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# Immunity against COVID-19: What Do We Know So Far? A Comprehensive Review on the Immunity Against COVID-19

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Review Article

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## ABSTRACT

The Coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has produce a major global health emergency. Various studies have tackled host immune responses to SARS-CoV-2 and offered new insights into the immune system's role in severe COVID-19 patients. A deep understanding of the immunopathogenesis of COVID-19 constitutes a solid platform to develop novel immunotherapeutic approaches for the treatment of patients infected with SARS-CoV-2. This review provides an update on host immune responses to SARS-CoV-2 infection and explores various immunological facets of both the innate and adaptive immunity. Data on important innate immune response elements are summarized, including cytokines, complement system, and potential evasion mechanisms. Information about the adaptive immune response against COVID-19 is also reviewed, including $CD4^+/CD8^+$ T cells, T cell exhaustion and T-cell epitope recognition in SARS-CoV-2 infected patients. Highlights on possible associations between impaired immune response and disease progression are alluded to. Various vaccine platforms and their effectiveness against emerging variants are described.

## INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) first appeared in December 2019 in the animal markets in Wuhan city of mainland China, as a result of a zoonotic transfer from bats to humans [1,2]. Following its rapid spread and threating outcome, the World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) as a pandemic on March 2020 [3]. As of December 2021, WHO has reported 266,504,411 cases of COVID-19 and 5,268,849 deaths [4]. COVID-19 is a respiratory disease and has exhibited heterogeneous transmission dynamics. Some cases resulted in no additional transmission while others displayed "superspreading events" and derived a large number of secondary infections. The clinical presentation of SARS-CoV-2 infection in humans ranges from asymptomatic to symptomatic with severe respiratory failure. Classical COVID-19 symptoms include fever, dry cough, severe myalgias, and fatigue. The incubation period is one to fourteen days. Dyspnea and pneumonia commonly develop about eight days after symptom onset while critical disease and death may occur at approximately sixteen days into the infection [5,6]. Clinical manifestations differ with age: older individuals with co-morbidities are at a higher risk of developing severe respiratory disease 3, whereas most young individuals are either asymptomatic or exhibit mild pneumonia. However, these features are not uniform and variations have been witnessed. The urgent need to control SARS-CoV-2 resulted in efforts being focused on developing antiviral drugs and channeling established agents towards the fight against COVID-19. Clinical trials have shown that certain established antiviral drugs such as remdesivir and fenofibrate possess antiviral efficacy towards COVID-19 and can reduce its corresponding inflammation. Other drugs such as ganciclovir, acyclovir, and ribavirin did not display any significant antiviral activity against the virus. Many vaccine candidates are being investigated and several have been approved and are being administered globally [7,8]. However, many challenges accompany the current COVID-19 treatment modalities. The continuous rise of COVID-19 variants with different genomes questions the function of the recently developed vaccines. The immunological effect of novel vaccines is expected to wean with time and their long-term efficacy remains to be doubt [9].

Understanding the body's immune response against COVID-19 and its mechanism of action of is crucial for the development of preventive and long-term treatment methods. This will allow an in-depth knowledge of the viral infection and the consequent eradication of the disease.

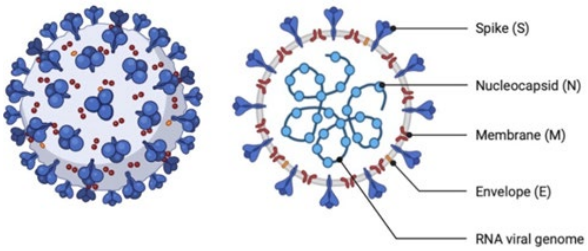
This review summarizes recent knowledge and provides a comprehensive update of the immunological aspect of SARS-CoV-2 infection, focusing on the functions and kinetics of the associated innate and adaptive immune responses. It also presents an overview about emerging SARS-CoV-2 variants, mutations in spike protein and potential mechanisms of immune escape.

## STRUCTURE, PATHOGENESIS AND TRANSMISSION OF SARS-COV-2

Seven strains of coronaviruses are known. Among these, four strains: 229E, NL63, OC43 and HKU1 infect the upper respiratory tract and generate mild symptoms. The remaining three strains: SARS-CoV-2, SARS-CoV, and middle east respiratory syndrome-related coronavirus (MERS-CoV) infect the lower respiratory tract and cause severe symptoms [10]. Coronaviruses are enveloped, single-stranded RNA viruses with a capped RNA genome ranging between 27-32 kb in size and 20 nm surface projections providing them with a crown shaped appearance [11-13]. Their diameter ranges between 60-140 nm. Four essential structural proteins are coded in one third of their

viral genome. The membrane protein M is the most abundant and central organizer interacting with all other coronaviral structural proteins. The nucleocapsid protein N is located on the endoplasmic reticulum region and aids in binding to the RNA genome, and possibly in virion formation. The enveloped protein E is the smallest protein and is commonly expressed in large amounts inside the infected cell. Small amounts of this protein are expressed on the virion envelope. The spike protein S aids in viral infectivity by allowing the attachment and fusion of the virus on the host membrane. Figure 1 illustrates the structure of the human coronavirus and its composites.

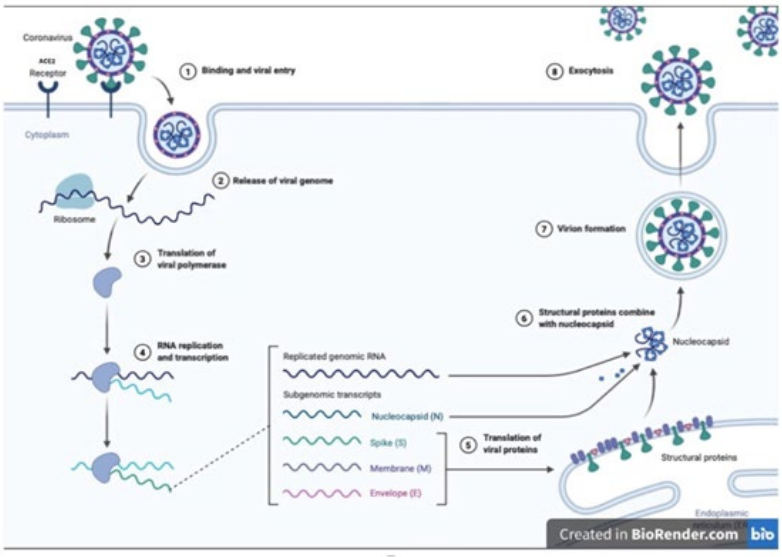
**Figure 1**. Structure of human coronavirus.




