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Identification of B-cell and T-cell specific peptide vaccine for *Pneumocystis jirovecii*

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Abstract:

Introduction:*Pneumocystis jirovecii* is a fungal pathogen that causes a severe lung infection primarily in individuals with compromised immune systems. The high morbidity and mortality associated with *Pneumocystis jirovecii pneumonia* (PJP) highlight the need for effective preventive strategies.Peptide vaccines offer several advantages, including their specificity, reduced risk of adverse reactions, and potential for customization. To develop an effective peptide vaccine, it is crucial to identify B-cell and T-cell specific epitopes that can stimulate the immune system to produce antibodies and activate T-cells against *Pneumocystis jirovecii*.

The aim of identifying B-cell and T-cell specific peptide vaccine for *Pneumocystis jirovecii* is to develop an effective immunization strategy against this fungal pathogen.

Materials and methods:Using the server https://www.uniprot.org/proteomes, the protein databases were analyzed for suitable targets for the identification of vaccine candidate.Inosine 5' monophosphate dehydrogenase located in genomic DNA was selected for the study. Pneumocystis jirovecii isolate T1 inosine 5' monophosphate dehydrogenase (IMPDH) gene, complete cds that is 2,070 bp size (Accession: MZ272376.1) was used for the study. Bepipred Linear Epitope Prediction 2.0 online server program was used to predict the B cell immunogenic epitopes.The resulted epitopes were analyzed.

Results: From the peptides obtained we can say that, the peptide GIGVIHHNCTIEEQTEMVRKVKKFEN of length 26mer can be considered an effective B cell peptide for preparing a B cell peptide vaccine against *Pneumocystis jirovecii*. From table 1, the peptide with length of 9/10mer and a high percentile rank can be an ideal t cell peptide. From the table obtained, we

can choose either VVDKGSLHVY or EEAKEKLKEY peptide as the ideal peptide for the vaccine against *Pneumocystis jirovecii*, due to its low percentile rank of 0.01 and ideal length of 10mer.
Discussion: Identifying appropriate antigens and epitopes from *Pneumocystis jirovecii's* complex proteome is a challenge. Genome and proteome analyses help identify vital proteins for the pathogen's survival. Using bioinformatics tools and prediction algorithms, potential B-cell and T-cell epitopes are screened based on antigenicity, conservation, and binding affinity.
Conclusion: In conclusion, the identification of B-cell and T-cell specific peptide vaccines for *Pneumocystis jirovecii* is a crucial research area for developing targeted immunization strategies against this fungal pathogen.

Keywords: Universal health, diseases, well being, health and international health policy,pneumonia, peptide vaccine

Introduction:

Pneumocystis jirovecii(formerly *P. carinii*) is an incentive-like fungus belonging to the family Pneumocystis(1).The causative agent of Pneumocystis pneumonia, it's an important mortal pathogen, especially among immunocompromised hosts. Before its discovery as a mortal-specific pathogen,*P. jirovecii* was known as *P.carnii*.*Pneumocystis jirovecii*, an opportunistic fungal pathogen, poses a significant trouble to immunocompromised individualities, particularly those with disabled T- cell function, similar as HIV/ AIDS cases, organ transplant donors, and those entering immunosuppressive remedy. The lack of an extensively effective vaccine against *Pneumocystis jirovecii* underscores the critical need for innovative immunization strategies(2). Cases presenting with PCP may show signs of fever, cough, dyspnea, and, in severe cases, respiratory failure.

Recent advances in vaccine development have shifted the focus to peptide- grounded vaccines that exploit the particularity of B- cell and T- cell responses. Peptide vaccines offer a targeted approach, minimize side goods and maximize the vulnerable response against the pathogen. In the environment of *Pneumocystis jirovecii*, the identification of specific B- cell and T- cell epitopes is crucial to the development of an effective and picky vaccine(3). B- cell epitopes responsible for converting humoral impunity are pivotal in negating extracellular pathogens. Meanwhile, T- cell epitopes play a crucial part in the activation of cellular impunity, which is particularly important for intracellular pathogens similar to *Pneumocystis jirovecii*. Integrating both B- cell and T- cell epitopes in a single vaccine medication can induce a comprehensive vulnerable response, furnishing strong protection against infection(4).

