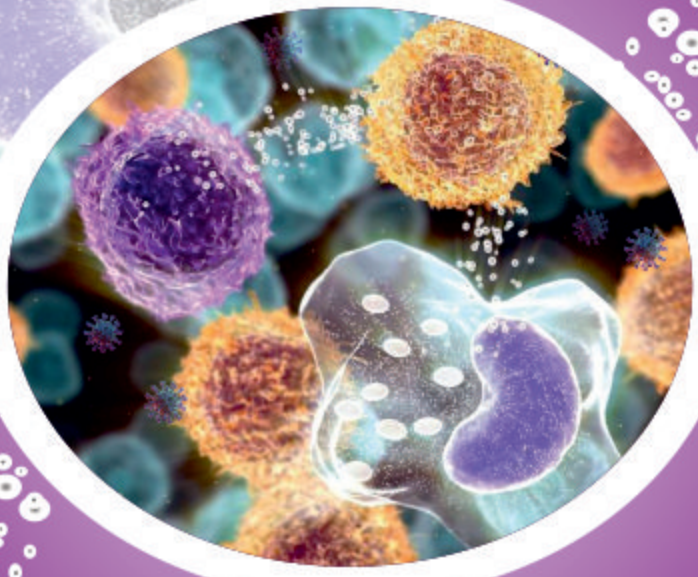
Cytokine Storm in COVID-19



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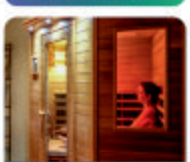
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PREFACE

COVID-19 was first detected in Wuhan, China, in December 2019; since then it rapidly spread and was declared a global pandemic within three months. It has infected over 25 million people and caused over 8,00,000 deaths worldwide; in India, it has affected over 9,00,000 people, and caused over 75,000 deaths. But, it has strengthened the indomitable human spirit to rise above any challenge thrown at it. Clinicians and scientists all over the world have banded together and taken charge of tackling every aspect of the disease. The World Health Organization's International Clinical Trials Registry Platform (ICTRP) lists a staggering 5459 clinical trials registered world over, within a mere 7 months (from February 2020 to September 2020)- a testament to this global teamwork. Trials range from small molecules that inhibit viral life cycle at different stages as well as deleterious host cell pathways, antibodies targeting the virus, and modalities like convalescent plasma, cellular therapy and ozone therapy, to psychological assessment of caregiver/clinician/patient outcomes following treatment, diagnostic techniques, prone position assessment of COVID-19 patients, and many more.

COVID-19 has a worldwide mortality rate of 3.5%. One of the most common reasons for mortality due to COVID-19 is the exaggerated inflammatory response, or the "cytokine storm", elicited by the body. This systemic inflammation precipitates the disease rapidly and worsens prognosis. Monitoring markers of inflammation and infection thus provide an opportunity to estimate the severity of the disease; and, limiting this inflammation will help clinicians mitigate the exacerbation of the disease. In this book, we describe the salient features of cytokines, the role they play upon viral infection, and the pathology of cytokine dysregulation. We then investigate the role of IL-6, a major pro-inflammatory cytokine. We elucidate the clinical picture and the laboratory findings of the cytokine storm, followed by management strategies. Finally, we discuss novel therapies such as ozone therapy and stem cell therapy to treat the cytokine storm.

Of note, modalities such as Ozone Therapy and Stem Cell Therapy have great potential in tackling the inflammatory processes. Ozone therapy works primarily by activating the inherent anti-inflammatory pathways of the cell. It also has potent anti-oxidative, antiviral, germicidal, and anti-coagulant activity, to say the least. Stem cells modulate the cellular microenvironment via various

paracrine mechanisms, and calm the immune storm that worsens the disease. Both these are powerful therapies that have been shown to resolve the clinical symptoms while improving the inflammatory profiles and other laboratory markers of inflammation, and correlate well with radiological improvements.

Our main aim for writing this book was to paint a consolidated picture for understanding the cytokine storm in COVID-19. This understanding will open up future directions to treat the disease. The field of COVID-19 healthcare and research is evolving at an unprecedented pace due to global collaboration. But, we hope to provide our readers with a starting point-and a solid foundation-to comprehend the cytokine storm, its ill effects, and effective management. This storm, too, shall pass.

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CONTENTS

CHAPTER 1

COVID-19: THE PANDEMIC

COVID-19 has so far affected a cumulative total of nearly 25 million people and caused 8,00,000 deaths worldwide as of 31st August 2020 according to the WHO.[1] India has 8,82,542 active COVID-19 cases with death toll reaching 71,642 as of 7th September 2020, according to the Ministry of Health and Family Welfare, Government of India. There is an urgent need to explore novel options of treatment for COVID-19. Despite standard treatment, 20% of patients progress to moderate stage out of which 5% deteriorate to severe stage. There is 3.5% worldwide mortality rate in COVID-19 patients.[2,3]

The first cases of COVID-19 started in Wuhan City, China, in December 2019[4], and was declared a pandemic on 11th March 2020. [5] There are currently no drugs or treatments for COVID-19. The Indian Government took a number of steps to ensure that COVID-19 spread is contained, including enforcing strict lockdowns, providing essential services, raising mass awareness and enforcing hygienic practices, such as hand hygiene, using masks and sanitizers, and employing social distancing in all areas. Patients were isolated and quarantined, as well as specialized hospitals were rapidly set up to respond to the surge in COVID-19 patients.[6–8]

Early studies[9] focused on unveiling the structure and function of the novel coronarvirus, including elucidating the genetic makeup of SARS-CoV-2; it was found to be highly homologous to the virus responsible for the 2003 pandemic, SARS-CoV. Pathological studies showed a drastic surge in the inflammatory conditions: elevated white blood cell counts with significant rise toward the end, vascular dysfunction, thromboemboli and coagulopathic damage, lymphocytopenia as well as pulmonary dysfuction, diffuse alveolar damage, bacterial bronchopneumonia, and fibrosis. The liver exhibits mild lobular infiltration by small lymphocytes, and centrilobular sinusoidal dilation and patchy necrosis; the heart shows focal mild fibrosis and mild myocardial hypertrophy. The causes of death from COVID-19 range from acute respiratory distress syndrome (ARDS), type I respiratory failure, sepsis, acute cardiac injury, heart failure, alkalosis, hyperkalemia, acute kidney injury, and hypoxic encephalopathy.[10–14]

Several studies show that increasing mortality was due to hyperinflammation and overstimulation of the immune system.[15,16] COVID-19 exhibits multimodal pathology by inducing a 'cytokine storm', or a flurry of cytokines that exacerbate the inflammatory response, depleted oxygenation and viral infection leading to pneumonia, edema, coagulopathy, respiratory failure and eventually, death. Curbing the cytokine storm (or cytokine release syndrome) is critical to improving survival[17]- and our main aim for writing this book.

Here, we discuss in depth what cytokines are, how SARS-CoV-2 invades the human body and the pathogenic mechanisms that engage upon viral entry, followed by the immune dysregulation and the major complications seen in COVID-19. We then focus on a major player, IL-6, elucidate the clinical presentation of the disease, the laboratory picture and ways to manage the cytokine storm. Finally, we discuss novel therapies to mitigate the cytokine storm.

CHAPTER 2

CYTOKINES

WHAT ARE CYTOKINES?

Cytokines are the means of communication within the immune system. They are cell-secreted signaling proteins that exert pleiotropic effects locally as well as systemically for modulating cellular responses. They may be membrane bound or soluble, and induce signals in cells that activate them or regulate certain enzyme activity, gene expression, and ultimately survival/death pathways. This affects the downstream behavior of the cells. Macrophages, natural killer cells, dendritic cells, and T- and B-lymphocytes produce cytokines that act in an integrated, finely calibrated manner to modulate the immune response.

For example, subsets of chemoattractant cytokines (called chemokines) mobilize and attract immune cells to the site of injury. Once at the site, immune cells differentiate based on the local signals to a "mature"-or activated-phenotype and relocate to the site of active infection/injury to fight the insult and repair damaged tissue. Cytokines-derived from the Greek words "cyto" meaning cell, and "kinesis" meaning movement-are thus critical in ensuring a controlled immune response in case of infection or injury.

