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TREATMENT OF COVID-19 INFECTION: A PHARMACOLOGICAL TARGET APPROACH

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1. ABSTRACT

Covids are a group of encompassed infections with positive sense, non fragmented, single abandoned RNA genomes. The subgroups of Covids family are alpha (α), beta (β), gamma (γ) and delta (δ) Covid. The ICTV named the infection as SARS-CoV-2, a gathering of virologists in China proposed renaming SARS-CoV-2 as human Covid 2019 (HCoV-19), taking into account that such a name would perceive the infection from SARS-CoV and keep it consistent with the WHO name of the malady it causes, Coronavirus. SARS-CoV-2 was accounted for to be an individual from the β gathering of Covids. All out six HCoVs are recognized in particular HCoV-229E, NL63, OC43, HKU1, SARS-CoV and MERS-CoV. But SARS-CoV AND MERS-CoV other output cause dangerous pneumonia and bronchiolitis particularly in old, kids and immunocompromised patients. They additionally have capacity to cause enteric and neurological infections. SARS CoV-2 spread astoundingly quick in China and after that then to the various numerous different nations, causing Covid 19 illness (Coronavirus). Coronavirus is the third-known zoonotic infection from Covid after SARS and MERS. The clinical possibilities of COVID19

essentially incorporate fever, hack and pneumonia subsequently we are introducing here the pharmacological focus for treatment of this malady.

KEYWORDS: Pharmacological Investigations, SARS-CoV, Treatment Adaptation, HCoVs, βgathering, immunocompromised.

2. INTRODUCTION

Novel coronavirus-induced pneumonia, which was identified on 11 February 2020 by the WHO as coronavirus disease 2019 (COVID-19). It has increased rapidly in epidemic scale it first emerged in December 2019 in Wuhan, China(Li, Geng et al. 2020, Zhou, Yang et al. 2020). Almost at the same day, the International Commission for the Classification of Viruses announced that the novel coronavirus was termed as severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2).COVID-19 is not the first serious outbreak of acute respiratory disease induced by coronavirus, the coronavirus has led three epidemic diseases within a short period of time (since the last two decades to so far), the first is recognized as severe acute respiratory syndrome (SARS) and the second is regarded as middle-eastern respiratory syndrome (MERS)(de Wit, van Doremalen et al. 2016) and finally the third is coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)(Li, Geng et al. 2020, Wu, Leung et al. 2020). It presents clinical symptoms such as fever, non-productive cough, dyspnea, myalgia, fatigue, reduced leukocyte counts and radiographic evidence of pneumonia, which are similar to the symptoms of SARS-CoV and MERS-CoV(Li, Geng et al. 2020, Wu, Zhao et al. 2020) infections. Currently COVID-19 cases have been found in many countries across the globe. According to the latest statistics, the number of confirmed cases in China reached 79,968 by March 1, 2020, of which 2,873 were dead, and 41,681 have been cured. Besides China, the number of confirmed cases in other countries also reached 7,041, of which 105 were dead, and 459 were cured. On 31 January 2020, the World Health Organization (WHO) reported that COVID-19 was considered as an international public health emergency (PHEIC), which means it may pose an immediate threat to several countries and needs an unified international response(Li, Geng et al. 2020). Hence currently we need to develop the appropriate pharmaceutical products as soon as possible for COVID-19 treatment. Therefore this study is focuses on the mechanism that leads to the COVID-19 pathophysiology, the pharmacological targets for the treatment of COVID-19 infection and potential outcomes of the study.

3. Epidemiology

As of 24:00, 20 February 2020 (UTC+8), there were 75 995 confirmed cases, including 2239 deaths in China (mainland: 75 891; Hong Kong: 68; Macao: 10; and Taiwan: 26), and 1200 confirmed cases, including eight life threatening cases outside China, in all five continents (Figure 1).

The epidemiology curve can roughly be divided into three phases.

1. The first step marks the local outbreak by exposure in the food wholesale market. A total of 41 cases have been confirmed, from the first case in December 2019 to the emergence of new cases outside of Wuhan by 13 January 2020. Epidemiological research found that person-to-person transmission had already occurred in this initial phase via near contact(Bai, Yao et al. 2020, Sun, He et al. 2020)**.**
2. The second phase began on January 13, marked by massive expansion and transmission of the virus in hospitals (hospital-acquired infection or nosocomial infection) and often transmitted via direct contact with family individuals(close-contact transfer or near-range transfer).The outbreak spread from Wuhan to other regions(Sun, He et al. 2020, Wang, Lu et al. 2020) during this phase. The first case outside of China was registered in Thailand on January 13 and was caused by a Wuhan resident travelling to that place. On January 19 cases were reported from outside of Wuhan, Beijing City, and Guangdong Province, revealing that the virus had spread inside China, and the total number of confirmed cases increased to 205.A total of 846 confirmed cases had already been registered by January 23,29 provinces, including six foreign countries, an approximately 20-fold increase from the first phase.In the meantime, Wuhan City has implemented a 'lock-down' (i.e., shutdown of all movement within and outside the city).Unfortunately, this period concur with the traditional mass migration of people before the Chinese New Year, a form of 'home-coming' and consequently more than 5 million people had left Wuhan already.
3. The third phase began on 26 January, marked by the sharp increase in cluster events.On February 10, retrospective analysis found that the number of clustered cases in Beijing, Shanghai, Jiangsu and Shandong accounted for 50-80% of all reported cases. The number increased 240-fold on January 30, approaching 9826 confirmed cases and this outbreak was declared a PHEIC by the WHO.By 11 February 44 730 confirmed cases and 16 067 suspected cases in about 1386 counties and districts in China(Sun, He et al. 2020, Surveillances 2020) were reported.But still only 441 confirmed cases were reported in 24

countries outside China. The death rate remained high in China, with a total of 1114 deaths, but in the Philippines, with just one death outside of China. By February12, following the adoption in Hubei province of a new clinical description for diagnosis, newly confirmed cases jumped to 14840, of which 13332 cases were focused solely on clinical diagnosis. 25 countries had registered 60 329 infections by that time, with 1471 times the initial number. Notably, February 3 appears to be a turning point of the outbreak, from which time the regular number of reported cases outside Hubei started decreasing. It is uncertain whether it represents a success of the 'Wuhan lockdown' and other public health initiatives, or decreased virus transmission for other reasons.

In addition, 85.8 % of the 37,269 confirmed cases either lived in or travelled to Wuhan, or were in close contact with individuals who had been to Wuhan (Guan, Ni et al. 2020, Sun, He et al. 2020, Surveillances 2020). Unfortunately, as of 11 February 1716 medical-related professionals were contaminated from 422 medical establishments, of which 1688 reported cases were examined. Among them, 64% were infected in the city of Wuhan, and 23.3% in the rest of Hubei, except Wuhan(Sun, He et al. 2020, Surveillances 2020). Further research is required into the particular causes of the medical personnel infection and lack of defence.

