Life and Times of COVID-19

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COVID-19: Onset of a pandemic

On 12 December 2019, a respiratory illness of unknown etiology appeared in the city of Wuhan, in central China (Zhou et al., 2020). Symptoms included dry cough, fever, and dyspnea, as well as pneumonia and alveolar damage (Zhou et al., 2020). By 26 January 2020, there were more than 2,000 confirmed cases, but most of these were limited to people in Wuhan (Lu et al., 2020; Zhou et al., 2020). Recognizing the potential of the disease to spread beyond China, WHO identified it as a Public Health Emergency of International Concern (PHEIC) five days later (Li et al., 2020).

Genome sequencing of samples from infected patients from Wuhan allowed WHO and the scientific community to identify the cause of the outbreak as a member of the *Coronaviridae* family (Lu et al., 2020). Tentatively, WHO termed this virus 2019-nCoV (2019 novel coronavirus) and the disease caused by it COVID-19; however, the International Committee on the Taxonomy of Viruses has since renamed the virus as SARS-CoV-2 (Xia et al., 2020).

After having been nearly restricted to Wuhan for several weeks, SARS-CoV-2 rapidly spread beyond central China soon after it was identified as a PHEIC (Li et al., 2020). By March 1, it was found that total confirmed cases in China had risen to nearly 30 times what they had been one month previously, and cases in other countries had risen from a few dozen to more than 7,000 (Li et al., 2020; Zhou et al., 2020). The number of cases has continued to grow, and more than 15 million total cases and 640,000 deaths have been reported by WHO as of 26 July 2019 (WHO, 2020).

The danger and economic damage associated with COVID-19 has resulted in an outpouring of new research. Much research is being carried out to characterize SARS-CoV-2 in spite of the fact that similar viruses have been studied for decades (Li & Cavanagh, 1997). This literature review seeks to provide a brief summary of existing molecular research on

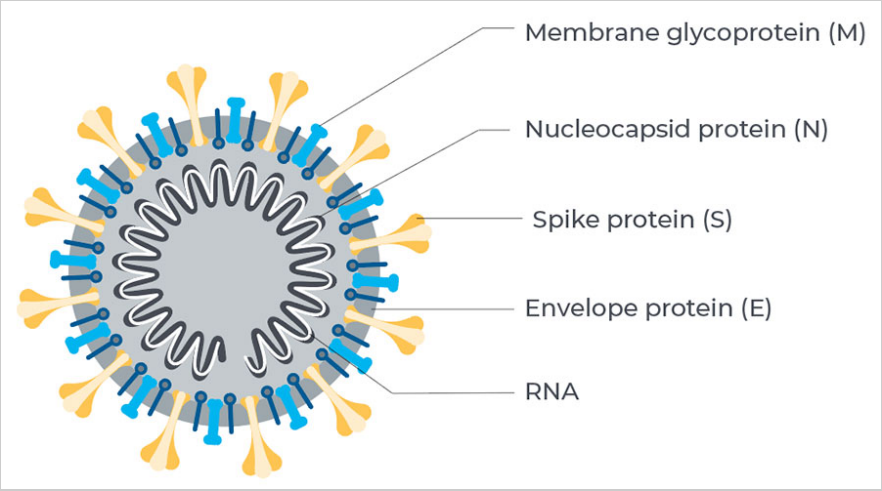
SARS-CoV-2 and its pathogenesis, as well as the current endeavors for development of novel therapeutics against the disease.

(The blackest sheep of) the Coronavirus family: COVID-19

Coronaviruses are a family of viruses, many of which were known and studied long before the COVID-19 pandemic (Cui et al., 2019). Among coronaviruses, many that were previously known to infect humans belong to the same genus as SARS-CoV-2, called *Betacoronavirus* (Cui et al., 2019). These viruses share a common structure with four major structural proteins: spike (S), a protruding oligomeric protein for host cell binding that is distributed across the virion's surface; membrane (M), which is gives shape to the lipid bilayer envelope; envelope (E), a smaller envelope-related protein; and nucleocapsid (N), which is in the center of each viral particle and which contains the viral RNA (Lai & Cavanagh, 1997). Relative to other RNA viruses, coronaviruses are large and complex with a diameter of perhaps 120 nm, and they are roughly spherical (Lai & Cavanagh, 1997). This means that SARS-CoV-2's diameter of 80-120 nm makes it slightly smaller than many coronaviruses (Monteil et al., 2020).

Figure 1

Major Structural Proteins of SARS-CoV-2 Viral Particle



Note. Image borrowed from 2020 article by Orland.

In spite of SARS-CoV-2's similarity to other SARS-related coronaviruses, there is a genetic discontinuity between SARS-CoV-2's lineage and that of any other known virus (Letko et al., 2020). In other words, there is no continuous evolutionary progression that led directly to the novel coronavirus as a whole. Instead, presumably due to recombination, SARS-CoV-2's genome contains motifs from three distinct clades of lineage B of betacoronaviruses, including the clade of SARS-CoV, the species that caused the 2002-2003 SARS epidemic (Letko et al., 2020). Recombination is not an uncommon source of novel viruses -- new forms of influenza, for instance, arise periodically through independent assortment -- although it is worth noting that coronaviruses are generally associated with a different mechanism of recombination from influenza that occurs at a lower frequency (Fleishmann, 1996).

