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In silico rational design of a novel tetra-epitope tetanus vaccine with complete population coverage using developed immunoinformatics and surface epitope mapping approaches

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ABSTRACT

Presentation of many unwanted epitopes within tetanus toxoid vaccine to lymphocyte clones may lead to production of many unwanted antibodies. Moreover an ideal vaccine must cover all individuals in a population that is dependent to the kinds of human leukocyte antigen alleles. Concerning these issues, our study was aimed to *in silico* design of a multi-epitope tetanus vaccine (METV) in order to improve population coverage and protectivity of tetanus vaccine as well as reduction of complications. Concerning these issues, a novel rational filtration was implemented to design a novel METV using immunoinformatics and surface epitope mapping approaches.

Prediction of epitopes for tetanus toxin was performed in the candidate country in which the frequency had been gathered from almost all geographical distributions. The most strong binder epitopes for major histocompatibility complex class II were selected and among them the surface epitopes of native toxin were selected. The population coverage of the selected epitopes was estimated. The final candidate epitopes had highly population coverage. Molecular docking was performed to prediction of binding affinity of our candidate epitopes to the HLA-DRB1 alleles.

At first, 680 strong binder epitopes were predicted. Among them 11 epitopes were selected. Finally, 4 epitopes had the most population coverage and suggested as a tetra-epitope tetanus vaccine. 99.41% of inessential strong binders were deleted using our tree steps filtration. HLA-DP had the most roles in epitope presentation. Molecular docking analysis proved the strong binding affinity of candidate epitopes to the HLA-DRB1 alleles.

In conclusion, we theoretically reduced 99.41% of unwanted antibodies using our novel filtration strategies. Our tetra-epitope tetanus vaccine showed 100% population coverage in the candidate country. Furthermore, it was demonstrated that HLA-DP and HLA-DQ had more potential in epitope presentation in comparison to HLA-DRB1.

Introduction

Tetanus is a vaccine-preventable disease but many reports of the disease have been documented in developing countries, especially in urban slums and rural areas [1]; and even in the vaccinated individuals [2–6]. Mortality rate varies from 6% to 72% depending on the quality of medical services in these regions but it rarely happens in unvaccinated individuals in developed countries [1]. Approximately 56,743 deaths have been reported due to tetanus in 2015[7].

Tetanus is a neurological disorder caused by a neurotoxin of *Clostridium tetani* named as tetanospasmin or tetanotoxin. The most obvious symptom of tetanus is rhythmic and severe muscle spasms which causes by this exotoxin. Tetanotoxin is produced subsequent to an infected deep injury in anaerobic condition. This neurotoxin consists of 1315 amino acids composed of a 100 kDa heavy and a 50 kDa light chains. The C-terminal domain of heavy chain binds to neural cell receptor and the N-terminal domain facilitates translocation of catalytic domain into the neuronal cytoplasm. The catalytic domain is responsible for all clinical signs located in light chain [8–11].

Since tetanus vaccine comes from wild strain, its production could be complex and costly. To vaccine production, the toxin is inactivated by treatment with formaldehyde or heating to get the toxoid antigen [12].

The first tetanus toxoid vaccine was produced in 1924 and a more

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effective adsorbed version of the vaccine created in 1938 [13]. The trivalent diphtheria–tetanus–pertussis (DTP) vaccine developed in the 1940s. In the U.S since the years 1930s and 1940s, due to introduction of tetanus vaccination, cases have declined more than 95% and deaths fell more than 99% [14].

Chemical and physical processing of a toxin could be led to denaturation of its structural epitopes. Accordingly, these conformational epitopes which can play a major role in immunization are not efficiently present to immune system. In this case, linear epitopes made production of inefficient antibodies that cannot strongly bind to structural epitopes in the surface of tetanus toxin. Moreover, production of many weak binder antibodies by many B cell clones that raised due to presentation of many unwanted epitopes could be led to increased metabolism. We speculate that febrile seizure is related to this issue after vaccination in children [15–20].

Due to current problems in production of tetanus vaccine, some efforts have been conducted to development of various recombinant vaccines based on the use of C-terminal domain of the recombinant toxin in animal model but none of them have been entered into clinical trials yet [21–27].

An ideal vaccine must cover all individuals in a population. Accordingly, during the past two decades, several studies have been focused on designing HLA-based vaccines [28–32], but there is no report concerning to design a tetanus vaccine based on all available HLA statistics in national or international levels so far.

To filling this gap, our study was aimed to *in silico* design of a multi-epitope tetanus vaccine (METV) in order to improve population coverage and protectivity of tetanus vaccine as well as reduction of complications. To achieve this goal, a novel rational filtration was innovated to design a novel multi-epitope tetanus vaccine using immunoinformatics and surface epitope mapping approaches.

