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Research Article

Epitope tailored vaccine construct for goat milk allergy

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Abstract

Goat milk is less allergic because concentration of milk proteins is lower than that of cattle milk. But we can't rule out the possibility of allergies from goat milk. This study, is conducted to check whether goat milk is allergic and antigenic epitopes are found. Six major milk proteins were downloaded from public domain. CTL, HTL and B cell epitopes were detected and 1 to 5 epitopes from each type in each study proteins crossed threshold, hence proving goat milk would also be allergic. Further topmost epitope from each category and linkers were used to develop vaccine against goat milk allergy. The designed vaccine was of the length 371 amino acid residues and was demonstrated to be strong antigenic while being non-allergic and non-toxic. Molecular docking of the epitopes was done against TLR3 and TLR4 and found to be very well interacting with the epitopes which was indicated in negative binding energies. Further immuno simulations were done and found that the designed vaccine was able to stimulate production of immune molecules.

Keywords: Goat; Milk allergy; Epitope; Vaccine; Immunoinformatic

Introduction

Goats are the preferred dairy animal for the poor due to their lower initial investment and ongoing production costs, as well as their quick generational turnover, which results in earlier milk production, shorter gestation periods, and a supply of milk in quantities suitable for immediate household consumption, thereby minimizing milk marketing and storage issues. Each species' milk has a unique composition, which results in a unique nutritional and physiological profile. Goat milk has several benefits over milk from other animal species, including being easier to digest, acting as a buffer, being naturally homogenized, and boosting immunity due to its antifungal and antibacterial qualities [1].

Goat milk and its byproducts have long been valued as immune system enhancers. Alpha s-1 casein levels in goat milk are 89% lower than those in cow milk [2]. It is hence less allergenic. Goat milk, however, may occasionally be dangerous. A common condition known as goat's milk allergy often affects very young children and is caused by an abnormal immune response to goat's milk. After drinking goat milk or products derived from milk, it may occur immediately or a few hours later. Anaphylactic shock, a life-threatening whole-body allergic reaction, is an uncommon but serious condition that requires immediate medical attention. Method of treatment for establishing long-term tolerance to goat milk allergies is immunotherapy. One of the most promising ways to silence disease-causing T cells and induce antigen-specific tolerance while maintaining the rest of the immune system is antigen-based immunotherapy, such as the approach used in the current study [3]. Since most persons who are sensitive to cow milk are also intolerant of goat and sheep milk, cow milk allergies frequently coexist with these allergies. According to Ehlayel *et al*. [4] only 18.4% of the children with cow-milk allergies were also allergic to camel milk, 63.2% of them to goat milk, and 15.8% to cow, goat, and camel milks. Keeping this in background, as a measure of prevention to goat milk allergy here we attempted to computationally design an active immunotherapy approach i.e., vaccination for goat milk allergy.

Methods

Sequences retrieval

The protein sequences of Alpha-lactalbumin (NCBI protein Id: AHG99152.1), Beta-lactoglobulin (NCBI protein Id: Z33881.1), Alpha-S1-casein (NCBI protein Id: AJ504712.2), Alpha-S2-casein (NCBI protein Id: P02663), Beta-casein (NCBI protein Id: P02666) and Kappa-casein (NCBI protein Id: P07498) (hereafter referred as AL, BL, CAS1, CAS2, BC and KC, respectively) were downloaded from Uniprot data bank (https://www.uniprot.org/uniprotkb) to identify potential epitopes and for further vaccine construction. The physiochemical properties of the proteins were assessed using the ExPASy database server's ProtParam programme [5]. The VaxiJen server was used to assess the antigenicity of the proteins [6]. AllerTOPv2.0 was used to assess the allergenicity of the proteins [7].

Designing of multi-protein multi-epitope vaccine construct

Five proteins' HTL, CTL, and B-cell epitopes were linked together using GPGPG, AAY, EAAAK, and KK linkers to form a multiprotein multi-epitope (MPME) vaccine construct. Defensin, universal memory T-cell helper peptide (TpD), PADRE (Pan HLA-DR reactive epitope), and an M-cell ligand were also added in the vaccine design through linkers. To improve immunogenicity, defensin was added to the N terminal, while M-cell ligand was added to the C terminal, followed by the addition of HHHHHH to make future purification experiments easier. The Chauhan *et al*. [8] technique was used to develop an MPME vaccination against milk protein allergies that met the following criteria: (a) be promiscuous; (b) overlap CTL and HTL epitopes; (c) be immunogenic; (d) have a strong affinity for HLA alleles; and (e) have no overlap with any human gene.