Initial assessment of the dynamics of COVID-19 transmission showed that the fundamental reproductive number(R0) of 2019-nCoV is estimated to be 1.4–3.9.The SARS-CoV in the absence of interventions was 2.3–3.7(Sun, He et al. 2020). By study of 55 of the first 64 laboratory-confirmed cases, Breban et al. estimated MERS-CoV R0 to be 0.50–0.92.With rapid diagnosis and effective patient isolation, the SARS-CoV R0 dropped to less than 1, and explaining why the outbreak of SARS-CoV could subsequently be regulated(Sun, He et al. 2020, Swerdlow and Finelli 2020). Nevertheless, it should be remembered, that measurements of R0 can differ from various biological, socio-behavioural, and environmental factors, and must be interpreted with caution(Sun, He et al. 2020).

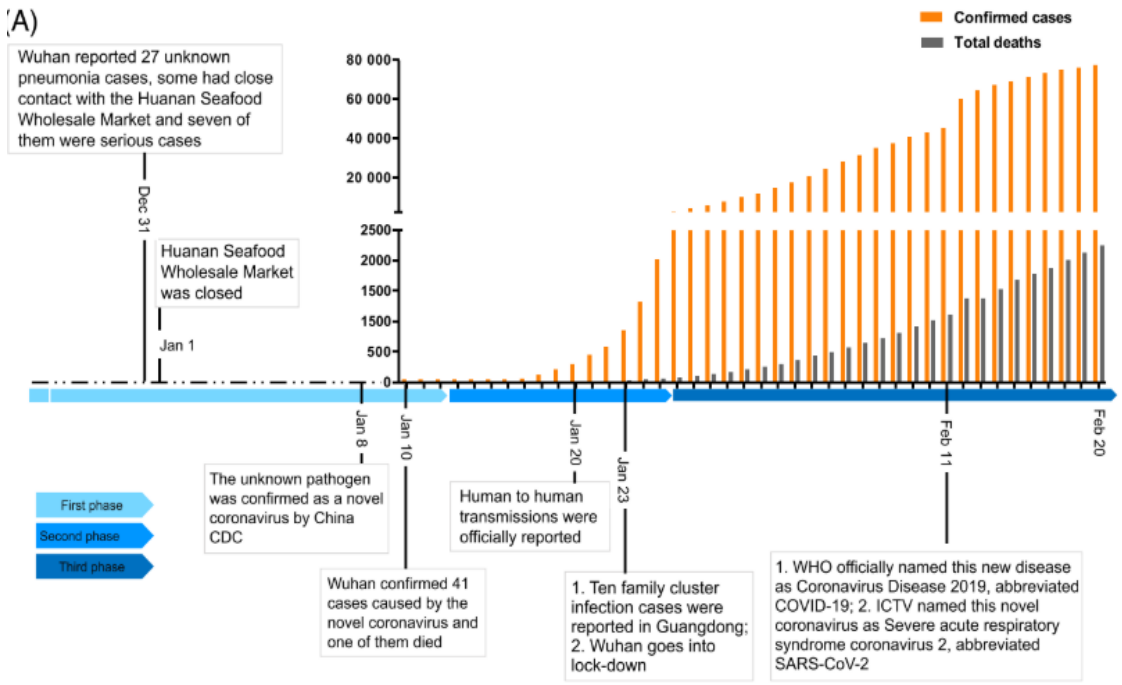

Figure 1: Timeline of events during 2019-nCoV epidemic (Shuo Su et. al, Trends in Molecular Medicine 2020).

4. Pathophysiology of COVID-19

The exact mechanism and Pathophysiology of COVID-19 is still unclear, but one thing is well understood by going through the literature, the clinical symptoms of COVID-19 include fever, chills, vomiting, unproductive cough, dyspnea, myalgia, fatigue, reduced leukocyte counts and pneumonia are identical to previous infections of corona virus such as SARS-CoV and MERS-CoV(Li, Geng et al. 2020). SARS-CoV and MERS-CoV mechanisms are well known, so we can get the information about the pathogenesis of SARS-CoV-2 infection that can aid in the COVID-19 diagnosis and treatment.

4.1 ACE-2 mediated pathology of COVID-19

Coronavirus spike (S) protein enables viral penetration into target cells. Entry is based on binding the surface component, S1, of the S protein to a cellular receptor that facilitates viral attachment to the target cell surface. Furthermore, entry requires S protein priming by cellular proteases, which includes S protein cleavage at the site of S1/S2 and S2 and enables viral and cell membrane fusion, a process driven by the S2 subunit (Figure 2). SARS-S requires angiotensin-converting enzyme 2 (ACE2)(Li, Moore et al. 2003, Hoffmann, Kleine-Weber et al. 2020) as an entry receptor and utilizes TMPRSS2 cell serine protease for S protein priming (Glowacka, Bertram et al. 2011, Hoffmann, Kleine-Weber et al. 2020). At the atomic

level, the SARS-S/ACE2 interface was explored, and the productivity of use of ACE2 was found to be a primary determinant of SARS-CoV transmissibility. Latest studies have shown that SARS-S and SARS-2-S share an origin of around 76% of amino acids. It has also been found that SARS-2-S also employs ACE2 and TMPRSS2 for entry of host cells just like SARS-S.

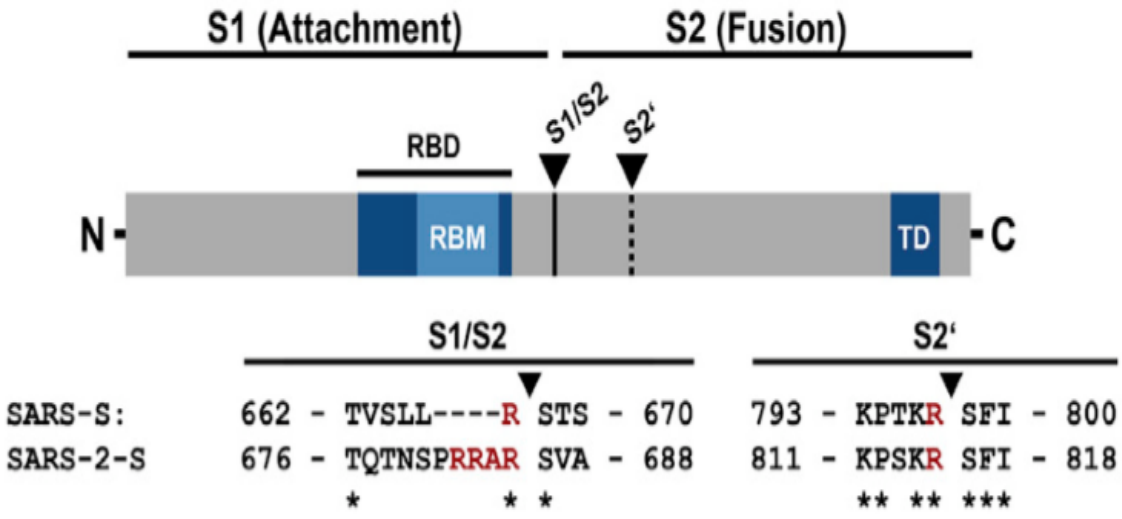

Figure 2: SARS-S schematic diagram including functional domains (RBD, binding receptor domain; RBM, binding receptor motif; TD, trans membrane domain) and proteolytic cleavage sites (S1/S2, S20); Sequences of amino acids around the two (red) protease recognition sites are shown for SARS-S and SARS-2-S (asterisks indicate conserved residues). Arrow heads signify the place of cleavage.