Two thirds of their genome in the first open reading frame (ORF 1a/b) code for sixteen non-structure proteins (NSPs). These proteins form the viral replicase transcriptase complex by rearranging membranes from the rough endoplasmic reticulum into double membrane vesicles. The virus recognizes and binds the receptor Angiotensin Converting Enzyme 2 (ACE2) through its crystal-structured receptor binding domain. The viral life cycle can be divided into three stages: entry, replication and exit. The entry stage is initiated by attachment to the host's cell surface followed by penetration. The virus then uncoats and sheds its capsid upon reaching the cytoplasm. Then, the naked viral genome is used for replication and gathered with the viral protein to form a virion and be released extracellularly. This constitutes the exit stage.

The spike protein of coronavirus has two functional units: S1, used for attachment on the host cell receptor through its Receptor Binding Domain (RBD) and for stabilizing the attachment of the second unit, S2, on the membrane. S2 is used for fusion with the host cellular membrane. It has been suggested that the functional ACE cellular receptor binds to the spike protein and allows viral entry in the host. The spike protein undergoes protease cleavage through lysosomal cathepsin L. Cleavage takes place at two sites, the S1/S2 site and the S2site. Importance of S1/S2 cleavage site might be due to its role in separating the spike protein's RBD and the fusion proteins. S2 uncovers the fusion peptides. The enzyme furin facilitates protein's entry. After that, translation of the replicase gene encoding for the ORF1a/b expressing terminal proteins pp1a and pp1ab occurs. RNA synthesis is the next step in viral life cycle, followed by translation of the structural proteins S, M and E. Virions are released through exocytosis with the help of transport vesicles (Figure 2). The virus then propagates through the respiratory tract. ACE2 are abundant in lung cells and regulate the Renin-Angiotensin System (RAS). Upon entry into the respiratory tract, the virus reduces the ACE2 expression affecting RAS the regulation. Consequently, blood pressure and electrolyte balance will be affected, leading to increased inflammation and vascular permeability in the respiratory tract. The rapid replication of SARS-CoV-2 in the lungs may trigger a strong immune response. The host immune system recognizes the whole virus or its surface epitopes, eliciting the innate or adaptive immune response.

**Figure 2.** The viral life cycle of SARS-CoV-2.




## INNATE IMMUNE RESPONSE IN SARS-COV-2 PATIENTS

The innate immunity is the body's first line of defense involved in the identification and the elimination of infections. Immune cells express specific Pattern Recognition Receptors (PRRs), able to recognize Pathogen Associated Molecular Patterns (PAMPs). PRRs stimulate a strong immune response to impede viral replication. Consequently, inflammation through downstream transcription factors causes the recruitment of proinflammatory cytokines and Interferons (IFN). This cascade of events manages to impede viral replication, stimulate immune cells of the adaptive immune response, and recruit other immune cells to the site of infection. Immune cells such as granulocytes, macrophages, Dendritic Cells (DC) and Natural Killer Cells (NK cells) are involved in the response against extracellular pathogens, while the Membrane Attack Complex (MAC) could help in lysis of infected cells. The complement system is also a major player in immune cell recruitment, activation and destruction of pathogens.

Currently, limited information is available concerning host innate immune responses against SARS-CoV-2. Reports revealed that pulmonary inflammation and lung injury lead to an increase in the levels of proinflammatory cytokines such as Interleukin 6 (IL-6), Interleukin 1 beta (IL-1β), Interleukin 12 (IL-12) and tumor necrosis factor alpha (TNF-α). This will lead to the activation of T-helper-1 (Th1) cells. Another report consisting of 99 COVID-19 patients showed elevated total neutrophils (38%), elevated serum IL-6 (52%), enhanced C-reactive protein (84%) and diminished total lymphocytes (35%). The neutrophil-to-lymphocyte ratio (NLR) is an established marker used in infections and systemic inflammation. COVID-19 patients displayed enhanced inflammatory responses in NLR marker. Furthermore, Sun, et al. showed peak neutrophil count and NLR as well as lower lymphocyte count in patients with severe COVID-19. Similarly, neutrophil infiltrates in COVID-19 patients were detected around pulmonary capillaries leaking into the alveolar space.

### Monocytes

Monocytes are essential players in the immune response to pathogens. Monocytes are classified into immature classical ($CD14^{++}CD16^{-}$), differentiated inflammatory transitional ($CD14^{+}$, $CD16^{+}$), and non-classical ($CD14^{-}$ $CD16^{++}$) subsets. Inflammatory transitional and non-classical monocytes were shown to migrate from blood to lungs in

patients with severe COVID-19 infection along with conventional DC ($CD1c^+$) subset. However, plasmacytoid DCs (CD123hi) and DCs ($CD141^+$) were depleted from blood but also absent in the lungs.

Because SARS viruses, especially SARS-CoV-2, employ ACE2 as a binding site, ACE2-expressing pulmonary macrophages may be able to penetrate lungs during SARS infection. Macrophages express ADAM17, a sheddase enzyme for the ACE2, as well as two other enzymes, TMPRSS2 and furin, involved in the exposure of the effusion and receptor binding sites of SARS virus on the S glycoprotein. Enhanced activity of pro-inflammatory macrophages in some COVID-19 patients may enhance the production of inflammatory cytokines and chemokines, among which CXCL10, leading to a "cytokine storm". Patients with chronic diseases like diabetes and Chronic Obstructive Pulmonary Disease (COPD) who were infected with COVID-19 and had increased alveolar macrophages showed higher mortality rates.