Nearly two decades after the initial SARS epidemic, the full sequence of hosts leading up to the SARS-CoV spillover to humans is not known with certainty (Cui et al., 2019), and it is possible that investigations of the source of SARS-CoV-2 will not bring greater clarity. However, it is believed that the viral strains that combined to create SARS-CoV-2 either came from bats or were directly descended from strains that could be found in bats (McKee et al., 2020). This can be inferred from the fact that bat populations in Chinese caves harbor genetically diverse lineage B betacoronaviruses (Cui et al., 2019). Unlike the masked civet cats which are believed to have been the source of the SARS spillover to humans, bats such as those in the *Rhinolophus* genus respond to SARS-CoV infection without apparent symptoms, making them a likely natural reservoir host for coronaviruses related to SARS (Li et al., 2005). The same has been found to be true of SARS-CoV-2 in bats (McKee et al., 2020). Furthermore, direct evidence has shown that bats harbor a virus of near-perfect sequence identity to SARS-CoV and other SARS-like coronaviruses within lineage B, termed SL-CoVs (Li et al., 2005). Indeed,

one of these SL-CoVs, RatG13, has 96.2% sequence identity with SARS-CoV-2 itself (Zhou et al., 2020). SARS-related coronaviruses are not the only human-pathogenic betacoronaviruses that have spilled over from bats, either: MERS-CoV is also believed to have originated in bats (Cui et al., 2019).

Because of their ability to cause life-threatening disease in humans, SARS-CoV and MERS-CoV are among the betacoronaviruses that are commonly used to inform research on SARS-CoV-2. Of the two, SARS-CoV is particularly relevant, as can be expected from the shared lineage that is apparent in phylogenetic analysis (Li et al, 2020). Much attention has been devoted to the mechanism of viral fusion to the host cell, and given the roughly 76% sequence identity between SARS-CoV-2 and SARS-CoV S protein, it is unsurprising that the two viruses have much in common in this regard (Hoffmann et al., 2020). Perhaps more importantly, the S proteins of both viruses have functional similarity as well: SARS-CoV-2 and the viruses in SARS-CoV's evolutionary clade of *Betacoronavirus* lineage B all target the angiotensin-converting enzyme 2 (ACE2) receptor when binding to a host cell (Letko et al., 2020). In contrast, it has been established that MERS-CoV binds to dipeptidyl peptidase-4 (DPP4), but not ACE2 (Letko et al., 2020). In spite of this difference, MERS-CoV is still used along with SARS-CoV in studies of SARS-CoV-2 for comparison, presumably because it is of the same genus and because it causes severe disease in humans (Lu et al., 2020). Another important common feature that makes MERS-CoV relevant to these studies is that, like the other two viruses, it seems to depend on the host cell protease TMPRSS2 to activate the cell-fusion-causing behavior of its S protein (Hoffmann et al., 2020).

These commonalities between the three viruses have been at the center of recent research of SARS-CoV-2 because of their potential to inform the development of drugs or vaccines for COVID-19. For example, in the receptor binding region (RBD), differences in

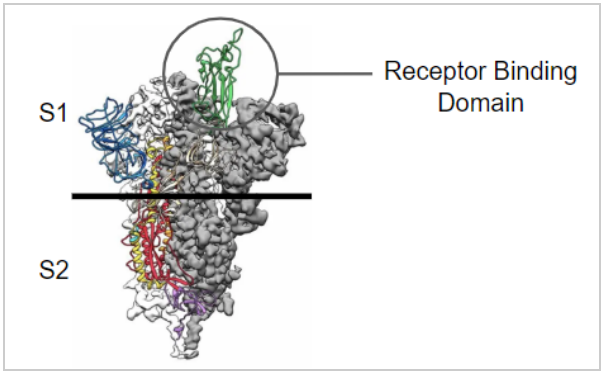
individual amino acid residues between SARS-CoV and SARS-CoV-2 have shed some light on the question of why the current pandemic seems to have spread more easily than the SARS epidemic. Although conflicting results have been found regarding the relative hACE2 binding affinities of the two RBDs, it is claimed that this confusion has been resolved based on the tendency of the SARS-CoV-2 to “hide” its RBD, and that SARS-CoV-2 RBD does indeed have the higher binding affinity (Shang et al., 2020a).

The Spike Protein: a Weapon and an Achilles Heel

The spike (S) protein on SARS-CoV-2 virions has 10 times the binding affinity than that of the SARS-CoV to human ACE2 host receptor cells (Wrapp et al. 2020). The S protein is a highly glycosylated trimeric type I transmembrane fusion protein with 2 functional subunits known as the S1 and S2 subunits (Wrapp et al., 2020). The attachment of the viral membrane to the cell membrane begins when the S1 subunit binds to a host cell receptor, ACE2 (Wrapp et al., 2020). The N-terminal S1 subunit recognizes and binds to receptors while the C-terminal S2 subunit is responsible for fusing the host cell membrane to the viral envelope with the help of fusion peptides (Wrapp et al., 2020).

Figure 2

3D visualization of SARS-CoV-2 Spike Protein



Note. Image borrowed from 2020 article by Wrapp et al.

