Designing a Novel Multi-epitope Subunit Vaccine Against *Trichuris trichiura* by Immunoinformatics-Guided Approach

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ABSTRACT

The prevalence of infections associated with *Trichuris trichiura* is highly significant across the world and has affected 429-508 million individuals, children in particular, according to the Centers for Disease Control and Prevention in 2024. Most cases occur in tropical regions, as the parasite can survive warm and humid temperatures exhibiting abdominal discomfort, chronic diarrhea, and may lead to rectal prolapse while also existing in co-infection with other soil-transmitted helminths.

To help combat this challenge, this study designed a multi-epitope subunit vaccine against *Trichuris trichiura* through immunoinformatics approach: a combination of immunology and bioinformatics. This computational immunology-based vaccine development employed the use of computer systems and predictive tools as well as databases in the retrieval of information about T. trichiura and its immunologic potential. In this study, 63 T. *trichiura* egg extract proteins with known molecular functions were screened via computational servers to identify the potential epitope candidates that can elicit an immune response in the human host. The proteins assessed and shortlisted based on their (1) immunogenicity; (2) antigenicity; (3) toxicity; and (4) allergenicity was connected by linkers forming the vaccine construct which is subjected to immune simulation as well as molecular docking and dynamics simulation studies to determine the affinity and stability of the vaccine-receptor interaction for further validation.

Accordingly, the simulation results have revealed that the construct is a non-allergen, non-toxic, antigenic, and immunogenic vaccine which is found to have the ability to be recognized and trigger immune responses without harm for the host.

INTRODUCTION

- Estimated to affect 429-508 million people globally (CDC, 2024).
- Children are most vulnerable due to soil-eating (pica).
- Warm climate
- Improper sanitation
- Lack of clean water supply
- MOT: Ingestion of eggs from contaminated food or water sources.
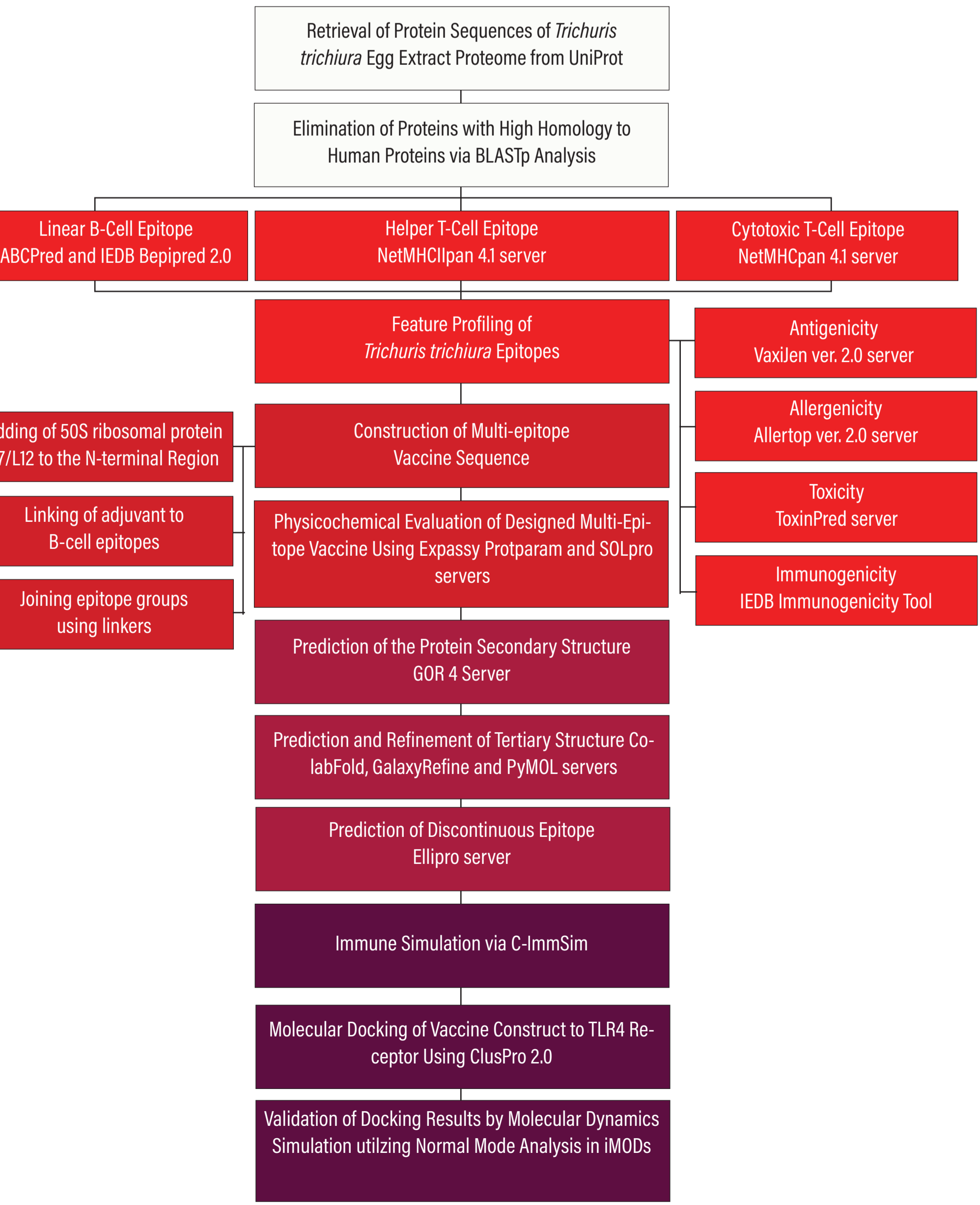- Causes lower GI infection, diarrhea, abdominal pain, and rectal prolapse.
- Uses computational immunology in designing a multi-epitope vaccine.