Interferons were the first class to be discovered. Back in 1957, Isaacs and Lindenmann studied the chick chorio-allantoic membrane infected with heat-inactivated influenza virus. A factor ran 'interference' in this infection-and was aptly named Interferon.[18]

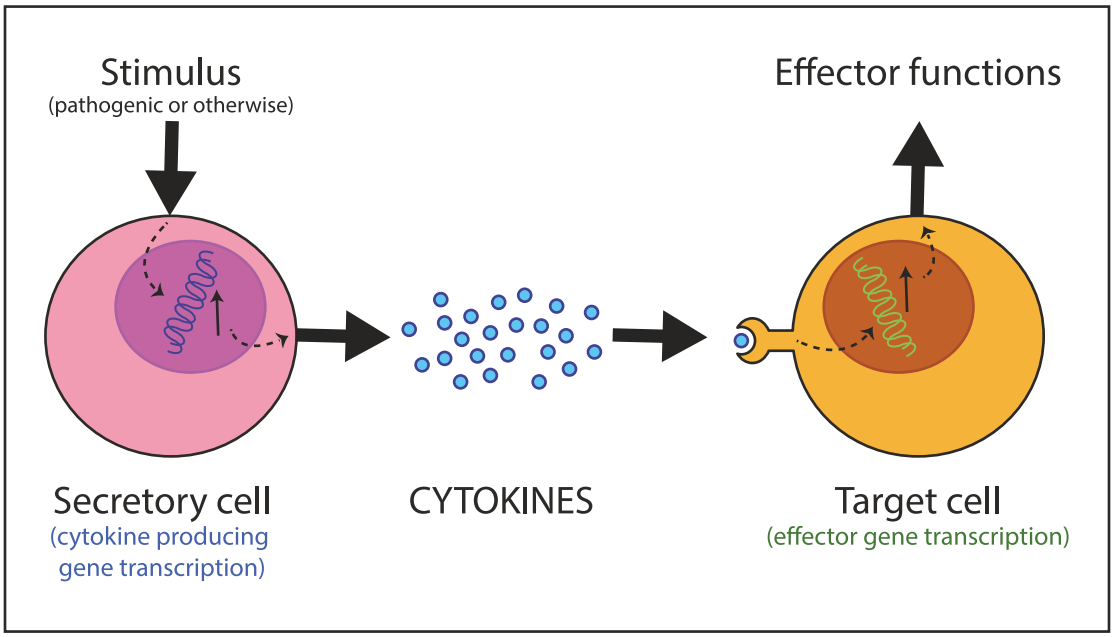

Figure 1: Cytokines are small molecules that tightly regulate a variety of biological processes, most notably immune regulation.

TYPES OF CYTOKINES

Cytokines have been categorized based on different aspects: receptor interactions, structure, function, and signaling pathway involvement:

BASED ON RECEPTOR FAMILIES

Cytokines engage with typical receptors and can be categorized on their interactions with specific receptors:

INTERLEUKIN (IL) - 1 RECEPTOR FAMILY

The cytokines of the Interleukin (IL)-1 family are typically pro-inflammatory in nature. The canonical members include IL-1α and IL-1β, other members include IL-18 and IL-33. Ten structurally similar receptors exist: ligand-binding chains

(IL-1R1, IL-1R2, IL-1R4, IL-1R5, and IL-1R6), 2 types of accessory chains (IL-1R3, IL-1R7), molecules that act as inhibitors of signaling (IL-1R2, IL-1R8, and the structurally distant IL-18BP), and 2 orphan receptors (IL-1R9, IL-1R10).[19]

HEMATOPOIETIN (CLASS I) RECEPTOR FAMILY

Four anti-parallel helix bundle motifs are the defining feature of this class of cytokines. IL-2, IL-3, IL-4, IL-6, IL-12, Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Granulocyte Colony Stimulating Factor (G-CSF) are a part of this family of cytokines. This class of receptors is further divided into 3 sub-families based on their specific receptors: the γ-chain bearing (IL-2 receptor) subfamily, the β-chain bearing, or GM-CSF, receptor subfamily, and the gp130 receptor subfamily.[20]

INTERFERON (CLASS II) RECEPTOR FAMILY

Interferon (IFN) was the first class of cytokines to be discovered, named after their ability to "interfere" with viral replication in rabbits. It is subdivided into 3 classes: type I IFNs, type II IFN and type III IFNs. The canonical type I IFNs are IFN-α and IFN-β. IFN-α has 13 different subtypes: IFN-α1, IFN-α2, IFN-α4, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α13, IFN-α14, IFN-α16, IFN-α17 and IFN-α21. Type I IFNs inhibit viral replication, increase natural killer (NK) cell activity and the expression of Major Histocompatibility Complex (MHC)-class I molecules on virus-infected cells, and stimulate the development of T_H1 cells. Type II IFN has only one representative, IFN-γ: an effective macrophage activator during immune responses. Type III IFNs include IL-28/29, and are similar in activity to type I IFNs.[21]

TUMOR NECROSIS FACTOR RECEPTOR FAMILY

The tumor necrosis factor (TNF) and TNF receptor (TNFR) superfamilies (TNFSF/TNFRSF) include 19 ligands and 29 receptors reviewed in great detail by Dostert et al.[22] TNFα is an acute phase pro-inflammatory cytokine, and engages the transcription factor Nuclear Factor-κB (NF-κB): a master regulator of multiple divergent processes such as cell survival, inflammation, and apoptotic or necroptotic death. TNFSF6, a member of the TNFSF, induces cell death via multiple pathways, and is present on both activated T-cells as well as NK cells. Another member, CD95, induces activation-induced cell death (AICD) in T-cells. It is also a pro-inflammatory cytokine, as a chemoattractant for

neutrophils, macrophages, and T-cells.[22]

INTERLEUKIN (IL) - 17 RECEPTOR FAMILY

The IL-17 family includes interleukins 17A, 17B, 17C, 17D, 17E and 17F. IL-17A is released by activated T (T_H17) cells and stimulates the production of IL-6, CXCL8, and granulocyte colony-stimulating factor (G-CSF) generating an inflammatory state. Members of this family generally coordinate the release of proinflammatory and neutrophil-mobilizing cytokines, except for IL-17E: it promotes the differentiation of the anti-inflammatory T_H2 cell subclass, while suppressing T_H17 cells. The receptors in this family act via the NF-κB as well as the mitogen-activated protein kinase (MAPK or MAP kinase) pathways to induce an inflammatory state.[23]

CHEMOKINE RECEPTOR FAMILY

Chemokines are cytokines that bind cell-surface receptors and drive the movement of leucocytes up a concentration gradient. Such a molecule is known as a "chemoattractant", and the cellular migration is called "chemotaxis". Two major chemokine sub-families exist, based on the position of cysteine residues: CXC and CC. Generally, CXC chemokines like IL-8 target neutrophils; whereas CC chemokines such as monocyte chemoattractant protein (MCP)-1 target monocytes and sub-set of lymphocytes (with exceptions).[24] Chemokines are also involved in homeostatic migration and tissue repair- for example, by inducing angiogenesis[25]- in addition to inducing a pro-inflammatory state by signaling T-cell differentiation.[26]

BASED ON SOLUBILITY

SOLUBLE

Cytokines are small molecules, and can be soluble or membrane bound. Soluble cytokines, such as IL-6, are released by the producing cells (in this case-macrophages), and exhibit myriad functions, such as the following: they activate cytotoxic T-cells, promote differentiation of B-cells into activated B cells (or plasma cells), and recruit monocytes to the site of inflammation, among others.[27]

MEMBRANE BOUND

Membrane-bound cytokines, such as those in the TNF superfamily, work by cell-to-cell contact. These transmembrane glycoproteins trimerize and transduce signals, often bidirectionally between the cytokine producing and target cells.[28]

BASED ON FUNCTION

Cytokines primarily modulate immune function by promoting or reducing inflammatory responses. This classification of cytokines is based loosely on the activity of the cytokines that was first noticed:

PRO-INFLAMMATORY CYTOKINES

Inflammation is essential for fighting infection and clearing pathogens from the site of injury. Pro-inflammatory cytokines are effectors of inflammation, and do so by: increasing capillary permeability, recruiting immune cells at the site of inflammation, inducing cells into producing more quantities and types of cytokines, activating differentiation of immune cells into mature phenotypes, and so forth. The two most well known effectors of the pro-inflammatory cascade are TNFα and IL-6.[29]

