

Medical Countermeasures Analysis of 2019-nCoV and Vaccine Risks for Antibody-dependent Enhancement (ADE)

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Summary

Background In 80% of patients, COVID-19 presents as mild disease^{1,2}. 20% of cases develop severe (13%) or critical (6%) illness. More severe forms of COVID-19 present as clinical severe acute respiratory syndrome, but include a T-predominant lymphopenia³, high circulating levels of proinflammatory cytokines and chemokines, accumulation of neutrophils and macrophages in lungs, and immune dysregulation including immunosuppression⁴.

Methods All major SARS-CoV-2 proteins were characterized using an amino acid residue variation analysis method. Results predict that most SARS-CoV-2 proteins are evolutionary constrained, with the exception of the spike (S) protein extended outer surface. Results were interpreted based on known SARS-like coronavirus virology and pathophysiology, with a focus on medical countermeasure development implications.

Findings Non-neutralizing antibodies to variable S domains may enable an alternative infection pathway via Fc receptor-mediated uptake. This may be a gating event for the immune response dysregulation observed in more severe COVID-19 disease. Prior studies involving vaccine candidates for FCoV^{5,6} SARS-CoV-1⁷⁻¹⁰ and Middle East Respiratory Syndrome coronavirus (MERS-CoV)¹¹ demonstrate vaccination-induced antibody-dependent enhancement of disease (ADE), including infection of phagocytic antigen presenting cells (APC). T effector cells are believed to play an important role in controlling coronavirus infection; pan-T depletion is present in severe COVID-19 disease³ and may be accelerated by APC infection. Sequence and structural conservation of S motifs suggests that SARS and MERS vaccine ADE risks may foreshadow SARS-CoV-2 S-based vaccine risks. Autophagy inhibitors may reduce APC infection and T-cell depletion^{12 13}. Amino acid residue variation analysis identifies multiple constrained domains suitable as T cell vaccine targets. Evolutionary constraints on proven antiviral drug targets present in SARS-CoV-1 and SARS-CoV-2 may reduce risk of developing antiviral drug escape mutants.

Interpretation Safety testing of COVID-19 S protein-based B cell vaccines in animal models is strongly encouraged prior to clinical trials to reduce risk of ADE upon virus exposure.

Introduction

COVID-19 is caused by the SARS-CoV-2 (2019-nCoV) betacoronavirus. The SARS-CoV-2 is a novel betacoronavirus with sequenced genomes ranging from 29.8k to 29.9k RNA bases. The SARS-CoV-2 genome encodes replicase proteins, structural proteins, and accessory proteins¹⁴ (Table 1). The ORF1a and ORF1ab polyproteins are proteolytically cleaved into 16 non-structural proteins designated nsp1-16¹⁴ (Table 1). Like SARS, COVID-19 manifests as a virulent zoonotic virus-mediated disease in humans with currently 81,191 confirmed cases and 2,768 deaths as of Feb. 26, 2020¹⁵.

Zoonotic MERS-CoV, SARS-CoV-1, and SARS-CoV-2 are evolutionarily related, and share many similarities in human disease characteristics and progression. The mild variant first phase of viral progression generally presents with mild flu-like symptoms. Most patients never progress beyond this phase, and typically recover quickly and uneventfully. In a mouse animal model, phagocytic cells contribute to the antibody-mediated elimination of SARS-CoV-1¹⁶, and it may be that innate responses are sufficient to suppress MERS-CoV and SARS-CoV-2 in the majority of patients. For some individuals (18.5%¹⁷), infection progresses to a second severe-critical variant phase. Progression to the second phase often coincides with the typical timing of onset of adaptive humoral immunity antibody response (approximately 7-14 days post infection). MERS-CoV can infect monocyte-derived macrophages (MDMs), monocyte-derived dendritic cells (MoDCs), and T-cells^{18,19}, but the infectivity of SARS-CoV-2 in these cell populations (with or without non-neutralizing antibody) has not been characterized. For patients with severe and critical symptoms, the pathophysiology is consistent with increased infection of phagocytic immune cells (immature MDMs and MoDCs); see Figure 1 for a diagram of the postulated cascade mechanism. Chemokines released from infected cells may attract additional dendritic cells and immature macrophages that are susceptible to infection, leading to a possible infection amplifying cascade of immune cell infection and dysregulation. For some patients with severe symptoms, excessive activation of macrophages may contribute to a chemokine and cytokine storm²⁰⁻²². Individuals with SARS have pronounced peripheral T-cell lymphocytopenia with reduced CD4⁺ and CD8⁺ T-cells^{23,24}, just as is observed with COVID-19³. MERS-CoV and SARS-CoV are also associated with T-cell apoptosis^{25,26}. Infection of macrophages and some T-cells along with viral dysregulation of cellular pathways result in compromised innate and humoral immunity in patients during this second and more severe phase of infection²⁷. High virus titer in blood plus the possibility of infected immune cell migration throughout the body may account for the additional disease pathophysiologic and clinical observations observed with these viruses. MHC I and interleukin (IL)-12 receptor B1 (IL-12RB1) genetic differences associated with disease progression has been characterized for SARS²⁸⁻³⁰. Patients with low or deficient serum levels of the innate immune response pattern recognition molecule mannose-binding lectin (MBL) have increased frequency in SARS patients versus controls³¹. MHC downregulation by epigenetic modifications seen with MERS-CoV infections may enhance avoidance of T-killer cell responses, and direct infection of some T-cells¹⁸ may play a role in increased mortality rate seen for MERS³². Other disease differences may simply be the different population of cells with target host receptors angiotensin I converting enzyme 2 (ACE2) for

