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

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Abstract:	Summary Background In 80% of patients, COVID-19 presents as mild disease. 20% of cases develop severe (13%) or critical (6%) illness. More severe forms of COVID-19 present as clinical severe acute respiratory syndrome, T-predominant lymphopenia, high circulating levels of proinflammatory cytokines and chemokines, accumulation of macrophages and neutrophils in lungs, and immune dysregulation including immunosuppression. 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 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 SARS-CoV-1 and Middle East Respiratory Syndrome coronavirus (MERS-CoV) 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 disease and may be accelerated by APC infection. Sequence and structural conservation of S suggests that SARS and MERS vaccine ADE risks may foreshadow SARS-CoV-2 vaccine risks. Autophagy inhibitors may reduce APC infection and T-cell depletion. Amino acid residue variation analysis identifies multiple constrained domains suitable as T cell vaccine targets. Evolutionary constraints on 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

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Summary

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

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 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 SARS-CoV-1 and Middle East Respiratory Syndrome coronavirus (MERS-CoV) 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 disease and may be accelerated by APC infection. Sequence and structural conservation of S suggests that SARS and MERS vaccine ADE risks may foreshadow SARS-CoV-2 vaccine risks. Autophagy inhibitors may reduce APC infection and T-cell depletion. Amino acid residue variation analysis identifies multiple constrained domains suitable as T cell vaccine targets. Evolutionary constraints on 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.

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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 proteins[1] (Table 1). The ORF1a and ORF1ab polyproteins are proteolytically cleaved into 16 non-structural proteins designated nsp1-16[1] (Table 1). Like SARS, COVID-19 manifests as a virulent zoonotic virus-mediated disease in humans with currently 82,555 confirmed cases and 2,810 deaths as of Feb. 27, 2020[2].

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-1[3], 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%[4]), 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 dendric cells (MoDCs), and T-cells[5,6], but the infectivity of SARS-CoV-2 in these cell populations (with or without non-neutralizing antibody) has not been characterized. For patients with moderate and severe 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 storm[7-9]. Individuals with SARS have pronounced peripheral T-cell lymphocytopenia with reduced $CD4^+$ and $CD8^+$ T-cells[10,11], just as is observed with COVID-19[12]. MERS-CoV and SARS-CoV are also associated with T-cell apoptosis[13,14]. 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 infection [15]. High virus titer in blood plus the possibility of infected immune cell migration throughput 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 SARS[16-18]. 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 controls[19]. MHC downregulation by epigenetic modifications seen with MERS-CoV infections may enhance avoidance of T-killer cell responses, and direct infection of some T-cells[5] may play a role in increased mortality rate seen for MERS[20]. 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-2[21], and dipeptidyl peptidase IV (DPP4) for MERS-CoV. ACE2 is expressed in high density in lungs[22].

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 against[23]. Neutral mutations[24] 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 residues[25]. The divergence model of protein evolution proposes that the number of critical residues for a protein is consistent for evolutionarily closely related proteins[26]. 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; 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 vaccines[27]. 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[3,28]. Peptides and antibodies targeting HR2 and cell fusion have been shown to block SARS-CoV-1 and MERS-CoV infections in cell lines[29-35] and animal models[36-38]. 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 program[39]. Additional 2019-nCoV sequences were added to existing alignments with the Jalview program[40]. Identified protein structures were downloaded from RCSB PDB database[41]. Dawn variation results were visualized with the Jmol program[42].

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 factions 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)[43] 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)[44,45]; this is the initial route for infecting host cells. To take advantage of antibody responses, coronaviruses also leverage antibody Fc uptake to infect immune cells[46]. 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 fusion[29]. Jaume et al.[46] 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 cells[47]. 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)[48-51].

Macrophages and Immune Dysregulation

Lymphopenia is a common feature in patients with SARS[10,52] or COVID-19[53,54]. Two receptors have been identified for SARS-CoV-1 including ACE2[55] and C-type lectin domain family 4 member M (CLEC4M, CD209L, CD299, DC-SIGN2, DC-SIGNR, HP10347, and L-SIGN)[56] with CLEC4M expressed in human lymph nodes[57]. Individuals homozygous for CLEC4M tandem repeats are less susceptible to SARS infection[58]. In a mouse model, depletion of CD4+ T cells resulted in an enhanced immune-mediated interstitial pneumonitis when challenged with SARS-CoV-1[59]. 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 dysregulation[59]. 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 response[60]. 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 infection[61]. These observations are likely linked by antibody-dependent enhancement of coronavirus infection of macrophages[46,62]. In SARS patients, severe SARS was associated with a more robust IgG response[63]; early responders (antibody detectable within 2 weeks) had a higher death rate[64,65]. The pathophysiology of moderate and severe 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 moderate and severe symptoms, and may explain the observed dysregulated immune responses[66] 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 mechanisms[49]. For influenza A H1N1, vaccine-induced anti-HA2 antibodies promote virus fusion causing vaccine-associated enhanced respiratory disease (VAERD)[67]. ADE was observed for the respiratory syncytial virus (RSV) in the Bonnet monkey model[48]. Van Erp et al.[48] recommends avoidance of induction of respiratory syncytial virus (RSV) nonneutralizing 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-1[68]; 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 protein[69]. Jaume et al.[46] 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 vaccines[70], MERS-CoV vaccines[71], 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 technology[72] or targeting strategy selected due to predicted phagocytic immune cell infections upon antibody uptake.

Convalescent plasma therapy has been provided to SARS[73] and COVID-19[74] 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 therapy[74].

