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Design of Multi-Epitope Vaccine against SARS-CoV-2

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Abstract**:** *The ongoing COVID-19 pandemic requires urgently specific therapeutics and approved vaccines. Here, the four structural proteins of the Severe Acute Respiratory Syndrome CoronaVirus 2* (*SARS-CoV-2*)*, the causative agent of COVID-19, are screened by in-house immunoinformatic tools to identify peptides acting as potential T-cell epitopes. In order to act as an epitope, the peptide should be processed in the host cell and presented on the cell surface in a complex with the Human Leukocyte Antigen* (*HLA*)*. The aim of the study is to predict the binding affinities of all peptides originating from the structural proteins of SARS-CoV-2 to 30 most frequent in the human population HLA proteins of class I and class II and to select the high binders* (IC_{50} *< 50 nM*)*. The predicted high binders are compared to known high binders from SARS-CoV conserved in CoV-2 and 77% of them coincided. The high binders will be uploaded onto lipid nanoparticles and the multi-epitope vaccine prototype will be tested for ability to provoke T-cell mediated immunity and protection against SARS-CoV-2.*

Keywords**:** *COVID-19, multi-epitope vaccine, SARS-CoV, SARS-CoV-2, EpiJen, EpiTOP, EpiDOCK, high binders, HLA class I, HLA class II*.

1. Introduction

COVID-19 is ongoing pandemic with almost 30 million confirmed cases and more than 900,000 deaths (September 2020). It is caused by the Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) which is closely related to SARS-CoV and Middle East Respiratory Syndrome CoronaVirus (MERS-CoV), both of which caused local epidemic in 2003-2004 and 2012-2018, respectively. The novel CoV-2 shows a higher contagiousness than the previous coronaviruses due to a stronger binding to human Angiotensin-Converting Enzyme 2 (ACE2) receptor, the functional receptor for CoVs [1]. T a i et al. [2] have shown that the SARS-CoV-2 spike protein binds to ACE2 with EC_{50} = 0.14 μg/mL, while the SARS-CoV spike protein – with EC_{50} = 1.32 μg/mL.

SARS-CoV-2 is a beta coronavirus with positive sense single-stranded RNA encoding 4 structural and 22 non-structural proteins [3]. The structural proteins are Spike glycoprotein (S), Membrane protein (M), Envelope protein (E) and Nucleocapsid glycoprotein (N) (Fig. 1). The S protein consist of 1273 amino acids divided into two subunits and several domains [6]. Three S monomers arrange to form a spike. Multiple spikes cover the virus surface, giving it the appearance of a crown (corona in Latin) [7]. The S1 subunit binds the host ACE2 receptor through its Receptor-Binding Domain (RBD), followed by conformational changes in the S2 subunit, which allows the fusion peptide to insert into the host target cell membrane. The E protein is a small protein, only 75 amino acids long, incorporated in the viral membrane. It was found that five monomer E proteins form an ion channel [8]. The E protein takes part in viral assembly, budding, envelope formation, and pathogenesis. Deletion of SARS-CoV E gene attenuates the virus [9]. The M protein consists of 222 amino acids and is the most abundant structural protein in CoVs. It is located among the S and E proteins in the viral envelope and is the primary driver of the virus budding process [10]. It is shown that the M protein acts as a dominant immunogen and elicits a strong humoral response [11]. The N protein forms the viral capsid, which incorporates the viral RNA genome. It consists of 419 amino acids and is required for RNA synthesis and replication [12]. The N protein acts as well as a regulatory protein interfering with the host cell cycle, inhibits the INF production, up-regulates COX2 production and AP1 activity [13].

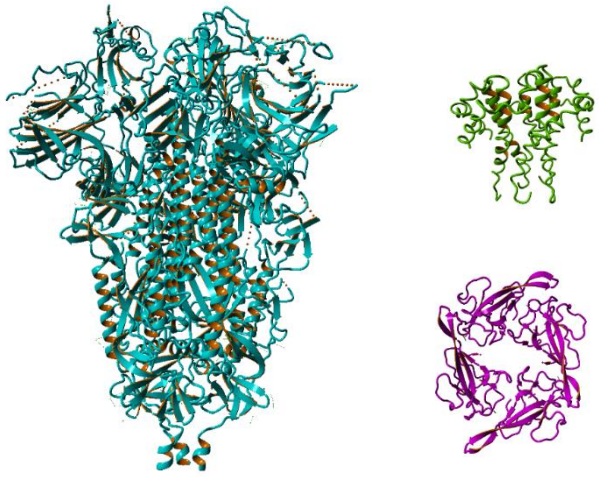

Fig. 1. Structure of S protein (left, pdb code: 6vyb [4]), E protein (upper right, pdb code: 5×29 [5]) and N protein (lower right, pdb code: 6vyo). The structure of M protein is not solved yet

