Sampling SARS-CoV-2 proteomes for predicted CD8 T-cell epitopes as a tool for understanding immunogenic breadth and rational vaccine design

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Abstract

Predictive models for vaccine design have become a powerful and necessary resource for the expeditiousness design of vaccines to combat the ongoing SARS-CoV-2 global pandemic. Here we use the power of these predicted models to assess the sequence diversity of circulating SARS-CoV-2 proteomes in the context of an individual's CD8 T-cell immune repertoire to identify potential. defined regions of immunogenicity. Using this approach of expedited and rational CD8 T-cell vaccine design, it may be possible to develop a therapeutic vaccine candidate with the potential for both global and local coverage.

Introduction

The COVID-19 pandemic is a worldwide health emergency. The first cases were believed to have occurred earlier than December 2019, and as of the middle of September there have been

more than 31,000,000 cases of SARS-CoV-2 infection have been reported worldwide and in excess of 965,000 deaths (Update, ecdc.2020). Vaccine development against SARS-CoV-2 is accelerating, and currently there are more than 130 vaccines in development with some progressing to clinical trials in man (Mullard, 2020). Of these vaccines the majority are for a prophylactic indication with the spike glycoprotein being the preferred antigen target.

The full repertoire of immune responses to COVID-19 in patients is still being evaluated but recent publications indicate a significant role for cell mediated immunity in clearing SARS-CoV-2 and conferring some level of protective immunity (Grifoni, Weiskopf, Ramirez, Mateus, et al., 2020). This mirrors observations made for other coronavirus infections, SARS (Li et al., 2008), MERS (Zhao et al., 2017) and other viral infections including Ebola (Sakabe et al., 2018) and Lassa virus (Sullivan et al., 2020). Concurrent with vaccine design and understanding the immune response in patients, significant efforts have been invested in understanding the virus phylogeny as it progresses around the globe to identify key sequence changes that may influence vaccine design. The data from this analysis appears to show that the majority of circulating mutations are neutral or deleterious (van Dorp et al., 2020), although a D614G mutation identified in the spike glycoprotein may cause an increase in susceptibility to infection (Korber et al., 2020)

Linking novel sequence data to the use of existing predictive *in silico* tools for epitope identification offers an intriguing approach that can complement existing vaccine design strategies. Previously we hypothesized that incorporating potential immune recognition information into established models may increase the likelihood of success. We have shown that within a population, although HLA sequences show high levels of polymorphism, there are

43 conserved, over-represented alleles that can be used as representative of larger allele diversity
44 (Buggert et al., 2012; Hare, Fiore-Gartland, et al., 2020)

45 Previously we have applied NetMHCpan (Nielsen & Andreatta, 2016) as a proxy to identify
46 putative CD8 T-cell epitopes contained within the HIV transmitted founder virus (TFV)
47 identified from the Protocol C clinical cohort of sub Saharan and East Africa. We have shown
48 that it is possible to stratify and rank protein and/or proteome sequences for their contributions of
49 potential T-cell epitopes (Ed McGowan et al., 2020) . Here we propose to use the same approach
50 to evaluate a subset of global circulating SARS-CoV-2 sequences and, using the predefined
51 analysis applied to modeling HIV diversity, identify key regions within the SARS-CoV-2
52 proteome that could be included within a therapeutic T-cell vaccine.

53 **Methods**

54 For genes from each SARS-CoV-2 virus proteome sequence all 8-11mer peptides were
55 generated. The binding affinity of each peptide to the HLA alleles described above was predicted
56 using NetMHCpan-4.1.

57 Binding predictions below the peptide conservation threshold were read into PostgreSQLR for
58 analysis. The evaluated set of predicted binders was determined as peptides that appear in any
59 virus proteomes, above the peptide conservation threshold of 2.1%. First the virus proteome with
60 the largest number of unique predicted binders was identified. Next, the proteome that, when
61 combined with the previously selected proteome gave the highest increase in coverage of all
62 predicted peptide binders was included. This iterative process was repeated until 100% of all
63 predicted T-cell epitopes were accounted for. For comparison, set-building was performed a

64 second time using randomly selected proteomes instead of choosing the proteome that resulted in
65 the greatest increase of peptide coverage. For more information go to dataspace.iavi.org

66 **Results**

67 Our approach uses NetMHCpan to predict the HLA/peptide binding affinity for the selected
68 virus strains and then identify a set of close binding peptides that are common across the strains.
69 This algorithm was used to analyze 287 SARS-CoV-2 proteome sequences collected between
70 and 08 January and 02 April 2020 and downloaded from NCBI SARS-CoV-2 virus database.
71 The model parameters assessed included a minimum peptide conservation threshold of 2.1% (as
72 previously defined ((Ed McGowan et al., 2020)) and a rank binding cut-off of 2.0. Figure 1
73 summarizes the output dataset which includes only those peptides that are present in 6 or more
74 virus proteomes (epitope frequency) and have low rank binding scores although they may
75 represent multiple HLA/peptide interactions (see Table 1 for input sample data and full model
76 parameters).

