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Computational Mapping of Class I HLA-A Specific Cytotoxic T Lymphocyte Epitopes of *Mycobacterium tuberculosis* and Their Potential Role in Vaccine Design

Aşı Tasarımında *Mycobacterium tuberculosis*'in Sınıf I HLA-A'ya Özgü Sitotoksik T Lenfosit Epitoplarının İşlemsel Haritalaması

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Abstract

Objective: Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (M. tb) is one of the deadliest diseases causing millions of deaths worldwide. Bacillus Calmette-Guérin (BCG) is the only vaccine that has been used in many countries where TB is prevalent. Despite vaccination, this disease prevails in many of the developing countries, necessitating the development of an effective vaccine against TB. Since M. tb acts as an intracellular pathogen, cell-mediated immune response plays an important role in disease control. Therefore, screening of CD8+ T cell epitopes of M. tb antigens could aid in the development of an effective vaccine against TB. In the current study, a reverse vaccinology approach was utilized to predict and map cytotoxic T lymphocyte (CTL) epitopes in the virulent proteins that are also essential for M. tb.
Materials and Methods: Database of Essential Genes and Virulence Factor Database were used for identifying the virulent proteins of M. tb and their antigenicity was assessed using VaxiJen server. Various immunoinformatics tools were used to predict MHC class I binding, MHC processing, immunogenicity, toxicity and allergenicity.
Results: Twelve M. tb antigens were selected for the prediction analyses using various tools. The results indicated the presence of 20 novel CTL epitopes predicted against human HLA-A alleles. This study has also screened for multiple allele binding epitopes that could be used as a vaccine component.
Conclusion: This study has yielded a few hitherto unreported CTL epitopes binding to class I HLA-A alleles. Further experimental validation is necessary for confirming their potential as vaccine candidates.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, CTL epitopes

Öz

Amaç: *Mycobacterium tuberculosis* (M. tb) kaynaklı tüberküloz (TB), dünya çapında milyonlarca insanın ölümüne neden olan en amansız hastalıklardan biridir. TB'nin yaygın olduğu çoğu ülkede kullanılmakta olan tek aşı Bacillus Calmette-Guérin'dir (BCG). Aşılamaya rapmen bu hastalık, TB'ye karşı etkin bir aşının geliştirilmesini gerektiren şekilde gelişen ülkelerin çoğunda halen etkilidir. M. tb'nin intraselüler bir patojen olarak hareket etmesinden ötürü, hücre aracılıklı immün cevap, hastalığın kontrolünde önemli bir rol oynamaktadır. Bu nedenle M. tb antijenlerinin CD8+ T hücresi epitoplarının taranması, TB'ye karşı etkin bir aşının geliştirilmesine yardımcı olabilir. Mevcut çalışmada, M. tb için de gerekli olan virülan proteinlerdeki cytotoxic T lymphocyte (CTL) epitoplarının öngörülmesi ve haritalanması için ters aşı yaklaşımı kullanılmıştır.
Gereç ve Yöntem: M. Tb'nin virülan proteinlerinin tanımlanması için Temel Genler Veritabanı ve Virülans Faktörü Veritabanı kullanıldı ve antijenlikleri, VaxiJen sunucusu kullanılarak değerlendirildi. MHC sınıf I bağlayıcılığı, MHC işleme, immünojenisite, toksisite ve alerjiklik hakkında tahminde bulunmak üzere çeşitli immünoinformatik araçlar kullanıldı.
Bulgular: Tahmin analizleri için farklı araçlar kullanılarak M. tb antijenleri seçildi. Sonuçlar, insan HLA-A allellerine karşı öngörülen 20 yeni CTL epitopunun olduğunu gösterdi. Bu çalışmada aynı zamanda bir aşı bileşeni olarak da kullanılabilecek olan çoklu allel bağlayıcı epitop için de tarama yapıldı.
Sonuç: Bu çalışma, sınıf I HLA-A allellerine bağlanan ve şimdiye kadar bildirilmemiş birkaç CTL epitopunu ortaya koymuştur. Aşı adayları olarak potansiyellerini doğrulamak için daha fazla deneysel validasyon gereklidir.

Anahtar kelimeler: Tüberküloz, *Mycobacterium tuberculosis*, CTL epitopları

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Introduction

Mycobacterium tuberculosis (M. tb) is an airborne pathogen that causes tuberculosis (TB) in human beings. The bacterium, first isolated by Robert Koch in 1882, mainly affects the lungs and causes a severe form of the disease called pulmonary tuberculosis.[1] TB is one of the top 10 causes of death. It has been estimated that more than 1.4 million people died from TB in 2019. Among them, 208,000 people were HIV patients.[2] Although TB has been reported to be a complication of other diseases, M. tb is one of the fatal infectious agents in recent years followed by HIV/AIDS.[3] To date, Bacillus Calmette-Guérin (BCG) is the only vaccine available for TB, which is a live attenuated strain of *Mycobacterium bovis*.[4] Though it confers protection against TB in children, the efficiency of the vaccine in adults is still debatable.[4] Although several subunit vaccines, viral vector vaccines, inactivated whole-cell vaccines, and DNA vaccines have been developed, they have not effectively eradicated the disease.[4] Hence, there is a need for a potent vaccine with the ability to prevent TB infection at any stage of the disease.