OBJECTIVES

This study aims to design a multi-epitope subunit vaccine against T. trichiura through an immunoinformatics-methodical approach.

METHODOLOGY

- Retrieval of Protein Sequences of *Trichuris trichiura* Egg Extract Proteome from UniProt
- Elimination of Proteins with High Homology to Human Proteins via BLASTp Analysis
  - Linear B-Cell Epitope ABCPred and IEDB Bepipred 2.0
  - Helper T-Cell Epitope NetMHCIIpan 4.1 server
  - Cytotoxic T-Cell Epitope NetMHCpan 4.1 server
- Feature Profiling of *Trichuris trichiura* Epitopes
  - Antigenicity VaxiJen ver. 2.0 server
- Construction of Multi-epitope Vaccine Sequence
  - Allergenicity AllerTop ver. 2.0 server
- Adding of 50S ribosomal protein L7/L12 to the N-terminal Region
- Linking of adjuvant to B-cell epitopes
  - Toxicity ToxinPred server
- Physicochemical Evaluation of Designed Multi-Epitope Vaccine Using Expassy Protparam and SOLpro servers
  - Immunogenicity IEDB Immunogenicity Tool
- Joining epitope groups using linkers
- Prediction of the Protein Secondary Structure GOR 4 Server
- Prediction and Refinement of Tertiary Structure ColabFold, GalaxyRefine and PyMOL servers
- Prediction of Discontinuous Epitope Ellipro server
- Immune Simulation via C-ImmSim
- Molecular Docking of Vaccine Construct to TLR4 Receptor Using ClusPro 2.0
- Validation of Docking Results by Molecular Dynamics Simulation utilizing Normal Mode Analysis in iMODs