ANTI-INFLAMMATORY CYTOKINES

Anti-inflammatory cytokines are vital for de-escalating the acute phase immune response and preventing an uncontrolled, excessive flurry of cytokines: the infamous "cytokine storm". Major anti-inflammatory cytokines include members of the Interleukin 1 family- IL-4, IL-10, IL-11, IL-13, IL-1 receptor antagonist (IL-1Ra), and ironically IL-6.[30]

However, these functions can be performed by the same cytokine: such activity is based on the phase of the immune response, the cellular microenvironment, the quantity of cytokine(s) present, the target cell, and the nature of the downstream cytokines.[31]

MECHANISM OF ACTION

Cytokines undertake vast variety of functions, brought about by a multitude of mechanisms. These may be understood based on site of their action or based on the various properties of cytokines:

BASED ON THE SITE OF ACTION

Cytokines can be classified on the basis of the distance they cover, between the cells that secrete them (secretory cells) and those that receive them (target cells) (Figure 2):

AUTOCRINE

Cytokines exert effects on the cells that secrete them. This function often promotes the proliferation of the secretory cell itself.[32,33]

PARACRINE

Cytokines secreted by cells have local effects on the cells in the surrounding areas, usually by diffusing across a few Ångstroms. Their concentrations, target cells and cellular microenvironment dictates their activity.[32,33]

ENDOCRINE

Cytokines have systemic effects on distant groups of cells, for which they must travel via the bloodstream to reach their targets. These functions may be classified as endocrine activity of the cytokine. Interestingly, IL-2 is capable of all the above functions in its role within innate immunity.[34]

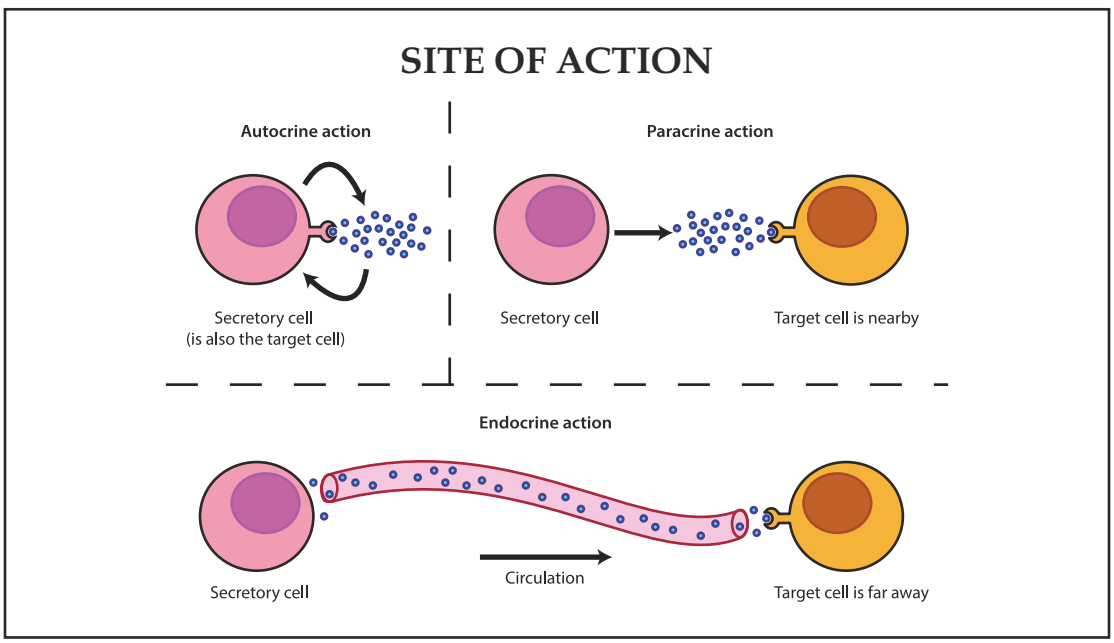

Figure 2: Action of cytokines based on the target site.

BASED ON PROPERTIES OF THE CYTOKINES

Cytokines exhibit certain characteristics: cascade induction, synergy, antagonism, redundancy and pleiotropy. These properties of cytokines are thought to contribute to the fine-tuning of the cytokine response. These may also serve as compensatory mechanisms for cytokinetic activities that would otherwise turn pathogenic.

CASCADE INDUCTION

The primary function of all cytokines is to induce signaling cascades in cells. These cascades are usually induced in phases, and involve multiple cytokines over the course of the insult (Figure 3). Stacey et al. sought to determine the nature and kinetics of cytokine levels across various stages in HIV-1, HBV or HCV infection. They found that the increase in plasma viremia in acute HIV-1 infection was associated with rapid and transient elevations in alpha interferon (IFN)-α and (IL-15); a large increase in inducible protein (IP)-10; rapid and sustained increases in TNFα and monocyte chemotactic protein (MCP)-1; slow elevations in additional pro-inflammatory factors including IL-6, IL-8, IL-18, and IFN-γ; and a late-peaking increase in IL-10. They also observed that plasma cytokine levels were only slightly affected during the same phase of HBV infection and a delayed response of more intermediate magnitude was observed in acute HCV infection.[35] This suggests that in different types of infections elicit a different cytokinetic milieu.

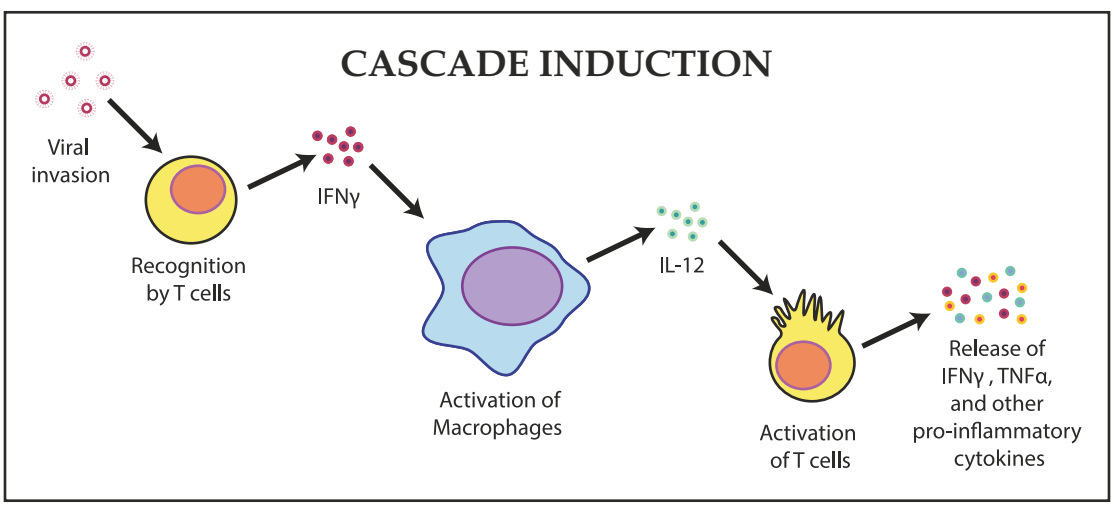

Figure 3: Cytokines induce cascades that end up releasing further cytokines, cellular activation, and other pleiotropic effects.

