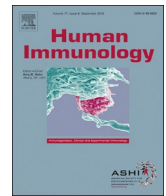




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Short Communication

Conserved HLA binding peptides from five non-structural proteins of SARS-CoV-2—An *in silico* glance

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ARTICLE INFO

Keywords:

Non-structural proteins
 COVID-19
 SARS
 MERS
 Epitopes
In silico

ABSTRACT

Coronavirus Disease 2019 (COVID-19) is a dangerous global threat that has no clinically approved treatment yet. Bioinformatics represent an outstanding approach to reveal key immunogenic regions in viral proteins. Here, five severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) non-structural proteins (NSPs) (NSP7, NSP8, NSP9, NSP12, and NSP13) were screened to identify potential human leukocyte antigen (HLA) binding peptides. These peptides showed robust viral antigenicity, immunogenicity, and a marked interaction with HLA alleles. Interestingly, several peptides showed affinity by HLA class I (HLA-I) alleles that commonly activates to natural killer (NK) cells. Notably, HLA binding peptides are conserved among SARS-CoV-2, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle Eastern respiratory syndrome coronavirus (MERS-CoV). Interestingly, HLA-I and HLA class II (HLA-II) binding peptides induced humoral and cell-mediated responses after *in silico* vaccination. These results may open further *in vitro* and *in vivo* investigations to develop novel therapeutic strategies against coronaviral infections.

1. Introduction

SARS-CoV-2 is the causative agent of Coronavirus Disease 2019 (COVID-19) [1] and is classified in the family *Coronaviridae* and the genus *Betacoronavirus*. This genus includes other pathogenic coronaviruses for human that share a marked sequence identity to SARS-CoV-2 such as severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle Eastern respiratory syndrome coronavirus (MERS-CoV), whose explosive outbreak in recent years has also led to global public health crisis—severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), respectively [2,3].

The SARS-CoV-2 single-stranded positive sense RNA genome encodes for both structural and non-structural proteins [2]. The former comprises the spike glycoprotein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N) [3]. The latter, on the other

hand, includes the open reading frame 1ab (ORF1ab), ORF3a, ORF6, ORF7a, ORF8, and ORF10. In turn, the ORF1ab expresses a polyprotein whose proteolytical cleaved forms 16 non-structural proteins (NSPs, numbered 1 to 16) [3]. The expression and activity of several NSPs constitute a fundamental aspect for SARS-CoV-2 pathogenesis [2]. For instance, the protein complex formed by NSP7, NSP8, and NSP12 give rise to the RNA polymerase, which is necessary for genome replication [4], whereas NSP13 represents the virus helicase, which unwinds duplex RNA [4]. On the other hand, it is thought that NSP9 binds to RNA, thereby improving viral replication [5].

Several works have shown that NSPs from other RNA viruses are fundamental factors to elicit effective immune responses [6,7]. For instance, the presentation of conserved helicase peptides from several flaviviruses in the context of human leukocyte antigen class I (HLA-I) is sufficient to inhibit viral replication [6]. Therefore, peptides derived

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle Eastern respiratory syndrome coronavirus; S, spike glycoprotein; E, envelope protein; M, membrane protein; N, nucleocapsid protein; ORF1ab, open reading frame 1ab; ORF3a, open reading frame 3a; ORF6, open reading frame 6; ORF7a, open reading frame 7; ORF8, open reading frame 8; ORF10, open reading frame 10; NSP, non-structural proteins; HLA, human leukocyte antigen; HLA-I, human leukocyte antigen class I; HLA-II, human leukocyte antigen class II; NSP7, non-structural protein 7; NSP8, non-structural protein 8; NSP9, non-structural protein 9; NSP12, non-structural protein 12; NSP13, non-structural protein 13; IFN- γ , interferon-gamma; IEDB-AR, immune epitope database and analysis resource; 3D, three-dimensional; PDB, protein data bank; P, peptide; Ig, immunoglobulin; CTC, cytotoxic T cell; TH1, T helper cell; IL-2, interleukin 2; SARS, severe acute respiratory syndrome; MERS, Middle Eastern respiratory syndrome; aKIR, activating killer immunoglobulin-like receptors; NK, natural killer cell; CTRL+, control positive; NCBI, National Center for Biotechnology Information

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<https://doi.org/10.1016/j.humimm.2020.08.001>

Received 2 July 2020; Received in revised form 8 August 2020; Accepted 10 August 2020

Available online 13 August 2020

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Table 1
Potential HLA-I binding peptides identified in five non-structural proteins of SARS-CoV-2.

