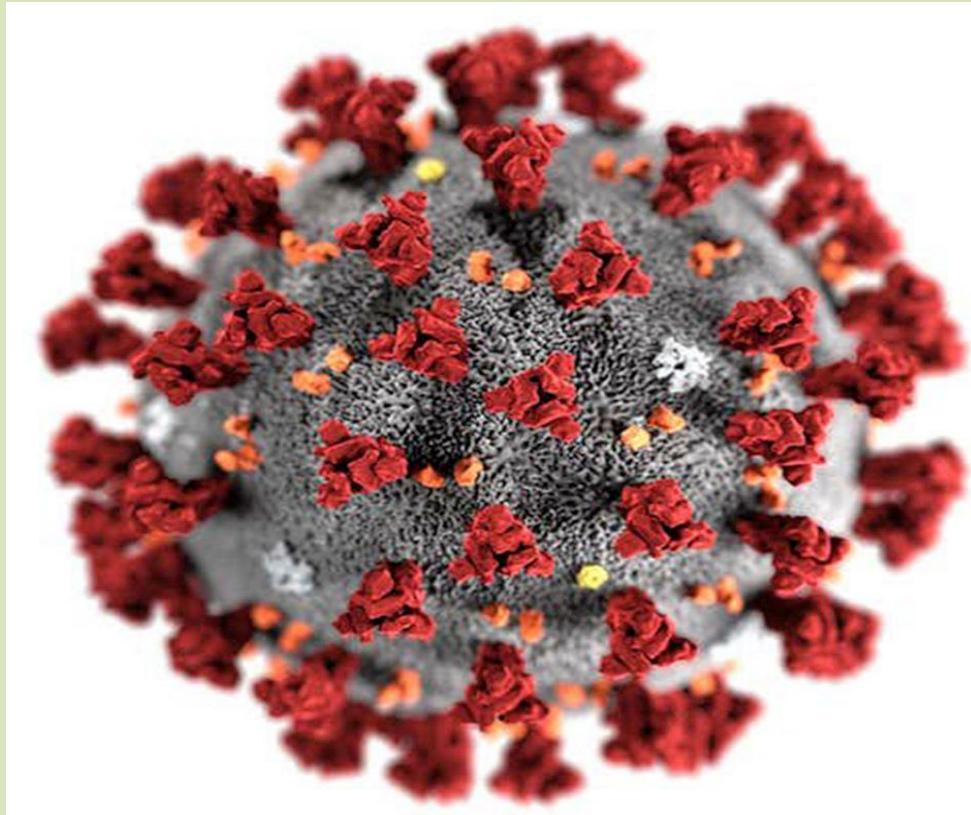


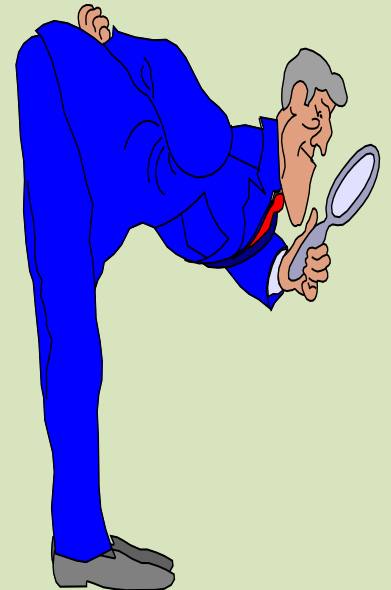
COVID -19

How Does It Work?



Objectives

- History
- Virus classification
- Clinical Manifestations
- Pathogenesis
- Potential Therapeutic Targets



History of COVID-19

On 31st December 2019, 27 cases of pneumonia of unknown etiology, were identified in Wuhan City, Hubei province in China.

Wuhan is the most populous city in central China with a population exceeding 11 million.

These patients most notably presented with clinical symptoms of dry cough, dyspnea, fever, and bilateral lung infiltrates on imaging.

History of COVID-19

Cases were all linked to Wuhan's Huanan Seafood Wholesale Market, which trades in fish and a variety of live animal species including poultry, bats, marmots, and snakes.

The causative agent was identified from throat swab samples conducted by the Chinese CDC (CCDC) on 7th January 2020.

History of COVID-19

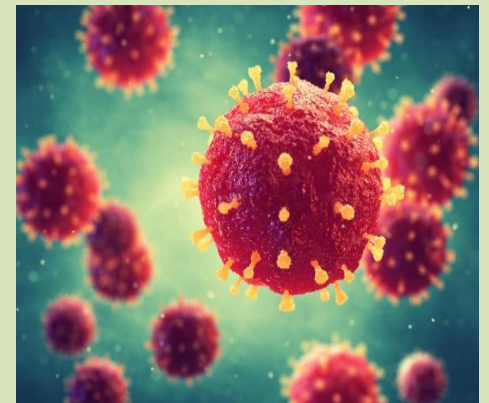
It was subsequently
named Severe Acute
Respiratory Syndrome
Coronavirus 2
(SARS-CoV-2).



The disease was
named COVID-19
by the WHO.

Coronaviruses

- Enveloped
- Positive (mRNA), single-stranded RNA viruses
- Rough spherical or multi-faceted crystal shape
- The surface: prominent club-shaped projections= Spike protein
- Inside: genome wrapped in a nucleocapsid



Coronaviruses

Infect many animals



Human-adapted viruses likely are introduced through zoonotic transmission from animal reservoirs



Most known human coronaviruses are associated with mild upper respiratory illness.

Respiratory and intestinal infections

Birds, humans and some other vertebrates.

- Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 9, September 2020
- Q. Ye, B. Wang and J. Mao, Journal of Infection 80 (2020) 607–613

SARS- CoV-2

Among all known coronavirus sequences, SARS- CoV-2 is most similar to **bat coronavirus** (RaTG13), with **98% similarity**

Coronavirus sequences in the **pangolin** (a scaly anteater) also share high similarity

Coronaviruses

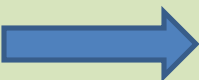
Belonging to the
Coronavirinae subfamily,
Coronaviridae family,
Nidovirales order

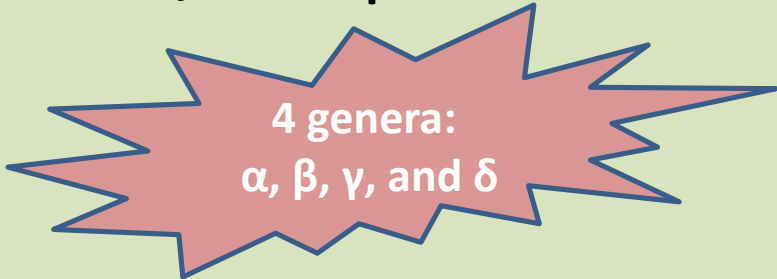
The International Committee
on Taxonomy of Viruses
(ICTV) classifies the CoVs into
four categories: α , β , γ , and δ

CoVs are the largest known RNA viruses.

- *Q. Ye, B. Wang and J. Mao, Journal of Infection 80 (2020) 607–613*
- *International Journal of Surgery 76 (2020) 71–76*

Coronaviruses

- **α coronavirus:** Human coronaviruses (229E and NL63)  common cold/croup
- **β coronaviruses:**
 - ♠ SARS-CoV
 - ♠ Middle East respiratory syndrome coronavirus (MERS-CoV)
 - ♠ SARS-CoV-2

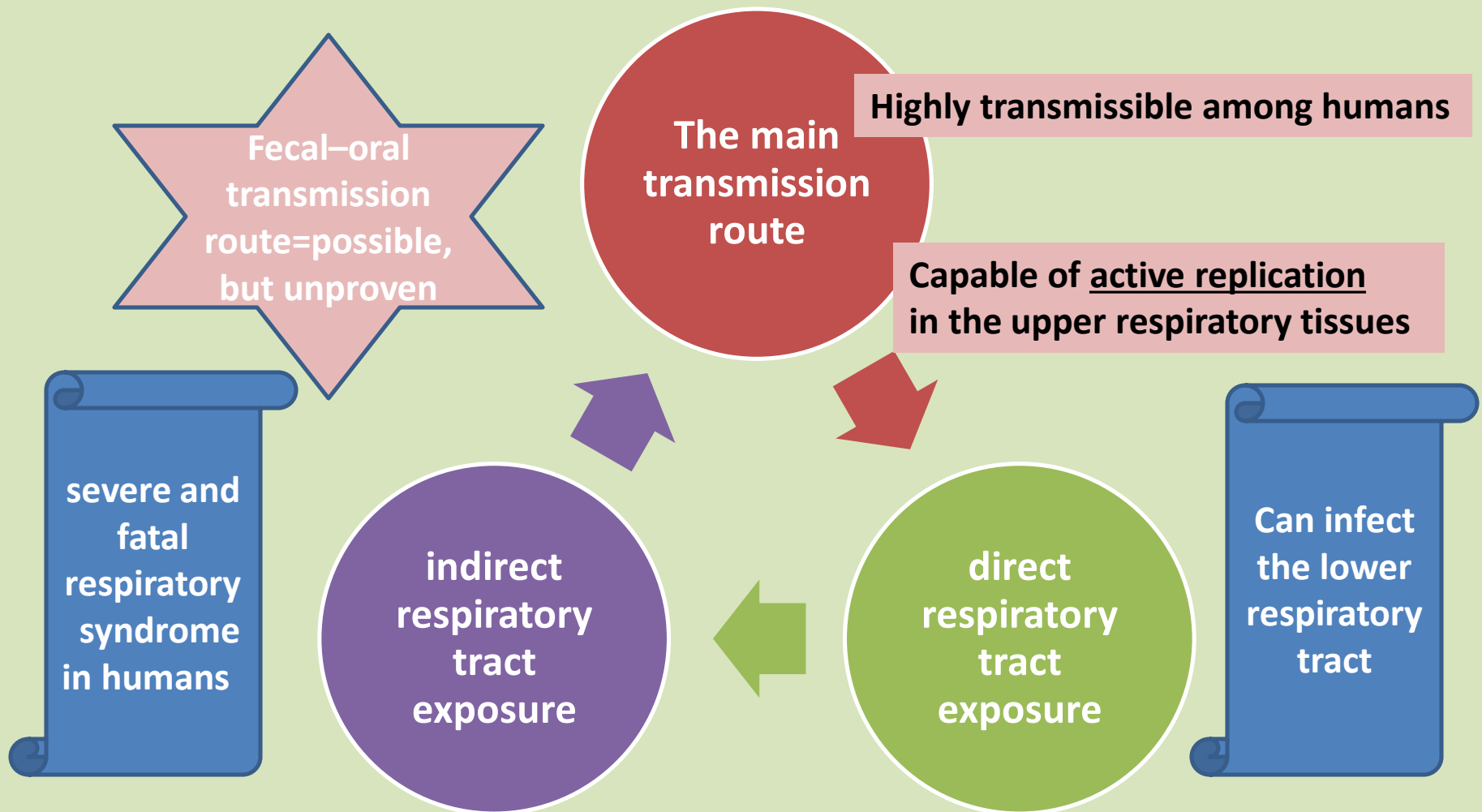


4 genera:
 α , β , γ , and δ



α and β coronaviruses
infect only mammals

SARS-CoV-2 Transmission Route



- Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 9, September 2020
- Cell Research (2020) 30:367–369; <https://doi.org/10.1038/s41422-020-0327-4>
- Q. Ye, B. Wang and J. Mao, Journal of Infection 80 (2020) 607–613

SARS-CoV-2

- On infection, the median incubation period is approximately 4–5 days before symptom onset
- 97.5% of symptomatic patients developing symptoms within 11.5 days

Within 5–6 days of symptom onset, SARS- CoV-2 viral load reaches its peak

ARDS & COVID

**Severe COVID-19 cases progress to ARDS,
on average around 8–9 days after symptom onset**

**ARDS is the main
cause of death in
COVID-19 disease**

The cytokine storm

**Disease severity in patients is due to not only the
viral infection, but also the host response**

ARDS & COVID

ARDS may lead directly to respiratory failure, which is the cause **of death in 70% of fatal** COVID-19 cases

ARDS is the main cause of death in COVID-19 disease

The cytokine storm

Release of cytokines by the immune system in response to the viral infection and/or secondary infections can result in a cytokine storm and symptoms of sepsis that are the cause of death in **28% of fatal** COVID-19 cases

The Systemic & Respiratory Disorders Caused by COVID-19 Infection

- *Muscle and/or joint pain
- *Dizziness
- *Nausea

Systemic Disorders

Fever, Cough, Fatigue,
Sputum Production,
Headache

Haemoptysis,

Acute Cardiac Injury

Hypoxemia

Dyspnoea,
Lymphopenia

Diarrhoea

Respiratory Disorders

Rhinorrhoea,
Sneezing, Sore Throat

Pneumonia

Ground-glass Opacities

RNAemia, Acute
Respiratory Distress
Syndrome

Organ failure:
*cardiac
*hepatic
*renal systems

**Most
who
progressed to
renal failure
eventually
died**

Journal of Autoimmunity 109 (2020) 102433
Nature Reviews, Immunology, volume 20, June 2020 , 363-374

Classification of COVID-19 Patients

Asymptomatic: PCR +, Without any clinical symptoms and signs and the chest imaging is normal

Mild: Symptoms of acute URTI (fever, fatigue, myalgia, cough, sore throat, runny nose, sneezing) or digestive symptoms (nausea, vomiting, abdominal pain, diarrhea)

Moderate: Pneumonia (frequent fever, cough) with no obvious hypoxemia, chest CT with lesions.

Severe: Pneumonia with hypoxemia ($\text{SpO}_2 < 92\%$)

Critical: ARDS, may have shock, encephalopathy, myocardial injury, heart failure, coagulation dysfunction and acute kidney injury.