Methods

Vaccine design strategies

At first, the frequency distributions of HLA alleles in almost all countries, for which HLA allele frequencies were available, investigated. Among them, a country in which its HLA frequencies data for HLA-DP, HLA-DQ, and HLA-DRB1 alleles had been obtained from almost all its geographical distributions and ethnicities was selected for prediction of interaction between the pools of epitopes and HLA alleles.

The second strategy was to find a filtration method for reduction of the numbers of predicted epitopes to selection of the best immunogenic epitopes. In this regards, at first, the overlapped epitopes having the overlapped cores with the highest affinity for MHCII alleles were selected. To narrow filtration, structurally external epitopes having superficial cores, were then selected. The third filtration step was selection of epitopes with the highest population coverage. Finally, the binding affinity of candidate epitopes was estimated using molecular docking. The general process of our filtration methods to selection of candidate epitopes is illustrated in Fig. 1.

Evaluation of HLA allele frequencies

The frequency of MHC class II alleles in all countries of the world for which HLA allele frequencies were available in allele frequencies server (www.allelefrequencies.net), evaluated [33]. There is HLA frequencies data from 126 countries gathered in this server. In each country, the frequency of all types of HLA alleles (DP, DQ and DR) was evaluated.

Our goal was selection of a country in which the frequency of all HLA alleles had been obtained from almost all its geographical regions.

Prediction of MHCII epitopes

The amino acid sequence of *Clostridium tetani* (Massachusetts Strain/E88) was obtained from the uniprot server [34]. In order to predict the strong binder epitopes for each allele, the NetMHCIIpan 3.1 server (http://www.cbs.dtu.dk/services/NetMHCIIpan/) was used [35].

Regarding to vaccine designing, in terms of antigen presentation, many studies had been focused on just some HLA-DRB1 alleles. But, we hypothesized that HLA-DP and HLA-DQ have substantial effects on antigen presentation. Thus, in order to prove our hypothesis and to perform a precise epitope prediction in NetMHCIIPan 3.1 server, we used all HLA-DP, DQ, and DRB1 alleles.

In the initial inputs, the peptide length was considered as 15 amino acid residues. The types of HLAs based on their frequencies in the desired country were inserted as input data. The data for HLA-DPA, HLA-DPB, HLA-DQA, and HLA-DQB alleles in the allele frequencies database had been reported separately, so we manually insert all the alleles one by one as HLA-DPA1*01:03 & HLA-DPB1*01:01, HLA-DPA1*01:03 & HLA-DPB1*01:02, HLA-DPA1*01:03 & HLA-DPB1*02:02, and so on.

The NetMHCIIpan 3.1 server predicts the binding affinity of epitopes to the MHCII molecules using a scoring system at nanomolar scale so that the strong and weak binders had a binding value of less than 50 and 50–500 nm, respectively [35].

Filtration strategies

Two main filtration steps were considered in order to reduce the numbers of predicted epitopes and also to obtain the more specific epitopes located in the surface of tetanus toxin.

Selection of strong binder epitopes having identical or overlapped cores

At the first step, the sequences of predicted epitopes that located adjacent together were classified regarding their identical or overlapped cores and also sorted from the highest to lowest affinities. Among the predicted epitopes, the identical or overlapped ones with significant affinity were then selected.

All selected strong binder epitopes for each individual allele (DP, DQ, and DR) was listed and categorized based on the kind and the numbers of their corresponding alleles.

Surface epitope and core mapping

Two previously determined three-dimensional structures corresponding to the light (PDB ID: 1z7h) and heavy (PDB ID: 1af9) chains of tetanus toxin were used in this study.

This filtration step was based on this rational fact that antibodies are just attached to the surface epitopes. Thus, among the previous selected epitopes, surface epitopes were selected using UCSF Chimera software (v.1.10.2) [36]. Among surface epitopes, the epitopes with surface cores were selected as candidate epitopes.

Analysis of the population coverage of the selected epitopes

The population coverage of candidate epitopes in the candidate country and in the world were determined reference to the frequency of each kind of HLA allele and in combinations as well. This assay was performed to elucidate that what kind of HLA is responsible for maximum epitope presentation. The HLA alleles that were able to present the selected epitopes used for estimation of the population coverage using IEDB server (http://tools.immuneepitope.org/tools/population/iedb_input) [37]. This server uses 'allelefrequencies.net' database to calculate the population coverage.

Molecular docking study of HLA-epitope interaction

To confirm the binding affinity of candidate epitopes with the relevant HLA allele, molecular docking was performed using AutoDockVina (ADV, v.1.1.2) [38]. The 3D structure of candidate epitopes were predicted in I-Tasser server and subjected for molecular

