An Insight about SARS-CoV-2 Infection

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Abstract

Several different zoonotic viruses cause acute respiratory tract infections in western and developing countries. Annually, there are an estimated one billion zoonotic positive cases every year, and up to millions of deaths yearly. In comparison to SARSCoV-1, SARS-CoV-2 has an evolutionary gain of Furin cleavage site (FCS) on the S protein. Infection caused by this virus can be spread with contact/droplet, airborne, and fomite transmission, along with other methods of transmission. Contact and droplet transmission is spread with respiratory droplets through coughs, sneezes, and talks with infected people. Touching contaminated surfaces and then eyes, nose or mouth, can lead to fomite transmission. Fomite of liable SARS-CoV-2 virus or RNA analyzed with RT-PCR has shown that SARSCoV-2 can be found on these surfaces for hours to days, depending on the environment (humidity and temperature). In the first few first months of 2021, vaccines have been developed and rapid vaccination is happening, although limitations of doses slowdowns the process of developing herd immunity. However, patients who need acute treatment get immunosuppressives, such as dexamethasone, prednisolone, or other types of glucocorticoids. If the course of the disease is severe, treatment with antiviral remdesivir can be given.

Keywords: SARS-CoV-2 Infection

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Introduction

Several different zoonotic viruses cause acute respiratory tract infections in western and developing countries. Annually, there are an estimated one billion zoonotic positive cases every year, and up to millions of deaths yearly. **(1)**

Coronaviruses are identified as a zoonotic virus-containing single stranded RNA, that transmit infection between people and vertebrate animals and is found throughout this world. The three big coronaviruses that have caused fatal consequences have started twice in China and once in middle east. **(1)**

SARS-CoV-2 in human and betacoronaviruses in bats are proven until date to be the most closely related, but the intermediate host leading to transmission in humans is still unknown. (2)

To survive the mammal immune system, SARS viruses develops virulence factors that manipulate and suppress the immune system. SARS-CoV-2 has developed postponement and hindrance of IFN mediated production of neutralizing antibodies. (3)

In comparison to SARSCoV-1, SARS-CoV-2 has an evolutionary gain of Furin cleavage site (FCS) on the S protein. Infection caused by this virus can be spread with contact/droplet, airborne, and fomite transmission, along with other methods of transmission. Contact and droplet transmission is spread with respiratory droplets through coughs, sneezes, and talks with infected people. Touching contaminated surfaces and then eyes, nose or mouth, can lead to fomite transmission. Fomite of liable SARS-CoV-2 virus or RNA analyzed with RT-PCR has shown that SARSCoV-2 can be found on these surfaces for hours to days, depending on the environment (humidity and temperature). (4)

Additionally, the scant and insufficient information suggests that although highly unlikely, maternal-fetal transmission of SARS-CoV-2 is possibly conceivable. (5)

Recent studies revealed that SARS-CoV-2 was found in stools, along with its nucleocapsid protein, which was found in gastrointestinal tissues. Live SARS-CoV-2 was also grown from stools. It should be noted that SARSCoV-2 might also be found in sputum, urine, blood/serum, ocular surface, saliva, and aerosol. (6)

Some individuals have higher risk of getting severe case history, depending on health factors, such as diabetes, high blood pressure, asthma, immune deficiency, age, obesity, dementia, stroke, chronic liver diseases, and stroke. (7)

Comorbidities have an impact on the disease course and on which scale the disease can be harmful. Mild to moderate infections leads to fever, dry cough, and tiredness. Aches, pain, sore throat, diarrhea, conjunctivitis, headache, loss of taste or smell, rashes on the skin, and discoloration of fingers or toes are more infrequent symptoms. In severe cases, loss of speech or movement, acute respiratory distress syndrome, multi-organ failure, difficulty breathing, or shortness of breath, and chest pain can occur. In critical severe cases, the disease can lead to respiratory failure and death. (8)

New studies suggest that almost one in five infected people are asymptomatic for SARS-CoV-2. Reliable figures are difficult to develop since a clear difference between the asymptomatic patients and pre-symptomatic patients is not fully determined and a standardized definition is not made. (9)

One out of five patients with COVID-19 develops long-term effects that last more than 12 weeks, called long COVID. (10)

Patients who experience a severe disease progression and require intensive care have a higher risk to develop long COVID. However, it is still unclear whether symptoms are caused by COVID-19 or intensive care consequences. Patients who have been undergoing intensive care have normally post-intensive care syndrome (PICS), which has similarities with COVID-19 symptoms. Older age, female sex, and disease severity is a typical risk factor for PICS. Long COVID studies focus

on the symptom prevalence, but to what extent these remaining symptoms effect life quality is unknown. **(11)**

SARS-CoV-2 Structure

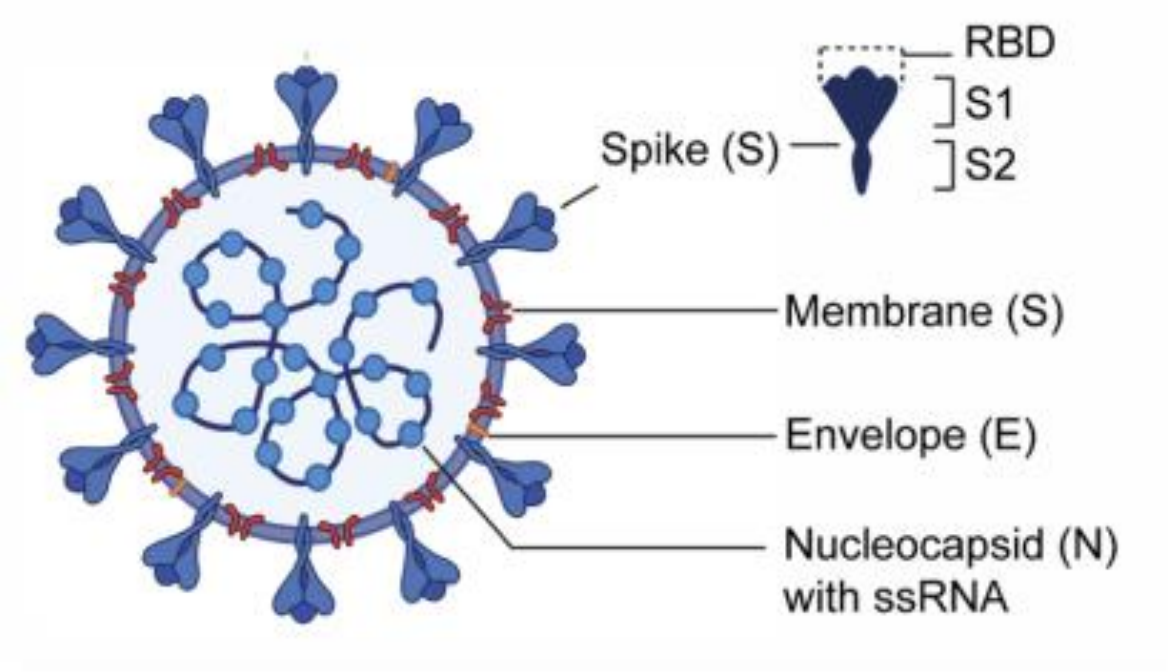

Figure 1. Schematic structure of the SARS-CoV-2 viral particle.

