RESEARCH
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IDENTIFICATION OF B-CELL AND T-CELL SPECIFIC PEPTIDE VACCINE FOR MYCOBACTEROIDES ABSCESSUS

Running title- Peptide vaccine for Mycobacteroides abscessus

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Abstract
Introduction- Illnesses of the skin and soft tissues, infections of the central nervous system, bacteremia, as well as ophthalmic and other illnesses are all brought on by a group of rapidly proliferating, multidrug-resistant, nontuberculous mycobacteria called the Mycobacterium abscessus complex. The current antimicrobial medications of preference for treatment include clarithromycin, amikacin, and cefoxitin. New treatment plans, however, are urgently required, as are quick and affordable diagnostic techniques, as well as steps to stop nosocomial transmission and epidemics. For many years, extremely effective methods for creating conventional vaccinations have been used to reduce infectious disease-related mortality and morbidity.
Materials and methods- Fasta sequences was used for B cell and T cell epitope prediction. Bepipred Linear Epitope Prediction 2.0 online server program was used to predict the B cell immunogenic epitopes. IEDB analysis resource program were used to analyse the results.
Results- 20 amino acids length B cell epitope and T cell epitope with the highest score were identified as the best candidates for peptide vaccine.
Discussion-Traditional vaccinations based on whole organisms or big proteins, however, are no longer recommended due to the unnecessary antigenic load that results in a lackluster protective immunity and exacerbates the problem by triggering reactogenic and/or allergic responses. On the other hand, peptide vaccines are a desirable alternative for triggering highly tailored immune responses and delaying the start of allergic and/or reactogenic events.
Conclusion- In this study we have identified B and T cell specific peptides against Mycobacteroides abscessus. They proved to have high efficacy against the bacteria as the amino acid length proved to be excellent.

Keywords- peptide vaccine, immunogenic epitopes, Mycobacteroides abscessus, b cell epitopes, T cell epitopes

Introduction

A rapidly proliferating nontuberculous mycobacterium (NTM) known to be connected to an increasing number of illness cases globally is Mycobacteroides abscessus (MAB)(1). It requires extensive and multiple treatment regimens to eradicate due to both its intrinsic resistance mechanisms to antibiotics and disinfectants (i.e., innate, not acquired during the course of antibiotic exposure). The majority of people have one of multiple dominant circulating clones, according to recent studies, which raises the possibility of a possible global transmission even if transmission mechanisms are not yet clearly documented and MAB infections are believed to be acquired from the environment (2). Muco-cutaneous and pulmonary infections are brought on by Mycobacterioides abscessus (formerly known as Mycobacterium abscessus). M. abscessus-infected skin is typically erythematous, warm to the touch, tender to the touch, swollen, and/or painful. Additionally, boils or vesicles packed with pus may form on infected sites. Fever, chills, aches in the muscles, and a general feeling of being sick are other symptoms of M. abscessus infection. In individuals with cystic fibrosis (CF), M. abscessus is a common pathogenic mycobacterium and one of the most drug-resistant mycobacterial species to antimicrobial drugs. It is still unknown how these clones developed and spread around the world, and it is possible that they were transmitted from person to person indirectly (3). The typical medications used to treat skin infections usually have little effect on this bacterium's illness.

A vaccination is an immunobiological material derived from a pathogen that causes sickness and prompts the immune system to mount a successful defense against that particular infection. Similar to natural immunity, they reduce the lethality of an infectious bacterium. Worldwide, infectious diseases brought on by microbial pathogens such as viruses, bacteria, and fungus are to blame for rising morbidity and death (4). For many years, extremely effective methods for creating conventional vaccinations have been used to reduce infectious disease-related mortality and morbidity. Conventional vaccinations based on whole organisms or big proteins, however, are no longer recommended due to the unnecessary antigenic load that results in a lackluster protective immunity and exacerbates the problem by triggering reactogenic and/or allergic responses. On the other hand, peptide vaccination offers a desirable alternative for triggering highly tailored immune responses and delaying the development of allergic and/or reactogenic events. Multi-epitope-based peptide vaccine design and development, which focuses on exploiting particular elements of a pathogen, known as epitopes, to construct a vaccine, is now a growing field of study (5). Short amino acid sequences called epitopes are identified by the immune system and cause an immunological reaction. Epitopes allow vaccinations to target particular components of a disease, resulting in a more focused and efficient immune response.Due to their easier synthesis, lack of infectiousness, and chemical stability, epitope-based peptides are sought-after vaccination candidates. As it only requires the production of a small number of antigenic peptides rather than the entire pathogen, epitope-based peptide vaccine design has the potential for relatively quick, inexpensive, and rapid development, making them ideal for use in response to emerging infectious diseases. This sort of vaccine's potential for increased safety is another benefit. Traditional vaccinations employ either inactivated or attenuated forms of the pathogen, which in some people might result in negative reactions and/or autoimmune responses. Conversely, epitope-based peptide vaccinations are extremely successful at generating the required immune response while being biologically safe (6,7).