SARS-CoV-1 and SARS-CoV-2³³, and dipeptidyl peptidase IV (DPP4) for MERS-CoV. ACE2 is expressed in high density in lungs³⁴.

Characterizing variability and evolution of viral proteins must inform medical countermeasure (MCM) design and development strategies for RNA viruses such as SARS-CoV-2. For viral progeny, deleterious mutations are rapidly selected against³⁵. Neutral mutations³⁶ provide a framework for antigenic drift to facilitate escape from immune responses; these residues will continue to mutate over time. The critical-spacer model proposes that proteins have either amino acid residue side-chains critical for function or have variable side-chains which may function for positioning/folding of critical residues³⁷. The divergence model of protein evolution proposes that the number of critical residues for a protein is consistent for evolutionarily closely related proteins³⁸. Herein, these concepts are applied to SARS-CoV-2 proteins by leveraging closely related coronavirus protein sequences to provide insights into viral vulnerabilities that can be exploited when designing MCMs. The majority of the SARS-CoV-2 proteins exhibit very high proportions of critical residues to total residues (see Table 2 and Figure 2); hence, these viral enzymes are excellent small molecule targets. Such small molecule drug therapeutics or prophylactics have good chances of being effective against SARS-CoV, SARS-CoV-2, and SARS-like CoVs if they target these highly conserved domains. Non-exposed replicase and accessory proteins have abundant highly conserved long peptide targets for selecting continuous segments of critical residues for T-cell epitope vaccines³⁹. In contrast, the extracellular domain of the S protein exhibits exposed surface areas with high amino acid residue variability. Increased risk for antibody-dependent enhancement (ADE) from vaccines targeting SARS-CoV-2, SARS-CoV-1, and MERS-CoV exposed residues is indicated by observed ADE in animal models and the antibody facilitated infection of phagocytic immune cells frequently observed with coronaviruses^{16,40}. Peptides and antibodies targeting HR2 and cell fusion have been shown to block SARS-CoV-1 and MERS-CoV infections in cell lines⁴¹⁻⁴⁷ and animal models⁴⁸⁻⁵⁰. Based on the conservation of these domains observed with divergence-based modeling, testing of similar peptides and antibodies to these targets for SARS-CoV-2 may yield new insights and opportunities for MCM development. Likewise, drugs that target the phagocytic pathway associated with Fc-receptor mediated endocytosis are promising candidates for blocking the cascade of immune cell infections that results in immune dysregulation in COVID-19 patients.

Methods

2019-nCoV protein sequences from GenBank entry MN908947.3 were searched against the non-redundant (nr) and PDB database using the NCBI BLASTP web interface. Hit protein sequences were downloaded. Protein multiple sequence alignments were created with the Dawn program⁵¹. Additional 2019-nCoV sequences were added to existing alignments with the Jalview program⁵². Identified protein structures were downloaded from RCSB PDB database⁵³. Dawn variation results were visualized with the Jmol program⁵⁴.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Dawn variation results for 2019-nCoV amino acid residues were classified into residues with no observed variability (candidate critical residues; colored dark green in Figure 2) and to residues with 5 or more amino acid substitutions (candidate spacer residues; colored dark blue in Figure 2). Amino acids residues colored yellow are considered constrained, allowing only a subset of possible amino acid substitutions. Amino acid residues with conservative substitutions are also considered critical residues, and are colored light green in Figure 2; positions with > 95% conservation of a single residue were included in this category to accommodate potential sequencing errors and possible adaptative mutations. Twelve of the nsp replicase proteins have a ratio of critical to total residues of 0.9 or higher (Table 1); this is illustrated in Figure 2 for 2019-nCoV proteins with high proportions of critical residues in Figure 2 (dark green residues). In sharp contrast, the S protein exhibits regions of extensive variability of exposed surface residues (Figure 2).

