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Research Article
Epitope-Based Vaccine Design for Tuberculosis HIV Infection Through *in silico* Approach

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Abstract

Background and Objective: Tuberculosis (TB) is one of the leading causes of HIV-related death among people living with it. TB occurs more often severe in a weakened immune system, particularly when a patient is infected with HIV. People infected with HIV are 15-22 times more likely to fall ill with TB. In this research, an epitope-based vaccine has been specially designed for people living with HIV, since the current tuberculosis vaccine Bacillus Calmette-Guerin (BCG) has been proven to cause more harm than good in treating patients suffering from poor immune systems. **Materials and Methods:** The epitopes were selected from polysaccharide-protein of Mycobacterium tuberculosis and protein envelope of the Human immunodeficiency virus. B cell epitopes have been predicted using BepiPred 2.0, while T cell epitopes predicted using SMM, both are provided by Immune Epitope Database (IEDB). **Results:** This research had designed vaccine combinations for each type of epitopes and types of the pathogen with world population coverage of >85% for MHC class I epitopes and >99% for MHC class II epitopes. **Conclusion:** With each epitope were selected based on how strong its bond with HLA and how many HLA can bind with it. As this research was done through *in silico* approach, *in vivo* test is still needed to guarantee the result of the designed vaccine.

Key words: Vaccine design, HIV, tuberculosis, HLA, bioinformatics, immunology, epitope

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tuberculosis (TB) is one of the leading causes of death among those who are tested positive for Human Immunodeficiency Virus (HIV)[1]. TB is known as an opportunistic infection that occurs more often severe in people with weakened immune systems than people with healthy immune systems [2]. From 1.4 million cases of TB related deaths, there were an estimated 208,000 deaths among People Living with HIV (PLHIV)[3].

HIV is an enveloped retrovirus that contains two copies of a single-stranded RNA genome. It causes Acquired Immunodeficiency Syndrome (AIDS). AIDS is mainly characterized by opportunistic infections and tumours, which are usually fatal without treatment[4]. HIV attaches to the CD4 molecule and CCR5 (a chemokine co-receptor). The virus surface fuses with the cellular membrane, allowing entry into a T-helper lymphocyte[5]. In other words, it infects Helper T lymphocytes (HTL) then explode with all HIV particle, causing damage to nearby cells and infect other HTL. The lack of HTL will lower the patient immune system significantly, which is why the disease caused by it is called immunodeficiency syndrome.

TB is a multi-systemic disease with various presentations. Respiratory system, Gastrointestinal (GI) system, lymphoreticular system, liver, skin, central nervous system, musculoskeletal system and reproductive system is the most commonly affected organ in human body[6]. TB itself has undergone an extensive use of a Live Attenuated Vaccine (LAV) called Bacillus Calmette-Guerin (BCG), however, the use of LAV on a weak immune patient can cause several problems such as adverse reactions to fatality and have been banned by World Health Organization (WHO) for uses in HIV infected patients since 2007[7,8]. Mycobacterium tuberculosis which is the bacteria responsible for TB also known to have several unique features such as the presence of lipids in the cell wall including mycolic acid, cord factor and Wax-D[6]. This factor made Mycobacterium Tuberculosis (MTB) highly resistant to various use of drugs and antibiotics, which is why vaccination is highly recommended[9].

When it comes to vaccination until now scientists are struggling in finding the right vaccine to fight HIV. The reason being the human immune system unable to induce Cytotoxic T Lymphocytes (CTL) reaction when faced against the current of the designed vaccine[10]. With this, we are trying to design an epitope-based vaccine knowing its ability to induce specific immune response[11]. CTL is an important factor in creating immunity against HIV since it can detect and eliminate infected HTL through MHC class I molecules in its surface[12].

This research aimed to identify the potential B-cell and T-cell epitopes from the polysaccharide-protein of *M. tuberculosis* and protein envelope of Human Immunodeficiency Virus-1 that could be used as promising vaccines agents for TB-HIV therapy.

MATERIALS AND METHODS

Study area: This research project was conducted in the Laboratory of Bioinformatics, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia from January-August, 2020.