RESULTS AND DISCUSSION

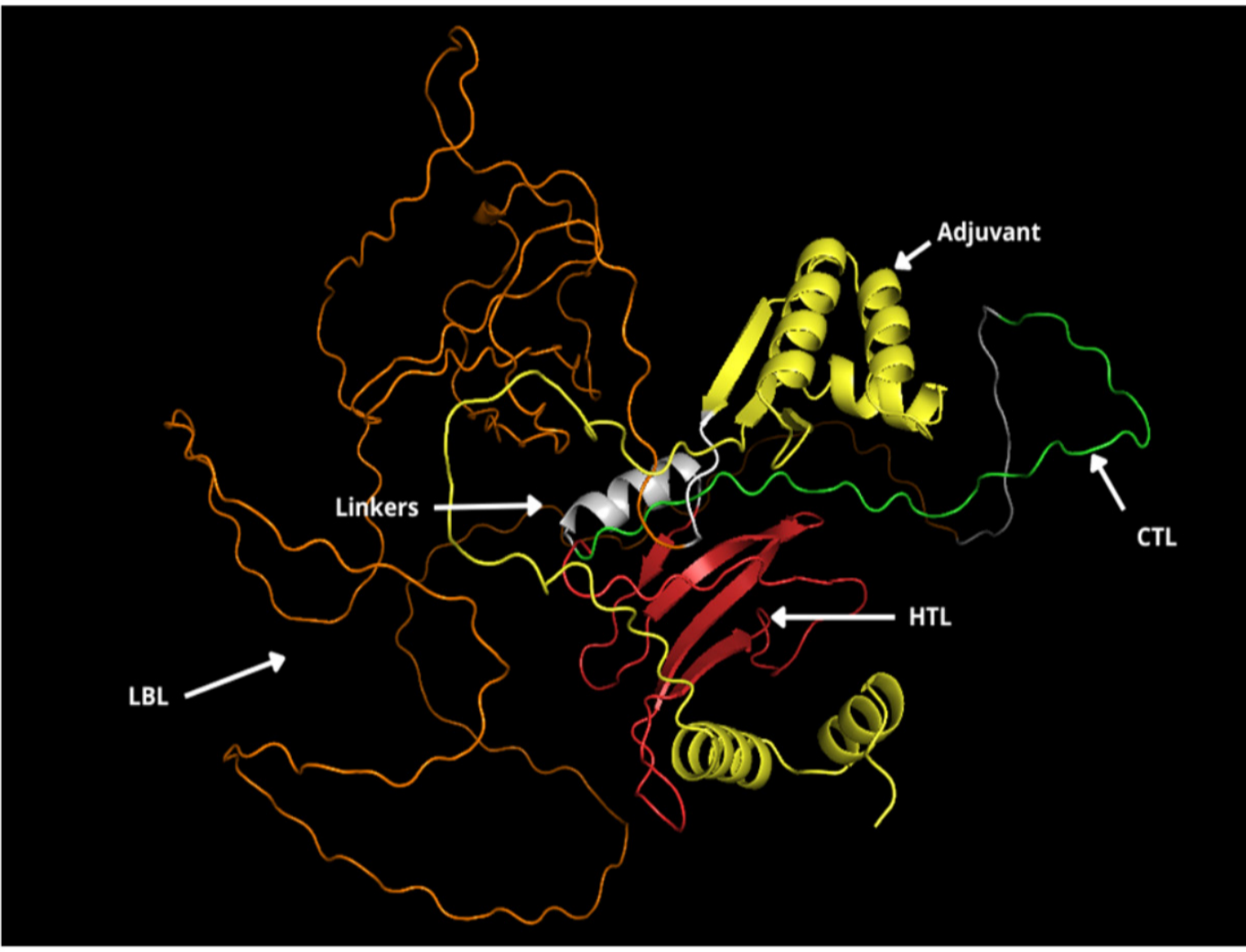### 1 Protein Retrieval & Homoginal Screening
- ✔ 167 protein sequences of T. trichiura egg extract were retrieved.
- ✔ 63 out of 167 have <50% homology with human proteins.