Scientists in the field of TB research have focused on T-cell responses, especially CD4+ T cells, for many years. But recently, the CD8+ subset of T cells has drawn the attention of researchers. The intracellular antigens in the cytoplasm of the cells are presented by MHC Class I molecules, which are then recognized by the CD8+ T cells [5,6]. Though M. tb resides in the vacuole of the cells, but not in the cytoplasm, studies suggest that it may gain access to the cytoplasm through pores in the membrane.[5,6] Escape of M. tb from the phagosome into the cytoplasm aids in the subsequent presentation by Class I MHC molecules.[7] Several other alternative mechanisms for MHC Class I loading of M. tb antigens have also been reported.[7] Other studies have also demonstrated MHC Class I presentation of M. tb antigens from infected cells to CD8+ T cells.[8-13] These experiments were conducted on human beings and mice, in which antigens recognized by CD8+ T cells were also identified.[8-13] MHC in human beings is encoded by different alleles of Human Leukocyte Antigen (HLA).[14] In general, the optimal length of cytotoxic T lymphocyte (CTL) epitopes is usually considered to contain 8 to 11 amino acids. The features of the peptides sequence help in the binding of the epitope to the specific anchor residues at the cleft of HLA molecules.[14]

The antigen-presenting cells process the M. tb specific antigens in the proteosomal compartment.[15] The final peptide-MHC complex interacts with the T cell receptor and activates the effector CTL.[15] Subsequently, the activated CTL produces different cell lysis enzymes and exerts a toxic effect on virus-infected cells.[15] Importantly, they also lyse M. tb infected macrophages and kill the infected cells in an antigen-specific manner.[15] Hence, CD8 T cells are a very potent subset of T cells that have the potential to prevent the disease and kill the M. tb cells.[15] Therefore, it is evident that activation of cell-mediated immune response is crucial to prevent infection caused by intracellular pathogens, especially M. tb.[16] This study aims to identify the CD8+ CTL epitopes for the development of an epitope-driven vaccine against TB.

The computational approach in such studies helps in the augmented discovery of potential epitopes for vaccine development.[17-20] Recently, various online resources and software have influenced the field of immunology.[17-20] Such resources have also provided insight into the immune system.[17-20] Immunoinformatics is a discipline that uses computational and mathematical approaches for a better understanding of large-scale immunological data.[21] It also helps in converting this data into an immunologically meaningful interpretation.[21] Immunoinformatic tools are used to study and design algorithms for B- cell and T-cell epitope mapping.

Several immunoinformatics tools are used to predict potential vaccine candidates.[22] These tools use various computational algorithms such as Artificial Neural Network (ANN), Stabilized matrix method (SMM), MHC binding energy covariance matrix (SMMPMBEC), and QM.[22] Most of the epitope prediction methods are based on the potential binding ability of peptides to MHC. Indeed, MHC-peptide binding is most responsive to the computational analysis.[23-25] Prediction of potent epitopes play a significant role in the development of candidate vaccines.[26]

In the current study, a reverse vaccinology approach is followed to predict the M. tb CTL epitopes by using immunoinformatics tools. The reverse vaccinology approach aided in the minimization of a very high number of peptides from virulent proteins of M. tb into selected potent epitopes.

Materials and Methods

Selection of proteins

The essential genes of M. tb were retrieved from DEG (Database of Essential Gene) online database.[27] Essential genes were further analyzed through VFDB (Virulence Factor Database) to predict the virulent proteins.[28]

VaxiJen v2.0 server was then used to identify the antigenic nature of the selected virulent proteins. A threshold value of ≥ 0.4 was set to consider any protein as an antigen.[29] CELLO2GO online server was used to predict the subcellular localization of antigenic proteins.[30] Finally, a set of essential, virulent, antigenic proteins with predicted subcellular localization were derived. These proteins were taken for further analysis.

CTL Epitope mapping

CTL epitopes in selected proteins were predicted using IEDB-AR Resource.[31] Based on the literature survey, HLA-A alleles *A*0101, A*0201, A*0206, A*0301, A*1101, A*2402, A*2601, A*3101,* and *A*3303* were used for CTL epitope mapping.[32]

Class I MHC Binding analysis

IEDB provides numerous tools such as ANN, SMM, SMMPMBEC, and scoring matrices derived from combinatorial peptide libraries to predict T and B cell epitopes.[31] Peptides of 8, 9, 10, and 11-mer length in the selected proteins were predicted for their ability to bind nine HLA-A alleles. Peptides with a percentile score of 1% or less were considered as potent epitopes and the peptides with lower percentiles were considered as strong binders.[22]

Multiple allele binding epitopes were also selected and analysed further.