To allow the virus to enter the cell, the S1 subunit must first bind to the host cell receptor. This induces two structural conformations, the first involving the transformation of an unstructured linker within the S2 subunit to become helical and the second change involving the inversion of the S2 subunit's C-helix to form a coil, resulting in the formation of a six-helix bundle (Wrapp et al., 2020). Like other Class I fusion proteins, SARS-CoV-2 S must undergo this change in order to extend to the host cell membrane, pull the two membranes toward each other, and create a fusion pore that will lead to complete membrane fusion (White et al., 2009).

Another possible conformational change of the S1 subunit can either hide or expose the receptor binding domain, and these two states are referred to as the "up" and "down" arrangements (Wrapp et al., 2020; Shang et al., 2020a). The "up" refers to the exposed domain, making the receptor accessible, while "down" refers to the hidden domain, making the receptor inaccessible (Wrapp et al., 2020; Shang et al., 2020a).

Because the S protein is the protein that allows for infection, it is the ideal target for developing vaccines (Padron-Regalado, 2020). Researchers have found that the S protein induces the formation of neutralizing antibodies (NAbs), which are produced naturally by the humoral immune system (Padron-Regalado, 2020). The S protein is highly sensitive to NAbs, so researchers hope to develop standardized agents that block the binding and fusion of the S protein of SARS-CoV-2 to host cells (Padron-Regalado, 2020).

ACE2 Receptor: COVID-19's ace in the deck

ACE2 is known to be the major receptor that SARS-related coronaviruses use to infect the cell (Letko et al., 2020). ACE2, or angiotensin converting enzyme 2, is a type 1 transmembrane protein expressed in both endothelial and epithelial cells around the body (Yan et al., 2020). It is most prevalent in the lungs, kidneys, liver, intestines, and heart (Monteil et al., 2020; Wong, 2016). ACE2 is an important component of the renal-angiotensin system (RAS),

which regulates blood pressure and fluid balance (Guo et al., 2020; Glowacka et al., 2009). ACE2 levels in patients are sex-dependent; the ACE2 gene is located at chromosome Xp22 in humans, so women possess two copies of the gene, while men only have one (Wong, 2016). In SARS patients, this is important because the binding of the SARS-CoV-2 RBD and ACE2 peptidase domain induces the infected cell to shed off other ACE2 proteins, reducing ACE2 expression in the cell (Glowacka et al., 2009). This decreased protein expression leads to an upregulation of angiotensin II, often resulting in severe acute respiratory distress among many other symptoms (Wong, 2016). It is believed that the COVID-19 affects the body through the same mechanism (Guo et al., 2020). The distribution of ACE2 in organ tissue around the body may help explain the multi-organ dysfunction and failure shown in those infected with SARS-CoV-2 (Monteil et al., 2020). Because of this, researchers have been considering the clinical implications of treatments that would increase ACE2 activity or inhibit ACE and angiotensin II receptors (McKee et al., 2020). Some have started studying the effects human recombinant soluble ACE2 (hrsACE2) has on SARS-CoV-2 infected cells and found promising results that show early stage infection may be significantly reduced through hrsACE2 treatment (Monteil et al., 2020). The experiments and research done around the ACE2 receptor hold great promise in helping uncover how SARS-CoV-2 may be treated.

Spike Protein Binding: Initiation of the Infection

Fusion proteins are responsible for fusing the membrane of the virus with that of the host cell (Harrison, 2015; White, 2008). There are three distinct classes of viral membrane fusion proteins, distinguished by protein structure. In addition, there are a plethora of mechanisms by which viral fusion proteins can undergo conformational changes (White et al., 2008). In spite of their diversity, all fusion proteins undergo changes transforming them from a fusion competent state (dimers or trimers, depending on the class) to a series of different structures resulting in

the union of viral and target membranes (White, 2008). Each fusion protein is present in two different forms: pre-fusion and post-fusion. The class of a fusion protein is determined by "whether the fusion subunit has a prominent cetra a-helical coiled- coil (Class 1 viral fusion proteins), whether it consists largely of a B-structure (Class II viral fusion proteins), or whether it displays a combination of a-helical and B-structure (Class III viral fusion protein)" (White et al., 2008).

The SARS-CoV-2 spike glycoprotein is categorized as a class I fusion protein, meaning it is a trimer whose major secondary structure is the alpha helix (Wrapp et al., 2020). The virus's entry into target cells is dependent on two events: the binding of the S1 subunit to a cellular receptor (ACE2), and the priming of the S protein by cellular proteases (Hoffman et al., 2020). TMPRSS2, a specific protease classified as a type II transmembrane-bound protease, is one of the proteases responsible for priming the S protein (Hoffmann et al., 2020). The cleavage of the S protein, brought about by the protease, occurs in two different locations, the first one being within the S1/S2 subunit, and the second being within the S2 subunit (Hoffmann et al., 2020). Following the cleavage between the S1 and S2 subunit, the S1 subunit dissociates from the protein and S2 subunit undergoes a large structural change, transitioning to the post-fusion structure. It was observed that the RBD (receptor binding domain) of the S1 subunit underwent a "hingelike" transformation, this transformation likely plays a part in the dissociation of the S1 subunit and the S2 subunit's increased stability (Wrapp et al., 2020).

