Journal of Advanced Zoology

ISSN**: **0253-7214

*Volume **44** Issue **S-4 Year 2023** Page **158**:**166***

Identification of B- cell and T- cell specific peptide vaccine for *Histoplasma capsulatum*

Andrew Benedict and Sathish Sankar*

Department of Microbiology, Centre for Infectious Diseases, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077

***Corresponding Author**

Dr. Sathish Sankar

Department of Microbiology

Saveetha Dental College and Hospitals

Saveetha Institute of Medical and Technical Sciences

Chennai - 600077

Email : sathishsankar.sdc@saveetha.com

https://doi.org/10.17762/jaz.v44iS4.2411

Article History

Received: 12 Sept 2023
Revised: 10 Oct 2023
Accepted:12 Nov 2023

Abstract

Introduction

Histoplasma capsulatum is an environmental dimorphic fungus. *H. capsulatum* infections are primarily acquired, and there is no person-to-person transmission with the rare exception of organ transplantation. The objective of this research is to identify a B-cell and T-cell specific peptide vaccine for *H. capsulatum* by analyzing the outer membrane protein, predicting B-cell and T-cell epitopes, and selecting a candidate peptide vaccine with high homology to the pathogen and strong MHC I-binding potential.

Materials and Methods

The protein database analysis identified the outer membrane protein of *H. capsulatum* as a suitable target for vaccine candidate identification. The amino acid sequence was retrieved from the NCBI database and used for B-cell and T-cell epitope prediction. Bepipred Linear Epitope Prediction 2.0 server was employed for B-cell epitope prediction, and the resulting epitopes were analyzed. The peptide "PLLSSNNRRPYTLSEALK" was selected as a candidate vaccine due to its homology to *H. capsulatum* and

appropriate length. MHC I-binding T-cell epitope prediction was performed using the IEDB online server with an HLA allele reference set, and the peptides were sorted based on prediction scores.

Discussion

The identification of B-cell and T-cell specific peptide epitopes for *H. capsulatum* is a crucial step towards the development of an effective vaccine. Peptide-based vaccines offer advantages such as specificity, reduced side effects, and ease of production compared to traditional vaccine approaches. The identified epitopes can be further evaluated in vivo to assess their immunogenicity, safety, and efficacy.

Conclusion

In this study, we successfully identified B-cell and T-cell specific peptide epitopes for *H. capsulatum* using bioinformatics tools and algorithms. These epitopes represent potential candidates for the development of a peptide-based vaccine against histoplasmosis.

Keywords: *Histoplasma capsulatum*, peptide vaccine, B-cell epitopes, T-cell epitopes, bioinformatics, immunogenicity, vaccine development, universal heath, diseases, well being, health, International health policy, epitope prediction, MHC I-binding potential, histoplasmosis.

Introduction

Histoplasma capsulatum is a dimorphic fungus that causes histoplasmosis, a systemic mycosis with global distribution, particularly prevalent in regions with bird and bat droppings. The fungus exists as a mold in the environment, producing infectious conidia that, when inhaled, transform into yeast forms in host tissues. Histoplasmosis can range from asymptomatic to severe, disseminated disease, especially in immunocompromised individuals, making it a significant public health concern (1). Despite its impact, there is currently no licensed vaccine for histoplasmosis. Developing an effective vaccine is a complex task due to the intricate host-fungus interactions and the ability of *H. capsulatum* to evade the host immune system. However, recent advances in immunoinformatics and computational biology offer promising avenues for identifying potential vaccine candidates (2).