Antibody Targets

Analyzing the Cryo-EM structures of MERS-CoV and SARS-CoV-1 spike (S) glycoproteins, Yuan et al. [75] 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 virus[46]. This has been demonstrated for SARS-CoV-1 for antibodies to the HR2 region[76-78]. 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 RBD[79] 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 entry[30-34]. Specific combinations of two peptides show synergistic viral inhibition[31]. An HR2 peptide was effective in a mouse model administered intranasally against human coronavirus 229E (HCoV-229E)[36]. An HR2 peptide combined with human interferon-α (IFN-α) also have significant synergistic antiviral effect against feline coronavirus (FCoV)[80]. Based on anti-HIV-1 peptide, T-20[81], Lambert et al. demonstrate that analogous peptides inhibit respiratory syncytial virus (RSV), human parainfluenza virus type 3 (HPIV-3), and measles virus (MV)[82]. An HR2 peptide can effectively inhibit MERS-CoV replication[37]. Gao et al.[29] 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 solubility[38]. 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[38]. Similar results are demonstrated for additional mouse models[83,84]. Lipopeptides have been design to target cell fusion peptides[35]. An analogous fusion inhibitor, enfuvirtide (T-20), has been approved for treatment of HIV-1 infections[81]. 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 autophagy[85]. SKP2 attenuates autophagy through Beclin1-ubiquination; its inhibition by the licensed drug niclosamide, a treatment for tapeworms, drastically reduced the replications of MERS-CoV in cell culture[86]. 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 available[87] 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[68,71], 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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References

1. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol* 2020. DOI: https://10.1002/jmv.25681
2. Coronavirus COVID-19 Global Cases by Johns Hopkins CSSE. https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6 (accessed Feb 27, 2020).
3. Yasui F, Kohara M, Kitabatake M, et al. Phagocytic cells contribute to the antibody-mediated elimination of pulmonary-infected SARS coronavirus. *Virology* 2014; **454-455**: 157-68.
4. Zhang Y. Vital Surveillances: The Epidemiological Characteristics of an Outbreak of 2019 Novel Coronavirus Diseases (COVID-19) — China, 2020. http://weekly.chinacdc.cn/en/article/id/e53946e2-c6c4-41e9-9a9b-fea8db1a8f51 (accessed Feb 24, 2020).
5. Chu H, Zhou J, Wong BH-Y, et al. Middle East Respiratory Syndrome Coronavirus Efficiently Infects Human Primary T Lymphocytes and Activates the Extrinsic and Intrinsic Apoptosis Pathways. *J Infect Dis* 2015; **213**(6): 904-14.
6. Zhou J, Chu H, Chan JF-W, Yuen K-Y. Middle East respiratory syndrome coronavirus infection: virus-host cell interactions and implications on pathogenesis. *Virol J* 2015; **12**(1): 218.
7. Huang K-J, Su I-J, Theron M, et al. An interferon-γ-related cytokine storm in SARS patients. *J Med Virol* 2005; **75**(2): 185-94.
8. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* 2012; **76**(1): 16-32.
9. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol* 2017; **39**(5): 529-39.
10. Wong RSM, Wu A, To KF, et al. Haematological manifestations in patients with severe acute respiratory syndrome: retrospective analysis. *BMJ* 2003; **326**(7403): 1358-62.
11. Li T, Qiu Z, Zhang L, et al. Significant Changes of Peripheral T Lymphocyte Subsets in Patients with Severe Acute Respiratory Syndrome. *J Infect Dis* 2004; **189**(4): 648-51.
12. Nicholls JM, Poon LLM, Lee KC, et al. Lung pathology of fatal severe acute respiratory syndrome. *Lancet* 2003; **361**(9371): 1773-8.
13. Yang Y, Xiong Z, Zhang S, et al. Bcl-xL inhibits T-cell apoptosis induced by expression of SARS coronavirus E protein in the absence of growth factors. *Biochem J* 2005; **392**(Pt 1): 135-43.
14. Li G, Fan Y, Lai Y, et al. Coronavirus infections and immune responses. *J Med Virol* 2020. DOI: https://doi.org/10.1002/jmv.25685
15. Gu J, Korteweg C. Pathology and pathogenesis of severe acute respiratory syndrome. *Am J Pathol* 2007; **170**(4): 1136-47.
16. Ng MHL, Lau K-M, Li L, et al. Association of Human-Leukocyte-Antigen Class I (B*0703) and Class II (DRB1*0301) Genotypes with Susceptibility and Resistance to the Development of Severe Acute Respiratory Syndrome. *J Infect Dis* 2004; **190**(3): 515-8.
17. Lin M, Tseng H-K, Trejaut JA, et al. Association of HLA class I with severe acute respiratory syndrome coronavirus infection. *BMC Med Genet* 2003; **4**: 9.
18. Tang F, Liu W, Zhang F, et al. IL-12 RB1 genetic variants contribute to human susceptibility to severe acute respiratory syndrome infection among Chinese. *PLoS One* 2008; **3**(5): e2183.