SYNERGY

Two or more cytokines within the same family or across different families work synergistically to induce a signal cascade within cells[34] (Figure 4). For example, IFN-I and TNFα raise a potent antiviral response by synergistic action[36] as demonstrated by Wang et al. on primary human fibroblasts.[37] They show that infecting human macrophages with Myxoma virus activates the cytoplasmic retinoic acid inducible gene I (RIG-I). This in turn initiates a sustained TNFα induction through the sequential involvement of the downstream IFN-regulatory factors 3 and 7 (IRF3 and IRF7). Additionally, evidence suggests that receptors also interact synergistically to mediate signal transduction: IL-2 mediated cell proliferation involves synergistic action of different interleukin 2 receptor beta-chain tyrosines, which engage at least two different pathways to induce cell proliferation.[38]

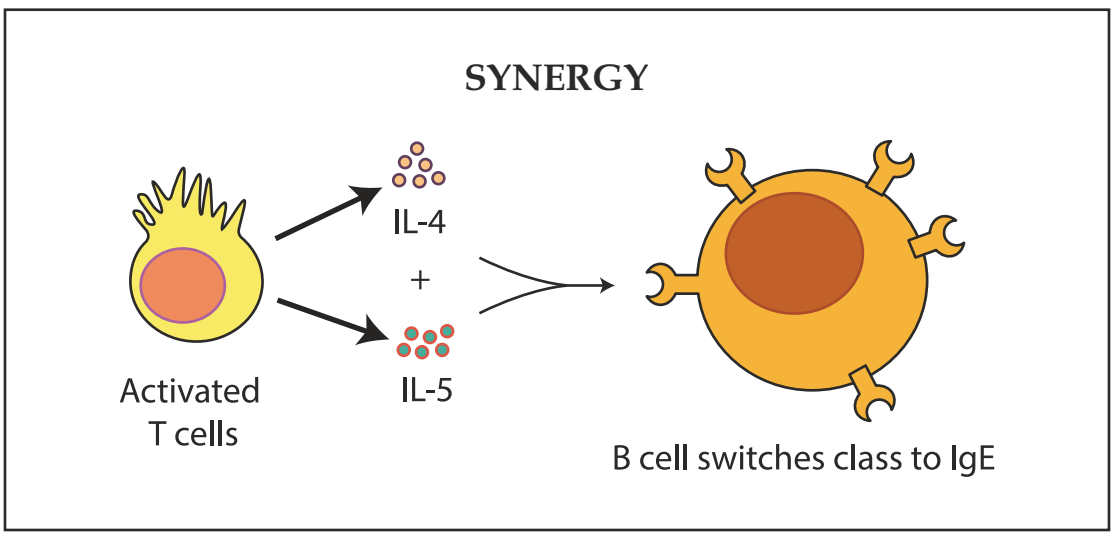

Figure 4: Cytokines often act synergistically, which fine-tunes their downstream activity.

ANTAGONISM

Cytokines can also have antagonistic effects on each other as well as the signal transduction pathways (Figure 5); classic examples are the IL-1 and IL-1Ra pair as well as the IL-18 and IL-18 binding protein (IL-18BP).[39] The receptor for IL-1 is a dimer, composed of IL-1RI (interleukin-1 receptor type I) and IL-1RAcp (interleukin-1 receptor accessory protein).[40] IL1-Ra interacts exclusively with IL-1RI, and prevents its dimerization with the IL-1RAcp.

IL-18BP, however, is a secreted antagonist that binds IL-18 with extremely high affinity[41], and prevents it from interacting with its receptor. These antagonistic cytokines serve as checkpoints for controlling cellular signal transduction and subsequent effector function.

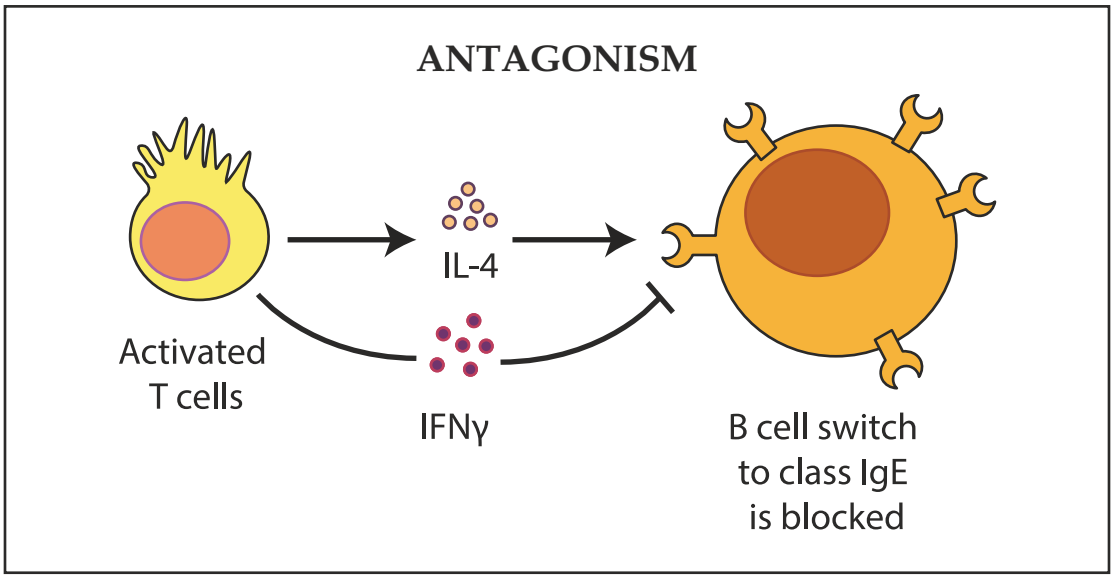

Figure 5: Cytokines act as antagonists and serve as checkpoints for downstream processes.

REDUNDANCY & PLEIOTROPY

Several cytokines induce the same signaling pathway within cells- a property known as redundancy. Conversely, when a single cytokine induces multiple pathways, it is known as pleiotropy[42] (Figure 6). Cytokines often have pleiotropic effects, in which they participate in multiple pathways, often in contradictory or confounding ways. This is the pleiotropic property of cytokines-depending on the context, the cytokines can perform different functions. The same cytokine can have either pro-inflammatory or anti-inflammatory functions.[31]

Redundancy in cytokines and their receptors is seen in varied processes, such as hematopoiesis[43], and embryonic implantation[44] apart from immune function. For example, IL-2 has several pleiotropic effects: it drives T-cell growth and differentiation, augments natural killer cytolytic activity, and mediates activation-induced cell death.[34] It performs these functions via the JAK/STAT pathway, the Ras/Mitogen-Activated Protein Kinases (MAPK) pathway, and

the Phosphoinositol-3 Kinase (PI3K)/Akt pathway.[45] However, it also has redundant functions, such as induction of the JAK/STAT pathway along with IL-4, IL-7, IL-13, and IL-15.[46]

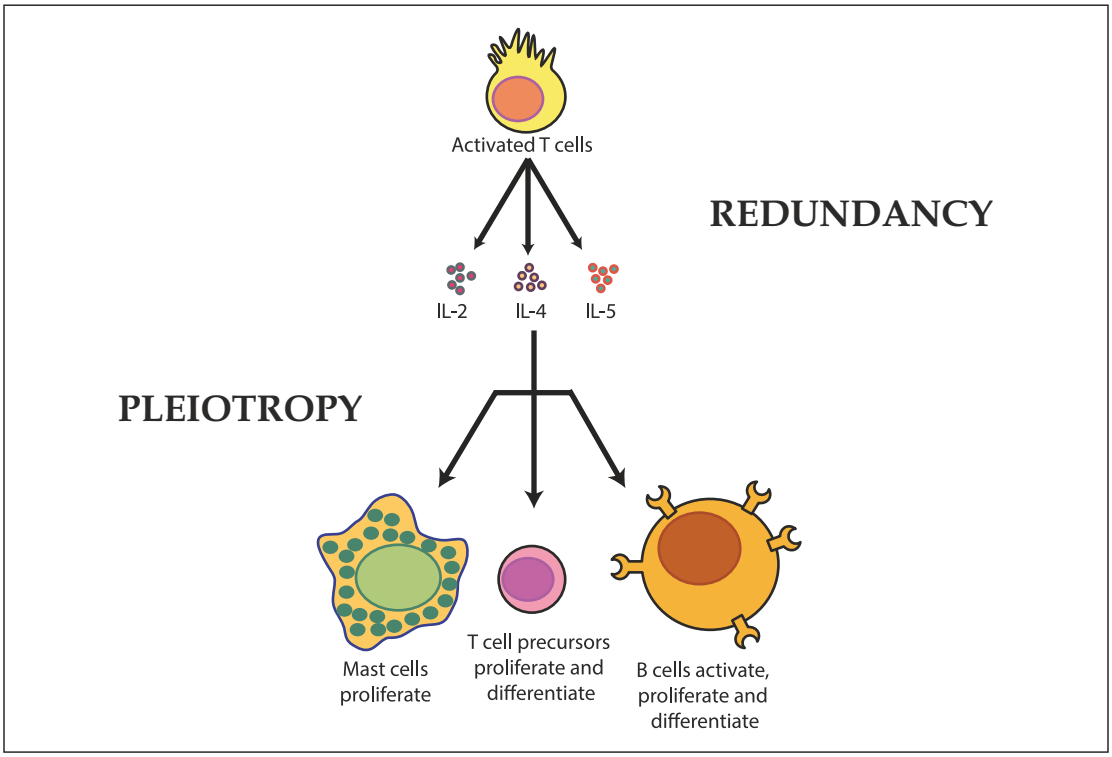

Figure 6: Redundancy and pleiotropy allow for fine-tuning of the cytokinetic response by providing compensatory mechanisms for effector processes. It also creates a feedback mechanism to calibrate the cellular microenvironment as required.