Class I MHC processing analysis

The default prediction method of class I MHC processing analysis was followed to analyse multiple allele binding CTL epitopes (8 to 11-mer). The combined score of MHC binding, proteasomal processing, and TAP Transport was considered to select potent T cell epitopes.[33,35]

Class I MHC Immunogenicity analysis

Peptides presented on the MHC molecule are recognized by T cells and provoke the immunity.[36] Although Class I immunogenicity analysis predictions can be made for any epitope lengths, it mainly authorizes 9-mer epitopes. A positive score indicates the immunogenicity of epitopes, and so others were excluded from this study. Therefore 8-mer and 11-mer peptides were eliminated from this study whereas 9-mer and 10-mer peptides were further analysed.

Non-Toxic, Antigenic, Non-allergic Peptides and BLAST with human proteome

A potential vaccine candidate should be non-toxic, non-allergic, and antigenic. Therefore, peptides that are toxic, allergic, and non-antigenic were eliminated from this study. ToxinPred v2.0 server was used to predict toxic peptides from the predicted immunogenic epitopes. The toxicity prediction is based on the physicochemical properties of amino acids.[37] VaxiJen v2.0 tool was used to predict the antigenic peptides. The peptides with a threshold value of ≥0.4 were considered to be antigens.[29] AllerTop v2.0 server was used to predict the allergic epitopes. Based on this predictions the selected peptides were classified as allergen and non-allergen.[38] Finally, the predicted epitopes were compared with human proteome (taxid:9606) using protein BLAST tool to rule out similarity with human proteome thereby reducing the possibility of autoimmunity. A total of 1242 M. tb essential proteins were retrieved from the Database of Essential Gene database. These proteins were further analyzed through VFDB (Virulence Factor Database). We have selected 9 HLA-A alleles (HLA-A*01:01, -A*02:01, -A*02:06, -A*03:01, -A*11:01, -A*24:02, -A*26:01, -A*31:01 and -A*33:03) that have been already reported as high frequency HLA-A alleles with worldwide distribution.

Results

Selection of target proteins

Twenty-five out of 1242 essential proteins, were confirmed as virulent proteins. Interestingly, all the 25 proteins scored above the threshold value (0.4) and they were confirmed as antigens when tested using VaxiJen server (Figure 1). Fifteen out of 25 proteins analyzed using CELLO2GO are localized in the cytoplasm, 6 in the membrane, 3 in the extracellular region, and 1 in the periplasmic region.

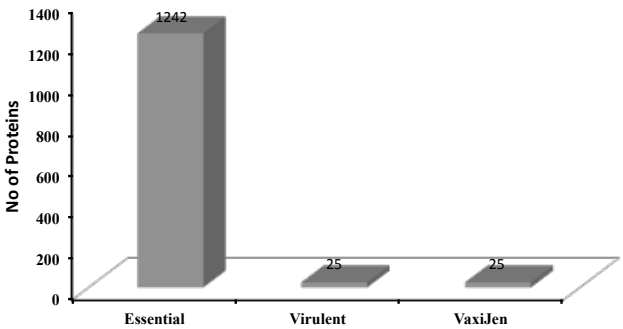

Figure 1. Selection of target proteins by manual screening using DEG, VFDB, and VaxiJen.

Class I MHC binding analysis

A total of 1814 CTL epitopes were predicted from 25 selected M. tb target proteins using 9 different HLA alleles. Peptides were predicted to be of various lengths such as 8-mer (3.03%), 9-mer (50.60%), 10-mer (39.69%), and 11-mer (6.67%) peptides with ≥1 percentile score. The number of peptides predicted in HLA-A*33:03 were 604, 204 in HLA-A*02:06, 186 in HLA-A*31:01, 170 in HLA-A*02:01, 148 in HLA-A*26:01, 138 in HLA-A*11:01, 133 in HLA-A*24:02, 120 in HLA-A*01:01, and 111 in HLA-A*03:01 (Figure 2).

The highest number of peptides were predicted in the protein, eccC3 (n=246), followed by rel A (147), eccD5 (138), eccD3 (133) proteins. Lowest number of peptides were predicted in PE/PE5 protein (n=9) (Figure 3).

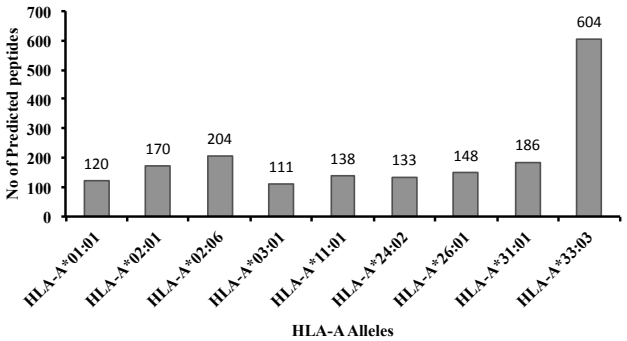

Figure 2. Distribution of predicted epitopes binding to various HLA-A alleles.

