A Review of SARS-CoV-2 Virology, Immunology with focus on Macrophages, T cells and Lymphopenia, Pathology and Symptoms

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Abstract

The common coronavirus family of single-stranded RNA viruses (+ssRNA) can be isolated in different animal species. However, unlike many of its family, SARS-CoV-2 is highly virulent and also lethal to certain age groups, causing its declaration as a pandemic by the World Health Organization in March 2020.[1] This review of the SARS-CoV-2 is a compilation of research on its virology, proteins, effects on immunology with a highlight on alveolar macrophages, T cells and lymphopenia, pathology, and symptoms. Thus, when faced with SARS-CoV-2, patients who were immunocompromised prior to infection are most severely affected, as they have lowered T cell count. The T cells respond to the infection by releasing a "cytokine storm", which has a debilitating effect on the lungs. Furthermore, long ICU stays lead to T Cell exhaustion, which reduces T Cell function, making risk of death higher. This exhaustion of T and B cells causes lymphopenia.[2] However, for patients who were healthy prior to infection, several studies have shown that the CD4+ T cells were especially responsive to the viral spike glycoprotein, and almost all COVID-19 patients develop some degree of antibodies to SARS-CoV-2 as a result of T-cell activation. Few studies also suggest that a majority of the unexposed population already has T_H and some T_c Cells that target SARS-CoV-2 proteins, including but not limited to, Spike proteins, Membrane proteins, and Nucleocapsid proteins.[4,5] However, scientists still do not have a definitive answer to the paradox between recovering patients' decreasing antibody count, and the few cases of reinfection. This review will attempt to find this answer, or answers, by compiling internationally sourced research efforts.

T Cell immunity may be the answer to the paradox between convalescing patients' weekly decreasing antibody count, and the rare cases of reinfection. A hypothesis for the emergence of this T Cell immunity is that people who have been infected by less dangerous coronaviruses in the past still carry T cells that recognize particular protein regions that have similar homologies to the newer SARS-CoV-2.

However, T Cell immunity is activated by alveolar macrophages, which are the first cells to be infected by SARS-CoV-2 due to their expression of ACE2 receptors on their cell surfaces. If macrophage function is inhibited, then few T helper cells will be activated, leading to low B cell activation and impaired adaptive immunity.

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Introduction:

According to the World Health Organization (WHO), viral diseases continue to emerge and represent a serious issue to public health. In fact, in the twenty-first century alone there have already been records of viral epidemics including the severe acute respiratory syndrome coronavirus (SARS-CoV) from 2002 to 2003 involving two dozen countries with approximately 8000 cases and 800 deaths, the H1N1 swine influenza in 2009, and the Middle East respiratory syndrome coronavirus (MERS-CoV) identified in 2012 with has approximately 2,500 cases and 800 deaths, and still causes sporadic cases as of 2020.[6]

In late December 2019, inexplicable cases with low respiratory infections were first detected in Wuhan, China. As they were unable to identify the causative agent, these first cases were classified as "pneumonia of unknown etiology." The cause of this illness, named COVID-19 in February 2020 by the WHO Director-General Dr.Tedros Adhanom Ghebreyesus, is caused by a novel virus of the coronavirus (CoV) family. COVID-19 is an acronym for "coronavirus disease 2019".

The novel coronavirus is highly virulent, as shown by its rapid spread; in a WHO meeting held on January 30, 2020, as it had already spread to 18 countries, with four countries reporting human-to-human transmission. On February 26, 2020, the first case of COVID-19 that was not transmitted from China was recorded in the United States.[7]

Initially, the new virus was called 2019-nCoV. Subsequently, the International Committee on Taxonomy of Viruses termed it the SARS-CoV-2 virus due to its 79.5% genetic similarity to SARS-CoV that caused the outbreak in 2003.[8]

The CoVs are a large family of single-stranded RNA viruses (+ssRNA) that can be isolated in different animal species. They are capable of crossing species barriers, causing disease ranging from the common cold, or the flu, to severe diseases like SARS. Interestingly, these latter viruses have probably originated from bats and then moved into other mammalian hosts — the Himalayan palm civet for SARS-CoV, and the dromedary camel for MERS-CoV — before jumping to humans. The dynamics of SARS-Cov-2 are speculated to have a bat origin.