19. Ip WKE, Chan KH, Law HKW, et al. Mannose-Binding Lectin in Severe Acute Respiratory Syndrome Coronavirus Infection. *J Infect Dis* 2005; **191**(10): 1697-704.
20. Menachery VD, Schäfer A, Burnum-Johnson KE, et al. MERS-CoV and H5N1 influenza virus antagonize antigen presentation by altering the epigenetic landscape. *Proc Natl Acad Sci U S A* 2018; **115**(5): E1012-E21.
21. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *J Virol* 2020: JVI.00127-20.
22. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. *bioRxiv* 2020. DOI: https://doi.org/10.1101/2020.01.26.919985
23. Darwin C. On the Origin of Species; 1859.
24. Kimura M. Evolutionary Rate at the Molecular Level. *Nature* 1968; **217**(5129): 624-6.
25. Bottema CDK, Ketterling RP, Li S, Yoon H-S, III JAP, Sommer SS. Missense mutations and evolutionary conservation of amino acids: evidence that many of the amino acids in factor IX function as "spacer" elements. *Am J Hum Genet* 1991; **49**: 820-38.
26. Ricke DO. Divergence Model of Protein Evolution. *bioRxiv* 2016. DOI: https://doi.org/10.1101/045930
27. Liu WJ, Zhao M, Liu K, et al. T-cell immunity of SARS-CoV: Implications for vaccine development against MERS-CoV. *Antiviral Res* 2017; **137**: 82-92.
28. Maier HJ, Britton P. Involvement of autophagy in coronavirus replication. *Viruses* 2012; **4**(12): 3440-51.
29. Gao J, Lu G, Qi J, et al. Structure of the fusion core and inhibition of fusion by a heptad repeat peptide derived from the S protein of Middle East respiratory syndrome coronavirus. *J Virol* 2013; **87**(24): 13134-40.
30. Yuan K, Yi L, Chen J, et al. Suppression of SARS-CoV entry by peptides corresponding to heptad regions on spike glycoprotein. *Biochem Biophys Res Commun* 2004; **319**(3): 746-52.
31. Liu IJ, Kao C-L, Hsieh S-C, Wey M-T, Kan L-S, Wang W-K. Identification of a minimal peptide derived from heptad repeat (HR) 2 of spike protein of SARS-CoV and combination of HR1-derived peptides as fusion inhibitors. *Antiviral Res* 2009; **81**(1): 82-7.
32. Lai S-C, Chong PC-S, Yeh C-T, et al. Characterization of neutralizing monoclonal antibodies recognizing a 15-residues epitope on the spike protein HR2 region of severe acute respiratory syndrome coronavirus (SARS-CoV). *J Biomed Sci* 2005; **12**(5): 711-27.
33. Bosch BJ, Martina BEE, Van Der Zee R, et al. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. *Proc Natl Acad Sci U S A* 2004; **101**(22): 8455-60.
34. Zhu J, Xiao G, Xu Y, et al. Following the rule: formation of the 6-helix bundle of the fusion core from severe acute respiratory syndrome coronavirus spike protein and identification of potent peptide inhibitors. *Bioc Biophy Res Commun* 2004; **319**(1): 283-8.
35. Wang C, Zhao L, Xia S, et al. De Novo Design of α-Helical Lipopeptides Targeting Viral Fusion Proteins: A Promising Strategy for Relatively Broad-Spectrum Antiviral Drug Discovery. *J Med Chem* 2018; **61**(19): 8734-45.
36. Xia S, Xu W, Wang Q, et al. Peptide-Based Membrane Fusion Inhibitors Targeting HCoV-229E Spike Protein HR1 and HR2 Domains. *Int J Mol Sci* 2018; **19**(2): 487.

37. Lu L, Liu Q, Zhu Y, et al. Structure-based discovery of Middle East respiratory syndrome coronavirus fusion inhibitor. *Nat Commun* 2014; **5**(1): 3067.
38. Xia S, Liu Q, Wang Q, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) entry inhibitors targeting spike protein. *Virus Res* 2014; **194**: 200-10.
39. Ricke DO, Shcherbina A. Dawn: Rapid large-scale protein multiple sequence alignment and conservation analysis. *2015 IEEE High Performance Extreme Computing Conference (HPEC)* 2015. DOI: https://10.1109/HPEC.2015.7322463
40. Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 2009; **25**: 1189-91.
41. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. *Nucleic Acids Res* 2000; **28**(1): 235-42.
42. Jmol: an open-source Java viewer for chemical structures in 3D. www.jmol.org. (accessed Jan 3, 2020).
43. Pyrc K, Berkhout B, Hoek Lvd. Antiviral Strategies Against Human Coronaviruses. *Infect Disorders - Drug Targets* 2007; **7**(1): 59-66.
44. Xu X, Chen P, Wang J, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci* 2020.
45. Letko M, Munster V. Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV. *bioRxiv* 2020: 2020.01.22.915660. DOI: https://doi.org/10.1101/2020.01.22.915660
46. Jaume M, Yip MS, Cheung CY, et al. Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcγR pathway. *J Virol* 2011; **85**(20): 10582-97.
47. Wang FS, Chu FL, Jin L, et al. Acquired but reversible loss of erythrocyte complement receptor 1 (CR1, CD35) and its longitudinal alteration in patients with severe acute respiratory syndrome. *Clin Exp Immunol* 2005; **139**(1): 112-9.
48. van Erp EA, van Kasteren PB, Guichelaar T, et al. In Vitro Enhancement of Respiratory Syncytial Virus Infection by Maternal Antibodies Does Not Explain Disease Severity in Infants. *J Virol* 2017; **91**(21): e00851-17.
49. Smatti MK, Al Thani AA, Yassine HM. Viral-Induced Enhanced Disease Illness. *Front Microbiol* 2018; **9**: 2991.
50. Jares Baglivo S, Polack FP. The long road to protect infants against severe RSV lower respiratory tract illness. *F1000Res* 2019; **8**: F1000 Faculty Rev-610.
51. Winarski KL, Tang J, Klenow L, et al. Antibody-dependent enhancement of influenza disease promoted by increase in hemagglutinin stem flexibility and virus fusion kinetics. *Proc Natl Acad Sci* 2019; **116**(30): 15194.
52. Panesar NS. Lymphopenia in SARS. *Lancet* 2003; **361**(9373): 1985.
53. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. DOI: https://doi.org/10.1016/S2213-2600(20)30076-X
54. Guan W-j, Ni Z-y, Hu Y, et al. Clinical characteristics of 2019 novel coronavirus infection in China. *medRxiv* 2020: DOI: https://doi.org/10.1101/2020.02.06.20020974.

55. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003; **426**(6965): 450-4.
56. Jeffers SA, Tusell SM, Gillim-Ross L, et al. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. *Proc Natl Acad Sci U S A* 2004; **101**(44): 15748.
57. Liu H, Yu W, Liou L-Y, Rice AP. Isolation and characterization of the human DC-SIGN and DC-SIGNR promoters. *Gene* 2003; **313**: 149-59.
58. Chan VSF, Chan KYK, Chen Y, et al. Homozygous L-SIGN (CLEC4M) plays a protective role in SARS coronavirus infection. *Nat Genet* 2006; **38**(1): 38-46.
59. Chen J, Lau YF, Lamirande EW, et al. Cellular Immune Responses to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection in Senescent BALB/c Mice: CD4+ T Cells Are Important in Control of SARS-CoV Infection. *J Virol* 2010; **84**(3): 1289.
60. Zhao J, Zhao J, Van Rooijen N, Perlman S. Evasion by stealth: inefficient immune activation underlies poor T cell response and severe disease in SARS-CoV-infected mice. *PLoS Pathog* 2009; **5**(10): e1000636.
61. Liu L, Wei Q, Lin Q, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. *JCI Insight* 2019; **4**(4): e123158.
62. Dandekar AA, Perlman S. Immunopathogenesis of coronavirus infections: implications for SARS. *Nat Rev Immunol* 2005; **5**(12): 917-27.
63. Lee N, Chan PKS, Ip M, et al. Anti-SARS-CoV IgG response in relation to disease severity of severe acute respiratory syndrome. *J Clin Virol* 2006; **35**(2): 179-84.
64. Ho M-S, Chen W-J, Chen H-Y, et al. Neutralizing Antibody Response and SARS Severity. *Emerg Infect Dis* 2005.
65. Zhang L, Zhang F, Yu W, et al. Antibody responses against SARS coronavirus are correlated with disease outcome of infected individuals. *J Med Virol* 2006; **78**(1): 1-8.
66. Channappanavar R, Fehr AR, Vijay R, et al. Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. *Cell Host Microbe* 2016; **19**(2): 181-93.
67. Khurana S, Loving CL, Manischewitz J, et al. Vaccine-Induced Anti-HA2 Antibodies Promote Virus Fusion and Enhance Influenza Virus Respiratory Disease. *Sci Transl Med* 2013; **5**(200): 200ra114.
68. Tseng C-T, Sbrana E, Iwata-Yoshikawa N, et al. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. *PLoS One* 2012; **7**(4): e35421.
69. Weingartl H, Czub M, Czub S, et al. Immunization with Modified Vaccinia Virus Ankara-Based Recombinant Vaccine against Severe Acute Respiratory Syndrome Is Associated with Enhanced Hepatitis in Ferrets. *J Virol* 2004; **78**(22): 12672.
70. Severe Acute Respiratory Syndrome (SARS) Vaccine. 2020. https://www.bcm.edu/departments/pediatrics/sections-divisions-centers/tropical-medicine/research/vaccine-development/sarsvaccine (accessed Feb. 5, 2020).
71. Agrawal AS, Tao X, Algaissi A, et al. Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus. *Hum Vaccin Immunother* 2016; **12**(9): 2351-6.
72. Rauch S, Jasny E, Schmidt KE, Petsch B. New Vaccine Technologies to Combat Outbreak Situations. *Front Immunol* 2018; **9**: 1963.

73. Cheng Y, Wong R, Soo YOY, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. *Eur J Clin Microbiol Infect Dis* 2005; **24**(1): 44-6.
74. Ravindranath P. Convalescent plasma therapy tested on critically ill COVID-19 patients. https://journosdiary.com/2020/02/15/convalescent-plasma-therapy-covid-19/ (accessed Feb 24, 2020.
75. Yuan Y, Cao D, Zhang Y, et al. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nat Commun* 2017; **8**: 15092.
76. Lip K-M, Shen S, Yang X, et al. Monoclonal Antibodies Targeting the HR2 Domain and the Region Immediately Upstream of the HR2 of the S Protein Neutralize In Vitro Infection of Severe Acute Respiratory Syndrome Coronavirus. *J Virol* 2006; **80**(2): 941.
77. Tripet B, Kao DJ, Jeffers SA, Holmes KV, Hodges RS. Template-based coiled-coil antigens elicit neutralizing antibodies to the SARS-coronavirus. *J Struct Biol* 2006; **155**(2): 176-94.
78. Keng ECT, Zhang A, Shen S, et al. Amino Acids 1055 to 1192 in the S2 Region of Severe Acute Respiratory Syndrome Coronavirus S Protein Induce Neutralizing Antibodies: Implications for the Development of Vaccines and Antiviral Agents. *J Virol* 2005; **79**: 3289-96.
79. Tian X, Li C, Huang A, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg Microbes Infect* 2020; **9**(1): 382-5.
80. Liu IJ, Tsai W-T, Hsieh L-E, Chueh L-L. Peptides corresponding to the predicted heptad repeat 2 domain of the feline coronavirus spike protein are potent inhibitors of viral infection. *PLoS One* 2013; **8**(12): e82081.
81. Kilby JM, Hopkins S, Venetta TM, et al. Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat Med* 1998; **4**(11): 1302-7.
82. Lambert DM, Barney S, Lambert AL, et al. Peptides from conserved regions of paramyxovirus fusion (F) proteins are potent inhibitors of viral fusion. *Proc Natl Acad Sci* 1996; **93**(5): 2186.
83. Channappanavar R, Lu L, Xia S, et al. Protective Effect of Intranasal Regimens Containing Peptidic Middle East Respiratory Syndrome Coronavirus Fusion Inhibitor Against MERS-CoV Infection. *J Infect Dis* 2015; **212**(12): 1894-903.
84. Jiang S, Tao X, Xia S, et al. Intranasally administered peptidic viral fusion inhibitor protected hDPP4 transgenic mice from MERS-CoV infection. *Lancet* 2015; **386**: S44.
85. Prentice E, Jerome WG, Yoshimori T, Mizushima N, Denison MR. Coronavirus Replication Complex Formation Utilizes Components of Cellular Autophagy. *J Biol Chem* 2004; **279**(11): 10136-41.
86. Gassen NC, Niemeyer D, Muth D, et al. SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. *Nat Commun* 2019; **10**(1): 5770.
87. Liu X, Zhang B, Jin Z, Yang H, Rao Z. The crystal structure of COVID-19 main protease in complex with an inhibitor N3. 2020. http://www.rcsb.org/structure/6LU7 (accessed 02-20-2020).
88. Narayanan K, Huang C, Lokugamage K, et al. Severe Acute Respiratory Syndrome Coronavirus nsp1 Suppresses Host Gene Expression, Including That of Type I Interferon, in Infected Cells. *J Virol* 2008; **82**(9): 4471.