SARS-CoV2 Infection & Disease



I. an asymptomatic phase with or without detectable virus

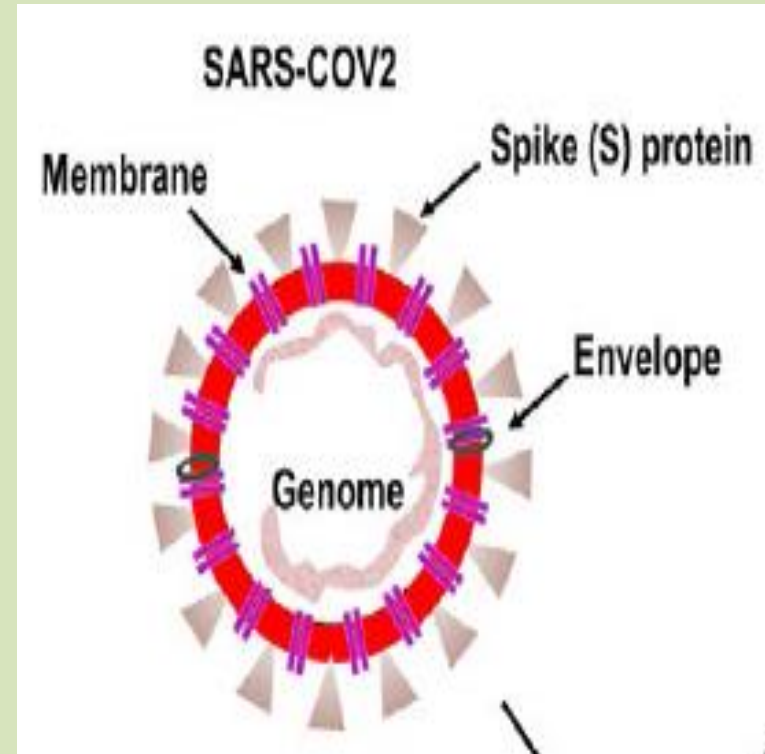
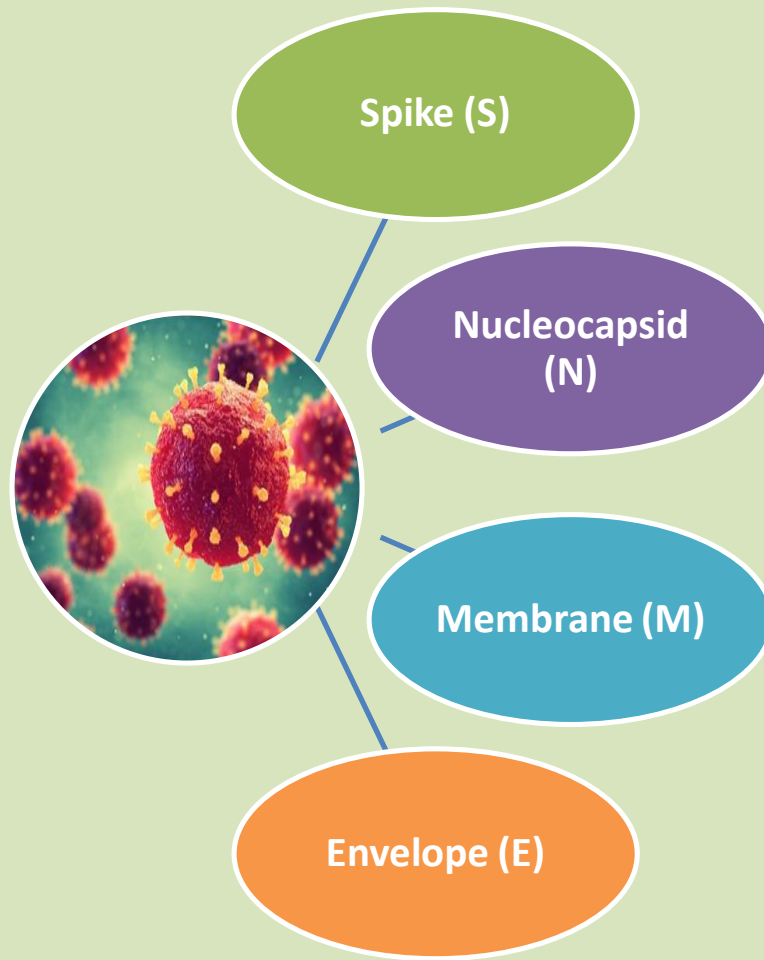


II. a non-severe symptomatic phase with upper airway involvement



III. a severe, potentially lethal disease with hypoxia, 'ground glass' infiltrates in the lung, and progression to ARDS with high viral load

The Coronavirus Genome Encodes 4 Major Structural Proteins



Spike

- **Spike** = a transmembrane trimetric glycoprotein protruding from the viral surface,

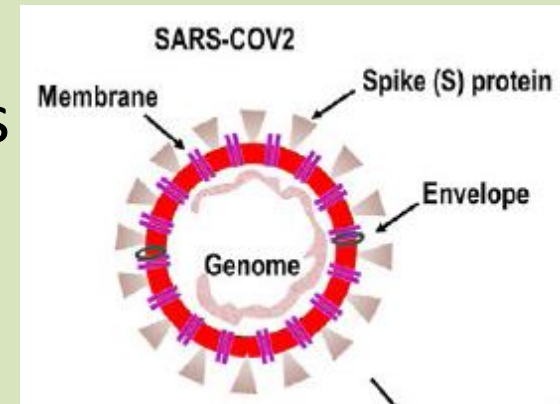


- the diversity of coronaviruses
- host tropism

- Spike functional subunits:

S1 subunit → binding to the host cell receptor

S2 subunit → the fusion of the viral and cellular membranes

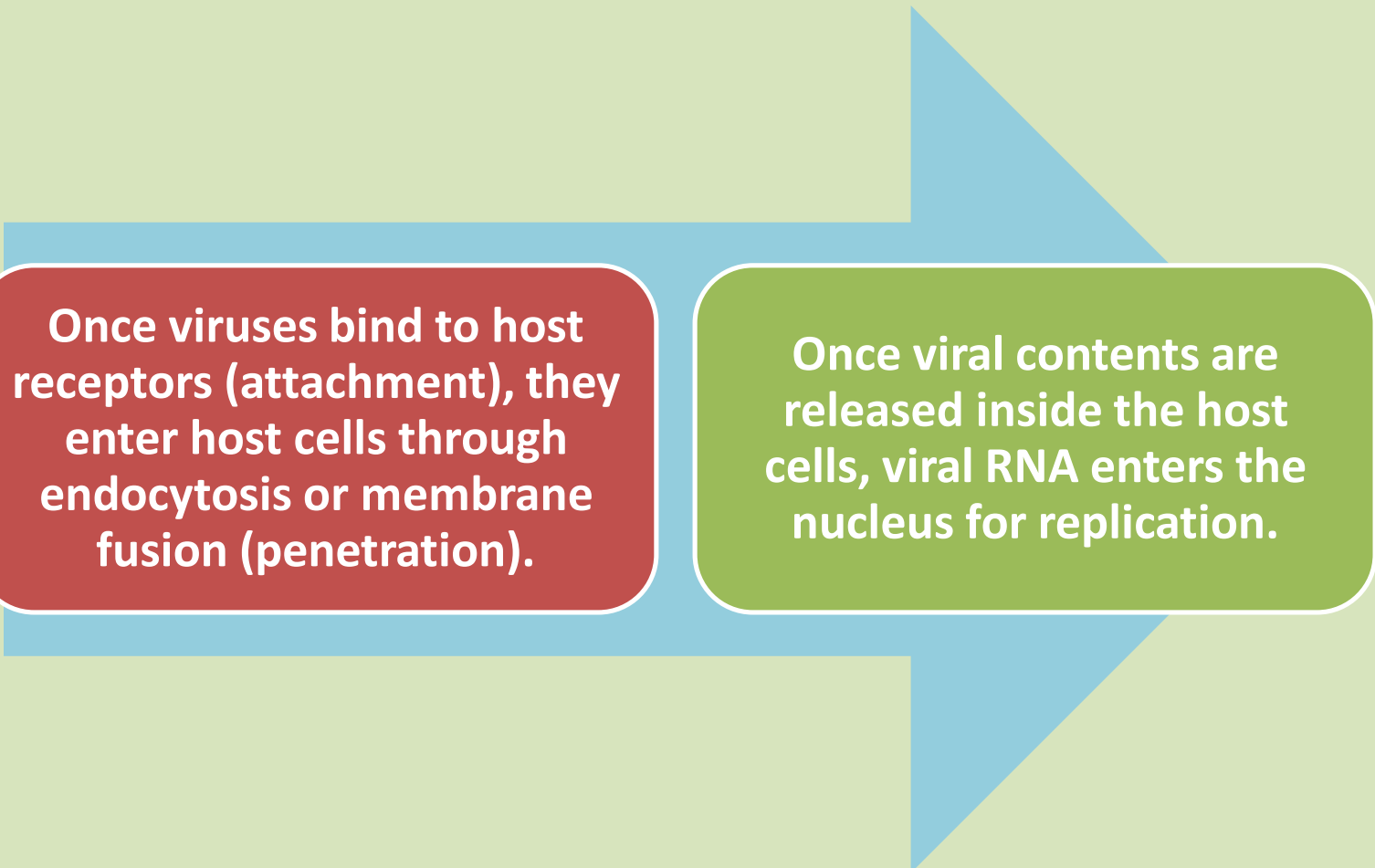


The Life Cycle Of The Virus

The life cycle of the virus with the host consists of the following 5 steps:



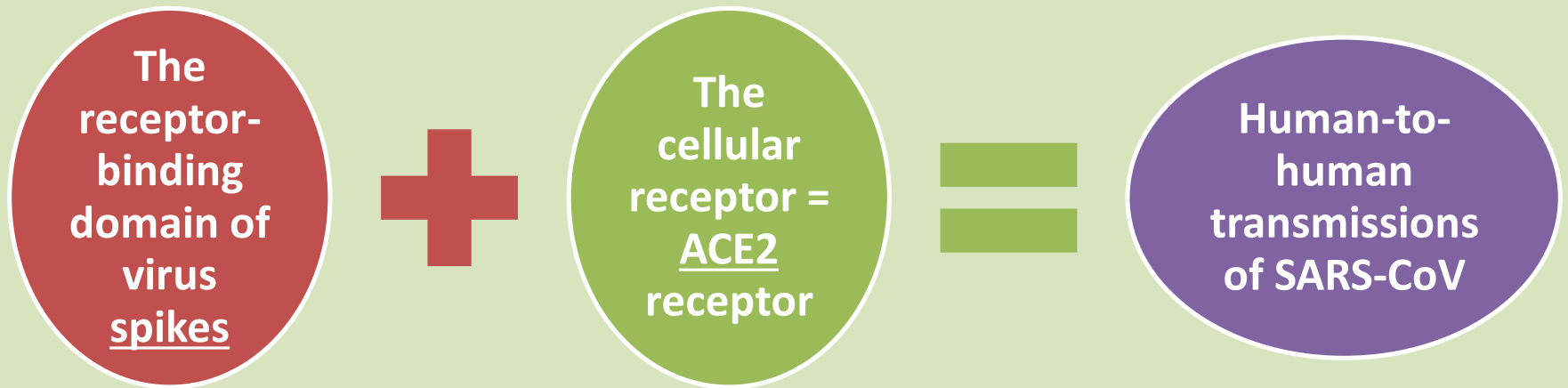
The Life Cycle Of The Virus



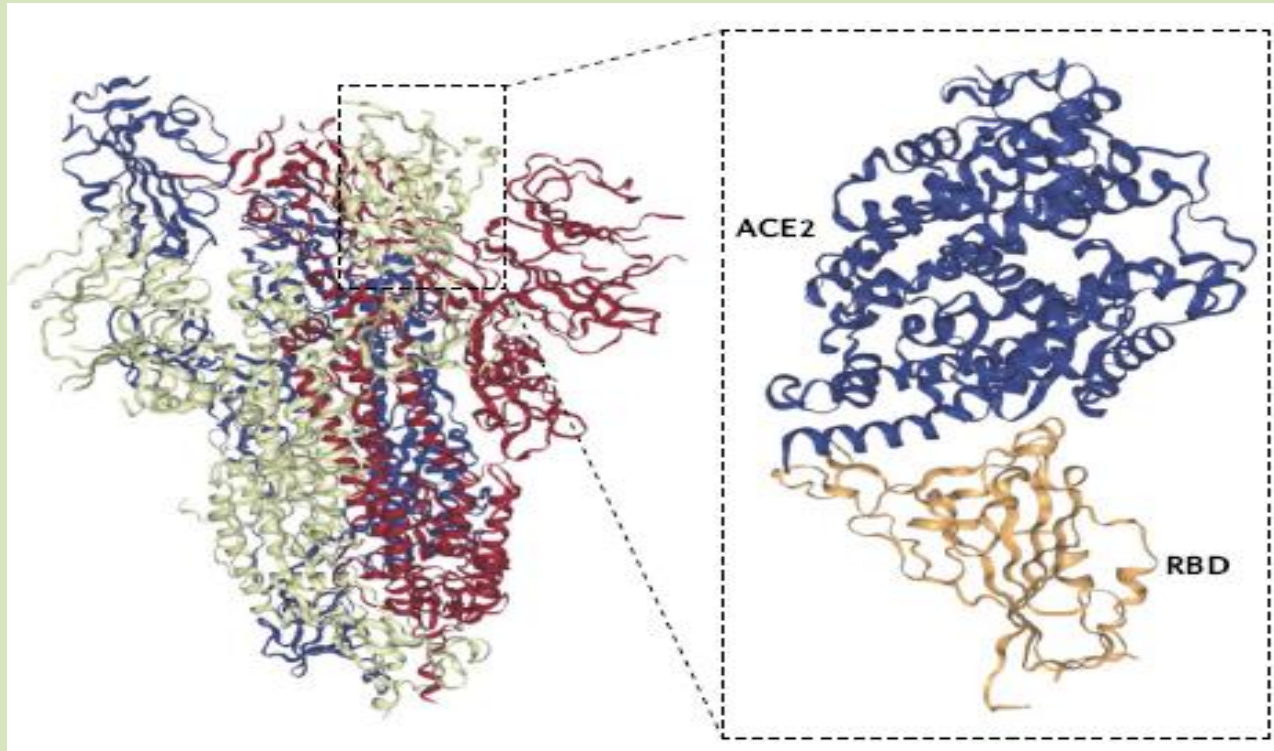
Once viruses bind to host receptors (attachment), they enter host cells through endocytosis or membrane fusion (penetration).

Once viral contents are released inside the host cells, viral RNA enters the nucleus for replication.