Discussion

Variation Results

The observed amino acid variations in SARS-CoV-2 proteins are consistent with expected natural variations in the context of random mutations and selection in the context of host immune responses. For the nonstructural replicase proteins, the majority have fractions of critical residues above 88% (Table 2). Long continuous stretches of invariant residues are excellent candidates for T-cell vaccines epitope selection, and also for exploratory anti-viral small inhibitory RNA (siRNAs)⁵⁵ development. With a large RNA genome, the virus has evolved over time by deleting unnecessary spacer residues. The S protein S1 extended domain shows the highest number of exposed surface highly variable residues, in sharp contrast to the replicase enzymes (Figure 2). These spacer residues may function as exposed antigens for antibody responses with the possible adaptive benefit of suppressing immune responses to less immunogenic surface antigens. Many of these S protein antigens may lead to non-neutralizing antibodies. Alternately, evolutionary selection for mutations to these residues may facilitate antigenic drift to escape immune responses. It seems unusual to have the excessive number of spacer residues on the S1 extended domain, unless it provides 2019-nCoV with an additional selective advantage associated with non-neutralizing antibodies bound to this domain.

Coronaviruses have Multiple Options for Cell Infection

The 2019-nCoV S protein contains receptor-binding domains (RBD) targeting human angiotensin I converting enzyme 2 (ACE2)^{56,57}; this is the initial route for infecting host cells. To take advantage of antibody responses, coronaviruses also leverage antibody Fc uptake to infect immune cells⁵⁸. Coronaviruses use the S protein subunit 2 FP, HR1, and HR2 to infect immune cells upon proteolytic cleavage of S within endosomes. HR1 and HR2 form a canonical 6-helix bundle involved in membrane fusion⁴¹. Jaume et al.⁵⁸ found that antibody-mediated infection was dependent on Fc receptor II and not the endosomal/lysosomal pathway utilized by ACE2 targeting. Viral infection of complement receptor (CR) cells is an additional possible route of infecting cells⁵⁹. This multi-pronged approach provides coronaviruses like SARS-CoV-1, MERS-CoV, and SARS-CoV-2 with more than one mechanism for infecting host cells. This leads to the hypothesis that antibody mediated uptake of virus is the potential mechanism that

induces ADE to vaccines and can also be mediated by maternally transferred antibodies (matAbs)⁶⁰⁻⁶³.

Macrophages and Immune Dysregulation

Lymphopenia is a common feature in patients with SARS^{23,64} or COVID-19^{65,66}. Two receptors have been identified for SARS-CoV-1 including ACE2⁶⁷ and C-type lectin domain family 4 member M (CLEC4M, CD209L, CD299, DC-SIGN2, DC-SIGNR, HP10347, and L-SIGN)⁶⁸ with CLEC4M expressed in human lymph nodes⁶⁹. Individuals homozygous for CLEC4M tandem repeats are less susceptible to SARS infection⁷⁰. In a mouse model, depletion of CD4+ T cells resulted in an enhanced immune-mediated interstitial pneumonitis when challenged with SARS-CoV-1⁷¹. In contrast, depletion of CD4+ and CD8+ T cells as well as antibodies enabled innate defense mechanisms to control the SARS-CoV-1 virus without immune dysregulation⁷¹. Similar results were also observed in mice with SARS-CoV-1 challenge, but treatment with liposomes containing clodronate, which deplete alveolar macrophages (AM), prevented immune deficient virus-specific T cell response⁷². In a macaque model, anti-spike IgG causes acute lung injury by skewing macrophage response towards proinflammatory monocyte/macrophage recruitment and accumulation during acute SARS-CoV-1 infection⁷³. These observations are likely linked by antibody-dependent enhancement of coronavirus infection of macrophages^{58,74}. In SARS patients, severe SARS was associated with a more robust IgG response⁷⁵; early responders (antibody detectable within 2 weeks) had a higher death rate^{76,77}. The pathophysiology of severe and critical SARS and COVID-19 diseases fits a proposed model of antibody-dependent infection of macrophages as the key gate step in disease progression from mild to severe and critical symptoms, and may explain the observed dysregulated immune responses⁷⁸ including apoptosis contributing to development of pan-T cell lymphopenia, proinflammatory cascade with macrophage accumulation, and cytokine and chemokine accumulations in lungs with a cytokine storm in some patients.