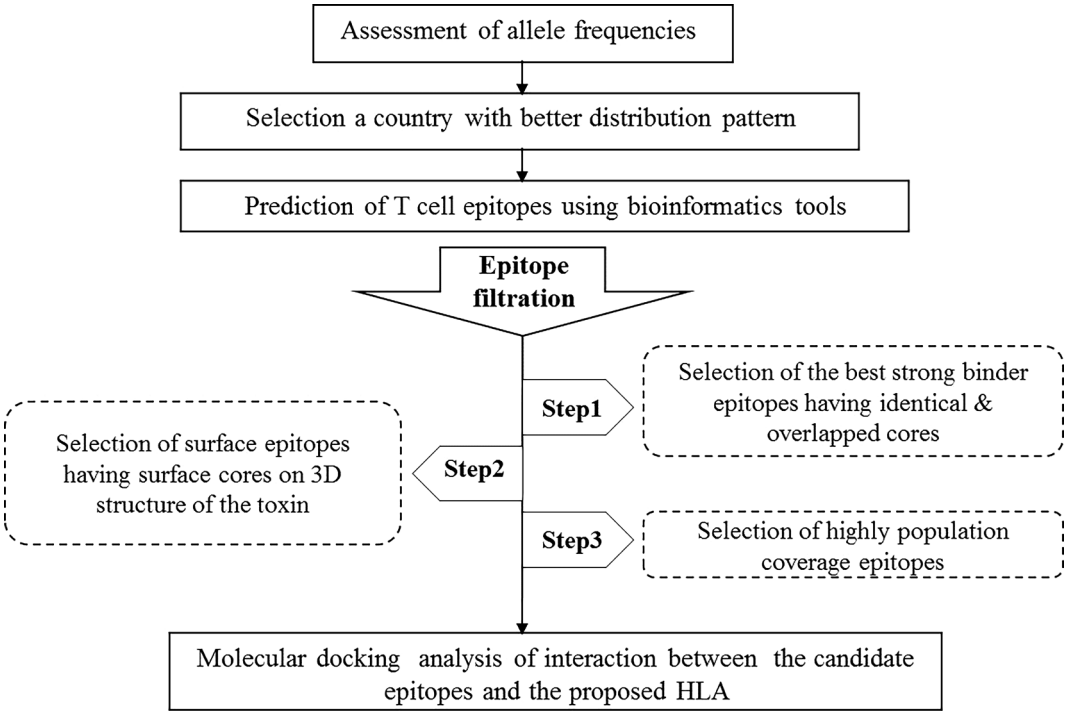

Fig. 1. Sequential filtration steps to selection of candidate epitopes.

docking against the predetermined 3D structure of HLA-DRB1 alleles (PDB ID: 3L6F) [39] which was the only available allele for our candidate epitopes in PDB server.

The HLA-DRB1 and the candidate epitopes were treated as the receptor and ligand, respectively. In order to prepare receptor and ligand for AutoDock Vina, AutoDock Tools (ADT, v.1.5.6) was used [40]. All hydrogen atoms were added and all side chains of the epitope were defined as flexible using ADT. The docking was performed with a grid/spacebox center of 18.149, 18.244, and 9.332 Å in the x-, y-, and z-axes to allow the epitope to freely bind to the epitope binding groove of HLA-DRB1*01:01. After docking simulation, the best output model with minimum binding energy was selected for analysis and visualization in 'BIOVIA discovery studio visualizer' (v.4.5) [41] and 'UCSF Chimera' (v.1.10.2) [36].

Results

Selection of strong binder epitopes with identical or overlapped cores

A total of 680 strong binder epitopes were predicted by NetMHCIIPan 3.1 server. At the first step of filtration, 183 strong binder epitopes with identical and overlapped cores were selected. Some selected epitopes demonstrated in Tables 1 and 2, respectively.

Surface epitope and core mapping

In the second step, among 183 selected epitopes, 11 epitopes with surface core were selected using 3D structure analysis (Fig. 2). All our selected epitopes were located in the surface of the light and heavy chains of the tetanus toxin. The locations of epitopes on 3D structure of tetanus toxin are shown in Fig. 2. It was also proved that some strong binder epitopes were structurally located inside the toxin (Fig. 3).

The selected surface epitopes and their corresponding HLA allele types are listed in Table 3. These alleles were also used for prediction of population coverage in the desired countries.

Population coverage analysis

In the third step of filtration, four epitopes (i.e. epitope 5, 6, 8, and 11) with the highest population coverage in France were selected for designing a multi-epitope tetanus vaccine. The percent of population coverage for each epitope is shown in Fig. 4.

The population coverage of four candidate epitopes in the world, France, and other countries are shown in Fig. 5. The final candidate epitopes had 95.8 and 100% coverage by HLA-DP alleles solely in the world and France, respectively. Combination of HLA-DP with HLA-DQ reached this value to 99.43% in the world. The population coverage of the candidate epitopes reference to each kind of HLA alleles solely and in combinations is demonstrated in Fig. 6.

Molecular docking analysis

The binding affinity of epitope5, 6, 8, and 11 against the antigen binding groove of HLA-DRB1*01:01were determined as −6.4, −6.3, −5.8, and −6.3 kcal/mole, respectively (Fig. 7).