It is also important to note that SARS-CoV-2 can also make use of the "endosomal cysteine proteases cathepsin B and L" (Hoffman, 2020); however, TMPRSS2 is essential to the viral entry and pathogenesis in the infected host. Recent clinical trials have shown that a TMPRSS2 inhibitor blocked entry of the virus cell into the host cells, making it a potential drug target (Schroeder, 2020).

Risk Factors

In one particularly informative study, Chakladar et al. attempted to determine whether smokers' increased risk of developing COVID-19 could be explained by the effect of smoking on ACE2 and TMPRSS2 expression (2020). Using RNA-sequencing data from the genes of the lung epithelial tissues of smokers and past-smokers, they found that the expression of ACE2 receptors was upregulated in smokers compared to non-smokers. In addition, Chakladar et al. used Gene Set Enrichment Analysis (GSEA) to uncover evidence for a link between the upregulation of ACE2 receptors and immune cell population dysregulation in smokers. This suggests that the increased expression of ACE2 and the accompanying dysregulation of the immune cell population in smokers may be the reason they have a higher vulnerability to COVID-19 (Chakladar et al., 2020).

Smoking is known to cause an increase in androgens, which are hormones that can stimulate TMPRSS2 expression, so Chakladar et al. utilized GSEA in the same 2020 study to try to find a link between upregulation of androgen signaling pathways and risk of SARS-CoV-2 infection. Their results indicate that the upregulation of the androgen and androgen-regulating pathways is linked to the upregulation of ACE2 and TMPRSS2 in smokers. In their study, application of the same procedures to oral epithelial cells also showed similar results, with an upregulation of ACE2 and TMPRSS2 in smokers compared to non-smokers. The upregulation of genes such as EP300 or CDK6 that regulate the androgen signaling pathway also indicated that that there was a link between the upregulation of the androgen pathway and elevated ACE2/TMPRSS2 expression in oral epithelial cells, making them more susceptible to SARS-CoV-2 due to smoking (Chakladar et al., 2020). Thus, they concluded that the upregulation of TMPRSS2 due to an increase in the androgen signaling pathway and increased ACE2 expression caused by smoking leads to a higher risk of the SARS-CoV-2 in smokers.

Cardiovascular diseases may increase the risk of COVID-19 because the treatment of cardiovascular diseases include angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs), which upregulate ACE2 (Guo et al., 2020). Thus, more pathways are created for the virus to enter the cell, theoretically making patients treated for cardiovascular disease more susceptible to SARS-CoV-2 (Guo et al., 2020). However, scientists are still debating whether to stop these treatments because the increased expression of ACE2 is necessary to reduce risk of cardiovascular diseases such as heart failure (Guo et al., 2020).Thus, ACEIs and ARBs can protect the cardiovascular system, but they also may make one more susceptible to SARS-CoV-2 infection.

How the Virus Multiplies

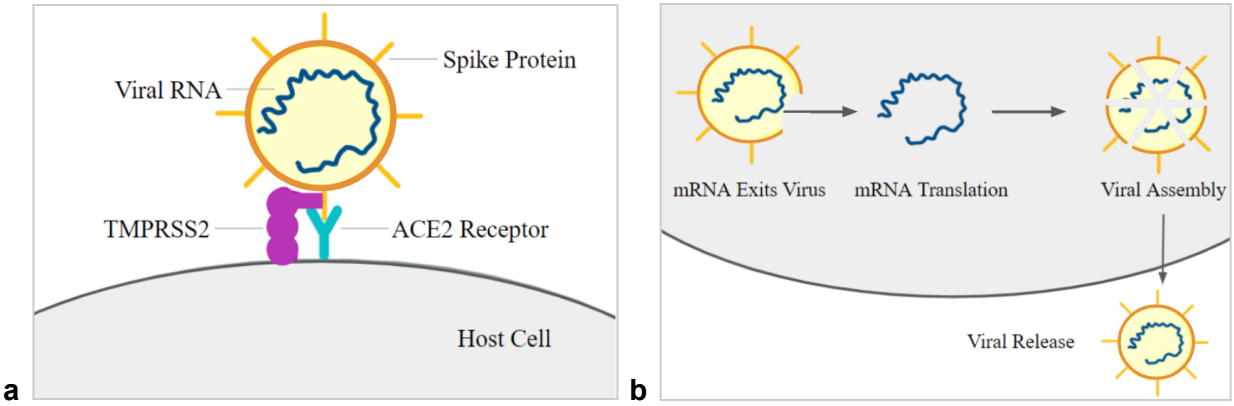
In order for the virus to be able to spread, the host must become infected. The first step is for the virus to reach an area where there are large amounts of the cell-surface receptor angiotensin-converting enzyme 2 (ACE2) (Datta et al., 2020). Using the virus's spike protein it has the ability to attach to the host cell. This is all happening in the S1 unit but the S2 unit is where the fusion peptide and transmembrane domains are located which are needed to fuse the viral and host membranes (Wrapp et al., 2020). However, the S2 unit can only be activated by being cleaved at the border between the S1 and S2 unit (Datta et al., 2020). This splitting is possible due to the enzyme transmembrane protein serine protease 2 (TMPRSS2) once the interaction between the ACE2 and spike protein occurs (Datta et al., 2020). Then the virus has just the S2 unit intact so it can end up fusing together both the membranes of the infected cell and the host cell so the infected cell RNA can be injected (Datta et al., 2020).