Protein	Peptide ID	Peptide sequence	Position (star-end)	Percentile Rank	Viral antigenicity	Toxicity and Allergenicity	HLA-I interacting allele
NSP7	P1	LRVSSSKL	20–28	1.60	0.60	Negative	C*06:02
	P2	LSMQGAVDI	60–68	1.50	0.78	Negative	C*01:02, C*02:02, C*04:01, C*12:02
	P3	EMLDNRTL	74–82	0.17	0.63	Negative	B*08:01, C*01:02, C*02:02, C*06:02, C*12:02,
NSP8	P4	AAFATAQEA	13–21	1.10	0.53	Negative	C*12:02, C*02:02,
	P5	CVPLNIPL	114–122	0.87	1.37	Negative	C*01:02
	P6	LAWPLIVTA	180–188	0.38	1.02	Negative	A*02:01, C*02:02, C*04:01, C*12:02, B*35:01
NSP9	P7	GLNNLNRGM	93–102	1.80	1.01	Negative	A*02:01, C*02:02, C*02:09, C*12:02
	P8	NNLNRGMVL	95–103	0.09	0.52	Negative	B*08:01, C*06:02
	P9	GMVLGSLAA	100–108	1.20	0.60	Negative	A*02:01
	P10	VLGSLAATV	102–100	0.20	0.66	Negative	A*02:01, A*02:05, C*02:09, C*02:02
NSP12	P11	WEPEFYEAM	116–124	0.21	0.53	Negative	A*02:01
	P12	MRNAGIVGV	196–204	0.02	1.63	Negative	C*06:02
	P13	LVYAADPAM	372–380	0.02	0.52	Negative	C*03:02, C*12:02, B*35:01, C*02:09, C*02:02, C*08:01
	P14	TVKPGNFNK	409–417	0.02	1.38	Negative	A*11:01
	P15	NAAISDYDY	447–455	0.07	1.29	Negative	B*35:01, A*01:01, C*03:03
	P16	FAYTKRNV	538–546	0.06	1.03	Negative	B*08:01, C*03:02, C*12:02, C*08:01, C*02:02, C*02:09, C*01:02, C*06:02
	P17	IAATRATV	579–587	0.16	0.89	Negative	B*08:01, C*01:02, C*02:02, C*03:02, C*02:09, C*08:01, C*12:02,
	P18	ATVVIGTSK	585–593	0.02	0.76	Negative	A*11:01
	P19	RLANCAQV	654–662	0.16	0.93	Negative	A*02:01
	P20	MLDMYSVML	899–907	0.08	0.56	Negative	A*02:01, A*01:01, C*01:02, C*08:01,
NSP13	P21	HVISTSHKL	33–41	0.10	0.69	Negative	C*01:02, C*02:02, C*03:02, C*12:02, B*35:01,
	P22	TEETFKLSY	141–149	0.65	0.65	Negative	A*01:01
	P23	YGIATVREV	149–157	0.37	1.43	Negative	C*02:02, C*03:02, C*12:02,
	P24	PTLVPQEHY	238–246	0.43	0.78	Negative	A*01:01
	P25	TLVPQEHYV	239–247	0.04	0.54	Negative	A*02:01, C*02:02
	P26	EHYVRITGL	244–252	0.33	0.57	Negative	B*08:01
	P27	SHFAIGLAL	289–297	0.35	1.39	Negative	C*02:02, C*03:02, C*12:02, B*40:01,
	P28	VVFDEISMA	371–379	0.11	0.74	Negative	A*02:01, A*02:05, C*02:02, C*12:02
	P29	VVNARLRAK	386–394	0.08	1.93	Negative	A*03:01, A*11:01
	P30	EIVDTVSAL	447–455	0.26	0.66	Negative	C*02:02, C*03:02, C*04:01, C*12:02, B*35:01
	P31	ITRAKVGIL	565–573	0.25	0.91	Negative	B*08:01

from critical SARS-CoV-2 NSPs such as those involved in viral replication and pathogenesis (e.g., NSP7, NSP8, NSP9, NSP12, and NSP13) represent optimal targets to develop future prophylactic and therapeutics approaches against COVID-19. Recent studies have reported promising viral peptides from other SARS-CoV-2 NSPs, including ORF3a [8], ORF6, ORF7, and ORF8 [9]. Likewise, SARS-CoV-2 structural proteins (S, E, M, and N) have been extensively explored for potential immunogenic peptides [8–13]. In fact, increasing data suggest that peptides from these structural proteins can be efficiently used in multi-epitope vaccine models [12,13]. Regardless of these remarkable contributions, our knowledge on the existence of similar peptides in SARS-CoV-2 NSP7, NSP8, NSP9, NSP12, and NSP13 is still poor. Therefore, this immunoinformatics work aimed to identify HLA-I and HLA class II (HLA-II) binding peptides from these key SARS-CoV-2 NSPs, thereby providing more immunogenic targets with strong potential to fight COVID-19.

2. Materials and methods

2.1. Data collection and availability

Amino acid sequences from NSPs of SARS-CoV-2, SARS-CoV, and MERS-CoV were downloaded from the National Center for Biotechnology Information (NCBI) using the following accession numbers: [YP 009725303.1](#) (NSP7), [YP 009725304.1](#) (NSP8), [YP 009725305.1](#) (NSP9), [YP 009725307.1](#) (NSP12), and [YP 009725308.1](#) (NSP13) for SARS-CoV-2; [NP 828865.1](#) (NSP7), [NP 828866.1](#) (NSP8), [NP 828867.1](#) (NSP9), [NP 828869.1](#) (NSP12), and [NP 828870.1](#) (NSP13) for SARS-CoV; [YP 009047235.1](#) (NSP7),

[YP 009047236.1](#) (NSP8), [YP 009047237.1](#) (NSP9), [YP 009047223.1](#) (NSP12), [YP 009047224.1](#) (NSP13) for MERS-CoV.

2.2. Prediction of HLA binding peptides from SARS-CoV-2 NSPs

A recent bioinformatics SARS-CoV-2 research [8] with minor modifications was used to identify HLA binding peptides. Briefly, HLA-I binding peptides (9 amino acids of length) were predicted using the NetMHCpan EL 4.0 algorithm [14]. The HLA-I alleles used for this analysis represent some of the most common alleles in human population: HLA-A*01:01, HLA-A*02:01, HLA-A*02:05, HLA-A*03:01, HLA-A*03:02, HLA-A*11:01, HLA-A*24:02, HLA-B*07:02, HLA-B*07:05, HLA-B*08:01, HLA-B*13:02, HLA-B*14:02, HLA-B*35:01, HLA-B*40:01, HLA-B*44:02, HLA-B*44:03, HLA-C*01:02, HLA-C*02:02, HLA-C*03:03, HLA-C*03:04, HLA-C*04:01, HLA-C*06:02, and HLA-C*12:02. HLA-II epitopes (15 amino acids of length) were predicted using the Consensus method [15]. The HLA-II alleles considered in the study were HLA-DRB1*01:01, HLA-DRB1*01:02, HLA-DRB1*03:01, HLA-DRB1*03:06, HLA-DRB1*03:07, HLA-DRB1*04:01, HLA-DRB1*04:02, HLA-DRB1*04:04, and HLA-DRB1*04:05, HLA-DRB1*07:01. In general, HLA-I and HLA-II binding peptides were selected with a percentile rank of prediction cut-off < 20. Peptides with lower percentile values have a higher affinity by HLA molecules [16].