The SARS-CoV-2 pandemic worldwide is a serious public health risk, as on February 28, 2020, the WHO raised the threat to the CoV epidemic to the "very high" level. As of January 15, 2021, 2.0 million deaths have been reported worldwide, out of 93.5 million reported cases.[9]

World governments are at work to establish countermeasures to stem possible devastating effects. Scientists are collaborating in a world-wide effort to collect information regarding transmission mechanisms, the clinical spectrum of disease, and develop new diagnostics, vaccines, and therapeutic strategies. Many uncertainties remain with regard to both the virus-host interaction and

when the epidemic will reach its peak, although projected death rates have been published in many countries, including Canada, where 28 790 COVID-19 deaths are projected by May 2021.[10]
At the moment, the therapeutic strategies to deal with the infection are only supportive, and prevention aimed at reducing transmission in the community is our best weapon. Several vaccines have emerged as preventative measures, but distribution is selective and slow in most parts of the world. The phrase "flatten the curve" corresponds to slowing the spread of infection as to give health measures more time to catch up. Aggressive isolation measures in China have led to a progressive reduction of cases, while other countries enforced quarantine measures as well.

The aim, therefore, is to collect information and scientific evidence and to provide an overview of the topic that will be continuously updated, specifically focusing on T cells and Lymphopenia. T cells play a number of important roles in the immune system. CD8 T cells' cytotoxicity is elicited in response to the binding of the CD8 T cell receptor to MHCI complexes on pathogen invaded cells. They kill virally infected cells. CD4 Helper T cells (T_H cells) are activated by MHCII complexes on macrophages, and some subtypes promote activation and proliferation of specific B cells.

Virology

General Virology
CoVs are positive-stranded RNA viruses with a crown-like appearance under an electron microscope (coronam is the Latin term for crown) due to the presence of spike glycoproteins on the envelope. The subfamily Orthocoronavirinae of the Coronaviridae family (order Nidovirales) classified into four genera of CoVs: Alphacoronavirus (alphaCoV), Betacoronavirus (betaCoV), Deltacoronavirus (deltaCoV), and Gammacoronavirus (gammaCoV). Genomic characterization has shown that probably bats and rodents are the gene sources of alphaCoVs and betaCoVs.[11]

Members of this large family of viruses can cause respiratory, enteric, hepatic, and neurological diseases in different animal species, including camels, cattle, cats, and bats. To date, seven human CoVs (HCoVs) — capable of infecting humans — have been identified. The most common human CoVs include HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63. These CoVs cause mild illnesses that many refer to as common colds, and cause respiratory infections.

SARS-CoV-2 belongs to the betaCoVs category. It has round or elliptic and often pleomorphic form, and a diameter of approximately 60–140 nm. Like other CoVs, it is sensitive to ultraviolet rays and heat. Furthermore, these viruses can be effectively inactivated by lipid solvents including ether (75%), ethanol, chlorine-containing disinfectant, peroxyacetic acid and chloroform except for chlorhexidine.[12]

In genetic terms, Chan et al. have proven that the genome of the new HCoV, isolated from a cluster-patient with atypical pneumonia after visiting Wuhan, had 89% nucleotide identity with bat SARS-like-CoVZXC21 and 82% with that of human SARS-CoV.[13] For this reason, the new virus was called SARS-CoV-2. Its single-stranded RNA genome contains 29891 nucleotides, encoding for 9860 amino acids, coding for 28 proteins (16 non-structured proteins (NSP)).[14]