After the first host cell is successfully infected then multiplication can begin. The virus will use the host's natural translation machinery where ribosome frameshifting occurs creating the polyproteins 1a and 1ab (Nakagawa et al., 2016). Once these go through proteolysis and are

turned into smaller proteins they will later be used for replication and transcribing. To do this these proteins will connect with the RNA of the coronavirus which creates the replication but this new copy will have genomic RNA(-) compared to the original genomic RNA(+) (Nakagawa et al., 2016). If the genomic RNA(-) goes through replication it will end up creating a genomic RNA(+) which can be released to infect more cells (Nakagawa et al., 2016). However, the genomic RNA(-) can also go through a discontinuous transition where the RNA polymerase, which is responsible for transcribing, can start transcribing at random points creating different subgenomic mRNAs which are the codes for different proteins (Nakagawa et al., 2016). The translation in which these mRNA codes turn into viral proteins occurs within the Rough ER and will then be sent with a genomic RNA(+) to the Golgi Apparatus (Nakagawa et al., 2016). Once in the Golgi Apparatus the newly created virus will be packaged in a vesicle which will be released and have the ability to go and infect more host cells (Alberts et al., 2002).

Figure 3

Process of Host Cell Infection and Replication



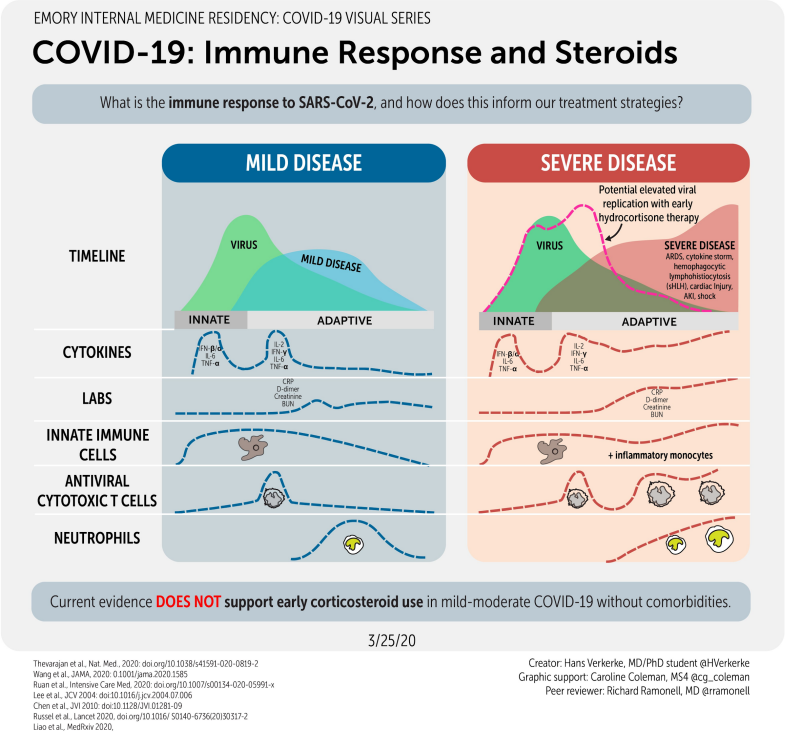
Cytokine Storm Associated with COVID-19

The cytokine storm, also called cytokine release syndrome (CRS), is a condition where cytokines, small secreted proteins that are heavily involved in intercellular communications, are

overproduced in response to microbial invasions in the body (Tisoncik et al., 2012; Zhang et al., 2007). The term was first used in 1993 in a paper about graft-versus-host disease, and it came to attention with the H5N1 ‘bird flu’ in 2005 (Tisoncik et al., 2012). It is now prevalent in the current COVID-19 pandemic and its sister diseases, SARS-CoV and MERS-CoV (Li et al., 2020). Cytokines are mainly produced by helper T cells (Th) and macrophages, and they play a crucial role in antimicrobial effector functions (Zhang & An, 2007, Mangalmurti & Hunter, 2020). In addition to their role in amplifying immune system response and increased production of immune cells such as neutrophils, monocytes, and T-cells, cytokines also regulate the immune response (Mangalmurti & Hunter, 2020). After the production of inflammatory cytokines -- IL-1, IL-2, IL-6, granulocyte-macrophage colony-stimulating factor [GM-CSF], interferon [IFN]γ, tumor necrosis factor [TNF], for instance -- a series of immunoregulatory molecules called anti-inflammatory cytokines including IL-4, IL-10, etc. are produced to keep internal organs from being damaged and maintain bodily homeostasis (Mangalmurti & Hunter, 2020, Zhang et al., 2007).

Figure 4

Normal and Pathogenic Progression of Immune Response



Note. Image created by Verkerke (2020).