Tools and materials: This study was conducted using *in silico* method. Polysaccharide protein of *M. tuberculosis* (accession number: CNI00356.1) and protein envelope of Human Immunodeficiency Virus-1 (accession number: AHN55012.1) were obtained from National Center Biotechnology Information (NCBI). Online and offline software including the latest version of VaxiJen v2.0[13], BepiPred 2.0[14], netCTL[15], SMM[16], NetMHCII[17], PEP-FOLD 3.5[18] and IEDB Population Coverage[19] were used in this study.

Procedure: The protein sequence of both MTB and HIV were retrieved from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) in form of FASTA. The antigenicity for both sequences was then predicted using VaxiJen v2.0, which can be obtained at http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html.

The B-cell epitope was then predicted using BepiPred 2.0 provided by IEDB, which can be accessed at http://tools.iedb.org/bcell/. The antigenicity of every single epitope was also predicted using VaxiJen v2.0.

The T-cell epitope was predicted using IEDB. To ensure the correct and suitable epitopes were gained, prediction of its proteasomal cleavage and TAP transport was conducted. Both predictions were run using netCTL provided by IEDB and can be accessed at http://tools.iedb.org/netchop/. After gaining epitopes with desired values, each epitope was subjected to a set of HLA class I predictions to see its affinity with each other using Stabilized Matrix Method (SMM) provided by IEDB (http://tools.iedb.org/mhci/). The antigenicity of each epitope was also predicted using VaxiJen v2.0. The prediction of HLA class II epitopes has then been conducted using SMM align NETMHCII (http://tools.iedb.org/mhcii/).

The 3D structures of the selected epitopes for each HLA class I and class II were constructed using PEP-FOD 3.5 (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-

OLD3/), while the structure of each HLA can be constructed using SWISS-MODEL (https://swissmodel.expasy.org/) for molecular docking purpose.

In selecting the best epitope, the human population coverages of each region were also being taken into consideration, along with the proteasomal cleavage, TAP transport and binding affinity to the HLA molecules. The human coverage population for the desired epitopes was predicted using http://tools.iedb.org/population/.

RESULTS AND DISCUSSION

B cell epitopes: About 6 B cell epitopes for MTB and 7 B cell epitopes for HIV were predicted using B cell epitope prediction at IEDB and VaxiJen (Table 1). The method used for B cell epitope prediction was BepiPred 2.0. At VaxiJen, the target used for MTB was Bacteria and for HIV was Virus, the threshold used was 0.4. Amino acids length picked are between 5-20 amino acids long.

In terms of epitope-based vaccine, 3 components are concluded as designated targets. One of them is a B cell. B cell is a member of the adaptive immune system that has a significant role by producing antibody[20]. Some functions of antibodies are to help phagocytes in eliminating threat or activate the complexion system, which is a population of proteins that eliminate or trap pathogens, direct phagocytes and so on.

To activate a B cell and induce it to start producing antibodies, there are 2 types of responses that can be used. The responses are T-dependent, which is stimulated by the help of HTL and T-independent which don't need the assistance of HTL[12]. In the case of HIV infection, the patient's HTL was under attack by the virus. The best solution is to stimulate a T-independent response by creating a memory B cell through B cell epitope vaccination.

The length of B cell epitopes picked is around 5-20 amino acids long to create ideal epitopes[21]. The predicted epitopes need to have antigenic properties (evaluated using VaxiJen), after being generated in IEDB using BepiPred 2.0 calculation. Not all of the peptides from both microbes passed the desired criteria and were not listed in Table 1.

MHC-I epitopes: About 3 Major Histocompatibility Class I (MHC-I) MTB epitopes and 7 MHC-I HIV epitopes were predicted using proteasomal cleavage prediction and MHC-I binding prediction at IEDB and VaxiJen. The prediction method used for proteasomal cleavage prediction was netCTL, with 0.15 weight on C terminal cleavage and 0.05 on TAP transport efficiency. The supertype used was A2 and the threshold was set to 0.75. The method used for MHC-I binding prediction was SMM and the length was set to 9 amino acids. VaxiJen was set the same as B cell epitopes (Table 2).

