Preliminary identification of potential vaccine targets for 2019-nCoV based on SARS-CoV immunological studies

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Abstract

The beginning of 2020 has seen the emergence of the 2019 novel coronavirus (2019-nCoV) outbreak. Since the first reported case in the Wuhan city of China, 2019-nCoV has spread to other cities in China as well as to multiple countries across four continents. There is an imminent need to better understand this novel virus and to develop ways to control its spread. In this study, we sought to gain insights for vaccine design against 2019-nCoV by considering the high genetic similarity between 2019-nCoV and the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), and leveraging existing immunological studies of SARS-CoV. By screening the experimentally-determined SARS-CoV-derived B cell and T cell epitopes in the immunogenic structural proteins of SARS-CoV, we identified a set of B cell and T cell epitopes derived from the spike (S) and nucleocapsid (N) proteins that map identically to 2019-nCoV proteins. As no mutation has been observed in these identified epitopes among the available 2019-nCoV sequences (as of 29 January 2020), immune targeting of these epitopes may potentially offer protection against 2019-nCoV. For the T cell epitopes, we performed a population coverage analysis of the associated MHC alleles and proposed a set of epitopes that is estimated to provide broad coverage globally, as well as in China. Our findings provide a screened set of epitopes that can help guide experimental efforts towards the development of vaccines against 2019-nCoV.

Introduction

The ongoing outbreak of 2019 novel Coronavirus (2019-nCoV) in the Wuhan city (Hubei province) of China (C. Wang, Horby, Hayden, & Gao, 2020) and its alarmingly quick transmission to 25 other countries across the world (Centers-of-Disease-Control-and-Prevention, 2020) has resulted in World Health Organization (WHO) declaring a global health emergency on 30 January 2020 (World-Health-Organization, 2020b). This came just one month after the first reported case on 31 December 2019 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). WHO, in its first emergency meeting (World-Health-Organization, 2020a), estimated the fatality rate of 2019-nCov to be around 4%. Worldwide collaborative efforts from scientists are underway to understand this novel and rapidly spreading virus, and to develop effective interventions for controlling and preventing it (Heymann, 2020; Huang et al., 2020; Xin Liu & Wang, 2020; Zhou et al., 2020).

Coronaviruses are positive-sense single-stranded RNA viruses belonging to the family Coronaviridae. These viruses mostly infect animals, including birds and mammals. In humans, they generally cause mild respiratory infections, such as those observed in the common cold. However, some recent human coronavirus infections have resulted in lethal endemics, which include the SARS-CoV (Severe Acute Respiratory Syndrome coronavirus) and MERS-CoV (Middle East Respiratory Syndrome coronavirus). Both of these zoonotic coronaviruses belong to the genus Betacoronavirus within Coronaviridae. SARS-CoV originated from Southern China and caused an endemic in 2003. A total of 8,098 cases of SARS-CoV infection were reported globally including 774 associated deaths, and an estimated case-fatality rate of 14-15% (https://www.who.int/csr/sars/archive/2003_05_07a/en/). The first case of MERS-CoV occurred in Saudi Arabia in 2012. Since then, a total of 2,494 cases of MERS-CoV infection have been reported including 858 associated deaths, and an estimated high case-fatality rate of 34.4% (https://www.who.int/emergencies/mers-cov/en/). While no case of SARS-CoV has been reported since 2004, MERS-CoV has been around since 2012 and caused multiple sporadic outbreaks in different countries.

Like SARS-CoV and MERS-CoV, the recent 2019-nCoV belongs to the Betacoronavirus genus (Lu et al., 2020). It has a genome size of ~30 kilobases which, like other coronaviruses, encodes for multiple structural and non-structural proteins. The structural proteins include the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleocapsid (N) protein. With 2019-nCoV being discovered very recently, there is currently a lack of immunological information available about the virus (e.g., information about immunogenic proteins and immunogenic epitopes eliciting antibody or T cell responses). Preliminary studies suggest that the 2019-nCoV is quite similar to SARS-CoV based on the full-length genome phylogenetic analysis (Lu et al., 2020; Zhou et al., 2020), and the putatively similar cell entry mechanism and human cell receptor usage (Hoffmann et al., 2020; Letko & Munster, 2020; Zhou et al., 2020). Due to this apparent similarity between the two viruses, previous research

that has provided an understanding of protective immune responses against SARS-CoV may potentially be leveraged to aid vaccine development for 2019-nCoV.

Various reports related to SARS-CoV suggest a protective role of both humoral and cell-mediated immune responses. For the former case, antibody response generated against the S protein, the most exposed protein of SARS-CoV, has been shown to protect from infection in mouse models (Deming et al., 2006; Graham et al., 2012; Yang et al., 2004). In addition, multiple studies have shown that antibodies generated against the N protein of SARS-CoV, a highly immunogenic and abundantly expressed protein during infection (Lin et al., 2003), were particularly prevalent in SARS-CoV-infected patients (X Liu et al., 2004; J. Wang et al., 2003). While being effective, the antibody response was found to be short-lived in convalescent SARS-CoV patients (Tang et al., 2011). In contrast, T cell responses have been shown to provide long-term protection (Fan et al., 2009; Peng et al., 2006; Tang et al., 2011), even up to 11 years post-infection (Ng et al., 2016), and thus has attracted more interest for a prospective vaccine against SARS-CoV [reviewed in (W. J. Liu et al., 2017)]. Among all SARS-CoV proteins, T cell responses against the structural proteins have been found to be the most immunogenic in peripheral blood mononuclear cells of convalescent SARS-CoV patients as compared to the non-structural proteins (C. K.-F. Li et al., 2008). Further, of the structural proteins, T cell responses against the S and N proteins have been reported to be the most dominant and long-lasting (Channappanavar, Fett, Zhao, Meyerholz, & Perlman, 2014).

Here, by analysing available experimentally-determined SARS-CoV-derived B cell epitopes (both linear and discontinuous) and T cell epitopes, we identify and report those that are completely identical and comprise no mutation in the available 2019-nCoV sequences (as of 29 January 2020). These epitopes have the potential, therefore, to elicit a cross-reactive/effective response against 2019-nCoV. We focused particularly on the epitopes in the S and N structural proteins due to their dominant and long-lasting immune response previously reported against SARS-CoV. For the identified T cell epitopes, we additionally incorporated the information about the associated MHC alleles to provide a list of epitopes that seek to maximize population coverage globally, as well as in China. Our presented results can potentially narrow down the search for potent targets for an effective vaccine against the 2019-nCoV.