When cytokines are overproduced in a cytokine storm, the resulting enhanced immune system activities and response cause collateral damage in the host (Mangalmurti & Hunter, 2020). Some hallmarks of the cytokine storm are fever, weight loss, joint and muscle pain, fatigue and headache (Mangalmurti & Hunter, 2020). When cytokine response crosses the normal threshold, inflammation progresses through the entire body, resulting in the loss of vascular tone, leading to decreased blood pressure, vasodilatory shock, and organ failure (Mangalmurti et al., 2020, Tisoncik et al., 2012). Cytokine IL-2 causes capillary leak syndrome, a condition where capillary permeability is increased and plasma leaks from blood vessels, and leads to hypotension, edema, acute respiratory failure, and kidney failure (Mangalmurti & Hunter, 2020). IL-1 causes disseminated intravascular coagulation (DIC) where blood clots throughout the body, which makes the host more susceptible to internal bleeding and abrasions. Another consequence of the cytokine storm is Acute Lung Injury (ALI), caused by the excess

pro-inflammatory cytokine IL-1β signaling increased mononuclear/ neutrophilic response (Tisoncik et al., 2012). In a cytokine storm, the extreme lung inflammation also influences other organs and can progress to Acute Respiratory Distress Syndrome (ARDS), which results in increased permeability and fluid in lungs, leading to respiratory insufficiency. This was shown in SARS-CoV and influenza, and might be worse for COVID-19 since the primary binding enzyme ACE2 for SARS-CoV-2 is located on the epithelial cells of the lungs (Tisoncik et al., 2012, Coperchini et al., 2020). IL-10 cytokines are produced in response to the cytokine storm (coined 'immunoparalysis') but it has been suggested that patients who survive the cytokine storm might die from immunoparalysis because of its constant downregulation of immune cells (Tisoncik et al. 2012).

In COVID-19, high levels of ARDS are observed in severe cases along with sepsis, pneumonia, pancreatitis, and blood transfusion (Coperchini et al., 2020). COVID-19 patients also show high levels of pro-inflammatory cytokines and chemokines, a type of cytokine that plays a role in immune cell recruitment: patients admitted to ICU had higher levels of chemokine CXCL10, CCL2 and cytokine TNFα, IL-6 (Huang et al, 2020., Coperchini et al., 2020). Viral infections are linked to interferon (IFN) levels, which are involved in rapid and efficient response against viruses (Coperchini et al., 2020). Chemokines CXCL10 and CXCL8 are key players in respiratory infection response (Coperchini et al., 2020). While increased immune response is usually a good sign for the host, it is suggested that SARS-CoV-2 1) mimic the chemokine system to cause signals that lead to disorganized immune response and 2) inhibit antiviral response by impairing the receptor system and modifying intracellular RNA sensors (Coperchini et al., 2020). SARS-CoV-2 interference combined with overproduced cytokines and lower numbers of T-lymphocytes (CD4+ and CD8+) could result in increased inflammation and collateral organ damage to the whole body, leading to death in severe cases (Coperchini et al.,

2020, Chen et al., 2020). The detrimental effects of the cytokine storm might suggest anti-cytokine therapy and interleukin (IL) inhibitors as possible ways to ameliorate COVID-19 symptoms (Il-6 inhibition in animals SARS-CoV showed decrease in mortality) (Coperchini et al., 2020). This, however, should be done cautiously since it might interfere with normal antiviral responses (Mangalmurti et al., 2020).

COVID-19 Pathogenesis

The novel coronavirus has devastating effects on the body, beginning in the patient's lungs. From there, the virus may have the ability to spread to other organs, such as the stomach, liver, and kidney (Zhang et al., 2020; Diao et al., 2020). COVID-19's effect on the body can be divided into the same three phases that WHO associated with SARS in 2006: the "viral replication phase," the "immune hyper-reactive phase," and the "pulmonary destruction phase" (WHO, 2006). During viral replication, the virus uses ciliated epithelial cells in the respiratory tract to make copies of itself (Lukassen et. al., 2020). In the second phase, the immune system jumps into action to face the detected threat of the virus, potentially unleashing a cytokine storm (Li et al., 2020). Cytokines are small proteins that are released by the immune system that can affect the signaling between cells, making them vital in the process of recruiting immune cells to the site of infection and other inflammatory responses (Zhang & An, 2007). Although these proteins are extremely important in cellular communication, the steep influx of cytokines being released into the bloodstream can have adverse effects. The immune response in a cytokine storm is unregulated and is associated with immune cells that target not only the infected cells, but healthy tissue as well in an uncontrolled effort to eliminate the virus (Li et al., 2020). A cytokine storm in the lungs can cause severe inflammation, which helps break down the functionality of the lungs even further by weakening blood vessels, which allows fluid to spill in through the alveoli (Tisoncik et. al., 2012).

It has also been hypothesized that one reason why COVID-19 is associated with damage to organs elsewhere in the body, such as the stomach, kidney, and liver, is that the bloodstream allows

for the virus to travel efficiently (Zhang & Zhang, 2020). Nearly half of patients that have been hospitalized have damaging enzyme levels in their liver (Zhang & Zhang, 2020), and kidney impairment is common in the most severe COVID-19 cases (Diao et al., 2020). Additionally, the lower gastrointestinal tract has an abundance of ACE2 receptors (Wong, 2016), which suggests that the virus could also target that area as a replication site. During the pulmonary destruction phase, bronchopneumonia or diffuse alveolar damage, which can lead to death (Menter et al., 2020). The virus, although very dangerous in itself, is not always the sole killer, as the immune response to the disease can be more dangerous than the culprit to which it reacts.