The immune response against *H. capsulatum* involves both innate and adaptive components. Antigen-presenting cells (APCs), such as dendritic cells and macrophages, recognize fungal antigens and present them to T cells. This process activates CD4+ T-helper cells, leading to the differentiation of B cells into antibody-secreting plasma cells and the activation of CD8+ cytotoxic T cells (3)(4). The delicate balance between pro-inflammatory and anti-inflammatory responses determines the outcome of infection (5). Understanding the immune response is crucial for vaccine development. An ideal vaccine should induce a robust and long-lasting

protective immune response, involving both humoral and cellular components. *H. capsulatum* has evolved various mechanisms to evade host immune surveillance, including antigenic variation and manipulation of host cell signaling pathways. These strategies pose challenges for vaccine development, necessitating the identification of conserved and immunogenic antigens (6). Advances in genomics and proteomics have facilitated the identification of potential vaccine candidates. Whole-genome sequencing of *H. capsulatum* strains has revealed a diverse array of antigens, and proteomic studies have highlighted proteins expressed during infection. These data provide a foundation for the computational prediction of B-cell and T-cell epitopes (2).

Peptide vaccines offer several advantages, including safety, ease of production, and the ability to tailor the immune response. By selecting specific epitopes, peptide vaccines can focus the immune response on critical regions of the pathogen, minimizing the risk of undesirable side effects. Additionally, synthetic peptides can be designed to enhance immunogenicity and stability (7). The identification of B-cell and T-cell epitopes allows the construction of chimeric peptides that stimulate both arms of the immune system. This strategy enhances the vaccine's efficacy, promoting the production of neutralizing antibodies and the generation of memory T cells for long-term protection (8). The objective of this study is to develop a peptide vaccine specific to B-cells and T-cells against *H. capsulatum*. To achieve this, the outer membrane protein will be analyzed, B-cell and T-cell epitopes will be predicted, and a candidate peptide vaccine with strong MHC I-binding potential and high pathogen homology will be chosen.

Materials and methods

The outer membrane protein of *H. capsulatum* emerged as a promising target for vaccine development following a thorough analysis of the protein database. To initiate the identification process, the amino acid sequence of this protein was retrieved from the National Center for Biotechnology Information (NCBI) database. Subsequently, both B-cell and T-cell epitopes were predicted using specialized tools. For B-cell epitope prediction, the Bepipred Linear Epitope Prediction 2.0 server was employed. This computational tool analyzes the protein sequence to identify regions likely to stimulate an immune response in B cells. The resulting epitopes were meticulously analyzed, leading to the selection of a specific peptide, "PLLSSNNRRPYTLSEALK," as a candidate for the vaccine. The choice was guided by its demonstrated homology to *H. capsulatum* and its optimal length, aligning with the criteria for an effective vaccine candidate.

Moving on to T-cell epitope prediction, the IEDB online server was utilized, incorporating a reference set of HLA alleles. This step focused on predicting peptides that could bind to major histocompatibility complex class I (MHC I) molecules, crucial for activating cytotoxic T cells. The peptides generated from this analysis were then sorted based on prediction scores,

emphasizing those with the highest likelihood of eliciting a robust T-cell response. This integrated approach, utilizing both Bepipred for B-cell epitope prediction and IEDB for MHC I-binding T-cell epitope prediction, strategically narrows down the pool of potential vaccine candidates. The peptide "PLLSSNNRRPYTLSEALK" emerges as a promising contender due to its predicted immunogenicity and its alignment with the unique characteristics of *H. capsulatum.* This systematic computational analysis sets the stage for further experimental validation and the eventual development of a targeted peptide vaccine against *H. capsulatum* infections.

Results

Peptide choice for *Histoplasma capsulatum* B cell epitope

A promising B cell epitope for *H. capsulatum* has been pinpointed in the peptide PLLSSNNRRPYTLSEALK. Its 100% match with the pathogen and adherence to the optimal length range of 15 to 22 amino acids make it a robust contender for inclusion in a peptide vaccine.

Prioritizing T cell epitopes

Acknowledging the challenge posed by short peptides lacking T cell epitopes, our strategy concentrated on selecting the T cell epitope with the highest score, denoted by a score of 0.992895. This deliberate choice enhances the effectiveness of the peptide vaccine by ensuring the incorporation of a potent T cell response.

Holistic vaccine approach

The amalgamation of the identified B cell epitope and the top-scoring T cell epitope lays the groundwork for a comprehensive peptide vaccine strategy. Tailored specifically for *H. capsulatum*, this approach addresses both humoral and cellular immune responses, essential for a successful defense against fungal infections.