Materials and Methods

Acquisition and processing of sequence data. A total of 39 whole genome sequences of 2019-nCoV were downloaded on 29 January 2020 from the GISAID database (https://www.gisaid.org/CoV2020/) (Table 1) excluding five sequences (EPI_ISL_ 402120, EPI_ISL_402121, EPI_ISL_402126, EPI_ISL_403928 and EPI_ISL_403931) that likely have spurious mutations resulting from sequencing errors (http://virological.org/t/novel-2019-coronavirus-genome/319/18, http://virological.org/t/clock-and-tmrca-based-on-27-genomes/347, and http://virological.org/t/novel-2019-coronavirus-genome/319/11). These nucleotide sequences were aligned to the GenBank reference sequence (accession ID:

NC_045512.2) and then translated into amino acid residues according to the coding sequence positions provided along the reference sequence for the 2019-nCoV proteins (orf1a, orf1b, S, ORF3a, E, M, ORF6, ORF7a, ORF8, N, and ORF10). These sequences were aligned separately for each protein using the MAFFT multiple sequence alignment program (Katoh & Standley, 2013). Reference protein sequences for SARS-CoV and MERS-CoV were obtained following the same procedure from GenBank using the accession IDs NC_004718.3 and NC_019843.3, respectively.

Acquisition and filtering of epitope data. SARS-CoV-derived B cell and T cell epitopes were searched on the NIAID Virus Pathogen Database and Analysis Resource (ViPR) (https://www.viprbrc.org/; accessed January 29, 2020) (Pickett et al., 2012) by querying for the virus species name: "Severe acute respiratory syndrome-related coronavirus" from human hosts. We limited our search to include only the experimentally-determined epitopes that were associated with at least one positive assay: (i) B cell positive assays (e.g., enzyme-linked immunosorbent assay (ELISA)-based qualitative binding) for B cell epitopes and (ii) either T cell positive assays (such as enzyme-linked immune absorbent spot (ELISPOT) or intracellular cytokine staining (ICS) IFN-γ release) or major histocompatibility complex (MHC) positive binding assays for T cell epitopes. The number of B cell and T cell epitopes obtained from the database following the above procedure is listed in Table 2.

Population-coverage-based T cell epitope selection. Population coverages for sets of T cell epitopes were computed using the tool provided by the Immune Epitope Database (IEDB) (http://tools.iedb.org/population/; accessed January 29, 2020) (Vita et al., 2019). This tool uses the distribution of MHC alleles (with at least 4-digit resolution, e.g., A*02:01) within a defined population (obtained from http://www.allelefrequencies.net/) to estimate the population coverage for a set of T cell epitopes. The estimated population coverage represents the percentage of individuals within the population that are likely to elicit an immune response to at least one T cell epitope from the set. To identify the set of epitopes associated with MHC alleles that would maximize the population coverage, we adopted a greedy approach: (i) we first identified the MHC allele with the highest individual population coverage and initialized the set with their associated epitopes, (ii) we progressively added epitopes associated with other MHC alleles that resulted in the largest increase of the accumulated population coverage. We stopped when no increase in the accumulated population coverage was observed by adding epitopes associated with any of the remaining MHC alleles.

Constructing the phylogenetic tree. We used the publicly available software PASTA v1.6.4 (Mirarab et al., 2015) to construct a maximum-likelihood phylogenetic tree of each structural protein using the available sequence data of SARS-CoV, MERS-CoV, and 2019-nCoV. We additionally included the Zaria Bat coronavirus strain (accession ID: HQ166910.1) to serve as an outgroup. The appropriate parameters for tree estimation are automatically selected in the software based on the provided sequence data. For visualizing the constructed phylogenetic trees, we used the publicly available

software Dendroscope v3.6.3 (Huson & Scornavacca, 2012). Each constructed tree was rooted with the outgroup Zaria Bat coronavirus strain, and circular phylogram layout was used.

Results and Discussion

Structural proteins of 2019-nCoV are genetically similar to SARS-CoV, but not to MERS-CoV. The 2019-nCoV has been observed to be close to SARS-CoV—much more so than MERS-CoV—based on full-length genome phylogenetic analysis (Lu et al., 2020; Zhou et al., 2020). We checked whether this is also true at the level of the individual structural proteins (S, E, M, and N). A straightforward reference-sequence-based comparison indeed confirmed this, showing that the M, N, and E proteins of 2019-nCoV and SARS-CoV have over 90% genetic similarity, while that of the S protein was notably reduced (but still high) (Fig. 1a). The similarity between 2019-nCov and MERS-CoV, on the other hand, was substantially lower for all proteins (Fig. 1a); a feature that was also evident from the corresponding phylogenetic trees (Fig. 1b). We note that while the former analysis (Fig. 1a) was based on the reference sequence of each coronavirus, it is indeed a good representative of the virus population, since very few amino acid mutations have been observed in the corresponding sequence data (Supplementary Fig. 1). It is noteworthy that while MERS-CoV is the more recent coronavirus to have infected humans, and is comparatively more recurrent (causing outbreaks in 2012, 2015, and 2018) (https://www.who.int/emergencies/mers-cov/en/), 2019-nCoV is closer to the SARS-CoV which has not been observed since 2004.

Given the close genetic similarity between the structural proteins of SARS-CoV and 2019-nCoV proteins, we attempted to leverage immunological studies of the structural proteins of SARS-CoV to potentially aid vaccine development for 2019-nCoV. We focused specifically on the S and N proteins as these are known to induce potent and long-lived immune responses in SARS-CoV (Channappanavar et al., 2014; Deming et al., 2006; Graham et al., 2012; W. J. Liu et al., 2017; X Liu et al., 2004; J. Wang et al., 2003; Yang et al., 2004). We used the available SARS-CoV-derived experimentally-determined epitope data (see Materials and Methods) and searched to identify T cell and B cell epitopes that were identical—and hence potentially cross-reactive—across SARS-CoV and 2019-nCoV. We first report the analysis for T cell epitopes, which have been shown to provide a long-lasting immune response against SARS-CoV (Channappanavar et al., 2014), followed by a discussion of B cell epitopes.

Mapping the SARS-CoV-derived T cell epitopes that are identical in 2019-nCoV, and determining those with greatest estimated population coverage. The SARS-CoV-derived T cell epitopes used in this study were experimentally-determined from two different types of assays (Pickett et al., 2012): (i) T cell-positive assays, which tested for a T cell response against epitopes, and (ii) MHC-positive assays, which tested for epitope-MHC binding. We aligned these T cell epitopes across the 2019-nCoV protein sequences. Among the 115 T cell epitopes that were reported in T cell-positive assays (Table 2), we found that 27 epitope-sequences were identical within 2019-nCoV proteins and comprised no

mutation in the available 2019-nCoV sequences (as of 29 January 2020) (Table 3). Interestingly, all of these were present in either the N (16) or S (11) protein. However, these 27 epitopes were associated with only five distinct MHC alleles (at 4-digit resolution): HLA-A*02:01, HLA-B*40:01, HLA-DRA*01:01, HLA-DRB1*07:01, and HLA-DRB1*04:01. Consequently, the accumulated population coverage of these epitopes (see Materials and Methods for details) is estimated to be low for the global population (59.76%) as well as for China (32.36%). Note that for 6 of these 27 epitopes, the associated MHC alleles were not reported, and hence they could not be used in the population coverage computation.