The 29.9 kb SARS-CoV-2 genome encodes a number of structural proteins, including the envelope glycoprotein spike (S), the envelope protein, the membrane protein, and the nucleocapsid protein. It also encodes non-structural and auxiliary proteins. **(12)**

The S1 subunit and S2 subunit make up the S protein. By binding to ACE2, which is largely expressed by respiratory epithelial cells, the receptor binding domain (RBD) of S1 facilitates viral attachment and entrance of the virus. In order to effectively prevent viral infection, antibodies or other substances inhibiting the RBD of the SARS-CoV-2 S protein are needed. The assembly and release of virion are regulated by the N protein. The virus's RNA genome is bundled with the N protein and the M protein forms the virion while safeguarding it. **(12)**

SARS-CoV-2 Lifecycle

Coronaviruses have a proofreading mechanism that prevents the virus to be weakened. Compared to influenza viruses, coronaviruses swap RNA chunks with other coronaviruses, which gives the coronavirus new unknown sequences for the human immunity. The S1 protein on SARS-CoV-2 surface binds to angiotensin-converting enzyme II (ACE2) on epithelial cells located in the lungs. S2 mediates then the spike cleaving by transmembraneprotease serine 2 (TMPRSS2) and fusion of the virus into the host cell occurs. Newly discovered is the FURIN proteases helping/priming activity ensuring that the virus enters the host cell. **(13)**

Endosomal cysteine proteases cathepsin B (CatB) and CatL assists in the fusion process in a minor order. **(13)**

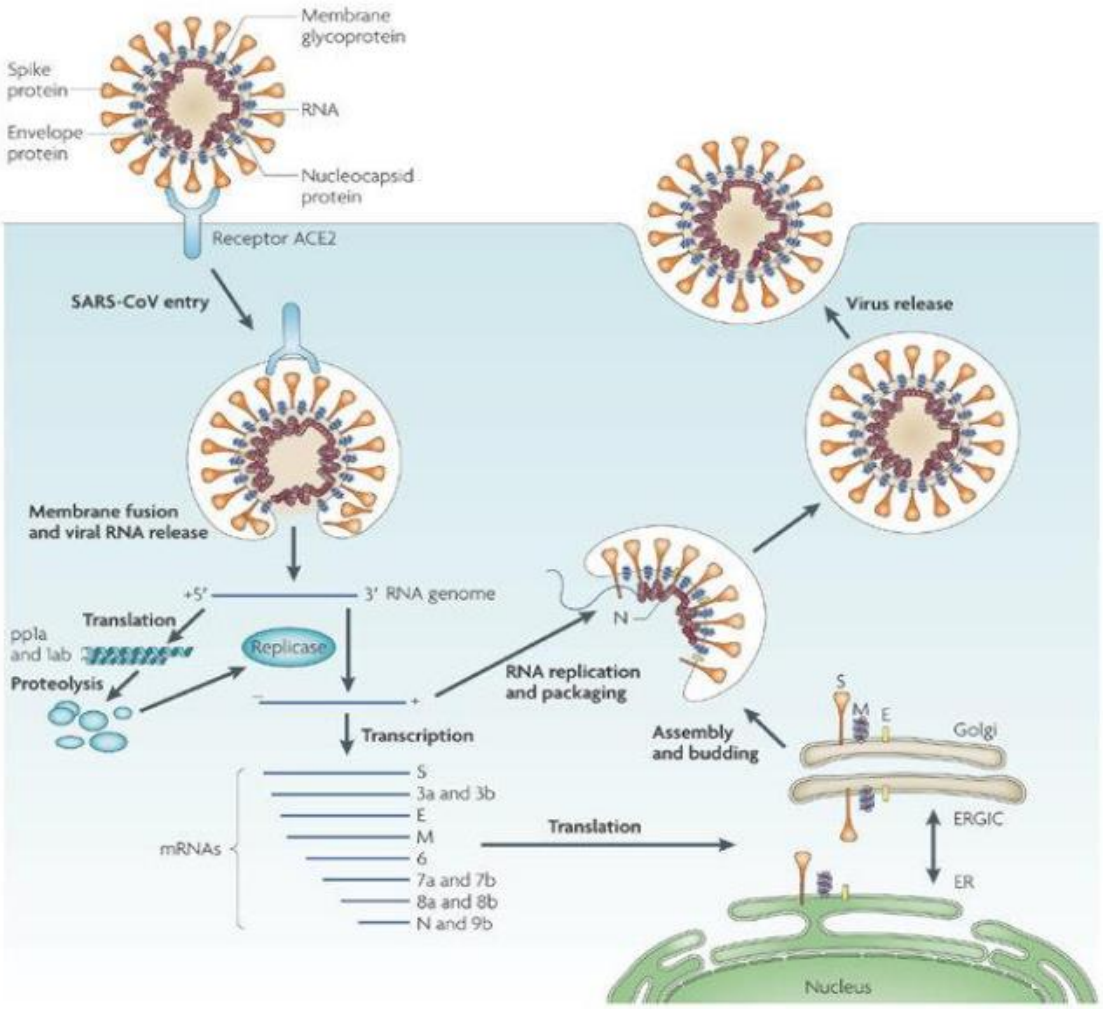

Figure 2. SARS-CoV-2 life cycle phases.

