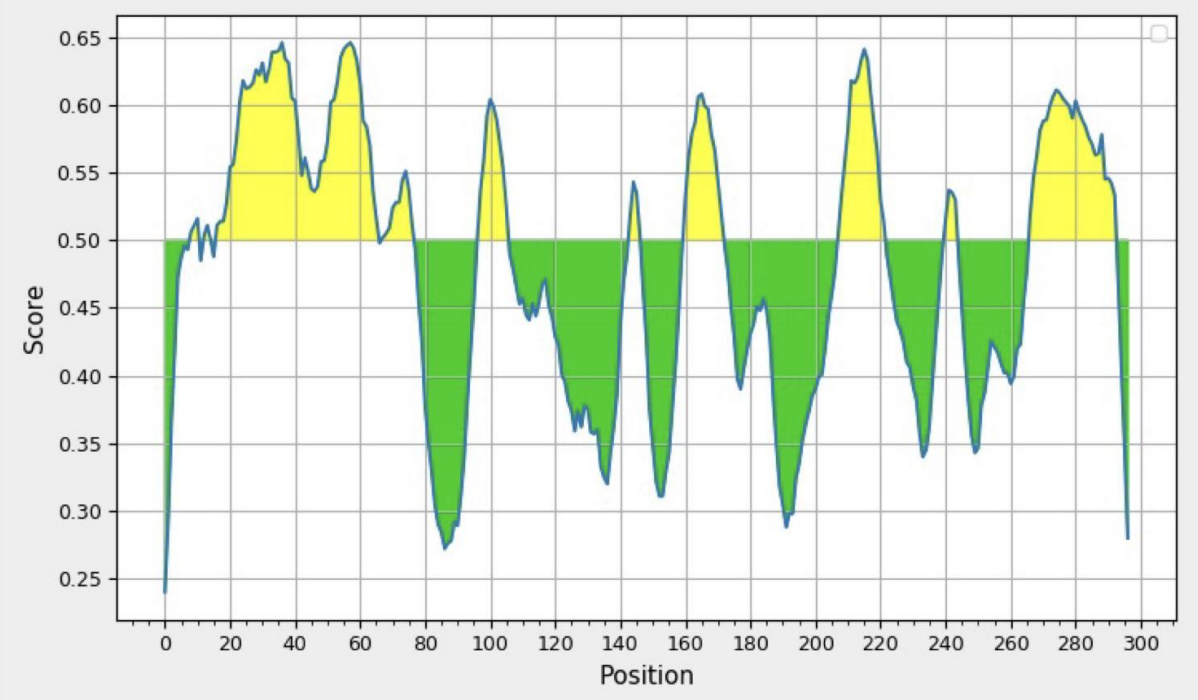

Figure 3. Graphical representation of immunogenicity regions of Ross River Virus

Discussion:

The emergence of mutant strains of pathogenic microbes, modifications to host lifestyles, and the host and pathogen surrounding habitats all contribute to the emergence and reemergence of diseases. A few of these diseases have begun to propagate throughout the world as a result of growing global trade and tourism, rather than remaining isolated to the nations where they originated.(Rosello et al 2017)(10,11)

Vaccines are critical prophylactic measures against potentially fatal diseases. The development of computer tools and databases opened the way for the design of vaccines. Traditional vaccine creation methods, which are time-consuming and expensive, are being replaced by more basic and cost-effective computer technologies.(Manzumder et al 2022)(10–12)

The prediction of T and B cell epitopes, which was covered in the current work, is an important stage in the development of vaccines. The cell surface binding protein of the Ross River virus contained 10 B cell and 31 T cell epitopes, according to the study. Following epitope identification, an excellent T cell epitope

was considered for vaccine design since it can generate long-lasting protection. Predicting the antigenicity of all protein sequences is a key part of vaccine development since only antigenic peptides can elicit an immune response in the host.

According to Grifoni et al 2020, the found B- and T-cell epitopes may aid in the creation of powerful peptide-based vaccines to combat the SARS-CoV-2 challenge. Those epitopes without mutations from conserved areas, in particular, may develop immunity that is not only cross-protective across Beta coronaviruses but also reasonably resistant to ongoing virus evolution.(10–13)

Lincon et al 2023 proposed that the postulated T cell epitope "ILFLMSQRY" showed increased affinity for interacting with the receptor of its target and would elicit a powerful immune response and operate as a therapeutic agent against monkeypox virus infection in another silico investigation. This highly immunogenic and nonallergenic epitope can also efficiently interact with the human leukocyte antigen HLA-B15:01.

Though no study has been done on identification of B cell and T cell epitopes for Ross River Virus similar studies employed as above had similar results to the present study on the basis of efficacy of T cell and B cell epitope vaccine against the treatment of a virus

Limitation of the study:
Extensive research and efficacy can be obtained by conducting the study in Vivo .

Conclusion:
A B cell peptide vaccination can induce the development of antibodies designed to recognize and neutralize the virus by targeting specific surface proteins or epitopes unique to RRV. This method can keep RRV from infecting host cells and spreading throughout the body.T cells play an important part in the immune response by attacking infected cells directly and coordinating the overall immune defence. A T cell-specific peptide vaccination can activate these cells to recognize and kill RRV-infected cells, lowering viral replication and infection severity. Furthermore, using both B cell and T cell peptide vaccines in a dual approach for comprehensive immune response can provide a comprehensive resistance against RRV.

Conflict of interest:
The author reported the conflict of interest while performing this study to be nil.

Funding Agency:
Saveetha dental college and hospitals, Chennai
Victoria Perch ltd, Tanzania

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