Design of an Epitope-Based Synthetic Long Peptide Vaccine to Counteract the Novel China Coronavirus (2019-nCoV)

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Abstract

In this report, we demonstrate that it is possible to design epitope-based peptide vaccine candidates to counteract the novel China coronavirus (2019-nCoV) by using an approach similar to the one used in cancer neoantigen vaccination therapy.

We identified multiepitope peptide vaccine candidates against 2019-nCov that can potentially trigger both $CD4^+$ and $CD8^+$ T cell immune response with increased efficiency due to the presence of $CD4^+$ and $CD8^+$ T cell epitopes and a cathepsin-sensitive linker.

Furthermore, we suggest that the peptide design strategy should incorporate population-specific HLA alleles in order to optimize binding specificity of the peptides. We refer to this as populationalized vaccinomics.

Keywords: 2019-nCoV; coronavirus; peptide vaccine; $CD4^+$ epitope; $CD8^+$ epitope.

Introduction

Wuhan seafood market pneumonia virus, also known as 2019 Novel Coronavirus (2019-nCoV) is a relative of both the deadly severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) viruses. According to the World Health Organization, by January 30, the number of confirmed cases has jumped to more than 9692 with more than 200 people dead as a result of the infection. By comparison, SARS spread globally in 2002-03, infecting 8000 and killing more than 700 people (Chen et al., 2017).

Traditional vaccines for viral diseases had limited success in the case of viral epidemics with high mutation rates. Many vaccination strategies are based on antibody-mediated protection which can be very efficient in preventing virus infection, but due to the variability of many virus surface proteins (facilitated by the high mutation rates), the virus can escape and infect host cells (Rosendahl Huber, van Beek, de Jonge, Luytjes, & van Baarle, 2014).

In addition, classical antibody-based vaccines are often poor inducers of T cell responses. By including small protein fragments (peptides), in a vaccine, which can be presented by major histocompatibility complex (MHC) molecules to $CD4^+$ and $CD8^+$ T cells, specific T cell responses can be induced (Rosendahl Huber et al., 2014). $CD8^+$ T cells are cytotoxic and can kill virus-infected cells, helping to clear out the infection, while $CD4^+$ T cells function as „helper" cells and can direct the activity of other immune cells against a viral threat by releasing specific mediators.

The approach that we used for designing potential peptide vaccine candidates takes a community-level personalized strategy, that we refer to as populationalized vaccinomics, which enables the design of peptides based on the genome sequence of the target virus and the immune characteristics of the target populations, variable at the gene level. Based on the human leukocyte antigen (HLA) gene allele variation, the peptide vaccines can be made more efficient for a given community or individual. The selection of the peptides that could act as vaccines is determined by the binding of the processed viral peptide with the MHC class I and II molecules, and the relevant HLA alleles.

We identified multiepitope peptide vaccine candidates against nCov that can potentially trigger both CD4+ and CD8+ T cell immune response.

Methods

We have used the genome sequence of the coronavirus isolate Wuhan-Hu-1 (MN908947.3/ Ref Seq NC_045512: https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2) to identify conserved proteins with a reduced potential for mutation, that could represent the antigen source.

We have identified spike (S-type) proteins important for adhesion and fusion, and membrane (M-type) proteins important for replication and virion exocytosis.

The bioinformatics tool from the Immune Epitope Database (IEDB) server was used to identify potent T cell epitopes from the sequences of the target proteins.

For $CD8^+$ T cell epitopes, the prediction method integrated MHC class I binding, proteasomal cleavage and TAP transport efficiency.

Results

The coronavirus isolate Wuhan-Hu-1 is an enveloped, positive-sense, single-stranded poly-adenylated RNA virus, with a genome of 29.9 kb (29903 bp), among the largest of all RNA virus genomes.

Based on the published genome sequence of the 2019-nCoV (RefSeq NC_045512), we have identified spike and membrane proteins as target for the nCoV vaccine.

The bioinformatics tool from the Immune Epitope Database (IEDB) server was used to identify potent T cell epitopes from the sequences of these proteins.

As previously mentioned, an important consideration when vaccinating with peptides is HLA specificity. Since processed peptides bind directly into the MHC class I binding groove the peptide has to match the HLA type of the vaccinated individual (Rosendahl Huber et al., 2014). In order to overcome the need for individualized vaccination, we have selected short epitopes with the capacity to bind to multiple HLA alleles, and we chose in particular for this example the most frequent alleles in Romania (Table 1), according to Constantinescu et al. (Constantinescu et al., 2016).

Table 1. HLA allele frequencies in the Romanian population

HLA allele	Frequency
HLA-A*02	29%
HLA-A*01	14.3%
HLA-A*24	11.2%
HLA-B*35 (supertype B07)	16%
HLA-B*18 (supertype B44)	11%
HLA-DRB1*11	18.5%
HLA-DRB1*03	11.3%
HLA-DRB1*13	10.5%

Several $CD8^+$ T cell epitopes were selected for further analysis based on high overall score and if they showed high MHC binding affinity (IC_{50} <50 nM). A list of selected $CD8^+$ T cell epitopes is shown in Table 2.