In critical patients SARS-CoV-2 will withdraw newly produced ACE2 and spread lung tissue infection. By promoting viral cell entry, the up-regulated ACE2 will intensify the rate of novel tissue invasion of SARS-CoV-2. The ACE2 receptor internalized in this way cannot degrade angiotensin II. The up-regulation of ACE2 by elevated angiotensin II-in hypothesis a replenishment for inflamed and increasingly fibrotic lung tissue-is converted into the opposite of the intended effect: additional lung tissue infections. In the competition for the new ACE2 sites, we suggest that if this occurs at a sufficiently high rate, SARS-COV-2 will outbalance even extremely high levels of angiotensin II. A cycle of destruction has emerged there, at the end of which lung failure occurs and patient death as a result. The enhanced angiotensin II described above is an issue in and of itself, as patients with unstable lung conditions would really benefit from strong activation of the AT1 receptors. But blocking AT1 receptors with ARBs may also be counterproductive in some patients: this does nothing per se to decrease

high levels of angiotensin II, which in effect drives expression of ACE2 which leads to SARS-CoV-2(Mancini and Fürst).

4.2 Immunopathology of COVID-19

In addition, in severe cases COVID-19 to ACE-2, mediated pathogenesis, the observation of 41 hospitalized patients with elevated levels of proinflammatory cytokines including IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A and TNFα was observed. These findings are consistent with SARS and MERS, because there is lymphopenia and "cytokine storm" which plays a major role in COVID-19 pathogenesis. The so-called "cytokine storm" can cause inflammation-induced viral sepsis and lung injury, leading to other complications such as pneumonitis, acute respiratory distress syndrome (ARDS), respiratory failure, shock, organ failure and eventually death.

Innate immune responses to SARS-CoV-2

There is currently only scant information on the innate immune state of patients infected with SARS-CoV-2 as a host. In one study, in which 99 cases were investigated in Wuhan, total neutrophils increased (38 %), total lymphocytes decreased (35 %), serum IL-6 increased (52 %), and c-reactive protein increased (84 %)(Lillie, Samson et al. 2020, Prompetchara, Ketloy et al. 2020). In a different study also from Wuhan, it reported that in 41 patients, total neutrophils increased, total lymphocyte decreases were found to be statistically different in patients with ICU vs. non-ICU treatment. Enhanced neutrophils and reduced lymphocytes also associate with seriousness of disease and death(Wu, Zhao et al., Prompetchara, Ketloy et al. 2020). In addition, the plasma levels of several endogenous cytokines, IP-10, MCP-1, MIP-1A, and TNFα(Huang, Wang et al. 2020, Prompetchara, Ketloy et al. 2020) were higher in patients requiring ICU treatment. Such clinical features indicated the possibility of strongly pro-inflammatory condition being involved in the development and seriousness of the disease. This early high increase in serum levels of pro-inflammatory cytokines was also observed in infection with SARS-CoV and MERS-CoV, indicating a potential like severity of cytokine storm-mediated disease(Mahallawi, Khabour et al. 2018, Prompetchara, Ketloy et al. 2020).

Potent innate immune response to viral infection relies a lot on the type I responses of interferon (IFN) and its downstream cascade that culminates in viral replication regulation and induction of successful adaptive immune response. Although SARS-CoV and SARS-CoV-2 tend to share the ACE2 entrance receptor, MERS-CoV uses dipeptidyl peptidase

(DPP)-4 as a particular receptor(Lillie, Samson et al. 2020, Prompetchara, Ketloy et al. 2020). The putative SARS-CoV-2, ACE2 receptor is expressed primarily in a small subset of alveolar cells in the lung called type 2(Prompetchara, Ketloy et al. 2020, Zhu, Zhang et al. 2020). Reportedly, SARS-Co-V directly infects macrophages and T cells, a central aspect of pathogenesis mediated by SARS-CoV(Dandekar and Perlman 2005, Prompetchara, Ketloy et al. 2020). It is still not clear whether SARS-CoV-2 infects any immune cells. ACE2 shared only small percentages of the monocytes / macrophages in the lung(Dandekar and Perlman 2005, Prompetchara, Ketloy et al. 2020). If ACE2 is expressed limitedly in the possible target immune cells, other receptors can exist or other mode of cell entry such as antibody-dependent enhancement is used (Figure 3).

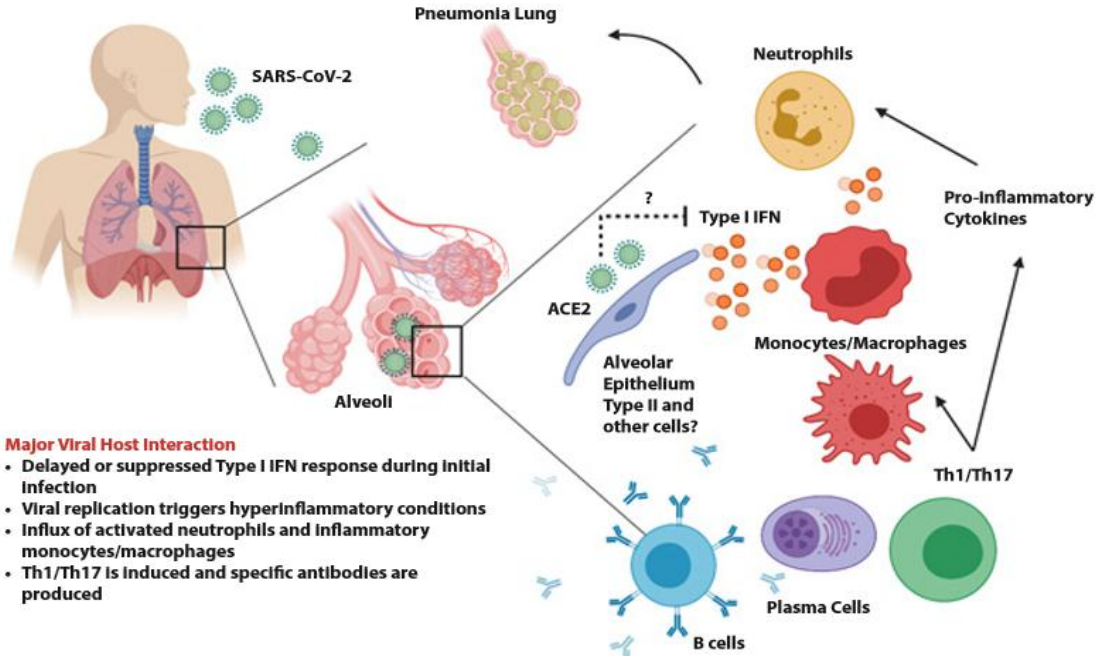

Figure 3: Proposed immune responses of SARS-CoV-2 (Palaga et. al., Asian Pac J Allergy Immunol 2020; 38: 1-9 DOI 10.12932/AP-200220-0772).

Uptake of ACE2 leads to infection with SARS-CoV-2 expressing target cells including alveolar type 2 cells or other unknown target cells. Virus will diminish IFN anti-viral responses leading to unregulated replication of the viruses. The influx of neutrophils and monocytes / macrophages is triggering overproduction of pro-inflammatory cytokines. Lung immunopathology can result from "cytokine storms" It can trigger particular th1/Th17 and help to intensify inflammatory responses. B cells / plasma cells produce different SARS-CoV-2 antibodies that can help neutralize viruses. The question marks indicated items that are unknown or unclear, too.