Vaccine Risks for Antibody-dependent Enhancement (ADE)

Many of the viruses associated with ADE have cell membrane fusion mechanisms⁶¹. For influenza A H1N1, vaccine-induced anti-HA2 antibodies promote virus fusion causing vaccine-associated enhanced respiratory disease (VAERD)⁷⁹. ADE was observed for the respiratory syncytial virus (RSV) in the Bonnet monkey model⁶⁰. Van Erp et al.⁶⁰ recommends avoidance of induction of respiratory syncytial virus (RSV) non-neutralizing antibodies or subneutralizing antibodies to avoid ADE. In a mouse model, attempts to create vaccines for SARS-CoV-1 lead to pulmonary immunopathology upon challenge with SARS-CoV-1⁹; these vaccines included inactivated whole viruses, inactivated viruses with adjuvant, and a recombinant DNA spike (S) protein vaccine in a virus-like particle (VLP)-based vaccine. Enhanced hepatitis was observed in a ferret model with a vaccine with recombinant modified vaccinia virus Ankara (rMVA) expressing the SARS-CoV-1 S protein⁸⁰. Jaume et al.⁵⁸ point out the potential pitfalls associated with immunizations against SARS-CoV-1. This leads to the prediction that new attempts to create either SARS-CoV-1 vaccines⁸¹, MERS-CoV vaccines¹¹, or SARS-CoV-2 vaccines have potentially higher risks for inducing ADE in humans facilitated by antibody infection of phagocytic immune cells. This potential ADE risk is independent of the vaccine technology⁸² or targeting strategy selected due to predicted phagocytic immune cell infections upon antibody uptake.

Convalescent plasma therapy has been provided to SARS⁸³ and COVID-19⁸⁴ patients. Candidate patients for convalescent plasma therapy are already experiencing advanced clinical disease symptoms, potentially mitigating ADE risk. For Hong Kong SARS patients, convalescent plasma therapy had improved outcomes (6.4% mortality rate) when it was provided before day 14 versus after (21.9% mortality rate) compared to the overall SARS-related mortality rate in of 17%. This is also being seen for initial COVID-19 patients treated with convalescent plasma therapy⁸⁴.

Antibody Targets

Analyzing the Cryo-EM structures of MERS-CoV and SARS-CoV-1 spike (S) glycoproteins, Yuan et al.⁸⁵ suggest that the fusion peptide (FP) and the heptad repeat 1 region (HR1) are potential targets for eliciting broadly neutralizing antibodies based on exposure on the surface of the stem region, lack of N-linked glycosylation sites in this region, and sequence conservation. Antibodies that interrupt virus-cell fusion will likely block the infection of immune cells using Fc-mediated uptake of virus⁵⁸. This has been demonstrated for SARS-CoV-1 for antibodies to the HR2 region⁸⁶⁻⁸⁸. Likewise, 2019-nCoV antibodies that block cell fusion are predicted to not share the same ADE risk of other 2019-nCoV antibodies. Antibodies that target the S RBD⁸⁹ may have an ADE risk unless combined with a second cell fusion blocking antibody.

Targeting Cell Fusion

In addition to antibodies, peptides targeting HR2 have been shown to effectively block infection in cell and animal models. Multiple peptides based on the heptad repeat regions (HR1 and HR2) have been shown to suppress SARS-CoV-1 cell entry⁴²⁻⁴⁶. Specific combinations of two peptides show synergistic viral inhibition⁴³. An HR2 peptide was effective in a mouse model administered intranasally against human coronavirus 229E (HCoV-229E)⁴⁸. An HR2 peptide combined with human interferon- α (IFN- α) also have significant synergistic antiviral effect against feline coronavirus (FCoV)⁹⁰. Based on anti-HIV-1 peptide, T-20⁹¹, Lambert et al. demonstrate that analogous peptides inhibit respiratory syncytial virus (RSV), human parainfluenza virus type 3 (HPIV-3), and measles virus (MV)⁹². An HR2 peptide can effectively inhibit MERS-CoV replication⁴⁹. Gao et al.⁴¹ identified an HR2 peptide that inhibits MERS-CoV fusion in their pseudotyped-virus system. MERS-CoV HR1 entry inhibitor peptides have been modified to form intra-molecular salt-bridges and increase peptide solubility⁵⁰. The peptide MERS HP2P-M2 protected C57BL/6 mice and mice deficient for VDJ recombination-activating protein 1 (RAG1); this protection was enhanced by combing this peptide with interferon- β ⁵⁰. Similar results are demonstrated for additional mouse models^{93,94}. Lipopeptides have been design to target cell fusion peptides⁴⁷. An analogous fusion inhibitor, enfuvirtide (T-20), has been approved for treatment of HIV-1 infections⁹¹. This provides a path forward for peptide-based MCMs for 2019-nCoV. A set of SARS-CoV-1 inhibitory peptides that could be adapted or directly tested on SARS-CoV-2 are illustrated in Figure 3. The SARS-CoV-1 HR2 peptides can be directly tested on 2019-nCoV without modification due to sequence identity in this region of the S protein.

B cell Vaccine Designs

B cell vaccines that target the S protein cell fusion mechanisms have the highest chance of raising neutralizing antibodies with minimal or no ADE risk. Antibodies targeting other portions

of the S protein or other 2019-nCoV exposed proteins may enable infection of phagocytic immune cells even if they are neutralizing.