Antigenicity, allergenicity, and physiochemical properties of MPME vaccine construct

The antigenicity of the vaccine was assessed using the VaxiJen server. The allergenicity of the vaccination was assessed using AllerTOPv2.0. The physiochemical characteristics of the vaccine were assessed using the ExPASy database service's ProtParam tool.

Structure prediction, validation, and docking of Vaccine construct with the receptor

The Phyre2 server was used to estimate the vaccine construct's tertiary structure, while PSIPred 4.0 Protein Sequence Analysis Workbench [9] was used to predict its secondary structure. To validate the vaccine construction model with the highest TMscore, web servers from PROCHECK v. 3.5 [10] and ProSA [11] were employed. The Cluspro v. 2 protein-protein docking web service [12] was used to dock vaccine receptors to determine the vaccination's affinity for the TLR3 receptor (PDB ID: 2A0Z) and TLR4 receptor (PDB ID: 3FXI). The C-ImmSim server [13] was utilized to explain the MPME vaccination's real-world immunogenic profiles and immunological response.

Results and Discussion

Physiochemical properties of proteins used for vaccine construction.

The ProtParam programme was used to predict the physicochemical characteristics of six proteins under investigation. Table 1 displays the results. Among the proteins under investigation, AL had the fewest amino acid residues (142), whereas BC had the most (224). Except for AL, all six proteins were projected to be unstable in the instability index, and in the antigenicity index, AL, BC, and KC did not qualify as antigens since their scores were less than the 0.4 threshold specified by the VaxiJen server. Furthermore, with the exception of KC, the AllerTOPv2.0 webtool projected that all proteins were allergens.

T cell and B cell epitope prediction

Prediction of B-cell and T-cell epitopes is a critical stage in vaccine development [14]. The NetCTL1.2 server was used to predict CTL epitopes for all of the proteins, and the VaxiJen server was used to assess antigenicity. For the AL, the server predicted 134 potential CTL epitopes, 5 of which exceeded the threshold established by the NetCTL1.2 server's prediction model. For BL, three epitopes out of 172, none out of 107 for CAS1, eight out of 214 for CAS2, four out of 214 for BC, and six out of 184 for KC met the threshold value and were thus deemed possible epitopes. However, in order to shorten the length of the vaccine design, the top one epitope from each protein was evaluated (Table 2). HTL epitopes, a critical component of the adaptive immune response, were predicted using the IEDB MHC II server, and one possible epitope from each of the proteins was incorporated in the vaccine design (Table 3). The web server ABCpred was used to predict B-cell epitopes, which are reported in table 4. MHC class II binding peptides typically have a length of 12-25 amino acids, whereas MHC class I binding peptides have a length of 8-11 amino acids. The chosen T-cell epitopes appeared to have high scores for antigenicity, allergenicity, immunogenicity,

Properties	Alpha-lactalbumin	Beta-lactoglobulin	Alpha-S1-casein	Alpha-S2-casein	Beta-casein	Kappa-casein
Number of amino acids	142	180	169	223	222	192
Molecular weight (KDa)	16.246	19.975	19.046	26.363	24.865	21.441
Asp + Glu	20	23	20	30	23	18
Arg + Lys	14	20	21	32	16	14
Instability index	28.31(Stable)	35.96(Stable)	56.99(Unstable)	53.60(Unstable)	97.84(Unstable)	46.72(Unstable)
Aliphatic index	92.68	109.56	92.31	66.91	98.65	79.27
Grand average of hydropathicity (GRAVY)	-0.196	0.070	-0.170	-0.829	-0.123	-0.328
Allergenicity (AllerTOPv2.0)	Allergen	Allergen	Non-Allergen	Allergen	Allergen	Non-Allergen
Nearest protein (AllerTOPv2.0)	UniProtKB accession number P00712	UniProtKB accession number P02756	UniProtKB accession number P98196	NCBI gi number 162929	UniProtKB accession number P11839	UniProtKB accession number P02670
Antigenicity (VaxiJen threshold: 0.4)	0.2973 (Probable NON-ANTIGEN)	0.4736 (Probable ANTIGEN)	0.5603(Probable ANTIGEN)	0.4688 (Probable ANTIGEN)	0.3553 (Probable NON-ANTIGEN)	0.6326(Probable Non-ANTIGEN)