The cytokine network is a delicately balanced system that fine-tunes the immune system: it maintains normal wear and tear, and engages the immune system meaningfully in disease conditions. The cytokinetic system going awry directly affects the organs, and determines the disease progression. The chapter "Cytokine Storm in COVID-19" discusses these issues in greater detail.

CHAPTER 3

SARS-CoV-2 INVASION

The WHO reports a cumulative total of over 2,30,00,000 cases and 8,00,000 deaths by SARS-CoV-2 as of August 23rd, 2020 with a fatality rate of 3.5%.[47] It spreads mainly via community transmission as it is less severe, but more infectious.[48] The reproduction number (R_0) of COVID-19 ranges from 1.4 to 6.49, with a mean of 3.28[49]: higher than both SARS (3)[50] and MERS (<1)[51]. Like SARS and MERS, SARS-CoV-2 may transmit through direct or contact, and presents similar clinical symptoms.[52]

GENETICS OF SARS-CoV-2

The CDC lists four main subgroups of coronaviruses: α, β, γ, and δ.[53] SARS-CoV-2 is a novel coronavirus of the genus *Betacoronavirus*, which also includes SARS-CoV (caused the 2003 SARS pandemic) and MERS-CoV (caused the 2012 MERS pandemic).[54]

Genetic sequence analysis of SARS-CoV-2 has revealed shared homology: 50% with MERS-CoV[2], and over 79% with SARS-CoV.[2,3] Furthermore, it has 96% similarity to the bat coronavirus BatCoV RaTG13, attesting to its zoonotic origins.[3]

Variants of viral isolates may exhibit differences in pathogenicity and viral load. However, this does not seem to be the case for SARS-CoV-2[55] even though SARS-CoV-2 genomes have evolved in different clusters worlwide.[56]

STRUCTURE OF SARS-CoV-2

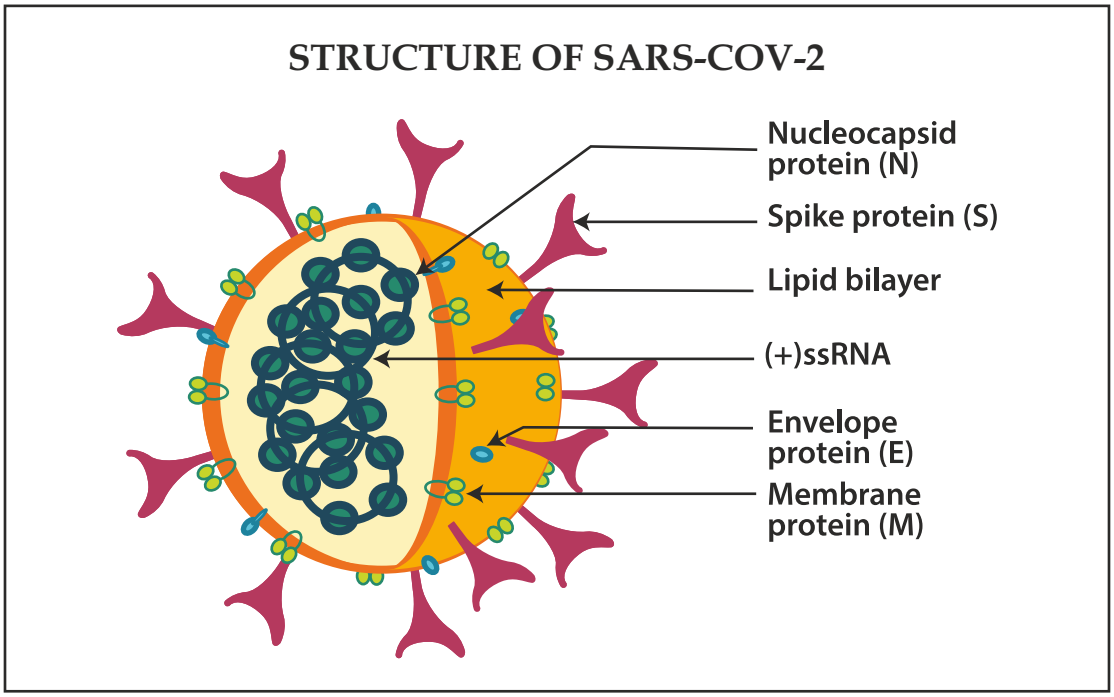

Figure 7: Structure of the mature SARS-CoV-2 virion.

SARS-CoV-2 is an enveloped, positive-stranded RNA virus with nucleocapsid (N). It has an envelope protein (E), a critical player in assembly, budding, and envelope formation[57]; and a membrane protein (M), the most abundantly distributed glycoprotein in the virion important for viral budding.[58] The spike protein (S) is responsible for recognizing and binding to the host cells, discussed below. Figure 7 elucidates the structure of the mature SARS-CoV-2 virion.

SARS-CoV-2 RECOGNIZES THE ACE2 RECEPTOR

THE SPIKE PROTEIN: S

SARS-CoV-2 has a transmembrane Spike (S) glycoprotein ectodomain that homotrimerizes[59] and protrudes from the viral surface.[60] It specifically

recognizes and binds to the angiotensin I converting enzyme (ACE)-2 receptor[2,61,62] with very high affinity.[63]

This spike protein consists of two functional subunits: the distal, N-terminal S1 that contains a receptor-binding domain (RBD); and the proximal, membrane-bound C-terminal S2 core that contains the fusion machinery.[64] In the pre-fusion state, the S1 and the S2 units are non-covalently bound.[65,66]

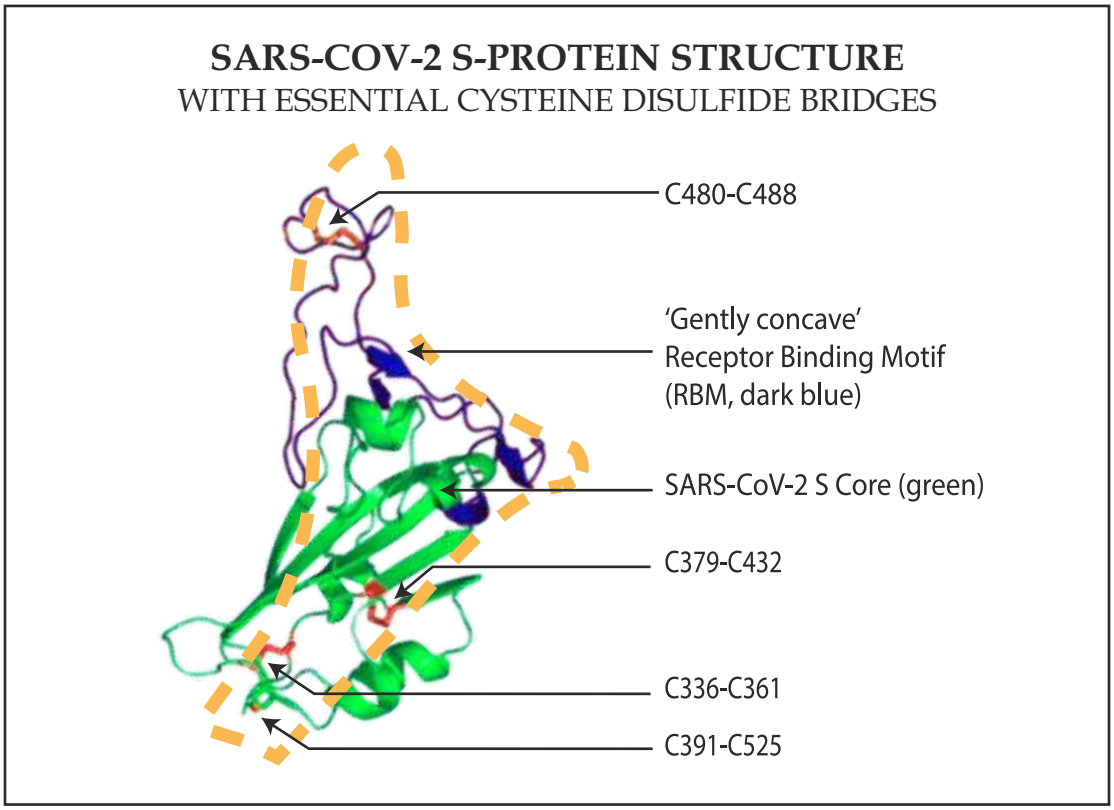

Figure 8: Structure of the Spike (S) protein shows the receptor binding motif in blue and the core in green, along with essential disulfide bridges between cysteine residues (red), as shown by Lan et al, Chen et al, Yan et al, etc.