## Dendritic cells

Dendritic cells play a profound role in the inflammatory process due to their abilities to uptake, process, and present viral antigens to naïve T-cells. Reports revealed an elevation in the number of mature DC in patients' Bronchoalveolar Lavage (BAL), suggesting a possible role of these cells in the lung response to the SARS-CoV-2 viral infection. Previous studies showed that MERS-CoV was associated with elevated levels of RANTES, IL-12, IFN-γ and IP-10 through infection of monocyte-derived DC. MERS-CoV acquires entry through Dc-Dependent Dipeptidyl Peptidase 4 (DPP4), a protein strongly expressed by DC. Both SARS-CoV and SARS-CoV-2 generally employ ACE2 for cell entry and a comparison between the two strains is particularly relevant. Many studies showed that the SARS-CoV is able to use another DC receptor known as DC-SIGN, with a critical role in ACE2-mediated viral infection. Monocytes migrate into tonsils and draining lymph nodes within few days of infection and differentiate into DC. This provides a systemic dissemination of SARS-CoV. DC may also be involved in hypercoagulability in COVID-19 patients through thrombin-PAR-1 signaling. This interaction may induce the activation of DC, inducing their migration to form an amplificatory loop. This loop is involved in lung inflammation and hypercoagulation in COVID-19 patients with pneumonia.

## Natural killer cells

While NK cells are considered significant players in modulating the immune response, several studies have indicated a drop in their levels, mainly in severe COVID-19. NK cells exhibit an exhaustion phenotype by downregulation of markers such as LAG3, PDCD1 and HAVCR2. In contrast, reports revealed an increased expression of NKG2A; an inhibitory marker of NK cells that downregulates the levels of granzyme B, IL-2, IFNγ and TNFα. NK cell genes such as FCGR3A and FGFBP2 are also downregulated in COVID-19 patients inducing an inflammatory NK cells phenotype. Similar to DC, NK cells are also associated with the coagulation cascade in COVID-19 patients through the IL-1 *via* regulation of thromboxane-A2 levels. Recruited to the lungs by chemokines such as MCP-1 and IP-10, NK cells produce IFN-γ and TNF-α causing cytolysis through NF-κB-dependent ICAM-1 upregulation, which might explain the reduced function of NK cells in COVID-19 patients.

## The complement system

The complement system is a crucial player connecting the innate and the adaptive immune responses. Three complement pathways lead to the production of complement components (C3 and C5): mast cell and neutrophil activation by anaphylatoxins (C3a and C5a), the development of the Membrane Attack Complex (MAC), and opsonization. The activation of the complement proteins was previously reported in the pathophysiology of MERS-CoV and SARS-CoV. Gralinski, et al. studied the complement system in SARS-CoV-infected mice where C3 products, C3a and C3b were observed during the first day of infection. Wild type C3-deficient mice experienced reduced levels of lung injury, neutrophils, inflammatory monocytes, chemokines and cytokines. Mice were protected from MERS-CoV through anti-C5a antibodies. This might explain the antagonistic role of C3a and C5a, as a tool for treating severe life-threatening lung diseases. It has also been evident that levels of complement activation proteins such as plasma C5a and C5b-9 were higher in COVID-19 patients with moderate and severe disease. In COVID-19 patients, the complement system was shown to cause Acute Respiratory Distress Syndrome (ARDS) and hypercoagulability. The latter is identified through the elevated levels of activated Partial Thromboplastin Time (aPTT), fibrinogen, platelet count, factor VIII and protein C. This is accompanied with normal levels of Prothrombin Time (PT) and near-normal levels of antithrombin. COVID-19 patients had higher compliance and dead space ventilation, characterizing microthrombi. An amplifying loop might be also observed with elevated C-reactive proteins and TNF-α, which might induce C3 expression, sequentially initiating the activation of the complement and the coagulation state.

A malfunction in the complement cascade can result in “cytokine storm”, inflammation, and degeneration of alveolar lining cells. The “cytokine storm” exhibited a crucial role in the development of the most severe forms of COVID-19. A “cytokine storm” is a severe form of an immune reaction where the body releases increased cytokine levels at a faster rate. Macrophage-released IL-1 *via* inflammasome formation is the primary contributor in this process, detected in the most hard-hitting forms of COVID-19. The role of IL-1 is most apparent in context of clinical studies. In one case series, 8 out of 9 individuals with refractory COVID-19 pneumonia experienced improvement, whether clinically or by decreased circulating inflammatory markers in the blood, following treatment with Anakinra, a competitive IL-1 type 1 receptor antagonist. IL-1B, one of the major cytokines released during the COVID-19 related cytokine storm, has a systematic effect on the body, such as shock and organ failure. As previously mentioned, pro-inflammatory cytokines and chemokines released during the immune response evoked by COVID-19 are IFNγ, IL-6, MCP1, TNFα and IP-10. This demonstrates a T-helper 1 (Th1) cell mediated response. Thus, T cells and monocytes are recruited, among other immune cells, but not neutrophils. This response explains the lymphocyte depletion and the high neutrophil to lymphocyte ratio exhibited in the majority of COVID-19 patients. COVID-19 patients express increased levels of IL-6 throughout the course of their infection, and are at higher levels in non-survivors compared to survivors.