Discussion

Conventional tetanus vaccine consists of a large detoxified protein that injects to children under the vaccination programs in many countries for a long time [13]. Some efforts have been conducted regarding the use of recombinant technology as a replacement strategy instead of toxoid vaccine [21–27,42–44]. In this regard, some studies had been focused on the use of recombinant tetanotoxin C-terminal domain (TeNT-Hc) [21–27]. Immunoprotectivity of this part of tetanus toxin has proven in animal model but it has not entered into clinical trial yet [21,23,25,26,44,45].

One of a novel strategy of vaccine developments is the implementation of responsible epitopes instead of using a whole pathogenic protein [46–74].

Multi-epitope vaccine was introduced in 1985 for the first time to develop a vaccine against cholera and *E. coli* heat-labile toxin [75].

Table 1
A group of strong binder epitopes with identical cores. Among a group of epitopes with similar cores, the one with the highest affinity (sequence: 668–703) was selected.

Sequence	Allele	Peptide	Core	Affinity (nM)	Binding level
668-703	DRB1*01:01	TE**YYLIPVASS**SKDV	**YYLIPVASS**	**2.50**	*SB
666-701	DRB1*01:01	YDTE**YYLIPVASS**SK	**YYLIPVASS**	3.00	SB
667-702	DRB1*01:01	DTE**YYLIPVASS**SKD	**YYLIPVASS**	3.06	SB
669-704	DRB1*01:01	E**YYLIPVASS**SKDVQ	**YYLIPVASS**	3.50	SB
665-700	DRB1*01:01	RYDTE**YYLIPVASS**S	**YYLIPVASS**	4.57	SB
664-669	DRB1*01:01	LRYDTE**YYLIPVASS**	**YYLIPVASS**	10.00	SB

*Strong Binder.

Recently, many multi-epitope vaccines have been suggested as a new effective approach against some infectious diseases or cancers, i.e. human immunodeficiency virus (HIV), hepatitis C virus (HCV), human papilloma virus (HPV), Neisseria meningitidis, malaria, tuberculosis, swine fever, influenza, foot and mouth disease, anthrax, and melanoma [54,57–74]. Identification of T-cell epitopes in a desired protein had been the key point in this kind of studies [75–78].

None of studies relating to tetanus toxoid or TeNT-Hc has mentioned on the existence of excess epitopes. This issue is an important disadvantage which mainly originated from the large size of protein.

It could be rationally speculated that a multi-epitope vaccine leads to more specific induction of immune responses. Our analyses regarding epitope prediction and structural studies showed that many strong binder epitopes are located in the depth of toxin conformation. Thus, it can be proposed that injection of the first dose of tetanus vaccine

Table 2
A group of strong binder epitopes with overlapped cores. As shown, the 7th epitope (sequence: 708–723) has a core that exists in other epitopes. In this case, the epitope with two cores and the strongest affinity (sequence: 706–721) was preferentially selected (underlined and bold cores).

Sequence	Allele	Peptide	Core	Affinity (nM)	Binding level
705-720	DRB1*01:01	NIYYRRLYNGLKFII	YRRLYNGLK	11.19	*SB
706-721	DRB1*01:01	IY**YRRLYNGLK**FIIK	**YRRLYNGLK**	11.89	SB
704-719	DRB1*01:01	LNIYYRRLYNGLKFI	YRRLYNGLK	12.42	SB
707-722	DRB1*01:01	YYRRLYNGLKFIIKR	YRRLYNGLK	16.84	SB
703-718	DRB1*01:01	KLNIYYRRLYNGLKF	YRRLYNGLK	17.22	SB
702-717	DRB1*01:01	GKLNIYYRRLYNGLK	YRRLYNGLK	34.05	SB
708-723	DRB1*01:01	YRRL**YNGLKFIIK**RY	**YNGLKFIIK**	43.63	SB