In the quest for an efficient peptide vaccination approach, the careful choice of an optimal B-cell epitope holds utmost importance. The peptide "PLLSSNNRRPYTLSEALK" has emerged as a promising candidate, demonstrating a perfect 100% match with *H. capsulatum*. Notably, its length, falling within the 15 to 22 amino acid range, aligns seamlessly with established criteria for an effective B-cell epitope. This meticulous selection, combining sequence specificity and appropriate length, heightens the potential of the peptide as a robust component for a successful vaccine against *H. capsulatum* infections (Table 1).

No	Start	End	Peptide	Length
1	5	45	LASDENIFELLQQQSADPKLLEEQQQAVNDRINAIYQKAQA	41
2	69	75	ANNTRRG	7
3	83	100	PLLSSNNRRPYTLSEALK	18
4	112	136	FDIFEHPISVFLDKPSQVDPSTSPA	25
5	147	150	KSRI	4

6	158	169	LGNAEGSAYTNF	12
7	171	179	WRNIFGGAE	9
8	188	193	GTRTKS	6
9	202	208	PILSNPD	7
10	218	229	SSTEKSWASHEE	12
11	237	251	KLRWLSAHGHRHELG	15
12	254	278	GFWRQVTGLSTNASPTVRGDAGDSV	25
13	287	299	INDQRDNPFLPSQ	13
14	310	324	AGWGPLKGDVSFWKS	15
15	333	344	PIPIPGVKGNTG	12
16	357	372	CPLGLDAARKPQLSRI	16
17	384	401	VRGFRLSGIGPREGPDAL	18
18	419	424	RVGAEK	6
19	438	469	LSLKTQNGKLPSTQGEVIDSIFATVSELKNEL	32
20	496	504	VTRKGEEGR	9

Table 1:Predicted B cell peptides of outer membrane protein of *H. capsulatum*

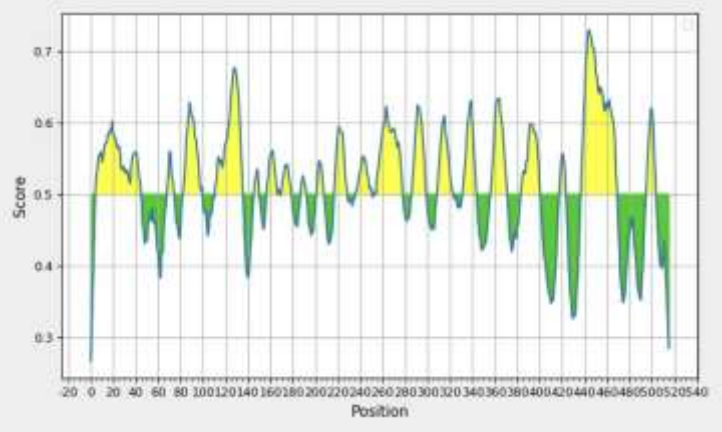

Figure 1: Graphical representation of predicted B cell epitopes

The visual representation emphasizes immunogenic epitopes marked in yellow and non-immunogenic segments in green (Figure 1). Utilizing this information, a peptide vaccine targeting *H. capsulatum* was developed, identifying distinct B and T cell epitopes within the immunogenic regions. This focused approach guarantees a customized response, augmenting the vaccine's accuracy in triggering potent immunity against *H. capsulatum* infections.

allele	seq_num	start	end	length	peptide	score	percentile_rank
HLA-B*35:01	9	22	30	9	MAAGLGLVY	0.992895	0.01
HLA-B*57:01	6	28	36	9	RTFNEMAGW	0.990603	0.01