## SARS-COV-2 EVASION STRATEGIES OF TYPE 1 IFN RESPONSE

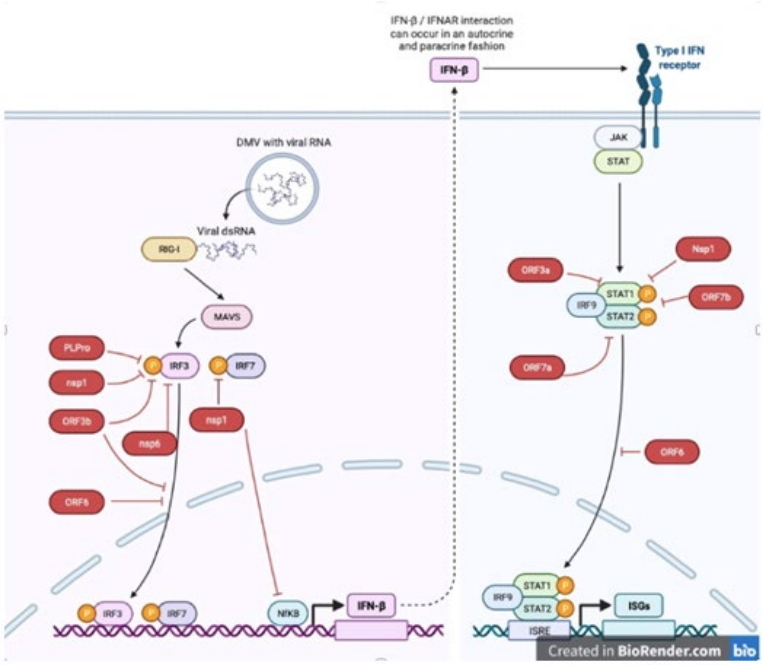
IFN type 1 response with its downstream cascade is a crucial factor for effective innate immunity against viral replication and the onset of adaptive immune responses. IFN-1 stimulates many functions, such as transcription factor binding, Ifn-Stimulated Genes (ISGs) expression, viral clearance, and tissue repair. After the identification of PAMP by PRRs, such as Retinoic Acid-Inducible Gene I (RIG-I), the Caspase Activation and Recruitment Domain (CARD) of the RIG-1 binds to the CARD of the Mitochondrial Antiviral Signaling Protein (MAVS). MAVS then activates

TANK binding kinase 1, which in turn phosphorylates the Interferon Regulatory Factor3 (IRF3) needed for IFN-1activation. Overexpression of IFN-1 can lead to deleterious inflammatory diseases in the host. Research efforts focused on the relationship between SARS-CoV-2 and IFN-1. Studies were conducted on chicken, mice models and patients, and revealed a reduced IFN-response induced by SARS-CoV-2 in comparison to other respiratory RNA viruses. Replication and entry of SARS-CoV mediated by S protein are restricted by the IFN-inducible transmembrane proteins. A comparable effect may be exhibited on SARS-CoV-2. Consequently, SARS-CoV-2 has cultivated many mechanisms to avoid the anti-viral properties of type 1 IFN.

SARS-CoV-2 employs an armamentarium of evasive mechanisms, several of which are in its ORFs. ORF9b interacts with MAVS through its association with the mitochondrial import receptor Tom70 to evade IFN-1 similar to the mechanism used by SARS-CoV. ORF3b inhibits IFN induction while ORF3a inhibits IFN-α signaling through prevention of STAT1 phosphorylation. ORF3a has also a PDZ-binding motif allowing regulation of cell signaling and use of lysosomal deacidification to reach plasma membranes. ORF6 of SARS-CoV-2 inhibits the IFN-β production through binding to karyopherin α, preventing the nuclear translocation of IRF3. It also blocks STAT1 nuclear translocation through amino acids 53-61 on its C terminus. In addition, ORF7 assists SARS-CoV-2 in evading the immune IFN-1 response. ORF7a inhibits STAT2 phosphorylation whereas ORF7b inhibits STAT1 and STAT2 phosphorylation, with both leading to ISGs transcription inhibition. ORF8 inhibits IFN-β promoter activation in a dose-dependent fashion and downregulates MHC-1. Another mechanism used by SARS-CoV-2 to evade immune responses includes NSP. Several studies have highlighted NSP1's role in binding to the 18S rRNA in the mRNA entry channel of the 40S subunit in SARS-CoV to inhibit mRNA translation. Banerjee, et al. hypothesized that a similar mechanism is observed in SARS-CoV-2 since its NSP1 shares 85% similarity with the SARS-CoV NSP1. Thus, when infected ISGs were stimulated by IFN-β with NSP1, the innate immune system was suppressed through the interaction between NSP1 and 18S rRNA. Moreover, NSP3 plays a role in evasion through IFN-β activation delay, providing additional viral replication time. In SARS-CoV, NSP3 blocks IFN-1 immune response through its Papain-Like Protease (PLpro) domain used in cleaving the replicase polyprotein. It also interacts with the Stimulator of Interferon Genes (STING) and obstructs TLR7-mediated cytokine induction. A similar mechanism involving PLpro interaction with the STING and TLR7 signaling pathways occurs in SARS-CoV-2. Concerning the NSP5, a recent study proved its role in inhibiting innate antiviral immunity through its restriction of histone deacetylase 2 nuclear translocation. ACE-2 mediated cells displayed enhanced ability of entry due to the previous mentioned mechanism. SARS-CoV-2 NSP6 inhibits IRF3 activation through its interaction with the TANK-binding kinase 1, but does not affect its phosphorylation. NSP7 and NSP8 in SARS-CoV-2 are like those found in SARS-CoV. They form a complex enhancing viral genome replication, with both NSP14 and NSP12. NSP8 regulates transcription of the viral genes through its interaction with the ribonucleoprotein 7 (LARP7). Even though NSP12 interacts with the complex to prevent IFN-β activation, it stimulates ISRE and ISG56 in immune responses. Banerjee, et al. showed that NSP8 and NSP9 bind to 7SL of the Signal Recognition Particle (SRP) in order to suppress membrane protein trafficking in cells infected with SARS-CoV-2. This in turn suppresses IFN immune response. Another complex, formed between NSP10 and NSP16, is involved in the methylation of the 5′guanosine viral cap, allowing the virus to escape detection and immune responses. NSP14 inhibits IFN-β production through IRF3 nuclear localization prevention and aids in viral replication and prevention of degradation by acting like a (guanine-N7)-methyltransferase. NSP15 is an endoribonuclease responsible for cleaving the 5′-polyuridines of the negative-sense viral RNA, leading to

antagonistic effects against IFN-1 function. The SARS-CoV-2 uses NSP15 and NSP13 to antagonize IFN induction through the binding of these NSPs with TBK1 and the TBK1 activator Ring Finger Protein 41 (RNF41)/Nrdp1. TBK1 phosphorylation is inhibited in this case thereby decreasing IFN production and IRF3. This is displayed and summarized in Figure 3. Using autocrine and paracrine signaling, IFN-1s are key players in the restriction of viral replication and spread. Coronaviruses usually target IFN responses for dysregulation as part of their effective immune-modulatory strategies. As summarized above, this armamentarium allows SARS-CoV-2 replication furtively within host cells during the incubation period, without triggering detectable IFN responses, thus leading to high viral loads. This ability of evading the innate immunity in the first 7-10 days of infection causes widespread inflammation.