Table 1: Physiochemical properties, allergenicity and antigenicity of proteins used for vaccine construction.

and toxicity, according to the study's predictions and evaluations of T cell epitopes based on features such as these. Furthermore, B-cell epitopes were classed as either discontinuous or conformational or continuous or linear. The antigenicity, allergenicity, and toxicity of the predicted linear B-cell epitopes with higher cut-off values (0.8 and above) were assessed, and the epitopes with the highest ratings were chosen for MPVC. A multi-epitope vaccine comprising a succession of peptides that activate humoral and adaptive immune responses is an attractive technique for viral or tumour infection prevention and therapy [8]. A vaccination must elicit memory immune responses capable of identifying the vaccine's intended target. These immune responses should be focused on pathogen-expressed highly conserved structures. The use of highly conserved antigens in vaccines can reduce the pathogen's ability to achieve immunological escape, which can occur when hypervariable areas are employed as vaccine antigens [15].

Designing of multi protein multi epitope vaccine construct

The highly antigenic 6 HTL and 5 CTL epitopes with the strongest affinity for the HLA alleles, as well as 6 B-cell epitopes with non-allergenic, non-toxic, and immunogenic features, were chosen for inclusion in the MPVC. Following the EAAAK linker coupling of the adjuvant defensin with the B cell epitope at the N terminal,

Protein	Epitopes	C terminal Amino acid number	NetCTL Score
Alpha-lactalbumin	CTAFHTSGY	47	3.0206
Beta-lactoglobulin	VLDTDYKKY	112	1.9230
Alpha-S2-casein	ALNEINQFY	97	2.0147
Beta-casein	FAQAQSLVY	67	2.1048
Kappa-casein	LINNQFLPY	71	1.7484

Table 2: CTL epitopes used for MPME vaccine construct for milk allergy.

Protein	Start	End	Peptide	Adjusted Rank	Epitope core
Alpha-lactalbumin	1	15	MMSFVSLLLVGILFH	4.9	FVSLLLVGI
Beta-lactoglobulin	2	16	KCLLLALGLALACGI	0.56	LLLALGLAL
Alpha-S1-casein	1	15	MKLLILTCLVAVALA	2.9	LTCLVAVAL
Alpha-S2-casein	3	17	FFIFTCLLAVALAKH	0.16	FTCLLAVAL
Beta-casein	201	215	QAFLLYQEPVLGPVR	2.1	FLLYQEPVL
Kappa-casein	60	74	GLNYYQQRPVALINN	0.01	YYQQRPVAL

Table 3: HTL epitopes used for MPME vaccine construct for milk allergy.

Protein	Sequence	Start	Score
Alpha-lactalbumin	KVGINYWLAHKALCSE	117	0.09
Beta-lactoglobulin	AVFKIDALNENKVLVL	98	0.83
Alpha-S1-casein	FPIVLSRSTFKRKMCP	87	0.91
Alpha-S2-casein	PQYLQYPYQGPIVLNP	109	0.95
Beta-casein	ETMVPKHKEMPFPKYP	115	0.87
Kappa-casein	YGLNYYQQRPVALINN	59	0.91

Table 4: B cell epitopes used for MPME vaccine construct for milk allergy.