Proteins

Virulence mechanisms of CoVs, and therefore also of SARS-CoV-2 have links to the function of the NSPs and structural proteins. For instance, ORF1ab is a chain consisting of 16 NSPs, which are cut by NSP3 to become 16 different proteins. NSP1 is able to block the host innate immune response. The protein NSP1 is a cellular saboteur that hijacks the cell machinery, preventing it from creating antiviral proteins while making more viral ones. Among functions of structural proteins, the envelope has a crucial role in virus pathogenicity as it promotes viral assembly and release. However, many of these features (e.g., those of NSP 2, and 11 have not yet been described. NSP3 is an untagging and cutting protein, which is a large protein which cuts loose viral proteins so they can perform their functions, and untagging proteins that have healthy cell tags for destruction, thus changing the balance of proteins, and possibly reducing the cell's ability to remove viral proteins. NSP4 is one of the proteins that build fluid-filled bubbles within the infected cells, where new viral parts are produced. NSP5 makes the majority of the cuts which free other NSP proteins to perform their own functions. NSP6 works with NSP3 and NSP4 to build bubbles. NSP7 and NSP8 assist NSP12 (RNA polymerase) to create copies of the SARS-CoV-2 genome. NSP9 infiltrates the nucleus, and is thought to influence molecule movement in and out of the nucleus. This may affect the host cell's mRNA transcripts that are exported from the nucleus. NSP10 and NSP16 work together to camouflage viral genes, as the hosT cells usually have antiviral proteins that find and shred viral RNA. NSP11 is an endoribonuclease associated with multiple functions, such as RNA processing and suppression of the infected host innate immunity system. The drug remdesivir is being tested as a treatment for COVID-19, as it is found to interfere with NSP12 in other CoVs. NSP13 unwinds intricately wound viral RNA so that NSP12, NSP7, and NSP8 can read the viral RNA and make proteins. NSP14 is the proofreader for NSP12; it cuts out incorrect nucleotides, and replaces them with correct ones. Scientists suspect that NSP15 cuts up leftover viral RNA to try to hide viral evidence in the cell. NSP16 works with NSP10 to help viral RNA to camouflage/hide from antiviral proteins that chop up viral RNA.[15]

Among the 4 surface structural proteins of CoVs (S, E, M, N), there are the spike glycoproteins composed of two subunits (S1 and S2). The S1 subunit is receptor binding, while the S2 subunit fuses with the host cell membrane. Spike proteins are grouped in 3, forming trimeric spikes on the viral surface, guiding SARS-CoV-2 to bind to host receptors ACE2 (Angiotensin Converting Enzyme II) on human type II pneumocytes and macrophages. ACE2 are surface enzymes that produce small proteins that normally cut the protein Angiotensin II into multiple pieces, serving as Angiotensin II regulator. ACE2 maintains blood pressure, aids with wound healing, and controls

inflammation and organ damage. The TMPRSS2 transmembrane protein serves to activate the S proteins on the SARS-CoV-2, priming it to bind to ACE2 receptors more efficiently. The SARS-CoV-2 spike proteins undergo an insertion mutation of 12 nucleotides, which increases the proteins' affinity for human receptors. Of note, in SARS-CoV-2, the S2 subunit — containing a fusion peptide, a transmembrane domain, and cytoplasmic domain — is highly conserved. Thus, it could be a target for antiviral (anti-S2) compounds. On the contrary, the spike receptor-binding domain presents only a 40% amino acid identity with other SARS-CoVs. The envelope (E) proteins help form the oily bubbles within the infected cells. The membrane (M) proteins make the SARS-CoV-2 membrane. Many nucleocapsid (N) proteins link together to form a spiral, wrapping and coiling the viral RNA, protecting the viral RNA and keeping it stable within the virus. Other structural elements on which research must necessarily focus on are the ORF3b that has no homology with that of SARS-CoV and a secreted protein (encoded by ORF8), which is structurally different from those of SARS-CoV. Meanwhile, ORF3a is another accessory protein that pokes a hole in the host cell membrane, allowing newly made viruses to escape. ORF3a is also responsible for inflammation of the lungs, one of the most lethal symptoms of COVID-19. Meanwhile, ORF6 blocks signals sent from the infected cells to the immune system, as well as blocking some of the infected cell's antiviral proteins. ORF7a is the virus liberator, which cuts down on the cell's tetherin supply (which the cell uses to pull escaping new viruses back to the cell), as well as inducing apoptosis in hosT cells, which leads to the death of lung cells. ORF8 is a mystery accessory protein. ORF9b blocks interferon, a key molecule used in antiviral defense. ORF9c and ORF10 are mystery proteins. ORF10 is unique to SARS-CoV-2.[16]