T cell Vaccine Designs

Variation results identified multiple continuous linear segments of critical residues from which T cell epitopes can be selected in SARS-CoV-2 replicase enzymes and accessory proteins (Figure 2). Antibodies developed against these epitopes are highly unlikely to enable antibody enhanced infection of phagocytic immune cells because they are not exposed on the surface of 2019-nCoV.

Targeting Autophagy

Coronavirus replication exploits aspects of normal cellular autophagy⁹⁵. SKP2 attenuates autophagy through Beclin1-ubiquitination; its inhibition by the licensed drug niclosamide, a treatment for tapeworms, drastically reduced the replications of MERS-CoV in cell culture¹³. Compounds that block autophagy are worth investigating as SARS-CoV-2 MCM.

Targeting Viral Enzymes

2019-nCoV enzyme proteins are highly conserved with minimal spacer residues (Table 2 and Figure 2). The variation results indicate that available SARS-CoV-1 protein structures (Table 2) can be directly used for in silico docking and high throughput compound screens. SARS-CoV-2 protein structures are becoming rapidly available⁹⁶ for compound screening approaches. The high conservation around enzyme pockets holds promise that compound inhibitors against SARS-CoV-2 will also be effective against SARS-CoV-1 and SARS-like CoV enzymes.

Summary

Given past data on multiple SARS-CoV-1 and MERS-CoV vaccine efforts which have failed due to ADE in animal models^{9,11}, it is reasonable to hypothesize a similar ADE risk for SARS-CoV-2 vaccine efforts unless they specifically target domains which will block virus-immune cell fusion. MCMs based on vaccines, antibodies, or peptides that block cell fusion could minimize predicted ADE risks. Synergy has been observed for combinations of CoV countermeasures including interferon- α and - β . Small molecules targeting viral enzymes should also be pursued.

Data Availability

Protein multiple sequence alignments and associated variation files are included in Ricke, Darrell, 2020, "Medical Countermeasures Analysis of 2019-nCoV / SARS-CoV-2 for COVID-19", <https://doi.org/10.7910/DVN/XWVOA8>, Harvard Dataverse, V1.

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Conflicts of Interest

Dr. Ricke and Dr. Malone have nothing to disclose.

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Tables

Table 1. 2019-nCoV proteins*.

Protein	Function	Cofactors	References
nsp1	cellular mRNA degradation, inhibiting type I interferon (IFN) expression		97,98
nsp2	unknown		
nsp3	multidomain protein		
nsp3a	interacts with single-stranded RNA		99
nsp3b	ADP-ribose 1"-phosphatase		100
nsp3d	papain-like protease (Plpro), deubiquitinating enzyme (DUB)		101
nsp4	double-membrane vesicles (DMV) formation		102
nsp5	3C-like protease (3CLpro)		103
nsp6	restricting autophagosome expansion, DMV formation		104,105
nsp7	RNA binding	nsp8:nsp12	106,107
nsp8	RNA binding; primase	nsp7:nsp12, nsp9	106,107
nsp9	RNA binding, dimerization	nsp8	108
nsp10	scaffold cofactor	nsp10, nsp16	109,110
nsp11	unknown		
nsp12	RNA-dependent RNA polymerase (RdRp)	nsp7:nsp8, nsp14	106
nsp13	RNA helicase, 5' triphosphatase		111
nsp14	3'-5' exoribonuclease (ExoN), guanine-N7 methyl transferase (N7-Mtase) for mRNA capping, nsp12:nsp14 RNA synthesis and proofreading		109
nsp15	endoribonuclease		112
nsp16	nsp16:nsp10 RNA cap 2'-O-methyltransferase, negatively regulates innate immunity		110,113
E	forms homopentameric ion channels (IC) with poor ion selectivity, Golgi complex-targeting signal, PDZ-binding motif (PBM)		114-120
M	membrane protein		121
N	packages viral RNA		122
ORF3a			
ORF6			
ORF7a	Ig-like domain, ER retention signal		123-126
ORF7b			
ORF8			
ORF10	unknown		
S	receptor binding, cell fusion		85

*The E protein IC releases calcium from the endoplasmic reticulum intermediate compartment (ERGIC), leading to NLRP3 inflammasome activation^{117,118}. The E protein has a PDZ-binding motif (PBM)¹¹⁶ that interacts with syntenin PDZ motifs to activate p38 mitogen-activated protein kinase (MAPK) pathway and promotes an acute proinflammatory response¹¹⁹ and a virus PBM domain is required for virulence¹²⁰. The E protein PDZ-binding motif binds to PALS1 and alters tight junction formation and epithelial morphogenesis¹²⁷. The envelope (E) protein includes two pathways to promote inflammation; these may contribute to the ADE response. ORF7a protein has Ig-like domain¹²³. Hänel et al.¹²⁴ suggest that this ORF7a possess binding activity for α_L integrin I domain of LFA-1 suggesting that this might block newly synthesized LFA-1 molecules from reaching the cell surface because ORF7a contains an ER retention signal¹²⁵. Loss of LFA-1 negatively impacts immune responses¹²⁶. This suggests possible interference of ORF7a with immune surveillance mechanisms.