*. Strong Binder, Gray highlighted sequence: overlapped core

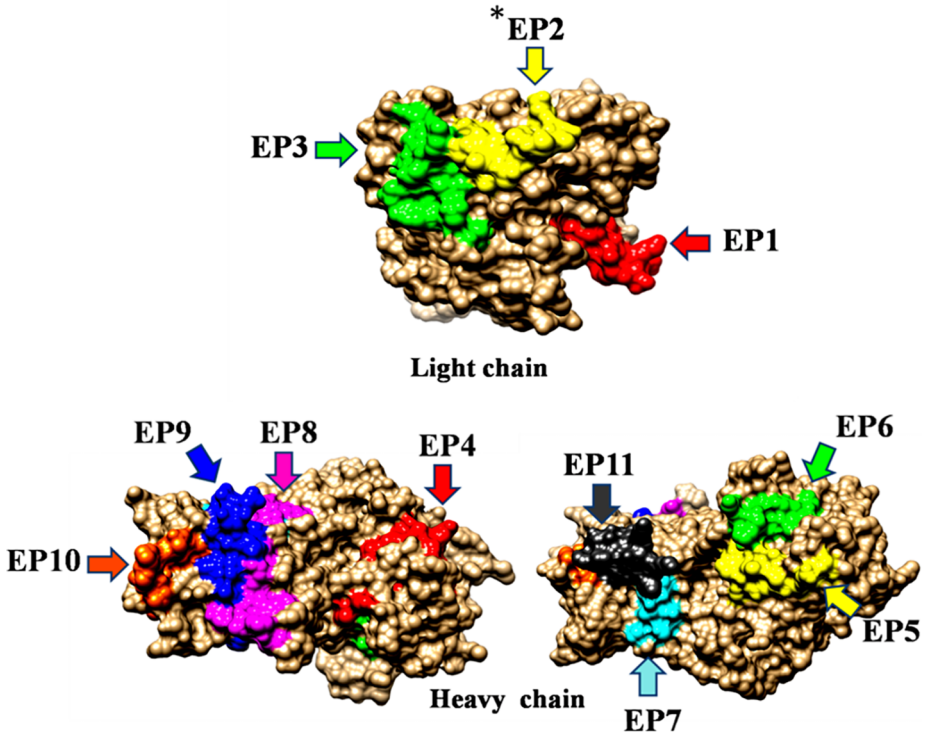

Fig. 2. The location of the candidate superficial epitopes on 3D structure of the light and heavy chains.*EP: Epitope.

bearing excess epitopes leads to production of some desired and many unwanted antibodies. Subsequently, hyperthermia and loss of energy would be caused by overstimulation of immune system. Invasive hyperthermia could be led to complicated febrile seizure that frequently reported after vaccination [15–20].

From the other point of view, we speculated that by increasing in the concentration of antibodies in bloodstream, an in vivo prozone phenomenon could be happened. It is rational that the efficiency of circulating antibodies would be reduced in terms of protectivity so that the possibility of getting tetanus would be possibly increased in deep injuries.

Moreover, in terms of autoimmunity, elevation of the concentration of circulating antibodies provides the chance for interaction with tissue antigens. Multiple injection of tetanus vaccine according to vaccination program can aggravate this issue so that it may lead to autoimmunity [79–81]. National vaccination program is a five-time vaccination in 2,

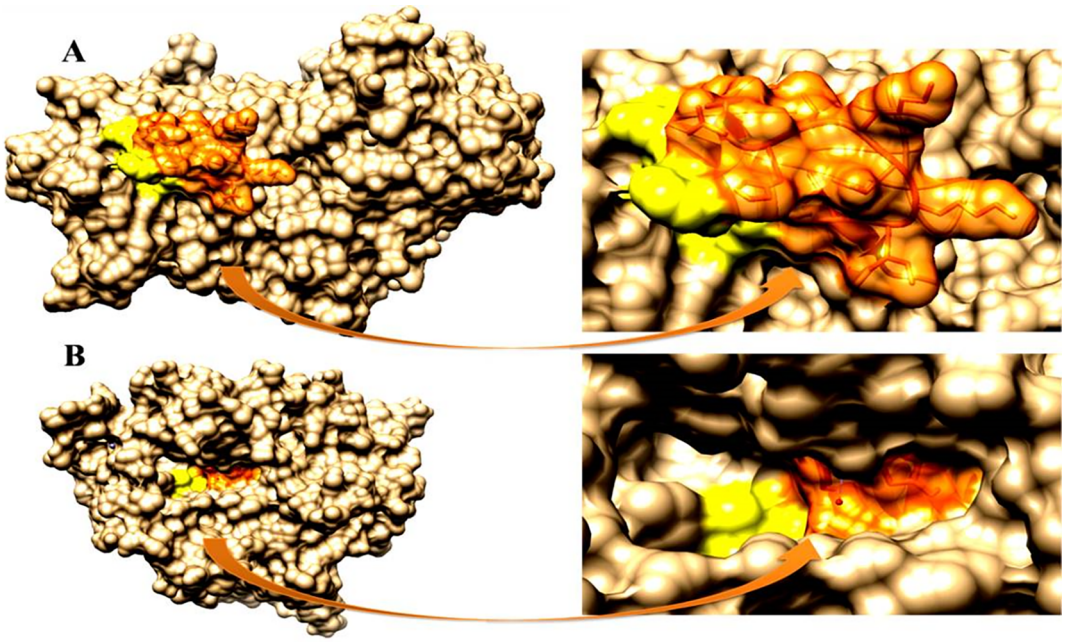

Fig. 3. Comparison of structural locations of a candidate epitope (EP11) and a rejected one on the 3D structure of tetanus toxin. A. Structural demonstration of side chains in the core sequence of a candidate epitope. B. An internal strong binder epitope. This epitope was predicted as strong binder epitope but it is not structurally accessible for antibodies. In both Fig A and B, the epitopes are colored in yellow and the cores with their side chains are orange highlighted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
The candidate epitopes with their corresponding HLA alleles.