There have been previous studies on designing of peptide vaccine for Mycobacteroides abscessus, although there’s no previous literature on identification of B-cell and T-cell specific epitopes for a peptide vaccine. By utilizing a number of computational databases and bioinformatics tools and software, this study aims to discover the B and T cell epitopes on the cell surface binding protein and to create an epitope-based peptide vaccination against M.abscessus. Additionally, this study aims to anticipate the protein's three-dimensional structure, as well as its refinement and quality assessment, and explore the evolutionary relationship between this virulence protein and other proteins of a similar type. The stability of the vaccination as well as its interactions with the host cell receptor were further examined in this study using molecular docking analysis and molecular dynamic modeling. Thus, the findings of this study will advance our understanding of potential therapies for emerging and reemerging infections, serve as a foundation for more in silico investigations and be of use to the researchers in future.

Materials and methods

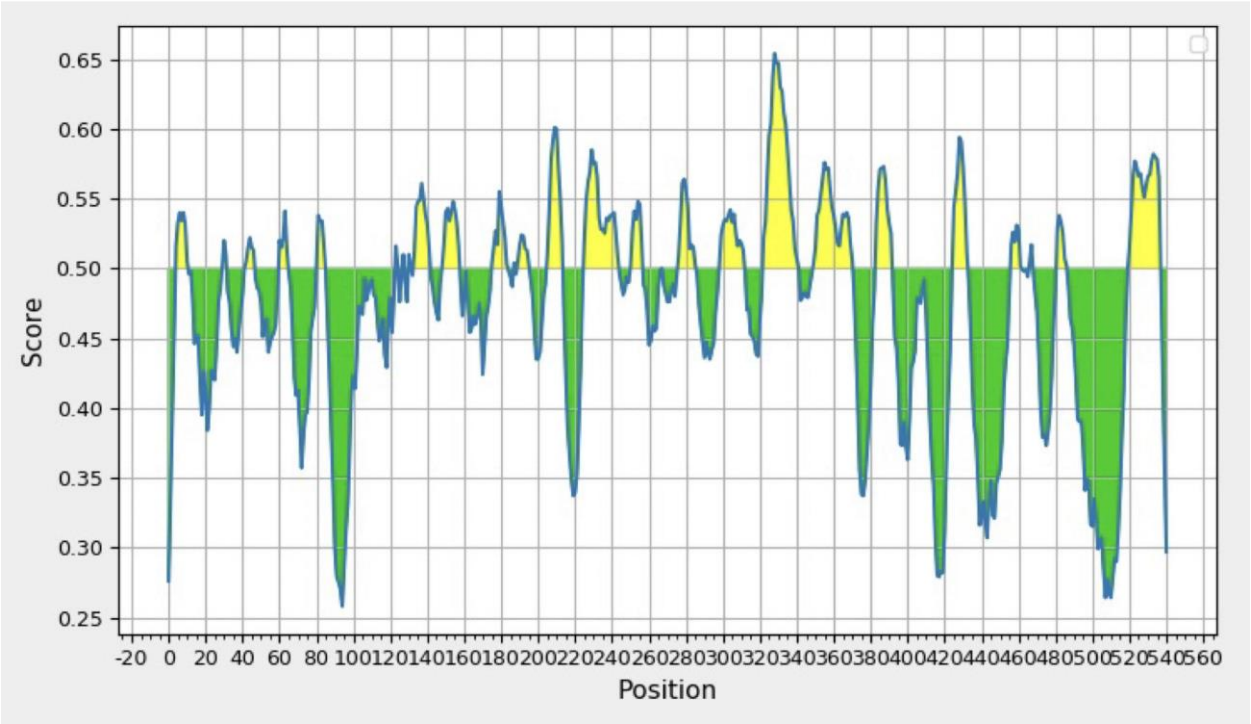
The study aims to identify and validate B and T cell peptide epitopes against Mycobacteroides abscessus.