Table 2. Selected $CD8^+$ T cell epitopes, on the basis of overall score, predicted by IEDB

Protein	Allele	Epitope	MHC IC_{50} (nM)	Overall score (IEDB)
S (Spike)	A*0201	YLQPRTFLL	4.6	1.17
		KIADYNYKL	15.9	0.99
		FQFCNDPFL	8.9	0.99
		SIIAYTMSL	15.3	0.82
		VLNDILSRL	19.7	0.43
	A*0101	LTDEMIAQY	5.2	1.71
		WTAGAAAYY	40.1	0.88
	A*2402	NYNYLYRLF	23.4	1.01
		QYIKWPWYI	8.9	0.45
	B*3501	IPFAMQMAY	2.3	2.24
		LPFNDGVYF	3.5	1.90
		VASQSIIAY	7.2	1.80
		FAMQMAYRF	6.3	1.70
		LGAENSVAY	11.5	1.66
M (Membrane)	A*0201	GLMWLSYFI	4.5	0.86
		FVLAAVYRI	11	0.45
		KLLEQWNLV	7.3	0.18
	A*0101	ATSRTLSYY	48.2	0.92
	A*2402	YFIASFRLF	9.9	1.48
		SYFIASFRL	22.5	0.72
	B*3501	YANRNRFLY	6.8	1.66
		VATSRTLSY	23.9	1.27
		FAYANRNRF	23.6	0.99

For $CD4^+$ T cell epitopes, the highest ranking peptide based on MHC class II binding was chosen (Table 3).

Table 3. Selected CD4$^+$ T cell epitopes, on the basis of MHC class II binding, predicted by IEDB combined method

Protein	Allele	Epitope
S (Spike)	DRB1*1101	GNYNYLYRLFRKSN
	DRB1*1301	IRAAEIRASANLAA
	DRB1*0301	INLVRDLPQGFSAL
M (Membrane)	DRB1*1101	SYFIASFRLFARTRS
	DRB1*1301	AVILRGHLRIAGHH
	DRB1*0301	EITVATSRTLSYYK

We designed 15-30 aa synthetic long peptides (SLPs), as proposed by Rabu et al. (Rabu et al., 2019), using a cathepsin-sensitive linker (LLSVGG) for linking MHC class I-restricted epitopes to MHC class II-restricted epitopes, with the MHC class II epitope located always at the N-terminal end, to stimulate both „cytotoxic" T lymphocytes (CTLs) and „helper" T lymphocytes (together with the innate immune system).

Our synthetic long peptide vaccine candidates are listed in Figure 1.

Synthetic Long Peptide Vaccine candidate for S protein

GNYNYLYRLFRKSN**LLSVGG**YLQPRTFLL

YLQPRTFLL HLA-A*0201 epitope

GNYNYLYRLFRKSN HLA-DRB1*1101 epitope

LYRLFRKSNLLSVG HLA-DRB1*1301 epitope

Synthetic Long Peptide Vaccine candidate for M protein

SYFIASFRLFARTRS**LLSVGG**GLMWLSYFI

GLMWLSYFI HLA-A*0201 epitope

SYFIASFRLFARTRS HLA-DRB1*1101 epitope

ASFRLFARTRSLLS HLA-DRB1*1301 epitope

Synthetic Long Peptide Vaccine candidate for S and M proteins

SYFIASFRLFARTRS**LLSVGG**YLQPRTFLL

YLQPRTFLL HLA-A*0201 epitope

SYFIASFRLFARTRS HLA-DRB1*1101 epitope

ASFRLFARTRSLLS HLA-DRB1*1301 epitope

Figure 1. Synthetic long peptide vaccine candidates for the 2019-nCoV

Discussion

In this example peptide vaccine design, we demonstrate that it is possible to design epitope-based peptides that could counteract the novel China coronavirus by eliciting both $CD4^+$ and $CD8^+$ T cell responses using a technology for personalized vaccination in cancer, based on long neoantigen peptides, currently in clinical trials (Peng et al., 2019). This approach is similar to the one previously reported by Oany et al. who used a computational approach to identify a multiepitope vaccine candidate for the Human coronavirus (HCoV) (Oany, Emran, & Jyoti, 2014).

Our proposed candidate peptides are applicable in particular to the Romanian population. Based on this model, additional peptide candidates can be identified for populations from all over the world, based on the characteristic HLA alleles.

The current approach is a combination between an industrial large-scale global vaccination strategy and an individually-targeted personalized vaccination strategy and has several advantages (Table 4): the production process of these vaccines is relatively easy; moreover, peptide vaccines have increased safety as they are the result of a highly pure and well-controled manufacturing process, while classical protein and live attenuated vaccines pose a higher risk of contamination with other agents and proteins (Rosendahl Huber et al., 2014).

Table 4. Comparison of classical protein vaccination, live attenuated vaccination, and peptide vaccination (reproduced from (Rosendahl Huber et al., 2014))

	Classical protein vaccine	Live attenuated vaccine	Peptide vaccine
Composition	Inactivated split virion or purified subunit	Attenuated virus, capable of replication	Synthetic, small protein fragments
Humoral response	Yes, induces humoral response	Yes, mimics natural infection	Possible, depends on peptides included
CD4 response	No	Yes	Yes
CD8 response	No	Yes	Yes
Preexisting response	Not important	Important, Ab can capture vaccine	Not important
Adjuvant	Required for cellular response	Not required	Required
Production	Biological	Biological	Synthetic
Safety	Risk of contamination with extraneous agents and proteins of the production substrate	Risk of contamination with extraneous agents and proteins of the production substrate	Well controlled and highly pure production process
Flexibility to match escape variants	Not easy	Not easy	Easy
Target conserved components	No, primarily strain-specific response	To some extent, limited cross-reactivity	Yes, capable of inducing a broad response

Protein vaccines are a form of inactivated vaccines that consist of purified subunit or subvirion products. Live attenuated vaccines are attenuated viruses, derived from disease-causing virus. These attenuated viruses still replicate in the host, but do not cause disease. Peptide vaccines are completely synthetic vaccines, comprised of small protein fragments.

Future *in vitro* studies should evaluate $CD4^+$- and CD8+-specific responses induced by the candidate peptides.

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