	*EPs	Epitope sequence	Core	Alleles	
Light chain	**EP1**	EVRGIVLRVDNKNYF	VLRVDNKNY	DRB1	*08:06,*08:10,*12:01,*14:06
	EP2	ENITSLTIGKSKYFQ	ITSLTIGKS	DRB1	*01:02,*08:06,*08:10,*11:04,*12:01,*12:02,*13:21,*14:06,*14:12
	EP3	KGQNMRVNTNAFRNV	MRVNTNAFR	DRB1	*01:02,*08:06,*08:10,*11:04,*13:03, *13:10,*13:21,*14:06,*14:12
Heavy chain	**EP4**	GKAIHLVNNESSEVI	IHLVNNESS	DRB1	*01:02,*08:06,*08:10
	EP5	NLYINGVLMGSAEIT	INGVLMGSA	DRB1	*01:01,*01:02,*08:06,*08:10
				DQ	DQA1*01:02-DQB1*06:03, DQA1*01:03-DQB1*06:02, DQA1*01:03-DQB1*06:03, DQA1*05:01-DQB1*06:02, DQA1*05:01-DQB1*06:03, DQA1*05:01-DQB1*03:01, DQA1*05:01-DQB1*03:03, DQA1* 05:01-DQB1*03:04, DQA1*05:01-DQB1*06:01
	EP6	IDKFRIFCKALNPKE	FRIFCKALN	DRB1	*01:01,*01:02,*08:01,*08:03,*08:06,*08:10,*11:01,*11:04,*11:09,*11:12,*11:29,*12:01,*12:02,*13:03,*13:05,*13:21,*14:02,*14:04,*14:06,*14:12,*16:02
				DP	DPA1*01:03-DPB1*03:01, DPA1*01:03-DPB1*14:01, DPA1*01:03-DPB1*15:01, DPA1*01:04-DPB1*03:01, DPA1*01:04-DPB1*14:01, DPA1*01:04-DPB1*15:01, DPA1*02:01-DPB1*03:01, DPA1*02:01-DPB1*14:01, DPA1*02:01-DPB1*15:01, DPA1*02:02-DPB1*14:01, DPA1*02:02-DPB1*15:01,
	EP7	YYLIPVASSSKDVQL	YYLIPVASS	DRB1	*01:02,*08:10,*13:21,*14:06
				DQ	DQA1*05:01-DQB1*03:01
	EP8	LYNGLKFIIKRYTPN	FIIKRYTPN	DRB1	*01:02,*08:01,*08:03,*08:04,*08:06,*08:10,*11:04,*11:12,*11:29,*12:01,*12:02,*13:05,*13:21,*14:02,*14:06,*14:12,*16:02
				DP	DPA1*01:03-DPB1*15:01, DPA1*01:04-DPB1*15:01, DPA1*01:04-DPB1*14:01, DPA1*02:01-DPB1*15:01, DPA1*02:02-DPB1*15:01, DPA1*01:03-DPB1*14:01
	EP9	DFIKLYVSYNNNEHI	IKLYVSYNN	DRB1	*08:06,*14:06,*12:01,*12:02,*08:10
	EP10	LDRILRVGYNAPGIP	LDRILRVGY	DRB1	*01:02,*12;02,*08:06,*08;10
	EP11	IASNWYFNHLKDKIL	FNHLKDKIL	DRB1	*01:01,*01:02,*08:06,*08:10,*11:04,*12:01,*12:02,*13:21,*14:06,*14:02
				DP	DPA1*01:03-DPB1*15:01, DPA1*01:04-DPB1*15:01, DPA1*02:01-DPB1*15:01, DPA1*02:02-DPB1*15:01

*Epitopes.

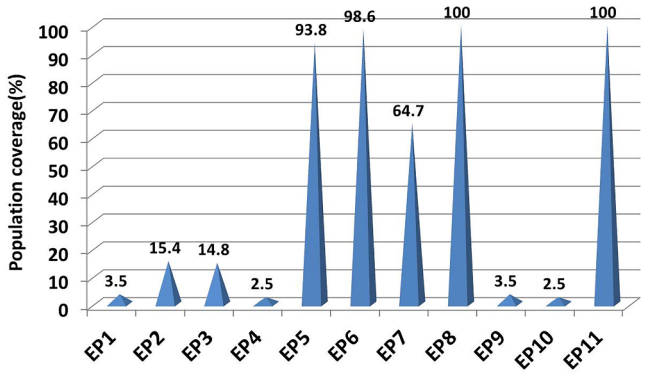

Fig. 4. The population coverage of all selected surface epitopes in France. EP5, EP6, EP8, and EP11 had the maximum population coverage ranged from 93.8 to 100%. These epitopes were selected as the final candidate epitopes.