Innate immune cells must recognize invasion of the virus, often via pathogen-associated molecular patterns (PAMPs), in order to mount an antiviral response. For RNA viruses including coronavirus, it is understood that either theendosomal RNA receptors, TLR3 and TLR7, and the cytosolic RNA sensor, RIG-I / MDA5, identify PAMPs in the form of viral genomic RNA or the intermediates during viral replication like dsRNA. This recognition event triggers downstream signalling cascade to activate, i.e. NF-ÿB and IRF3, with their nuclear translocation. Such transcription factors induce type I IFN expression and other pro-inflammatory cytokines in the nuclei and these initial responses are the first line protection against viral infection at the entry site(de Wit, van Doremalen et al. 2016, Prompetchara, Ketloy et al. 2020).In addition, type IFN through IFNAR stimulates the JAK-STAT cascade, where JAK1 and TYK2 kinases STAT1 and STAT2 phosphorylate. STAT1/2 form a complex with IRF9, and together travel into the nucleus to initiate the transcription of IFN-stimulated genes (ISGs) under the guidance of the promoter-containing IFN-stimulated response factor (ISRE)(de Wit, van Doremalen et al. 2016, Prompetchara, Ketloy et al. 2020). Successful mounting of this type IFN response should be capable of suppressing early on viral replication and dissemination.

For SARS-CoV and MERS-CoV, IFN type I response to viral infection is disabled. Both coronaviruses use several strategies to interfere with the signalling that leads to IFN production of type I and/or downstream IFNAR signals. This damping technique is closely related to the severity of the disease(Channappanavar and Perlman 2017, Prompetchara, Ketloy et al. 2020). At the induction stage of type IFN, SARSCoV interferes directly or indirectly with the downstream signalling of RNA sensors, such as ubiquitisation and degradation of MAVS and TRAF3/6 RNA sensor adapter molecules and inhibits IRF3 nuclear translocation(Kindler, Thiel et al. 2016, Prompetchara, Ketloy et al. 2020).MERS-CoV also uses some of these approaches with additional methods such as repressive modulation of the histone(Kindler, Thiel et al. 2016). These two viruses are equipped with mechanisms that inhibit IFN signalling such as decreasing STAT1 phosphorylation(de Wit, van Doremalen et al. 2016, Prompetchara, Ketloy et al. 2020) once Type I IFN is secreted. The viral proteins involved in this host type IFN response modulation are both structural proteins (such as M, N) and non-structural proteins (ORF proteins), respectively.

Based on the comparison of the genomic sequence, SARS-CoV-2 shares overall genomic similarity with SARS-CoV or MERSCoV, roughly 79% and 50% respectively. The SARS-

CoV-2 genome also comprises additional regions of the genes (10b, 13, 14). Furthermore, the amino acid sequences of certain putative SARS-CoV-2 proteins show just 68 % resemblance to SARS-CoV(Lu, Zhao et al. 2020, Prompetchara, Ketloy et al. 2020). Consequently, careful sequence comparison of each gene region can yield a better prediction as to how SARS-CoV-2 interferes with innate immune response from host. It is partly plausible that SARS-CoV-2 employs similar strategies to modulate the innate immune response of the host, in particular by dampening the response of type IFN but additional novel mechanismmay be uncovered (Figure 3).

The increased influx of neutrophils and monocyte-macrophages is reliably observed (Dandekar and Perlman 2005, Zumla, Hui et al. 2015, Prompetchara, Ketloy et al. 2020) in serious or lethal cases of SARS-CoV or MERS-CoV infection. Dysregulated type IFN and inflammatory monocyte-macrophages are the major cause of lethal pneumonia(Channappanavar and Perlman 2017, Prompetchara, Ketloy et al. 2020) in a mouse model of SARSCoV infection. Excessive type IFN with infiltrated myeloid cells are also the main cause of lung dysfunction and have a detrimental effect on the outcome of the infection. It is hypothesized that delayed type 1 IFN compromise early viral regulation upon SARS-CoV or MERS-CoV infection, contributing to the accumulation of hyperinflammatory neutrophils and monocyte-macrophages. The rise in these innate immune cells, like pneumonia or acute respiratory distress syndrome, contributes to worsening consequences for infected host that manifested in lung immunopathology. Ironically, virus transmission is reported to occur even in people infected with asymptomatic conditions. This may be representative of the inborn immune response being suppressed early.

Based on the cumulative evidence for previous infection with coronavirus, innate immune response plays a critical role in defensive or destructive responses, which may open a window for immune intervention. Later on, successful viral replication results in type IFN hyperproduction and the release of neutrophils and macrophages which are the key sources of pro-inflammatory cytokines. During COVID19, with related changes in total neutrophils and lymphocytes, SARS-CoV-2 is likely to cause delayed type IFN and viral control loss in an early infection. Individuals vulnerable to CoVID19 are those with underlying conditions including diabetes, high blood pressure and cardiovascular disease(Huang, Wang et al. 2020, Prompetchara, Ketloy et al. 2020). Furthermore, while innate immune response is highly

effective, no severe cases have been reported in young children. These findings suggest strongly that an innate immune response is a vital factor in the outcome of diseases.

Adaptive immune responses to SARS-CoV-2

The immune response type Th1 typically plays a dominant role in an adaptive immunity to viral infections. Cytokine microenvironment produced by cell presenting antigen dictates the direction of cell responses in T. Helper T cells orchestrate the overall adaptive response, while cytotoxic T cells are necessary to destroy cells infected with viruses. Humoral immune response, particularly the development of neutralizing antibodies, plays a protective role by limiting later-phase infection and will prevent potential re-infection. In SARS-CoV the structural proteins, S, N, M and E proteins(Liu, Zhao et al. 2017, Prompetchara, Ketloy et al. 2020) were extensively mapped for both T and B cell epitopes.

Infection with SARS-CoV causes seroconversion as early as day 4 after the initiation of the disease and was observed by 14 days in most patients. Specific long-lasting IgG and neutralizing antibody was recorded for as long as 2 years after infection(Liu, Fontanet et al. 2006). Seroconversion is seen for MERS-CoV infection in the second or third week of onset of disease. Delayed and poor response to an antibody was correlated with serious outcome(Liu, Zhao et al. 2017, Prompetchara, Ketloy et al. 2020) for both forms of coronavirus infections. A limited description of SARS-CoV-2 serology has been published. One patient displayed peak-specific IgM in a preliminary analysis on day 9 after the initiation of the disease and the switch to week 2 IgG (Lillie, Samson et al. 2020, Prompetchara, Ketloy et al. 2020). Amusingly, sera from 5 confirmed COVID-19 patients show some cross-reactivity with SARS-CoV but not other coronavirus. In addition, both sera patients were able to neutralize SARS-CoV-2 in a plaque assay in vitro, indicating a possible positive mounting of the humoral responses(Lillie, Samson et al. 2020, Prompetchara, Ketloy et al. 2020). It remains to be studied whether the kinetic / titer of the specific antibody correlates with the extent of the disease.