the AAY, GPGPG, and KK linkers were used to connect the B cell epitopes, CTL epitopes, and B HTL epitopes, respectively. Adjuvants such as PADRE (Pan HLA-DR reactive epitope), Universal memory T-cell helper peptide (TpD), and a M cell ligand were connected into the vaccine formulation using EAAAK linkers. To facilitate vaccine purification, the HHHHHH and EAAAK linkers were joined at the C terminal (Figure 1). The purpose of the various adjuvants added to MPVC was to boost innate and adaptive immune responses as well as MPVC transit over the intestinal membrane barrier. The use of adjuvant in the developed multiepitope vaccines was intended to increase immunogenicity and activate multiple adaptive and innate immune mechanisms [16]. Immunogenic adjuvant boosts antibody synthesis and aids in long-term protection [17]. The EAAAK linker was employed to attach the adjuvant and CTL epitope to the vaccine structure's N-terminus, limiting contact with other protein sections and allowing for rapid separation [18]. GPGPG linkers play two roles in the vaccine design. First, it prevents the formation of junctional epitopes, which is a fundamental problem in the design of epitope vaccines; and second, it simplifies vaccination and presentation of HTL epitopes [19]. To connect the CTL epitopes, the AAY motif was employed as a linker [20]. The antigenic, allergenic, and physiochemical properties of the vaccination were then validated. The design demonstrated strong antigenicity while being non-allergic and non-toxic.

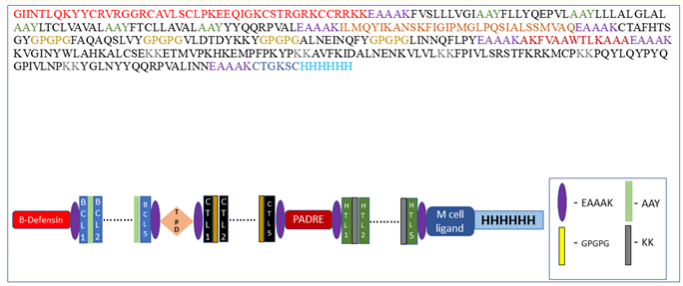

Figure 1: Multiprotein multi epitope vaccine construct with different linkers and adjuvants. Different sequences of vaccines are color coded and diagrammatically represented.

Physiochemical properties, antigenicity, and allergenicity of multi epitope vaccine construct

The MPVC's physiochemical properties were determined using the Protparam tool. The final MPVC includes 371 amino acid residues, 17 negatively charged residues (Asp + Glu), and 49 positively charged residues (Arg + Lys). The construct's stability index was 34.55, indicating that the vaccine was generated from a stable protein. The aliphatic index and the grand average of hydropathicity (GRAVY) were calculated to be -0.001 and 90.89, respectively. GRAVY score in positive designates its hydrophobic nature of vaccine [21]. The theoretical isoelectric point (PI) was calculated to be 9.67. When the vaccine construct sequence was analysed in the VaxiJen server, it was determined to be antigenic in nature, with an overall prediction score of 0.4786. Furthermore, this vaccine design was shown to be non-allergenic, and the anticipated closest protein to AllerTOPv2.0, with the UniProtKB accession number P04920, was discovered which is a Sodium-independent anion exchanger which mediates the electroneutral exchange of chloride for bicarbonate ions across the cell membrane [22].

Secondary and tertiary structure prediction and validation

The SOPMA server was utilised to study the secondary structure of the vaccine construct, which revealed a 42.886% (159 amino acid residues) helix, a 17.52% (65 amino acid residues) extended strand, a 5.93% (22 amino acid residues) turn coil, and a 33.69% (125) random coil (Figure 2). The Phyre2 webtool and Galaxy Refine predicted and refined the tertiary structure of the MPVC (Figure 3A). The top-performing model has GDT-HA, RMSD, and MolProbity scores of 0.9389, 0.480, and 2.156, respectively. PROCHECK was used to check the stereochemical quality, and Ramachandran plot analysis of the modelled structure revealed that 71.3% of the residues were in the most favoured regions, 11.2% were in the additional allowed regions, 9.5% were in the generously allowed region, and 0.1% were in the disallowed region (Figure 3B). Ramachandran plots serve as indirect verification tools of the stereochemistry and geometry of the complex by establishing that none of the geometries are in the forbidden electrostatically unfavored regions of the plot [23]. A ProSA webtool was used to look for potential flaws in the protein 3D model, and it predicted a negative Z-score of -4.75 (Figure 3C). The z-score indicates overall model