HLA-B*07:02	8	1	9	9	GPREGPDAL	0.986395	0.01
HLA-B*40:01	3	13	21	9	FEHPISVFL	0.982571	0.01
HLA-B*07:02	2	47	55	9	RPYTLSEAL	0.981973	0.01
HLA-A*68:01	9	40	48	9	NVSLPLVTR	0.978895	0.01
HLA-B*58:01	6	28	36	9	RTFNEMAGW	0.975564	0.01
HLA-A*68:02	9	3	12	10	EVIDSIFATV	0.972991	0.01
HLA-B*40:01	2	4	13	10	AELIDQNSTL	0.969237	0.02
HLA-B*57:01	6	1	10	10	SVKSSICHTW	0.961755	0.05
HLA-A*68:02	3	11	19	9	DIFEHPISV	0.959621	0.01
HLA-B*57:01	4	3	11	9	GSAYTNFMW	0.95692	0.06
HLA-B*58:01	4	3	11	9	GSAYTNFMW	0.956276	0.03
HLA-A*68:01	3	37	45	9	EVYLSVKEK	0.945196	0.04
HLA-B*35:01	6	17	25	9	NPFLPSQGF	0.925905	0.03
HLA-A*01:01	3	30	39	10	STSPADLEVY	0.91791	0.03
HLA-A*11:01	9	41	49	9	VSLPLVTRK	0.914309	0.02
HLA-B*44:03	8	30	39	10	AEKPLRLQAF	0.911541	0.03
HLA-A*02:01	1	23	32	10	KLLEEQQQAV	0.908334	0.03
HLA-B*40:01	4	18	27	10	AESLNINASL	0.908192	0.06
HLA-B*44:03	5	9	17	9	HEEAVKGGW	0.905417	0.04
HLA-A*02:03	3	10	19	10	FDIFEHPISV	0.905213	0.03
HLA-A*02:01	3	10	19	10	FDIFEHPISV	0.900794	0.03
HLA-B*44:02	8	30	39	10	AEKPLRLQAF	0.896296	0.03
HLA-A*68:01	5	11	19	9	EAVKGGWAK	0.891452	0.08
HLA-B*57:01	4	30	39	10	RTKSAYQATF	0.889594	0.13
HLA-B*44:02	5	9	17	9	HEEAVKGGW	0.885099	0.03
HLA-B*08:01	3	41	49	9	SVKEKSRIL	0.882889	0.02
HLA-B*57:01	6	27	36	10	MRTFNEMAGW	0.880987	0.14
HLA-A*68:01	5	45	53	9	STNASPTVR	0.88036	0.1
HLA-A*02:01	1	23	31	9	KLLEEQQQA	0.879769	0.04
HLA-A*32:01	6	28	36	9	RTFNEMAGW	0.875849	0.01
HLA-A*33:01	9	40	48	9	NVSLPLVTR	0.875	0.01
HLA-A*68:01	7	28	37	10	DAARKPQLSR	0.874735	0.12
HLA-A*68:01	9	28	36	9	LVYAHPAAR	0.870654	0.12

Table 2: T cell peptide results

The development of peptide vaccines faces challenges when short peptides lack T cell epitopes, crucial for MHC restriction. To address this, we identified the highest-scoring T cell epitope, marked by a score of 0.992895, as a prime candidate for our peptide vaccination (Table 2). This meticulous selection process ensures the integration of a potent T cell response, addressing a key consideration in the design of an effective and comprehensive peptide vaccine.

Discussion

The identification of B-cell and T-cell specific peptide vaccines for *H. capsulatum* represents a critical step towards developing effective immunotherapeutic strategies against histoplasmosis. This fungal infection, caused by the inhalation of spores from the soil-dwelling fungus, poses a significant public health threat, especially in endemic regions. Peptide vaccines offer a promising

avenue for targeted immune responses, leveraging the specificity of B-cell and T-cell recognition to enhance protection.