Table 2. 2019-nCoV Variance Analysis

Protein	V1: Critical	V2	V3	V4	V5+: Spacers	Residues	Fraction	Structure
nsp1	112	40	19	3	7	181	0.84	2GDT:A ¹²⁸
nsp2	279	187	101	46	25	638	0.73	
nsp3	996	514	239	115	92	1,956	0.77	2GRI:A ⁹⁹
nsp3a	82	35	20	22	12	171	0.68	2ACF:A ¹⁰⁰
Pl _{pro}	212	68	24	10	5	319	0.88	5Y3E:A ¹²⁹
nsp4	337	112	34	13	4	500	0.90	
nsp5	254	46	4	2	0	306	0.98	6LU7 ⁹⁶
nsp6	209	64	15	2	0	290	0.94	
nsp7	69	13	1	0	0	83	0.99	2AHM:A ¹⁰⁷
nsp8	170	26	2	0	0	198	0.99	2AHM:G ¹⁰⁷
nsp9	95	16	2	0	0	113	0.98	1UW7:A ¹⁰⁸
nsp10	109	27	3	0	0	139	0.98	3R24:B ¹³⁰
nsp12	5,226	1374	346	105	50	7,101	0.93	
nsp13	538	61	2	1	0	602	1.00	6JYT:A ¹¹¹
nsp14	442	78	7	0	0	527	0.99	5C8T:B ¹³¹
nsp15	246	76	17	6	1	346	0.93	2GTH:A ¹³²
nsp16	230	55	8	1	2	296	0.96	3R24:A ¹³⁰
E	24	33	17	5	3	82	0.70	5X29:A ¹¹²
M	178	29	11	4	0	222	0.93	
N	294	76	33	15	4	422	0.88	2OFZ:A ¹³³
ORF3a	107	79	54	20	15	275	0.68	
ORF6	17	21	22	3	0	63	0.60	
ORF7a	55	28	30	10	4	127	0.65	1XAK:A ¹²³
ORF7b	5	33	11	4	1	54	0.70	
ORF8	59	39	15	8	0	121	0.81	5O32:I ¹³⁴
ORF10	38	0	0	0	0	38	1.00	
S	650	263	123	107	152	1,295	0.71	6CRZ:A ¹³⁵

Figures

Figure 1. Disease progression model with normal immune responses during the initial mild symptoms phase (see 1-3). Antigen presenting cells migrate to the lymph nodes to activate T-cells (2a). The progression gate to severe and critical disease is the infection of phagocytic immune cells (3a) leading to immune dysregulation (4b). In the lungs, chemokines attract additional dendritic cells and immature macrophages that are subsequently infected in an positive feedback-loop infection cascade (4b). Virus and infected phagocytic immune cells disseminate throughout the body infecting additional organs (5 & 6). Levels of chemokine and cytokines in the lungs from infected cells can create a cytokine storm (6).