Immediately after entry into the host cell, the virus releases RNA. The two large genomes ORF1a and ORF1b undergo translation and results in the production of two large polyproteins, pp1a and pp1ab. Papin-like protease (PLpro) and chymotrypsine protease (a serine type Mpro), 3CLpro, supplement the polyproteins. Non-structural proteins (nsp) with different functions are released by the pp1a and pp1ab **(14)**

Nsps are important for intracellular replication and can assemble into replicase-transcriptase complex (RTC). RTC is a key factor for viral RNA transcriptase, leading to S, E, M production in endoplasmic reticulum. Viral components get maturated in Golgi vesicle resulting in mature lipid enveloped virion. By exocytosis, the new virion leaves the cells and will bind to other host cells. **(15)**

SARS-CoV-2 Evolution/Mutation

Numerous viruses have high rates of mutation, which when combined with enormous population numbers can lead to great genetic diversity. Be aware that RNA viruses' ability to survive and cause disease depends heavily on their expanding genetic diversity. **(16)**

As a result, RNA viruses frequently possess extremely prone RNA polymerases that result in numerous mutations, however SARS-CoV-2 has so far demonstrated a modest acquisition of mutations. The greatest rate of SARS-CoV-2 was found in the S gene, while coronaviruses in general had a worldwide mutation rate of about 10-6 per base and each infection cycle (10-3 per site and year). **(17)**

The basic reproductive number (R0), transmissibility, and mortality of viruses can all be affected by mutations that occur in the viral surface proteins. These mutations are also crucial for producing antigenic variants that enable the virus to evade host immune surveillance. In fact, certain changes enable the virus to evade the effects of antiviral medications. **(18)**

Intriguingly, coevolving sites were found in important SARSCoV-2 proteins, such the spike protein, likely as a result of stresses inside the host that affected protein stability, affinity, and interaction patterns. Given that SARS-CoV-2 has just recently infected humans, we anticipate that its genetic diversity will steadily rise over time. **(18)**

Diagnosis

In this part of the review, we will discuss the major scope of investigations regarding COVID-19 without reference to the CBC, CRP, D-dimer and LDH that were performed during this study.

The rapid antigen tests (RAT) for SARS-CoV-2 can be done on-site on a large scale of the population. It's cost efficient, does not need the presence of sophisticated laboratory equipment and provide the results in a mean time of 15 minutes. **(19)**

RAT can provide up to 56.2% sensitivity and 99.5% specificity, making it a valuable testing technique for mass screening. **(19)**

Diagnosis and detection of SARS-CoV-2 in patients are done by nasopharyngeal and oropharyngeal swabs. Samples are then carried in a transport media and sent to laboratories to detect the virus in the samples. In most countries, the recommendation of diagnosis in people has changed multiple times. Depending on the capacity of the testing that could be conducted. However, it is recommended that everyone experiencing acute respiratory infection symptoms and other symptoms for more than two days should be tested. **(7)**

The diagnostic procedures are done in two ways.

Firstly, direct detection of virus in patient samples with for example detection of proteins from the virus by culturing it, with using nucleic acid amplification tests, such as RT-PCR, transcription-mediated amplification, or Loop mediated isothermal amplification (LAMP).

Secondly, immunological diagnostics used for identifying the virus-specific antibodies after having the viral infection. **(20)**

Global standards of detecting SARS-CoV-2 in patient samples are done using real-time polymerase chain reactions (RT-PCR) assays that have high specificity and sensitivity. The specificity is estimated around 99% and sensitivity around 80%. Sensitivity is slightly lower than the specificity because that parameter is dependent on the stage of the disease. The sensitivity of RT-PCR increases if the test is conducted within the onset of symptoms. **(7)**

However, alternatively, SARS-CoV-2 infections can be detected with antigen rapid-tests. Most of the rapid-tests are mainly based on detecting specific viral antigens depending on the particular test kit. Antigen tests has lower sensitivity hence low antibody count in early phase detection of COVID-19. (7)

Treatment of COVID-19

Treatment of SARS-CoV-2 is not fully specific; however, guidelines are developed that are updated regularly.

In the first few first months of 2021, vaccines have been developed and rapid vaccination is happening, although limitations of doses slowdowns the process of developing herd immunity. However, patients who need acute treatment get immunosuppressives, such as dexamethasone, prednisolone, or other types of glucocorticoids. If the course of the disease is severe, treatment with antiviral remdesivir can be given.