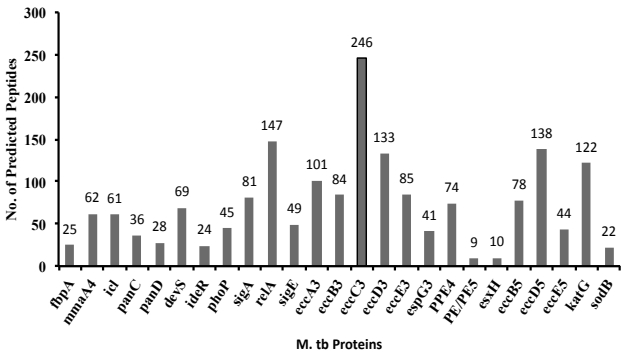

Figure 3. Distribution of predicted peptides in various target proteins of *M. tuberculosis*.

Multiple Allele Binders

A total of 268 multiple allele binding CTL epitopes were identified out of 1814 class I MHC binders. The maximum number of multiple allele binding peptides was observed in 9-mer (Total: 164) followed by 10-mer (Total: 95), 8-mer (Total: 6), and 11-mer peptides (Total: 3) (Figure 4).

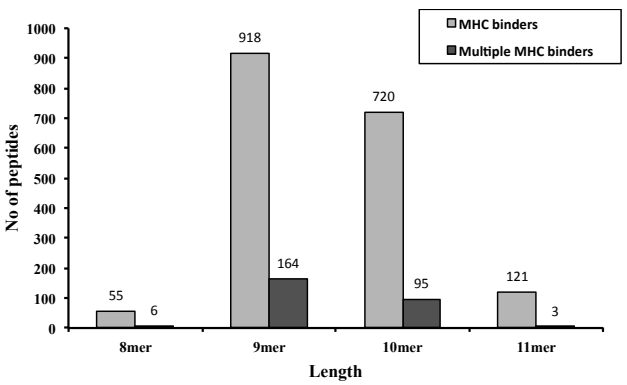

Figure 4. Presence of multiple allele binders among the predicted epitopes of varying length.

Class I MHC Processing analysis

A total of 157 MHC Class I processing epitopes were predicted out of 268 multiple allele binding epitopes. Among them 106 epitopes were 9-mer in length; and 51 epitopes predicted in 10-mer in length; whereas, none of the epitopes showed positive processing score in 8-mer and 11-mer in lengths. Epitopes with negative scores were eliminated and those with positive scores were analysed further (Figure 5).

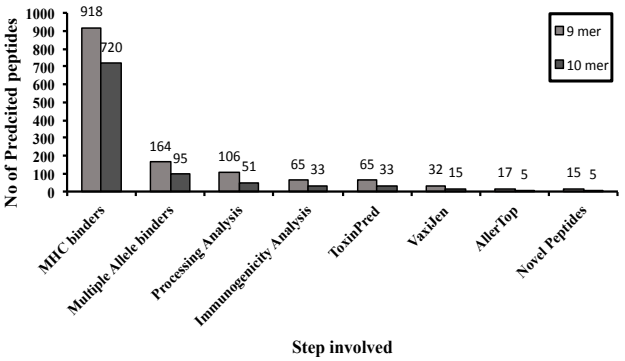

Figure 5. Peptides of 9-mer and 10-mer in length predicted in each step of the pipeline.

Class I MHC immunogenicity analysis

The pMHC prediction tool was used to identify the immunogenicity of processed M. tb CTL epitopes. This tool does not contain an option for HLA-A*33:03. Therefore, immunogenicity prediction was performed against the remaining eight HLA-A alleles. A total of 106 (9-mer) and 51 (10-mer) epitopes were analysed. Among them, 65 (9-mer) and 33 (10-mer) epitopes have shown positive immunogenicity scores (Figure 5).

Non-Toxic, Antigenic, Non-allergic Peptides and BLAST with human proteome

A total of 98 immunogenic epitopes were further analysed in the ToxinPred server to predict non-toxic peptides. None of the epitopes were predicted as toxic.

All the non-toxic peptides were further analysed using the VaxiJen server to predict antigenic peptides. The antigenicity analysis using VaxiJen server predicted 47 peptides as antigenic (32 9-mer and 15 10-mer). All the 47 antigenic epitopes were further analysed using the AllerTop server to predict the non-allergic peptides. A total of 22 peptides were predicted to be non-allergic (17 9-mer and 510-mer).

Twenty out of 22 peptides (15 9-mer and 5 10-mer) were found to be novel immunodominant epitopes (Table 1; Table 2; Figure 5). Further, the predicted novel epitopes were analysed for similarity with human proteome using BLAST and none of the epitopes have shown 100% similarity with human proteome ruling out the possibility of autoimmunity.

Table 1. Novel *M. tuberculosis* 9-mer CTL epitopes.