2.3. General assessment of predicted HLA binding peptides

Viral antigenicity (cut-off ≥ 0.5), allergenicity and toxicity predictions were conducted on Vaxijen (www.ddg-pharmfac.net/vaxijen/) [17], AlergenFP (<http://ddg-pharmfac.net/AllergenFP/index.html>)

Table 2
Potential HLA-II binding peptides identified in five non-structural proteins of SARS-CoV-2.

Protein	Peptide ID	Peptide sequence	Position (star-end)	Percentile Rank	Viral antigenicity	Toxicity and Allergenicity	IFN-g induction	HLA-I interacting allele
NSP7	P32	VVLLSVLQQLRVES	11–25	0.60	0.50	Negative	Positive	DRB1*01:01, DRB1*01:02, DRB1*03:01, DRB1*03:07, DRB1*04:01, DRB1*04:02, DRB1*04:04,
NSP8	P33	IASEFSSLPYAAFA	2–16	0.13	0.54	Negative	Positive	DRB1*01:01, DRB1*04:01, DRB1*04:04
	P34	SEFSSLPYAAFATA	4–18	0.15	0.54	Negative	Positive	DRB1*01:01, DRB1*04:01, DRB1*04:04
	P35	FSSLPYAAFATAQE	6–20	7.20	0.51	Negative	Positive	DRB1*01:01, DRB1*04:01, DRB1*04:04
	P36	GCVPLNIPLTTAAK	113–127	3.00	1.12	Negative	Positive	DRB1*03:06, DRB1*03:07, DRB1*04:04, DRB1*04:01
	P37	PLNIPLTTAAKLMV	116–130	6.10	0.82	Negative	Positive	DRB1*03:06, DRB1*03:07, DRB1*04:04, DRB1*04:01
	P38	WEIQQVVDADSKIVQ	154–168	7.60	0.53	Negative	Positive	DRB1*03:01, DRB1*03:06, DRB1*03:07
	P39	IQQVVDADSKIVQLS	156–170	7.20	0.59	Negative	Positive	DRB1*03:01, DRB1*03:06, DRB1*03:07
	P40	PNLAWPLIVTALRAN	178–192	8.50	0.92	Negative	Positive	DRB1*01:01
	P41	NLAWPLIVTALRANS	179–193	3.50	0.99	Negative	Positive	DRB1*03:06, DRB1*03:07, DRB1*04:04
	P42	LAWPLIVTALRANSA	180–194	3.10	0.80	Negative	Positive	DRB1*01:01, DRB1*03:06, DRB1*03:07, DRB1*04:04
	P43	AWPLIVTALRANSAV	181–195	3.30	0.72	Negative	Positive	DRB1*01:01, DRB1*03:06, DRB1*03:07, DRB1*04:04
	P44	WPLIVTALRANSAVK	182–196	2.60	0.74	Negative	Positive	DRB1*01:01, DRB1*03:06, DRB1*03:07, DRB1*04:04
	P45	PLIVTALRANSAVKL	183–197	0.45	0.66	Negative	Positive	DRB1*01:01, DRB1*01:02, DRB1*03:01, DRB1*03:06, DRB1*03:07, DRB1*04:01, DRB1*04:04
	NSP9	P46	GPKVKYLYFIKGLNN	82–96	7.20	0.70	Negative	Positive
P47		PKVKYLYFIKGLNNL	83–97	6.10	0.62	Negative	Positive	DRB1*01:01, DRB1*04:04
P48		KVKYLYFIKGLNNLN	84–98	4.40	0.91	Negative	Positive	DRB1*04:01, DRB1*01:01, DRB1*04:04
P49		KYLYFIKGLNNLNRG	86–100	0.38	0.64	Negative	Positive	DRB1*01:01, DRB1*04:01, DRB1*04:04
P50		YLYFIKGLNNLNRGM	87–101	0.31	0.52	Negative	Positive	DRB1*01:01, DRB1*04:01, DRB1*04:04
P51		LYFIKGLNNLNRGMV	88–102	0.31	0.52	Negative	Positive	DRB1*01:01, DRB1*04:01, DRB1*04:04
P52		NNLNRGMVGLSAAAT	95–109	1.20	0.82	Negative	Positive	DRB1*03:06, DRB1*03:07, DRB1*04:02
P53		NLNRGMVGLSAAATV	96–110	1.20	0.83	Negative	Positive	DRB1*03:06, DRB1*03:07, DRB1*04:02
P54		LNRGMVGLSAAATVR	97–111	1.20	0.92	Negative	Positive	DRB1*04:04
NSP12		P55	NAGIVGVLTLDNQDL	198–212	1.70	1.30	Negative	Positive
	P56	RLSFKELLVYAADPA	365–379	3.50	0.59	Negative	Positive	DRB1*04:01, DRB1*04:03 DRB1*01:01
	P57	LSFKELLVYAADPAM	366–380	3.00	0.65	Negative	Positive	DRB1*04:01 DRB1*04:03, DRB1*01:01 DRB1*04:05
	P58	AASGNLLLDKRTTCF	382–396	2.40	0.89	Negative	Positive	DRB1*03:01
	P59	ASGNLLLDKRTTCFS	383–397	1.70	0.94	Negative	Positive	DRB1*03:01
	P60	SGNLLLDKRTTCFSV	384–398	1.40	1.04	Negative	Positive	DRB1*03:01
	P61	GNLLLDKRTTCFSVA	385–399	1.90	1.20	Negative	Positive	DRB1*03:01
	P62	NLLLDKRTTCFSVAA	386–400	3.80	1.20	Negative	Positive	DRB1*03:01
	P63	KHFFFAQDGNAAISD	438–452	1.10	0.54	Negative	Positive	DRB1*04:01, DRB1*01:03 DRB1*04:03, DRB1*01:01
	P64	KRNVIPTITQMNLY	532–546	0.96	1.25	Negative	Positive	DRB1*04:03
	P65	ITQMNLYAISAKNR	539–553	1.20	1.51	Negative	Positive	DRB1*04:03, DRB1*01:03 DRB1*04:01
	P66	QMNLYAISAKNRAR	541–555	0.32	1.50	Negative	Positive	DRB1*04:03, DRB1*01:03, DRB1*04:01, DRB1*04:05
	P67	MNLKYAISAKNRART	542–556	0.44	1.44	Negative	Positive	DRB1*04:03, DRB1*04:01 DRB1*01:03
	P68	NLKYAISAKNRARTV	543–557	0.96	1.34	Negative	Positive	DRB1*04:03, DRB1*01:03 DRB1*04:01
	P69	MLRIMASLVLARKHT	629–643	0.43	0.53	Negative	Positive	DRB1*04:03, DRB1*03:01 DRB1*01:01, DRB1*01:03 DRB1*04:01, DRB1*04:05
	P70	LRIMASLVLARKHTT	630–644	0.53	0.67	Negative	Positive	DRB1*03:01, DRB1*04:03 DRB1*01:01, DRB1*01:03, DRB1*04:01
	P71	ASQGLVASIKNFKSV	771–785	0.85	0.58	Negative	Positive	DRB1*04:03, DRB1*04:01 DRB1*03:01
NSP13	P72	EVLSDRELHLSWEVG	156–170	2.90	1.54	Negative	Positive	DRB1*03:01
	P73	TTYKLNVDGYFVLTS	215–229	2.50	0.60	Negative	Positive	DRB1*03:02
	P74	LNVDGYFVLTSHTVM	219–233	3.00	0.61	Negative	Positive	DRB1*01:01, DRB1*01:03
	P75	DYFVLTSHTVMPLSA	223–237	0.96	0.56	Negative	Positive	DRB1*01:01, DRB1*01:03
	P76	RFNVAITRAKVGILC	560–574	3.60	1.32	Negative	Positive	DRB1*04:02, DRB1*01:03
	P77	KVGILCIMSDRDLVD	569–583	0.98	1.51	Negative	Positive	DRB1*03:01, DRB1*03:02