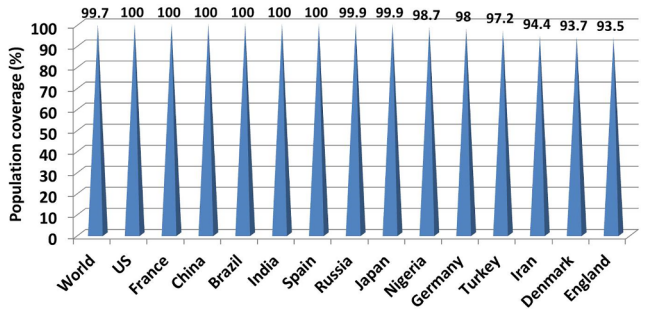

Fig. 5. The population coverage of tetra-epitope tetanus vaccine in the world and other countries. Our METV showed the maximum coverage ranged from 93.5 to 100%.

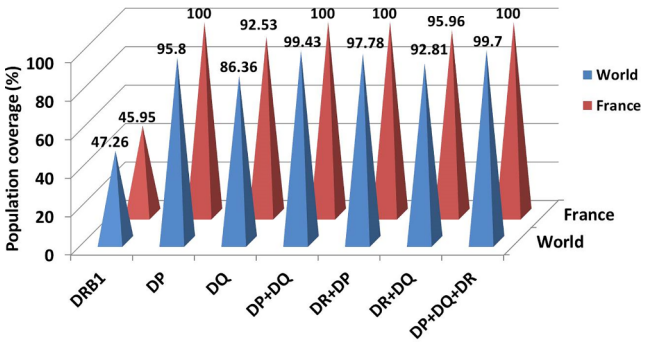

Fig. 6. Determination of population coverage of the candidate epitopes reference to each kind of HLA alleles solely and in combination in the world and France. The final candidate epitopes had 95.8 and 100% coverage by HLA-DP alleles in the world and France, respectively. Combination of HLA-DP and HLA-DQ reached this value to 99.43% in the world. The minimum coverage was seen for HLA-DRB1.

4, 6, 15–18 months old and 4–6 years old, revaccination every ten years, and vaccination after deep injuries or during pregnancy [13] which may expose anyone to those abovementioned unwanted antibodies over and over again throughout the life.

As a rational insight, subsequent overload of antibodies may negatively affect the human blood homeostasis. As an overall approach, it can be said that there is a difference between blood homeostasis of humans in the present and past century.

It is expected that by injection of an approved multi-epitope vaccine in future, the human blood homeostasis approaches to its previous normal condition. Furthermore, by activation of specific lymphocyte clones, specific protective antibodies will be produced. With this aspect, we will approach a more specific immune response in humans.

Based on our literature review on all published papers about multi-epitope vaccines, we found that almost all studies neglect the role of HLA-DP and HLA-DQ alleles [51,82–97]. In this project, we suggested that HLA-DP and HLA-DQ have further roles in the presentation of epitopes to immune cells.

To prove our hypothesis, all countries in the world were evaluated regarding the frequency of all HLA alleles. Among them, France was one of the countries in which not only all HLA-DP, HLA-DQ, and HLA-DRB1 alleles were considered but also the samples for determination of HLA frequencies had been gathered from almost all geographical regions.

Since the different HLA alleles have different frequencies in different populations, we considered all available allele frequencies information in France to give an equal possibility to all people to be protected by our multi-epitope vaccine. According to our best of knowledge, reference to our heavy search in databanks i.e. PubMed and Google, unfortunately nobody or no company have pointed to the issue of population coverage in the administered vaccines in terms of HLA alleles frequencies.

Using our filtration strategies, among 680 strong binder epitopes, 183 epitopes having overlapped cores were selected. In this step, many similar strong binder epitopes (73%) were filtered. Our structural analyses showed that many surface epitopes had no surface cores. In this step, 11 epitopes were selected so that 25.38% of the previous selected epitopes were filtered. Using two filtration steps, 98.38% of inefficient strong binder epitopes were filtered.

To applicability of a multi-epitope based vaccine, several ideal epitopes is critically necessitated to complete coverage in the candidate country or the world population. Estimation of population coverage for those 11 selected epitopes from the previous filtration step showed that only 4 epitopes had the maximum coverage and therefore selected as candidate epitopes. According to results, the three filtration steps made 99.41% (676 epitopes) reduction in the numbers of strong binder epitopes. In comparison to conventional tetanus vaccine, we theoretically suggest that by using our METV, 99.41% of unwanted antibodies will not produce. It has already been suggested that eleven different HLA molecules can cover 90% of population in several ethnic groups [92] whereas we showed that 4 epitopes can cover 100% of a candidate population, in this case, France. Interestingly, the coverage in USA, China, Brazil, India, and Spain was 100% too.

Further analyses showed that 99.7% of the world population can be theoretically protected against tetanus by our designed tetra-epitope vaccine. In other countries with good allele frequencies data, the population coverage ranged between 93.5 and 99.9%.