To expand the search and identify potentially effective T cell targets covering a higher percentage of the population, we next considered the set of T cell epitopes that were experimentally-determined using MHC-positive assays (Table 2), derived from either the S or N protein. Epitopes determined using MHC-positive assays may also generate a positive T cell response. This has been reported for some of the identified epitopes (Table 3); however, establishing this more broadly requires further experimental investigation. Of the 959 epitopes reported in MHC-positive assays, we found that 253 epitope-sequences have an identical match in 2019-nCoV proteins (listed in Supplementary Table 1), with 80% being MHC class I restricted epitopes (Supplementary Table 2). Importantly, 109 of the 253 epitopes were derived from either the S (71) or N (38) protein. Interestingly, mapping the 71 S-derived epitopes onto the resolved crystal structure of the SARS-CoV S protein (Fig. 2) revealed that 3 of these (GYQPYRVVVL, QPYRVVVLSF, and PYRVVVLSF) were located entirely in the SARS-CoV receptor-binding motif, known to be important for virus cell entry (F. Li, 2005).

Similar to previous studies on HIV and HCV (Ahmed, Quadeer, Morales-Jimenez, & McKay, 2019; Dahirel et al., 2011; Quadeer et al., 2014), we estimated population coverages for various combinations of these 107 epitopes. Our aim was to determine sets of epitopes with maximum population coverage, potentially aiding the development of subunit vaccines against 2019-nCoV. For selection, we adopted a greedy computational approach (see Materials and Methods), which identified a set of T cell epitopes estimated to maximize population coverage in China, the country most affected by the 2019-nCoV outbreak. This set comprised of multiple T cell epitopes associated with 15 distinct MHC class I alleles and was estimated to provide an accumulated population coverage of 85.89% (Table 4). We also identified another set of T cell epitopes, associated with 14 distinct MHC class I alleles, that was estimated to maximize global population coverage (94.4%) (Table 5). We note that the estimated population coverage of this specific set of epitopes was lower for China (83.65%) as certain MHC alleles (e.g., HLA-A*02:01) associated with some of these epitopes are less frequent in the Chinese population (Table 5).

A recent study predicted T cell epitopes for 2019-nCoV that may be presented by a population from the Asia-Pacific region (Ramaiah & Arumugaswami, 2020). This study has multiple differences to our work. First, the authors focused on MHC Class II epitopes, while here we considered both MHC Class I and II epitopes. Interestingly, while we found a few MHC Class II epitopes using our approach

(Supplementary Table 2), none of these appeared in our identified epitope set (Tables 4 and 5), due to their comparatively low estimated population coverage. Second, computational tools were used to predict MHC Class II epitopes in (Ramaiah & Arumugaswami, 2020), while here we analysed the SARS-CoV-derived epitopes that have been determined experimentally using MHC-positive assays, and which match identically with the available 2019-nCoV sequences (as of 29 January 2020).

Since the identified T cell epitopes (Tables 4 and 5) were determined experimentally using MHC-positive assays, we believe this provides motivation to conduct further experimental studies to determine their specificity to natural T cell responses against 2019-nCoV. Furthermore, as the epitopes are estimated to provide a broad population coverage (globally as well as in China), they present potentially useful candidates for guiding experimental efforts towards developing universal subunit vaccines against 2019-nCoV.

Mapping the SARS-CoV-derived B cell epitopes that are identical in 2019-nCoV. Similar to T cell epitopes, we used in our study the SARS-CoV-derived B cell epitopes that have been experimentally-determined from B cell-positive assays (Pickett et al., 2012). These epitopes were classified as: (i) linear B cell epitopes (antigenic peptides), and (ii) discontinuous B cell epitopes (conformational epitopes with resolved structural determinants).

We aligned the 298 linear B cell epitopes (Table 2) across the 2019-nCoV proteins and found that 61 epitope-sequences, all derived from structural proteins, have an identical match and comprised no mutation in the available 2019-nCoV protein sequences (as of 29 January 2020). Interestingly, a large number (56) of these were derived from either the S (32) or N (24) protein (Table 6), while the remaining (5) were from the M and E proteins (Supplementary Table 3).

On the other hand, all 6 SARS-CoV-derived discontinuous B cell epitopes obtained from the ViPR database (Table 7) were derived from the S protein. Based on the pairwise alignment between the SARS-CoV and 2019-nCoV reference sequences (Supplementary Fig. 2), we found that none of these mapped identically to the 2019-nCoV S protein, in contrast to the linear epitopes. For 3 of these discontinuous B cell epitopes there was a partial mapping, with at least one site having an identical residue at the corresponding site in the 2019-nCoV S protein (Table 7).

Mapping the residues of the B cell epitopes onto the available structure of the SARS-CoV S protein revealed that the linear epitopes (Table 6) map to seemingly less-exposed regions, away from the most exposed region, the “spike head” (Fig. 3a). In contrast, all discontinuous B cell epitopes (Table 7) map onto the spike head region (Fig. 3b, *top panel*), which contains the receptor-binding motif of the SARS-CoV S protein (F. Li, 2005). We observe that few residues of the discontinuous epitopes that lie within the receptor-binding motif are identical within SARS-CoV and 2019-nCoV (Fig. 3b). It has been reported recently that 2019-nCoV is able to bind to the same receptor as SARS-CoV (ACE2) for cell entry,

despite having multiple amino acid differences with SARS-CoV's receptor-binding motif (Hoffmann et al., 2020; Letko & Munster, 2020; Lu et al., 2020; Zhou et al., 2020). Whether the antibodies specific to this motif maintain their binding and elicit an immune response against 2019-nCoV warrants further experimental investigation.

A related preliminary analysis of linear B cell epitopes has been reported online on the ViPR database website (https://www.viprbrc.org/brcDocs/documents/announcements/Corona/2019-nCov-ViPR-report_24JAN2020.pdf). Different from our analysis, which is focused on linear and discontinuous SARS-CoV-derived epitopes, they considered linear B cell epitope data for all Betacoronaviruses from human hosts. While only a summary of their results has been provided so far, preventing direct comparison of the individual epitopes, the number of linear B cell epitopes reported to map identically to 2019-nCoV is comparable to our findings.