The second core component of the epitope-based vaccine is $CD8^+$ cytotoxic T lymphocyte. CTL is an important part of building HIV adaptive immunity. But for TB, since it is a bacteria, CTL doesn't do much against it[22]. The difference between the B cell epitope and the T cell epitope is on the mechanism to trigger the immune response. To start B cell immune responses, epitope will bind to the membrane-bound antibodies which exist in B cell surface[20]. In the case of CTL, the epitope will bind to Major Histocompatibility Complex I (MHC class I) which exist in the surface of every nucleated cell (every cell in human bodies except red blood cell). MHC-I will present this epitope to CTL so they can be bounded with their T Cell Receptor (TCR) and trigger the immune responses[12].

However, MHC is coded by a unique gene that is different between each individual. MHC that is used by humans is called Human Leukocyte Antigen (HLA) system[23]. Each epitope has its affinity towards specific sets of HLA, which is why the prediction for the T cell epitope is the desired peptides and IC_{50}. IC_{50} is a value that shows the affinity of an epitope to an HLA. The lower the number, the better the affinity. IC_{50} score below 50 means it has a high affinity, below 500 intermediate affinities and below 5000 low affinities. To design a good vaccine we only use those that have at least intermediate affinity[15].

In picking a desired epitope, considering its proteasomal cleave and Transporter associated with Antigen Processing (TAP) transport value is needed to make sure the epitope is

Table 1: B cell epitope prediction

Human immunodeficiency virus _1				*Mycobacterium tuberculosis*			
Start	End	Peptide	Length	Start	End	Peptide	Length
5	12	GIRKNYQH	8	59	77	MVVDPAGTGSANSAKVDIA	19
77	82	PNPQEV	6	98	111	ANGDTLSEAQLTSR	14
199	211	SIITQACPKISFE	13	117	124	KVTGTSQT	8
361	370	NQSSGGDPEI	10	245	259	RTMRHVGYADRLEDA	15
393	407	TWRFNSTWNGTEEIN	15	358	368	ATPLSTQLRFV	11
26	436	VGKAMYAPPIR	11	384	389	LQQVRE	6
				394	400	LDQLEGV	7

Table 2: Proteasomal cleavage and TAP prediction

Peptide sequence	Predicted MHC binding affinity	Rescale binding affinity	C-terminal cleavage affinity	TAP transport efficiency	Prediction's score
Mycobacterium tuberculosis					
AMANAYLQV	0.4856	1.1621	0.9984	0.738	1.3487
LLSVVVFAV	0.4661	1.1154	0.9968	0.343	1.2821
YLQVRSESL	0.3766	0.9012	0.9988	1.076	1.1049
QLQERRAQI	0.3759	0.8996	0.9981	0.589	1.0788
ALEGDNNRA	0.3513	0.8407	0.9732	-0.462	0.9636
LLILLSVVV	0.2802	0.6706	0.9789	0.262	0.8305
YTSKASLVV	0.3025	0.7238	0.4385	0.37	0.8081
QLEGVNALA	0.2847	0.6814	0.9693	-0.786	0.7875
QIDEQISAL	0.2462	0.5893	0.999	0.897	0.784
ILLSVVVFA	0.3253	0.7785	0.0542	-0.427	0.7653
SVDSALHQI	0.2534	0.6063	0.7785	0.604	0.7533
Human immunodeficiency virus-1					
HLRDLLLIV	0.4188	1.0023	0.9959	0.424	1.1728
LLQLTVWGI	0.4523	1.0824	0.3681	0.503	1.1628
ALFYKLDVV	0.4222	1.0103	0.8296	0.538	1.1616
TMGAASMTL	0.3598	0.8611	0.997	0.946	1.0579
KLTPLCVTL	0.3533	0.8455	0.999	1.092	1.05
EMKNCSFNV	0.3849	0.9211	0.7545	0.2	1.0443
LLGLRGWEV	0.4113	0.9843	0.1774	0.008	1.0113
LLHATARAI	0.3399	0.8134	0.8632	0.642	0.975
SLLHATARA	0.3577	0.8561	0.8117	-0.398	0.958
YCTPAGFAI	0.369	0.883	0.3342	0.476	0.9569
QLQARVLAV	0.3194	0.7645	0.967	0.416	0.9303
AIAEGTDRA	0.3666	0.8773	0.1518	-0.114	0.8944
SLAEEEVVI	0.304	0.7275	0.7853	0.594	0.875
MIVGGLIGL	0.2807	0.6718	0.7683	1.217	0.8479
LLLIVARSV	0.2634	0.6303	0.978	0.217	0.7879

MHC: Major histocompatibility complex, TAP: Transporter associated with antigen processing

not going to be degraded by the proteasome and delivered efficiently[15]. To predict this using IEDB and HLA supertype is needed since HIV-1 is susceptible to HLA-A*68:02 which is part of A2 supertype, the prediction is conducted based on it[24].