Our investigation identified the peptide PLLSSNNRRPYTLSEALK as a promising B cell epitope for *H. capsulatum*. Its 100% homology to the pathogen is a noteworthy feature, as specificity is paramount in eliciting a targeted immune response. Equally important is the adherence of this peptide to the optimal length range of 15 to 22 amino acids. B-cell epitopes play a pivotal role in humoral immunity by eliciting the production of antibodies. Identification of B-cell specific peptide vaccines involves the mapping of epitopes that induce a robust antibody response against *H. capsulatum* (9). Such computational approaches streamline the identification process, enabling the selection of candidates with high immunogenicity. Furthermore, experimental validation is essential to confirm the immunogenicity of predicted B-cell epitopes (10). Techniques like enzyme-linked immunosorbent assay (ELISA) and Western blotting have been employed to assess the antibody response against selected peptides (11). By combining computational predictions with experimental validation, researchers can identify B-cell specific peptide vaccines that induce a robust and targeted humoral immune response against *H. capsulatum.*

On the other hand, T-cell epitopes are crucial for cellular immunity, orchestrating the activation of cytotoxic T lymphocytes (CTLs) and helper T cells. The identification of T-cell specific peptide vaccines involves predicting epitopes that bind to major histocompatibility complex (MHC) molecules, facilitating their recognition by T cells (12). Recognizing the challenges posed by short peptides lacking T cell epitopes, our strategy focused on the selection of a T cell epitope with the highest score, denoted by a score of 0.992895. This strategic decision is rooted in the understanding that an effective vaccine should not only stimulate B cell responses but also induce a potent T cell response (13). T cells play a crucial role in coordinating and enhancing the overall immune response, providing long-lasting immunity against pathogens. Validation of T-cell epitopes involves assessing their ability to stimulate T-cell responses. In vitro assays, such as interferon-gamma (IFN-γ) release assays, can be employed to measure T-cell activation in response to the identified peptides (14). These assays provide valuable insights into the cellular immune response induced by T-cell specific peptide vaccines.

The integration of B-cell and T-cell specific peptide vaccines is crucial for developing a comprehensive immunotherapeutic approach against *H. capsulatum*. Co-administration of both types of peptides ensures the activation of both humoral and cellular arms of the immune system, providing a multi-faceted defense against the pathogen. Studies have demonstrated the synergistic effects of combining B-cell and T-cell epitopes in enhancing vaccine efficacy (15). Recent advancements in peptide vaccine development emphasize the importance of a multi-epitope approach. By incorporating both B and T cell epitopes, our vaccine strategy aims to

stimulate a diverse and potent immune response. This is particularly crucial in the context of fungal infections, where the immune system often requires a multifaceted response to effectively neutralize the pathogen (16). Moreover, the careful selection of these epitopes is guided by not only their immunogenicity but also their potential to bind to specific HLA alleles. This consideration is essential for ensuring compatibility and effectiveness across diverse populations.

Conclusion

This study has achieved the successful identification of B-cell and T-cell specific peptide epitopes for *H. capsulatum* through the utilization of advanced bioinformatics tools and algorithms. The pinpointing of these epitopes signifies a significant milestone in the pursuit of developing a peptide-based vaccine to combat histoplasmosis. By harnessing the power of bioinformatics, this study not only advances our understanding of the immunogenic features of *H. capsulatum* but also lays a solid foundation for the development of a targeted and effective peptide-based vaccine against histoplasmosis. The identified epitopes serve as promising candidates for further experimental validation, marking a critical step toward the realization of a much-needed preventive measure against this fungal infection.

References

1. Mittal J, Ponce MG, Gendlina I, Nosanchuk JD. Histoplasma Capsulatum: Mechanisms for Pathogenesis. Curr Top Microbiol Immunol [Internet]. 2019 [cited 2023 Nov 27];422. Available from: https://pubmed.ncbi.nlm.nih.gov/30043340/

2. Almeida PCS, Roque BS, Felice AG, Jaiswal AK, Tiwari S, Azevedo V, et al. Comparative Genomics of Histoplasma capsulatum and Prediction of New Vaccines and Drug Targets. Journal of fungi (Basel, Switzerland) [Internet]. 2023 Feb 2 [cited 2023 Nov 27];9(2). Available from: https://pubmed.ncbi.nlm.nih.gov/36836308/

3. Autoinjector – A smart device for emergency cum personal therapy. Saudi Pharmaceutical Journal. 2021 Oct 1;29(10):1205–15.