Immunity against SARS-CoV-2

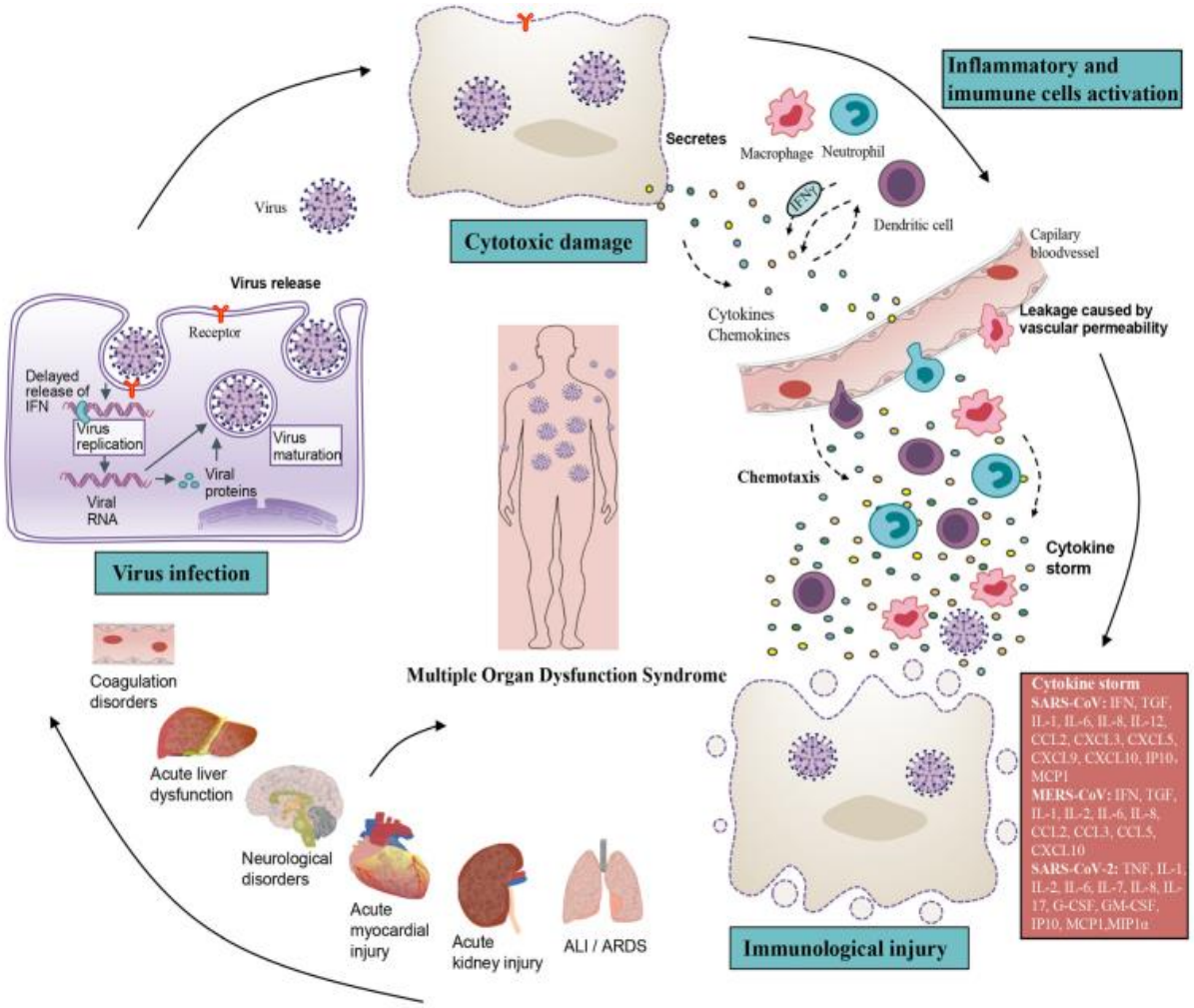

Figure 3. The mode by which lethal hCoVs lead cytotoxic damage (direct) and immunological injury (indirect) to host cells and cause multiple organ dysfunction syndrome

Understanding of the SARS-CoV-2 immune response is limited and updating rapidly. Along with lack of information about alterations occurring in convalescent patients' immune system. However, the antiviral response against SARS-CoV-2 is believed to be like other CoVs since the homology is similar. (21)

ssRNA and dsRNA from the virus engage the PRR receptors, such as RIG-I like receptors (RLRs) and TLRs, and initiates downstream signaling cascades resulting in cytokine production. In antiviral responses cytokines as INF type I/III, TNF-α, IL-1, IL-6, and IL-18 get released. IFN-1 is believed to limit the CoV infection. (34) However, studies have shown that SARS-CoV-2 has a mutated difference compared to other CoVs, resulting in the ability to block the IFN type I/III production. (21)

IFN levels are delayed in SARS-CoV-2 infected patients. IFN levels correspond to the severity of COVID-19. Due to poor initial IFN response, the recruitment of neutrophils can be postponed. Late recruitment of neutrophils can result in increased viral load. (23)

In severe COVID-19 patients increased neutrophils was reported. (23)

Also, elevated inflammatory cytokines such as IL-6, IFN-γ, TNF-α, IL- 8, MCP-1 (CCL2 chemokine ligand 2), and IL-10 have been detected and show resemblance to an inflammatory phenomenon called cytokine storm. Reasons for the cytokine storm occurring are yet to be established. One theory is the viral PAMPs and host danger signals trigger the phenomenon. (21)

The knowledge about T cells against SARS-CoV-2 is limited. In peripheral blood, the total number of CD4+ and CD8+ T cells, B cells, and NK cells has been low. COVID19 disease outcome is associated with the Th1/Th2 balance. Th1 can discreetly clear the viral infection and lack of its function can lead to exacerbated reaction leads to the cytokine storm. Th2 cells are associated with poor prognosis for the disease. (23)

Acute phases of SARS-CoV-2 infections are associated with significantly marked lymphopenia with low numbers of circulating CD4+ T cells and CD8+ T cells. Lymphopenia can be caused by reduction in APCs functions and impaired migration of DCs that lead to limited T cell proliferation, this includes decreasing numbers of γδ T cells.

In SARS-CoV-2 infections γδ T cells act as a bridge between the adaptive and innate immune system since it has an antigen presenting role. Furthermore, these cells have antiviral effects by secreting IFN-γ. (24)

CD4+ T cells and CD8+ T cells are associated with viral clearance in primary infections. In a previous study, it has been shown that S-reactive CD4+ T cells are present in patients and healthy donors, suggesting that these T cells are already present in our human body. (25).

A possible explanation for this is the previously CoV epidemics. The S reactive CD4+ T cells in COVID-19 patients co-expresses CD38 and HLA-DR (25).Some studies have shown that GMCSF and IL-6 expressing CD4+ T cells are more abundant in severe COVID-19 patients that do not need intensive care. (21)

T cells memory has been shown that it can last up to 17 years after SARS-CoV infection, resulting in protection in long-term cross. (21)

However, if this is reliable for SARSCoV-2 is still unknown. Follicular helper T cells (TFH) play an important role in antibody mediated humoral immunity. In viral infections, TFH aids long-lived memory B cells and plasma cells, which is important for virus-specific neutralizing antibodies. In a case study it has been seen that TFH, APCs, activated CD4+ and CD8+ T cells, IgG and IgM had an increasing tendency post symptom. **(21)**

To understand if SARS-CoV-2 has an impact on TFH must be investigated by analyzing the immune response in peripheral blood. IgM and IgA have been detected in blood samples from patients as early as 5 days after infection, while IgG has been measured within 14 days in most patients. Seroconversion starts on day 6 after symptom onset. SARS-CoV-2 neutralizing antibodies are directed mainly to the viral surface S protein and the N-protein. These antibodies neutralize viral infections of ACE-2 expressing human cells and tissues. **(26)**

In recovered COVID-19 patients, considerable amounts of IgG antibody against SARS-CoV-2 have been detected.

Comparison between the 3 Coronaviruses

	SARS-CoV-2	SARS-CoV	MERS-CoV
Genus	Clade I, lineage B	Clade I, lineage B	Clade II, lineage C
Length of nucleotides	29.9 kilobases	29.75 kilobases	30.11 kilobases
First emergence	7 December 2019, Wuhan, China	16 November 2002, Foshan, China	4 April 2012, Zarqa, Jordan
Virus identification	January 2020	March 2003	June 2012
Causative agent declaration	January 2020	April 2003	September 2012
Recent status	Pandemic ongoing	Completely control	Sporadic continuous
Number of infected cases	Above 12.7 million[a]	8096	2553
Male-to-female ratio	1.27:1	1:1.13	1.78:1
Number of attributed deaths	Above 566 thousand[a]	774	876
Number of viral Footprint	213 countries or regions[a]	29 countries or regions	27 countries or regions
Case fatal rate	4.4%	9.6%	34.3%

NA Not available. [a] According to the data released by the WTO on 13, July, 2020

Table 1. The phylogenetic origin, crucial events and basic demographic information of SARS-CoV-2, SARS-CoV and MERS-CoV. (27)