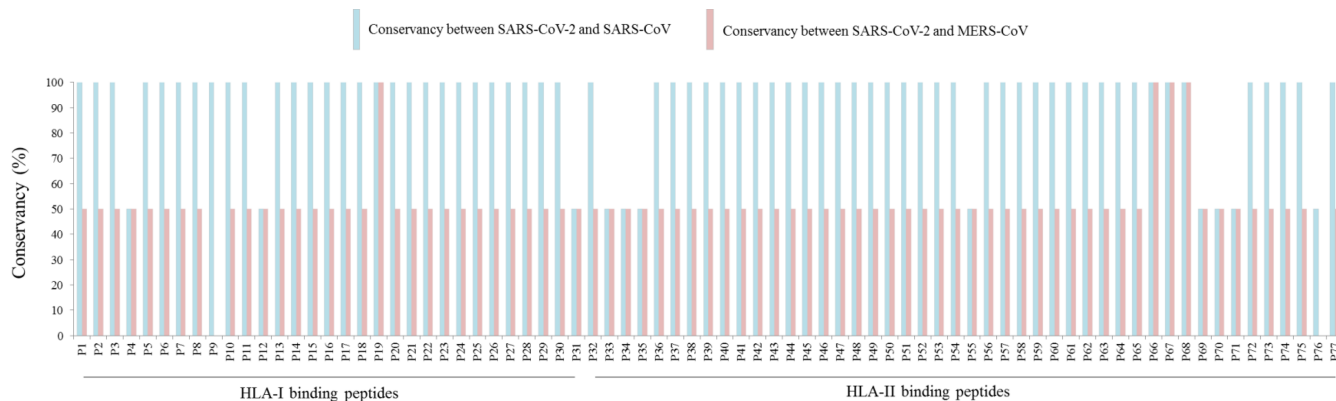


Fig. 1. Conservancy analysis of HLA binding peptides.