Conclusion

To summarize, motivated by the high genetic similarity between the structural proteins of SARS-CoV and 2019-nCoV, we analysed the available experimentally-determined SARS-CoV-derived T cell and B cell epitopes and identified sets of epitopes that map identically to 2019-nCoV proteins. The absence of any mutation in these identified epitopes among the available 2019-nCoV sequences (as of 29 January 2020) suggests their potential for eliciting an effective T cell and antibody response in 2019-nCoV. We also screened the identified T cell epitopes using a population coverage criterion and reported a set of epitopes that can provide an estimated population coverage of 94.4% globally and 83.65% in China.

We acknowledge that this is a preliminary analysis based on the limited sequence data available for 2019-nCoV (as of 29 January 2020). As the virus continues to evolve and as more data is collected, it is expected that additional mutations will be observed. Such mutations will not affect our analysis, provided that they occur outside of the identified epitope regions. If mutations do occur within epitope regions, then these epitopes may be further screened (in line with the conservative filtering principle that we have employed), thereby producing a more refined epitope set.

Data and code availability

All sequence and immunological data, and all scripts for reproducing the results are available at https://github.com/faraz107/2019-nCoV-T-Cell-Vaccine-Candidates.

Acknowledgements

We thank all the authors, the originating and submitting laboratories (listed in Supplementary Table 4) for their sequence and metadata shared through GISAID, on which this research is based.

M.R.M. and A.A.Q. were supported by the General Research Fund of the Hong Kong Research Grants Council (RGC) [Grant No. 16204519]. S.F.A. was supported by the Hong Kong Ph.D. Fellowship Scheme (HKPFS).

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ahmed, S. F., Quadeer, A. A., Morales-Jimenez, D., & McKay, M. R. (2019). Sub-dominant principal components inform new vaccine targets for HIV Gag. *Bioinformatics*, *35*(20), 3884–3889. https://doi.org/10.1093/bioinformatics/btz524

Centers-of-Disease-Control-and-Prevention. (2020). Confirmed 2019-nCoV cases globally. Retrieved January 31, 2020, from https://www.cdc.gov/coronavirus/2019-ncov/locations-confirmed-cases.html

Channappanavar, R., Fett, C., Zhao, J., Meyerholz, D. K., & Perlman, S. (2014). Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. *Journal of Virology*, *88*(19), 11034–11044. https://doi.org/10.1128/jvi.01505-14

Dahirel, V., Shekhar, K., Pereyra, F., Miura, T., Artyomov, M., Talsania, S., … Chakraborty, A. K. (2011). Coordinate linkage of HIV evolution reveals regions of immunological vulnerability. *Proceedings of the National Academy of Sciences*, *108*(28), 11530–11535. https://doi.org/10.1073/pnas.1105315108

Deming, D., Sheahan, T., Heise, M., Yount, B., Davis, N., Sims, A., … Baric, R. (2006). Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS Medicine*, *3*(12), e525. https://doi.org/10.1371/journal.pmed.0030525

Fan, Y.-Y., Huang, Z.-T., Li, L., Wu, M.-H., Yu, T., Koup, R. A., … Wu, C.-Y. (2009). Characterization of SARS-CoV-specific memory T cells from recovered individuals 4 years after infection. *Archives of Virology*, *154*(7), 1093–1099. https://doi.org/10.1007/s00705-009-0409-6

Graham, R. L., Becker, M. M., Eckerle, L. D., Bolles, M., Denison, M. R., & Baric, R. S. (2012). A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. *Nature Medicine*, *18*(12), 1820–1826. https://doi.org/10.1038/nm.2972

Heymann, D. L. (2020). Data sharing and outbreaks: best practice exemplified. *The Lancet*. https://doi.org/10.1016/S0140-6736(20)30184-7

Hoffmann, M., Kleine-Weber, H., Kruger, N., Muller, M., Drosten, C., & Pohlmann, S. (2020). The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *BioRxiv 2020.01.31.929042*. https://doi.org/10.1101/2020.01.31.929042

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. https://doi.org/10.1016/S0140-6736(20)30183-5

Huson, D. H., & Scornavacca, C. (2012). Dendroscope 3: An interactive tool for rooted phylogenetic trees and networks. *Systematic Biology*, *61*(6), 1061–1067. https://doi.org/10.1093/sysbio/sys062

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Letko, M., & Munster, V. (2020). Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV. *BioRxiv 2020.01.22.915660*. https://doi.org/10.1101/2020.01.22.915660

Li, C. K.-F., Wu, H., Yan, H., Ma, S., Wang, L., Zhang, M., … Xu, X.-N. (2008). T cell responses to whole SARS coronavirus in humans. *The Journal of Immunology*, *181*(8), 5490–5500. https://doi.org/10.4049/jimmunol.181.8.5490

Li, F. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science*, *309*(5742), 1864–1868. https://doi.org/10.1126/science.1116480

Lin, Y., Shen, X., Yang, R. F., Li, Y. X., Ji, Y. Y., … He, Y. Y. (2003). Identification of an epitope of SARS-coronavirus nucleocapsid protein. *Cell Research*, *13*(3), 141–145. https://doi.org/10.1038/sj.cr.7290158

Liu, W. J., Zhao, M., Liu, K., Xu, K., Wong, G., Tan, W., & Gao, G. F. (2017). T-cell immunity of SARS-CoV: Implications for vaccine development against MERS-CoV. *Antiviral Research*, *137*, 82–92. https://doi.org/10.1016/j.antiviral.2016.11.006

Liu, X, Shi, Y., Li, P., Li, L., Yi, Y., Ma, Q., & Cao, C. (2004). Profile of antibodies to the nucleocapsid protein of the severe acute respiratory syndrome (SARS)-associated coronavirus in probable SARS patients. *Clinical and Vaccine Immunology*, *11*(1), 227–228. https://doi.org/10.1128/cdli.11.1.227-228.2004

Liu, Xin, & Wang, X.-J. (2020). Potential inhibitors for 2019-nCoV coronavirus M protease from clinically approved medicines. *BioRxiv 2020.01.29.924100*. https://doi.org/10.1101/2020.01.29.924100

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., … Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*, *6736*(20), 1–10. https://doi.org/10.1016/S0140-6736(20)30251-8

Mirarab, S., Nguyen, N., Guo, S., Wang, L.-S., Kim, J., & Warnow, T. (2015). PASTA: Ultra-large multiple sequence alignment for nucleotide and amino-acid sequences. *Journal of Computational Biology*, *22*(5), 377–386. https://doi.org/10.1089/cmb.2014.0156

Ng, O.-W., Chia, A., Tan, A. T., Jadi, R. S., Leong, H. N., Bertoletti, A., & Tan, Y.-J. (2016). Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. *Vaccine*, *34*(17), 2008–2014. https://doi.org/10.1016/j.vaccine.2016.02.063