COVID Transmission



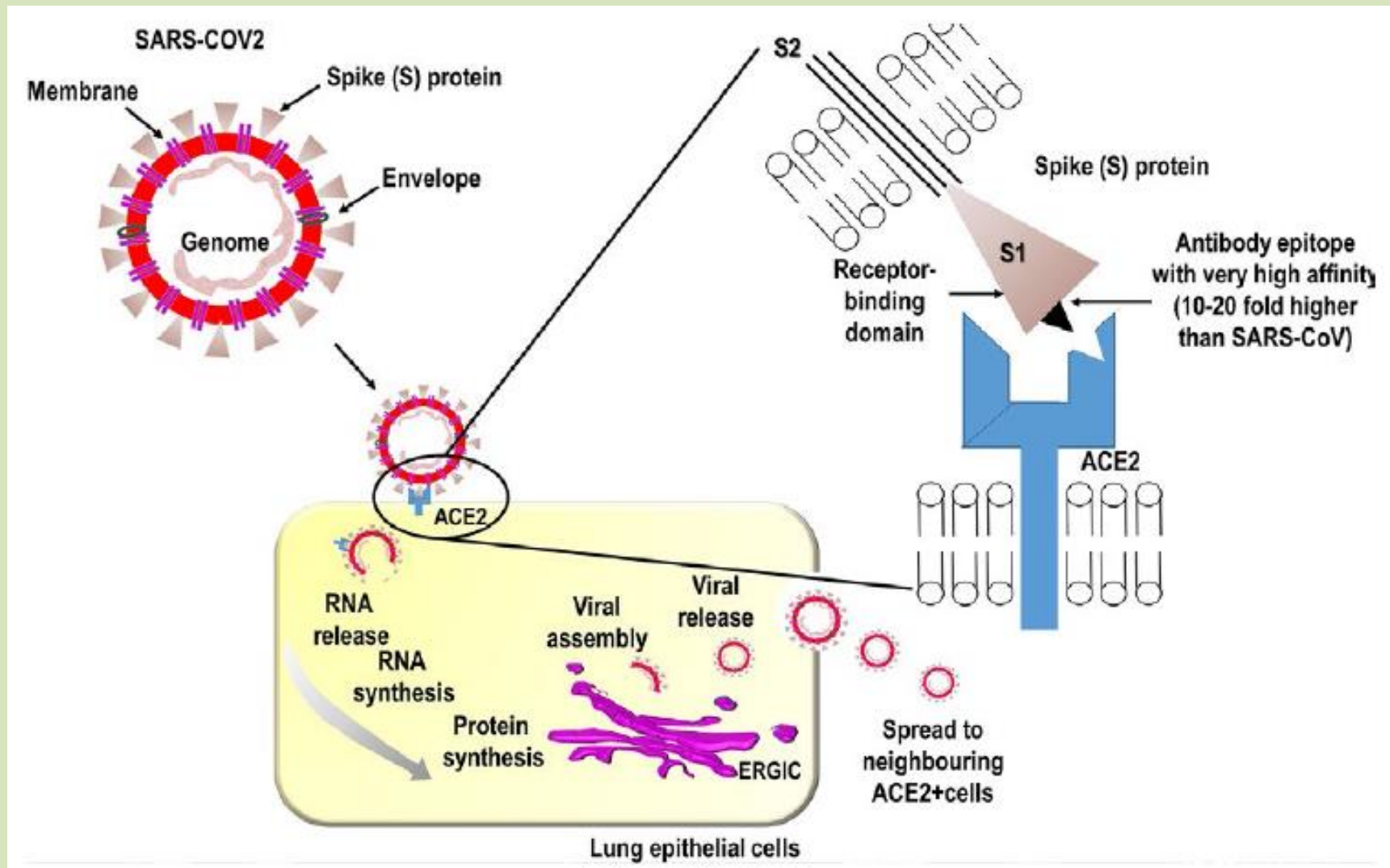
The Structure Of The Trimeric Spike Protein Of SARS-CoV-2



The receptor binding domain (RBD) is shown interacting with its receptor, human ACE2.
Adapted from Protein Data Bank IDs 6VSB42 and 6VW1

- Nature Reviews, Immunology, volume 20, June 2020 , 363-374

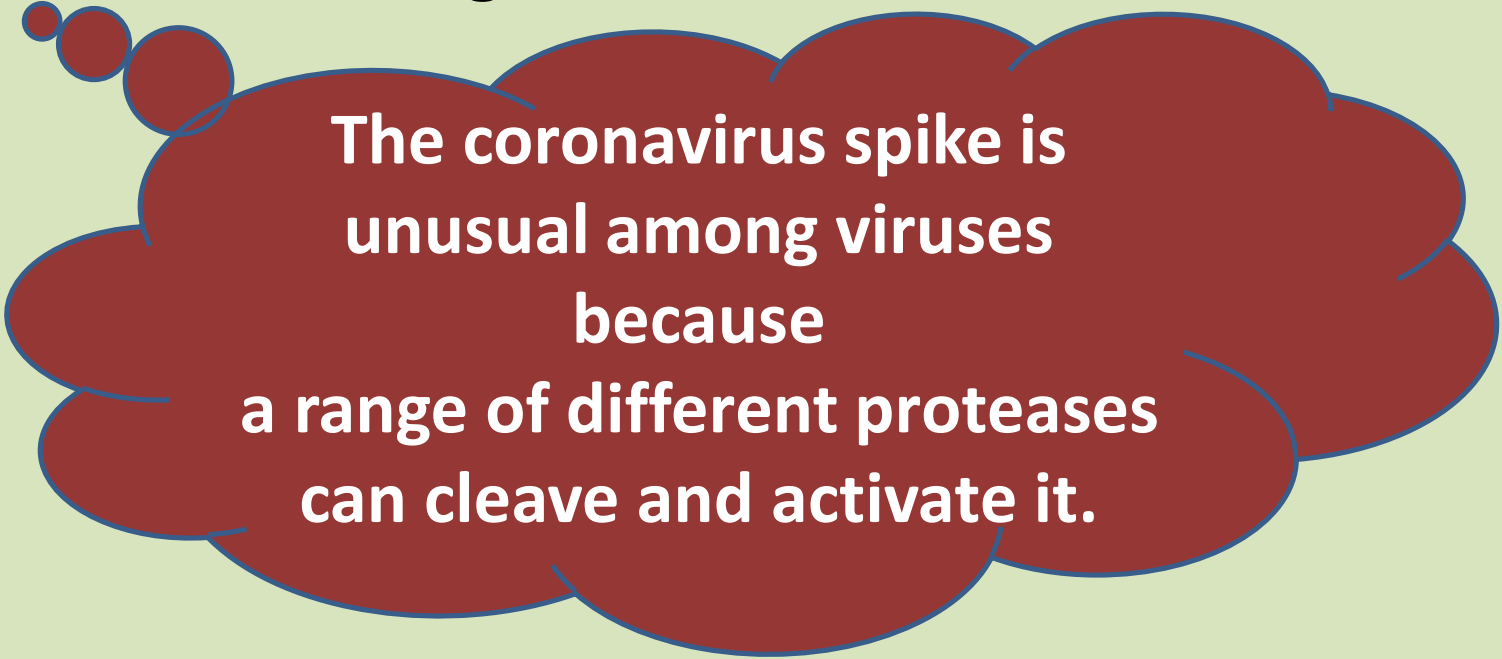
Structural Proteins of SARS-COV-2



Mechanism of SARS-CoV-2

Invasion Into Host Cells

- Following the binding of SARS-CoV-2 to the host protein, the spike protein undergoes protease cleavage.



The coronavirus spike is unusual among viruses because a range of different proteases can cleave and activate it.

Mechanism of SARS-CoV-2 Invasion Into Host Cells

- The characteristics unique to SARS-CoV-2 among coronaviruses is the existence of **furin cleavage site (“RPPA” sequence)** at the S1/S2 site.



Makes this virus very pathogenic

SARS-CoV-2, Lung



- SARS-CoV-2 uses ACE2 as its main receptor, which is broadly expressed in:

- ◆ Vascular endothelium
- ◆ Respiratory epithelium
- ◆ Alveolar monocytes
- ◆ Macrophages

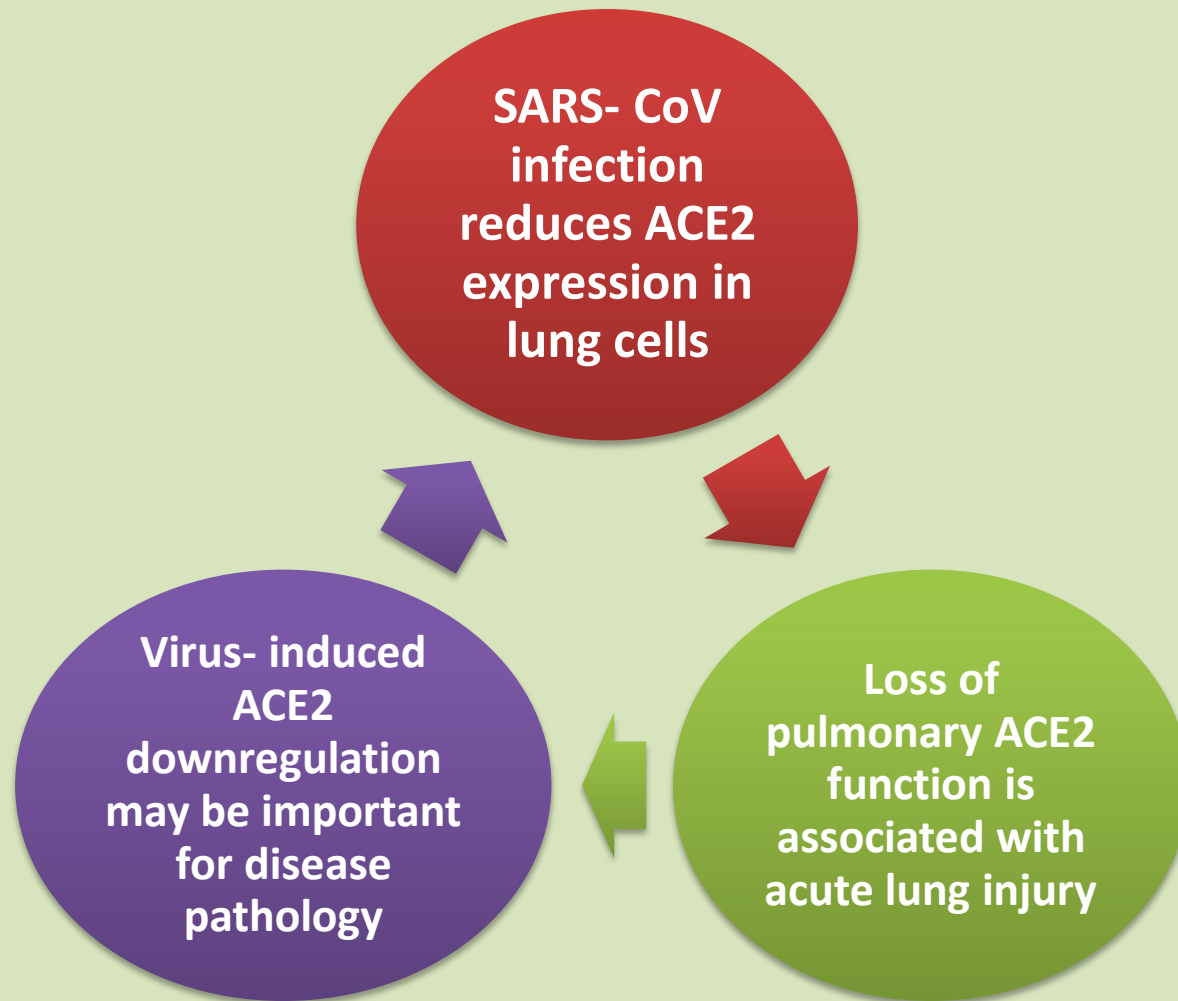
ACE2 is highly expressed on the apical side of lung epithelial cells in the alveolar space, this virus can likely enter and destroy them.

Early lung injury, often in the distal airway

SARS-CoV-2 Infection

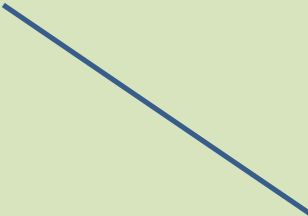
- ACE2 has been shown to regulate the renin–angiotensin system (RAS):

- A reduction in ACE2 function after viral infection could result in a dysfunction of the RAS, which influences:

 - Blood pressure
 - Fluid/electrolyte balance
 - Enhance inflammation
 - Enhance vascular permeability in the airways.

SARS-CoV-2, Lung



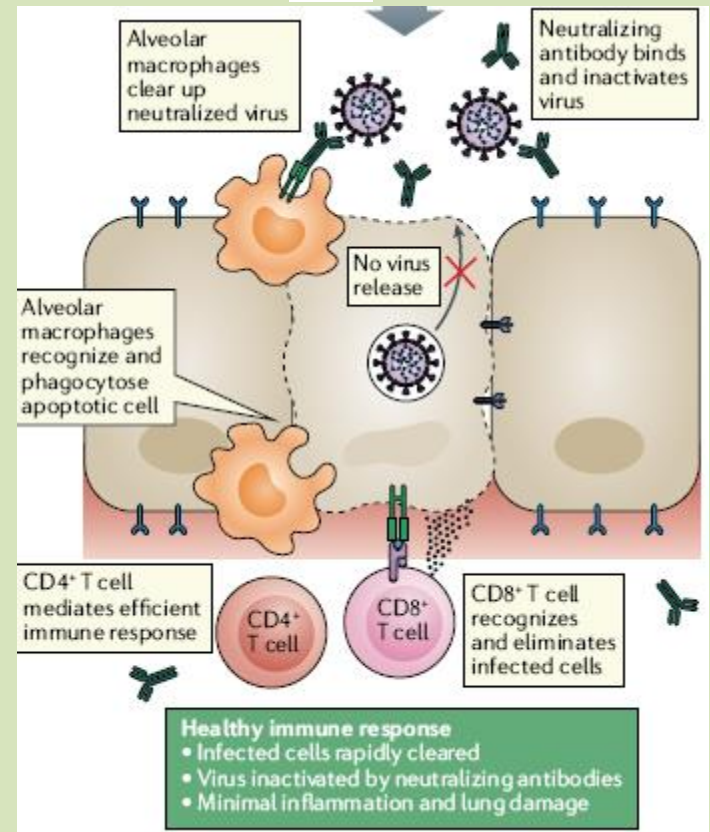
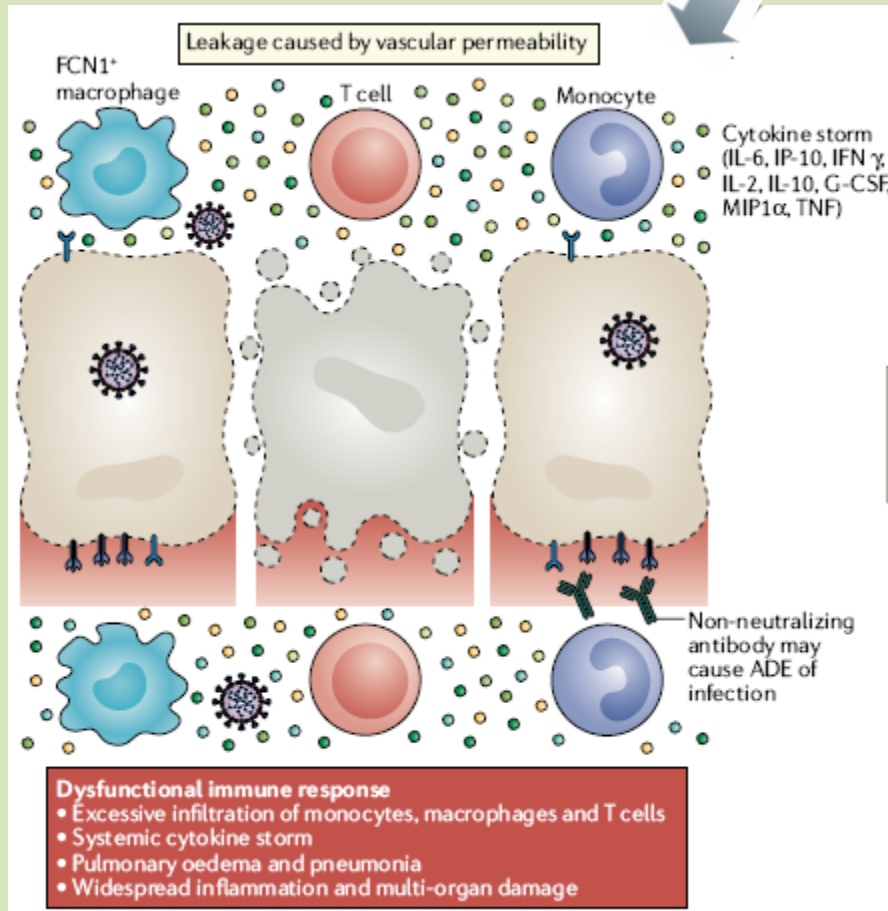
Spike