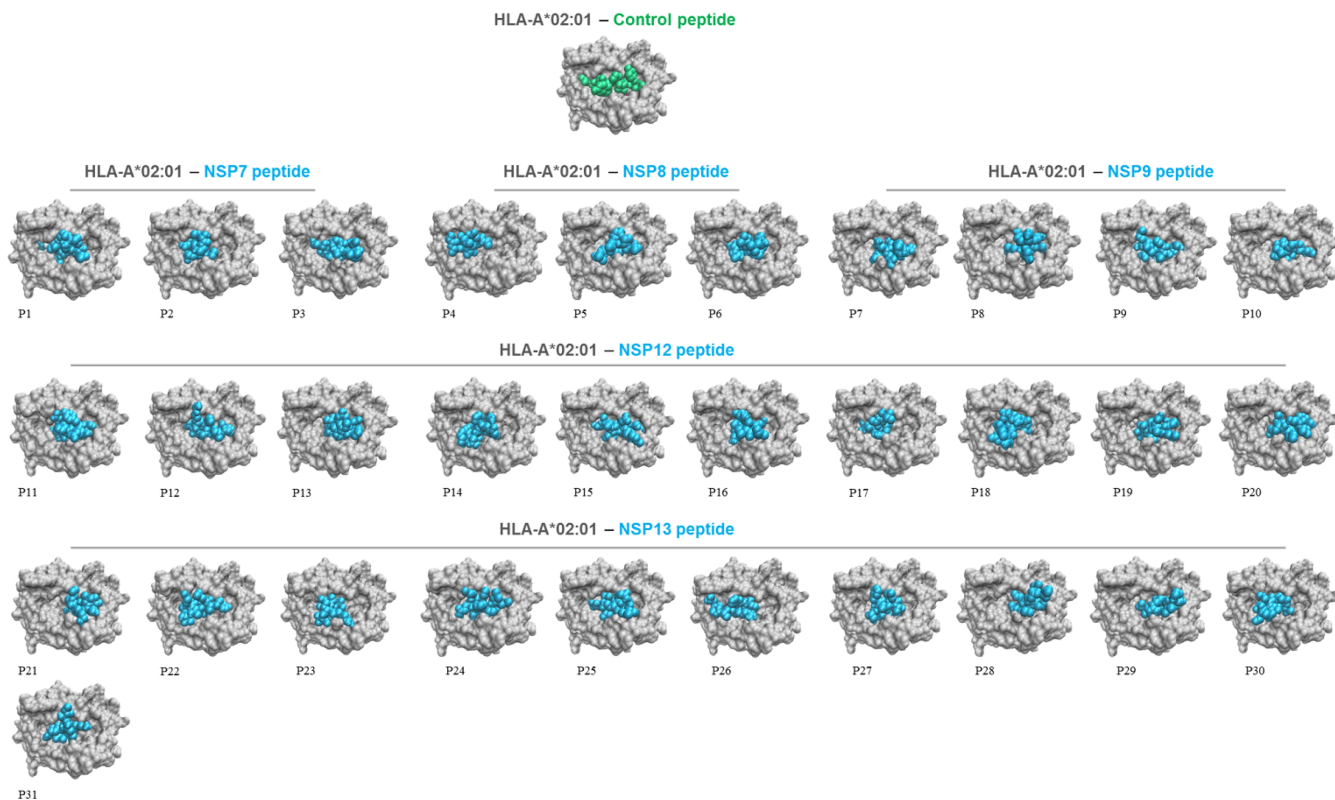


Fig. 2. Top view of HLA-A*02:01 presenting 9-mer viral peptides (numbered, P1 to P31) from selected SARS-CoV-2 NSPs. HLA alleles, viral peptides, and control peptides are shown in grey, cyan, and green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[18], and ToxinPred (<http://crdd.osdd.net/raghava/toxinpred/>) [19], respectively. Moreover, HLA-II binding peptides were also selected by their potential capability to induce interferon-gamma (IFN- γ), which was evaluated on IFNepitope web server (<http://crdd.osdd.net/raghava/ifnepitope/>) [20].

2.4. Conservancy analysis

Conservation of HLA binding peptides within NSPs (NSP7, NSP8, NSP9, NSP12, and NSP13) across SARS-CoV-2, SARS-CoV and MERS-CoV was studied by an epitope conservancy analysis on the Immune Epitope Database and Analysis Resource (IEDB-AR) (<http://tools.immuneepitope.org/main/>) [21]. Conservancy is described as the fraction of protein sequences that have the peptide whereas the identity is referred to the degree of correspondence (similarity) between several

sequences [21]. Results were represented as a barplot using Rstudio software (Version 3.5.3).

2.5. Interaction between HLA alleles and viral peptides: molecular docking

HLA-I and HLA-II binding peptides were docked with representative HLA-I and HLA-II alleles. The molecular docking simulation study was performed as follows. First, the three-dimensional (3D) structures of 9-mer and 15-mer peptides were obtained from PEPFOLD server (<https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/>) [22]. Second, the receptor-ligand docking simulations were conducted on ClusPro (version 2.0) server (<https://cluspro.bu.edu/>) [23]. For this purpose, the Protein Data Bank (PDB) accession numbers of HLA-A*02:01 (1DUZ) [24] and DRB1*01:01 (1AQP) [25] along with the PDB format file of each peptide were used as input on ClusPro server. Third, the