Peng, H., Yang, L.-T., Wang, L.-Y., Li, J., Huang, J., … Lu, Z.-Q. (2006). Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-recovered patients. *Virology*, *351*(2), 466–475. https://doi.org/10.1016/j.virol.2006.03.036

Pickett, B. E., Sadat, E. L., Zhang, Y., Noronha, J. M., Squires, R. B., Hunt, V., … Scheuermann, R. H. (2012). ViPR: An open bioinformatics database and analysis resource for virology research. *Nucleic Acids Research*, *40*(D1), D593–D598. https://doi.org/10.1093/nar/gkr859

Quadeer, A. A., Louie, R. H. Y., Shekhar, K., Chakraborty, A. K., Hsing, I.-M., & McKay, M. R. (2014). Statistical linkage analysis of substitutions in patient-derived sequences of genotype 1a hepatitis C virus nonstructural protein 3 exposes targets for immunogen design. *Journal of Virology*, *88*(13), 7628–7644. https://doi.org/10.1128/JVI.03812-13

Ramaiah, A., & Arumugaswami, V. (2020). Insights into cross-species evolution of novel human coronavirus 2019-nCoV and defining immune determinants for vaccine development. *BioRxiv 2020.01.29.925867*. https://doi.org/10.1101/2020.01.29.925867

Tang, F., Quan, Y., Xin, Z.-T., Wrammert, J., Ma, M.-J., Lv, H., … Cao, W.-C. (2011). Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: A six-year follow-up study. *The Journal of Immunology*, *186*(12), 7264–7268. https://doi.org/10.4049/jimmunol.0903490

Vita, R., Mahajan, S., Overton, J. A., Dhanda, S. K., Martini, S., Cantrell, J. R., … Peters, B. (2019). The immune epitope database (IEDB): 2018 update. *Nucleic Acids Research*, *47*(D1), D339–D343. https://doi.org/10.1093/nar/gky1006

Wang, C., Horby, P. W., Hayden, F. G., & Gao, G. F. (2020). A novel coronavirus outbreak of global health concern. *The Lancet*. https://doi.org/10.1016/S0140-6736(20)30185-9

Wang, J., Wen, J., Li, J., Yin, J., Zhu, Q., Wang, H., … Liu, S. (2003). Assessment of immunoreactive synthetic peptides from the structural proteins of severe acute respiratory syndrome coronavirus. *Clinical Chemistry*, *49*(12), 1989–1996. https://doi.org/10.1373/clinchem.2003.023184

World-Health-Organization. (2020a). Statement on the meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). Retrieved January 31, 2020, from https://www.who.int/news-room/detail/23-01-2020-statement-on-the-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov)

World-Health-Organization. (2020b). Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). Retrieved January 31, 2020, from https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov)

Yang, Z.-Y., Kong, W.-P., Huang, Y., Roberts, A., Murphy, B. R., … Subbarao, K. (2004). A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. *Nature*, *428*(6982), 561–564. https://doi.org/10.1038/nature02463

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. https://doi.org/10.1038/s41586-020-2012-7

Figures

a

Percentage sequence identity with 2019-nCoV

	S protein	N protein	M protein	E protein
SARS-CoV	76.0%	90.6%	90.1%	94.7%
MERS-CoV	4.6%	7.6%	6.3%	30.5%

b

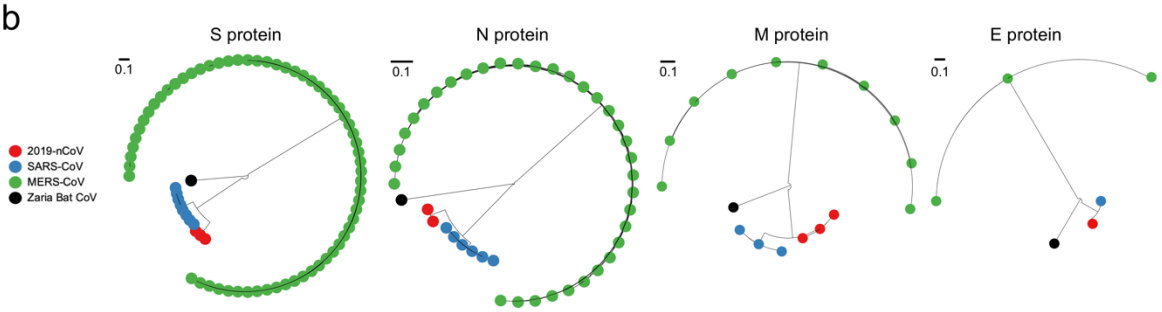


Figure 1. Comparison of the similarity of structural proteins of 2019-nCoV with the corresponding proteins of SARS-CoV and MERS-CoV. (a) Percentage genetic similarity of the individual structural proteins of 2019-nCoV with those of SARS-CoV and MERS-CoV. The reference sequence of each coronavirus (Materials and Methods) was used to calculate the percentage genetic similarity. (b) Circular phylogram of the phylogenetic trees of the four structural proteins. All trees were constructed using PASTA (Mirarab et al., 2015) and rooted with the outgroup Zaria Bat CoV strain (accession ID: HQ166910.1).

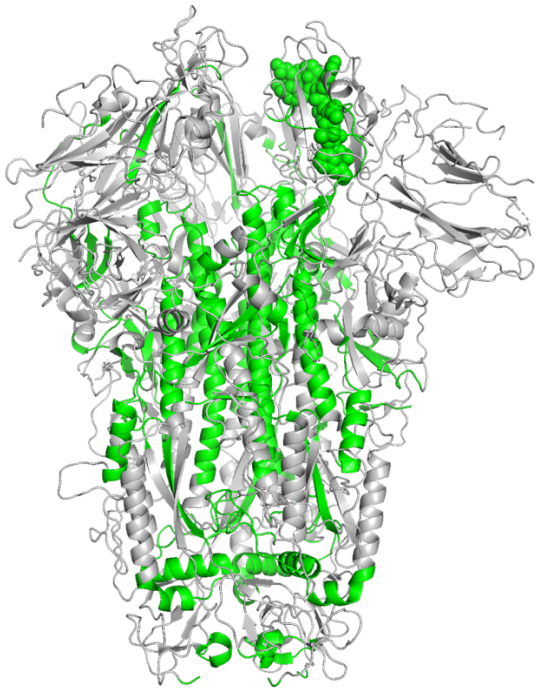


Figure 2. Location of identified T cell epitopes on the SARS-CoV S protein structure (PDB ID: 5XLR). Residues of the SARS-CoV-derived T cell epitopes (determined using MHC-positive assays that were identical in 2019-nCoV) are shown in green color. The 3 overlapping epitopes (GYQPYRVVVL, QPYRVVVLSF, PYRVVVLSF) that lie within the SARS-CoV receptor-binding motif are shown as spheres.