2 Feature Profiling
- ✔ Antigenic
- ✔ Non-toxin
- ✔ Non-allergen
- ✔ Immunogenic
- ✔ IL-4 inducers

3 Multi-Epitope Vaccine Construction

Figure 1. Epitope groups and linkers in the 3D structure of T. trichiura vaccine construct visualized through PyMOL.

- Adjuvant
- Linker
- LBL
- HTL
- CTL

Adjuvant: 50s ribosomal protien L7/L12

4 Physicochemical Properties

Properties	Results	Interpretation
Number of Amino Acids	499	
Molecular Weight	55.28 kDa	
Theoretical Isoelectric Points (pI)	9.21	Basic
Instability Index	27. 84	Protein Stable
Aliphatic Index	73. 01	Thermostable
GRAVY Value	-0. 614	Water-soluble
Antigenic	Non-toxic	Non-allergen

5 Secondary and Tertiary Structure and Discontinuous Epitopes Prediction

Secondary
Residues:
- ✔ 42.89% in the alpha helices
- ✔ 41.28% from the random coils
- ✔ 15.83% from the extended strands

Tertiary
Refined Model 5
- ✔ Highest rama-favored score
- ✔ Low MolProbity
- ✔ Z-score of -2.39

Discontinuous
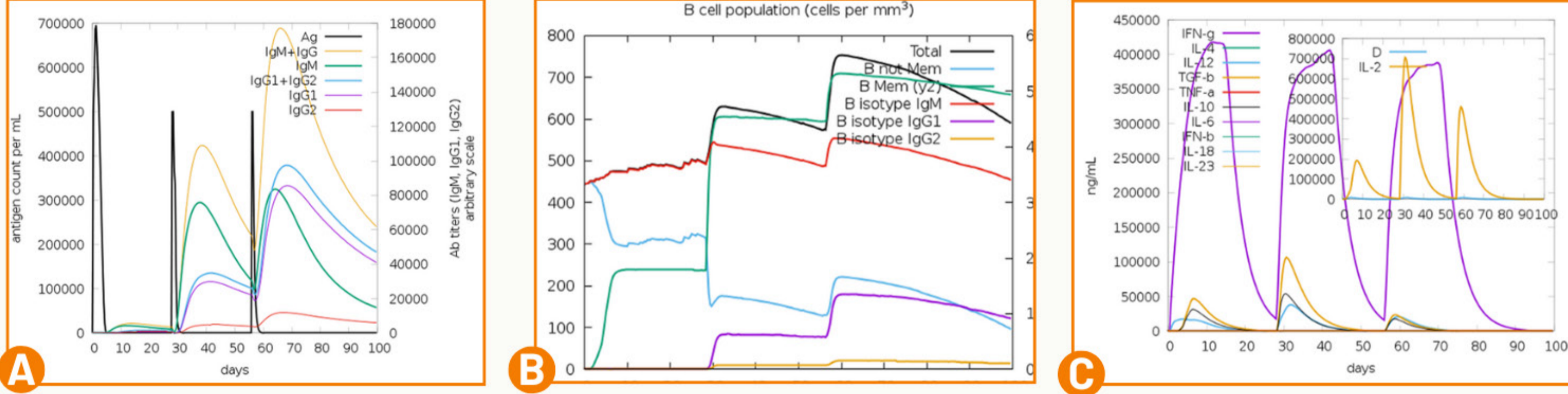- ✔ 27 epitopes
- ✔ 133 residues scoring from 0.712 to 0.964

6 Immune Simulation

Figure 2.