89. Narayanan K, Huang C, Lokugamage K, et al. Severe acute respiratory syndrome coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in infected cells. *J Virol* 2008; **82**(9): 4471-9.
90. Serrano P, Johnson MA, Almeida MS, et al. Nuclear Magnetic Resonance Structure of the N-Terminal Domain of Nonstructural Protein 3 from the Severe Acute Respiratory Syndrome Coronavirus. *J Virol* 2007; **81**(21): 12049.
91. Saikatendu KS, Joseph JS, Subramanian V, et al. Structural Basis of Severe Acute Respiratory Syndrome Coronavirus ADP-Ribose-1"-Phosphate Dephosphorylation by a Conserved Domain of nsP3. *Structure* 2005; **13**(11): 1665-75.
92. Ratia K, Saikatendu KS, Santarsiero BD, et al. Severe acute respiratory syndrome coronavirus papain-like protease: structure of a viral deubiquitinating enzyme. *Proc Natl Acad Sci U S A* 2006; **103**(15): 5717-22.
93. Beachboard DC, Anderson-Daniels JM, Denison MR. Mutations across murine hepatitis virus nsp4 alter virus fitness and membrane modifications. *J Virol* 2015; **89**(4): 2080-9.
94. Yang H, Yang M, Ding Y, et al. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. *Proc Natl Acad Sci* 2003; **100**(23): 13190.
95. Angelini MM, Akhlaghpour M, Neuman BW, Buchmeier MJ. Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. *mBio* 2013; **4**(4): e00524-13.
96. Cottam EM, Whelband MC, Wileman T. Coronavirus NSP6 restricts autophagosome expansion. *Autophagy* 2014; **10**(8): 1426-41.
97. Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat Commun* 2019; **10**(1): 2342.
98. Zhai Y, Sun F, Li X, et al. Insights into SARS-CoV transcription and replication from the structure of the nsp7–nsp8 hexadecamer. *Nat Struct Mol Biol* 2005; **12**(11): 980-6.
99. Sutton G, Fry E, Carter L, et al. The nsp9 Replicase Protein of SARS-Coronavirus, Structure and Functional Insights. *Structure* 2004; **12**(2): 341-53.
100. Ma Y, Wu L, Shaw N, et al. Structural basis and functional analysis of the SARS coronavirus nsp14-nsp10 complex. *Proc Natl Acad Sci U S A* 2015; **112**(30): 9436-41.
101. Decroly E, Debarnot C, Ferron F, et al. Crystal Structure and Functional Analysis of the SARS-Coronavirus RNA Cap 2'-O-Methyltransferase nsp10/nsp16 Complex. *PLoS Pathog* 2011; **7**(5): e1002059.
102. Jia Z, Yan L, Ren Z, et al. Delicate structural coordination of the Severe Acute Respiratory Syndrome coronavirus Nsp13 upon ATP hydrolysis. *Nucleic Acids Res* 2019; **47**(12): 6538-50.
103. Surya W, Li Y, Torres J. Structural model of the SARS coronavirus E channel in LMPG micelles. *Biochim Biophys Acta Biomembranes* 2018; **1860**(6): 1309-17.
104. Shi P, Su Y, Li R, Liang Z, Dong S, Huang J. PEDV nsp16 negatively regulates innate immunity to promote viral proliferation. *Virus Res* 2019; **265**: 57-66.
105. Wilson L, Gage P, Ewart G. Hexamethylene amiloride blocks E protein ion channels and inhibits coronavirus replication. *Virology* 2006; **353**(2): 294-306.
106. Li Y, Surya W, Claudine S, Torres J. Structure of a conserved Golgi complex-targeting signal in coronavirus envelope proteins. *J Biol Chem* 2014; **289**(18): 12535-49.

107. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. *Virol J* 2019; **16**(1): 69.
108. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, et al. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS Pathog* 2014; **10**(5): e1004077.
109. Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virology* 2015; **485**: 330-9.
110. Jimenez-Guardeño JM, Nieto-Torres JL, DeDiego ML, et al. The PDZ-Binding Motif of Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Is a Determinant of Viral Pathogenesis. *PLoS Pathog* 2014; **10**(8): e1004320.
111. Jimenez-Guardeño JM, Regla-Nava JA, Nieto-Torres JL, et al. Identification of the Mechanisms Causing Reversion to Virulence in an Attenuated SARS-CoV for the Design of a Genetically Stable Vaccine. *PLoS Pathog* 2015; **11**(10): e1005215.
112. Tseng Y-T, Chang C-H, Wang S-M, Huang K-J, Wang C-T. Identifying SARS-CoV Membrane Protein Amino Acid Residues Linked to Virus-Like Particle Assembly. *PLoS One* 2013; **8**(5): e64013.
113. Chen C-Y, Chang C-k, Chang Y-W, et al. Structure of the SARS Coronavirus Nucleocapsid Protein RNA-binding Dimerization Domain Suggests a Mechanism for Helical Packaging of Viral RNA. *J Mol Biol* 2007; **368**(4): 1075-86.
114. Nelson CA, Pekosz A, Lee CA, Diamond MS, Fremont DH. Structure and Intracellular Targeting of the SARS-Coronavirus Orf7a Accessory Protein. *Structure* 2005; **13**(1): 75-85.
115. Hänel K, Stangler T, Stoldt M, Willbold D. Solution structure of the X4 protein coded by the SARS related coronavirus reveals an immunoglobulin like fold and suggests a binding activity to integrin I domains. *J Biomed Sci* 2006; **13**(3): 281-93.
116. Fielding BC, Tan Y-J, Shuo S, et al. Characterization of a unique group-specific protein (U122) of the severe acute respiratory syndrome coronavirus. *J Virol* 2004; **78**(14): 7311-8.
117. Arnaout MA. Leukocyte Adhesion Molecules Deficiency: Its Structural Basis, Pathophysiology and Implications for Modulating the Inflammatory Response. *Immunol Rev* 1990; **114**(1): 145-80.
118. Teoh K-T, Siu Y-L, Chan W-L, et al. The SARS coronavirus E protein interacts with PALS1 and alters tight junction formation and epithelial morphogenesis. *Mol Biol Cell* 2010; **21**(22): 3838-52.
119. Almeida MS, Johnson MA, Herrmann T, Geralt M, Wüthrich K. Novel β-Barrel Fold in the Nuclear Magnetic Resonance Structure of the Replicase Nonstructural Protein 1 from the Severe Acute Respiratory Syndrome Coronavirus. *J Virol* 2007; **81**(7): 3151.
120. Lin M-H, Moses DC, Hsieh C-H, et al. Disulfiram can inhibit MERS and SARS coronavirus papain-like proteases via different modes. *Antiviral Res* 2018; **150**: 155-63.
121. Chen Y, Su C, Ke M, et al. Biochemical and Structural Insights into the Mechanisms of SARS Coronavirus RNA Ribose 2'-O-Methylation by nsp16/nsp10 Protein Complex. *PLoS Pathog* 2011; **7**(10): e1002294.
122. Ma Y, Wu L, Shaw N, et al. Structural basis and functional analysis of the SARS coronavirus nsp14–nsp10 complex. *Proc Nat Acad Sci U S A* 2015; **112**(30): 9436.