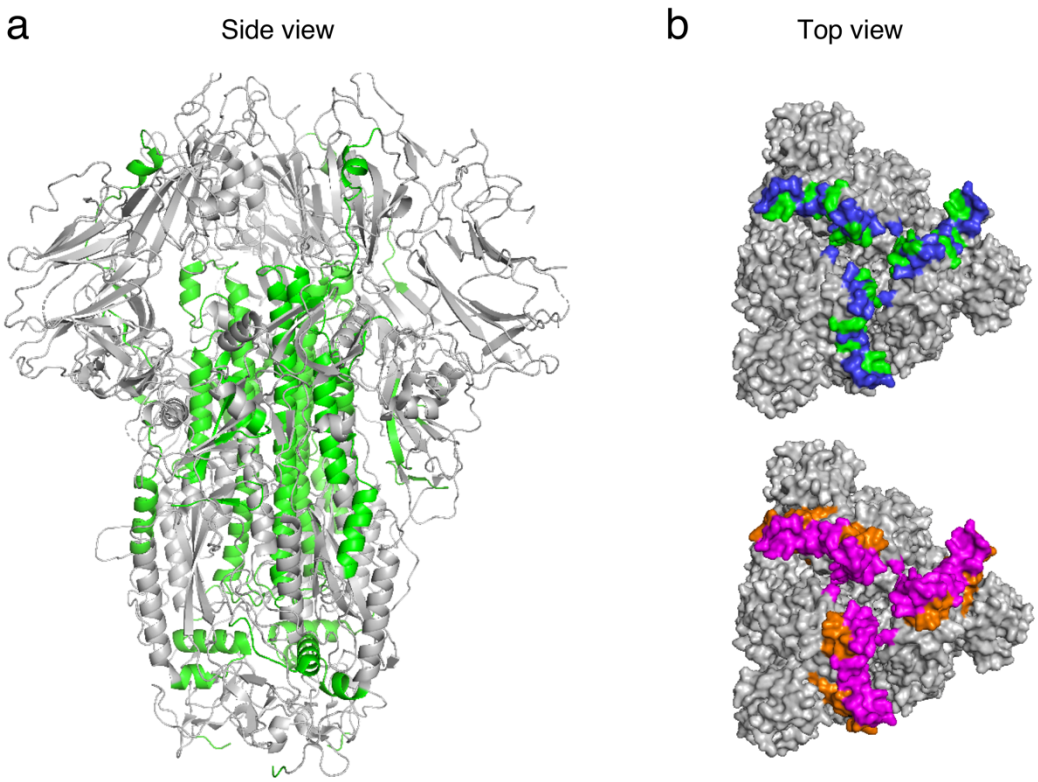


Figure 3. Location of SARS-CoV-derived B cell epitopes on the SARS-CoV S protein structure (PDB ID: 5XLR). (a) Residues of the linear B cell epitopes, that were identical in 2019-nCoV (Table 6), are shown in green color. (b) (*Top panel*) Location of discontinuous B cell epitopes that share at least one identical residue with corresponding 2019-nCoV sites (Table 7). Identical epitope residues are shown in green color, while the remaining residues are shown in blue color. (*Bottom panel*) Location of the receptor-binding motif of SARS-CoV. Residues in discontinuous B cell epitopes within the motif are indicated in magenta color, while the remaining motif residues are shown in orange color.

Tables

Table 1. GISAID accession IDs of the 39 whole genome sequences of 2019-nCoV used in this analysis.

EPI_ISL_402119	EPI_ISL_402132	EPI_ISL_403937	EPI_ISL_406034	EPI_ISL_406538
EPI_ISL_402123	EPI_ISL_403929	EPI_ISL_403962	EPI_ISL_406036	EPI_ISL_406592
EPI_ISL_402124	EPI_ISL_403930	EPI_ISL_403963	EPI_ISL_406223	EPI_ISL_406593
EPI_ISL_402125	EPI_ISL_403932	EPI_ISL_404227	EPI_ISL_406531	EPI_ISL_406594
EPI_ISL_402127	EPI_ISL_403933	EPI_ISL_404228	EPI_ISL_406533	EPI_ISL_406595
EPI_ISL_402128	EPI_ISL_403934	EPI_ISL_404253	EPI_ISL_406534	EPI_ISL_406596
EPI_ISL_402129	EPI_ISL_403935	EPI_ISL_404895	EPI_ISL_406535	EPI_ISL_406597
EPI_ISL_402130	EPI_ISL_403936	EPI_ISL_406031	EPI_ISL_406536	

Table 2. Filtering criteria and corresponding number of SARS-CoV-derived epitopes obtained from the ViPR database.

Filtering criteria		Number of epitopes
B cell positive assays	Linear B cell epitopes	298
	Discontinuous B cell epitopes	6
MHC positive assays	T cell epitopes	959
T cell positive assays	T cell epitopes	115

Table 3. SARS-CoV-derived T cell epitopes obtained using T cell-positive assays that are identical in 2019-nCoV.

Protein	IEDB ID	Epitope	MHC Allele[1]	MHC Allele Class[1]
N	125100	ILLNKHID	HLA-A*02:01	I
N	1295	AFFGMSRIGMEVTPSGTW	NA	NA
N	190494	MEVTPSGTWL	HLA-B*40:01	I
N	21347	GMSRIGMEV	HLA-A*02:01	I
N	27182	ILLNKHIDA	HLA-A*02:01	I
N	2802	ALNTPKDHI	HLA-A*02:01	I
N	28371	IRQGTDYKHWPQIAQFA	NA	NA
N	31166	KHWPQIAQFAPSASAFF	NA	NA
N	34851	LALLLLDRL	HLA-A*02:01	I
N	37473	LLLDRLNQL	HLA-A*02:01	I
N	37611	LLNKHIDAYKTFPPTEPK	NA	NA
N	38881	LQLPQGTTL	HLA-A*02:01	I
N	3957	AQFAPSASAFFGMSR	NA	II
N	3958	AQFAPSASAFFGMSRIGM	NA	NA
N	55683	RRPQGLPNNTASWFT	NA	I
N	74517	YKTFPPTEPKKDKKKK	NA	NA
S	100048	GAALQIPFAMQMAYRF	HLA-DRA*01:01 DRB1*07:01	II
S	100300	MAYRFNGIGVTQNVLY	HLA-DRB1*04:01	II
S	100428	QLIRAAEIRASANLAATK	HLA-DRB1*04:01	II
S	16156	FIAGLIAIV	HLA-A*02:01	I
S	2801	ALNTLVKQL	HLA-A*02:01	I
S	36724	LITGRLQSL	HLA-A2	I
S	44814	NLNESLIDL	HLA-A*02:01	I
S	50311	QALNTLVKQLSSNFGAI	HLA-DRB1*04:01	II
S	54680	RLNEVAKNL	HLA-A*02:01	I
S	69657	VLNDILSRL	HLA-A*02:01	I
S	71663	VVFLHVTYV	HLA-A*02:01	I

[1]NA: Not available

Table 4. Set of SARS-CoV-derived S and N protein T cell epitopes that maximize estimated population coverage for China.