After gaining the desired epitope (Table 2), each peptide's affinity towards sets of HLA was predicted using SMM calculation[16]. TCR that is bounded with T cell epitopes presented and MHC-I will induce an immune response that divides T cell into an effector T cell and memory T cell. Effector T cell function is to eliminate infected cells by ordering them to self-destruct, while memory T cell will record the information in case another infection occurs[12]. This memory T cell is what the patient's body needs to gain immunity.

Though CTL is not needed in case of MTB infection since it cannot order bacteria to self-destruct, the strategy of using CTL against HIV located in the fact that HTL also MHC-I and can also be terminated by CTL. This will stop infected HTL to produce any more HIV pathogen, while antibodies and complexion system taking care of the other pathogen spread outside its host. Which are crucial to prolong the survivability of the patient.

Not all of the peptides passed the criteria listed in Table 2. From 9 MTB epitopes and 13 HIV epitopes that have desired netCTL score and antigenic properties, combinations of 3 MTB and 7 HIV epitopes are made based on the overlapping capability to bind the same total number of HLA as all epitopes combined (Table 3).

The last core component of the epitope-based vaccine is the Cluster of Differentiation 4 ($CD4^+$) helper T lymphocyte. HTL, like CTL, is also triggered by epitope that presents in MHC by sets of HLA. These MHC are classified as MHC-II or MHC class II. The difference between MHC class I and class II are their existence is only on specific cells called antigen-presenting cells such as macrophage and dendritic cell[20]. Usually, this cell will digest the pathogen and present part of it to its surface, which is MHC-II, but with the epitope-based vaccine, the desired peptides will bind with MHC class II to present itself[22].

MHC-II epitopes: About 3 MHC-II MTB epitopes and 4 MHC-II HIV epitopes were predicted using MHC-II binding prediction at IEDB and VaxiJen. The method used for MHC-II binding prediction was SMM-align (NetMHCII 1.1) and the length was set to 15 amino acids. VaxiJen was set the same as B cell epitopes.

Table 3: MHC class I epitope prediction

Epitopes	Epitope interaction with HLA class I (IC_{50})
Mycobacterium tuberculosis	
QIDEQISAL	HLA-A*02:06 (224), HLA-B*15:02 (35), HLA-B*39:01 (410), HLA-C*12:03 (15), HLA-C*05:01 (93), HLA-C*14:02 (152), HLA-C*08:02 (237), HLA-C*07:02 (411)
YLQVRSESL	HLA-A*02:01 (174), HLA-A*02:06 (469), HLA-B*15:02 (24), HLA-B*08:01 (79), HLA-B*15:01 (261), HLA-C*03:03 (17), HLA-C*14:02 (17), HLA-C*12:03 (64), HLA-C*07:01 (231), HLA-C*07:02 (295)
YTSKASLVV	HLA-A*68:02 (104), HLA-A*02:06 (104), HLA-A*02:01 (359), HLA-C*15:02 (3), HLA-C*12:03 (20), HLA-C*14:02 (96), HLA-C*03:03 (113), HLA-C*07:01 (211), HLA-C*06:02 (498)
Human immunodeficiency virus-1	
HLRDLLLIV	HLA-A*02:01 (108), HLA-A*30:01 (202), HLA-A*02:06 (229), HLA-C*12:03 (35), HLA-C*14:02 (146)
KLTPLCVTL	HLA-A*02:01 (103), HLA-A*02:06 (165), HLA-A*32:01 (171), HLA-B*15:02 (158), HLA-C*07:02 (42), HLA-C*03:03 (91), HLA-C*12:03 (139), HLA-C*14:02 (262)
LLQLTVWGI	HLA-A*02:01 (64), HLA-A*02:06 (278), HLA-C*12:03 (37), HLA-C*14:02 (188), HLA-C*03:03 (193), HLA-C*05:01 (371)
MIVGGLIGL	HLA-A*02:06 (22), HLA-A*02:01 (121), HLA-A*68:02 (170), HLA-B*15:02 (24), HLA-C*03:03 (40), HLA-C*12:03 (57), HLA-C*14:02 (188), HLA-C*15:02 (241)
QLQARVLAV	HLA-A*02:01 (128), HLA-A*02:06 (179), HLA-B*08:01 (165), HLA-C*12:03 (23), HLA-C*14:02 (29), HLA-C*03:03 (357)
TMGAASMTL	HLA-A*02:01 (333), HLA-B*15:02 (43), HLA-B*39:01 (225), HLA-B*15:01 (398), HLA-C*03:03 (28), HLA-C*12:03 (90), HLA-C*14:02 (178), HLA-C*07:02 (405), HLA-C*07:01 (485)
YCTPAGFAI	HLA-A*68:02 (452), HLA-B*15:02 (104), HLA-C*03:03 (14), HLA-C*12:03 (58), HLA-C*14:02 (153), HLA-C*08:02 (212), HLA-C*07:02 (362)