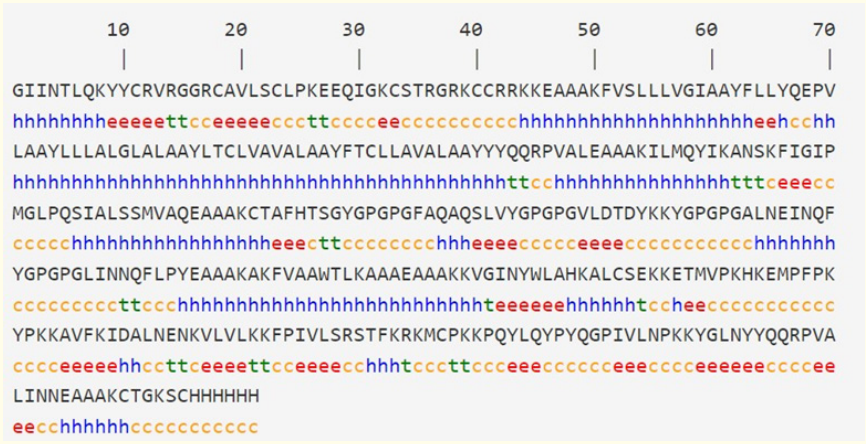

Figure 2: Secondary structure of Vaccine construct.

quality and measures the deviation of the total energy of the structure with respect to an energy distribution derived from random conformations [11]. These observations corroborated the correctness of the expected model.

Docking of multi epitope vaccine construct with receptors

The 3D structures of human TLR3 and TLR4 were retrieved from the protein data repository (PDB ID: 2A0Z and 3FXI). To improve the model's accuracy, CTL, HTL, and B cell epitopes were built individually before docking. Cluspro version 2 predicted ten models for each epitope group-TLR4 complex and epitope group-TLR3 complex based on their related cluster scores. The best-docked complex among these models was picked based on the models with the lowest energy (Table 5). This indicates that a molecular interaction between the expected vaccine design and TLR3 and TLR4 recep-

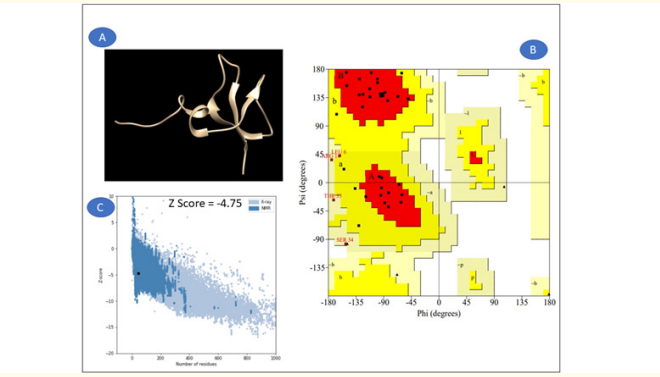

Figure 3: A) Predicted tertiary structure using Phyre2 webtool. B) Ramachandran plot and plot statistics predicted by PROCHECK. C) Quality analysis of the predicted vaccine construct structure by ProSA.

Macromolecule for docking	Epitope	Best model	Representative	Weighted Score
TLR3	Merged CTL epitopes	Model 0	Center	-603.6
			Lowest Energy	-721.2
	Merged B cell epitopes	Model 0	Center	-1037.5
			Lowest Energy	-1060.3
	Merged HTL epitopes	Model 0	Center	-595.3
			Lowest Energy	-684.4
TLR4	Merged CTL epitopes	Model 0	Center	-750.4
			Lowest Energy	-974.5
	Merged B cell epitopes	Model 0	Center	-1112.7
			Lowest Energy	-1176.2
	Merged HTL epitopes	Model 0	Center	-1452.3
			Lowest Energy	-1452.3

Table 5: Docking results with binding energy.

tors is possible. Docking studies on the molecular interactions of vaccines with TLR3 and TLR4 revealed that the vaccine generated had a high affinity for the toll-like receptors to identify pathogen molecular patterns and start the immune response. As a TLR agonist, the adjuvant defensin in the current MPVC can interact with a variety of TLRs to increase both innate and adaptive immunity. As a result, when combined with the defensin adjuvant, the MPVC has the potential to trigger an immunological response effective in the treatment of milk allergy. Molecular docking gives information on the vaccine-receptor complex's interaction, stability, and dynamics [24]. The findings point to beneficial intermolecular interactions between the vaccination protein and the TLR receptors.