**Figure 3.** SARS-CoV-2 action on type 1 IFN-response.




## TYPE I IFN SIGNALING DEFECTS, ANTI-IFN ANTIBODIES AND SEVERE COVID-19 INFECTIONS

Immunodeficient individuals have a higher risk of severe SARS-CoV-2 infection. Zhang, et al. performed a genetic study of 659 individuals with life threatening COVID-19 pneumonia and investigated. Their data indicated that at least 23 out of 659 patients harbored genetic defects at eight loci. The latter are associated with Toll-like receptor signaling molecules or the type I IFN pathway identified in cases of severe influenza and other viral infections. At the mentioned loci, about 3.5% of SARS-CoV-2 patients had loss-of-function variants underlying autosomal-recessive or autosomal-dominant deficiencies. However, Povysil, et al. observed no enrichment of predicted loss-of-function variants in genes involved in the type I IFN pathway predisposing to severe disease. Therefore, additional studies were needed to substantiate the hypothesis of a genetic immune predisposition to severe COVID-19, and to highlight the importance of experimental design when implicating a monogenic basis for severe disease.

In addition to congenital errors, auto-Antibodies (auto-Abs) against type I IFNs can be a risk factor of life-threatening COVID-19 pneumonia. Some authors compared auto-abs against type I IFN in patients with life-threatening SARS-

CoV-2 pneumonia, asymptomatic or mildly affected SARS-CoV-2 individuals and healthy controls. Results showed that 13.7% of patients with life-threatening COVID-19 harbored auto-Abs against type I IFN, mostly against IFN-α2 and IFN-ω, with most showing neutralizing capacity *in vitro*. Such antibodies were only detected in 0.3% of healthy individuals. Among those patients, males account for the vast majority. Moreover, 49.5% of patients with auto-Abs were over 65 years old. These findings suggest that gender and aging may bring on an increased frequency of auto-Abs. Future studies should elucidate the reason and mechanism of auto-Abs production. Identification of other immune defects in patients with severe COVID-19 pneumonia will provide essential clues for the determination of clinical treatment.

## THE ADAPTIVE IMMUNE RESPONSE TO SARS-COV-2

Following a viral infection, cell-mediated and humoral adaptive immune responses are facilitated through DC that can be either monocyte-derived DC or resident DC. After picking up viral antigens in the respiratory tract and moving into draining lymph nodes, DC led to T cell differentiation. Afterwards, T cells begin their various functions characterized by cell lysis, cytokine production and IgG antibody production.

### T-cell response

T-cell immunity against SARS-CoV-2 is not yet fully understood. Recent data highlighted the heterogeneity of $CD4^+$ T-cells subsets in inducing anti-viral immunity across patients with differing severity of COVID-19. In most cases, $CD4^+$ T-cells target highly expressed SARS-CoV-2 ORFs S, M, and N proteins through production of IL-2 and IFN-γ. This indicates a classical Th1 response activation. $CD8^+$ T-cells reacted mostly to S and N proteins, through co-expressing IFN-γ+ cells and granzyme B. When T-cell response was further assessed in COVID-19 patients with a mild, moderate and severe infection, a significant increase in the activation of circulating Tfh ($CD4^+$ $CXCR5^+$ $ICOS^+$ $PD1^+$) and Th1-polarised cTfh ($CD4^+$ $CXCR5^+$ $CXCR3^+$ $ICOS^+$ $PD1^+$) was shown in both moderate and severe patient groups compared to healthy controls. This demonstrates a specific contribution of Tfh cells in immune protection against the virus. Moreover, Th2/Th17-polarised subset ($CD4^+$ $CXCR5^+$ $CXCR3^-$ $ICOS^+$ $PD1^+$) was markedly activated, but only in severely infected patients. However, it has been shown that cTfh cells in hospitalized COVID-19 patients dampen the B cell response and reduce anti-spike antibodies levels early in the course of illness during hospitalization *via* dampening of germinal canter responses. Post-mortem examination of thoracic lymph nodes and spleens in those who had an acute SARS-CoV-2 infection demonstrated an absence of germinal centres and a stark reduction in Bcl-66 germinal centres. These findings emphasize the need of assessing the dynamics of the T-cell and B-cell response against the virus. Polyfunctional TH1 and TH17 cell were present in lower frequencies among reactive T cells, showing weak contribution of these two subsets. Therefore, the main mechanism through which T-cell immunity is achieved is through the production of Tfh cells against different strains. However, more studies are needed to assess the interplay of both T and B-cell immunity in fighting off the virus.

### T-cell exhaustion

Patients of various age groups and comorbidities with SARS-CoV-2 infection exhibited depletion in the $CD4^+$ and $CD8^+$ levels with some T cells potentially remaining in an exhausted or activated status. This was demonstrated in a study by Diao, et al. that revealed a drastic reduction in T-cell count and cytokine levels in patients with COVID-19.