Prevention and Therapeutic Approaches

Research teams and universities around the world are working towards the development of more than 90 different vaccines (Callaway, 2020). Different approaches are being taken to safely present antigens for SARS-CoV-2. Some have employed strategies that have been successful when treating other epidemic viruses like polio and Ebola, while other researchers are looking to take advantage of modern technology and novel approaches to create a new type of vaccine for SARS-CoV-2 (Callaway, 2020).

At least seven research teams are developing vaccines using an attenuated or inactivated form of the virus itself. This is similar to other successful vaccines that have used live attenuated viruses, such as those used to treat measles and polio in the past (Griffin, 2018; Cossart, 1977). However, they face a downfall concerning the potential side-effects of the vaccine. It is possible for the attenuated form of the vaccine to still develop a way to infect host cells and cause the vaccinated patient to contract the illness (WHO, n.d.). Because of this risk, these types of drugs require extensive safety tests. Sinovac Biotech, based in Beijing, China, has recently begun testing the efficacy and safety of an inactivated version of SARS-Co-V-2 in humans (Held & Mak, 2020). Codagenix, based in New York, is currently collaborating with the

Serum Institute of India to make a weakened form of SARS-CoV-2 by altering its genetic code (Codagenix, 2020).

Approximately 25 research groups are developing viral-vector vaccines to combat COVID-19 (Callaway, 2020). This type of vaccine uses a different virus, such as measles or adenovirus, that is genetically engineered to produce coronavirus proteins, like the characteristic spike protein (Callaway, 2020). Unlike live attenuated vaccines, these vaccines tend to use viral vectors that are already proven safe, having been genetically engineered to eliminate pathogenicity and used in other vaccines (Ura et al., 2014). However, they still can typically elicit an immune response strong enough for sufficient antibodies to be produced (Ura et al., 2014; Callaway, 2020). However, existing immunity to the vector virus used could weaken the desired immune response (Callaway, 2020). The U.S.-based company Johnson & Johnson is currently utilizing this approach in hopes of creating an effective vaccine (Lovelace, 2020).

One of the simplest strategies being tested is protein-based vaccines. Researchers are looking to directly inject patients with the SARS-CoV-2 S protein, and other proteins that mimic the outer coat of the virus (Callaway, 2020). This would directly allow the immune system to act on the antigens and create specific antibodies, without the intermediate of human cells having to transcribe and translate DNA and RNA fragments (Francis, 2018). Nearly 30 groups are utilizing this viral protein subunit strategy, and most are focusing on the receptor binding domain of the spike protein (Callaway, 2020).

The rate of vaccine development in response to the COVID-19 pandemic is unprecedented. Due to the severity of the pandemic, national governments, international bodies, and private firms have all realized the importance of the vaccine, and have invested billions of dollars into its development (Brennan, 2020). With more being discovered about the virus every day and researchers accelerating the speed of their work, experts believe a vaccine

is likely able to become widely available to the public by mid-2021, merely 12 to 18 months after the virus first appeared (Gallagher, 2020).

Remdesivir

On April 10, 2020, a compassionate-use study provided tentative evidence that the use of Remdesivir (RDV), an antiviral developed for Ebola patients (Grein et al., 2020). The study was open-label, it included data from only 53 patients, and there was no clearly comparable group, either within the study or in any other study, that could be used as a control (Grein et al., 2020). However, researchers remarked that mortality seemed relatively low at 13 percent, given the baseline condition of the patients (Grein et al., 2020). This result was just one of multiple early indications that the drug could be effective in COVID-19 treatment (Tawfiq et al., 2020).

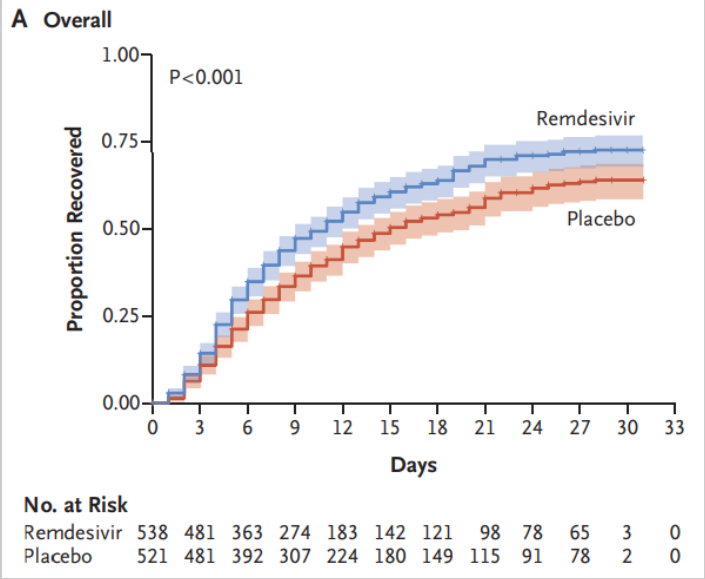
Researchers had provided theoretical support for the possibility that RDV could act against coronaviruses even as it was being developed for Ebola in early 2017 (Siegel et al., 2017). RDV was in fact hoped to slow replication of a variety of viruses because it is a nucleotide analogue that can be incorporated into viral RNA, thereby terminating the RNA chain (Gordon et al., 2020). As a prodrug, RDV must first be metabolized to produce a triphosphate, and then it is effective because it is selective for viral RNA polymerases in comparison with host polymerases (Siegel et al., 2020). It has been found that RDV triphosphate is in fact more optimal for the SARS-CoV-2 RNA polymerase than for the Ebola virus RNA polymerase by some measures, meaning that it can be incorporated more easily into SARS-CoV-2 RNA (Gordon et al., 2020). After incorporation, the RNA chain terminates within 3-5 nucleotides of RDV (Gordon et al., 2020).