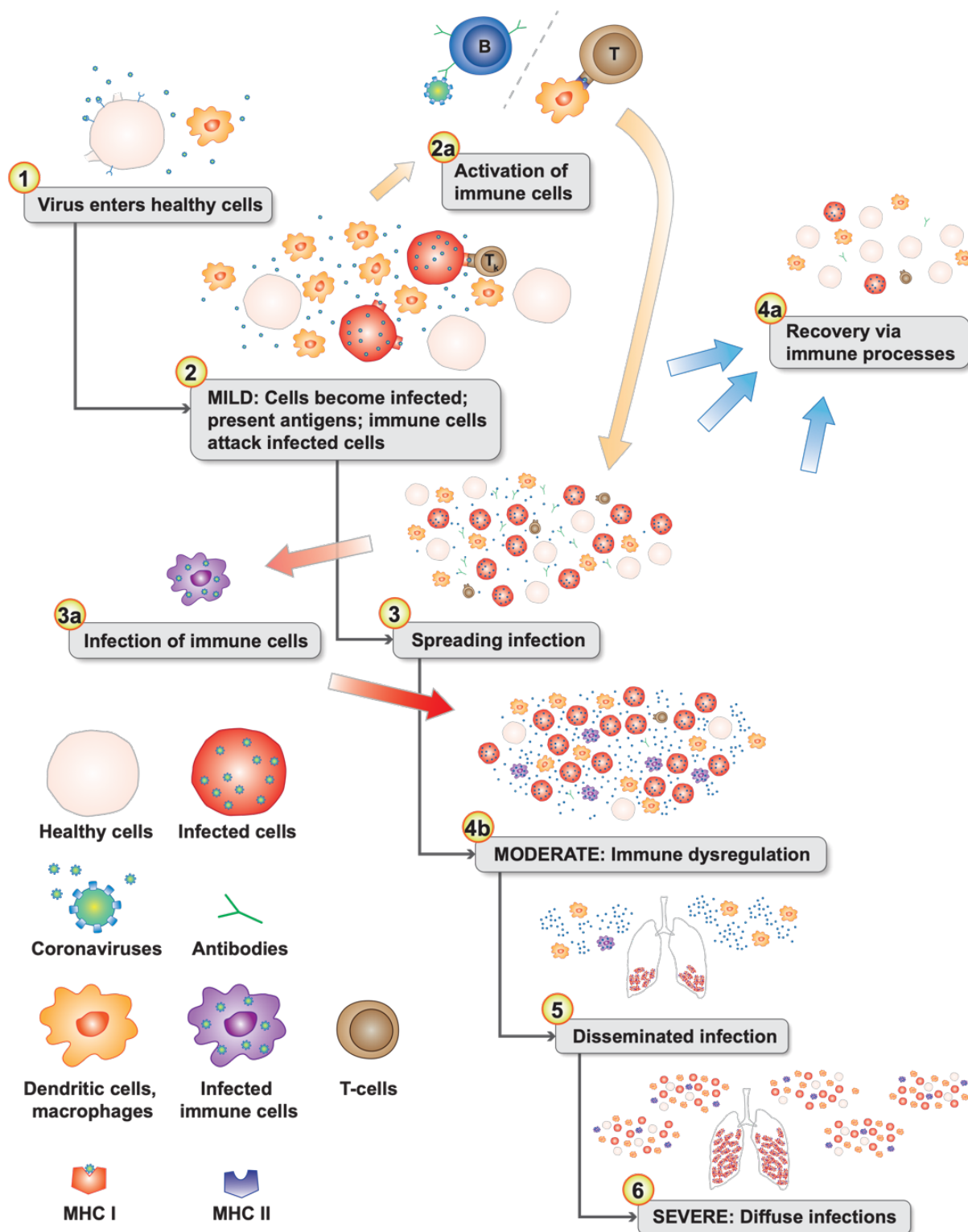
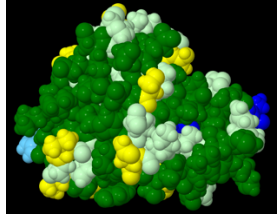


Figure 2. 2019-nCoV variation results. Amino acid residue color code: dark green (critical residues), light green (critical residues with conservative substitutions or variant in less than 10

sequences, yellow (3 variants), light blue (4 variants; likely spacer residues), and blue (5+ variants; spacer residues).

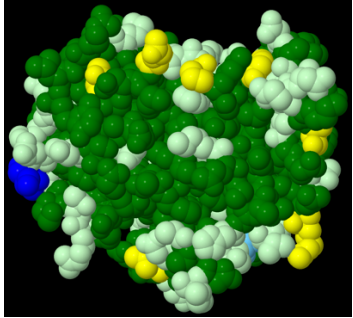
nsp1



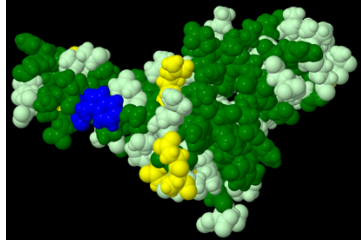
nsp9



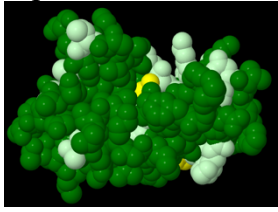
nsp16



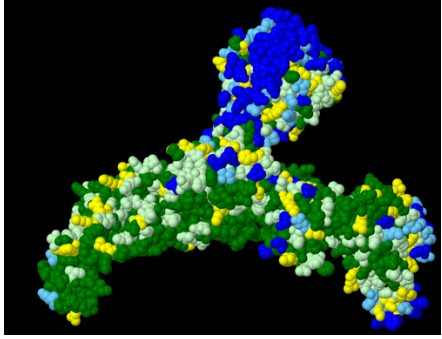
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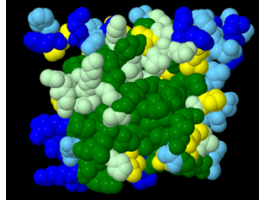
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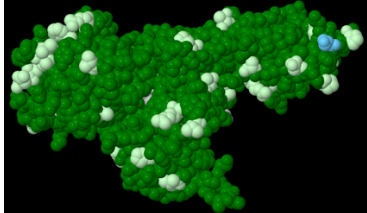
S