77 These frequency and binding thresholds identified 11267 unique SARS-CoV-2-specific
78 predicted CD8 T-cell epitopes and identified a range of predicted binding profiles for the
79 different peptide-HLA interactions with the majority of peptides associating to selected HLA-A
80 and HLA-B alleles (Figure 1A & B -HLA Ids 1-36) and few primary associations to HLA-C
81 alleles.

82 These predicted peptides can be used to assess diversity by assigning a coverage gain value to
83 each sequence. These values can then be used to rank each virus proteome for the coverage it
84 provides within the sample population and by extension identify the sequences that are necessary
85 to obtain the optimum level of epitope restricted sequence coverage (Figure 2).

86 This model may be used to target and prioritize individual proteomes from which vaccine targets
87 could be derived. In our population of 287 SARS-CoV-2 proteomes each proteome offers an
88 individual coverage gain of 72.2- 98.3%. Using this model 8 key proteomes are required to reach
89 100% epitope coverage within the sample population. It would require an 8-fold increase in
90 virus sequences to achieve 100% coverage if sequences had been selected at random (n=68
91 $p<0.0001$).

92 Interestingly, of the 11267 unique predicted CD8 T-cell epitopes, 409 are contained in all 287
93 proteomes. These peptides map to 4 genes within the proteome with 392 peptides contained in
94 ORF1ab polypeptide and spike glycoprotein (Figure 3 and Supplementary Table 1).

95 **Discussion**

96 Evaluating predicted CD8 T-cell epitopes within the SARS-CoV-2 proteome revealed a high
97 level of conservation between proteomes, with each proteome representing 72 - 98.3% of all
98 predicted T-cell epitopes; however, using our model it is possible to reach 100% epitope
99 coverage using 8 defined proteomes. This is in contrast to HIV where it would take 83 proteomes
100 to achieve 100% epitope coverage on a comparable sample size (Ed McGowan et al., 2020) . The
101 enhanced conservation of epitopes is in concordance with the observed sequence diversity to
102 date and indicates that no bias has been introduced in to the analysis through modeling with
103 reduced input data.

104 Moreover, the elevated conservation can be used as a guide to identify regions within the
105 proteome that should be included within a therapeutic T-cell vaccine. Recent data has indicated
106 that naturally occurring T-cell responses in convalescent COVID-19 patients preferentially target
107 the spike glycoprotein and ORF1a polypeptide (Dong et al., 2020; Grifoni, Weiskopf, Ramirez,

108 Smith, et al., 2020). However, limitations in both these studies including that ORF1ab peptides
109 were not examined in one study, the sample size in both was small, peptides were generated from
110 the reference strain and the HLA Class 1 distribution was limited, may mean that potential
111 regions of interest have been overlooked.

112 We identified 409 predicted CD8 peptides that have 100% conservation within our sample set
113 with >90% of the predicted epitopes contained within either the ORF1ab polypeptide or spike
114 glycoprotein. Furthermore, the predicted epitopes appear to cluster within a ~550 a.a region of
115 the spike glycoprotein (amino acid 319-865) and within 2 regions of the ORF1ab polypeptide
116 (amino acid positions 2750-3250 and 4500-5500). Future experimental testing of these epitopes
117 would confirm whether natural infection induces CD8 T-cell responses targeting these regions,
118 but from an *in silico* perspective they offer a potential target for developing a therapeutic T-cell
119 vaccine that warrants further investigation.

Parameter	Values
SARS-COV-2 Proteomes	Brazil (1), China (13), France (1), Greece (1), Iran (1), South Africa (1), Spain (10), Sweden (1), Taiwan (1), Turkey (1), USA (238), Unknown (16), Vietnam (2)
Binding Threshold	1%
HLA allele contributions	46 alleles (16 x HLA-A, 19 x HLA-B, 11 x HLA-C)
HLA haplotype weighting	None
Rank Binding	<2.0
Peptide Conservation threshold (%)	2.1%
Peptide length	8, 9, 10 & 11mers

120 Table 1 Model Parameters and proteome sources

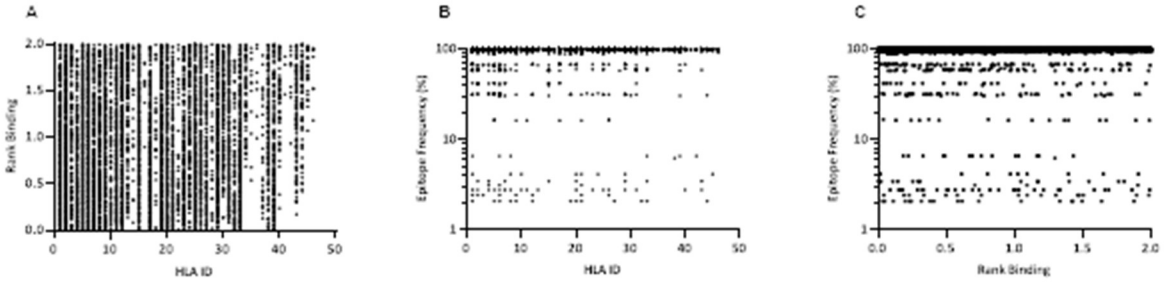


Figure 1. Affinity plots for all predicted peptides with conservation of ≥2.1% (n=11267). A – Predicted peptide affinity (Rank Binding) versus peptide frequency within transmitted founder proteome. B-Predicted peptide frequency versus primary associated HLA. C – Predicted peptide affinity (Rank Binding) versus primary associated HLA