The aim of this study is to identify and characterize immunogenic B- cell and T- cell epitopes within *Pneumocystis jirovecii*, paving the way for the development of a peptide vaccine. Using state- of- the- art bioinformatics tools, epitope vaticination algorithms and experimental confirmation styles, we aim to pinpoint the sequences that spark a strong and specific vulnerable response. Having decrypted the features of the vulnerable system of *Pneumocystis jirovecii*, we aim to contribute to the development of an acclimatized peptide vaccine suitable to strengthen host defenses against this fugitive fungal pathogen. Such a vaccine holds the pledge of enhancing defensive impunity in vulnerable populations and reducing morbidity and mortality associated with *Pneumocystis jirovecii* infections (5).

Materials and methods:

Epitope mapping is a process that involves identifying and characterizing the specific regions on an antigen (typically a protein or peptide) that are recognized and bound by antibodies, T-cell receptors (TCRs), or other immune system components. These recognized regions are known as epitopes, and the mapping of these epitopes is crucial for understanding the immune response to a particular antigen. Epitope mapping provides insights into the molecular interactions between the immune system components and the antigen, facilitating the design of targeted vaccines, diagnostics, and therapeutic antibodies. Peptide mapping is a comprehensive library of overlapping peptides spanning the entire sequence of the antigen. These peptides are then individually tested to identify the specific peptide sequences recognized by antibodies or TCRs.Antigen or peptide fragments are immobilized on a surface, and their interaction with antibodies or TCRs is detected using enzyme-conjugated secondary antibodies. This method allows for the identification of regions on the antigen that are bound by the immune components.Computational tools are used to analyze antigen sequences and predict potential epitopes based on structural and sequence data, as well as physicochemical properties.Epitope mapping is essential for designing effective vaccines, understanding autoimmune diseases, and developing targeted therapies. The knowledge gained from epitope mapping contributes to a deeper understanding of immune responses and aids in the development of immunotherapeutics with enhanced specificity and efficacy.

Using the server https://www.uniprot.org/proteomes, the protein databases were analyzed for suitable targets for the identification of vaccine candidates.

Inosine 5' monophosphate dehydrogenase located in genomic DNA was selected for the study.

Pneumocystis jirovecii isolate T1 inosine 5' monophosphate dehydrogenase (IMPDH) gene, complete cds that is 2,070 bp size (Accession: MZ272376.1) was used for the study

Bepipred Linear Epitope Prediction 2.0 online server program was used to predict the B cell immunogenic epitopes

The resulting epitopes were analyzed. Peptide SPGQYYYRDGQRLKSYRGMG was selected as it was homologous (100%) to the P. jiroveci strain and the length was 20 mers appropriate as a candidate vaccine.

MHC I – binding T cell epitope prediction was carried out using http://tools.iedb.org/mhci/ online server program

This tool will take in an amino acid sequence, or set of sequences and determine each subsequence's ability to bind to a specific MHC class I molecule.

HLA allele reference set were used for the prediction.

The peptides were sorted by the prediction score.

Results:

Figure 1 shows the immunogenic and non immunogenic response of the b cell peptide vaccine . The yellow part represents the immunogenic response and the green part represents the non immunogenic response. The table 1 shows the peptides identified by the program considering many factors including amino acid composition, their length,sequence number, score, percentile mark, etc. Table 2 shows B cell response. Peptide (9mer or 10mer) induces a good T cell immune response. Among these, peptides around 15-25mer induce ideal B cell response.

In the B cell response graph, the part highlighted in yellow shows immunogenic response and the part highlighted in green shows non-immunogenic response. Then we have represented the peptides for immunogenic response in the form of a table. The ideal peptide should be of length 20mer to induce B cell

response. From the peptides obtained we can say that, the peptide GIGVIHHNCTIEEQTEMVRKVKKFEN of length 26mer can be considered an effective B cell peptide for preparing a B cell peptide vaccine against *Pneumocystis jirovecii*. From table 1, the peptide with length of 9/10mer and a high percentile rank can be an ideal t cell peptide. From the table obtained, we can choose either VVDKGSLHVY or EEAKEKLKEY peptide as the ideal peptide for the vaccine against *Pneumocystis jirovecii*, due to its low percentile rank of 0.01 and ideal length of 10mer.