	COVID-19	SARS	MERS
Signs and symptoms			
Fever	56–99%	99–100%	81.7–100%
Fatigue	18–55%	31.2%	NA
Cough	39–81%	29.0–74.3%	75–85%
Sore throat	5–17%	11.0–23.2%	14
Dyspnea	12–41%	40–42%	72%
Myalgia	18–55%	49.3–60.9%	38
Diarrhea	3–17%	20–25%	26
Headache	4–23%	15.0–55.8%	NA
Complications			
ARDS	18–30%	20%	20–30%
AKI	3%	6.7%	41–50%
Laboratory findings			
Leukopenia ($< 4.0 \times 10^9$/L)	26.8%	23–35%	14%
Lymphopenia ($< 1.5 \times 10^9$/L)	55.3%	68–85%	32%
Thrombocytopenia ($< 150 \times 10^9$/L)	11.5%	40–45%	36%
Elevated LDH	55.5%	50–71%	48%
Elevated AST	17.9%	20–30%	14%
Elevated ALT	16.0%	20–30%	11%

LDH Lactate dehydrogenase, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase, *NA* Not available

Table 2. Clinical characteristics and laboratory findings of COVID-19, SARS and MERS patients. (28)

Immunology

The Immune System

The immune system has advanced mechanisms and many different important components that together protect an individual against harmful pathogens, such as viruses, bacteria, fungi, and parasites. The immune system is divided into two interconnecting branches, the innate immune system and the adaptive system. Both systems work cooperatively together. The innate immune system is congenital and is developed before birth and is the first line of defense. **(29)**

Since the innate immune system is the first line of defense it is dominating in the first hours/days of fighting an antigen. In contrast, the adaptive system must be slowly developed and is not inherited. This system has a great capacity to develop an immunological memory. Generally, the innate immune system is non-specific, while the adaptive immune system is more advanced and specific. **(29)**

Innate and adaptive immunity activates different immune cells that can result in cytokine production. Cytokines are messenger molecules that regulate the immune system by suppressing

or inducing the immune responses, and it has a major role in orchestrating the balance between innate and adaptive immunity. These molecules bind to cell receptors and induce complex signaling cascade. **(29)**

Cytokines can have autocrine (effect on the secreting cell), paracrine (effect on other cells), juxtracrine (on adjacent cells, needs membrane anchored proteins), or endocrine (distanced target cells) effects. These pleiotropic proteins are divided into proinflammatory (e.g., type 1 interferons/INF-1, tumor necrosis factor/TNF, interleukin/IL-1, IL-12) and anti-inflammatory cytokines (e.g., IL-4, IL-10, transforming growth factor β/TGFβ). However, a cytokine can have both activities dependent on the local microenvironment. **(29)**

Innate Immune System The innate immune system is congenital and has briefly three important functions: inflammation, antiviral response, and stimulation of the adaptive immune system. The innate immune response does not develop immunological memory in the same manner as the adaptive immune system. Responses provided by the innate immune system are essentially similar in every microbe encounter. **(29)**

The main components of the innate immune system are epithelial barriers, monocytes, dendritic cells (DCs), neutrophils, macrophages, natural killer (NK) cells, and the complement system. Innate immune cells recognize the pathogen-associated molecular patterns (PAMPSs) that have been evolutionarily conserved in microorganisms infecting humans. There are five main pattern recognition receptors (PRR) in the innate immune cells that recognize PAMPS: (Toll-like receptors (TLRs), nucleotide-binding oligomerization domains (NOD-)-like receptor, retinoic acid-inducible gene (RIG-I), DNA sensors, and C-type lectin. **(29)**

One way of activation of the innate immune system is through PAMPs are lipopolysaccharides binding to TLR on antigen presenting cells (APC) and other cells, like endothelial cells. This will lead to proinflammatory cytokines. PAMPs can also be digested and processed in the APCs and then bounded to major histocompatibility complex (MHC) and translocated on the surface of the APC. Dying or damaged cells can release endogenous molecules, called damage associated molecular patterns (DAMPs) that can also bind to PRR. **(29)**

A collection of cell membrane proteins, surface and intracellular, constitutes the complement system, which plays an important role in defeating inflammations and harmful microbes. Activated complement proteins can affect microbes through opsonization, cell lysis, or with triggering immune cells to produce molecules that result in an inflammation. **(29)**

Adaptive Immune System

In contrast to the innate immune system, the adaptive immune system needs to be gained and built up through exposure to different kinds of infections and antigens. When activated the adaptive system provides a specific response against specific pathogens/antibodies based on immunological memory. B lymphocytes and T lymphocytes are the important components in the adaptive immune system. B and T cells have specialized antigen-specific receptors. Adaptive immune system is divided into two response types, humoral immunity (mediated of B lymphocyte produce antibodies) and cell-mediated immunity (T lymphocytes). Upon recognition of a microbial antigen, naive lymphocytes proliferate and differentiate into effector or memory cells. **(29)**

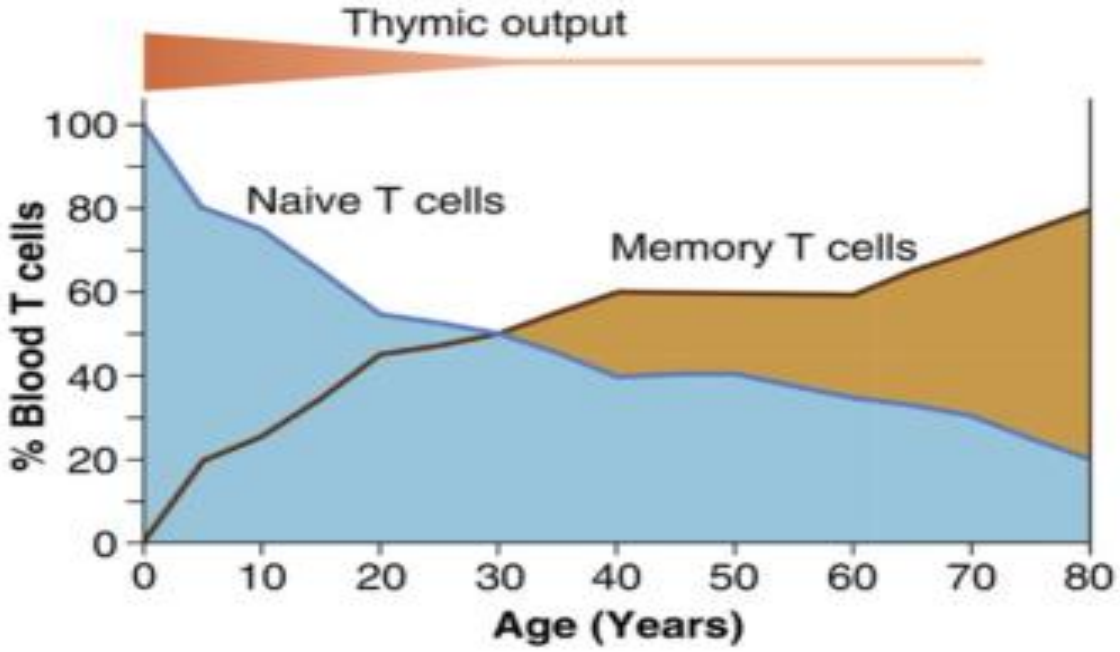

Figure 4. Age-dependent alteration of naive and memory cells

Naive T lymphocytes have a unique receptor not responding to self antigen and have not been presented to its specific antigen by an APC. When a naive T-cell, CD4+ or CD8+ is presented to its