123. Xu X, Zhai Y, Sun F, et al. New Antiviral Target Revealed by the Hexameric Structure of Mouse Hepatitis Virus Nonstructural Protein nsp15. *J Virol* 2006; **80**(16): 7909.
124. Saikatendu KS, Joseph JS, Subramanian V, et al. Ribonucleocapsid Formation of Severe Acute Respiratory Syndrome Coronavirus through Molecular Action of the N-Terminal Domain of N Protein. *J Virol* 2007; **81**(8): 3913.
125. Xue X, Wu J, Ricklin D, et al. Regulator-dependent mechanisms of C3b processing by factor I allow differentiation of immune responses. *Nat Struct Mol Biol* 2017; **24**(8): 643-51.
126. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020: eabb2507.
127. Sainz B, Jr., Mossel EC, Gallaher WR, et al. Inhibition of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) infectivity by peptides analogous to the viral spike protein. *Virus Res* 2006; **120**(1-2): 146-55.
128. Liu S, Xiao G, Chen Y, et al. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. *Lancet* 2004; **363**(9413): 938-47.
129. Ujike M, Nishikawa H, Otaka A, et al. Heptad Repeat-Derived Peptides Block Protease-Mediated Direct Entry from the Cell Surface of Severe Acute Respiratory Syndrome Coronavirus but Not Entry via the Endosomal Pathway. *J Virol* 2008; **82**(1): 588.

Tables

Table 1. 2019-nCoV proteins*.

Protein	Function	Cofactors	References
nsp1	cellular mRNA degradation, inhibiting type I interferon (IFN) expression	..	88,89
nsp2	Unknown	..	..
nsp3	Multidomain protein	..	..
nsp3a	interacts with single-stranded RNA	..	90
nsp3b	ADP-ribose 1"-phosphatase	..	91
nsp3d	papain-like protease (Plpro), deubiquitinating enzyme (DUB)	..	92
nsp4	double-membrane vesicles (DMV) formation	..	93
nsp5	3C-like protease (3CLpro)	..	94
nsp6	Restricting autophagosome expansion, DMV formation	..	95,96
nsp7	RNA binding	nsp8:nsp12	97,98
nsp8	RNA binding; primase	nsp7:nsp12, nsp9	97,98
nsp9	RNA binding, dimerization	nsp8	99
nsp10	scaffold cofactor	nsp10, nsp16	100,101
nsp11	Unknown	..	..
nsp12	RNA-dependent RNA polymerase (RdRp)	nsp7:nsp8, nsp14	97
nsp13	RNA helicase, 5' triphosphatase	..	102
nsp14	3'-5' exoribonuclease (ExoN), guanine-N7 methyl transferase (N7-Mtase) for mRNA capping, nsp12:nsp14 RNA synthesis and proofreading	..	100
nsp15	endoribonuclease	..	103
nsp16	nsp16:nsp10 RNA cap 2'-O-methyltransferase, negatively regulates innate immunity	..	101,104
E	forms homopentameric ion channels (IC) with poor ion selectivity, Golgi complex-targeting signal, PDZ-binding motif (PBM)	..	105-111
M	Membrane protein	..	112
N	packages viral RNA	..	113
ORF3a	..	..	..
ORF6	..	..	..
ORF7a	Ig-like domain, ER retention signal	..	114-117
ORF7b	..	..	..
ORF8	..	..	..
ORF10	Unknown	..	..
S	Receptor binding, cell fusion	..	75