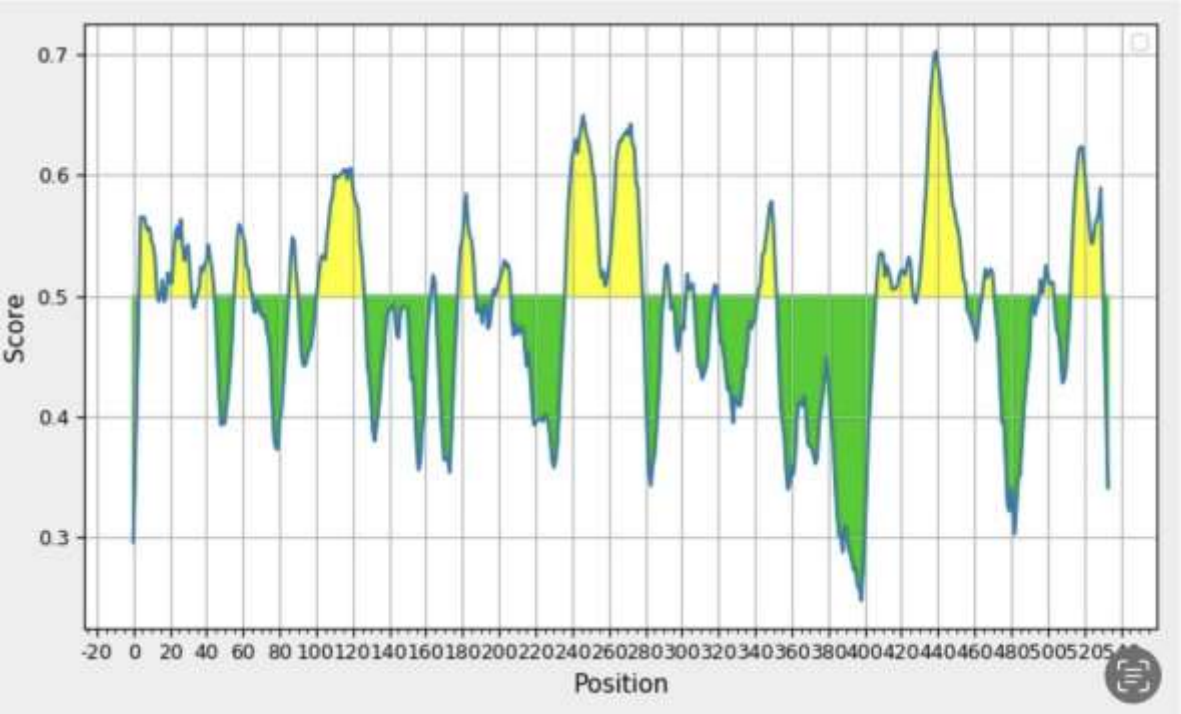

Figure 1: B cell response recording immunogenic and non immunogenic response

allele	seq_num	start	end	length	peptide	score	percentile_rank
HLA-B*44:03	8	14	22	9	AESPGQYYY	0.994255	0.01
HLA-B*44:02	8	14	22	9	AESPGQYYY	0.988311	0.01
HLA-B*44:03	1	16	25	10	EEAKEKLKEY	0.968063	0.01
HLA-A*68:01	1	32	40	9	DVNTLILSR	0.966199	0.02
HLA-A*68:01	6	34	43	10	EIIAGNVVTR	0.963551	0.02

HLA-A*01:01	9	16	25	10	VVDKGSLHVY	0.961982	0.01
HLA-B*44:03	8	49	57	9	GENAASSRY	0.960739	0.02
HLA-B*40:01	6	28	36	9	KEFPGLEII	0.959329	0.02
HLA-A*01:01	8	13	21	9	TAESPGQYY	0.957410.02	
HLA-B*44:02	1	16	25	10	EEAKEKLKEY	0.956645	0.01
HLA-A*31:01	4	8	16	9	KLLGIVTFR	0.953459	0.01
HLA-B*40:01	1	23	31	9	KEYAEKDGL	0.950008	0.03
HLA-A*01:01	8	12	21	10	GTASPGQ	0.946715	0.02
HLA-A*03:01	6	16	24	9	SIYQINMIK	0.934571	0.02
HLA-A*68:02	9	4	12	9	DTIRVAQGV	0.934480.01	
HLA-A*01:01	8	13	22	10	TAESPGQYYY	0.933958	0.02
HLA-A*03:01	5	22	31	10	KNLHFPLSSK	0.928198	0.02
HLA-A*33:01	1	32	40	9	DVNTLILSR	0.927888	0.01
HLA-A*24:02	6	17	25	9	IYQINMIKW	0.925912	0.02
HLA-A*03:01	4	26	34	9	SLLSEVMTK	0.920190.02	
HLA-B*44:02	8	49	57	9	GENAASSRY	0.917582	0.02
HLA-A*11:01	6	16	24	9	SIYQINMIK	0.917370.02	
HLA-A*68:01	10	13	21	9	NVHGLHSYK	0.914192	0.07
HLA-A*02:03	4	4	12	9	KLNGKLLGI	0.903507	0.03
HLA-A*23:01	6	17	25	9	IYQINMIKW	0.896079	0.02
HLA-A*68:01	2	16	25	10	DASSVSLESR	0.895826	0.07
HLA-A*24:02	8	20	28	9	YYYRD	0.89339	0.03