As a result, poor survival was observed. In addition, T-cell exhaustion markers PD-3 and Tim-3 were higher in symptomatic and intensive care. However, T cell numbers and serum concentrations of TNF-α, IL-6 and IL-10 were inversely correlated. Subsequent study of $CD8^+$ T-cell exhaustion markers showed an elevated expression of PD-1, CTLA-4, and TIGIT. Immune responses characteristic of $CD4^+$ and $CD8^+$ T cells are usually detected against the viral main protease, nucleoproteins and the spike-RBD. $CD4^+$ T cells specific to SARS-CoV stimulate the release of IFNγ, TNFα and IL-2, thus exhibiting a Th1 cell mediated response. Depletion of these specific $CD4^+$ T cells delayed the viral clearance and enhanced the inflammation of the lungs in SARS-CoV-2 infected mice. SARS-CoV-2 showed a more pronounced decline of IFNγ levels compared to the SARS-CoV, as indicated by a small cohort performed on middle-aged COVID-19 male patients, whereby granzyme B and TNFα also dropped. Exhausted $CD8^+$ T-cells induced the expression of different activation and exhaustion markers such as CD38, CD25, PD-1, TIGIT, Tim-3, HLA-DR, and NKG2A. Reduced $CD4^+$ T-cells, accompanied with an increase in CD38 and HLA-DR activating markers, PD-1 and CD57 exhaustion markers were also observed in COVID-19 patients. Thus, T-cell exhaustion is one of the main mechanisms used by SARS-CoV-2 to evade the immune system. An expression of several exhaustion markers and a significant reduction of several inflammatory mediators such as IFNγ, TNFl-2, granzyme B and others are observed.

## T-cell epitope recognition in SARS-CoV-2

HLA antigen loci are usually associated with various disease susceptibilities and can determine survival rates. $CD4^+$ and $CD8^+$ T cell antigen receptors can identify the antigen peptides and antigen grooves of the aforementioned structures. Results showed that the mild group, having the least comorbidities demonstrated HLA with higher binding capacity to SARS-CoV-2 peptides than other groups thereby influencing the evolution of the disease. These results need additional evidence due to the limited sample size and the presence of confounding variables. In another study, $CD8^+$ T-cells were probed for reactivity against ORF1ab, S, N, M, and ORF3a proteins. Most recognized epitopes were located in ORF1ab and mostly N protein, while only 3 epitopes of 29 resided in the S protein. Genome-wide based screening identified epitopes for HLA A*02:01, the most common HLA in convalescent and healthy patients. 9 HLA A*02:01 SARS-CoV-2 patients were then screened for shared epitopes, where 6 regions were recognized. KLW, YLQ, and LLY were the most common epitopes identified among patients and at least one of them was recognized in all screened individuals. Remaining HLA alleles were tested and most patients recognized at least one of the three most common epitopes. This epitope mapping could improve vaccine production and enhance $CD8^+$ immunity. The most commonly recognized epitopes for $CD8^+$ T-cells were KLW, YLQ and LLY. Therefore, targeting these epitopes in vaccination is critical to strengthen and prolong $CD8^+$ immunity.

## B-cell response

The humoral response driven by B cells is pivotal in response to SARS-CoV-2 infection. B cell levels are elevated alongside with T helper cells in COVID-19 patients. This was demonstrated in patients receiving B-cell-depleting drugs, such as IL-6 blockers, obstructing the cytokine storm leading to respiratory failure in COVID-19. SARS-CoV induced B cell-responses against the nucleocapsid protein, followed by S protein, 4-8 days after the onset of symptoms, then leading to the development of neutralizing antibodies. Similar evidence was detected in COVID-19 pediatric patients, where 5 out of 6 patients produced IgM and IgG antibodies targeting the nucleocapsid and S proteins. Long, et al. reported that 19 days after onset of COVID-19 symptoms, all patients tested positive for

antiviral IgG antibodies. These antibodies targeted specific viral epitopes through B cell receptor sequencing, as well as the RBD. This will prevent the interaction with the viral ACE2 receptor and possibly inhibit re-infection 24 months post-infection. In addition, the titers of neutralizing antibodies against SARS-CoV-2 were associated with virus-specific T cells, particularly those specific for the viral nucleocapsid. However, the extent of long-term immunity against COVID-19 remains unclear. Asymptomatic individuals who tested positive for SARS-CoV-2 exhibited lower levels of IgM and IgG, in comparison to those with severe symptoms of the disease. Patients who recovered from COVID-19 displayed TFH, in addition to memory B cells and spike specific neutralizing antibodies against SARS-CoV-2.

It was reported that antibodies against SARS-CoV-2 are maintained for two months. Afterwards a great decline in antibody titers against the virus was reported in severe cases, but remained within the detection limit. During the acute phase of the disease, antibody levels were rising after the second and third week of the disease onset 128, with a peak of IgM and IgG on day 28 and 49, respectively. Zhao, et al. noted that seroconversion took place at median time of 12 days for IgM, 14 days for IgG, and 11 days for neutralizing antibodies. The SARS-CoV was previously shown to induce anti-S neutralizing antibodies that facilitate the infection of APCs and immune cells in FcR-expressing cells. The virus was associated with Antibody-Dependent Enhancement (ADE) pathogenesis, whereby antibodies generate lung injury after targeting the S protein through macrophage viral shedding and myeloid cell activation. Although no similar evidence is present for SARS-CoV-2, it is of a great interest to study this possibility when dealing with vaccine-induced humoral responses. Recent studies showed that spike-, RBD and nucleocapsid-specific B-cells were undetected in unexposed patients and were significantly elevated around 4-5 months post-symptom onset. Early post-infection was characterized by an equal representation of IgM and IgG antibodies, followed by a decrease in IgM and an increase in IgG alongside a stable, low frequency of IgA antibodies for 8 months. However, Gaebler et al. found that RBD-specific IgG and IgM/Pylon-IgG and IgM were most prominent at 1.3 months, along with nucleocapsid-specific (anti-N) IgG. Anti-RBD IgM, IgG, and IgA isotypes decreased in frequency between 1.3 and 6.2 months (53%, 32% and 15%, respectively), but IgG antibodies persisted 6 months post-infection. B-cells involved in immunity are IgG, IgM and IgA antibodies, with IgG lasting for up to 6 months post-infection. These antibodies mainly target S-, RBD and N-specific proteins. As for memory B-cells, additional research is needed to study their kinetics.