antigen by an APC, activation of the T-cell will occur and start an immune response. Of the T-cell clonal expansion, some cells are programmed to be memory cells. Effector CD4+ T cells activate B cells, macrophages, and other cell types through the production of cytokines. Effector CD8+ T cells have the ability to kill infected host cells. B effector lymphocytes are antibody-secreting plasma cells located in the peripheral lymphoid organs, plasmablasts located in the blood, or long-lived plasma cells in the bone marrow. **(29)**

In contrast to effector cells, memory cells remain alive without the presence of antigen, resulting in a higher frequency of memory cells dependent on age. The peripheral blood of adults can contain 50% or more memory cells.

CD4+ T helper cells are divided into subtypes based on their cytokine production. These subsets have distinct functions and defend against different types of microbial infections. Subtypes of T helper (Th) cells are Th1, Th2, Th9, Th17, and Tfh. Th1 is essential in IL-2 and IFN-γ mediated activation of macrophage, effector cytotoxic cells, and NK cells. Th2 cells are essential for humoral response, and IL-4 and IL-6 mediate activation of eosinophils, basophils, and mast cells. **(29)**

B lymphocytes mediated the humoral immunity and produce antibodies. Antibodies, also known as immunoglobulins (Ig), can be membrane-bounded on B cells or secreted proteins. Different Ig isotypes (IgD, IgM, IgE, IgG, and IgA) have specific effector, physical, and biologic characteristics. Engagement of membrane-bounded antibodies starts B cell activation and triggers antibody secretion. Stimulation of antibodies triggers a range of effector mechanisms that eliminate the antigen/microbe. **(29)**

Although the immune system is broadly divided into two arms, the systems work together to provide an effective host response. APCs have major histocompatibility complex (MHC) presented on their surface that shows antigen fragments to cells in the adaptive immune system. MHC can be divided into two subclasses, MHC class I and MHC class II. MHC class I is present on every nucleated cell in our body, and MHC class II is only expressed on the DC, macrophages, and B lymphocytes. **(29)**

Even though the lymphocytes are mainly a part of the adaptive immunity, they have had some features that function in the innate immunity. These cells have the same morphological and functional characteristics similar to T cells, but the receptor diversity is limited. γδ T cells and NK-T cells are examples of lymphocytes with limited diversity. NK-T cells express T cell receptors with limited diversity along with NK-cell-specific surface molecules. **(29)**

Human Immune Responses to SARS-COV-2/IAV

Upon detection of the invading of respiratory virus like SARS-CoV-2 and IAV, the host immune system activates a network of cells and biomolecules to defend the host and clear the virus. The human immune system comprises of two arms: the innate and adaptive immune system. As the first line of defense, the innate immune system recognizes and rapidly responds to general patterns commons to groups of pathogens, known as pathogen associated molecular patterns (PAMPs) by secretion of anti-viral and pro-inflammatory mediators. **(30)**

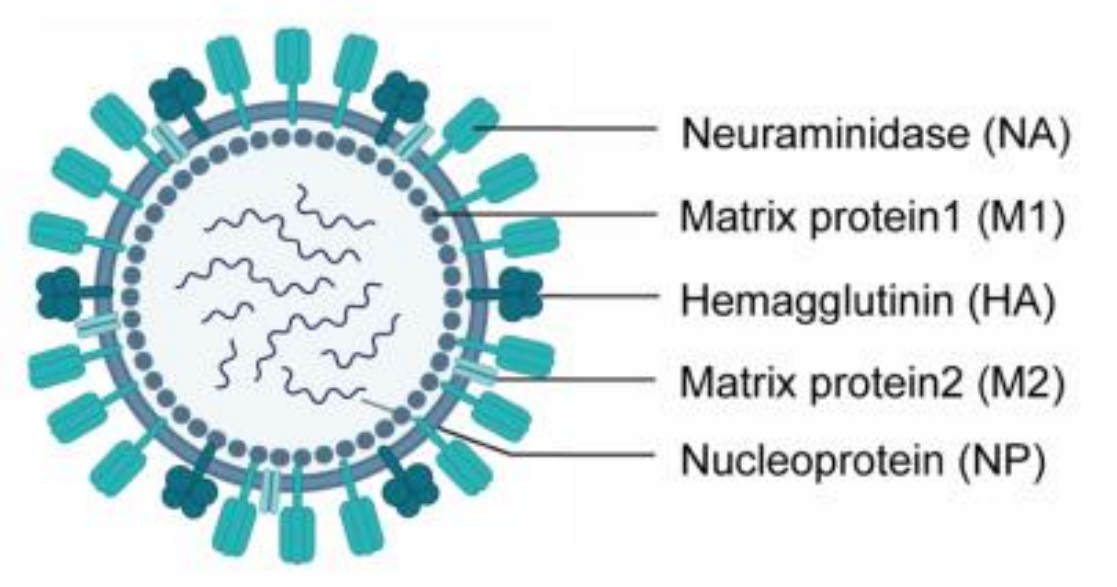

Figure 5. IAV structure

This provides time for the adaptive immune system to mount pathogen-specific protection, clear the pathogen and establish immune memory. Consequently, both the innate and adaptive immune responses are required for control and clearance of SARS-CoV-2 or IAV infection and resolution of symptoms. However, the dysregulated immune response would in turn cause immunopathogenesis and contribute to severe disease progression. **(31)**

SARS-CoV-2 enter the host via the airways and airway epithelial cells are the primary target of the virus. Upon viral exposure, infection epithelial cells but also resident mucosal innate immune cells

respond by rapidly producing cytokines and chemokines that facilitate recruitment of innate immune cells to the site of infection, including neutrophils, monocytes/macrophages, natural killer (NK) cells and DCs. **(32)**

These innate immune cells perform important effector roles to control infection by releasing reactive oxygen species, killing or phagocytosing infected cells directly. DCs together with monocytes and macrophages are collectively called mononuclear phagocytes (MNPs). In addition to providing innate protection, MNPs also play an important role in processing and presenting