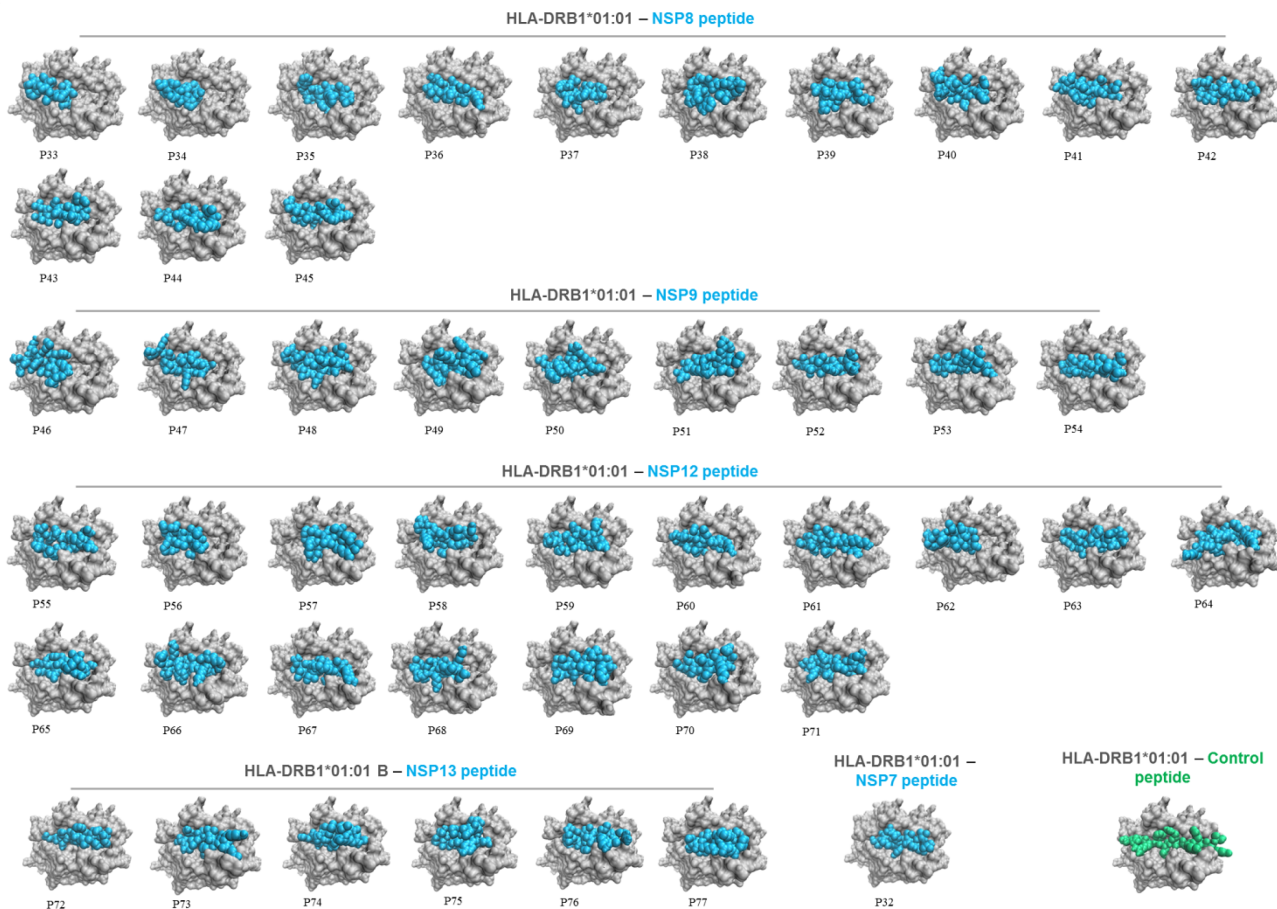


Fig. 3. Top view of HLA-DRB1*01:01 presenting 15-mer viral peptides (numbered, P32 to P77) from selected SARS-CoV-2 NSPs. HLA alleles, viral peptides, and control peptides are shown in grey, cyan, and green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

receptor-ligand docking complexes were visualized on VMD software (Version 1.9.3) [26]. The best allele-peptide complexes were selected based upon visual inspection and the ClusPro criteria, as well as they were compared with the co-crystal ligands of the HLA allele, which are considered as control peptides in this study. Finally, the Gibbs free energy (ΔG) of the each HLA-viral peptide complex was predicted on the PRODIGY server (<https://bianca.science.uu.nl/prodigy/>) [27]. Free energy values were represented as barplots using Rstudio software (Version 3.5.3).

2.6. Immune response simulations

To evaluate the potential use of HLA binding peptides on future vaccine trials, a vaccine amino acid sequence was developed with the predicted HLA-I and HLA-II binding peptides, which were linked using AAY and GPGPG linkers, respectively, as previously reported [28]. This vaccine construct was subjected to immune response simulations on the C-ImmSim server (<http://150.146.2.1/C-IMMSIM/index.php>) [29]. Three injections were applied four weeks apart as described by Nain et al. 2020 [28]. For immune interpretation purpose, the Simpson index D was used.

3. Results

3.1. Prediction of HLA binding peptides

31 HLA-I binding peptides were identified as the best (numbered, P1 to P31) (Table 1). These viral peptides showed robust viral antigenicity

(≥ 0.5) and absence of either allergenic or toxic residues (Table 1). The higher number of peptides was observed in NSP12 and NSP13 (10 and 11 peptides, respectively). Interestingly, overlapping residues were observed among P7, P8, and P9, and among P22, P23, P24, P25, and P26 (Table 1). Regarding the HLA-I interacting alleles, most of the viral peptides showed promiscuity by several HLA-I molecules including A*02:01, B*08:01, C*02:02, and C*12:02 (Table 1).

46 HLA-II binding peptides were predicted (numbered, P32 to P76). In this regard, 1 peptide was identified for NSP7, 13 peptides for NSP8, 9 peptides for NSP9, 17 peptides for NSP12, and 6 peptides for NSP13 (Table 2). Similar to HLA-I binding peptides, strong viral antigenicity and lack of allergenicity and toxicity were observed in these 15-mer viral peptides. Of note, each HLA-II binding peptide was classified as a potential inducer of IFN- γ (Table 2). Likewise, viral peptides from NSP8, NSP9, NSP12, and NSP13 showed the presence of overlapping residues. On the other hand, DRB1*01:01, DRB1*03:01, and DRB1*04:01 were identified as the most common HLA-II interacting alleles (Table 2).

Notably, HLA-I and HLA-II binding peptides are 100% conserved between SARS-CoV and SARS-CoV-2 with exception of P4, P12, P31, P33, P34, P35, P55, P69, P70, P71, and P76, that showed 50% of conservancy (Fig. 1). In contrast, most of the SARS-CoV-2 peptides common to MERS-CoV reached 50% of conservancy, with exception of P9 and P76 (0% of conservancy each) and P19, P66, P67, and P68 (100% of conservancy each) (Fig. 1).