For the retrieval of sequences, amino acid sequence for Mycobacteroides abscessus subsp. abscessus strain 0037-2 65kDa heat shock protein (hsp65) gene, complete cds (GenBank: OL704624.1) was retrieved from NCBI database.

Fasta sequences was used for B cell and T cell epitope prediction. Bepipred Linear Epitope Prediction 2.0 online server program was used to predict the B cell immunogenic epitopes. The resulted epitopes were analyzed. Peptide VTKDETTIVEGAGDSDAIAG was selected as it was homologous (100%) to the M. abscessus strain and the length was 20 mers appropriate as a candidate vaccine. MHC I - binding T cell epitope prediction was carried out using IEDB Analysis Resource 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- graph representing immunogenic and non-immunogenic epitopes



The yellow portion of the graph represents the immunogenic epitopes while the green portion represents the non-immunogenic portion. The b and T cell epitopes were identified under the immunogenic epitopes for making a peptide vaccine against Mycobacteroides abscessus.

Figure 2- B-cell epitopes

No.	Start	End	Peptide	Length
1	5	11	IAYDEEA	7
2	30	32	LGP	3
3	43	47	WGAPT	5
4	61	65	LEDPY	5
5	81	85	DDVAG	5
6	124	125	EK	2
7	128	128	E	1
8	131	132	LK	2
9	134	142	AKEVETKEQ	9
10	149	158	ISAGDQSIGD	10
11	176	184	EESNTFGLQ	9
12	188	188	T	1
13	190	196	GMRFDKG	7
14	206	214	AERQEAVLE	9
15	226	244	STVKDLLPLLEKVIQAGKP	19
16	251	257	DVEGEAL	7
17	277	286	PGFGDRRKAM	10
18	299	312	VSEEVGLSLETADI	14
19	**323**	**342**	**VTKDETTIVEGAGDSDAIAG**	**20**
20	350	371	EIENSDSDYDREKLQERLAKLA	22
21	384	392	EVELKERKH	9
22	426	433	ELSLTGDE	8

A good B cell epitope for the construction of a good peptide vaccine must be of the length of 15 to 20 amino acids in length. In our study we achieved a 20 amino acid length B cell epitope (highlighted in bold), making it a perfect candidate for the peptide vaccine.

Figure 3- T-cell epitopes

allele	seq_num	start	end	length	peptide	score	percentile_rank
HLA-B*07:(	1	31	39	9	GPKGRNVV	0.974452	0.02
HLA-A*03:(	5	38	46	9	ALSTLVVNK	0.957563	0.01
HLA-B*40:(	5	34	42	9	VEGEALSTL	0.945312	0.04
HLA-B*40:(	5	31	39	9	AEDVEGEA	0.940888	0.04
HLA-B*40:(	3	33	41	9	KEVETKEQI	0.932758	0.05
HLA-A*11:(	5	49	57	9	GTFKSVAVK	0.922334	0.01
HLA-A*03:(	5	49	57	9	GTFKSVAVK	0.913159	0.03
HLA-A*68:(	9	39	48	10	GVADPVKV	0.911851	0.07
HLA-A*68:(	2	45	53	9	TTATVLAQA	0.909609	0.02
HLA-A*02:(	7	28	36	9	KLQERLAKL	0.906067	0.03
HLA-A*02:(	8	24	32	9	ALLQAAPSL	0.888775	0.04
HLA-B*40:(	6	31	39	9	LETADITLL	0.88492	0.07
HLA-A*02:(	7	28	36	9	KLQERLAKL	0.879867	0.04
HLA-A*11:(	5	38	46	9	ALSTLVVNK	0.877062	0.04
HLA-A*03:(	7	35	44	10	KLAGGVAV	0.873611	0.04
HLA-A*30:(	6	41	49	9	TARKVVVTI	0.867312	0.01
HLA-A*30:(	1	33	41	9	KGRNVVLEI	0.864896	0.01
HLA-A*68:(	4	19	27	9	NTFGLQLEL	0.86436	0.03
HLA-B*40:(	4	15	23	9	VEESNTFGL	0.854627	0.08
HLA-A*11:(	2	4	13	10	ITNDGVSIA	0.846872	0.05
HLA-A*03:(	5	48	57	10	RGTFKSVAV	0.844015	0.05
HLA-A*02:(	7	35	43	9	KLAGGVAV	0.843387	0.05
HLA-A*11:(	8	49	57	9	KVAVEAPLK	0.835621	0.06
HLA-A*02:(	7	5	13	9	AIAGRVAQ	0.833235	0.05
HLA-A*11:(	2	48	56	9	TVLAQALVK	0.827505	0.06
HLA-A*03:(	8	49	57	9	KVAVEAPLK	0.827347	0.06
HLA-A*11:(	6	40	49	10	GTARKVVV	0.819956	0.06
HLA-B*40:(	3	21	29	9	VEKVTETLL	0.812049	0.1
HLA-A*02:(	5	2	10	9	LVSSKVSTV	0.804177	0.05
HLA-A*02:(	1	21	30	10	ALADAVKV	0.802747	0.08
HLA-A*68:(	5	37	46	10	EALSTLVVN	0.791338	0.21
HLA-B*07:(	8	29	37	9	APSLDELSL	0.777053	0.09
HLA-B*44:(	4	24	33	10	QLELTEGM	0.775918	0.09
HLA-A*68:(	8	41	49	9	EATGAAIVK	0.772513	0.23
HLA-B*58:(	4	13	21	9	ITVEESNTF	0.771798	0.14
HLA-B*44:(	3	33	41	9	KEVETKEQI	0.766208	0.1
HLA-B*15:(	9	24	32	9	GLNAATGE	0.764652	0.09
HLA-A*02:(	1	21	30	10	ALADAVKV	0.764221	0.07
HLA-B*44:(	7	15	24	10	SEIENSDSD	0.760767	0.1
HLA-B*40:(	5	36	44	9	GEALSTLVV	0.760748	0.13
HLA-A*31:(	9	39	48	10	GVADPVKV	0.750966	0.09
HLA-B*57:(	4	13	21	9	ITVEESNTF	0.750869	0.25