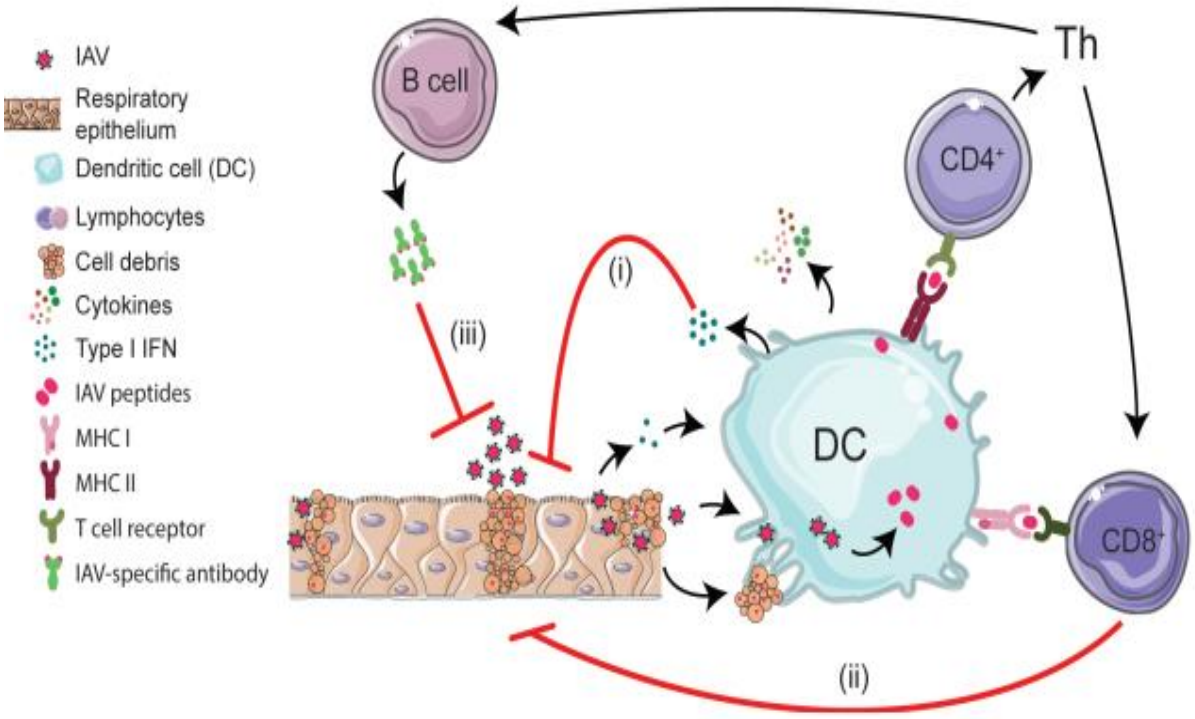

Figure 6. Mechanisms of human dendritic cell (DC) in restricting influenza A virus (IAV) infection of respiratory epithelium.

viral antigens to T cells, thus bridging innate and adaptive immunity. Among these MNPs, DCs exhibit the unique ability in activating naïve T cells. **(33)**

DCs are found both in circulation and residing in the airways, where they are well positioned to rapidly respond to incoming respiratory viruses. Upon SARS-CoV-2 infection, DCs recruited from blood or resident in the airways sense and restrict viral replication and transmission by producing antiviral proteins including type I IFNs. DCs also produce cytokines that aid the recruitment of other immune cells to the site of infection. In addition, DCs take up and process viral particles or infected cell debris, migrate to draining lymph nodes to present antigen to T cells. The antigen-specific T cells, activated by DCs, migrate to the site of infection, or provide efficient help to regulate humoral immune response in lymphoid tissues, to control and clear respiratory virus. DCs consist of plasmacytoid DCs (pDCs) that are potent the type I IFN producers and important in antiviral defense, and myeloid DCs (mDCs) with excellent antigen presenting function. **(34)**

mDCs are referred to as conventional DCs (cDCs) and can be further divided into cDC1s (CD141+) and cDC2s (CD1c+). Notably, CD1c+ mDCs are the most abundant mDC subset in

both blood and airways. CD1c+ mDCs are excellent at antigen presenting ability, and in initiating T helper (Th) cells responses, including Th1, Th2 and Th17. (35)

Their ability to present antigen on MHC II is not affected by IAV infection but their ability to cross-present antigen is impaired 64. CD141+ mDCs can efficiently cross-present endogenous antigen on MHC I to initiate CD8+ T cell responses. They express high levels of TLR3 and respond to poly(I:C) by producing TNF, IL-6, IL-12 and IFNb. (36)

CD141+ mDCs are less frequent in blood than CD1c+ mDCs, and rarer still, in tissues, but can be recruited rapidly to the site of infection, where they can take up the antigen, migrate to the draining lymph nodes and initiate CTL responses. Recent studies show that the cDC2 population contains a subset, that express CD163 but not CD5, named cDC3. pDCs have a superior ability to rapidly produce large quantities of type I IFN in response to TLR7/9 engagement. Type I IFNs mediate strong innate antiviral protection by transcriptionally regulating a multitude of interferon stimulated genes (ISGs). A well-studied ISG, MxA (human), is expressed at high levels in pDCs at steady state. pDCs also contribute to the activation of T cells, and B cells, and therefore, coordinate the innate and adaptive responses necessary for clearance of IAV infection. (37)

In contrast to DCs, there is a myeloid-derived innate immune cell population that displays remarkable efficiency in suppressing T cell response, called myeloid-derived suppressor cells (MDSCs), which will be described in further detail below.