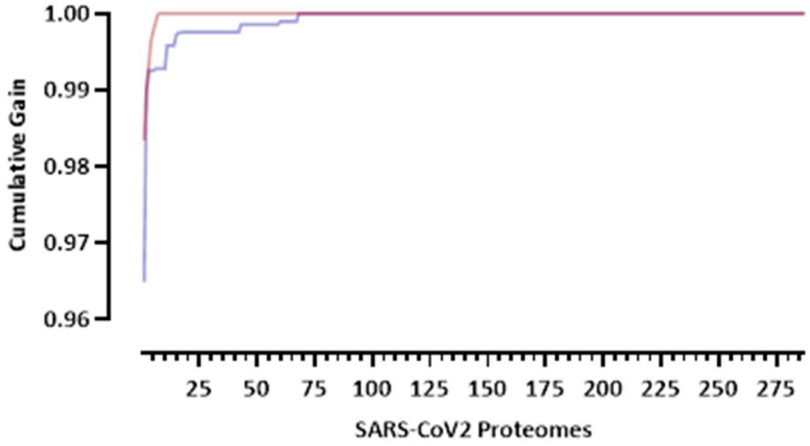
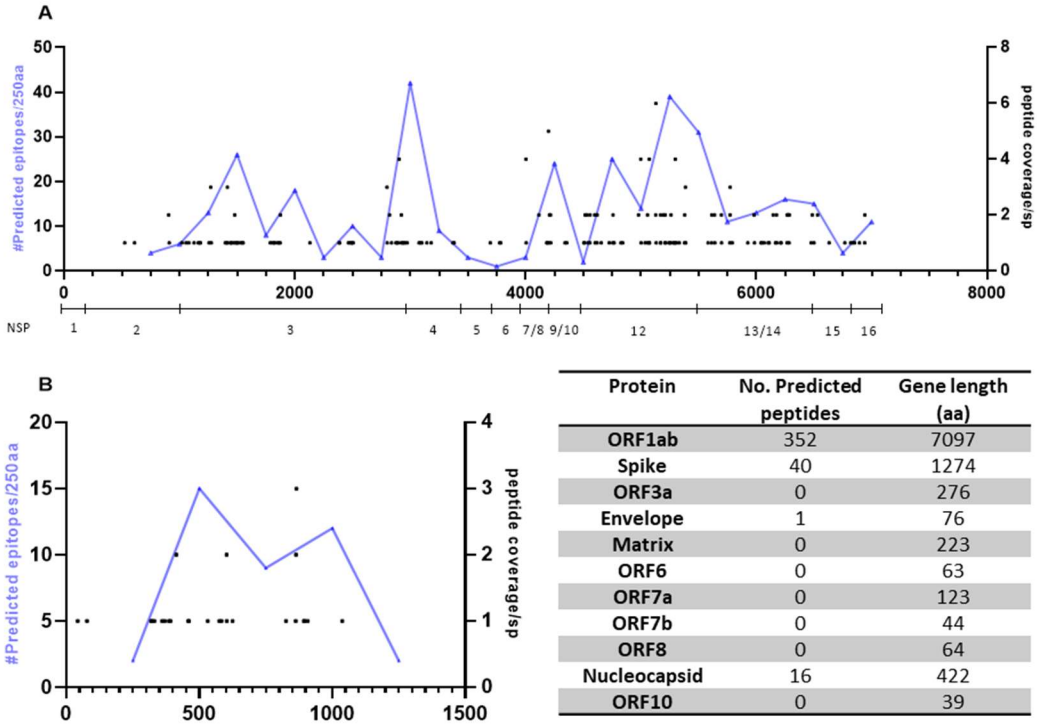


Figure 2. Cumulative coverage distribution plots of full length transmitted founder gag sequences using a 3-select coverage model and a 2% Binding Threshold, 3-Select best (red)) and 3-Select random (blue).



Protein	No. Predicted peptides	Gene length (aa)
ORF1ab	352	7097
Spike	40	1274
ORF3a	0	276
Envelope	1	76
Matrix	0	223
ORF6	0	63
ORF7a	0	123
ORF7b	0	44
ORF8	0	64
Nucleocapsid	16	422
ORF10	0	39

Figure 3. Mapping of predicted epitopes with 100% conservation showing location and number of predicted responses across the proteome and cumulative number of precited epitopes per 250aa. A – Predicted responses mapped across the ORF1ab polyprotein (with NSP genes identified). B - Predicted responses mapped across the spike glycoprotein.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JH wrote the manuscript and provided conceptual input. DM and MN developed and implemented the analysis scripts and contributed to developing the manuscript.

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