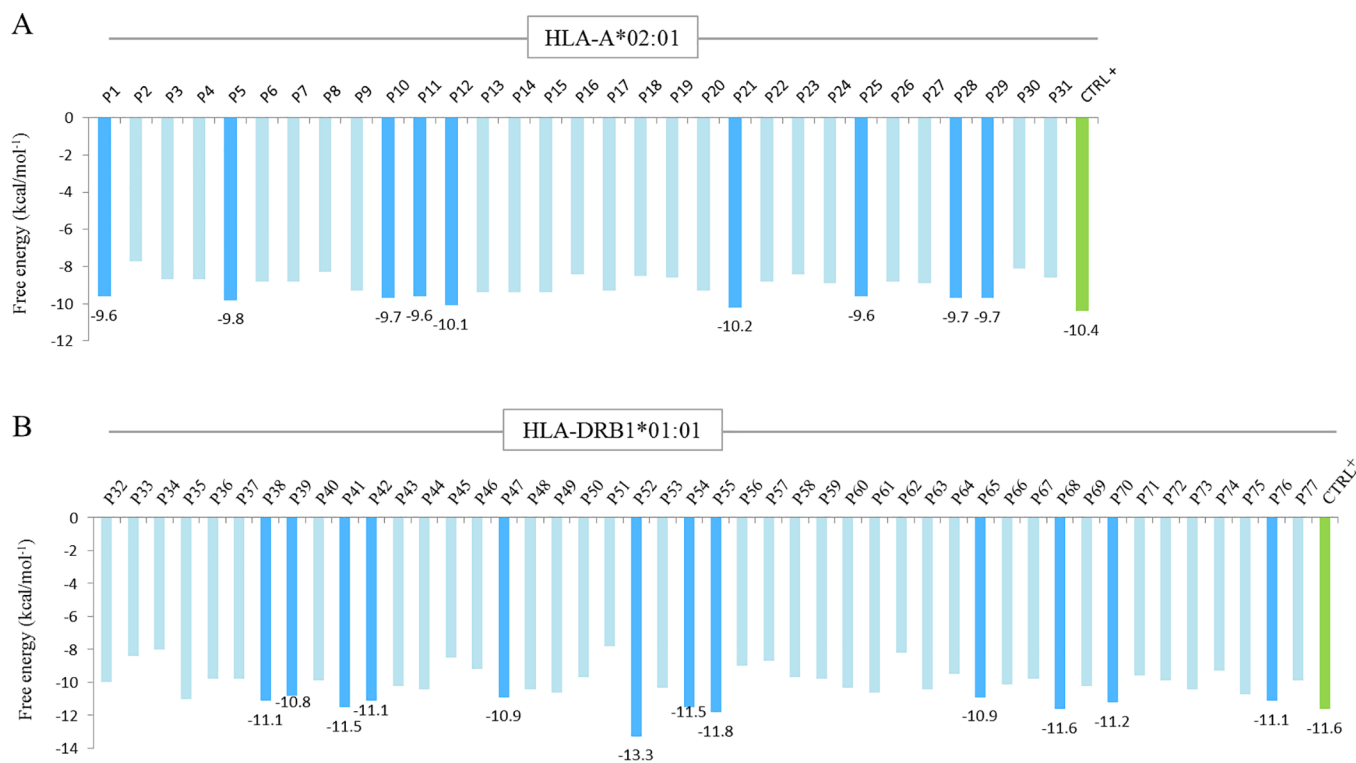


Fig. 4. Free energy for each HLA-viral peptide interaction. A: free energy values for the binding between HLA-A*02:01 and 9-mer viral peptides (numbered, P1 to P31). B: free energy values for the binding between HLA-DRB1*01:01 and 15-mer viral peptides (numbered, P32 to P377). Note that the lowest values are shown in cyan bars while values of control peptides (CTRL+) are shown in green bars. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. HLA allele-viral peptide interaction

Molecular docking simulations were conducted to evaluate the presentation of HLA binding peptides in the context of HLA molecules. To achieve this aim, A*02:01 and DRB1*01:01 were selected as representative HLA-I and HLA-II alleles, respectively. The analysis revealed that both 9-mer (Fig. 2) and 15-mer (Fig. 3) peptides rightly interact with the groove of their respective HLA molecule in a similar way to control peptides, as well as they showed different HLA binding patterns (Figs. 2 and 3).

Remarkably, these HLA-viral peptide complexes showed strong potential interactions (free energy values < -6 kcal/mol⁻¹) comparable to control peptides (Fig. 4A,B). In this regard, P1, P5, P10, P11, P12, P21, P25, P28, P29, P38, P39, P41, P42, P47, P52, P54, P55, P65, P68, P70, and P76 showed the lowest affinity values (Fig. 4A,B). Interestingly, P52 obtained the lower free energy value (-13.3 kcal/mol⁻¹) compared to other HLA-II binding peptides (Fig. 4B).

3.3. Immune responses simulations

The immune responses simulations with the vaccine construct showed high levels of immunoglobulin M (IgM) as well as isotypes IgG1 and IgG2 along with reduced immunogen levels during the secondary humoral response. Moreover, active B cell population increased after each dose (Fig. 5). Likewise, a marked increase of cytotoxic T cell (CTC) and T helper cell (THC) populations were observed along with effector cell generation, which decreased after immunogen clearance (Fig. 5). Finally, higher levels of key cytokines such as IFN-g and interleukin 2 (IL-2) were documented after each dose (Fig. 5). Taken together, these results suggest that HLA-I and HLA-II binding peptides could elicit humoral and cellular-mediated immune responses to SARS-CoV-2.

4. Discussion

The present study using an integrated *in silico* approach showed that five SARS-CoV-2 NSPs (NSP7, NSP8, NSP9, NSP12, and NSP13) harbour conserved viral peptides, whose properties—high viral antigenicity, absence of allergenic and toxic residues, potential IFN-g induction, interaction with HLA alleles, and suitable immune responses after *in silico* vaccination—make them potential targets for future prophylactic and/or therapeutics against the overwhelming COVID-19 pandemic. In this regard, HLA binding peptides from SARS-CoV-2 NSPs showed a robust viral antigenicity and immunogenicity similar to initial works based on peptide prediction from SARS-CoV-2 S, E, M, and N proteins [8,10,11]. Therefore, these peptides could successfully improve *in vitro* and *in vivo* immune responses against SARS-CoV-2. In fact, immune responses were observed after active immunization simulations, whose results (e.g., production of antibodies, T cell generation, and cytokines profile) are comparable to other vaccine candidates against infectious diseases [28,30]. On the other hand, conservancy analysis on IEDB-AR revealed that most of the peptides are strongly conserved across highly pathogenic betacoronaviruses—SARS-CoV-2, SARS-CoV, and MERS-CoV. This result is in agreement, at least partially, with a former report that has demonstrated a robust amino acid sequence identity between SARS-CoV-2 NSPs and SARS-CoV NSPs [4]. Hence, HLA binding peptides here predicted could evoke—perhaps—simultaneous immune responses against other betacoronaviruses such as SARS-CoV, and MERS-CoV.