					GQRL	6	
HLA-A*68:01	2	19	28	10	SVSLESRITR	0.878372	0.11
HLA-A*02:03	2	36	44	9	FMSSPMDTV	0.878189	0.03

Table 1: T cell response to identify ideal T cell peptide

No	Start	End	Peptide	Length
1	5	14	ETEEYLSECK	10
2	16	17	EE	2
3	19	32	KEKLKEYAEKDGLD	14
4	36	45	LILSRVNGGL	10
5	57	66	IDFDASSVSL	10
6	87	91	TVTES	5
7	102	127	GIGVIHHNCTIEEQTWMVRKVKKFEN	26
8	164	166	KLN	3
9	179	188	QFHVNDSSLL	10
10	198	207	TGSEGITLEE	10
11	237	279	DLMKNLHFPLSSKLPDSKQLICAATVGTRPEDRIRLKYLVDAG	43

Table 2: B cell immunogenic response to identify the ideal peptide

Discussion:

The success of a peptide vaccine is highly dependent on the identification of immunogenic epitopes capable of eliciting a strong immune response. In this study, we used advanced bioinformatics tools to predict and select B-cell and T-cell epitopes from the *Pneumocystis jirovecii* genome. The accuracy and reliability of these predictions were further confirmed by experimental tests, which ensured the inclusion of truly immunogenic sequences in vaccine design(6).

The integration of both B-cell and T-cell epitopes into the vaccine formulation aims to elicit a comprehensive immune response. B-cell epitopes facilitate the production of antibodies that are critical for neutralizing

extracellular pathogens and preventing infections. On the other hand, T-cell epitopes play an important role in the activation of cellular immunity, which enhances the host's ability to fight against intracellular pathogens such as *Pneumocystis jirovecii(7)* The synergy between these two immune systems probably provides a more effective and durable defense against the fungus(8). Immunocompromised individuals, such as patients with HIV/AIDS or immunosuppressive therapy, are particularly susceptible to *Pneumocystis jirovecii* infections. The peptide vaccine developed in this study addresses the specific challenges faced by these vulnerable populations. The vaccine aims to target both B-cell and T-cell epitopes to overcome the immunodeficiencies in these individuals and provide a tailored solution to improve protection.
Integrating bioinformatics tools for epitope prediction is an effective initial screening method(9). However, the importance of experimental validation cannot be overstated. Using methods such as peptide binding assays, T-cell proliferation assays and serological tests, we confirm the immunogenicity of selected epitopes. This approach ensures the conversion of in silico predictions into specific and reliable candidates for vaccine development (10).

Despite the promising aspects of this study, challenges remain, including possible differences in immune response between different populations and the need to continuously monitor *Pneumocystis jirovecii* strains for epitope conservation. Future studies should focus on optimizing the vaccine formulation, taking into account factors such as dose, administration methods, and possible adjuvants to improve immunogenicity(11). The successful development of a B-cell- and T-cell-specific peptide vaccine for *Pneumocystis jirovecii* could have a significant impact on population health, especially in immunocompromised individuals. Clinical trials are needed to assess the safety, efficacy and long-term immunity of the vaccine.If successful, the vaccine could become an important tool to prevent *Pneumocystis jirovecii* infections, reducing the burden on health systems and improving the quality of life of vulnerable populations (12)(13)
(14)
(15,16)

Conclusion:

In conclusion, identifying and incorporating B-cell and T-cell epitopes into a *Pneumocystis jirovecii* peptide vaccine is a promising way to address the challenges presented by this opportunistic fungal pathogen. The multidimensional immune response elicited by this vaccine can provide an effective and tailored defense strategy, representing an important advance in the fight against *Pneumocystis jirovecii***.**

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Conflict of Interest

The authors would like to declare no conflict of interest in the present study.

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