## IMMUNE ESCAPE, SARS-COV-2 VARIANTS AND SPIKE MUTATIONS

Multiple variants of SARS-CoV-2 have emerged and with some being classified as variants of concern by CDC. These variants affect the transmissibility, severity and immunity against the virus. Variants include the alpha (B.1.1.7), beta (B.1.351) 135, gamma (P.1), delta (B.1.617.2), and omicron (B.1.1.529). The alpha variant was first detected in the United Kingdom and exhibited 23 mutations with 17 amino acid changes. Such mutations include N501Y, D614G and P681H at the RBD of S protein. This variant is associated with higher viral RNA including Orf9b and its protein. Beta variant was first detected in South Africa and included 23 mutations and 17 amino acid changes such as K417N, E484K, N501Y, D614G and A701V. Gamma variant was first detected in Brazil and is characterized by 35 mutations and 17 amino acid changes such as K417T, E484K, N501Y, D614G and H655Y. While the delta variant was first detected in India and included L452R, T478K, D614G, P681R mutation. As for the omicron variant, it was first detected in South Africa and Botswana and includes a scary number of mutations such as A67V,

Δ69-70, T95I, G142D, Δ143-145, Δ211, ins214EPE, G339D, S371L, S373P, S375F, to name a few. Variants of interest are classified by the WHO as those that have predictable genetic changes and a threatening impact on transmission and prevalence. These include the Lambda variant (C.37) and the Mu variant () (B.1.621). D614G increased notably around April 2020, and assisted the virus in its infective and transmission properties. N439K is another substitution mutation, in the receptor-binding motif (RBM) that acts *via* increasing the binding affinity to ACE2 receptor. This will reduce the neutralization effects of monoclonal and polyclonal antibodies in the sera of recovering individuals. Like N439K, Y453F also enhances the binding affinity to ACE2 receptor. It was first discovered in Denmark where it was accompanied by an amino terminal domain (NTD) deletion. The K417T/N is responsible for the antibody escape characteristic of the virus, while the L452R mutation is associated with higher viral transmission and infectivity and a decrease in the neutralization by specific antibody therapies. E484 amino acid substitutions by K, Q, or P are main players in decreasing the viral neutralization by the immune system. Substitutions at the E484 residue occurred more frequently than at any other site and decreased the neutralizing ability of all four sera of convalescent Abs tested. These genomic variations increase the pathogenicity of SARS-CoV-2 by altering the essential immunological environment against the virus. Deletions in NTD are associated with SARS-CoV-2 evolution and mutations. Four recurrently deleted regions (RDRs), RDR1 (Δ69–70), RDR2 (Δ141–144 and Δ146), RDR3 (Δ210) and RDR4 (Δ243–244) have been recorded. RDR1, 2 and 4 correspond to loops N2, N3 and N5 while RDR3 corresponds to loop N4 and N5. E484K mutation associated with the Δ140 spike mutant resulted in a drop in the neutralization titer that was eventually abolished upon acquiring of an 11-residue insertion in the loop N5 between Y248 and L249. The aforementioned insertion led the RDR4 to acquire a glycosylation motif, with all these events leading to immune evasion. Disruption of disulfide bonds that are usually targeted by antibodies also play a role in immune evasion. In the NTD, mutations such as C136Y and S12P occurred in its signal peptide region and at cysteine residues 15 and 136, which in turn disrupt the disulfide bonds.

**SARS-CoV-2 vaccines:** The development of vaccines is imperative in absence of effective antiviral drugs to combat SARS-CoV-2 infection. Both humoral and cellular immune responses are involved in the clearance of the infection. To date, the most efficacious among the licensed vaccines are those with an ability to elicit antigen-specific antibodies and persistent memory B and T cell responses. The similarities between SARS/MERS and SARS-CoV-2 gave insight for the development of vaccines against the latter. Concerns regarding novel treatments and vaccines include Antigen-Dependent Enhancement (ADE). ADE occurs when the virus gains access to the host immune cell, causing infection through adherence to non-neutralizing antibodies, thus allowing the pathogen to enter the cell and hinder any immune response. Evidence shows that targeting S protein and its Receptor Binding Domain (RBD) can be effective in preventing viral attachments. It may enable an immune response by stimulating memory cells since it is found in different coronavirus strains exposed to humans. Recent evidence showed that $CD4^+$ cells of healthy individuals recognized and reacted upon exposure to the SARS-CoV-2 S protein 97. This suggests a cross reactivity between $CD4^+$ cells specific to SARS-CoV-2 and $CD4^+$ cells specific to previous coronavirus strains such as the SARS. The neutralizing antibodies are capable of binding and inhibiting viral infection of host cells. S1 unit has been shown to help in avoiding ADE. This displays that vaccines should be targeting certain epitopes in S protein inducing neutralizing rather than non-neutralizing antibodies.

According to the DRAFT landscape of COVID-19 candidate vaccines released in December 2020 by WHO, there were 52 candidates in clinical evaluation. The various vaccine candidates include whole virus vaccines, subunit vaccines and nucleic acid vaccines. The nucleic acid vaccines utilize nucleic-acid encoded antigens, which can promote both antibody and cell-mediated immune responses. Nucleic acid vaccines include both DNA and mRNA vaccines. DNA vaccines essentially contain a gene encoding the desired antigen. They are considered simple, safe, stable and relatively easy to produce as opposed to other methods. mRNA vaccines stimulate the immune system in a manner similar to a natural infection. They are better than inactivated vaccines due to their inability of reactivation. As compared to protein-based vaccines, mRNA vaccines are safer in terms that they do not carry the risk of contamination of *in vitro* Transcribed (IVT) mRNA by double stranded dsRNA through inaccurate IVT reaction. Protein vaccines would also require several years to purify; this renders mRNA vaccines more attractive. The first COVID-19 vaccine to be registered is the Sputnik V provided by Russia. This vaccine is based on the human adenovirus vector platform. (https://sputnikvaccine.com/about-vaccine/) Another is Moderna's mRNA-1273 which was administered to its first patient in January 2021. RNA-based mRNA-1273 vaccine is the first vaccine composed of a nucleoside-modified mRNA (modRNA) encoding a part of an S protein. It acts by stimulating a part of the S protein to induce an immune response. Oxford University conducted an interim analysis of AstraZeneca AZD1222 phase 3 trials which and it has been in use since September 2021.