T-cell response has been investigated extensively in SARS-CoV. During one study using 128 convalescent samples it was stated that responses to CD8 + T cells were more frequent with greater magnitude than responses to CD4 + T cells. In addition, virus-specific T cells from the extreme community appeared to be a core memory phenotype with a significantly higher frequency of polyfunctional CD4 + T cells (IFNÿ, TNFα, and IL-2) and CD8 + T cells (IFNπ, TNFα, and degranulated state) compared to the mild-moderate cells. Strong T-cell responses

associated significantly with higher neutralizing antibody, while the fatal community identified more serum Th2 cytokines (IL-4, IL-5, IL-10) (Li, Wu et al. 2008, Prompetchara, Ketloy et al. 2020). Most responses (70%) against the structural proteins (spike, shell, membrane, and nucleocapside) were used for epitope mapping. In MERS-CoV infection, early rise of CD8 + T cells correlates with severity of disease, and dominant Th1 type helper T cells are observed (Shin, Kim et al. 2019) at the convalescent period. In an animal model, conserved epitope-specific CD4 + T airway memory cells are defensive against lethal challenges and can cross-react with SARS-CoV and MERS-CoV (Zhao, Zhao et al. 2016, Prompetchara, Ketloy et al. 2020). The protective or destructive role of Th17 in human coronavirus infection remains unclear, as neutrophils play a destructive function in all infections.

Current evidence strongly suggested that the response to the Th1 case is a key to effective control of SARS-CoV and MERS-CoV, and possibly also valid for SARS-CoV-2. Although crucial, CD8 + T cell response needs to be well regulated so as not to cause lung pathology. Since the majority of epitopes found for both viruses are focused on the viral structural proteins, comparing those epitopes associated with SARS-CoV / MERS-CoV with those of SARS-CoV-2 will be informative. If one can distinguish overlapping epitopes among the three viruses, using convalescent serum from recovered SARS or MERS patients would be advantageous for application in passive immunization (Prompetchara, Ketloy et al. 2020).

Potential immune evasion mechanisms

Current studies suggest that coronaviruses are especially suited to avoid immune detection and to lessen the response of human immune systems. It explains in part why they appear to have a longer time of incubation, averaging 2-11 days compared to influenza, 1-4 days (Lessler, Reich et al. 2009, Prompetchara, Ketloy et al. 2020). The longer time of incubation is possibly due to their properties of immune evasion, essentially preventing host immune detection at the early stage of the infection. As a member of the genus Betacoronavirus, the mechanism of immune evasion probably resembles those of SARS-CoV and MERS-CoV. Mechanisms for how SARS-CoV and MERSCoV modulate host immune responses have been studied and discussed extensively (Figure 4) (Shokri, Mahmoudvand et al. 2019, Kikkert 2020, Prompetchara, Ketloy et al. 2020). In summary, most mechanisms depend on inhibiting innate immune responses, especially the recognition and signaling of type I interferons. The key molecules in host immune modulation are the viral proteins including

membrane (M) or non-structural (NS) proteins (e.g. NS4a, NS4b, NS15). In accordance with the aforementioned report, examination of two individuals with different severity infected with MERS-CoV showed that the response of type I interferon in the patient with poor outcome (death) was significantly lower than that of the recovered patient (Faure, Poissy et al. 2014, Prompetchara, Ketloy et al. 2020)Antigen presentation through MHC class I and MHC class II was de-regulated for adaptive immune evasion when the macrophages or dendritic cells were infected with MERS-CoV, which would significantly inhibit T cells activaton (Shokri, Mahmoudvand et al. 2019, Prompetchara, Ketloy et al. 2020).

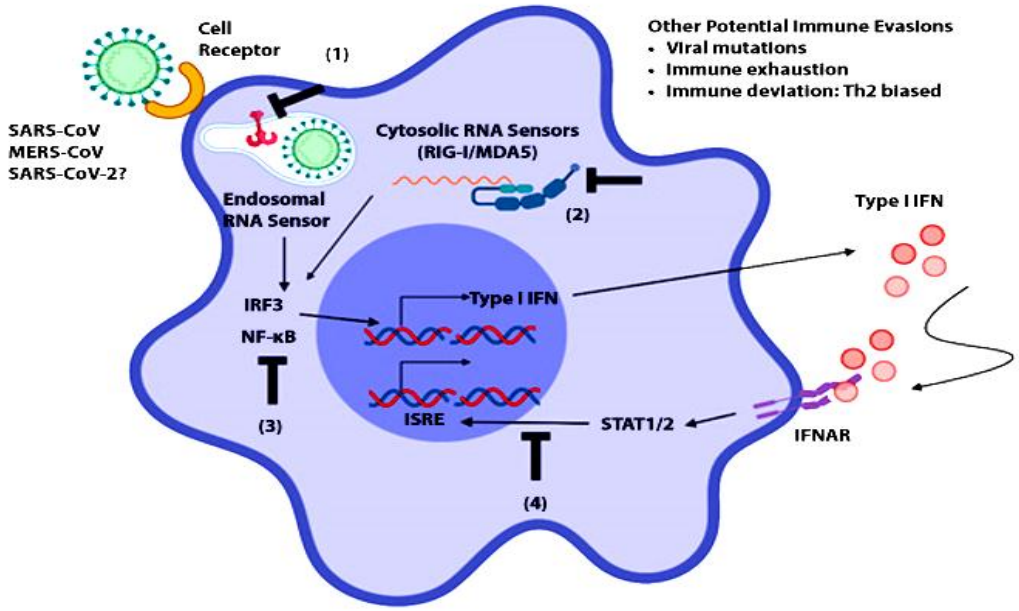

Figure 4: Potential mechanisms of immune evasion common to SARS-CoV, MERS-CoV, and SARS-CoV-2 (Palaga et. al., Asian Pac J Allergy Immunol 2020; 38: 1-9 DOI 10.12932/AP-200220-0772).

Coronaviruses interfere with multiple steps during initial innate immune response, including RNA sensing (1 and 2), Type I IFN production signaling pathway (3), STAT1/2 downstream activation of IFN / IFNAR (4), as indicated by suppressive signs. This type IFN response which is delayed or dampened impairs adaptive immune activation. Prolonged viral persistence exacerbates inflammatory responses as a regulatory feedback mechanism that can contribute to immune fatigue and immune suppression. Biased response of the Th2 type also favors poor disease outcome.

5. Pharmacological targets

Currently there is no specific target for COVID-19 treatment, based on COVID-19 pathogenesis as discussed above, as mentioned below are some of the targets.