Immune simulations of vaccine construct

TThe C-ImmSim simulator was used to test the final vaccine construct's capacity to induce an immunological response. The simulation's major focus is on three events: the binding of B-cell epitopes, the binding of HLA Class I and II epitopes, and the binding of the TCR, in which the interaction of the HLA peptide complex should be demonstrated. The combined findings of the immune responses after three antigen exposures revealed that there was an enhanced primary immune response to the antigenic fragments, as seen by the consistent rise in IgM level after each antigen exposure.The subsequent response, like the first, was defined by ad-

equate synthesis of IgM + IgG rather than IgM. Furthermore, it was discovered that IgG1 + IgG2 and IgG1 levels had increased (figure 4). When the vaccination was given again, antigen levels dropped, indicating the establishment of an immunogenic response in the form of immunological memory. Higher levels of all circulating immunoglobulins facilitate correct clonal proliferation of the B-cell and T-cell populations. Furthermore, an increase in the B-cell population was linked to an increase in immunoglobulin expression, which resulted in a decrease in antigen concentration. Furthermore, as memory development continued, the number of Th (helper) and Tc (cytotoxic) cells grew progressively. Dendritic cells, macrophages, and total NK cells increased as well. It was also discovered that the immunization increased IFN-gamma production. These data revealed that the MPVC proposed in this study had the ability to trigger a strong immune response that would last even after repeated exposure. The presence of B-cell and IFN-epitopes validates the construct's acquired humoral and cell-mediated immune responses [21]. In C-IMMSIM, a constant rise in IgG subclass levels as well as immunological cells (B and T cell population) revealed that humoral immunity had been activated. Because allergen-specific IgG antibodies appear to have a significant role in suppressing allergic immune responses, allergen-specific IgG antibody passive immunization has arisen as another strategy for allergy vaccination [25]. If more possible epitopes from other proteins are included, the MPVC's complexity may be limited, in addition to synthesis difficulties [26].

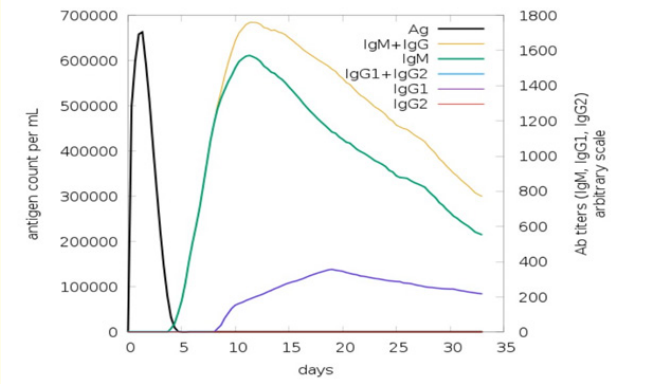

Figure 4: Vaccine response in inducing immunoglobulins.

Conclusion

Immunotherapy is generally recognised as a viable treatment option for food allergies, with data showing that it can change allergen-specific immune responses while fostering desensitisation. The six milk proteins used in the MPME vaccine in the current investigation must be investigated for oral vaccination as an active immunotherapy method. Because it was designed generically, this vaccine has the potential to protect a significant portion of the population from milk allergy if tested in vivo and followed by clinical trials.

Bibliography

1. Conesa C., *et al*. "Isolation of lactoferrin from milk of different species: calorimetric and antimicrobial studies". *Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology* 150.1 (2008): 131-139.

2. McCullough FS. "Nutritional evaluation of goat's milk". *British Food Journal* (2003).

3. Di Sabatino A and Corazza GR. "Epitope-specific immunotherapy: A vaccine for coeliac disease?" *The Lancet Gastroenterology and Hepatology* 2.7 (2017): 464-465.