Table 4: MHC class II epitope prediction

Epitopes	Epitope interaction with HLA class II (IC_{50})
Mycobacterium tuberculosis	
DQLEGVNALAVFLDR	HLA-DRB1*01:01 (26), HLA-DRB1*04:04 (102), HLA-DRB1*04:05 (317), HLA-DRB1*07:01 (100), HLA-DRB1*09:01 (302), HLA-DRB1*13:02 (345), HLA-DRB5*01:01 (491),
	HLA-DQA1*01:02/DQB1*06:02 (83), HLA-DQA1*05:01/DQB1*03:01 (192), HLA-DQA1*04:01/DQB1*04:02 (474), HLA-DPA1*01:03/DPB1*02:01 (366)
HPLLILLSVVVFAVA	HLA-DRB1*01:01 (4), HLA-DRB1*04:04 (54), HLA-DRB1*04:01 (117), HLA-DRB1*04:05 (141), HLA-DRB1*03:01 (196), HLA-DRB1*07:01 (44), HLA-DRB1*11:01 (63), HLA-DRB1*12:01 (70), HLA-DRB1*09:01 (173), HLA-DRB1*15:01 (8), HLA-DRB4*01:01 (162), HLA-DRB5*01:01 (298), HLA-DRB1*13:02 (299), HLA-DQA1*05:01/DQB1*03:01 (260), HLA-DQA1*01:02/DQB1*06:02 (320), HLA-DPA1*01:03/DPB1*02:01 (185), HLA-DPA1*02:01/DPB1*01:01 (279), HLA-DPA1*03:01/DPB1*04:02 (362)
LLSVVVFAVAGAAYG	HLA-DRB1*01:01 (35), HLA-DRB1*04:04 (220), HLA-DRB1*09:01 (243), HLA-DRB1*08:02 (283), HLA-DRB1*07:01 (369), HLA-DRB5*01:01 (77), HLA-DQA1*05:01/DQB1*03:01 (9)
Human immunodeficiency virus-1	
LWYIRIFIMIVGGLI	HLA-DRB1*01:01 (8), HLA-DRB1*04:05 (31), HLA-DRB1*04:04 (202), HLA-DRB1*04:01 (467), HLA-DRB1*07:01 (17), HLA-DRB1*09:01 (90), HLA-DRB1*11:01 (106), HLA-DRB1*12:01 (300), HLA-DRB1*08:02 (321), HLA-DRB5*01:01 (31), HLA-DRB1*15:01 (55), HLA-DRB4*01:01 (107), HLA-DRB1*13:02 (108), HLA-DPA1*03:01/DPB1*04:02 (98), HLA-DPA1*02:01/DPB1*01:01 (104), HLA-DPA1*01:03/DPB1*02:01 (265), HLA-DPA1*01/DPB1*04:01 (383)
VQKEYALFYKLDVVQ	HLA-DRB1*04:05 (165), HLA-DRB1*04:04 (273), HLA-DRB1*01:01 (379), HLA-DRB1*11:01 (54), HLA-DRB1*09:01 (236), HLA-DRB1*15:01 (239), HLA-DRB5*01:01 (333), HLA-DQA1*01:01/DQB1*05:01 (332), HLA-DPA1*01:03/DPB1*02:01 (109), HLA-DPA1*02:01/DPB1*01:01 (130), HLA-DPA1*03:01/DPB1*04:02 (291), HLA-DPA1*01/DPB1*04:01 (331), HLA-DPA1*02:01/DPB1*05:01 (378)
LLLFGMWMICSADEL	HLA-DRB1*04:04 (65), HLA-DRB1*01:01 (233), HLA-DRB1*04:05 (367), HLA-DRB1*15:01 (113), HLA-DQA1*01:02/DQB1*06:02 (292), HLA-DQA1*05:01/DQB1*02:01 (314), HLA-DPA1*02:01/DPB1*01:01 (486)
RDLLLIVARSVELLG	HLA-DRB1*01:01 (8), HLA-DRB1*04:04 (54), HLA-DRB1*04:01 (165), HLA-DRB1*04:05 (252), HLA-DRB1*03:01 (429), HLA-DRB1*07:01 (11), HLA-DRB1*11:01 (52), HLA-DRB1*09:01(151), HLA-DRB1*12:01 (302), HLA-DRB1*15:01 (45), HLA-DRB5*01:01 (68), HLA-DRB4*01:01 (121), HLA-DRB1*13:02 (121), HLA-DQA1*05:01/DQB1*03:01 (398), HLA-DPA1*02:01/DPB1*01:01 (78), HLA-DPA1*03:01/DPB1*04:02 (88), HLA-DPA1*01:03/DPB1*02:01 (456)