(A) Production of immunoglobulins and their isotypes and antigen levels after three vaccine doses.
(B) B-cell population.
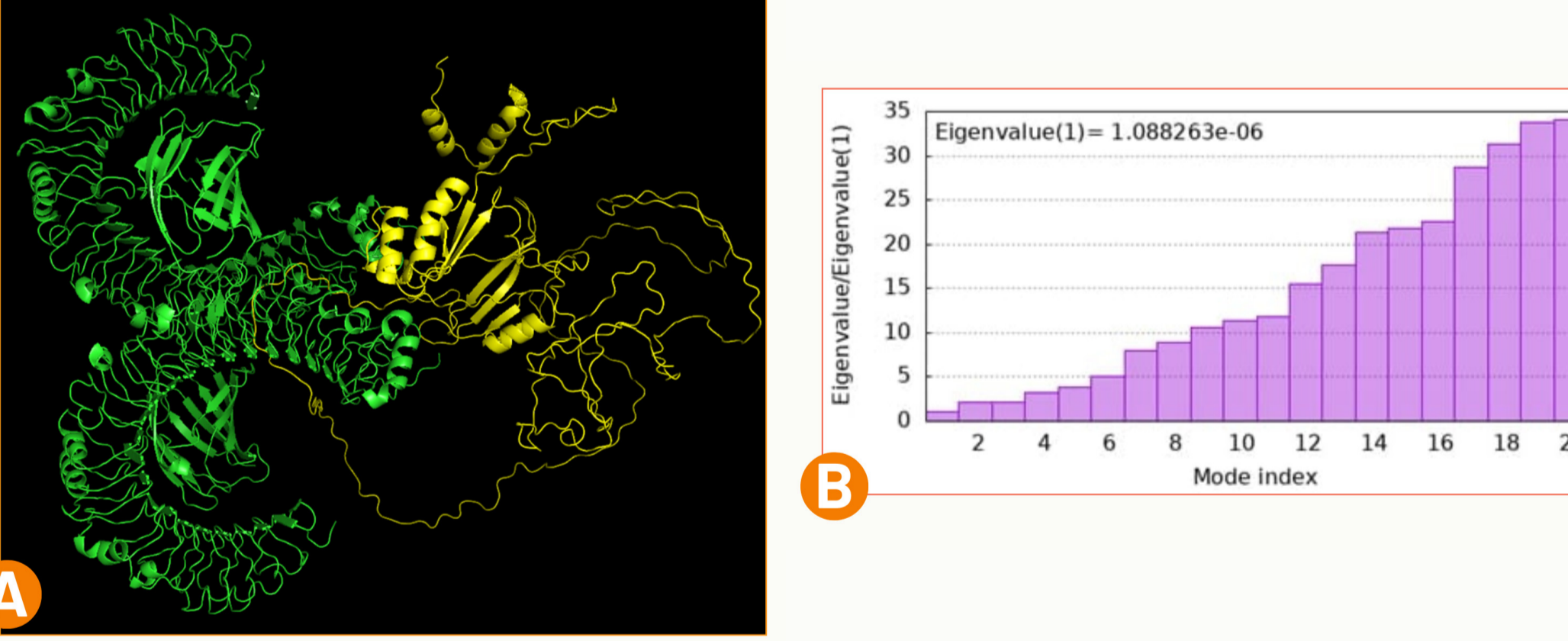(C) Concentration of various cytokines and interleukins.

7 Molecular Docking and Molecular Dynamics Simulation

Figure 3.

(A) Docked Complex of Multi-epitope Vaccine Construct (yellow) and TLR-4 Receptor (green).
(B) Eigenvalues graph representing motion stiffness.

CONCLUSION

The study designed a multi-epitope subunit vaccine against Trichuris trichiura using immunoformatics. The selected LBL, HTL, and CTL epitopes were non-allergens, non-toxins, immunogenic, and antigenic, making them potent candidates for the construction of the multi-epitope vaccine. The final vaccine construct was predicted to be antigenic, immunogenic, safe, protein-stable, and functional, with promising simulation results in interaction with TLR4. Further laboratory testing is recommended to confirm safety and efficacy.

RECOMMENDATION

- In silico Cloning
- Vaccine's Safety and Toxicity Assessment
- Further Validation through in vivo and in vitro studies
- Follow-up Research and Development

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