- The spike for SARS-CoV-2 bound to ACE2
- ACE2 expression, high in:
 - ▶ lung
 - ▶ heart
 - ▶ ileum
 - ▶ kidney
 - ▶ bladder

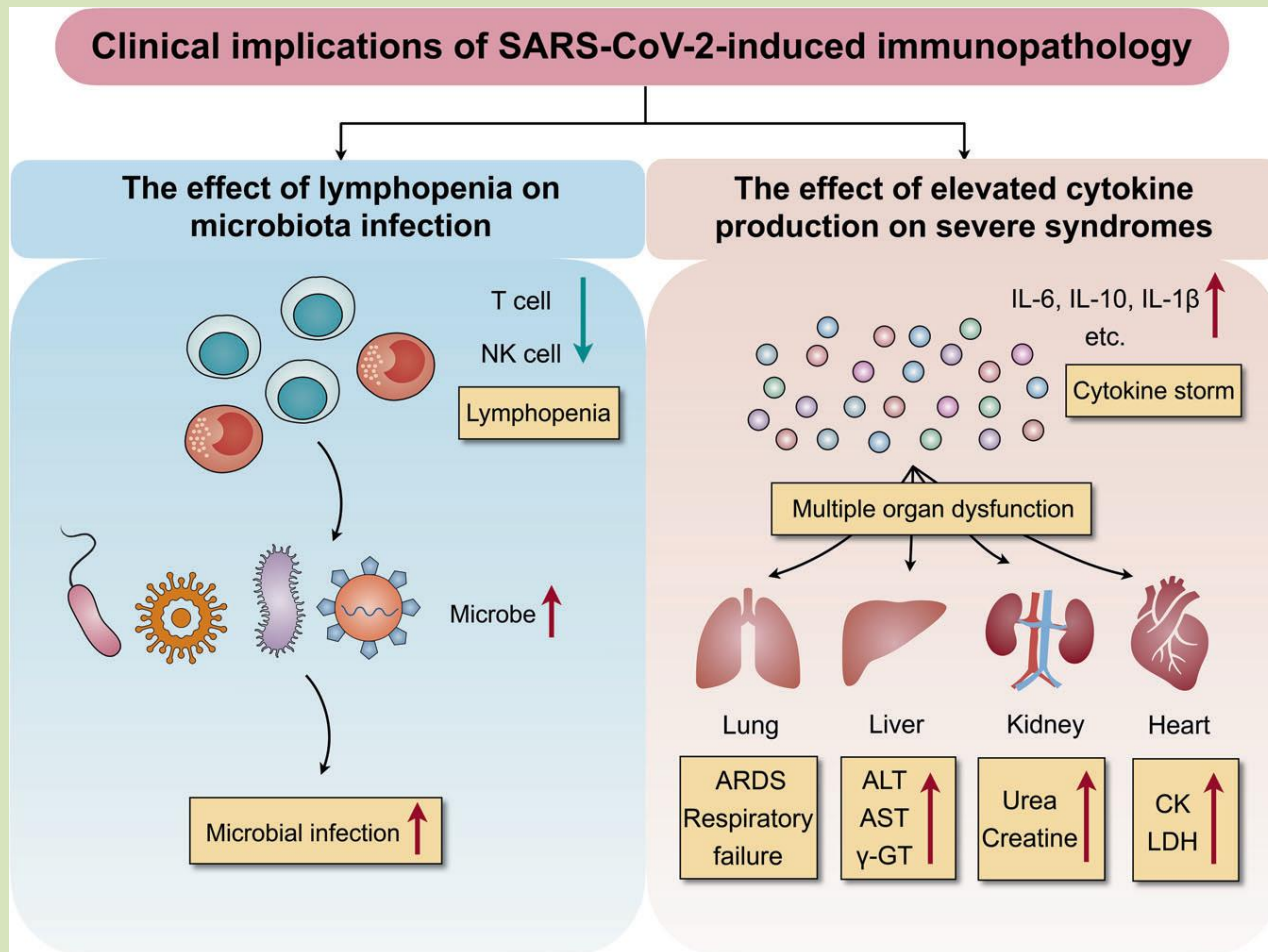


S is responsible for
viral entry into
target ACE2 expressing cells
of the body.

Chronology Of Events During SARS-CoV-2 Infection



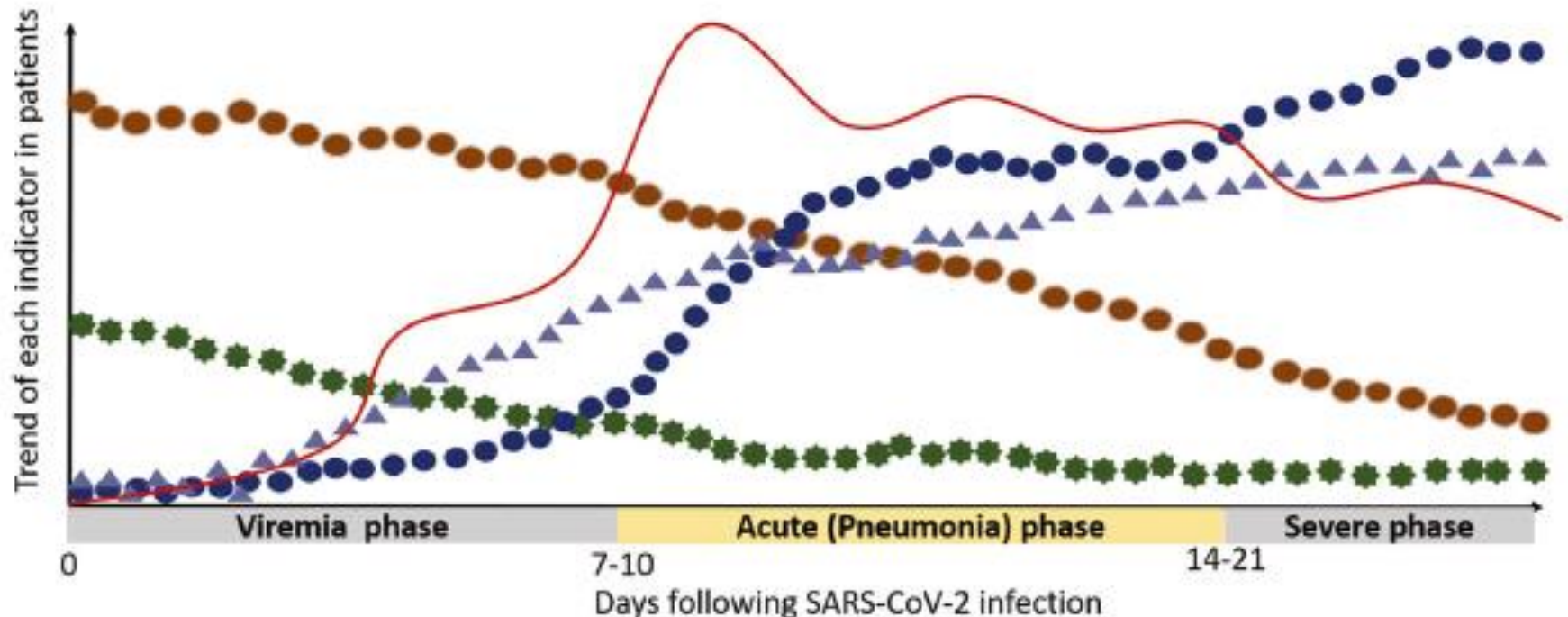
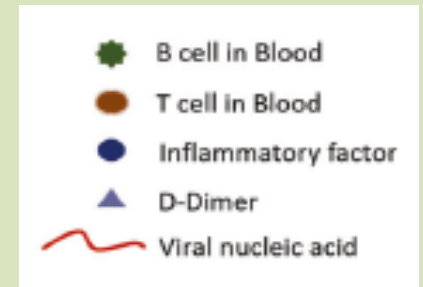
Clinical Implications of SARS-CoV-2-Induced Immunopathology