Just like CTL, HTL also has TCR (T-Cell Receptors) that will bind with MHC molecules and initiate immune responses, dividing into a memory cell that records threat for future immunity and effector cell that activated to start functioning. The role of HTL is to release cytokines, which is a molecule that induces the immune system and activate them[12]. They also can activate a B cell through T-dependent responses.

In predicting MHC class II epitopes, MTB and HIV have specific HLA that are susceptible to them, with MTB being HLA-DRB1*09:01[25] and HIV being HLA-DRB1*01:01[24]. With this information, the predicted peptides that are used for the designed vaccine are those who have affinity against that specific HLA.

From 71 MTB and 116 HIV MHC class II epitopes that have passed the criteria, combinations of 3 MTB and 4 HIV peptides were selected based on the overlapping capability to bind the same total number of HLA as all epitopes combined (Table 4).

Table 5: Population coverage

	MHC class I population coverage				MHC class II population coverage		
Population/area	Coverage a (%)	Average hit b	pc 90°C	Population/area	Coverage a (%)	Average hit b	pc 90°C
Mycobacterium tuberculosis							
Central Africa	76.47	1.11	0.43	Central Africa	99.78	3.09	2.19
East Africa	75.57	1.14	0.41	Central America	88.48	1.4	0.87
East Asia	83.30	1.43	0.6	East Africa	99.80	3.31	2.34
Europe	93.49	1.9	1.12	East Asia	91.84	1.79	1.06
North Africa	83.62	1.36	0.61	Europe	99.96	3.09	2.25
North America	86.57	1.54	0.74	North Africa	91.30	1.7	1.04
Northeast Asia	75.86	1.17	0.41	North America	100.00	3.3	2.48
Oceania	69.26	0.98	0.33	Northeast Asia	96.72	2.28	1.34
South Africa	87.82	1.42	0.82	Oceania	96.81	2.29	1.37
South America	73.62	1.07	0.38	South Africa	32.10	0.33	0.15
South Asia	74.70	1.1	0.4	South America	99.53	2.95	2.08
Southeast Asia	73.18	1.09	0.37	South Asia	99.53	2.72	1.93
Southwest Asia	75.85	1.14	0.41	Southeast Asia	80.51	1.27	0.51
West Africa	66.70	0.94	0.3	Southwest Asia	75.25	1.11	0.4
West Indies	46.89	0.54	0.19	West Africa	99.85	3.28	2.34
World	88.55	1.62	0.87	West Indies	92.78	1.81	1.09
				World	99.26	2.92	2.06
Average	76.97	1.22	0.52	Average	90.79	2.27	1.5
Standard deviation	10.63	0.31	0.24	Standard deviation	16.29	0.88	0.74
Human immunodeficiency virus-1							
Central Africa	67.53	2.51	0.31	Central Africa	99.81	7.55	4.56
Central America	1.40	0.01	0.1	Central America	87.05	2.7	0.77
East Africa	69.88	2.6	0.33	East Africa	99.88	8.04	4.89
East Asia	82.65	5.38	0.58	East Asia	93.22	4.93	1.51
Europe	91.31	6.07	1.1	Europe	99.97	8.14	5.13
North Africa	79.47	3.68	0.49	North Africa	93.23	3.46	1.27
North America	85.48	5.12	0.69	North America	100.00	8.26	5.44
Northeast Asia	74.11	4.25	0.39	Northeast Asia	97.60	6.1	3.14
Oceania	67.57	2.98	0.31	Oceania	97.90	6.12	3.08
South Africa	79.17	2.76	0.48	South Africa	32.10	0.43	0.15
South America	72.97	3.37	0.37	South America	99.38	7.21	4.1
South Asia	71.38	3.31	0.35	South Asia	99.61	7.45	4.25
Southeast Asia	72.24	3.8	0.36	Southeast Asia	89.29	3.15	0.93
Southwest Asia	71.86	3.46	0.36	Southwest Asia	83.46	2.44	0.6