Epitopes[1]	MHC Allele Class	MHC Allele	Accumulated Population Coverage in China (%)
AQALNTLVK, ASANLAATK, GSFCTQLNR, GVVFLHVTY, MTSCCSCLK, SFIEDLLFNK, SVLNDILSR, TQNVLYENQK, ATEGALNTPK, KTFPPTEPK, QQQGQTVTK, QQQGQTVTKK, QQQQGQTVTK, SASAFFGMSR	I	HLA-A*11:01	43.48
VYDPLQPEL	I	HLA-A*24:02	60.68
ALNTLVKQL, FIAGLIAIV, IITTDNTFV, LLFNKVTLA, LLLQYGSFC, LLQYGSFCT, NLNESLIDL, RLDKVEAEV, RLNEVAKNL, RLQSLQTYV, VLNDILSRL, VVFLHVTYV, ALNTPKDHI, GMSRIGMEV, ILLNKHIDA, LALLLLDRL, LLLDRLNQL, LLLLDRLNQL, LQLPQGTTL, TTLPKGFYA, VLQLPQGTTL, ILLNKHID, LLLLDRLNQ	I	HLA-A*02:01	69.63
LQIPFAMQM	I	HLA-B*15:01	73.9
DEDDSEPVL, SEPVLKGVKL, GMEVTPSGTWL, MEVTPSGTWL	I	HLA-B*40:01	77.23
DSFKEELDKY	I	HLA-A*26:01	78.98
VQIDRLITGR, ASAFFGMSR, SQASSRSSSR	I	HLA-A*31:01	80.62
LSPRWYFYY	I	HLA-A*01:01	82.03
VRFPNITNL	I	HLA-C*14:02	83.27
SLIDLQELGK, ALALLLLDR, KTFPPTEPKK, QLPQGTTLPK	I	HLA-A*03:01	84.4
FPNITNLCPF	I	HLA-B*35:01	85.07
GYQPYRVVVL, PYRVVVLSF	I	HLA-A*23:01	85.45
QELGKYEQYI, YEQYIKWPWY	I	HLA-B*44:02	85.64
LIDLQELGKY, RVDFCGKGY, GTTLPKGFY, VTPSGTWLTY	I	HLA-A*30:02	85.78
IGAGICASY, TPSGTWLTY	I	HLA-A*29:02	85.89

[1] Multiple SARS-CoV-derived epitopes that were reported in positive-MHC assays are shown for each allele.

Table 5. Set of SARS-CoV-derived S and N protein T cell epitopes that maximize estimated population coverage globally.

Epitopes[1]	MHC Allele Class	MHC Allele	Global Accumulated Population Coverage (%)	Accumulated Population Coverage in China (%)
ALNTLVKQL, FIAGLIAIV, IITTDNTFV, LLFNKVTLA, LLLQYGSFC, LLQYGSFCT, NLNESLIDL, RLDKVEAEV, RLNEVAKNL, VLNDILSRL, VLYQDVNCT, VVFLHVTYV, ALNTPKDHI, GMSRIGMEV, ILLNKHIDA, LALLLLDRL, LLLDRLNQL, LQLPQGTTL, TTLPKGFYA, VLQLPQGTTL, ILLNKHID, LLLLDRLNQ	I	HLA-A*02:01	39.08	14.62
VYDPLQPEL	I	HLA-A*24:02	55.48	36.11
CVADYSVLY, LSPRWYFYY	I	HLA-A*01:01	66.78	39.09
SLIDLQELGK, ALALLLLDR, KTFPPTEPKK, QLPQGTTLPK	I	HLA-A*03:01	76.14	41.68
AQALNTLVK, ASANLAATK, GSFCTQLNR, GVVFLHVTY, MTSCCSCLK, SFIEDLLFNK, SVLNDILSR, TQNVLYENQK, ATEGALNTPK, KTFPPTEPK, QQQGQTVTK, QQQGQTVTKK, QQQQGQTVTK, SASAFFGMSR	I	HLA-A*11:01	83.39	73.43
CMTSCCSCLK	I	HLA-A*68:01	85.71	74.25
DSFKEELDKY	I	HLA-A*26:01	87.86	76.39
GYQPYRVVVL, PYRVVVLSF	I	HLA-A*23:01	89.7	76.99
VQIDRLITGR, ASAFFGMSR, SQASSRSSSR	I	HLA-A*31:01	91.37	78.96
IGAGICASY, TPSGTWLTY	I	HLA-A*29:02	92.49	79.12
LIDLQELGKY, RVDFCGKGY, GTTLPKGFY, VTPSGTWLTY	I	HLA-A*30:02	93.14	79.33
LQIPFAMQM	I	HLA-B*15:01	93.72	82.23
QELGKYEQYI, YEQYIKWPWY	I	HLA-B*44:02	94.22	82.44
VRFPNITNL	I	HLA-C*14:02	94.4	83.65

[1] Multiple SARS-CoV-derived epitopes that were reported in positive-MHC assays are shown for each allele.

Table 6. SARS-CoV-derived linear B cell epitopes (total 56 epitopes) from S and N proteins that are identical in 2019-nCoV.

Protein	IEDB ID	Epitope
N	15814	FFGMSRIGMEVTPSGTW
N	21065	GLPNNTASWFTALTQHGK
N	22855	GTTLPK
N	28371	IRQGTDYKHWPQIAQFA
N	31116	KHIDAYKTFPPTEPKKDKKK
N	31166	KHWPQIAQFAPSASAFF
N	31692	KKSAAEASKKPRQKRTA
N	33669	KTFPPTEPKKDKKKK
N	37640	LLPAAD
N	38249	LNKHIDAYKTFPPTEPK
N	38648	LPQGTTLPKG
N	38657	LPQRQKKQ
N	48067	PKGFYAEGSRGGSQASSR
N	50741	QFAPSASAFFGMSRIGM
N	50965	QGTDYKHW
N	51483	QLPQGTTLPKGFYAE
N	51484	QLPQGTTLPKGFYAEGSR
N	51485	QLPQGTTLPKGFYAEGSRGGSQ
N	52117	QQQGQTVTKKSAAEASKK
N	55683	RRPQGLPNNTASWFT
N	60379	SQASSRSS
N	60669	SRGGSQASSRSSSRSR
N	63729	TFPPTEPK
N	75235	YNVTQAFGRRGPEQTQGNF
S	10778	DVVNQNAQALNTLVKQL
S	11038	EAEVQIDRLITGRLQSL
S	12426	EIDRLNEVAKNLNESLIDLQELGKYEQY
S	14626	EVAKNLNESLIDLQELG