In international gene banks including GenBank, multiple SARS-CoV-2 gene sequences have been published. This gene mapping allows researchers to determine the phylogeny of the virus, and pinpoint the mutations that cause differences in strains. A spike protein mutation that likely occurred in November of 2019 triggered jumping of the virus to humans. In particular, Angeletti et al. compared the SARs-Cov-2 gene sequence with that of SARs-CoV. They analyzed the transmembrane helical segments in the NSP2 and NSP3 and found that position 723 presents a serine instead of a glycine residue, while the position 1010 is occupied by proline instead of isoleucine.[17]

Pathogenesis

The pathogenic mechanism that produces pneumonia seems to be particularly complex. Clinical and preclinical research will have to explain many aspects that underlie the particular clinical presentations of the disease. Available data seems to indicate that viral infection may induce an excessive immune reaction in the host. In some cases, a reaction takes place which as a whole is labeled a cytokine storm. The effect is extensive tissue damage. Interleukin 6 (IL-6) is a cytokine that triggers this storm. IL-6 is produced by activated leukocytes and acts on a large number of cells

and tissues. It is able to promote the differentiation of B lymphocytes, promotes the growth of some categories of cells, and inhibits the growth of others. It also stimulates the production of acute phase proteins and plays an important role in thermoregulation, in bone maintenance and in the functionality of the central nervous system. Although IL-6 mainly plays a pro-inflammatory role in COVID-19, it can also have anti-inflammatory effects. In turn, IL-6 increases during inflammatory diseases, infections, autoimmune disorders, cardiovascular diseases and some types of cancer. It is also implicated in the pathogenesis of the cytokine release syndrome (CRS) that is an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction.[18]

Effect on Macrophages, T cells and Lymphopenia

T cells represent an important component of adaptive immunity against SARS-CoV-2. Understanding the effects of SARS-CoV-2 on T cells is crucial for vaccine development, interpreting the pathogenicity of the virus, as well as for adjustment of pandemic control techniques.

T cells also provide a clear indicator of severe COVID-19 cases; T cells release cytokines into the bloodstream in response to pathogens. Lymphopenia occurs when the body's lymphocyte count (most consistently of T cells, but B cells counts are affected as well) drops drastically. Lymphopenia is largely a cause of high serum levels of pro-inflammatory cytokines such as TNF-α and IL-6 which are released into the blood by CD4+ T_H cells.[19] As aforementioned, these floods are named cytokine storms, which vastly increase disease severity and is often witnessed in patients in the ICU. Cytokine storms are also suspected to be closely linked with infection of alveolar macrophages by SARS-CoV-2, as the S protein of the virus directly binds to the ACE2 expressed on the surface of alveolar macrophages.[21, 22]

Since macrophages use their MHC II complexes to activate CD4+ T_H cells, if macrophage activity is inhibited, the body's adaptive immune response is inhibited very early on. Due to high expression of ACE2, the ligand for SARS-CoV-2's S protein, on macrophages including alveolar macrophages, macrophage function is greatly inhibited. This causes lower levels of activated T_H cells. Since fewer T_H cells are activated, T cell and B cell counts drop drastically, inducing lymphopenia and consequently leading to cytokine storms as available T_H cells attempt to stimulate the compromised immune system. Pro-inflammatory cytokines such as IL-6, when combined with other inflammatory mediators such as histamines, cause high fever, inflammation, severe fatigue and nausea. These are all common symptoms of lymphopenia. In severe cases of SARS-CoV-2, patients experience lymphopenia and exhibit these symptoms, as lymphopenia causes a hyperimmune response in the form of cytokine storms, causing pulmonary, heart, lung damage, and possibly multiple organ failure.