4. Ehlayel M., *et al*. "Camel milk: An alternative for cow's milk allergy in children". *Allergy and Asthma Proceedings: The Official Journal of Regional and State Allergy Societies* 32 (2011): 255-258.

5. Gasteiger E., *et al*. "ExPASy: The proteomics server for in-depth protein knowledge and analysis". *Nucleic Acids Research* 31 (2003): 3784-3788.

6. Doytchinova IA and Flower DR. "VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines". *BMC Bioinformatics* 8 (2007): 1-7.

7. Dimitrov I., *et al*. "AllerTOP-a server for in silico prediction of allergens. In BMC Bioinformatics". *Bio Med Central* 14 (2013): 1-9.

8. Chauhan V., *et al*. "Designing a multi-epitope-based vaccine to combat Kaposi Sarcoma utilizing immunoinformatics approach". *Scientific Reports* 9 (2019): 1-15.

9. Geourjon C and Deleage G. "SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments". *Bioinformatics* 11.6 (1995): 681-684.

10. Laskowski R., *et al*. "PROCHECK: validation of protein-structure coordinates" (2006).

11. Wiederstein M and Sippl MJ. "ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins". *Nucleic Acids Research* 35.2 (2007): W407-W410.

12. Kozakov D., *et al*. "The ClusPro web server for protein-protein docking". *Nature Protocols* 12.2 (2017): 255-278.

13. Rapin N., *et al*. "Computational immunology meets bioinformatics: The use of prediction tools for molecular binding in the simulation of the immune system". *PloS One* 5.4 (2010): e9862.

14. Van Regenmortel MH. "Mapping epitope structure and activity: From one-dimensional prediction to four-dimensional description of antigenic specificity". *Methods* 9.3 (1996): 465-472.

15. Hoft DF., *et al*. "Optimizing vaccine development". *Cellular Microbiology* 13.7 (2011): 934-942.

16. Perrie Y., *et al*. "Vaccine adjuvant systems: Enhancing the efficacy of sub-unit protein antigens". *International Journal of Pharmaceutics* 364.2 (2008): 272-280.

17. Ojha R., *et al*. "Strategic development of a next-generation multi-epitope vaccine to prevent Nipah virus zoonotic infection". *ACS Omega* 4.8 (2019): 13069-13079.

18. Pandey RK., *et al*. "Exploring dual inhibitory role of febrifugine analogues against Plasmodium utilizing structure-based virtual screening and molecular dynamic simulation". *Journal of Biomolecular Structure and Dynamics* 35.4 (2017): 791-804.

19. Nezafat N., *et al*. "Designing an efficient multi-epitope peptide vaccine against Vibrio cholerae via combined immunoinformatics and protein interaction based approaches". *Computational Biology and Chemistry* 62 (2016): 82-95.

20. Bergmann CC., *et al*. "Flanking residues alter antigenicity and immunogenicity of multi-unit CTL epitopes". *The Journal of Immunology* 157.8 (1996): 3242-3249.

21. Shahid F., *et al*. "Immunoinformatics guided rational design of a next generation multi epitope based peptide (MEBP) vaccine by exploring Zika virus proteome". *Infection, Genetics and Evolution* 80 (2020): 104199.

22. Aranda V., *et al*. "Shared apical sorting of anion exchanger isoforms AE2a, AE2b1, and AE2b2 in primary hepatocytes". *Biochemical and Biophysical Research Communications* 319.3 (2004): 1040-1046.

23. Patil VM., *et al*. "Chapter 14 - Dengue Virus Polymerase: A Crucial Target for Antiviral Drug Discovery. In S. P. Gupta (Ed.), *Viral Polymerases* (2019): 387-428. Academic Press.

24. Kalita P., *et al*. "Design of a peptide-based subunit vaccine against novel coronavirus SARS-CoV-2". *Microbial Pathogenesis* 145 (2020): 104236.

25. Flicker S., *et al*. "Passive immunization with allergen-specific antibodies". *Vaccines against Allergies* (2011): 141-159.

26. Srinivasan S., *et al*. "Epitope identification and designing a potent multi-epitope vaccine construct against SARS-CoV-2 including the emerging variants". *Journal of Global Infectious Diseases* 14.1 (2022): 24.