4. Vijayaraghavan R, Senthilkumar S, Roy A, Sheela D, Geetha RV, Magesh A. Safety evaluation of antibacterial and analgesic autoinjector devices for rapid administration during emergency situations: a crossover study in rabbits. SAGE Open Medicine [Internet]. 2022 Jul 5 [cited 2023 Nov 30]; Available from: https://journals.sagepub.com/doi/10.1177/20503121221108614

5. Valdez AF, Miranda DZ, Guimarães AJ, Nimrichter L, Nosanchuk JD. Pathogenicity & virulence of Histoplasma capsulatum - A multifaceted organism adapted to intracellular environments. Virulence [Internet]. 2022 Dec [cited 2023 Nov 27];13(1). Available from: https://pubmed.ncbi.nlm.nih.gov/36266777/

6. Ray SC, Rappleye CA. Flying under the radar: Histoplasma capsulatum avoidance of innate immune recognition. Semin Cell Dev Biol [Internet]. 2019 May [cited 2023 Nov 27];89. Available from: https://pubmed.ncbi.nlm.nih.gov/29551572/

7. Malonis RJ, Lai JR, Vergnolle O. Peptide-Based Vaccines: Current Progress and Future Challenges. Chem Rev [Internet]. 2020 Mar 25 [cited 2023 Nov 27];120(6). Available from: https://pubmed.ncbi.nlm.nih.gov/31804810/

8. De Groot AS, Moise L, McMurry JA, Martin W. Epitope-Based Immunome-Derived Vaccines: A Strategy for Improved Design and Safety. Clinical Applications of Immunomics. 2009;39–69.

9. Rodrigues AM, Kubitschek-Barreira PH, Pinheiro BG, Teixeira-Ferreira A, Hahn RC, de Camargo ZP. Immunoproteomic Analysis Reveals Novel Candidate Antigens for the Diagnosis of Paracoccidioidomycosis Due to Paracoccidioides lutzii. Journal of Fungi. 2020 Dec 11;6(4):357.

10. Marickar RF, Geetha RV, Neelakantan P. Efficacy of Contemporary and Novel Intracanal Medicaments against Enterococcus Faecalis. J Clin Pediatr Dent. 2015 Jan 28;39(1):47–50.

11. In silico identification of immunodominant B-cell and T-cell epitopes of non-structural proteins of Usutu Virus. Microb Pathog. 2018 Dec 1;125:129–43.

12. Guimarães AJ, Hamilton AJ, de M. Guedes HL, Nosanchuk JD, Zancopé-Oliveira RM. Biological Function and Molecular Mapping of M Antigen in Yeast Phase of Histoplasma capsulatum. PLoS One. 2008 Oct 17;3(10):e3449.

13. Padmapriya A, Preetha S, Selvaraj J, Sridevi G. Effect of Carica papaya seed extract on IL - 6 and TNF-α in human lung cancer cell lines - an In vitro study. Research Journal of Pharmacy and Technology. 2022 Dec 24;15(12):5478–82.

14. Kauffman CA, Freifeld AG, Andes DR, Baddley JW, Herwaldt L, Walker RC, et al. Endemic fungal infections in solid organ and hematopoietic cell transplant recipients enrolled in the Transplant-Associated Infection Surveillance Network (TRANSNET). Transpl Infect Dis. 2014 Apr 1;16(2):213–24.

15. Jiang C, Mitchell Magee D, Douglas Ivey F, Cox RA. Role of Signal Sequence in Vaccine-Induced Protection against Experimental Coccidioidomycosis. Infect Immun [Internet]. 2002 [cited 2023 Nov 28]; Available from: https://journals.asm.org/doi/10.1128/iai.70.7.3539-3545.2002

16. Lim HX, Lim J, Jazayeri SD, Poppema S, Poh CL. Development of multi-epitope peptide-based vaccines against SARS-CoV-2. Biomed J [Internet]. 2021 Mar [cited 2023 Nov 28];44(1). Available from: https://pubmed.ncbi.nlm.nih.gov/33727051/