*The E protein IC releases calcium from the endoplasmic reticulum intermediate compartment (ERGIC), leading to NLRP3 inflammasome activation[108,109]. The E protein has a PDZ-binding motif (PBM)[107] that interacts with syntenin PDZ motifs to activate p38 mitogen-activated protein kinase (MAPK) pathway and promotes an acute proinflammatory response[110] and a virus PBM domain is required for virulence[111]. The E protein PDZ-binding motif binds to PALS1 and alters tight junction formation and epithelial morphogenesis[118]. The envelope (E) protein includes two pathways to promote inflammation; these may contribute to the ADE response. ORF7a protein has Ig-like domain[114]. Hänel et al.[115] 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 signal[116]. Loss of LFA-1 negatively impacts immune responses[117]. 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[119]
nsp2	279	187	101	46	25	638	0·73	..
nsp3	996	514	239	115	92	1,956	0·77	2GRI:A[90]
nsp3a	82	35	20	22	12	171	0·68	2ACF:A[91]
PI_{pro}	212	68	24	10	5	319	0·88	5Y3E:A[120]
nsp4	337	112	34	13	4	500	0·90	..
nsp5	254	46	4	2	0	306	0·98	6LU7[87]
nsp6	209	64	15	2	0	290	0·94	..
nsp7	69	13	1	0	0	83	0·99	2AHM:A[98]
nsp8	170	26	2	0	0	198	0·99	2AHM:G[98]
nsp9	95	16	2	0	0	113	0·98	1UW7:A[99]
nsp10	109	27	3	0	0	139	0·98	3R24:B[121]
nsp12	5,226	1374	346	105	50	7,101	0·93	..
nsp13	538	61	2	1	0	602	1·00	6JYT:A[102]
nsp14	442	78	7	0	0	527	0·99	5C8T:B[122]
nsp15	246	76	17	6	1	346	0·93	2GTH:A[123]
nsp16	230	55	8	1	2	296	0·96	3R24:A[121]
E	24	33	17	5	3	82	0·70	5X29:A[103]
M	178	29	11	4	0	222	0·93	..
N	294	76	33	15	4	422	0·88	2OFZ:A[124]
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[114]
ORF7b	5	33	11	4	1	54	0·70	..
ORF8	59	39	15	8	0	121	0·81	5O32:I[125]
ORF10	38	0	0	0	0	38	1·00	..
S	650	263	123	107	152	1,295	0·71	6CRZ:A[126]

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 moderate and server 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). 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).

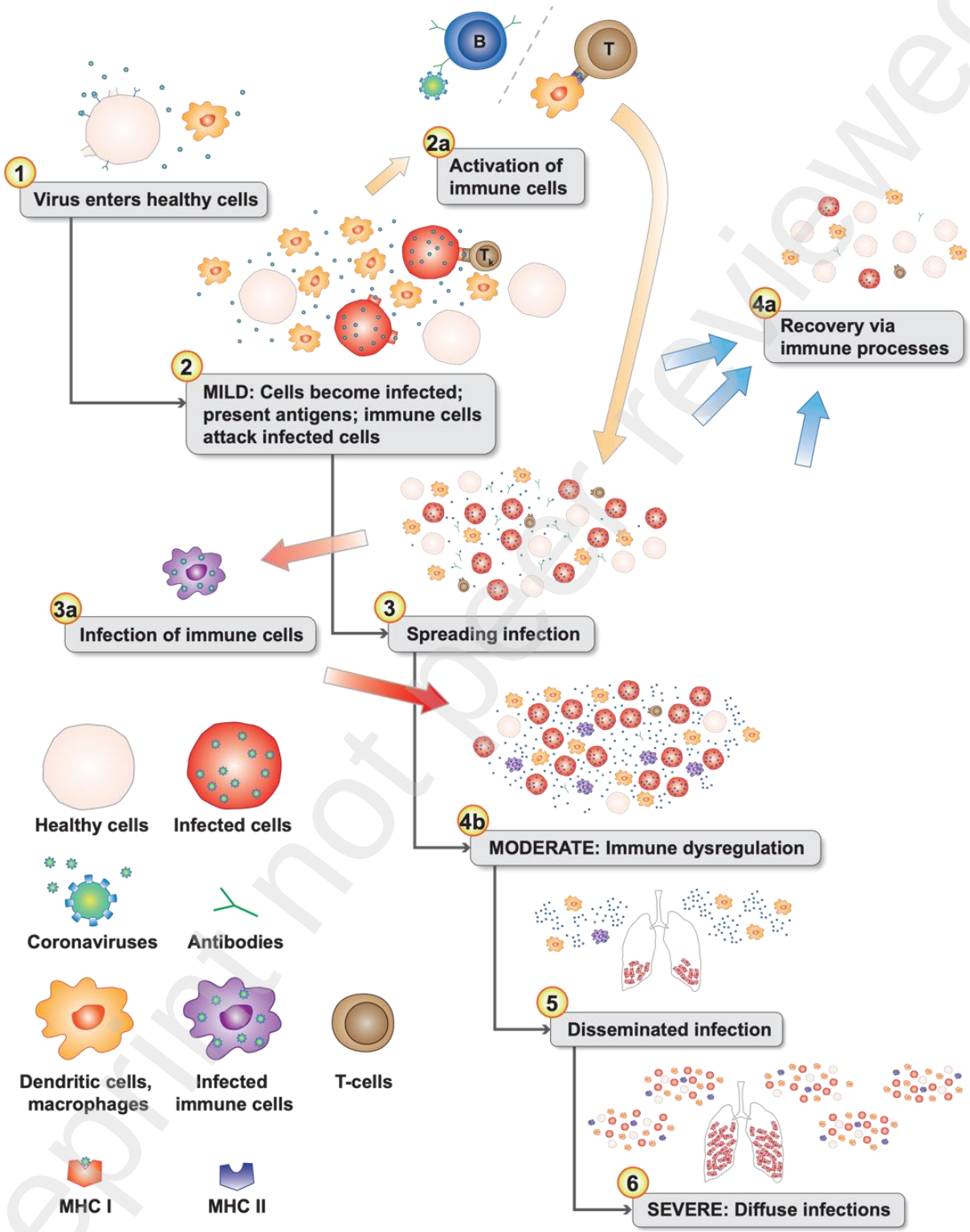
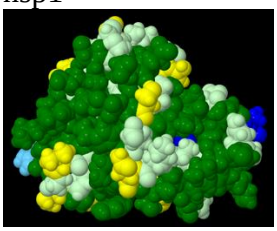
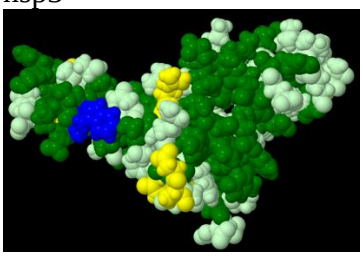


Figure 2. 2019-nCoV Variation results. Amino acid residue color code: dark green (critical residues), light green (critical residues with conservative substitutions or variant in less than 10 sequences, yellow (3 variants), light blue (4 variants; likely spacer residues), and blue (5+ variants; spacer residues).