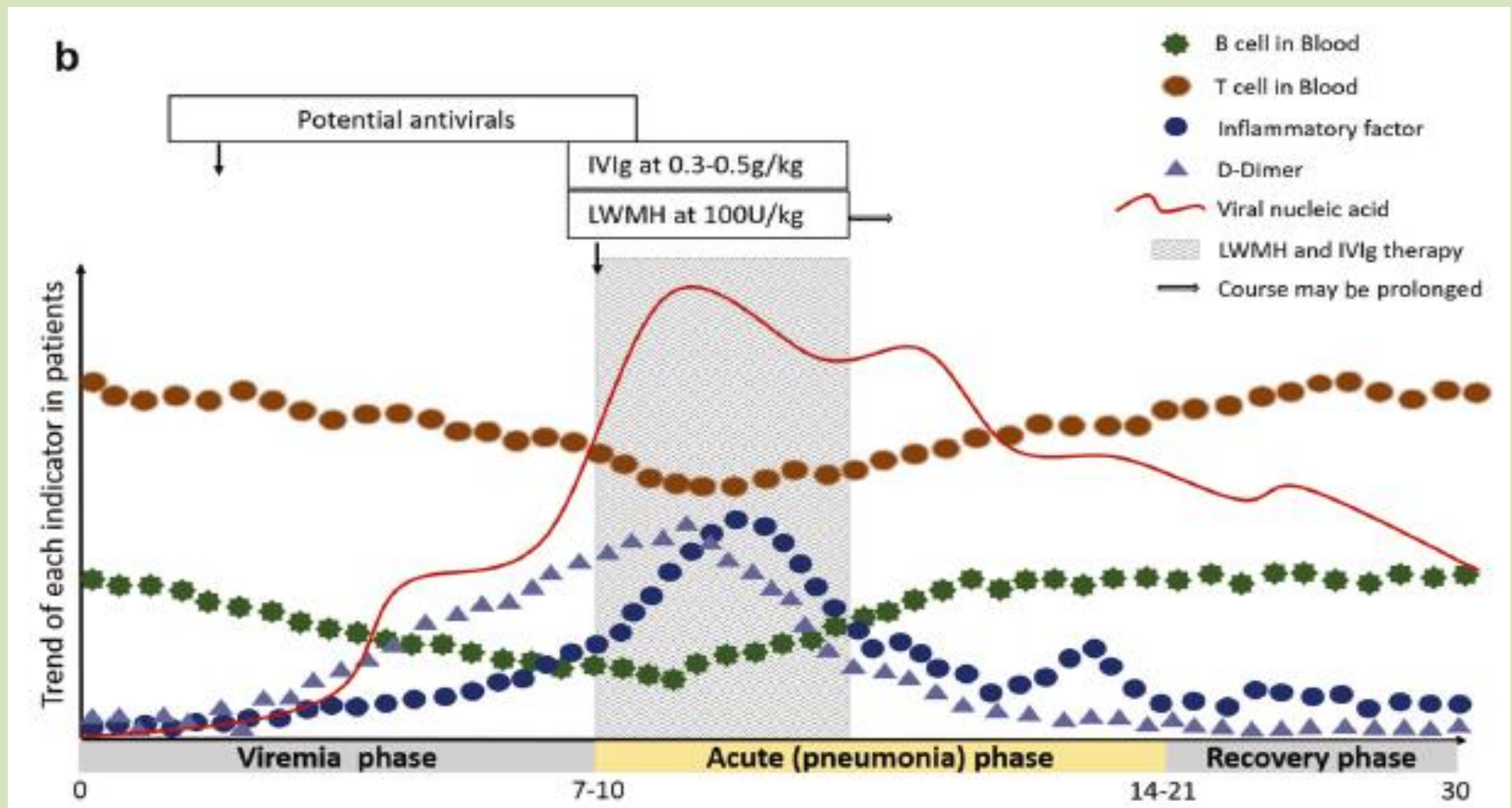
Hypothetical Pathogenesis of COVID-19

www.nature.com/cr

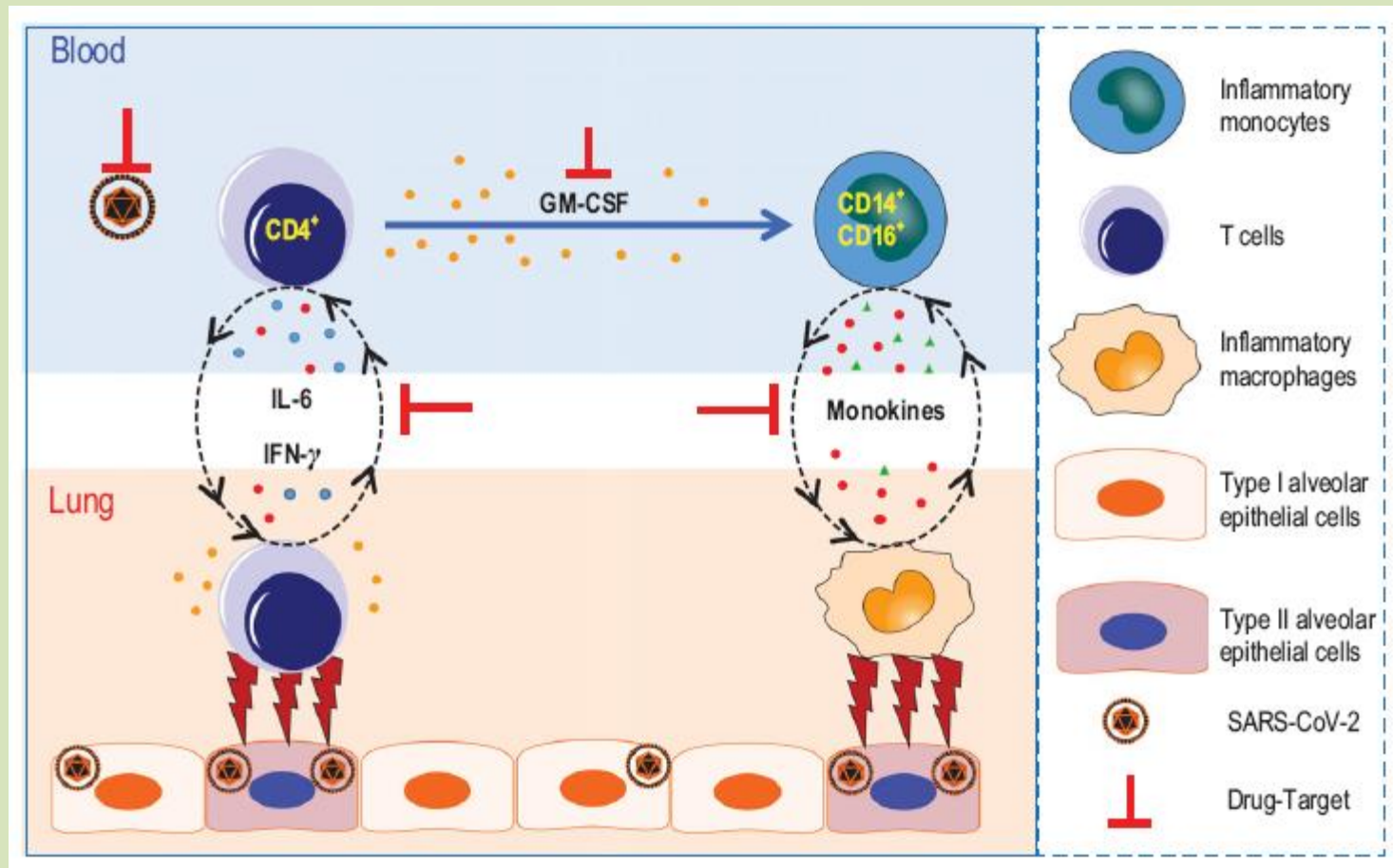
www.cell-research.com



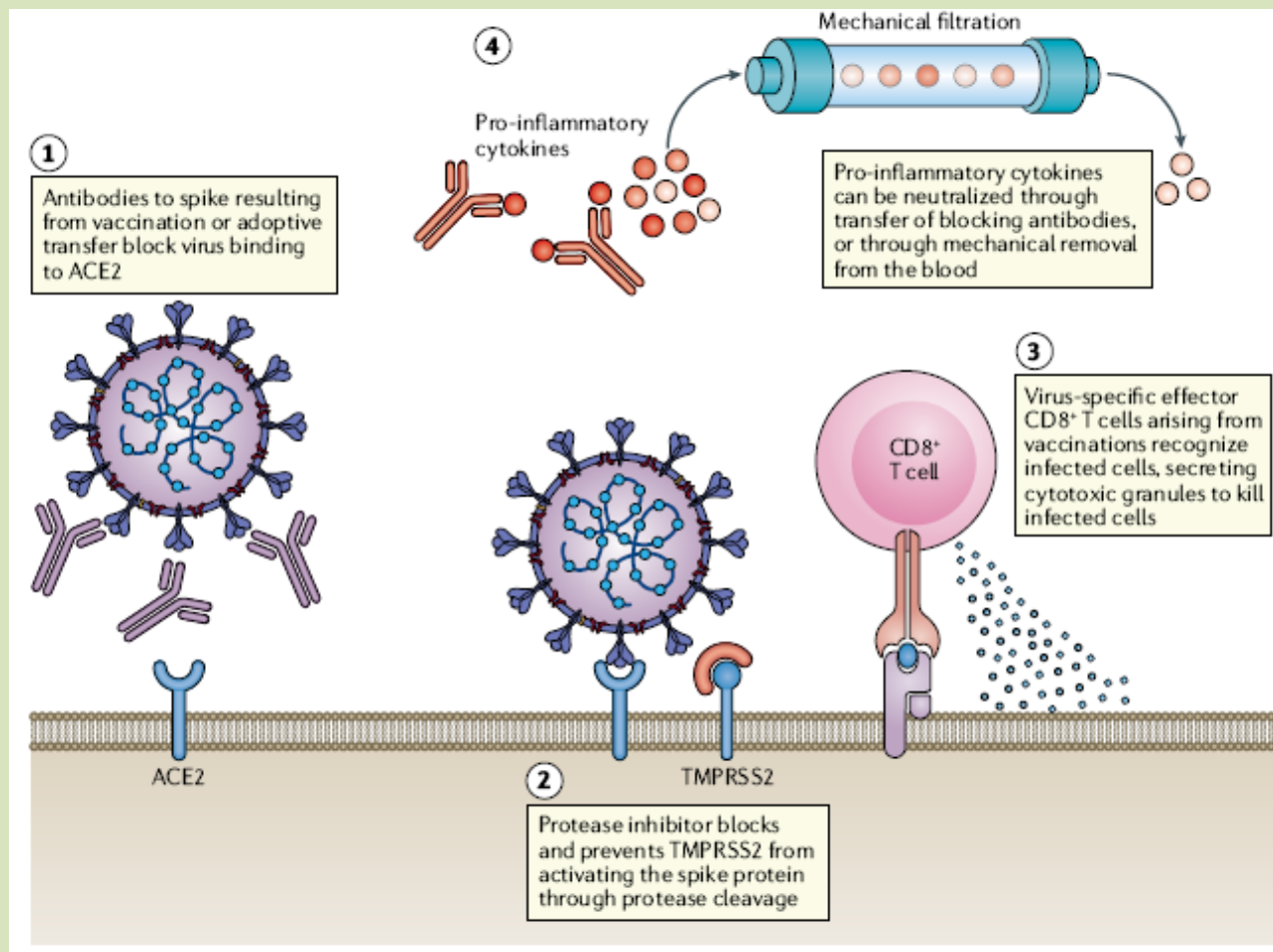
Hypothetical Pathogenesis of COVID-19



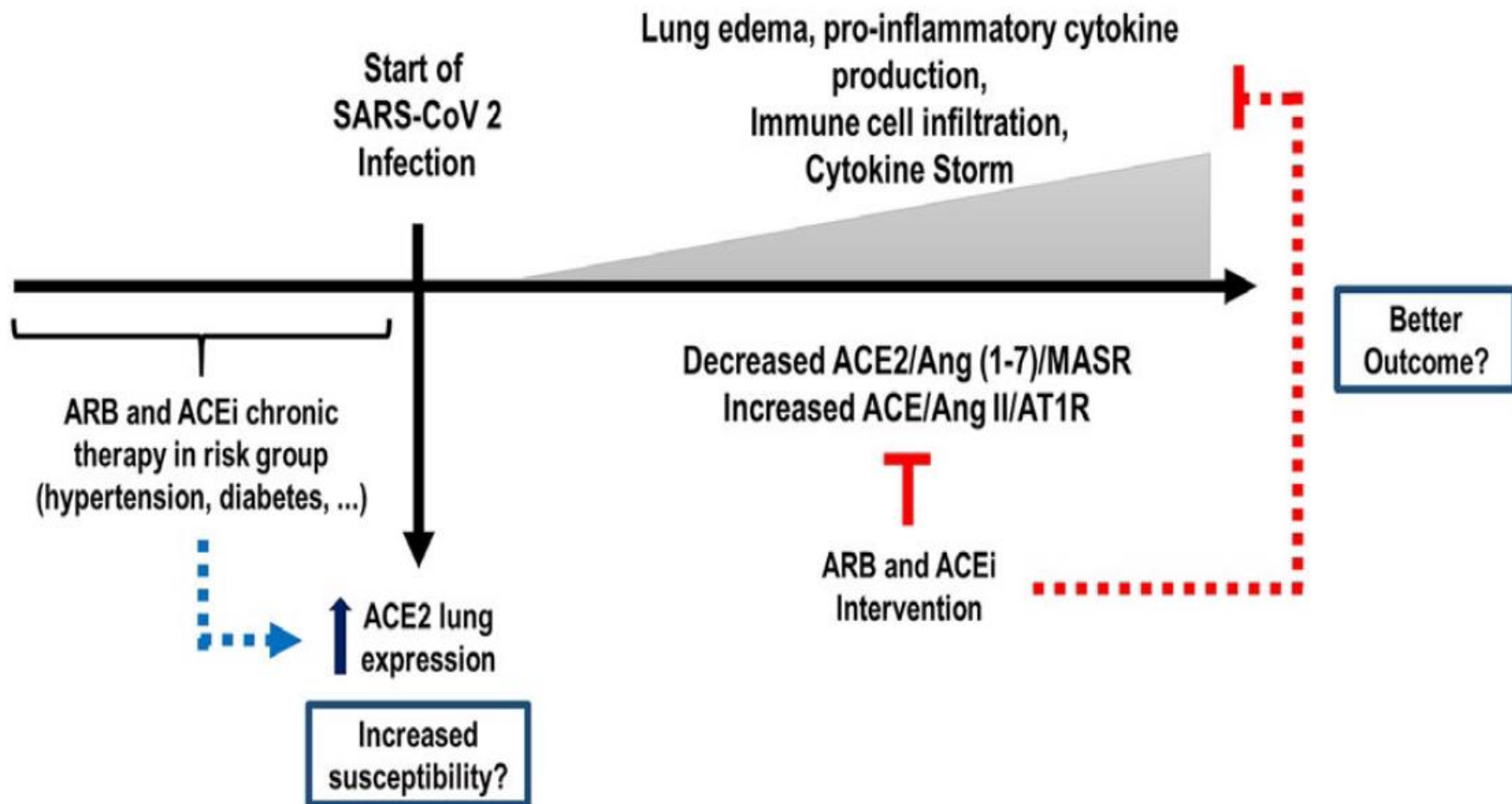
Pathogenic Th1 Cells & Inflammatory Monocytes In Severe COVID-19



Potential Therapeutic Approaches Against SARS-CoV-2



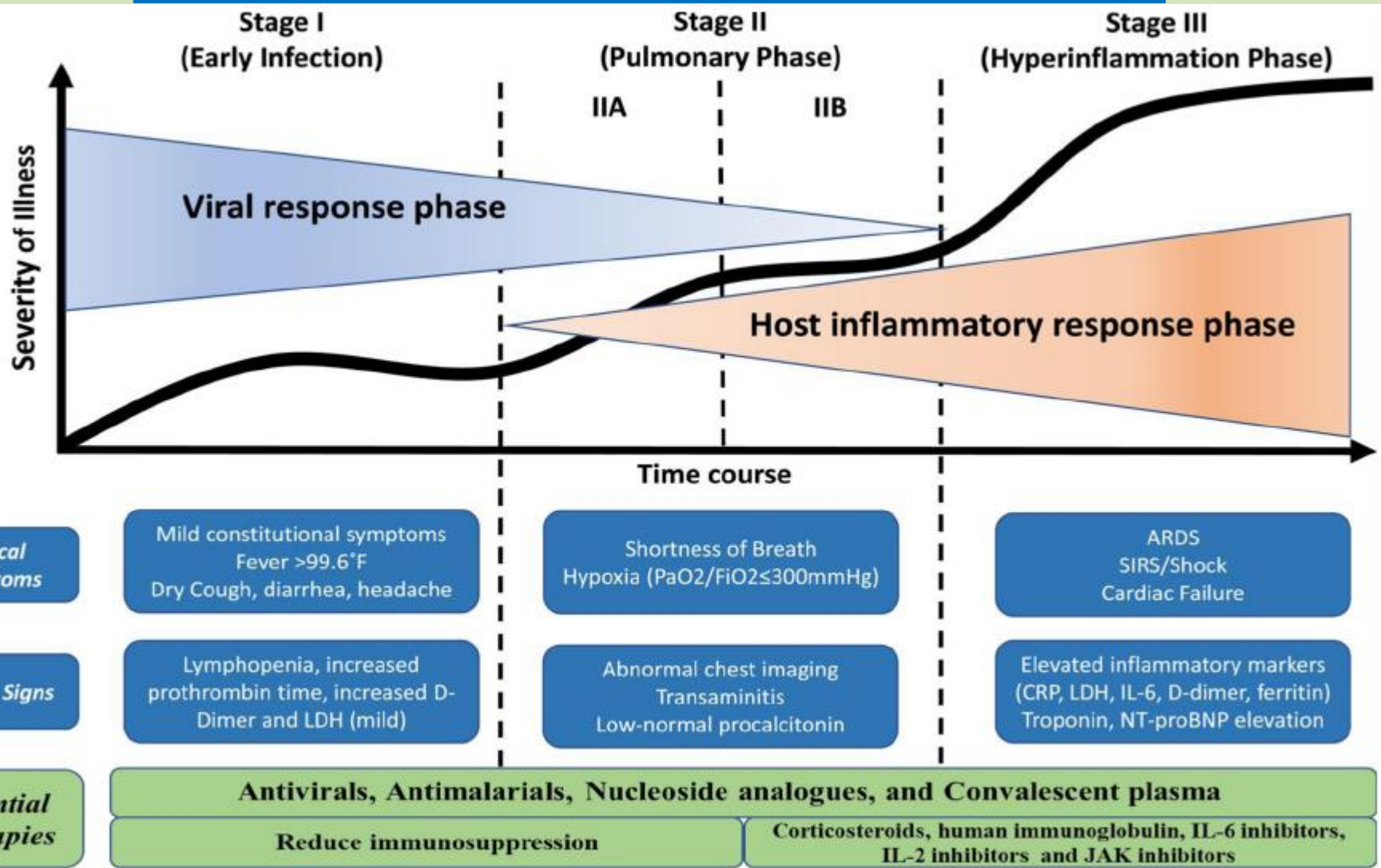
COVID-19 Disease Pathogenesis, ARB/ACEi-based Intervention



Potential Therapeutic Targets

- To block the host target ACE2 receptor or TMPRSS2 (Proteases such as transmembrane protease serine 2) , for example, a Janus kinase (JAK) inhibitor
- TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib

COVID-19 Pathogenic Phases & Potential Therapeutic Targets

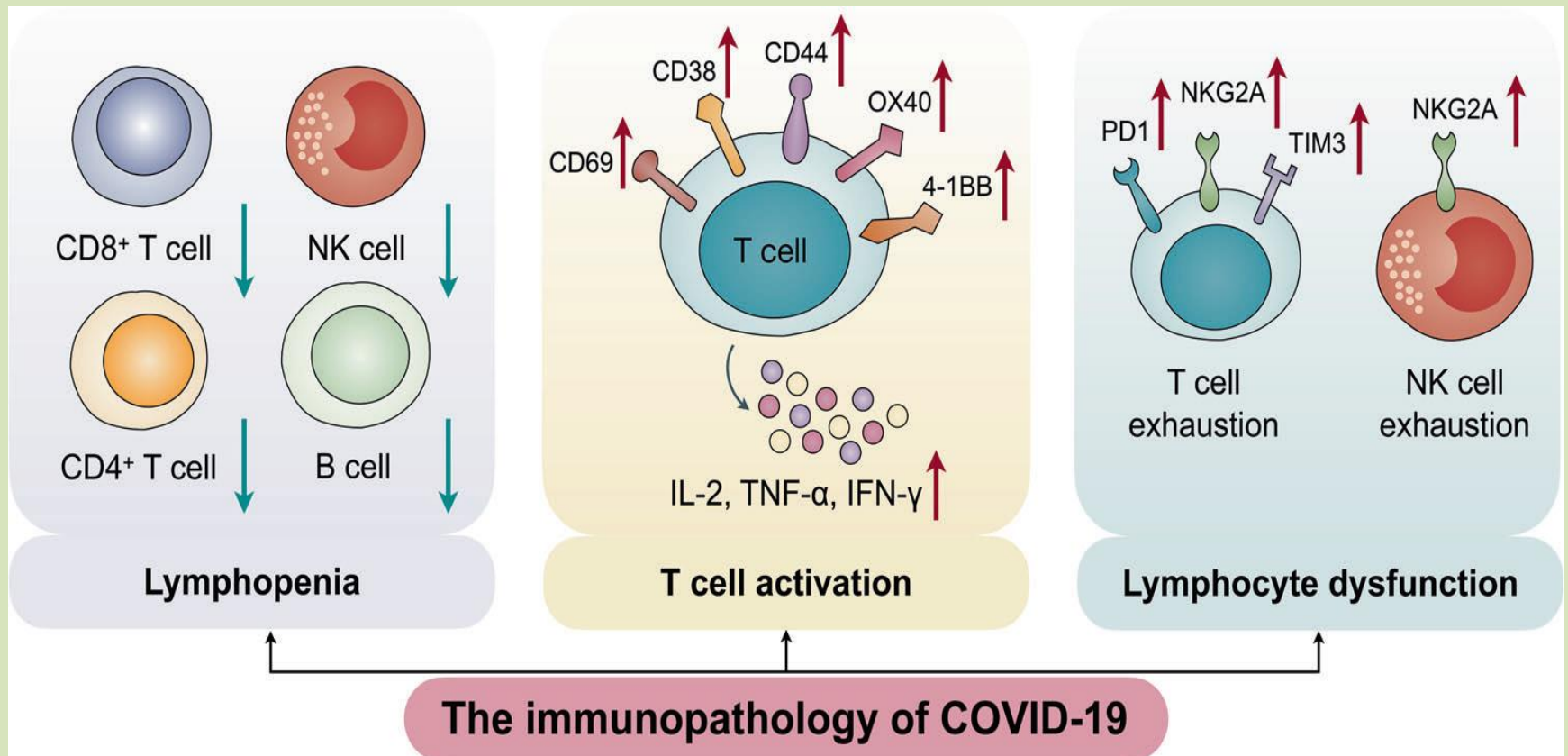




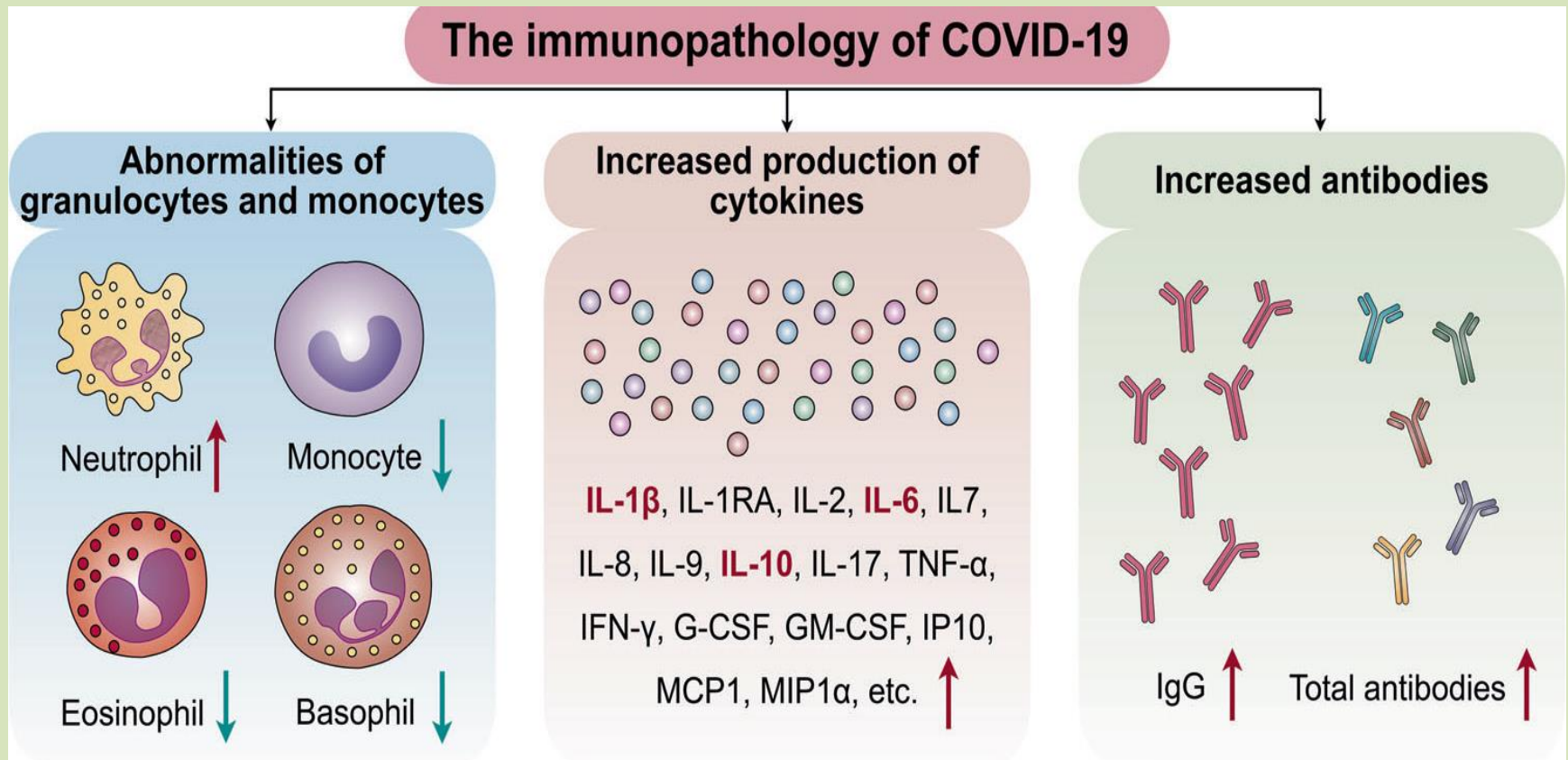
Abbreviations

- Monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein (MIP)
- Proteases such as transmembrane protease serine 2 (TMPRSS2) and cathepsin L
- NK group 2 member A (NKG2A)
- ACE2 was significantly expressed on innate lymphoid cells (ILC)2 and ILC3
- T cell receptor (TCR)
- ADE= Ab dependent enhancement

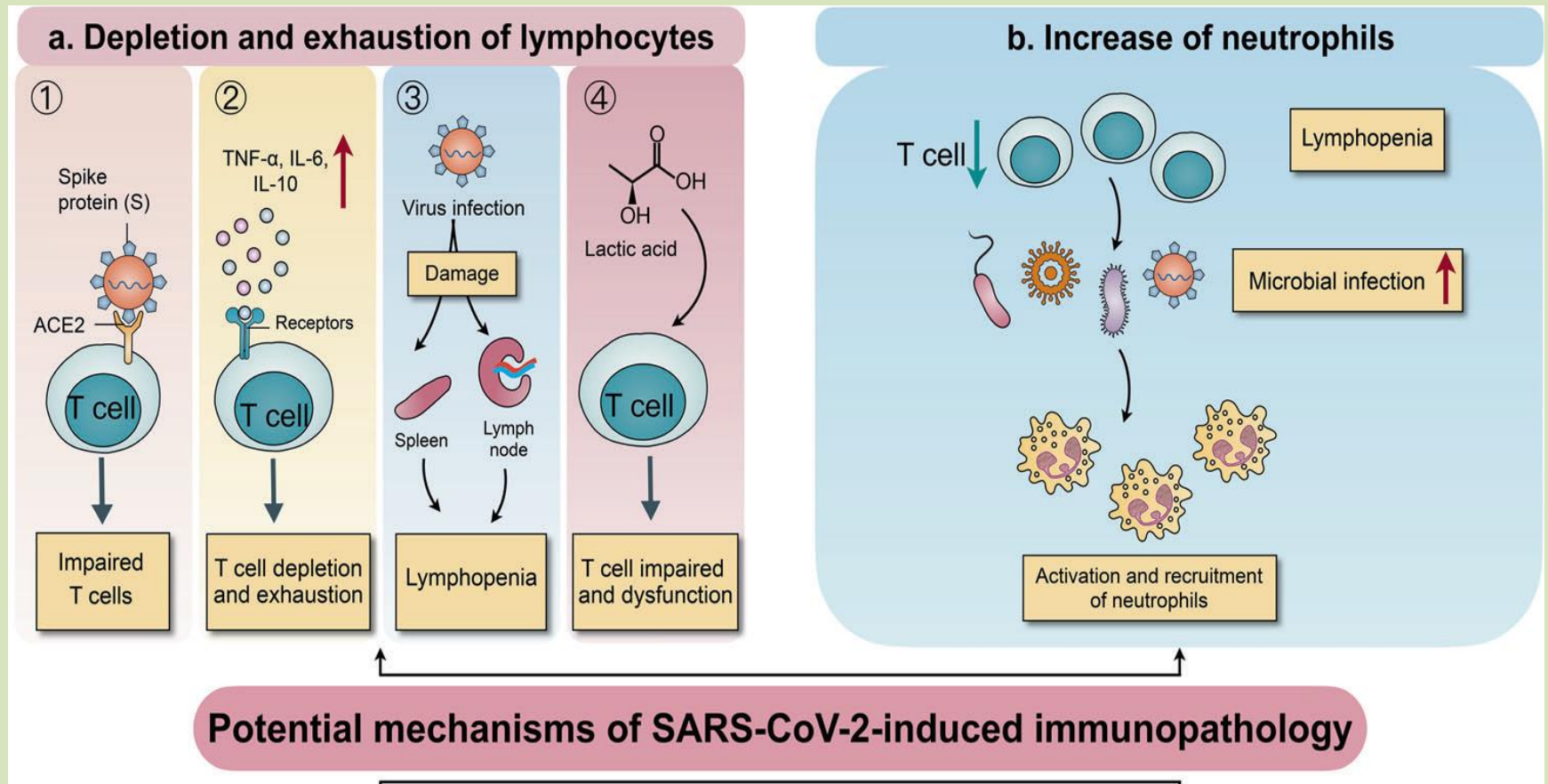
The Immunopathology of COVID-19



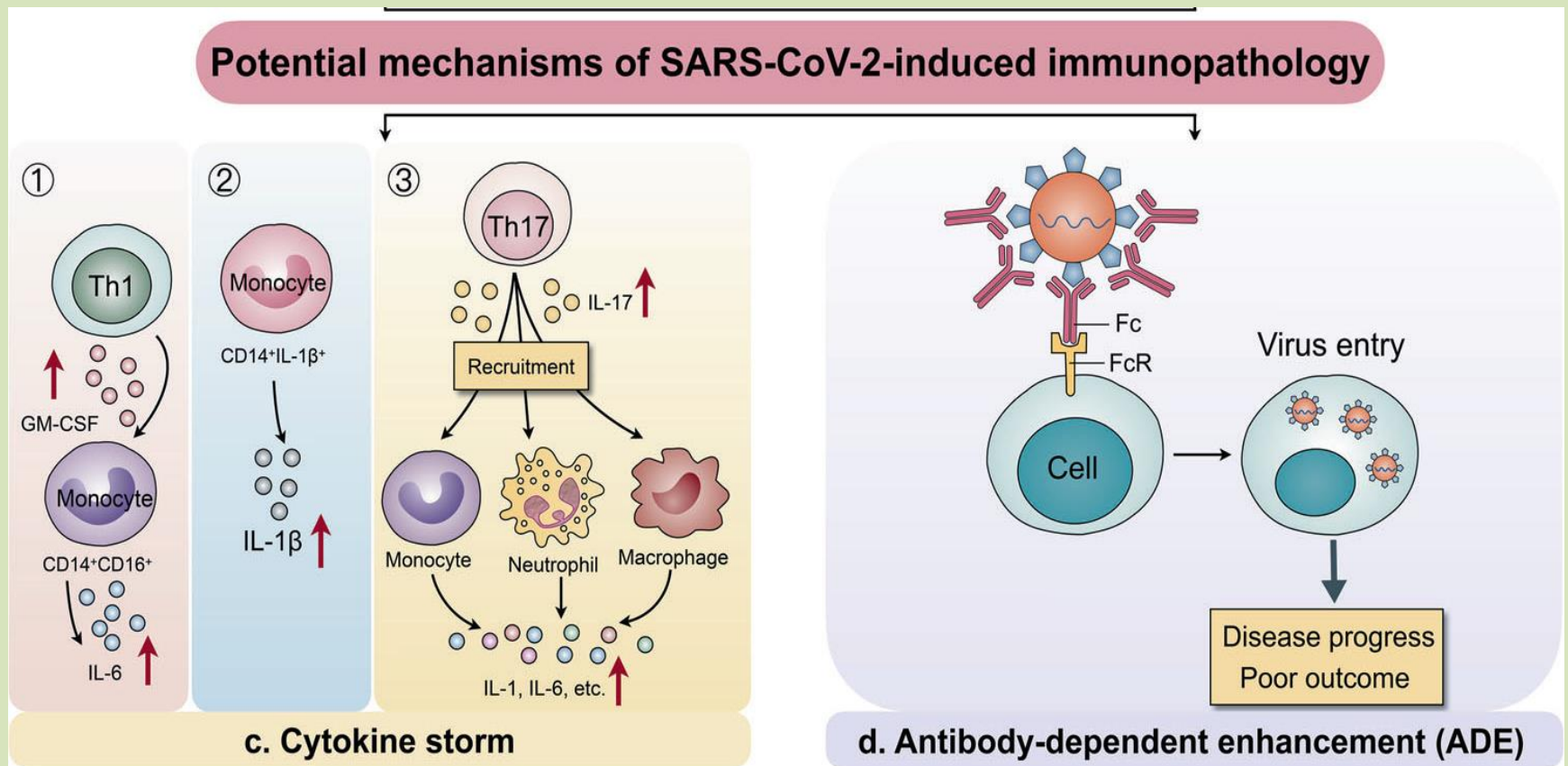
The Immunopathology of COVID-19



Potential Mechanisms of SARS-CoV-2-Induced Immunopathology



Potential Mechanisms of SARS-CoV-2-Induced Immunopathology



Pathogenic Th1 Cells & Inflammatory Monocytes In Severe COVID-19

- Pathogenic CD4⁺ Th1 (GM-CSF+IFN- γ +) *cells were rapidly activated to produce GM-CSF and other inflammatory cytokines* to form a cascade signature of inflammatory monocytes (CD14⁺CD16⁺ with high expression of IL-6) and their progeny. These activated immune cells may enter the pulmonary circulation in large numbers and played an immune-damaging role in severe-pulmonary-syndrome patients.
- The monoclonal antibodies that target the GM-CSF or interleukin-6 receptor may potentially prevent or curb immunopathology caused by COVID-19.