SARS-CoV-2 viral life cycle and potential drug targets

SARS-CoV-2, a single-stranded RNA-enveloped virus, targets cells via the angiotensin-converting enzyme 2 (ACE2) receptor-binding viral structural spike (S) protein. The virus particle uses host-cell receptors and endosomes to penetrate cells following binding of the receptor. A serine protease of host type 2 transmembrane, TMPRSS2, enables cell entry through the S-protein(Hoffmann, Kleine-Weber et al. 2020, Sanders, Monogue et al. 2020). once inside the cell, viral polyproteins are synthesized which encode for the complex replicase-transcriptase. Then the virus synthesizes RNA through its RNA-dependent RNA polymerase. Structural proteins are synthesized which lead to viral particle assembly and release completion(Bravo, Solano et al. 2014, Fehr and Perlman 2015, Sanders, Monogue et al. 2020). These steps in the viral life cycle offer potential targets (Figure 5), Promising drug targets includes non-structural proteins (e.g.,3-chymotrypsin-like protease, and RNA-dependent polymerase), and additional targets constitute pathways for viral entry and immune regulation(Savarino, Boelaert et al. 2003, Al-Bari 2017, Sanders, Monogue et al. 2020).Table.1 summarizes potential COVID-19 targets and proposed treatments or adjunctive therapies.

Ongoing clinical trials

On Clinical Trials, the search terms COVID OR coronavirus OR SARS-COV-2. The Government has conducted 351 active trials, with 291 COVID-19-specific trials as of April 2, 2020. For these 291 trials, about 109 trials (including those not yet enrolled, enrolled, involved or completed) provided pharmacological therapy for COVID-19 treatment in adult patients. of these 109 trials, 82 are interventional, with 29 placebo-controlled studies (Sanders, Monogue et al. 2020).

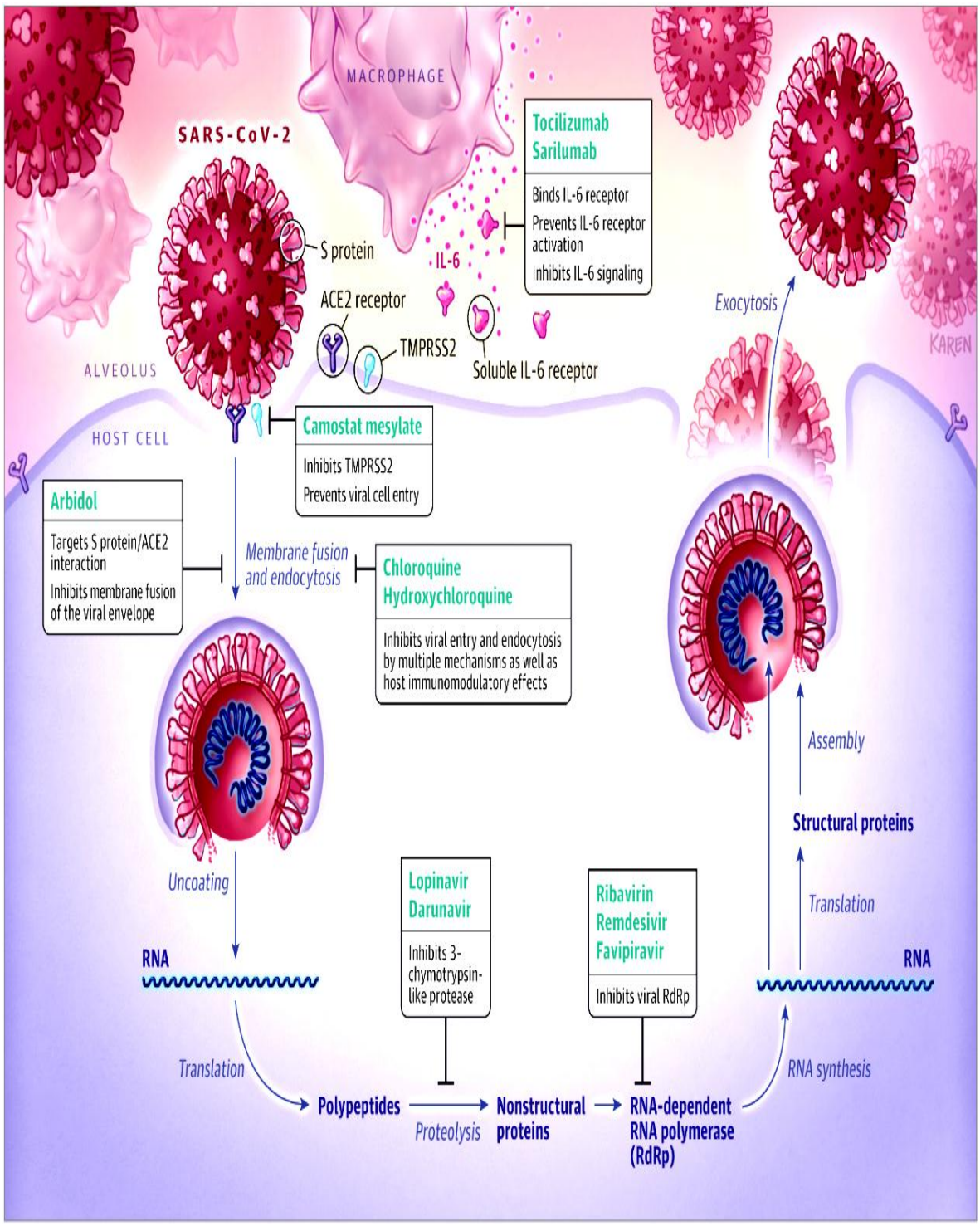

Figure 5: The virus induced response of the host immune system and the processing of viruses within target cells.ACE2, angiotensin converting enzyme2; S-protein, spike protein; and TMPRSS2, type2 transmembrane serine protease (Cutrel J.B. et. al Clinical Review& Education doi:10.1001/jama.2020.6019)

Table 1: Proposed targets and their repurposed and investigational drugs.

Target	Drugs	References
	Repurposed drugs	
Viral entry blockage by inhabiting host receptor glycosylation, proteolytic processing, and endosomal acidification. Additional immunomodulatory effects in host cells by inhibiting the cytokine production, autophagy, and lysosomal function.	Chloroquine phosphate (Aralen/generic) Hydroxychloroquinesulfate (Plaquenil/ generic)	(Devaux, Rolain et al. 2020, Sanders, Monogue et al. 2020, Zhou, Dai et al. 2020)
Inhibition of TMPRSS2 and prevent viral cell entry	Camostatmesylate	(Hoffmann, Kleine-Weber et al. 2020, Sanders, Monogue et al. 2020)
3-Chymotrypsin like protease	Lopinavir/ritonavir (Kaletra)	(Chu, Cheng et al. 2004, Sanders, Monogue et al. 2020)
S protein/ACE2, membranefusion inhibitor	Umifenovir (Arbidol)	(Khamitov, Loginova et al. 2008, Sanders, Monogue et al. 2020)
RNA polymerase inhibitor	**Investigational drugs**	(Al-Tawfiq, Al-Homoud et al. 2020, Sanders, Monogue et al. 2020)
	Remdesivir	
RNA polymerase inhibitor	Favipiravir	(Sanders, Monogue et al. 2020)
IL-6 inhibition- reduction in cytokine storm	**Adjuvant therapy**	(XuX, HanM, LiT,etal.2020(Sanders, Monogue et al. 2020))
	Tocilizumab (Actemra)	

6. Hypothesis

6.1 ACE-2 hypothesis

ACE2 receptors act as binding sites for anchoring spike (S) proteins on the outer surfaces of beta coronaviruses (Diaz 2020, Xu, Chen et al. 2020). Severe acute respiratory syndrome (SARS) is caused by the beta coronavirus SARS-CoV. The phylogenetically related beta coronavirus, SARS-Cov-2, induces novel coronavirus disease (nCoV-2019) or COVID-19 (Diaz 2020, Xu, Chen et al. 2020). S proteins attach all beta coronaviruses to ACE2 receptors in infected patients' lower respiratory tract in order to enter the lungs and induce viral pneumonia and potentially lethal respiratory failure (Diaz 2020, Xu, Chen et al. 2020).