Adaptive Immunity

While the innate immune system is greatly involved in antiviral protection, including initiating adaptive responses, typically adaptive immune responses are required for clearance of infection and induction of immune memory that will prevent or limit future infections. The adaptive immune response is mediated by B and T cells, and responsible for humoral and cellular immunity, respectively. (38)

Humoral Immunity

The humoral immune response consists of antigen-specific antibodies, which are produced by differentiated B cells, named short- and long-lived plasma cells. Antibodies efficiently limit respiratory viral infection by binding to neutralization sensitive epitopes on the virus and blocking it from entering into target cells or releasing from infected cells. (39)

In addition to directly neutralizing respiratory viruses, antibodies could promote efficient viral clearance by coordinating innate effector cells, including antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). Antibodies could induce the cytotoxic destruction of infected cells by medicating the degranulation and cytotoxicity of natural killer (NK) cells. (38)

Studies have shown the positive correlation between elevated ADCC with antibody-mediated protection against IAV both in vitro and in vivo. Antibodies could also promote the opsonophagocytosis of MNPs and granulocytes (including basophils, eosinophils, neutrophils and mast cells) to clear or process and present viral particles marked by immune complexes from the circulation.A study found that the IAV infection induced influenza-specific ADCP in humans and macaques could not only clear encountered IAV, but also display cross-reactivity to previously non-

exposed strains, highlighting the importance of ADCP in immunological memory. Based on the constant region of the heavy chain, antibodies can be divided into different isotypes including IgA, IgD, IgM, IgG and IgE. During a primary infection, i.e., when the immune system is exposed to a pathogen for the first time, IgM emergence precedes the appearance of IgA and IgG antibodies in the blood. This pattern was also seen in COVID-19 patients infected with the newly emerged SARS-CoV-2 virus. **(40)**

Anti-SARS-CoV-2 antibody responses have been revealed to positively correlate with disease severity, in that the most severely ill patients displayed the highest antibody titers and higher nasopharyngeal viral loads. In addition, a study found that COVID-19 patients with severe disease generated SARS-CoV-2 S- and N-specific antibodies with more extensive epitope spreading compared to patients with milder symptoms. **(41)**

The heterogeneity of antibody responses and COVID-19 disease severity suggests that patients that mount antibodies targeting non-neutralizing viral epitopes fail to limit virus replication during early infection, resulting in prolonged viral persistence and higher viral loads. This might contribute to the disease progression. Prolonged antigen availability and higher antigen load may keep fueling the germinal center reaction, to extend the period of antibody evolution and production. Consequently, patients with severe COVID-19 disease display a broader and more robust antibody response against SARS-CoV-2. In response to recurring pathogen exposure like IAV, that many are exposed to annually, studies have shown that individuals recovered from influenza displayed robust and broad antibody protection against not only currently infected strain, but also previous exposed strains from the same subtype. **(42)**

In addition, several influenza virus infection studies in mice demonstrated B cell synergy with T cells in optimizing protection against a primary viral challenge, indicating that B cells act as regulators of T dependent antiviral immunity.However, effective humoral immune responses depend on well-orchestrated cellular interactions between B and T cells, especially CD4 T helper cells. **(42)**

Cellular Immunity

Adaptive cellular immune response is efficient in controlling intracellular pathogens such as respiratory viruses. The cellular immunity is conducted by T cells, mainly including CD4 and CD8 T cells. CD4 T cells provide help for optimal B cell and CD8 T cell responses against viral infection as Th cells, as well as regulate the immune activities as T regulatory (Treg) cells. Upon virus infection, naïve CD4 T cells are activated by DCs and differentiate into effector subsets with signature transcription factor expression and cytokine production, including Th1 (T-bet+ IFNg+), Th2 (GATA-3+ IL-4+), Th17 (RORgt+ IL-17A+), T follicular helper (Tfh) cells (Bcl-6+ IL-21+) and Treg (FoxP3+ IL-10+). CD4 Th cell subsets play important role against different pathogens, including intracellular pathogens (Th1 dominated), extracellular parasites (Th2 dominated) and bacteria (Th17). **(43)**

Tfh cells aid in optimizing antibody production by providing signals to activate and select B cells in the germinal centre and finally to generate long-lived antibody-secreting cells. **(43)**

The role Tfh cells play in respiratory infection will be described in further detail below. Treg cells play an opposite role compared to other CD4 T cell subsets, in that Treg cells actively suppress T

cell activity. Th1 activation and expansion have been commonly observed in influenza infected patients. **(44)**

Meanwhile virus-specific CD4 T cell response to SARS-CoV-2 infection is complex and associated with disease severity. Studies have shown that COVID-19 patients who experienced severe disease demonstrated stronger virus-specific CD4 T cell responses including T cell activation and expansion compared to asymptomatic or mild patients. In addition, robust Th2 responses were found to be associated with severe or fatal outcome in COVID-19, which has also been shown to hamper recovery from influenza virus infection. Similar as CD4 T cells, naïve CD8 T cells are activated by DCs to proliferate and differentiate into cytotoxic T lymphocytes (CTLs). It has been reported that type I IFNs, IFN-y, IL-12 are involved in helping CD8 T cells to differentiate into CTLs after antigen encounter. CTLs produce cytokines and cytotoxic granules, including perforin and granzymes, to kill virus infected cells and restrict viral replication. **(45)**

In COVID-19 patients, virus-specific CTLs responses were observed within 7 days of symptom onset and peaking after 14 days, and early CTL response correlated with effective viral clearance and mild clinical outcome. After virus clearance, differentiated virus-specific CD8 T cells downregulate their expression of cytolytic molecules and provide long-term protection. **(46)**

Studies have shown that post-infection IAV-specific CTLs circulate in blood and lymphoid organs as memory CTLs and are efficient in response to secondary IAV infection. Sophie A. Valkenburg et al. revealed that IAV-specific CTLs can persist for up to 2 years but with significant decrease in cytotoxicity, due to the decreased cytolytic molecule expression. **(47)**

However, excessive CTLs responses have proved to be detrimental, which are associated with severe COVID-19 disease outcome and contribute to tissue damage in influenza-infected patients with poor clinical outcome. **(48)**

Immune dysregulation in COVID-19/IAV

Specific and potent immune responses to SARS-CoV-2 and IAV are critical for virus clearance and host protection, however, the immune dysregulation induced immunopathogenesis in turn contribute to the progression of severe disease. On one hand, excessive immune cell responses lead to immunological injury. For example, cytokine storm is commonly observed in COVID-19 and IAV infected patients with severe and fatal disease outcome. Cytokine storm is induced by over activation of white blood cells and resident tissue cells that produce excessive amount of circulating pro-inflammatory cytokines and cause life-threatening systemic inflammation, multiorgan failure and hyper-ferritinemia. **(49)**

On the other hand, functionality of immune cells could be impaired due to pathogen invasion, which reduces their efficiency in host protection. For example, human primary DCs displayed impaired ability in antigen cross-presentation due to IAV infection. Substantial function loss of DCs was also observed in COVID-19 patients, which lead to delayed T cell response. In addition, aberrant ratio of immune cell population may also lead to poor clinical outcome. For example, the neutrophil-to-lymphocyte ratio was dramatically increased in COVID-19 patients with higher disease severity, which has been considered as a marker to predict COVID-19 disease severity. Thus, studies on the immune dysregulation induced by SARS-CoV-2/IAV infection would help

to predict disease progression and support the development of therapeutic strategies/medicines for COVID-19 and IAV treatment. **(50)**

No Conflict of interest.

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