As obligate intracellular infectious agents, viruses use the host cell's apparatus for translation and replication. The newly synthesised viral proteins are detected as antigens and processed by the Major Histocompatibility Complex (MHC) class I antigen presentation pathway. This pathway exists in all nucleated cells and its role is to present intracellular proteins to CD8+ T cells. The pathway consists of several consecutive steps. Initially, the protein is cleaved into oligopeptides by the

immunoproteasome complex in the cytosol [14]. Next, the peptides enter the Endoplasmic Reticulum (ER) via the Transporter associated with Antigen Processing (TAP). Here, the peptides bind to MHC class I molecules and the complex is transported to the cell surface where is presented to the CD8+ T cells. The peptides recognised by the T cells are assigned as epitopes. The cytotoxic CD8+ T cells directly attack and kill the infected cells by various mechanisms. Long-living CoV-specific memory T cells have been found in the blood of patients recovered from SARS-CoV infection [15]. Many viruses have evolved mechanisms to escape the host processing pathway [16]. So far, there is no evidence that CoVs have developed such mechanisms. Even more, there is evidence that MHC class II pathway also is involved in the CoVs antigen processing [17]. The MHC class II pathway processes extracellular proteins and present them in complexes with MHC class II proteins on the surface of Antigen-Presenting Cells (APCs) for recognition by the CD4+ T cells. The CD4+ T cells direct B cell response and control the cytokine production but also are responsible for the cytokine storm damaging many organs after dysfunctional immune response [18].

In human, the MHC proteins are encoded by the Human Leukocyte Antigen (HLA) gene complex and are named HLA proteins. The HLA proteins contain a specific Binding Site (BS) for peptides. The BS has a polymorphic architecture and determines the extreme polymorphism of HLA alleles. There are more than 20,000 HLA class I and 7500 HLA class II alleles registered in the IPD-IMGT/HLA database (September 2020) [19]. The clinical outcome of viral infections is associated with HLA genotype [20]. These HLA proteins that make stable and long-living complexes with the viral peptides enable T-cell-based immunity development and are associated with mild severity or even resistance to viral infection. On the contrary, HLA proteins falling to complex with viral peptides are associated with susceptibility to and severity of viral infection. Several associations between HLA and the current SARS-CoV-2 infection are already described [21-23].

As the duration of ongoing COVID-19 pandemic is unknown, specific therapeutics and approved vaccines are needed immediately. Many different types of vaccines against SARS-CoV-2 are currently under development [24]. Here, we examined the protein sequences of the four structural proteins of SARS-CoV-2 for affinity to 30 most frequent in the human population HLA proteins of both classes by in-house predictive models and selected several high binders for uploading onto lipid nanoparticles as a prototype of a multi-epitope vaccine. For comparison, a set of known HLA high binders originating from SARS-CoV are collected as well.

2. Databases and servers

2.1. Databases

The protein sequences of the four structural proteins of SARS-CoV and SARS-CoV-2 were retrieved from GenBank [25]. After BLAST alignment, the closest corresponding sequences were used in the analyses. The known high affinity HLA binders of SARS-CoV were selected from the Immune Epitope DataBase (IEDB, **www.iedb.org**) using the following settings: Organism – SARS coronavirus Tor2,

Method – purified MHC/competitive/radioactivity/dissociation constant K_D, Positive-high, HLA proteins – A*01:01, A*02:01, A*02:02, A*02:03, A*02:06, A*03:01, A*11:01, A*23:01, A*24:02, A*26:01, A*29:02, A*30:02, A*31:01, A*68:01, A*68:02, B*07:02, B*13:01, B*18:01, B*35:01, B*40:01, B*44:02, B*45:01, B*51:01, B*53:01, B*54:01, DRB1*01:01.

2.2. Servers for HLA binding prediction

The HLA binding affinities of the peptides originating from the four structural SARS-CoV-2 proteins were predicted by the in-house developed servers EpiJen, EpiTOP v.3 and EpiDOCK.

EpiJen is a three-step algorithm for HLA class I binding prediction mimicking the antigen processing in the cells [26]. It is based on Quantitative Matrices (QMs) for proteasome cleavage prediction [27], Transporter-associated with Antigen Processing (TAP) binding prediction [28] and HLA class I binding prediction. The matrices are arranged successively and eliminate from 10% to 60% of the true non-binders at each step. EpiJen predicts peptides binding to 11 most frequent HLA-A (A*01:01, A*02:01, A*02:02, A*02:03, A*02:06, A*03:01, A*11:01, A*24, A*31:01, A*68:01, A*68:02) and 7 most frequent HLA-B proteins (B*07, B*27, B*35:01, B*40, B*44, B*51, B*53). It recognizes 85% of the true binders among the top 5% of the best-predicted peptides [26]. In the present study, EpiJen was used to predict peptides binding to HLA class I proteins.

