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Letter to the Editor

Response Comment on "A New Immunogenic Structure of Poly-epitopic Fusion against *Leishmania major*: In Silico Study"

Saeed Pirmoradi [1], Mohammad Darvish Khadem [1], Zohre Monjezi [2], *Somayeh Bahrami [3], Chukwunonso O. Nzelu [4,5]

1. *Department of Basic Sciences, Division of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
2. *Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran*
3. *Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
4. *Department of Microbiology, Immunology, and Infectious Diseases, Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada*
5. *Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada*

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*Correspondence Email: s.bahrami@scu.ac.ir

Dear Editor-in-Chief

The comments on our article "A New Immunogenic Structure of Polyepitopic Fusion against *Leishmania major*: In Silico Study" published in Iran J Parasitol: 2024; 19 (3) were noticed. Thank you for taking the time to review our manuscript and for providing your feedback. Unfortunately, some mistakes occurred, and the version published was not the final edited one. But about the scientific issues: Due to the large number of proteins, providing more details about each one and including additional background would significantly increase the length of the paper and potentially render it less engaging for readers. The next point is that the structure P72151.2 pertains only to the adjuvant used to increase immunogenicity.

It is worth mentioning that the protein named LPG63 in the response letter to the editor is not present in *Leishmania*. They likely meant LPG3 or GP63. In this regard, our general aim was to design a vaccine that could

be used for both *L. major* and *L. infantum*. The sequences of these two epitopes in the two species, despite differing in their accession numbers, are highly conserved, and their sequences are very similar to each other, which does not affect the results of our study.

Regarding the information about the type of HLA alleles, peptide lengths, prediction thresholds, adjuvants, etc. in epitope prediction and vaccine design sections, it should be noted that including information about various epitopes in the table was omitted due to the large number. The esteemed journal editor also requested we reduce certain sections of the analysis results due to the journal's limitations. In this study, we used several up-to-date analytical methods, from structural modeling and immunogenicity to cloning (1), and it was decided to avoid including such data.

Regarding the linker, as demonstrated in numerous studies, the design of vaccine constructs that connect various epitopes has successfully employed this method along with these linkers (2,3). The initial vaccine structure was formed for subsequent evaluations aimed at modeling. The results obtained from modeling this structure through the AlphaFold server (https://alphafold.ebi.ac.uk/), which is a reliable server for protein modeling based on artificial intelligence, indicate the suitability of the designed vaccine model. Furthermore, the validations of the obtained model using various servers, such as PROCHECK and ProSA, demonstrated the quality of the designed vaccine model.

The tertiary structure of the vaccine discussed in the article was validated by both PROCHECK and Prosa servers. The percentage and score affirming the structure of the vaccine are provided in the text of the article, and validation graphs are included as well (Fig. 6- our published article). It is noteworthy that our modeling was performed using the AlphaFold server. Furthermore, to enhance the accuracy percentage of our vaccine model, we utilized refinement tools. In Fig. 7 (our published article), we superimposed the two models before and after refinement using PyMOL, and we also calculated the RMSD to demonstrate the improved quality of the model. Minor corrective differences are illustrated in that image.

The analysis of IFN-γ and other cytokines is presented in chart 10E in the article, and data regarding the examination and prediction of IFN-γ cytokine induction has also been carried out. However, due to the limitations of the article's length and because the results were aligned with the findings of the immunological simulations of the vaccine, we decided not to include this data in the article. Using the IFNepitope server (http://crdd.osdd.net/raghava/ifnepitope/), prediction of IFN-γ inducing epitopes of MHC-II epitopes was performed. The server predicts the epitopes based on SVM-based, motif-based, and hybrid approaches (4).

The importance of B cells and humoral response in protective responses to *Leishmania* should not be fully discarded. The role of the humoral response can be influenced by *Leishmania* isolates, the host itself, the host's immune status, and the stage of the disease. Therefore, if both immune system components are mentioned as playing roles in the article, it is based on this premise. In different articles for *L. major*, B cell epitopes were considered for vaccine design and evaluation (5-7).

The next point to note is that not all CD8 T cells are involved in the production of IFN-γ. Our article presents results based on the outputs of the software used, which indicate an increase in IFN-γ, and our focus was generally on the subset of these cells that have beneficial roles. The effects assessed based on these cytokines are included in the immunological simulation section of the article, presented through immunological simulation charts that we have provided in full. Furthermore, two charts derived from IEDB highlight the vaccine's highly impactful sites in terms of epitope availability for the immune system and their high antigenicity (1,8).

In summary, a review of recent articles in the area of vaccine design would reveal that all respected tools and methods used by researchers have been applied in our study, collectively validating the reliability of our findings. Furthermore, all requested data are available and have been sent to the journal. It is important to note, however, that this study was conducted in silico; thus, further validation through in vitro and in vivo experiments and clinical trials is necessary for definitive confirmation.

Conflict of Interest

The authors declare that there is no conflict of interest.

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