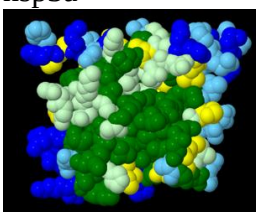
nsp1



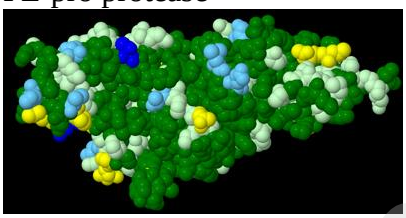
nsp3



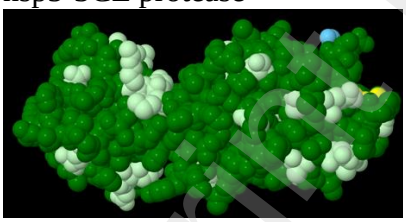
nsp3a



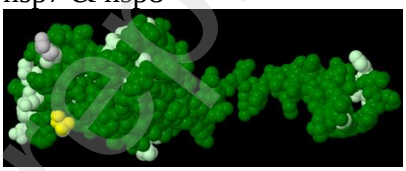
PL-pro protease



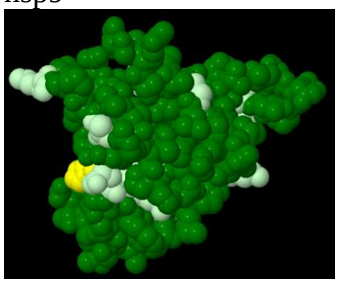
nsp5-3CL protease



nsp7 & nsp8



nsp9



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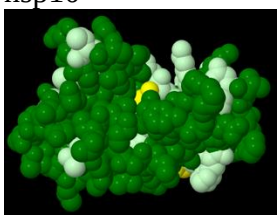

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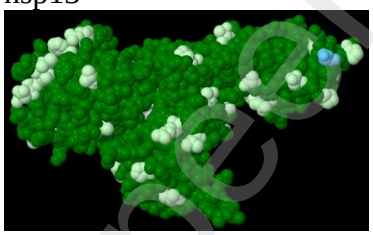

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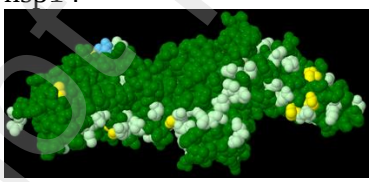

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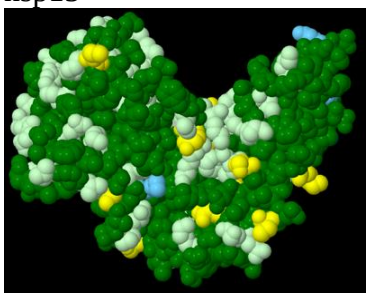

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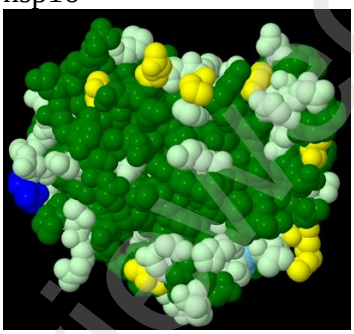

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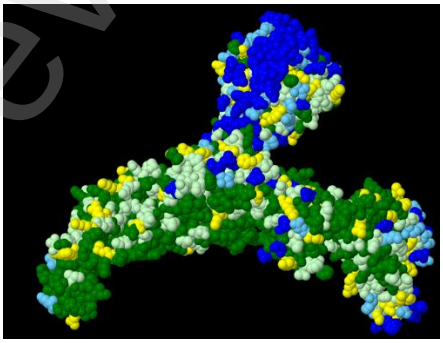

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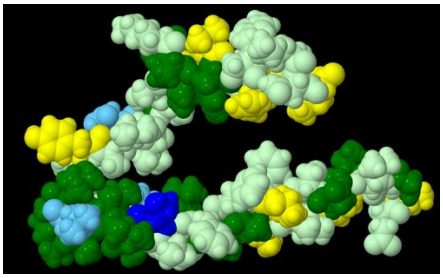

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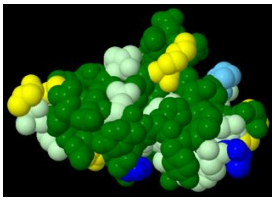

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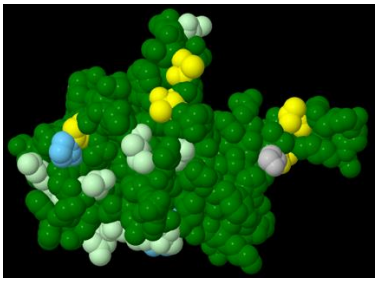

Figure 3. SARS-CoV-1 Inhibitory Peptides N46[31], HR1-1[30], HR2-18[30], WW-III[127], WW-IV[127], sHR2-2[33], sHR2-8[33], HRC1[77], HRC2[77], CP-1[128], SR9[129], P6[31], and CB-119[32]. SARS-CoV-2 residues different from SARS-CoV-1 are underlined for adapting SARS-CoV-1 inhibitory peptides.

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SARS2 907-NGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQ-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-ELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK-1211
SARS1 1126-ELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK-1193
sHR2-8     ELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK
sHR2-2         PKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE
HR2-18                                      IQKEIDRLNEVAKNLNESLIDLQELGK
HRC2                                         QKEIDRLNEVIKNLNESIIDLQEL
HRC1                                       NASIVNLQKEIDRLNEVIKNLNES
CP-1                                     GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE
P6                                       GINASVVNIQKEIDRLNEVAKNL
SR9                                    ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQEL
CB-119                           SPDVDLGDISGINAS
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