EpiTOP utilizes the method of proteochemometrics [29] to derive models for peptide binding prediction to HLA class II proteins [30]. It predicts binding to 12 most frequent HLA-DRB1, 5 HLA-DQ and 7 HLA-DP proteins. In the present study, EpiTOP v.3 was used to recognize the binding core of peptides binding to HLA-DRB1 proteins (*01:01, *03:01, *04:01, *04:04; *04:05, *07:01, *08:02, *09:01, *11:01, *12:01, *13:02, *15:01).

EpiDOCK prediction is based on QMs derived by molecular docking of combinatorial peptide libraries on the X-ray structures of HLA class II proteins from loci DRB1, DQ and DP [31]. It contains 23 QM for the most frequent HLA class II alleles. In the present study, EpiDOCK was used to identify the binding core of peptides binding to HLA-DRB1 proteins (*01:01, *03:01, *04:01, *04:04; *04:05, *07:01, *08:02, *09:01, *11:01, *12:01, *13:02, *15:01).

3. Results and discussion

3.1. Identification of HLA binders from SARS-CoV-2 similar to HLA binders from SARS-CoV

The BLAST alignment of the corresponding structural proteins of SARS-CoV and SARS-CoV-2 showed that the identity between them ranges from 76% for S protein to 95% for E protein (Table 1). The similarity spans from 87% for S protein to 96% for M and E proteins.

In the IEDB are registered 461 high binders to 26 HLA-A, -B and -DRB1 originating from the four structural proteins of SARS-CoV and no one from SARS-CoV-2 yet. As a high binder is defined a peptide with $IC_{50} < 50$ nM. Among the 243

high binders from the S protein of SARS-CoV, only 38 are identical to SARS-CoV-2 S peptides and 33 are similar. The 71 common high binders show 92% identity (Table 1). Most of the conserved binders are localized in the S2 subunit. Nineteen identical binders exist among the HLA high binders of both CoVs E proteins. All of them overlap from G10 to I46. The M proteins have 40 common HLA high binders, 17 of them are identical and 23 – similar. They span mainly in the first half of the protein sequence from L17 to T116. Another three fragments are available in the second part from R131 to I151, from P165 to K180 and from R198 to L206. The N proteins share 18 conserved HLA high binders, 13 of them are identical and 5 – similar. They are localised between T49 and Y173 and between A305 and K370.

Table 1. Comparison between the four structural proteins of SARS-CoV and SARS-CoV-2

SARS-CoV-2 CSD	Protein	Length		Identity %	Similarity %
		SARS-CoV	SARS-CoV-2		
YP009724390.1	Spike glycoprotein (S)	1255 UniProt: P59594	1273	76	87
YP009724392.1	Envelope protein (E)	76 UniProt: P59637	75	95	96
YP009724393.1	Membrane glycoprotein (M)	221 UniProt: P59596	222	91	96
YP009724397.2	Nucleocapsid phosphoprotein (N)	422 UniProt: P59595	419	91	94
Total		1974	1989	88	93

Table 2. HLA high binders from the four structural proteins of SARS-CoV-2, identical or similar to the HLA high binders from SARS-CoV published in IEDB

Protein	HLA High Binders		Identical	Similar	Average identity %
	SARS-CoV	SARS-CoV-2			
S	243 (0.19)[a]	71 (29%)[b]	38	33	92
E	42 (0.55)	19 (45%)	19	-	100
M	137 (0.62)	40 (29%)	17	23	94
N	39 (0.09)	18 (46%)	13	5	97
Total	461 (0.23)	148 (32%)	87	61	96

[a] Ratio between the number of high binders and the protein length. [b] The percentage shows the fraction of identical and similar HLA high binders.

Longer proteins generate more HLA binders. In order to compare proteins of different length, the ratio between the number of high binders and the protein length is considered. The average ratio for the four structural proteins of SARS-CoV is 0.23 (Table 2). The M protein shows the highest ratio of 0.62. Similarly, the ratio of high binders from the E protein is 0.55. The S and N proteins are relatively poor in high binding peptides – the ratios are 0.19 and 0.09, respectively. Proteins rich in high binders are good vaccine candidates. Recently, much effort has been put in the development of CoV vaccines encoding or containing the S protein [32]. This protein generates a great number of high binders because of its long sequence. The M and E

proteins are better choices as targets for vaccine candidates against SARS-CoV because they contain more high binders per amino acid.