S.No	Proteins	Start	End	Length	Peptide	Binding Allele	Percentile score
1	panD	56	64	9	VTYAITGER	HLA-A*31:01	0.64
2	devS	26	34	9	RLHELLVEV	HLA-A*02:01, HLA-A*02:06	0.3, 0.26
3	relA	421	429	9	FAYAVHTEV	HLA-A*02:06	0.28
4	eccB3	42	50	9	VTGWRFVMR	HLA-A*31:01	0.35
5	eccB3	43	51	9	TGWRFVMRR	HLA-A*31:01	0.78
6	eccC3	1190	1198	9	WTFAGHTHY	HLA-A*01:01, HLA-A*26:01	0.42, 0.69
7	eccD3	464	472	9	GLFAWVLNR	HLA-A*31:01	0.58
8	eccD3	362	370	9	ATAAAATLR	HLA-A*11:01	0.94
9	eccD3	461	469	9	YLVGLFAWV	HLA-A*02:01, HLA-A*02:06	0.2, 0.07
10	eccE3	106	114	9	RYLDRYGIR	HLA-A*31:01	0.28
11	eccE3	251	259	9	YTVAAACAL	HLA-A*02:06	0.82
12	PPE4	68	76	9	YVAAHLPYV	HLA-A*02:01, HLA-A*02:06	0.3, 0.06
13	eccB5	35	43	9	MALTRWRVR	HLA-A*31:01	0.33
14	eccB5	465	473	9	SLAPWVALR	HLA-A*31:01	0.39
15	eccE5	20	28	9	FLVDVLILA	HLA-A*02:01, HLA-A*02:06	0.2, 0.12

Table 2. Novel *M. tuberculosis* 10-mer CTL epitopes.

S.No	Proteins	Start	End	Length	Peptide	Binding Allele	Percentile score
1	eccB3	41	50	10	QVTGWRFVMR	HLA-A*31:01	0.85
2	eccC3	755	764	10	ATIGEQLARY	HLA-A*26:01	0.38
3	eccD3	287	296	10	ALWARFPLPV	HLA-A*02:01	0.13
4	eccB5	34	43	10	AMALTRWRVR	HLA-A*31:01	0.42
5	eccE5	20	29	10	FLVDVLILAV	HLA-A*02:01, HLA-A*02:06	0.13, 0.07

Discussion

Since M. tb infects and survives inside the macrophages, it is mandatory to activate the cell-mediated immunity in order to eliminate this intracellular pathogen. Instead of concentrating on B cell and antibody-mediated immune response, T cells should be induced to fight against M. tb.[39] There are several studies and reports on the response of human CD8+ T cells to M. tb antigens. These studies reveal that CD8+ T cells prevent the replication of M. tb in the alveolar macrophages.[40] Hence, the present study focused on the identification of the CD8+ CTL epitopes for the epitope-based vaccine development for TB.

The M. tb genome has a larger number of proteins.[41] Proteins developed for vaccine development should be essentially immune dominant. Therefore, 25 such proteins of M. tb were used to predict the potential epitope vaccine candidate against tuberculosis. Among these 25 proteins, PPE family protein (PPE4) showed the highest antigenic score of 0.7141. IEDB-AR was used to map M. tb CTL epitopes. In this study, the sequence-based epitopes prediction method for analysing CTL epitopes was chosen. Hence, the IEDB- recommended approach for MHC binding prediction was selected. Nine HLA-A alleles (HLA-A*01:01, HLA-A*02:01, HLA-A*02:06, HLA-A*03:01, HLA-A*11:01, HLA-A*24:02, HLA-A*26:01, HLA-A*31:01 and -A*33:03) that were already reported as the high frequency HLA-A allele that are distributed worldwide were selected.[32] Using this approach a total of 1814 class I MHC binding peptides were predicted against these nine different HLA-A alleles.

Both dominant epitopes for the same and different MHC alleles must be considered in epitope-based vaccine development trials. Although this collection of epitopes may not include every peptide derived from the protein, it includes adequate number of epitopes to induce an effective immune response.[42] In this study, the epitopes predicted in each allele were compared with one another for the prediction of multiple allele binding epitopes. The peptide of ESAT-6 Excretion system protein (EeccC3), $RVIPPSLLR_{32-40}$, binds to four HLA alleles with a low percentile score of 0.72, 0.38, 0.46, and 0.78 for HLA-A*03:01, HLA-A*11:01, HLA-A*31:01, and HLA-A*33:03, respectively.

The protein antigens are processed in proteasomes and further these peptides are transported to the surface of antigen-presenting cells by TAP.[22] This processing step is important for the expression of peptide- bound MHC molecules on antigen-presenting cells.[22] The T-cell receptors specific for peptides are also presented on antigen-presenting cells along with peptide bound MHC class I molecules during CTL activation. Therefore, antigen processing and

transport must also be considered for epitope-based vaccine development.[22] A total of 268 multiple allele binding peptides were analysed for MHC class I processing. This analysis resulted in 157 peptides with a positive score. All of them were either 9-mer or 10-mer in length. Five peptides from four different M. tb proteins were observed to be processed by three HLA-A alleles. They are mmaA4 - $TTMRRAVER_{86-94}$, icl - $HTYPDQSLY_{104-112}$, sigA - $STYATWWIR_{344-352}$, eccD5 - $HTIYSPLFR_{464-472}$, and katG - $KTFGFGFGR_{179-187}$. Immunogenicity is defined as the ability of peptides to stimulate T cells. Therefore, prediction of T-cell epitope aims to identify the immunogenicity of the shortest peptide within a protein that can induce CD8 T-cell response.[43,44] A total of 98 epitopes were predicted as immunogenic epitopes using the IEDB immunogenicity analysis tool. Unfortunately, the allele HLA-A*33:03 was not available in the prediction tools. Therefore, we could not include the epitopes predictedly bind to HLA-A*33:03 allele for further analysis.