THE BINDING SITE BETWEEN S AND ACE2

The S1 RBD stands erect for receptor binding; but lies down to evade the immune system.[67,68] It has 2 sub-domains: a receptor-binding motif (RBM) that makes contact with ACE2, and a core that presents a 'gently concave' outer surface. This concavity cradles the N-terminal helix of ACE2 (Figure 8).[69]

The RBM lies to one side of the core (reinforced by an essential disulfide bridge: C480–C488[69]) and contacts the loops between ACE2 helices 2 and 3. A ridge to the other side inserts between a short ACE2 helix (residues 329 to 333) and an -hairpin at ACE2 residue 353. Residues 445 to 460 of the RBD anchor the entire receptor-binding loop to the core of the RBD.[69]

A total of nine cysteine residues are found in the RBD, eight of which form four pairs of disulfide bonds. Three of these stabilize the core β-sheet structure (C336–C361, C379–C432 and C391–C525). The remaining pair (C480–C488) compacts the distal end loop of the RBM (Figure 8).[70]

THE VIRAL LIFE CYCLE

HIGH-AFFINITY BINDING: THE SARS-CoV-2 S-ACE2 COMPLEX

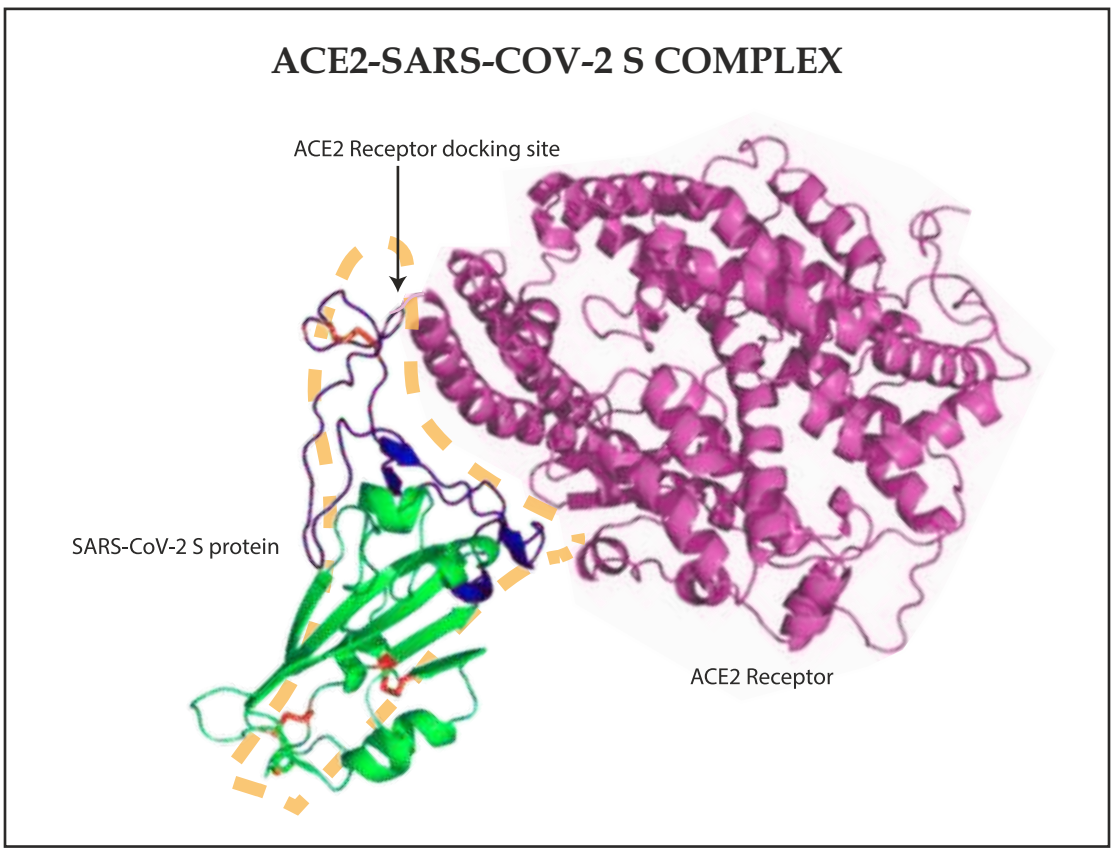

Figure 9: Lan et al, Chen et al, Yan et al etc. elucidate the binding site between S protein and ACE2.

Crystal structure of the SARS-CoV-2 spike protein-ACE2 complex shows a more compact conformation compared to SARS-CoV.[63] Similar to the SARS-CoV RBM, the SARS-CoV-2 RBM has an ACE2-binding ridge with the essential disulfide bridge mentioned above.

But, SARS-CoV has a three-residue motif: P-P-A, while SARS-CoV-2 has a four-residue motif: G-V/Q-E/T-G. This changes the SARS-CoV-2 ridge conformation such that an additional main-chain hydrogen bond forms within its RBD (between N487 and A475). The ridge compacts further and forms more contacts with the N-terminal of ACE2 helix.[63] This high-affinity binding within the SARS-CoV-2-ACE2 complex may worsen the infection rate of SARS-CoV-2.

THE CLEAVAGE OF S INTO S1 AND S2

The N-terminal ridge of the S1 unit binds to an ACE2 receptor helix. An essential[64], stoichiometrically contiguous protease, Transmembrane Protease Serine (TMPRSS)-2 then proteolytically cleaves S at the S1/S2 boundary.[71] This cleavage site is present at a locus closer to the S C-terminal region than is typical; it may be exposed only after the viral RBD binds the host ACE2.[72,73]

The S2 protein contains the fusion machinery: the fusion peptide (FP), a second furin-like proteolytic site (S2′), an internal fusion peptide (IFP) (identical to SARS-CoV), two heptad-repeat domains (HRs) and the transmembrane domain (TM).[74] The exact mechanisms remain unknown; however, multiple studies have confirmed increased virulence due to the presence of both ACE2 and TMPRSS2 on viral target cell surfaces.[64,72,73,75,76] This means that 2 cleavages must occur: one at the S1/S2 boundary and the other at the S2′ site.[77]

THE S2' CLEAVAGE

A four-residue insertion has been found in the S protein directly adjacent to the cleavage site (P681, R682, R683, and A684).[78] In combination with R685, this insertion can form an exposed loop, which may result in an increased sensitivity to proteases[79] like TMPRSS2.[64,78] This insert sequence is a cleavage site for the protease furin.[78,79] The insertion sequence is unique to the SARS-CoV-2.[3]

After the non-covalently bound S1 detaches, the S2 unit undergoes a conformational change. Cleavage at S2′ cleavage exposes a fusion peptide, which inserts into the host membrane. Then, two heptad repeats in S2 join,

forming an antiparallel six-helix bundle[80], fusing the virion with the host cell. The virus then releases its contents into the cell, uses the host replication machinery to multiply, bursts the host cell and eventually infects other cells.[75]