Despite of the short evolutionary distance between SARS-CoV and SARS-CoV-2 [33], the structural proteins of the two viruses share only 32% common high binders to the most frequent HLA alleles. The N and E proteins contain 46% and 45% common binders, respectively, while S and M proteins are more diverse sharing only 29% of the HLA high binders.

3.2. Prediction of HLA binders from SARS-CoV-2

The protein sequences of the four structural proteins of SARS-CoV-2 in fasta format were input in the in-house developed servers for HLA binding prediction as described in Databases and servers. The servers convert the protein sequences into sets of overlapping nonamers and the binding affinities to 30 HLA proteins are calculated according to the models implemented in the servers. EpiJen eliminates the peptides predicted as non-cleavable by the immunoproteasome and as non-binding to TAP protein. Then, the affinities of the remaining peptides to 18 HLA class I proteins of loci A and B are predicted. The top 2% of the high binders from each protein was selected. EpiTOP and EpiDOCK were used to predict the binding affinities of the nonamer binding cores of the peptides binding to 12 HLA class II proteins of locus DRB1. The top 10 high binders with $IC_{50} < 50$ nM from each protein were selected. The peptides binding to more than one HLA protein were counted only once.

The predicted HLA class I and class II high binders from the S protein are 131 and 108, respectively (Table 3). Twenty of them were predicted to bind to both classes HLA proteins. Thus, the unique binders are 219, 48 of them coincide with the binders from the experimental set (true positives) giving 68% sensitivity of the predictions. The E protein contains 20 high binders to HLA class I and 28 high binders to class II. The promiscuous binders are 9. Seventeen predicted binders are confirmed experimentally (89% sensitivity). The HLA class I high binders from the M protein are 56, 63 peptides bind to class II. The promiscuous binders are 20, the true positives 34 (85% sensitivity). The corresponding binders from the N protein are 83 for class I, 61 for class II, 34 promiscuous, 12 true positives and 67% sensitivity of the prediction.

The total amount of predicted HLA high binders from the four structural proteins of SARS-CoV-2 are close to the number of the experimentally determined ones from SARS-CoV – 467 *vs*. 461. The data derived from the experimental and predicted sets are not fully comparable because the number of tested/predicted HLA alleles are different – the experimental set includes binders to 26 HLA proteins, the predicted binders are for 30 HLAs, only 18 of them are common. Although this difference, the trends are the same. The proteins E and M are rich in high binders having ratios between the number of high binders and protein length of 0.52 and 0.45, respectively. The proteins S and N contain a great number of high binders – 219 and 110, respectively, but their corresponding ratios of binders to protein length are 0.17 and 0.26.

Table 3. HLA high binders from the four structural proteins of SARS-CoV-2, predicted by EpiJen, EpiTOP and EpiDOCK

Protein	HLA high binders from SARS-CoV-2				Sensitivity, %
	Identical or similar to SARS-CoV	Predicted as high binders to			
		HLA class I	HLA class II	Total	
S	71	131	108	219 (0.17)[a]	68
E	19	20	28	39 (0.52)	89
M	40	56	63	99 (0.45)	85
N	18	83	61	110 (0.26)	67
Total	148	290	260	467 (0.23)	77

[a] Ratio between the number of high binders and the protein length.

Most of the predicted SARS-CoV-2 high binders from the structural proteins are confirmed by the SAR-CoV high binders conserved in CoV-2 (Table 3). The sensitivity of predictions (true binders/total binders) ranges from 67% for the N protein to 89% for the E protein. This indicates that the predictions made in the present study identify effectively the strong HLA binders and are a reliable tool for the design of a multi-epitope based vaccine.

The great amount of peptides binding strongly to a wide variety of HLA proteins arising from the structural proteins of SARS-CoVs indicates that both viruses undergo an extensive processing in the host cells of diverse HLA genotypes and activate CD8+ and CD4+ T-cell based immunity. As a result, most of the infected people experience the disease mildly or even asymptomatically and the mortality rates are relatively low [WHO report]. The SARS-CoV-2-specific T-cell immunity plays an important role in the disease control, recovery and immune memory. The development of vaccines aiming to produce and boost the T-cell mediated immunity is an utmost strategy for prevention from CoV infections.

4. Conclusion

In the present study, in-house immunoinformatic tools are applied to predict the binding affinities to 30 HLA class I and class II alleles of peptides originating from the structural proteins of SARS-CoV-2. The best binding peptides (IC_{50} < 50 nM) were compiled and compared to known high binders from SARS-CoV conserved in CoV-2. Seventy seven percent of the conserved high binders are recognised. The predicted high binders are arranged in several sets according to their origin and HLA binding. The peptides will be uploaded onto lipid nanoparticles and will be tested as a multi-epitope vaccine prototype.

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