One of the major characteristics of the predicted peptide is that it should be non-toxic to cells. Therefore, toxic peptides were eliminated by using the ToxiPred tool. Prediction in ToxinPred is mainly based on machine learning techniques and quantitative matrix methods.[45] Interestingly, none of the epitopes were predicted to be toxic in our study. Another important characteristic of the predicted peptide is its antigenicity. Antigenicity is the ability of peptides to bind the immune cells or antibodies to provoke an immune response.[46] VaxiJen server was used to identify the antigenic peptides. The highest number of the antigenic score (1.2721) was observed for $VTGWRFVMR_{42-50}$, the peptide of eccB3.

The vaccine should not be allergic to the host. Hence, AllerTop server was used to remove the allergic peptides. Finally, 47 immunodominant epitopes were predicted using a reverse vaccinology approach. An epitope, $GTVRSRIHR_{223-231}$, from M. tb protein sigE induces IL4. This epitope was predicted in IL4Pred.[47] Similarly, $KTFGFGFGR_{179-187}$ epitope from katG protein has been reported to induce IFN gamma and IL4 in an *in vivo* study.[47,48]

In this study, 20 novel CTL epitopes were predicted from 12 different M. tb proteins. A similar study has been conducted with a protein called panD with epitope $VTYAITGER_{56-64}$. Rhis protein is involved in lipid biosynthesis and metabolism in M. tb., and it is crucial for the intracellular replication and persistence of M. tb.[49] Another protein relA, with epitope $FAYAVHTEV_{421-429}$, is required for the long time survival of M. tb.[50] The protein devS, with peptide $RLHELLVEV_{26-34}$, regulates the oxidative stress response. It plays an important role in mycobacterial dormancy response induced by oxygen starvation.[51] Similarly, PPE4 ($YVAAHLPYV_{68-76}$), eccB3 ($VTGWRFVMR_{42-50}$, and $TGWRFVMRR_{43-51}$), eccC3 ($WTFAGHTHY_{1190-1198}$), and eccD3 ($GLFAWVLNR_{464-472}$, $ATAAAATLR_{362-370}$, and $YLVGLFAWV_{461-469}$) were required for siderophore- mediated iron acquisition in M. tb.[52] In the present study, we predicted 20 novel CTL epitopes extracted from the various virulent proteins of M. tb using different prediction tools. Few of them were already reported to induce immunological response in vivo. Further study is needed to analyse the immunological response of this novel epitopes.

Ethics Committee Approval: As there are no animal or human studies involved, I have not enclosed the ethics committee approval.

Conflict of Interest: The authors declare that they have no conflict of interest regarding the publication of this article.

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Informed Consent: As there were no human studies involved, there is no need for informed consent.

Reference

1. Kaufmann SH. Robert Koch, the Nobel Prize, and the ongoing threat of tuberculosis. N Engl J Med 2005;353;23:2423-6. **[CrossRef]**
2. https://www. who .int /news -room /fact -sheets /detail / tuberculosis dated October 14, 2020)
3. World Health Organization, 2019. WHO guidelines on tuberculosis infection prevention and control: 2019 update (No. WHO/CDS/TB/2019.1). World Health Organization.
4. Kaufmann SH, Weiner J, von Reyn CF. Novel approaches to tuberculosis vaccine development. Int J Infect Dis. 2017;56:263-7. **[CrossRef]**
5. Mazzaccaro RJ, Gedde M, Jensen ER, Van Santen HM, Ploegh HL, Rock KL, Bloom BR. Major histocompatibility class I presentation of soluble antigen facilitated by Mycobacterium tuberculosis infection. Proc Natl Acad Sci 1996;93(21):11786-91. **[CrossRef]**
6. Teitelbaum R, Cammer M, Maitland ML, Freitag NE, Condeelis J, Bloom BR. Mycobacterial infection of macrophages results in membrane-permeable phagosomes. Proc Natl Acad Sci 1999;96(26):15190-5. **[CrossRef]**
7. Canaday DH, Ziebold C, Noss EH, Chervenak KA, Harding CV, Boom WH. Activation of human CD8+ αβ TCR+ cells by Mycobacterium tuberculosis via an alternate class I MHC antigen-processing pathway. J Immunol. 1999;162(1):372-9.
8. Lalvani A, Brookes R, Wilkinson RJ, Malin AS, Pathan AA, Andersen P, Dockrell H, Pasvol G, Hill AV. Human cytolytic and interferon γ-secreting CD8+ T lymphocytes specific for Mycobacterium tuberculosis. Proc Natl Acad Sci 1998;95(1):270-5. **[CrossRef]**
9. Bai X, Wang D, Liu Y, Xiao L, Liang Y, Yang Y, Zhang J, Lin