REPLICATION AND TRANSMISSION

The viral replicase gene is then transcribed: viral non-structural proteins (NSPs) assemble to form the replicase–transcriptase complex (RTC) that generates the viral RNA. In addition to viral genome replication, NSPs promote host mRNA degradation and blocks host cell translation, blocking innate immune response.[81]

Viral RNA is then translated. Translation produces both genomic and sub-genomic RNAs (necessary for downstream structural and accessory genes). The viral structural proteins, M, E, and S are eventually translated and are processed by the endoplasmic reticulum–Golgi intermediate compartment (ERGIC).[82,83] The viral genomes are encapsidated by N protein, and form mature virions by budding into membranes of the ERGIC containing viral structural proteins. Virions are then transported to the cell surface in vesicles and exocytosed.[84]

VULNERABLE ACE2 ENRICHED CELLS

CELLULAR DISTRIBUTION OF ACE2

The ACE2 receptor is widely distributed on the human cells surface, in over 150 different cell types corresponding to all major human tissues and organs: intestinal enterocytes, renal tubules, gallbladder, cardiomyocytes, male reproductive cells, placental trophoblasts, ductal cells, eye, and vasculature.[85] It is especially enriched in the Alveolar Type II cells (AT2) cells of the lung.[86]

ACE2+ CELLS EXPRESS GENES THAT PROMOTE SARS-CoV-2 VIRULENCE

Single-cell RNA sequencing of normal human donor AT2 cells reveals that these ACE2-abundant cells highly express genes required for viral assembly and transmission.[61] One of these is Caveolin (CAV)-2, responsible for specialized lipid rafts required for viral entry into host cells followed by replication.[87] Further, multiple genes involved in the endosomal sorting complex required for transport (ESCRT) machinery[88], are also enriched within the ACE2 abundant

cells.[61] ESCRT members are critical players in multiple pathways involved in viral budding and particle release.[89,90]

Additionally, ACE2 is an interferon-stimulated gene (ISG) in upper airway nasal epithelium.[91] ISGs are robustly upregulated by SARS-CoV-2[92]; this may worsen the vulnerability of ACE2 enriched cells towards infection.

SARS-CoV-2 BINDS ACE2 WITH VERY HIGH AFFINITY

SARS-CoV-2 also has higher ACE2-binding affinity compared to the SARS-CoV, the coronavirus responsible for the 2003 pandemic.[63] Thus, the ACE2-rich cells are prime targets for SARS-CoV-2 entry and infection. The downstream cellular dysregulation that follows the invasion of the host cells is discussed in the next chapter.

CHAPTER 4

CYTOKINE STORM: PATHOGENESIS

CYTOKINE DYSREGULATION: CAUSES OF THE CYTOKINE STORM

Cytokines operate in a finely tempered equilibrium to regulate the immune system. When viral pathogens invade the organism, this balance disrupted, leading to an excessive inflammatory response injurious to host cells, known as the "cytokine storm". Cytokine storms are prevalent in a variety of diseases and conditions, including hemophagocytic syndromes[93], infection[94], idiopathic multicentric Castleman disease[95], sepsis[96], pancreatitis[97], and more. In COVID-19 infection, dysregulated cytokine/chemokine responses and higher virus titers cause an inflammatory cytokine storm with lung immunopathological injury.[98,99] Such inflammation may begin at one local site but further spread throughout the body via systemic circulation.[100,101] Further, cytokines bind their receptors with a very high affinity[102], which allows small quantities of cytokines to produce a greatly magnified effect. This is a significant disadvantage during a cytokine storm, as excessive cytokine production snowballs rapidly and wreaks long-lasting systemic havoc.

THE ROLE OF INNATE IMMUNITY

Innate immunity forms the first line defense upon pathogen invasion. This first line of defense includes lymphocytes such as macrophages, and neutrophils (Figure 10). Pattern recognition receptors (PRRs) on these cells recognize foreign pathogen-associated molecular patterns (PAMPs).[82,83] The type-I interferon system specifically responds to viral pathogens.[103] Viral RNA is generated during coronavirus genome replication[104] and could be recognized by the following PRRs: NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), Toll-like receptors (TLRs), and C-type lectin-like receptor (CLRs). These stimulate the production of IFNs (especially type I[105]) and trigger anti-viral effectors such as T CD8+ cells, Natural Killer (NK) cells, and macrophages. Importantly, SARS-CoV-2 entry factors are also highly expressed in nasal epithelial cells along with innate immune genes.[106]

IFNs operate by engaging cell-autonomous immunity.[107] Further, the NF-κB signaling pathway is also engaged. NF-κB is a master regulator, and its signaling pathway-including critical players-like inhibitors of κB kinase (IKK)-related kinases, TANK binding kinase (TBK)-1 and inducible IκB kinase (IKKi)-is interwoven with inflammation.[108] The mechanisms are discussed below; however, it is imperative to note that every step in these tightly regulated mechanisms may be hyperinduced to produce an exorbitant amount of cytokines in COVID-19, causing systemic disruption and organ damage.

PATTERN RECOGNITION RECEPTORS AND SARS-CoV-2

NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN (NOD)-LIKE RECEPTORS (NLRs)

SARS-CoV-2 employs viroporin 3a, a facilitator of virulence, infectivity, ion channel formation, and virus release.[109] This viroporin activates the nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain-containing (NLRP)-3, which regulates the secretion of proinflammatory cytokines IL-1β and IL-18.[110] It does so by promoting TNF receptor-associated factor 3 (TRAF3)–mediated ubiquitination of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) in association with NF-κB.[111] NF-κB is dysregulated in a variety of inflammatory disorders, including cancer[112], Chronic Inflammatory Airway Disease[113], and neuroinflammation.[114] TNFα can also promote T-cell apoptosis via TNFR-1 and NF-κB[115], leading to