The other issue in this study was this matter whether HLA-DP or HLA-DQ alleles has significant role in epitope presentation or not. According to results, the population coverage of our tetra-epitope vaccine was 100% in France and 95.8% in the world that obtained by just HLA-DP alleles whereas for HLA-DRB1 was 45.95 and 47.26%, respectively. This value for HLA-DQ was 92.53 and 86.36, respectively. This result pointed to this issue that up to now, the critical role of HLA-DP and HLA-DQ alleles in epitope presentation has been neglected [51,82–97]. Despite the conventional notice to frequency determination of HLA-DR alleles, our result was also showed that the frequencies of all HLA alleles i.e. DRB1, DP, and DQ must be determined and considered in each country. In this case, in a world scale mega-project, a universal multi-epitope vaccine would be designed against all vaccine-preventable infectious diseases.

Significant affinity of the candidate epitopes (ranging from −5.8 to −6.4 kcal/mole) indicated that these epitopes can attach to antigen binding groove of HLA-DRB1*01:01 and theoretically they would be presented to immune system effectively. According to our results, concerning to the significance of HLA-DP and HLA-DQ alleles in presentation of the candidate epitopes, molecular docking experiments with these HLA alleles must be performed but no studies have been performed to determine the 3-dimensional conformation of HLA-DP and HLA-DQ alleles.

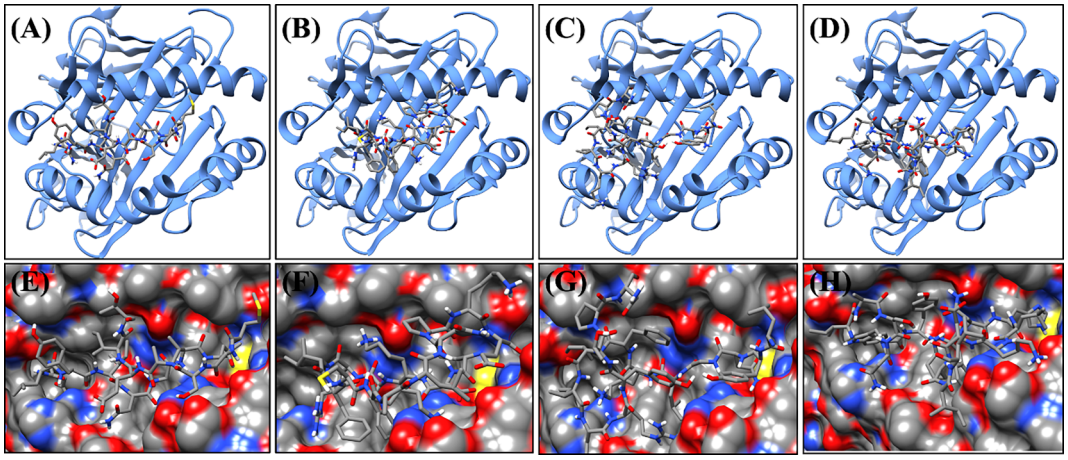

Fig. 7. Molecular docking of epitope 5, epitope 6, epitope 8, and epitope 11 against the HLA-DRB1*01:01 antigen binding groove. A ribbon diagram of HLA-DRB1*01:01 showing epitope 5 (A), epitope 6 (B), epitope 8 (C), and epitope 11 (D) with gray sticks for carbon, blue for nitrogen, red for oxygen, white for hydrogen, and yellow for sulfur. Epitope 5 (E), epitope 6 (F), epitope 8 (G), and epitope 11 (H) are shown in the top scoring binding orientation predicted by molecular docking using AutoDock Vina. The molecular surface of HLA-DRB1*01:01 is showing as gray for carbon, blue for nitrogen, red for oxygen, white for hydrogen, and yellow for sulfur. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In conclusion, our approach theoretically led to more specific immune response by reduction of unnecessary antibodies and also decreased the possibility of auto-antibodies which could be due to prozone phenomenon after vaccination by toxoids. The immunogenicity and protectivity of our designed tetra-epitope tetanus vaccine should be examined in a complementary study. Our insight may be a promising strategy to design multi-epitope vaccines against any desirable toxin or protein. *In silico* analyses showed that we successfully filtered many unwanted epitopes using our novel filtration strategies. Our tetra-epitope tetanus vaccine showed complete population coverage in the candidate country. Furthermore, the significant role of HLA-DP and HLA-DQ in epitope presentation was demonstrated.

Contribution

S.B performed all bioinformatics assays, analyses, and writing the manuscript. M.S and A.G contributed in analyzing bioinformatics assays. H.A performed molecular docking. K.P.B supervised the project and contributed in experimental design, writing, revision, and redaction of the manuscript. The idea for designing a multi-epitope tetanus vaccine and experimental design belong to the corresponding author, K.P.B. The following ideas including “in vivo prozone”, “post-vaccination autoimmunity”, “critical roles of HLA-DP and HLA-DQ alleles in epitope presentation”, “post-vaccination imbalance in blood homeostasis”, “some strong binder epitopes are structurally located inside the toxin”, and “suggestion of a universal multi-epitope vaccine for all infectious diseases” belong to the corresponding author, K.P.B.

Declaration of Competing Interest

None declared.

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