M, Wu X. Novel epitopes identified from Mycobacterium tuberculosis antigen Rv2629 induces cytotoxic T lymphocyte response. Immunol Lett 2018;203:21-8. **[CrossRef]**

10. Bastian M, Braun T, Bruns H, Röllinghoff M, Stenger S. Mycobacterial lipopeptides elicit CD4+ CTLs in Mycobacterium tuberculosis-infected humans. J Immunol 2008;180(5):3436-46. **[CrossRef]**
11. De Libero G, Flesch I, Kaufmann SH. Mycobacteria-reactive Lyt-2+ T cell lines. Eur J Immunol. 1988;18(1):59. **[CrossRef]**
12. Silva CL, Silva MF, Pietro RC, Lowrie DB. Protection against tuberculosis by passive transfer with T-cell clones recognizing mycobacterial heat-shock protein 65. Immunol. 1994;83(3):341.
12. Lewinsohn DM, Alderson MR, Briden AL, Riddell SR, Reed SG, Grabstein KH. Characterization of human CD8+ T cells reactive with Mycobacterium tuberculosis-infected antigen-presenting cells. J Exp Med. 1998;187(10):1633-40. **[CrossRef]**
14. Hung CF, Ma B, Monie A, Tsen SW, Wu TC. Therapeutic human papillomavirus vaccines: current clinical trials and future directions. Expert Opin Biol Ther. 2008;8(4):421-39. **[CrossRef]**
15. Lin PL, Flynn JL. CD8 T cells and Mycobacterium tuberculosis infection. Semin Immunopathol 2015;37(3):239-49. Springer Berlin Heidelberg. **[CrossRef]**
16. Seder RA, Hill AV. Vaccines against intracellular infections requiring cellular immunity. Nature 2000;406(6797):793-8. **[CrossRef]**
17. Raoufi E, Hemmati M, Eftekhari S, Khaksaran K, Mahmodi Z, Farajollahi MM, Mohsenzadegan M. Epitope Prediction by Novel Immunoinformatics Approach: A State-of-the-art Review. Int J Pept Res Ther. 2019;20:1-9.
18. Brusic V, Petrovsky N. Immunoinformatics and its relevance to understanding human immune disease. Expert Rev Clin Immunol. 2005;1(1):145-57. **[CrossRef]**
19. Gardy JL, Lynn DJ, Brinkman FS, Hancock RE. Enabling a systems biology approach to immunology: focus on innate immunity. Trends Immunol. 2009;30;6:249-62. **[CrossRef]**
20. Tomar N, De RK. Immunoinformatics: an integrated scenario. Immunol. 2010;131(2):153-68. **[CrossRef]**
21. Soria-Guerra RE, Nieto-Gomez R, Govea-Alonso DO, Rosales-Mendoza S. An overview of bioinformatics tools for epitope prediction: implications on vaccine development. J Biomed Inf. 2015;53:405-14. **[CrossRef]**
22. Mohan M, Haribalaganesh R, Coico R, Sundar K. HLA-directed bioinformatics approach for genome-wide mapping of dengue CTL epitopes. Future Virol. 2018;13(5):331-42. **[CrossRef]**
23. Nielsen M, Lund O, Buus S, Lundegaard C. MHC class II epitope predictive algorithms. Immunol. 2010;130(3):319-28. **[CrossRef]**
24. Wang P, Sidney J, Dow C, Mothé B, Sette A, Peters B. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoS Comput Biol 2008;4(4):e1000048. **[CrossRef]**
25. Farrell D, Jones G, Pirson C, Malone K, Rue-Albrecht K, Chubb AJ, Vordermeier M, Gordon SV. Integrated computational prediction and experimental validation identifies promiscuous T cell epitopes in the proteome of Mycobacterium bovis. Microb Genomics 2016;2(8). **[CrossRef]**
26. Rana A, Thakur S, Kumar G, Akhter Y. Recent trends in system-scale integrative approaches for discovering protective antigens against mycobacterial pathogens. Front Genet 2018;9:572. **[CrossRef]**
27. Zhang R, Ou HY, Zhang CT. DEG: a database of essential genes. Nucleic Acids Res 2004;32(suppl_1):D271-2. **[CrossRef]**
28. Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, Jin Q. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res 2005;33(suppl_1):D325-8. **[CrossRef]**
29. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinf 2007;8(1):4. **[CrossRef]**
30. Yu CS, Cheng CW, Su WC, Chang KC, Huang SW, Hwang JK, Lu CH. CELLO2GO: a web server for protein subCELlular LOcalization prediction with functional gene ontology annotation. PloS one. 2014;9(6):e99368. **[CrossRef]**
31. https://www.iedb.org/
32. Yao Y, Huang W, Yang X, Sun W, Liu X, Cun W, Ma Y. HPV-16 E6 and E7 protein T cell epitopes prediction analysis based on distributions of HLA-A loci across populations: an in silico approach. Vaccine 2013;31(18):2289-94. **[CrossRef]**
33. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, Wheeler DK, Gabbard JL, Hix D, Sette A, Peters B. The immune epitope database (IEDB) 3.0. Nucleic Acids Res 2015;43(D1):D405-12. **[CrossRef]**
34. Farrell D, Jones G, Pirson C, Malone K, Rue-Albrecht K, Chubb AJ, Vordermeier M, Gordon SV. Integrated computational prediction and experimental validation identifies promiscuous T cell epitopes in the proteome of Mycobacterium bovis. Microb Genomics 2016;2(8). **[CrossRef]**
35. Peters B, Bulik S, Tampe R, Van Endert PM, Holzhütter HG. Identifying MHC class I epitopes by predicting the TAP transport efficiency of epitope precursors. J Immunol. 2003;171(4):1741-9. **[CrossRef]**
36. Tenzer S, Peters B, Bulik S, Schoor O, Lemmel C, Schatz MM, Kloetzel PM, Rammensee HG, Schild H, Holzhütter HG. Modeling the MHC class I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class I binding. Cell Mol Life Sci 2005;62(9):1025-37. **[CrossRef]**
37. Calis JJ, Maybeno M, Greenbaum JA, Weiskopf D, De Silva AD, Sette A, Keşmir C, Peters B. Properties of MHC class I presented peptides that enhance immunogenicity. PLoS Comput Biol. 2013;9(10):e1003266. **[CrossRef]**
38. Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Raghava GP, Open Source Drug Discovery Consortium. In silico approach for predicting toxicity of peptides and proteins. PloS one 2013;8;9:e73957. **[CrossRef]**
39. Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v. 2-a server for in silico prediction of allergens. J Mol Model