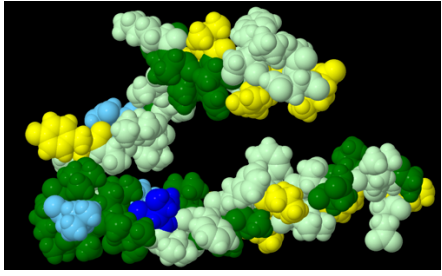
nsp3a



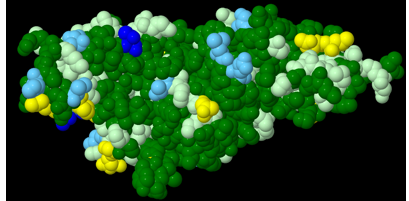
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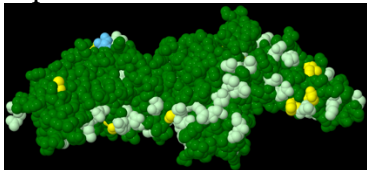
E



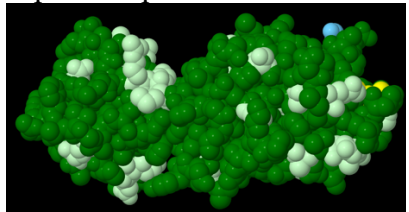
PL-pro protease



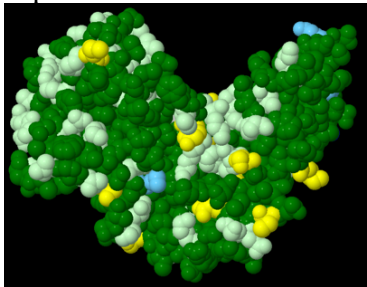
nsp14



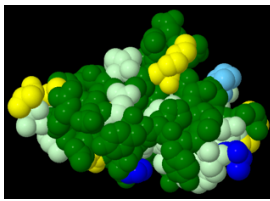
nsp5-3CL protease



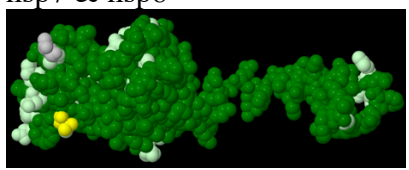
nsp15



ORF7a



nsp7 & nsp8



N

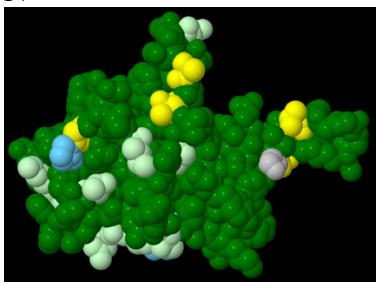


Figure 3. SARS-CoV-1 Inhibitory Peptides N46⁴³, HR1-1⁴², HR2-18⁴², WW-III¹³⁶, WW-IV¹³⁶, sHR2-2⁴⁵, sHR2-8⁴⁵, HRC1⁸⁷, HRC2⁸⁷, CP-1¹³⁷, SR9¹³⁸, P6⁴³, and CB-119⁴⁴. SARS-CoV-2 residues different from SARS-CoV-1 are underlined for adapting SARS-CoV-1 inhibitory peptides.

SARS2 907-NGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQ-965
SARS1 889-NGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQ-947
N46 QKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQ
HR1-1 NGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTA

SARS2 1046-GYHLMSFPQSAPHGVVFLHVTY-1067
SARS1 1028-GYHLMSFPQAAPHGVVFLHVTY-1049
WW-III GYHLMSFPQAAPHGVVFLHVTW

SARS2 1093-GVFVSNGTHWFVTQRNFYE-1111
SARS1 1075-GVFVFNGTSWFITQRNFFS-1093
WW-IV GVFVFNGTSWFITQRNFFS

SARS2 1144-ELDSFKEELDKYFKNHTSPDVLDGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK-1211
SARS1 1126-ELDSFKEELDKYFKNHTSPDVLDGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK-1193
sHR2-8 ELDSFKEELDKYFKNHTSPDVLDGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK
sHR2-2 PKEELDKYFKNHTSPDVLDGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE
HR2-18 IQKEIDRLNEVAKNLNESLIDLQELGK
HRC2 QKEIDRLNEVIKNLNESIIDLQEL
HRC1 NASIVNLQKEIDRLNEVIKNLNES
CP-1 GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE
P6 GINASVVNIQKEIDRLNEVAKNL
SR9 ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL
CB-119 SPDVDLGDISGINAS