Because of the existing clinical and theoretical evidence that RDV could be a successful treatment for COVID-19, multiple randomized, double blind, placebo-controlled trials have begun to test its efficacy (Tawfiq et al., 2020). One such study, published by Wang et al., failed

to find conclusive evidence that use of RDV was associated with faster clinical improvement than placebo (2020). Furthermore, the hazard ratio for clinical improvement calculated in that study was only 1.23, favoring RDV over placebo, although the 95 percent confidence interval for that result was wide (Wang et al., 2020). In his assessment of that result, Norrie has emphasized that 1.23 is lower than the anticipated hazard ratio, and suggested that the clinical significance of that result is questionable (2020). However, he also emphasized that the result was inconclusive (2020). More recently, a preliminary report from a larger study called ACTT-1 has concluded that RDV is associated with a faster recovery than placebo, with P<0.001 (Biegel et al., 2020). However, these data were not sufficient to conclude that RDV had an effect on mortality (Biegel et al., 2020).

Figure 5

Proportion Recovered Versus Time, ACTT Preliminary Report



Dexamethasone

For reducing mortality, dexamethasone has been found in a preliminary report released by RECOVERY to be effective at a 0.001 confidence level (Horby et al., 2020). The difference

between the effect of dexamethasone and the effect of remdesivir is well-defined: unlike remdesivir, dexamethasone was found to reduce deaths by approximately one-third, but to result in only a small reduction in time before release from the hospital (Horby et al., 2020). In response to this result, dexamethasone was hailed in the BMJ as the second “evidence based treatment” to become available for COVID-19, after Remdesivir (Johnson & Vinetz, 2020).

That assessment was not unjustified. Although the trial was open-label, its primary endpoint was 28-day mortality -- a metric that is unlikely to be strongly affected by lack of blinding (Horby et al., 2020). The fact that the trial was randomized, with 2104 patients receiving dexamethasone and 4321 receiving usual care, gives credence to the conclusion that administration of dexamethasone reduces 28-day mortality for patients receiving oxygen -- especially severely afflicted patients receiving invasive mechanical ventilation (Horby et al., 2020).

Dexamethasone, a glucorticosteroid, can be described as less targeted than remdesivir (Horby et al., 2020). This is because it treats inflammation, a dangerous symptom of severe COVID-19, instead of affecting the virus directly (Horby et al., 2020).

Moderna as a Leading Vaccine Candidate

One of the first vaccines to begin clinical trials was engineered by the company Moderna, based out of Cambridge, Massachusetts, in collaboration with the United States Institute of Allergy and Infectious Diseases (Callaway, 2020). This RNA-based vaccine consists of a segment of synthetic viral mRNA that codes for the coronavirus’s spike glycoprotein (Hussey, 2020). Upon entrance to human cells, the synthetic mRNA is translated by host ribosomes, resulting in the formation of the spike protein (Hussey, 2020). The immune system then has the opportunity to produce antibodies that are specific to the spike protein (Callaway, 2020).

The effects of the vaccine were first analyzed in animals samples, specifically mice. The SARS-CoV-2 virus, which naturally is unable to infect rodent cells, was genetically modified to let it attack mouse cells (Callaway, 2020). After the mice were infected, the vaccine was administered, and resulting data revealed that the spread of the lung infection was halted by vaccine (Callaway, 2020). This was an important indication that the RNA based vaccine was potentially able to prevent infection in human cells.

On May 18, Moderna revealed data from their first human trials which indicated that their vaccine was able to trigger an immune response in human patients (Hussey, 2020). After receiving two doses of the vaccine over the course of 43 days, all participants in the study had SARS-CoV-2 antibody levels consistent with patients who had recovered from the actual infection (Hussey, 2020). Adding to the promise of this being a safe vaccine is the fact that only 1 of the patients experienced a mild reaction (self-correcting redness around the site of injection) following the second dose (Hussey, 2020).

The company has quickly advanced to Phase 2 clinical trials, consisting of 600 participants (Forbes, 2020). This Phase 2 study is evaluating the safety, reactogenicity, and immunogenicity of the two-dose RNA vaccine (Forbes, 2020). In the near future, Moderna also hopes to begin their Phase 3 study, which will test the efficacy of the vaccine in preventing symptomatic COVID-19 disease and the prevention of severe cases of COVID-19 that require hospitalization (Hussey, 2020).

Given the early success of Monderna's RNA vaccine along with the rapid progression of each phase, it is plausible that it will be commercially available as early as 2021 (Callaway, 2020). Data about the efficacy of the vaccine is expected to be received by late November, which will allow the company to petition approval of the vaccine by late this year or early next year (Hussey, 2020). Moderna explains that it is on track to deliver between 500 million and 1

billion doses annually (Mishkin, 2020). However, the public release of the vaccine is mostly dependent on its approval by the U.S. Food and Drug Administration, and on the regulations set in place by national and international governing bodies.

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