Protein	IEDB ID	Epitope
S	15972	FGEVFNAT
S	16183	FIEDLLFNKVTLADAGF
S	18515	GAALQIPFAMQMAYRFN
S	18594	GAGICASY
S	2092	AISSVLNDILSRLDKVE
S	22321	GSFCTQLN
S	27357	ILSRLDKVEAEVQIDRL
S	30987	KGIYQTSN
S	3176	AMQMAYRF
S	32508	KNHTSPDVDLGDISGIN
S	41177	MAYRFNGIGVTQNVLYE
S	462	AATKMSECVLGQSKRVD
S	47479	PFAMQMAYRFNGIGVTQ
S	50311	QALNTLVKQLSSNFGAI
S	51379	QLIRAAEIRASANLAAT
S	52020	QQFGRD
S	53202	RASANLAATKMSECVLG
S	54599	RLITGRLQSLQTYVTQQ
S	558417	EIDRLNEVAKNLNESLIDLQELGKYEQY
S	558455	LYQDVN
S	558456	LYQDVNC
S	558457	LYQDVNCT
S	59425	SLQTYVTQQLIRAAEIR
S	6476	CKFDEDDSEPVLKGVKLHYT
S	67220	TVYDPLQPELDSFKEEL
S	70719	VRFPNITNLCPFGEVFN
S	7868	DDSEPVLKGVKLHYT
S	9094	DLGDISGINASVVNIQK

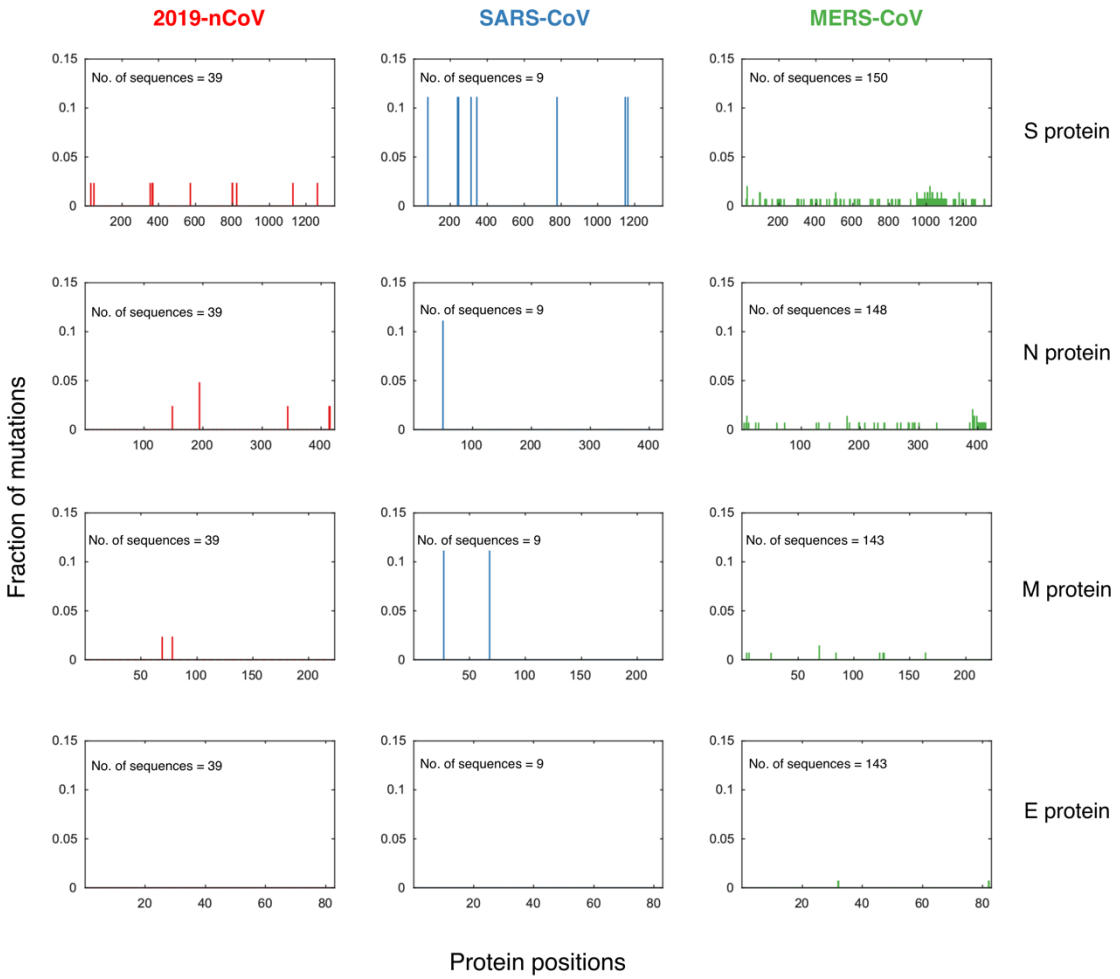
Table 7. SARS-CoV-derived discontinuous B cell epitopes that have at least one site with an identical amino acid to the corresponding site in 2019-nCoV.

IEDB ID	SARS-CoV S protein residues[1,2]
910052	G446, P462, D463, Y475
77444	T359, T363, K365, K390, G391, D392, R395, R426, Y436, G482, Y484, T485, T486, T487, G488, I489, G490, Y491, Q492, Y494
77442	R426, S432, T433, Y436, N437, K439, Y440, Y442, P469, P470, A471, L472, N473, C474, Y475, W476, L478, N479, D480, Y481, G482, Y484, T485, T486, T487, G488, I489, Y491, Q492

[1] Residues are numbered according to the SARS-CoV S protein reference sequence, accession ID: NP_828851.1.

[2] Residues in the epitopes identical in the 2019-nCoV sequences are underlined.

Supplementary Figures



Supplementary Figure 1. Fraction of mutations in the observed sequences of the structural proteins of the three coronaviruses. Mutation is defined here as an amino acid difference from the reference sequence of the respective coronavirus; accession IDs: NC_045512.2 (2019-nCoV), NC_004718.3 (SARS-CoV), and NC_019843.3 (MERS-CoV).

Supplementary Figure 2. Pairwise sequence alignment of the reference sequences of the S proteins of SARS-CoV and 2019-nCoV (accession ID: NP_828851.1 and YP_009724390.1, respectively). Identical residues are indicated by *.