In mild cases of COVID-19, upon being activated by MHC I complexes on mature dendritic cells, CD8+ T cells increase clonal expansion, creating more CD8+ T cells. This reflects viral clearance due to the induction of virus-specific cytotoxic T cells, as is seen in influenza virus infection. Overall, these data support therapeutic strategies that target the myeloid cell compartment, such as IL-6 inhibitors, to treat COVID-19-associated inflammation.[23] Unfortunately, T cell exhaustion is another known effect of COVID-19, where T cells express higher than normal levels of cell death proteins. T cell exhaustion is known to correlate with increased disease severity and need for intensive care.[24]

Research shows that 70 to 100% of recovering COVID-19 patients have specific CD8+ and CD4+ T cells to SARS-CoV-2. T_H cell responses to the S protein, the main target of most vaccine efforts, were strong and correlated with the magnitude of the anti-SARS-CoV-2 IgG and IgA titers.[25] Notably, the Pfizer and Moderna anti-SARS-CoV-2 vaccines both use the S protein as targets.

The M, S and N proteins each account for 11-27% of total T_H adaptive immune response, with other responses mainly focused towards NSP3, NSP4, ORF3a and ORF8. S and M proteins were recognized by CD8+ T_C cells. Importantly, SARS-CoV-2−reactive CD4+ T_H cells were detected in ~40-60% of unexposed individuals, suggesting cross-reactive T cell recognition between previous 'common cold' coronaviruses and SARS-CoV-2. This gives us hope for immunity prior to infection.[26]

Pathology and COVID-19 Symptoms

Of the first 41 cases of laboratory confirmed infections with 2019-nCoV, all had viral pneumonia and almost a third of the patients developed acute respiratory distress syndrome (ARDS) requiring intensive care and 6 patients (14.6%) died. Many SARS-CoV-2 deaths are attributed to acute respiratory distress syndrome (ARDS), caused by the host body's natural immune response to the viral infection. Since SARS-CoV-2 binds to the ACE2 receptor, common on type II pneumocytes in the lungs, the viral infection leads to inflammation of the entire lung.[27] Normally, diffusion occurs between the alveoli and the pulmonary capillaries through very thin membranes. However, the inflammation causes fluid build-up between the membranes, creating a barrier preventing gas exchange from occurring. Furthermore, the pulmonary capillaries become more permeable due to histamines released as part of the acute inflammatory response to the viral infection. Proteinaceous liquid begins to leak from the capillaries into the alveolar space, further preventing oxygen and air from entering the bloodstream.[28]

On Jan 27, 2020, about 3000 cases were confirmed in China, and cases were also reported in Japan, South Korea, Thailand, Singapore, the United States, and Australia, all of which were exported from China.[29] However, the highly virulent SARS-CoV-2 quickly became a global pandemic, and by the end of 2020, over 90 million cases were confirmed, as well as over two million deaths

worldwide.[30] The true numbers are likely much higher due to lack of testing in some areas of the world. The fatality rate is currently around 2.5% worldwide, and this rate has decreased dramatically from the outset of the pandemic, as treatment methods and self-isolating measures have improved.[31]

The most common symptoms of SARS-CoV-2 are high fever, dry cough, and tiredness. Less common symptoms include headaches, sore throat, aches and pains, and loss of taste and smell. Severe symptoms include chest pains, difficulty breathing, and loss of speech or movement.[32] Perform a detection test if experiencing some symptoms. Emergency medical care should be sought out immediately if any severe symptoms surface.[33]

Conclusion

In sum, SARS-CoV-2 is the largest global pandemic ever recorded in human history, with the most cases and deaths. This review of SARS-CoV-2 virology, proteins, effects on immunology focussing on alveolar macrophages, T cells, and lymphopenia, pathology, and symptoms. Further research regarding T cell memory in response to viruses with similar homologies to SARS-CoV-2, and on drugs that suppress the effects of cytokines, are strong paths forward in terms of research in treatments for COVID-19.

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