2014;20(6):2278. **[CrossRef]**

40. Flynn JL. Immunology of tuberculosis and implications in vaccine development. 2004;84(1-2):93-101. **[CrossRef]**
41. Canaday DH, Wilkinson RJ, Li Q, Harding CV, Silver RF, Boom WH. CD4+ and CD8+ T cells kill intracellular Mycobacterium tuberculosis by a perforin and Fas/Fas ligand-independent mechanism. J Immunol. 2001;167(5):2734-42. **[CrossRef]**
42. De Groot AS, Sbai H, Aubin CS, McMurry J, Martin W. Immuno-informatics: Mining genomes for vaccine components. Immunol Cell Biol. 2002;80(3):255-69. **[CrossRef]**
43. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and methods for T-and B-cell epitope prediction. J Immunol Res. 2017. **[CrossRef]**
44. Ahmed RK, Maeurer MJ. T-cell epitope mapping. In Epitope Mapping Protocols 2009:427-38. Humana Press. **[CrossRef]**
45. Adhikari UK, Tayebi M, Rahman MM. Immunoinformatics approach for epitope-based peptide vaccine design and active site prediction against polyprotein of emerging oropouche virus. J Immunol Res. 2018. **[CrossRef]**
46. Rao BS, Gupta KK, Kumari S, Gupta A, Pujitha K. Conserved HIV wide spectrum antipeptides-a hope for HIV treatment. Adv Tech Biol Med 2013. **[CrossRef]**
47. Dhanda SK, Vir P, Singla D, Gupta S, Kumar S, Raghava GP. A web-based platform for designing vaccines against existing and emerging strains of Mycobacterium tuberculosis. PloS one 2016;11(4):e0153771. **[CrossRef]**
48. Liu SD, Su J, Zhang SM, Dong HP, Wang H, Luo W, Wen Q, He JC, Yang XF, Ma L. Identification of HLA-A* 11: 01-restricted Mycobacterium tuberculosis CD 8+ T cell epitopes. J Cell Mol Med. 2016;20(9):1718-28. **[CrossRef]**
49. Smith I. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin Microbiol Rev. 2003;16(3):463-96. **[CrossRef]**
50. Primm TP, Andersen SJ, Mizrahi V, Avarbock D, Rubin H, Barry CE. The stringent response of Mycobacterium tuberculosis is required for long-term survival. J bacteriol 2000;182(17):4889-98. **[CrossRef]**
51. Saini DK, Malhotra V, Dey D, Pant N, Das TK, Tyagi JS. DevR-DevS is a bona fide two-component system of Mycobacterium tuberculosis that is hypoxia-responsive in the absence of the DNA-binding domain of DevR. Microbiol. 2004;150(4):865-75. **[CrossRef]**
52. Serafini A, Pisu D, Palù G, Rodriguez GM, Manganelli R. The ESX-3 secretion system is necessary for iron and zinc homeostasis in Mycobacterium tuberculosis. PloS one 2013;8(10):e78351. **[CrossRef]**