Clinical Immunology 215 (2020)

108427

- The coronavirus spike is unusual among viruses because a range of different proteases can cleave and activate it
- The characteristics unique to SARS-CoV-2 among coronaviruses is the existence of furin cleavage site (“RPPA” sequence) at the S1/S2 site.
- The S1/S2 site of SARS-CoV-2 was entirely subjected to cleavage during biosynthesis in a drastic contrast to SARS-CoV spike, which was incorporated into assembly without cleavage
- Although the S1/S2 site was also subjected to cleavage by other proteases such as transmembrane protease serine 2 (TMPRSS2) and cathepsin L, the ubiquitous expression of furin likely makes this virus very pathogenic.

Clinical Immunology 215 (2020)

108427

- viruses till adaptive immunity is involved.
- T cell responses are initiated by antigen presentation via DCs and macrophages. How does SARS-CoV-2 enter APCs?
- DCs and macrophages can phagocytize apoptotic cells infected by virus For example, virus-infected apoptotic epithelial cells can be phagocytized by DCs and macrophages, which leads to antigen presentation to T cells.
- DCs and macrophages may be infected with virus primarily? Based on the Immunological Genome database (<http://rstats.immgen.org>), the expression of ACE2 on (splenic) dendritic cells and alveolar macrophages is present but limited (Fig. 1).

Clinical Immunology 215 (2020)

108427

- Determining whether or not SARS-CoV-2 uses another protein to bind to APCs helps to answer this question. SARS-CoV can also bind to dendritic-cell specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) and DC-SIGN-related protein (DC-SIGNR, L-SIGN) in addition to ACE2. DC-SIGN is highly expressed on dendritic cells and macrophages.
- Another target for SARS-CoV-2, if any, can help the virus to directly infect DCs and alveolar macrophages. This needs future research.
- These antigen presenting cells move to the draining lymph nodes to present viral antigens to T cells. CD4+ and CD8+ T cells play a critical role. CD4+ T cells activate B cells to promote the production of virus-specific antibody, while CD8+ T cells can kill viral infected cells.

Clinical Immunology 215 (2020)

108427

- Immunological studies were mainly reported in severe COVID-19 patients. Patients with severe diseases showed lymphopenia, particularly the reduction in peripheral blood T cells.
- Patients with severe diseases were reported to have increased plasma concentrations of proinflammatory cytokines, including IL-6, IL-10, G-CSF, monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein (MIP)1 α , and TNF- α

Clinical Immunology 215 (2020)

108427

- The more severe conditions patients were in, the higher their IL-6 levels were.
- CD4+ and CD8+ T cells were activated in those patients as suggested by higher expression of CD69, CD38 and CD44. Higher percentage of checkpoint receptor Tm3+PD-1+ subsets in CD4+ and CD8+ T cells showed that T cells were also exhausted. NK group 2 member A (NKG2A), another marker for exhaustion was elevated on CD8+ T cells

Clinical Immunology 215 (2020)

108427

- Exhaustion of T cells could have led to the progression of the disease. Another interesting finding was that aberrant pathogenic CD4⁺ T cells with coexpressing interferon (IFN)- γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) were seen in COVID-19 patients with severe disease.
- GM-CSF production from T cells has been previously reported as a response to virus infection. GM-CSF can help to differentiate innate immune cells and augment T cell function, but it can initiate tissue damage at excess
- GM-CSF+IFN- γ + CD4⁺ T cells were previously seen upon strong T cell receptor (TCR) responses in experimental autoimmune encephalomyelitis (EAE) models, where CD8⁺ T cells expressing GM-CSF were found at higher percentage and secreted IL-6.
- It is worth mentioning that these immunological studies were exclusively reported from adult patients. Immunological responses in pediatric population needs to be examined.

Clinical Immunology 215 (2020)

108427

- The study of SARS-CoV showed that virus infected lung epithelial cells produced IL-8 in addition to IL-6.
- IL-8 is a well-known chemoattractant for neutrophils and T cells. Infiltration of a large number of inflammatory cells were observed in the lungs from severe COVID-19 patients, and these cells presumably consist of a constellation of innate immune cells and adaptive immune cells.
- Among innate immune cells, we expect the majority to be neutrophils. Neutrophils can act as double-edged sword as neutrophils can induce lung injury
- The majority of the observed infiltrating adaptive immune cells were likely T cells, considering that the significant reduction in circulating T cells was reported. CD8+ T cells are primary cytotoxic T cells.

Clinical Immunology 215 (2020)

108427

- Severe patients also showed pathological cytotoxic T cells derived from CD4+ T cells. These cytotoxic T cells can kill virus but also contribute to lung injury.
- Circulating monocytes respond to GM-CSF released by these pathological T cells. CD14+CD16+ inflammatory monocyte subsets, which seldom exist in healthy controls and were also found at significantly higher percentage in COVID-19 patients.
- These inflammatory CD14+CD16+ monocytes had high expression of IL-6, which likely accelerated the progression of systemic inflammatory response.

Clinical Immunology 215 (2020)

108427

- An interesting note is that ACE2 was significantly expressed on innate lymphoid cells (ILC)2 and ILC3 (Fig. 1).
- NK cells are a member of ILC1, which constitute a large portion of ILCs in the lung (~95%).
- ILC2 and ILC3 work for mucous homeostasis. So far there is a very limited study of ILC2 and ILC3 in coronavirus infection.

Clinical Immunology 215 (2020)

108427

- In addition to respiratory symptoms, thrombosis and pulmonary embolism have been observed in severe diseases.
- This is in line with the finding that elevated d-dimer and fibrinogen levels were observed in severe diseases.
- The function of the endothelium includes promotion of vasodilation, fibrinolysis, and anti-aggregation.
- Because endothelium plays a significant role in thrombotic regulation, hypercoagulable profiles seen in severe diseases likely indicate significant endothelial injury.
- Endothelial cells also express ACE2
- Of note, the endothelial cells represent the one third of lung cells
- Microvascular permeability as a result of the endothelial injury can facilitate viral invasion.

Clinical Immunology 215 (2020)

108427

- between the severity of COVID-19 and the amount of viral loads (or the duration of virus-shedding period) [63], children may have less virus loads even if they get COVID-19
- The first possibility is that the expression level of ACE2 may differ between adults and children. A previous study showed that ACE2 was more abundantly expressed on well-differentiated ciliated epithelial cells.
- Human lung and epithelial cells continue to develop following the birth. ACE2 expression may be lower in pediatric population

Clinical Immunology 215 (2020)

108427

- In addition, gender may also affect ACE2 expression. ACE2 gene is located on the X-chromosome.
- Circulating ACE2 levels are higher in men than in women
- This may be in part responsible for the difference in severity and mortality between men and women both in the adult and the pediatric population

Clinical Immunology 215 (2020)

108427

- The second possibility is that children have a qualitatively different response to the SARS-CoV-2 virus to adults.
- With ageing, continuous antigen stimulation and thymic involution lead to a shift in T cell subset distribution from naïve T cells to central memory T cells, effector T cells and effector memory T cells

Clinical Immunology 215 (2020)

108427

- This process is accompanied by the loss of expression of co-stimulatory molecules such as CD27 and CD28, with increased susceptibility to infections
- At the early stage after birth, CD4+ T cells are impaired in production of Th1 associated proinflammatory cytokines and skewed toward Th2
- CD8+ T cells reduced expression of cytotoxic and inflammatory mediators.
- Less killing ability by T cells at early stage after birth may explain susceptibility to SARS-CoV-2 in infants.
- The study comparing aged and young macaques infected with SARS-CoV showed that aged macaques had more robust proinflammatory responses with worse lung pathology

Clinical Immunology 215 (2020)

108427

- that inflammatory responses in adults and children are much different
- Ageing is associated with increasing proinflammatory cytokines that govern neutrophil functions and have been correlated with the severity of ARDS.
- So far there is no animal model for SARS-CoV-2

Clinical Immunology 215 (2020)

108427

- The third possibility is that the simultaneous presence of other viruses in the mucosa lungs and airways, common in young children, can let SARS-CoV-2 virus compete with them and limit its growth.
- At this point, we do not have study testing various viruses along with SARS-CoV-2 to determine this possibility.
- Rather a combination of these possibilities may explain pediatric and adult COVID-19 phenotypes. Understanding why children in general are less susceptible to severe COVID-19 would help to design immunotherapy to eradicate this virus.



Coronaviruses

- So far, seven HCoVs that can invade humans have been identified, including the α -type HCoV-229E and HCoV-NL63; the β -type HCoV-HKU1, SARS-CoV, MERS-CoV, and HCoV-OC43; and 2019-nCoV, causing the present epidemic.
- According to their pathogenicity, HCoVs are divided into mildly pathogenic HCoVs (including HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU) and highly pathogenic CoVs (including SARS-CoV, MERS-CoV and SARS-CoV-2).

Coronaviruses

- The positive-strand viral RNA consists of a cap structure at the 5' end and multiple poly(A) tails at the 3' end. It serves as messenger RNA (mRNA), allowing the translation of replicase/transcriptase and viral structural proteins. The replicase/transcriptase genes account for approximately 2/3 of the 5'-end RNA sequence and are composed of two overlapping open reading frames (ORFs): ORF1a and ORF1b. The ORFs encode 16 non-structural proteins. The remaining 1/3 of the RNA sequence encodes four classical viral structural proteins, namely, spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein.

Coronaviruses

- Structurally, the SARS coronavirus (SARS-CoV):



14 binding residues that directly
interact with human ACE2

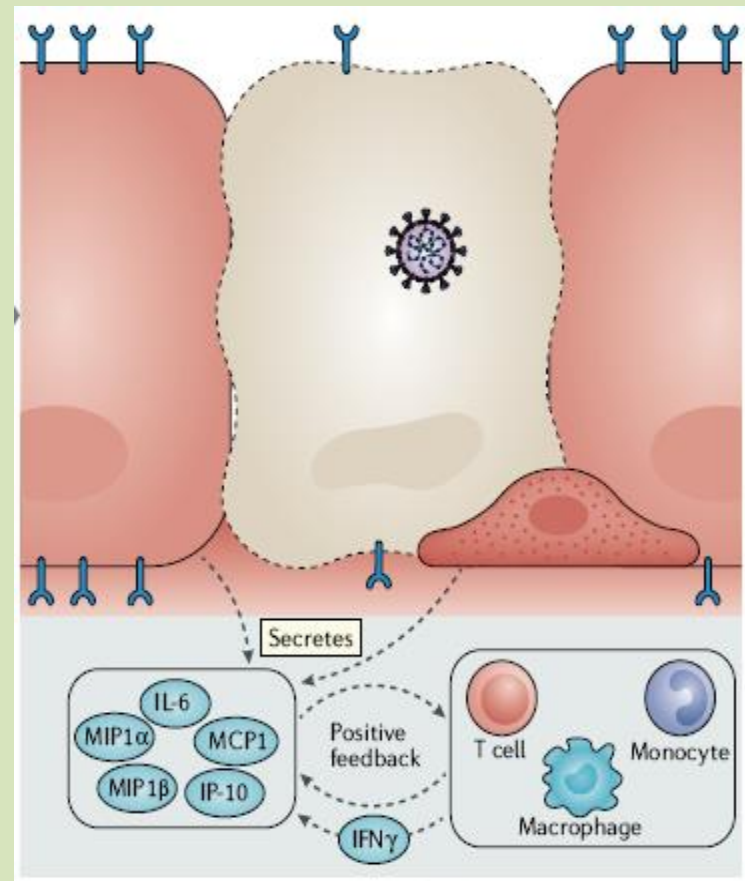
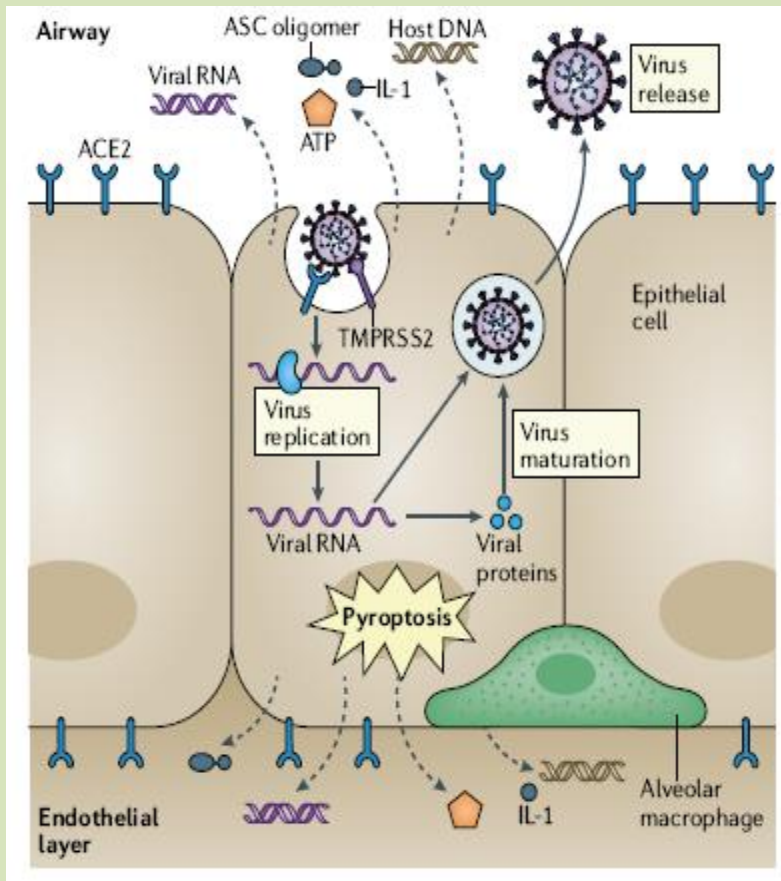
- Of these amino acids, 8 have been conserved in SARS-CoV-2

Clinical Immunology 215 (2020)