Infection with SARS-CoV-2 results in a major loss of overall physiological activity of the ACE2. It in turn leads to a decline in the rate of its products and an excess of its substrate. Hence, angiotensin I-9, angiotensin I-7, and thus activation of the mas receptor, will vanish. Meanwhile angiotensin I is gradually transformed by the remaining ACE into angiotensin II and angiotensin II is no longer degraded to angiotensin I-7. Both of these mechanisms

contribute directly to high levels of angiotensin II which contributes to ACE-2 upregulation(Mancini and Fürst , Huang, Li et al. 2010, Liu, Yang et al. 2020). The lack of angiotensin I-9 adversely affects both the activation of the mas receptor and the balance of the activation of the AT1/AT2 receptor, pushing it towards the AT1 (Figure 6).

Whereas patients treated with ACEIs and ARBs may have increased numbers of ACE2 receptors that bind to coronavirus S proteins in their lungs, they may be at increased risk for serious disease outcomes due to infections with SARS-CoV-2 (Diaz 2020). During the ongoing COVID-19 outbreak, patients treated with ACEIs and ARBs for cardiovascular conditions will avoid crowds, public gatherings, ocean cruises, lengthy air travel and all individuals with respiratory illnesses to reduce their risk of infection (Diaz 2020).

6.2 Cytokine hypothesis

SARS-CoV-2 binds to the alveolar epithelial cells, then the virus stimulates the innate immune system and the adaptive immune system, which results in the release of a large number of cytokines, including IL-6. However, due to the function of these inflammatory factors, vascular permeability enhanced, a significant number of fluid and blood cells entering the alveoli, leading to dyspnea and even respiratory failure(Knudsen and Ochs 2018, Leiva-Juárez, Kolls et al. 2018, Zhang, Wu et al. 2020)(Figure 7).

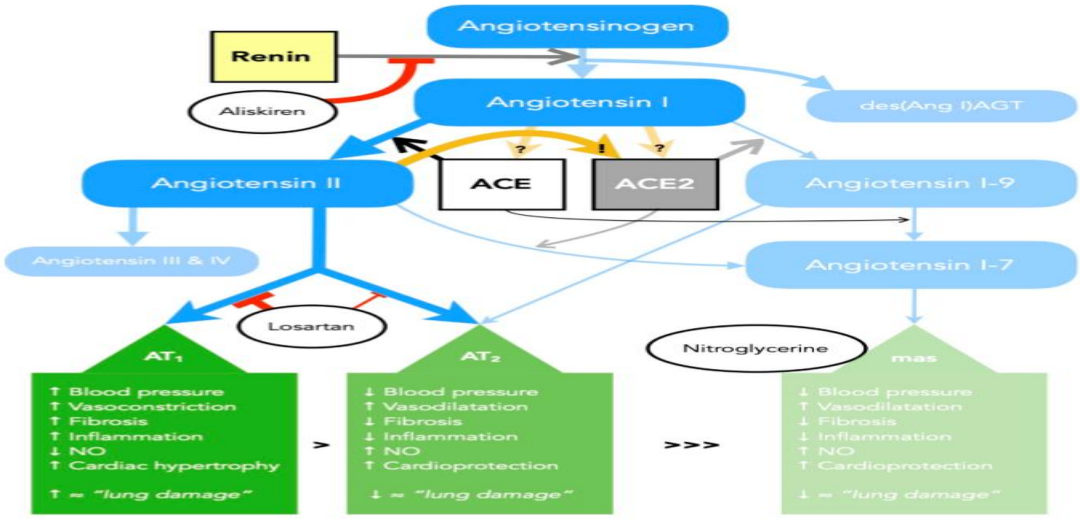

Figure 6: The entry of SARS-CoV-2 cells decreases the activity of ACE2, results in elevated angiotensin II and elevated angiotensin II upregulates ACE2. The AT1/AT2/mas activation moves to AT1 and away from mas. The treatment recommends aliskiren, losartan, and nitroglycerin. (Edele Mancini et. al, 2020 DOI:10.13140/RG.2.2.35010.94400)

In cytokine storm IL-6 plays a central role; IL-6 is a multi-effective cytokine with anti-inflammatory and pro-inflammatory effects. There are three major IL-6 signal transduction pathways(Hunter and Jones 2015, Zhang, Wu et al. 2020)(fig 8). 1. Classical signal transduction (fig 8A), 2. trans signal transduction (fig 8B) and 3. Trans presentation. (Fig 8C).

IL-6 binds to its receptor IL-6R to form a complex in the classical signal transduction pathway(Baran, Hansen et al. 2018), and then binds to the membrane proteins gp130 to activate intracellular signal transduction.