Pfizer and BioNTech developed the mRNA-based COVID-19 vaccine candidate BNT162b2. Clinical trials showed a significant elevated rate of efficiency against COVID-19 28 days after administration of the first dose. This efficiency was consistent across age, sex, race and ethnicity. The first primary objective analysis focused on 170 cases of COVID-19, with 162 out of the 170 placed in the placebo group and 8 cases in the BNT162b2 group. An efficiency reaching 94% was recorded in individuals of 65 years or older. Results also displayed no side effects among 43,000 enrolled patients except for fatigue with a frequency of 3.8% and a headache of 2% frequency. The BNT162b2 was then approved by FDA on December 11, 2020. Johnson & Johnson issued their adenovirus Ad26COVs1 vaccine which is now available as a primary and booster dose. COVAXIN was developed by Bharat biotech and reported 78% efficacy 14 days post the second dose. Novavax is in the second phase III trial recruiting 30,000 volunteers in the US and Mexico. It is currently seeking emergency authorization from the FDA. The BBIBP-CorV Chinese vaccine that is usually referred to as the Sinopharm vaccine introduces dead version of the virus into the host's body over a two dose regime separated by two to three weeks. Phase III was done in 4 trials in countries such the UAE, Egypt, Jordan, Bahrain and Argentina, with the UAE reporting 86% efficacy of the following trials 170. Another COVID-19 vaccine candidate is Co-VLP. It is currently undergoing phase III clinical trials with over 30,000 subjects enrolled in North and Latin America, and Europe.

Despite the unprecedented rise in vaccines targeting SARS-CoV-2, this virus has developed ways to enhance its infectivity and transmission. Accumulation of several RBD mutations has led to evidence speculating possible evasion of the immune response and a negative impact on antibody neutralizing capacity. As such, many studies examined the immune response of Pfizer BNT-161b2 vaccinated individuals against different SARS-CoV-2 strains. IgA saliva antibodies appeared the same among vaccinated and uninfected individuals, unlike saliva IgG level which was elevated among vaccinated individuals' more than both infected and uninfected groups. More importantly, the alpha, beta and lambda variants were examined to compare the immune response of vaccinated, infected and

uninfected individuals. Both vaccinated and infected individuals had a similar response for the alpha, and lambda variant compared to the wild-type variant, while the South African strain had a reduced neutralization capacity for both infected and vaccinated individuals. The use of pseudoviruses with specific spike protein mutations was used to assess vaccine efficacy against variants. There was a slight but significant decrease in neutralizing activity against viruses with mutations in the RBD, specifically the E484K, N501Y and the K417N+E484K+N501Y triple mutant variants. However, a combination of B.1.1.7 spike mutations and E484K, showed decreased neutralization in patients receiving BNT162b2 vaccine. On the other hand, there has been a good efficacy of the ChAdOx1 nCoV-19 vaccine against the B.1.1.7 variant, but not against the B.1.351 variant. Emerging variants posed a challenge for the existing developed vaccines, making it necessary for further studies to be conducted in-order to increase the efficacy against the virus. Table 1 displays the different SARS-CoV-2 variants, their associated mutations and efficacy of the developed vaccines against them.

**Table 1.** The different SARS-CoV-2 variants, their associated mutations and vaccine efficacy against them.

| WHO label | Lineage | Amino acids altered | Date of designation | Pfizer-BioNTech (BNT162b2) | Moderna mRNA-1273 | Oxford AstraZeneca (ChAdOx1 nCOV-19) | Novavax (NVX-CoV2373) |
|---|---|---|---|---|---|---|---|
| Alpha | B.1.1.7 | 23 mutations with 17 amino acid changes ex. N501Y, D614G and P681H at the RBD of S protein | 19-Dec-20 | 89.5% reduction in infection;100 % reduction in severity | 100% effectiveness ≥ 14 days after second dose | 74.5% effectiveness against infection | 86.3% reduction in symptomatic disease |
| Beta | B.1.351 | 23 mutations and 17 amino acid changes ex. K417N, E484K, N501Y, D614G and A701V | 18-Dec-20 | 75% effectiveness against infection;100 % reduction in severity | 96.4% effectiveness after second dose | 10.4% effectiveness against mild-moderate infection | 51% reduction in symptomatic disease |
| Gamma | P.1 | 35 mutations and 17 amino acid changes ex. K417T, | 11-Jan-21 | 60% efficacy against symptomatic after partial vaccination; Mid 90% efficacy against severe | 77% efficacy against symptomatic infection | 48-50% efficacy against symptomatic infections after partial vaccination | Insufficient data |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | E484K, N501Y, D614G and H655Y | | outcomes after full vaccination | | | |
| Delta | B.1.617.2 | L452R, T478K, D614G, P681R mutations | variants of interest: 4-Apr-2021; variants of concern: 11-May-2021 | 87.5% reduction in symptomatic disease after second dose | 76% efficacy against symptomatic infection | 67% effectiveness against infection ≥ after second dose | Insufficient data |
| Omicron | B.1.1.529 | | 26-Nov-21 | 33% efficacy against symptomatic infection; 70% efficacy against hospitalization | 36.7% efficacy against infection ≥ 14 days post 2nd dose | No efficacy >15 weeks post dose 2 | Insufficient data |
| **Note :** *: 67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F | | | | | | | |

## CONCLUSION

COVID-19 is characterized by extensive inflammation and involves various tissues depending on the disease severity. Elevated inflammatory markers indicate that the disease has progressed from moderate to severe. Ferritin levels serve to predict the severity and prognosis of patients with COVID-19. HBOT may be a beneficial adjunctive treatment when used in combination with standard pharmacological treatment approved by the Naval Hospital of the Ministry of the Navy in patients with COVID-19. HBOT significantly reduces inflammation and can become an important point in managing the disease. If used at an early stage of the infection, it would significantly improve the respiratory symptoms of patients with COVID-19.

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## ETHICAL APPROVAL

An approval from the Institutional Review Board (IRB) was not needed for conducting this review.

## CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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