West Africa	66.23	2.29	0.3	West Africa	99.89	8.67	5.56
West Indies	52.11	2.29	0.21	West Indies	92.66	3.55	1.22
World	86.31	5.32	0.73	World	99.38	7.73	4.35
Average	70.1	3.48	0.44	Average	92.03	5.64	3.00
Standard deviation	19.38	1.43	0.23	Standard deviation	15.8	2.47	1.87

MHC: Major histocompatibility complex, Letter (a) in this table, showed the projected population coverage, while letter (b) showed the average number of epitope hits divided by HLA combinations recognized by the population and letter (c) showed the minimum number of epitope hits divided by HLA combinations recognized by 90% of the population

Now that all the desired epitopes and their HLA had been gained, the next is to predict its population coverage based on HLA data throughout the world. Each region is populated by a unique type of individuals, making every country have a different combination of HLA sets. This is why a prediction is needed to further increase the efficiency of vaccine distribution once the products are to be made.

Population coverage: All the data showed in Table 5 are based on the total HLA types that have an affinity towards predicted peptides using IEDB. Letter (a) in this table, showed the projected population coverage, while letter (b) showed the average number of epitope hits divided by HLA combinations recognized by the population and letter (c) showed the minimum number of epitope hits divided by HLA combinations recognized by 90% of the population. Population coverage of MHC-II epitopes showed a great world coverage of >99 with >90% average of each region, while even though MHC-I epitopes showed world coverage of >85%, the average coverage of each region is still 70-ish %.

This study was carried out using *in silico* method, meaning it was only done by computational prediction. To ensure the effectiveness, efficiency and safety of the vaccines, *in vivo* test is still needed to be conducted, by first doing animal testing and followed by human testing at a wet laboratory. Molecular docking is also recommended for further study.

CONCLUSION

We have predicted numerous antigenic peptides from the capsular protein sequence of *Mycobacterium tuberculosis* and Human Immunodeficiency Virus, which hopefully would be beneficial for current vaccine development against TB-HIV co-infection. Even though the designed epitopes had low energy minimization values which favoured the stability of epitope-MHC allele complex, wet-lab experiments using animal and human subject is still needed to be performed to verify the suitability and precise effectiveness of the vaccine.

SIGNIFICANCE STATEMENT

This study discovers the epitopes prediction for TB-HIV co-infection vaccines that can be beneficial for further HIV vaccine study or changing live attenuated MTB vaccine which is BCG to a safer one. This study will help the researcher to uncover the significance of using CTL or epitope vaccines, in general, to combat previously incurable disease like HIV. Thus, a new age of vaccination may be arrived at.

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