The clear and proper interaction between conserved viral peptides and HLA alleles suggest that they could elicit not only adaptive immune responses by CD8+ CTC and CD4+ THC but also an innate immune response by Natural Killer (NK) cells—one of the first lines of defence against viruses []. In fact, it has been shown a decisive role of activating killer immunoglobulin-like receptors (aKIR) in viral recognition by NK cells [31]. Moreover, it has been reported that the interaction between aKIRs and HLA-C molecules is conditioned by C1 and C2 epitopes, for

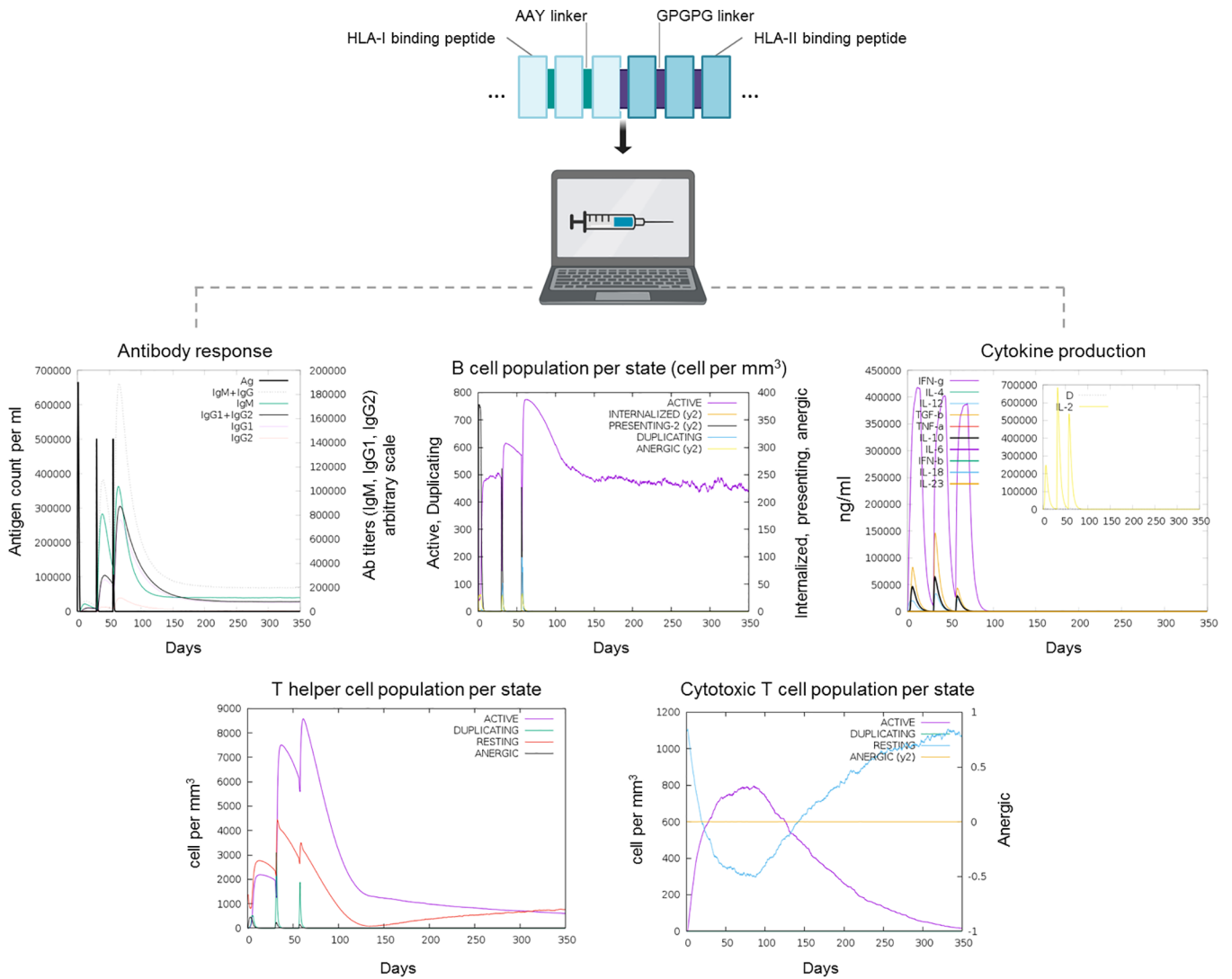


Fig. 5. Immune response simulations after *in silico* active immunization (3 doses) with the vaccine construct.

instance, KIR2DS2 may recognize HLA-C allotypes carrying the C1 epitope (N80), including HLA-C*01:02, HLA-C*14:02, and HLA-C*16:01, whereas KIR2DS4 binds to HLA-C allotypes with C1 (N80) or C2 (K80) epitopes, including HLA-C*02:01, HLA-C*04:01, and HLA-C*05:01 [31]. Interestingly, Nayer and colleagues reported that KIR2DS2 directly recognizes conserved viral peptides in the context of HLA-C*01:02, thereby leading to the inhibition of the hepatitis C virus and dengue virus replication [6]. In the present study, several 9-mer viral peptides showed affinity by HLA-C*01:02 as well as by HLA-C*02:02, which activates to KIR2DS4 [31]. However, further research is required to reveal peptide-specific recognition of SARS-CoV-2 and other betacoronaviruses by certain subsets within the NK cell compartment.

This study was limited by 1) all data only reflect immunoinformatics analyses. Future *in vitro* and *in vivo* investigations are required to ensure safety and efficacy of these peptides prior to their use in human trials; 2) Future studies should address other SARS-CoV-2 NSPs derived from ORF1ab to determine their probable immunogenicity. However, NSP7, NSP8, NSP9, NSP12, and NSP13 used in this research represent optimal targets for anti-SARS-CoV-2 immune responses as exposed earlier; and 3) although the vaccine construct showed robust humoral and cell-mediated responses, further investigations are necessary to assess its physicochemical properties, 3D structure, and refinement.

In summary, these initial results describing the immunogenic

potential of conserved peptides from SARS-CoV-2 NSPs are promising, and they could be strong candidates to evaluate functional NK cells, humoral, and T cell responses in experimental animal models. These results open a powerful window for future fundamental and translational studies focus on coronaviral diseases.

Declarations of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

I wish to thank the support of the Venezuelan Institute for Scientific Research (IVIC) – Venezuela, and Laboratory of Cellular and Molecular Pathology-IVIC.

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