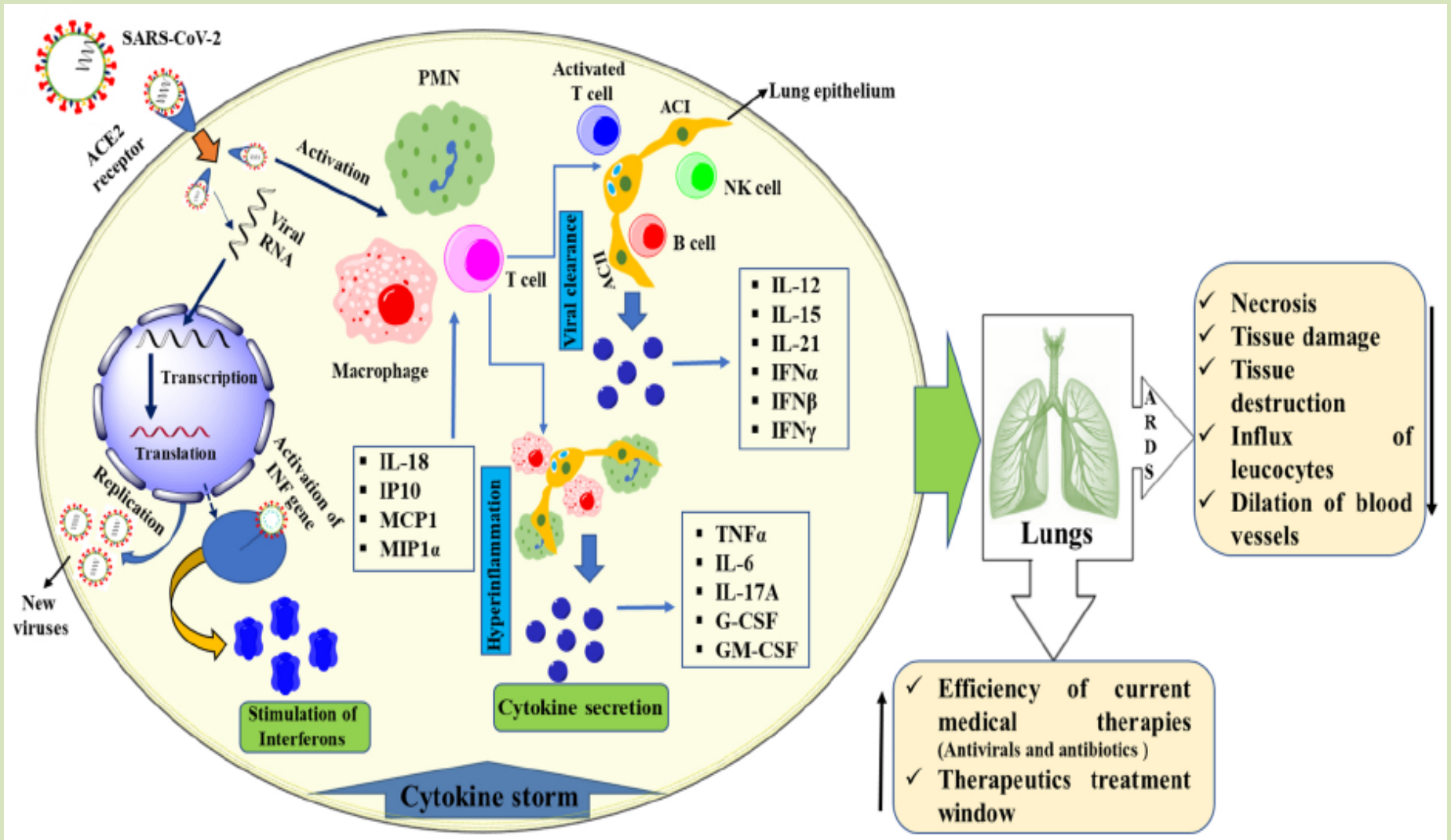
108427

- Following the binding of SARS-CoV-2 to the host protein, the spike protein undergoes protease cleavage.
- A two-step sequential protease cleavage to activate spike protein of SARSCoV and MERS-CoV was proposed as a model, consisting of cleavage at the S1/S2 cleavage site for priming and a cleavage for activation at the S'2 site, a position adjacent to a fusion peptide within the S2 subunit
- After the cleavage at the S1/S2 cleavage site, S1 and S2 subunits remain non-covalently bound and the distal S1 subunit contributes to the stabilization of the membrane-anchored S2 subunit at the prefusion state [32]. Subsequent cleavage at the S'2 site presumably activates the spike for membrane fusion via irreversible, conformational changes.

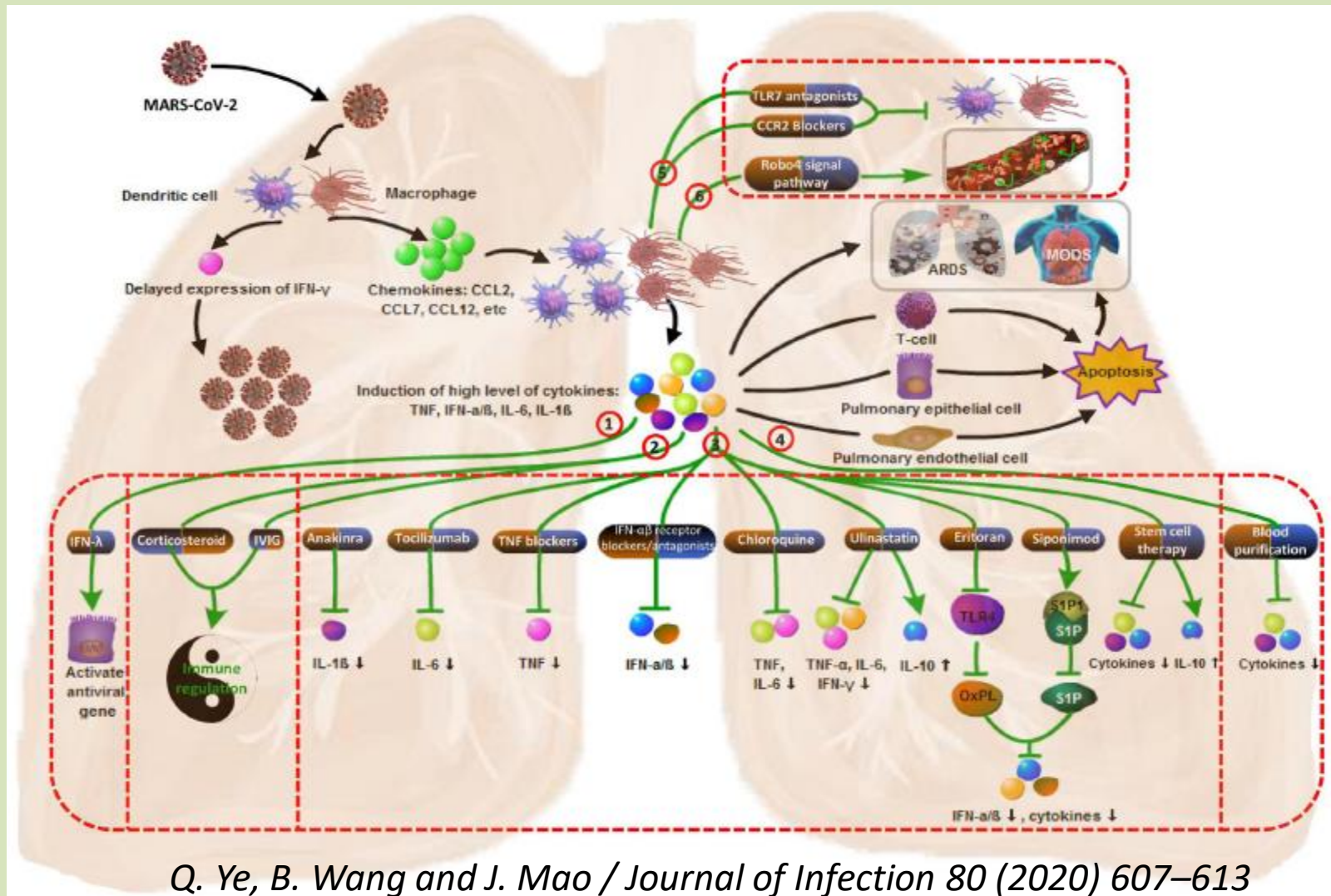
Chronology Of Events During SARS-CoV-2 Infection



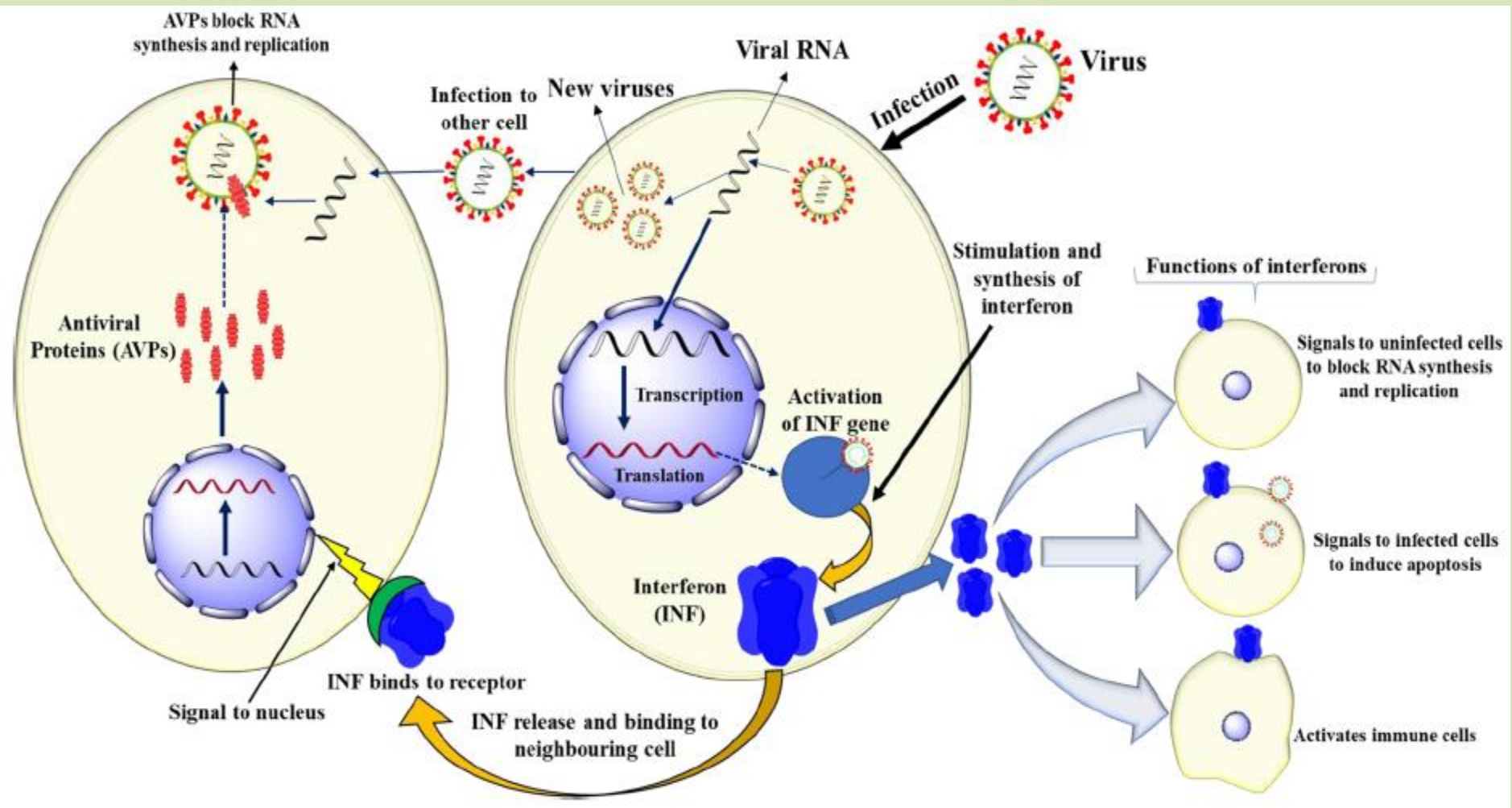
COVID-19 Pathogenesis & Cytokine Storm

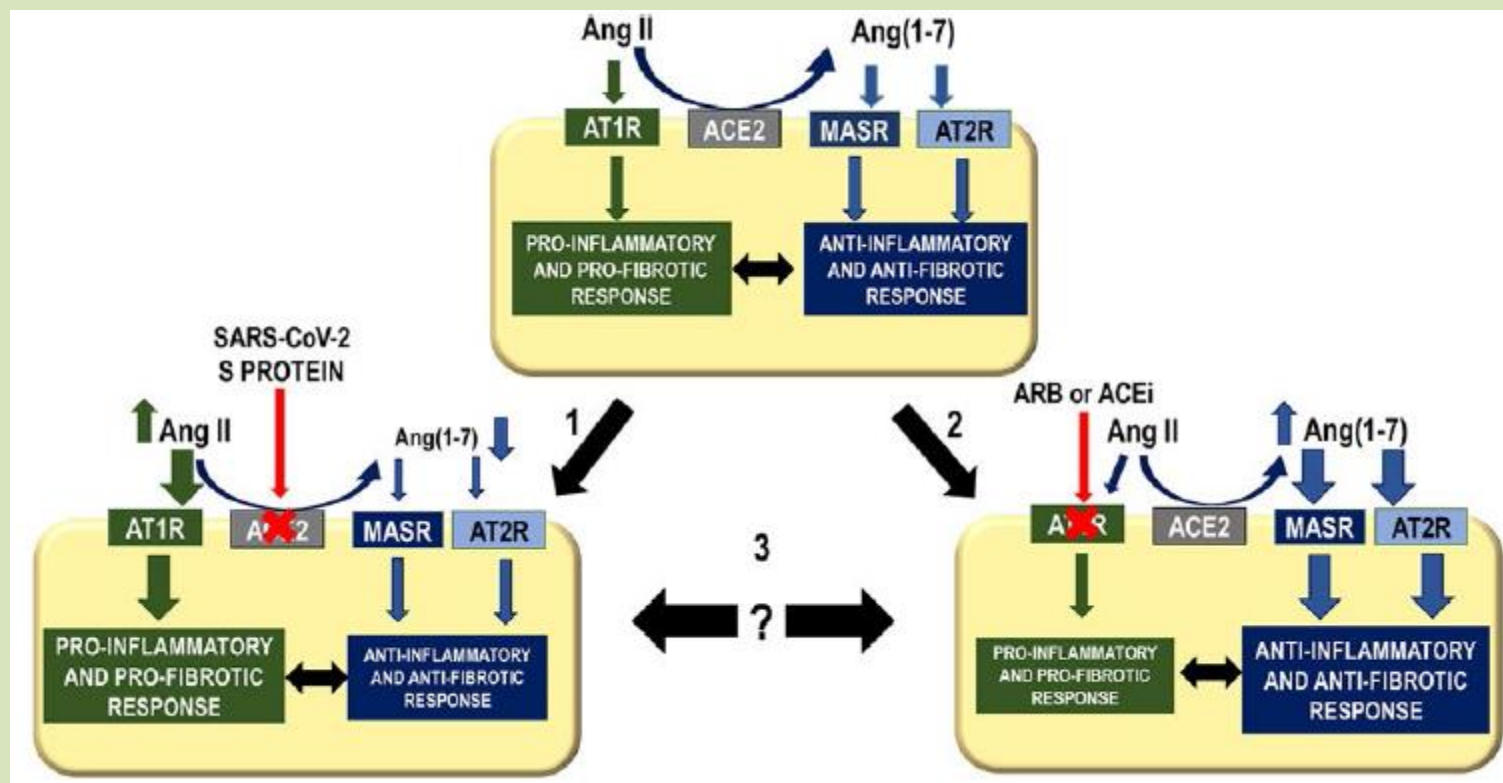


Mechanism of Cytokine Storm in COVID-19 & Potential Therapy



Mechanism of Interferon Biosynthesis & Their Functions





- Three main components for innate immunity in the airway:
- Epithelial cells
- Alveolar macrophages
- Dendritic cells (DCs)
- DCs reside underneath the epithelium
- Macrophages are located at the apical side of the epithelium.
- DCs and macrophages serve as innate immune cells to fight against viruses till adaptive immunity is involved.