It can be a potential hindrance in the making of peptide vaccine if there is no T cell epitope on short peptide such as those associated with MHC restriction. For the construction of peptide vaccine we chose the T cell epitope with the highest score as the candidate. (Score - 0.974452)

Discussion

Conventional vaccines based on whole organisms or big proteins are no longer recommended because of the needless antigenic load that results in a weak immune response that worsens matters by triggering reactogenic and/or allergic responses (9). By choosing the right B-cell epitopes, it may be able to create

efficient and secure peptide vaccines for a variety of diseases for humoral immunization. The development of peptide vaccines is severely hampered by the absence of T-cell epitopes on short peptides, such as those connected to MHC-restriction (8). Epitope identifications have resulted from recent improvements in bioinformatic analysis's predictions of B- and T-cell epitopes. The choice of effective epitopes to be included in peptide-based vaccinations requires evaluation of which peptide epitope can produce potent neutralizing antibodies and robust T-cell responses. We can further construct a multi epitope peptide vaccine as it can effectively engage the host immune system against Mycobacteroides abscessus (10).

A study by Hamza Arshad et al, formulated an in silico vaccine against Mycobacteroides abscessus which demonstrated to elicit both major humoral and cellular immunity (11). In another study by Rohit Satyam et al, a chimeric vaccine was constructed against multiple isolates of Mycobacteroides abscessus and the best candidates were identified after protein- protein docking studies. For restriction cloning, the final vaccine construct was backtranslated. This vaccination may provide new therapeutic options for battling this heinous microbe (12). There haven't been many studies on peptide vaccine against Mycobacteroides abscessus therefore more studies are required for further clinical trials and it's application for an effective treatment.

Conclusion

Emerging multi-drug resistance infectious pathogen M. abscessus has the potential to cause devastating infections in people. It would be revolutionary to create a vaccination that could shield people against contracting the aforementioned illness. It is difficult to build a vaccine against this virus because of the pathogen's intricate biology and the intimidating nature of traditional vaccine production. Therefore, we successfully identified B and T cell peptide vaccine against Mycobacteroides abscessus. We can also formulate an effective Multi epitope vaccine which can be yield higher results. The B- and T-cell candidate peptide epitopes discovered in our study would elicit both immune responses with a proper adjuvant and serve as a vaccine candidate against Mycobacteroides abscessus, according to all of the findings taken into consideration. To assess the biological protection effectiveness of the vaccination, in vitro immunological assays are advised due to the limitations of these commonly used tools and servers.

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Ethical clearance

Duration of study

5 months

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