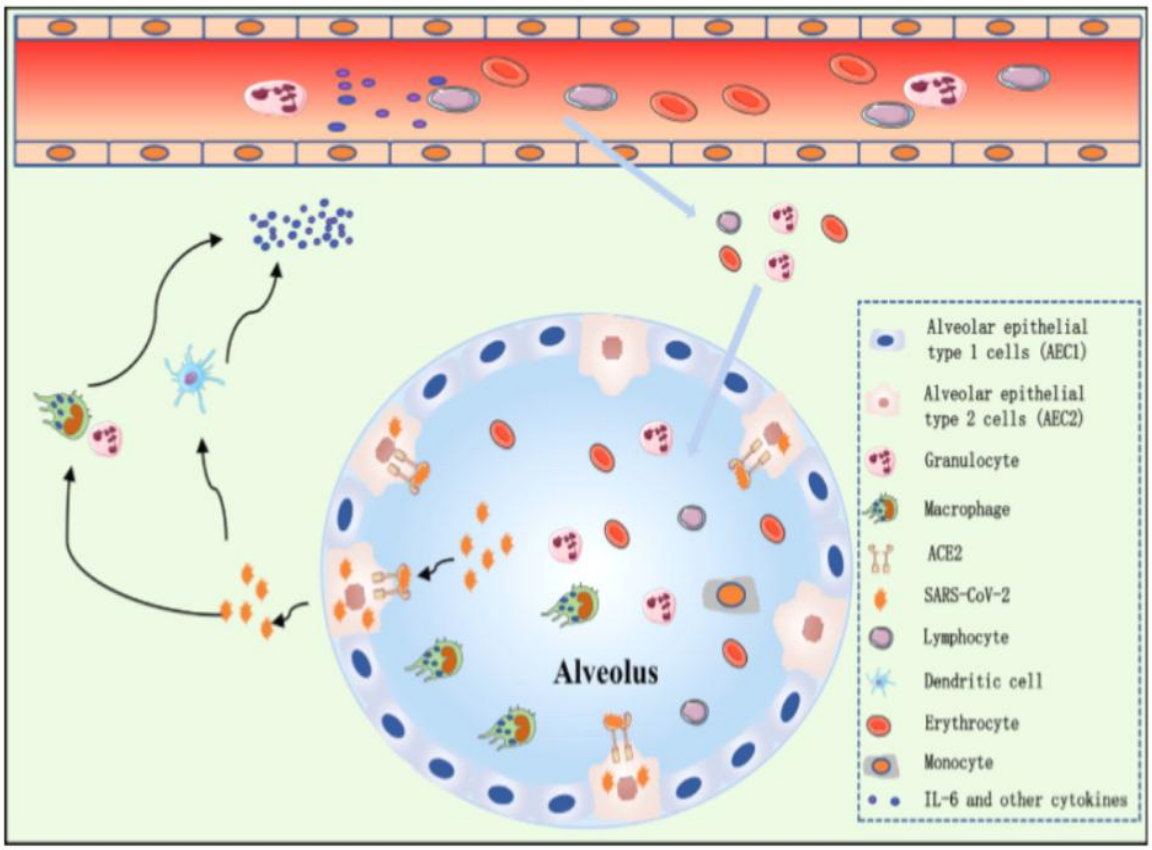

Figure 7: Probable cytokine-release mechanism in COVID-19. The SARS-CoV-2 infects epithelial alveolar cells (mainly alveolar epithelial type 2 cells (AEC-2)) via the receptor ACE-2. The destruction of epithelial cells and the rise of cell permeability lead to virus entry.

The SARS-CoV-2 stimulates the innate immune system, which not only traps the virus by macrophases and other innate immune cells, but also releases a significant number of cytokines and chemokines including IL-6. Adaptive immunity is often triggered by the cells (mainly dendritic cells) presenting antigen. T cell and B cell not only play antiviral function

but also facilitate the secretion of inflammatory cytokines directly or indirectly .A significant number of inflammatory exudates and erythrocytes enter and resultingdyspnea and respiratory failure, under the stimulation of inflammatory factors.

IL-6R not only occurs in transmembrane form, but also in soluble form. IL-6 binds to these 2 types, and then interacts with gp130, activating downstream signal transduction and gene expression(Briso, Dienz et al. 2008, Zhang, Wu et al. 2020). In the trans signal transduction pathway, the binding affinity of sIL-6R to IL-6 is close to that of IL-6, and this complex binds to gp130, which initiates intracellular signal transduction, many cells cannot respond to the IL-6 signal in the classical signal pathway because they do not express IL-6R, but some of these cells can be stimulated by the sIL-6R-IL-6 complex to respond to the IL-6 signal and cause signal transduction(Johnson, O'Keefe et al. 2018, Zhang, Wu et al. 2020).Extracellular sgp130 suppresses the trans-presentation signal, and sgp130 will form a sIL-6R complex to prevent sIL-6R from binding to the membrane bound gp130 (Jones and Jenkins 2018, Zhang, Wu et al. 2020).

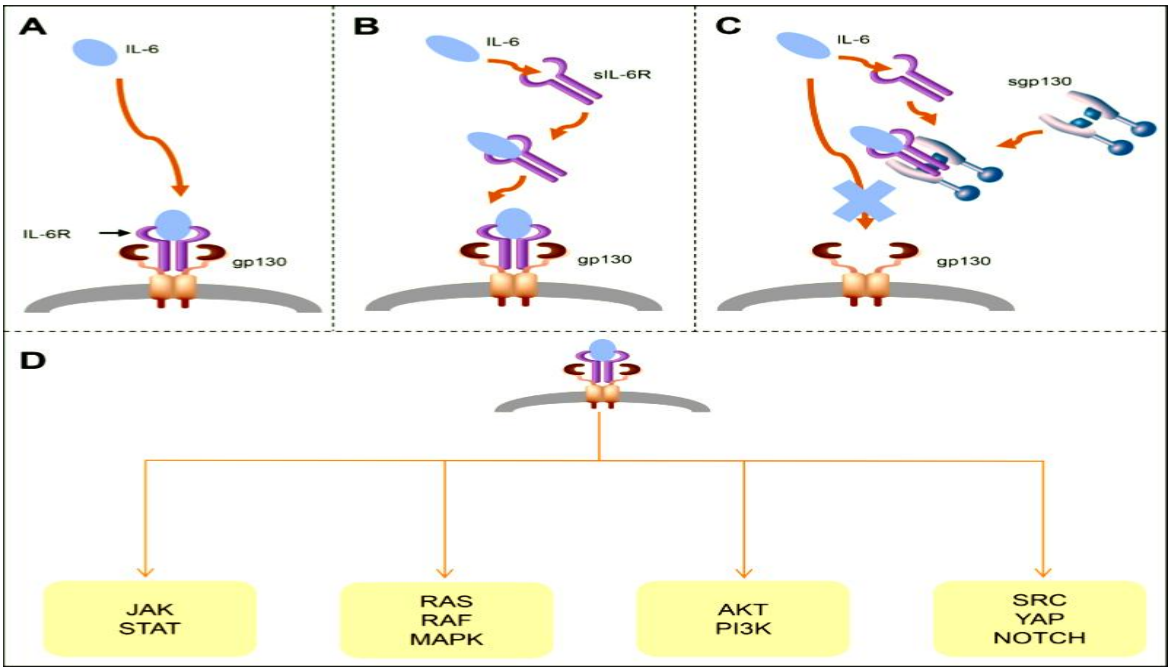

Figure 8: IL-6 signal transduction pathway. A. classical signal transduction B. trans signal transduction C. trans presentation D. activation of the JAK-STAT(STAT1,STAT3 and, to a lesser extent, STATS) pathway. In addition, RAS-RAF pathway, SRC-YAP-NOTCH pathway, and AKT-PI3K pathway also being activated, to facilitate complex biological functions such as proliferation, differentiation, oxidative stress, immune regulation.

The next move is to activate the pathway to JAK-STAT(STAT1,STAT3 and, to a lesser degree, STATS)(Villarino, Kanno et al. 2017, Johnson, O'Keefe et al. 2018). In addition, it also activates RAS-RAF pathway(Villarino, Kanno et al. 2017), SRC-YAP-NOTCH pathway(Taniguchi, Wu et al. 2015, Zhang, Wu et al. 2020), and AKT-PI3 K pathway, to encourage complex biological functions such as proliferation, differentiation, oxidative stress, immune regulation (Zhang, Wu et al. 2020).

7. Swot analysis of hypothesis

Strengths

- Previous coronaviruses i.e SARS-CoV and MERS-CoV information on ACE-2 and immune system will strengthen us to better interpretation of current COVID-19.

Weakness

- Patients treated with ACEIs and ARBs may have increased numbers of ACE2 receptors that bind to coronavirus S proteins in their lungs.

Opportunities

- Modulation of ACE-2 and immune system in COVID-19 are the opportunities for the treatment of COVID-19.

Threats

- Refractory hypertension, coronary artery disease, heart failure, and post-myocardial infarction can exacerbate the COVID-19.

8. Potential outcomes

- Possible therapeutic strategy to improve the prognosis of COVID-19 patients could be the modulation of ACE2 in the lung.
- Another therapeutic strategy for improving COVID-19 patients can be the suppression of the innate immune and adaptive immune responses, which resulting in cytokine release inhibition, including IL-6.

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