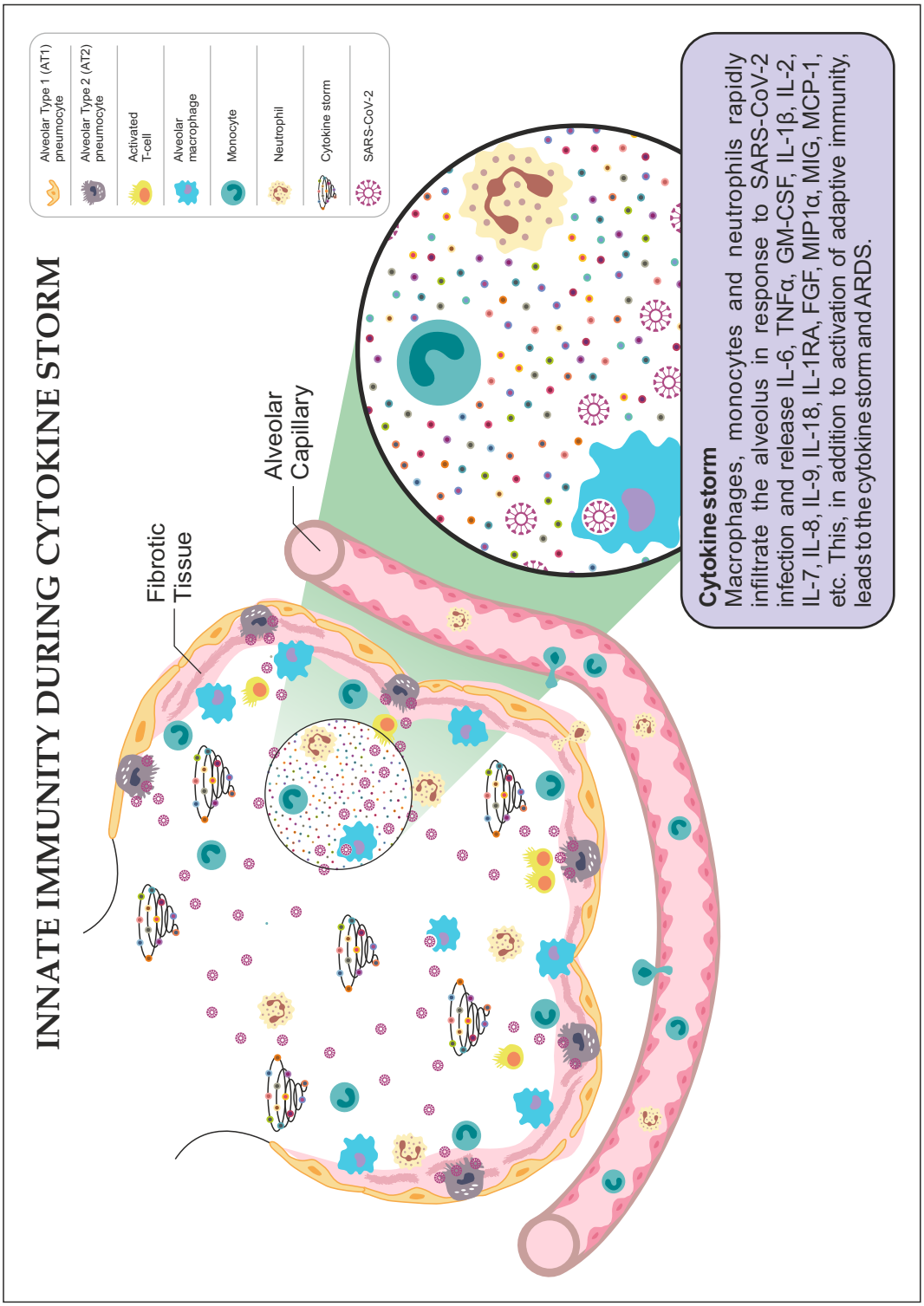

Figure 10: Role of Imate immunity in precipitating the cytokine storm.

dearth of antiviral machinery and excessive cytokine production.

RIG-I-LIKE RECEPTORS (RLRs)

The retinoic acid-inducible gene I (RIG-I) and its cognate protein receptors (the RIG-I-like receptor (RLR) protein family) is crucial in SARS-CoV-2 pattern recognition.[116] RIG-I induces type I IFNs by activating interferon regulatory transcription factors (IRF) via IKK-related kinases.[117] IKKs are intricately involved in the NF-κB inflammatory pathway.[108] The signaling pathway to induce IKKs and IRFs 3 and 7 is lead by the interferon-beta promoter stimulator 1 (IPS-1)[118] and TNF receptor associated factor family (TRAF) 3 and 6[119] (Figure 13). Excessive quantities of pro-inflammatory cytokines in such microenvironments may snowball into a much larger production of pro-inflammatory state, leading to the cytokine storm.

TOLL-LIKE RECEPTORS (TLRs)

The association of TRAFs with the IFN induction pathway is a fairly upstream event and proceeds through the toll-like receptor adaptors Toll/IL-1R domain-containing adapter inducing IFN- (TRIF) and IL-1R–associated kinase (IRAK)-1, another set of NLRP inflammasome activators[120] discussed above. TRAF3, a member of the TRAF family also associates with downstream IRF3/7 kinases TBK-1 and IKK-ε, suggesting that TRAF3 serves as a critical link between TLR adaptors and downstream regulatory kinases important for IRF activation and subsequent cytokine production that goes awry in COVID-19 (Figure 13). Toll-like receptor 5 (TLR5) activates dendritic cells, which produces interleukin IL-22, and IL-18 (via NLRs)- in turn eliminating rotavirus infection in immune-sufficient and immunocompromised mice.[121]

C-TYPE LECTIN-LIKE RECEPTORS (CLRs)

C-type lectin-like receptors sense glycolipids and glycoproteins present on viral surfaces, and activate antiviral immune responses: phagocytosis, antigen processing and presentation, induction of cytokines, and finally T-cell activation.[122] This proceeds via another pathway leading to NF-κB activation, and induce differentiation of CD4+ T-helper cells, B cells, and CD8+ cytotoxic T-cells.[123]

ROLE OF ADAPTIVE IMMUNITY

T CELLS

SARS-CoV-2 invades the host cells via the ACE2 receptor. Once inside, the viral peptides are recognized as foreign and presented by the cells to CD8+ cytotoxic T-cells through class I major histocompatibility complex (MHC-I) proteins.[124] CD8+ T-cells activate and proliferate as particle-detecting clones, and further stimulate virus-specific effector and memory T-cells. Antigen-presenting cells such as dendritic cells and macrophages recognize and present viral peptides to CD4+ T-cells through MHC-II proteins[124] (Figure 11).

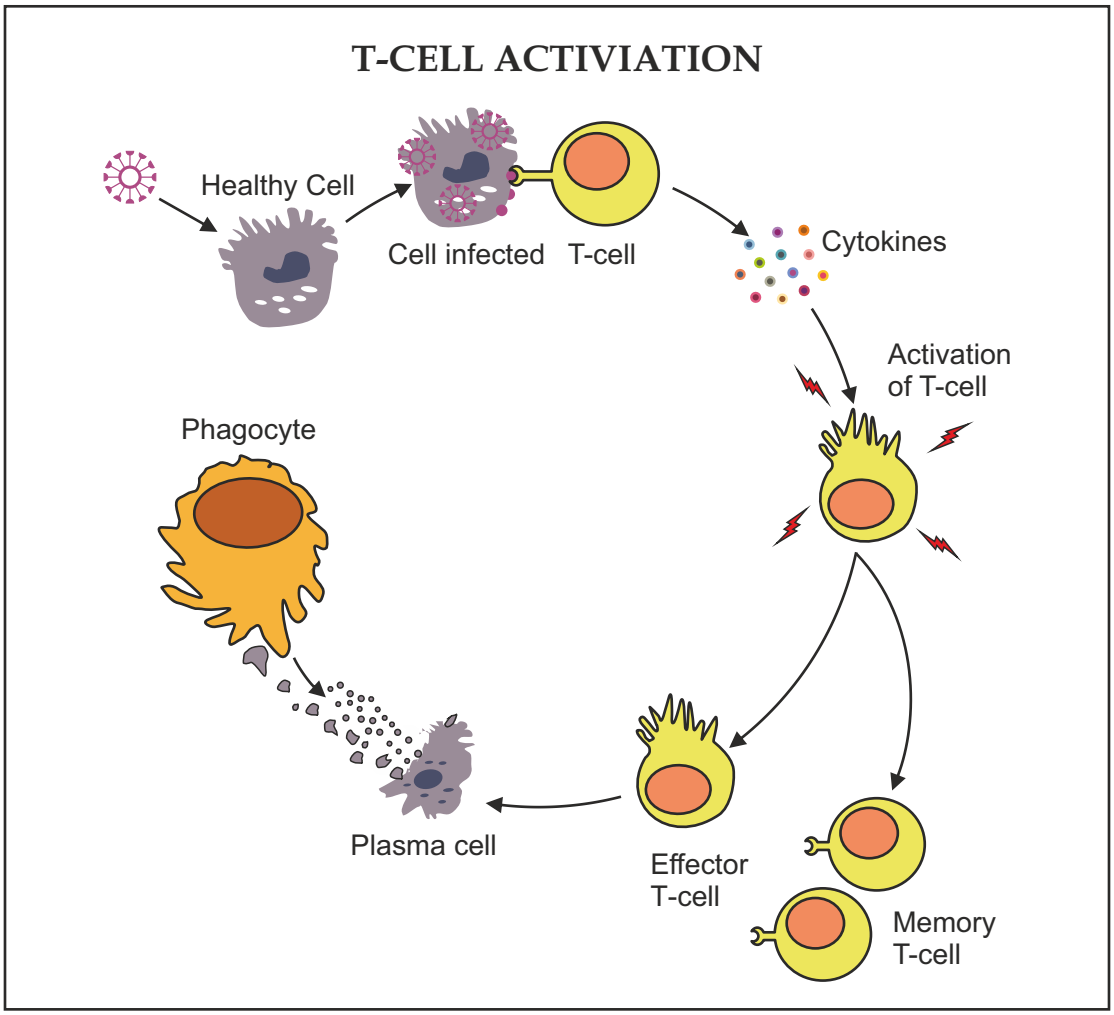

Figure 11: T-cell activation and recruitment.

S-reactive CD4+ T-cells are detected in 83% of COVID-19 patients, as well as in 34% of SARS-CoV-2 seronegative healthy donors. CD4+ T-cells from COVID-19 patients further co-expressed higher levels of CD38 and the MHC II protein Human Leukocyte Antigen – DR isotype (HLA-DR), indicating their recent activation.[125]

B CELLS

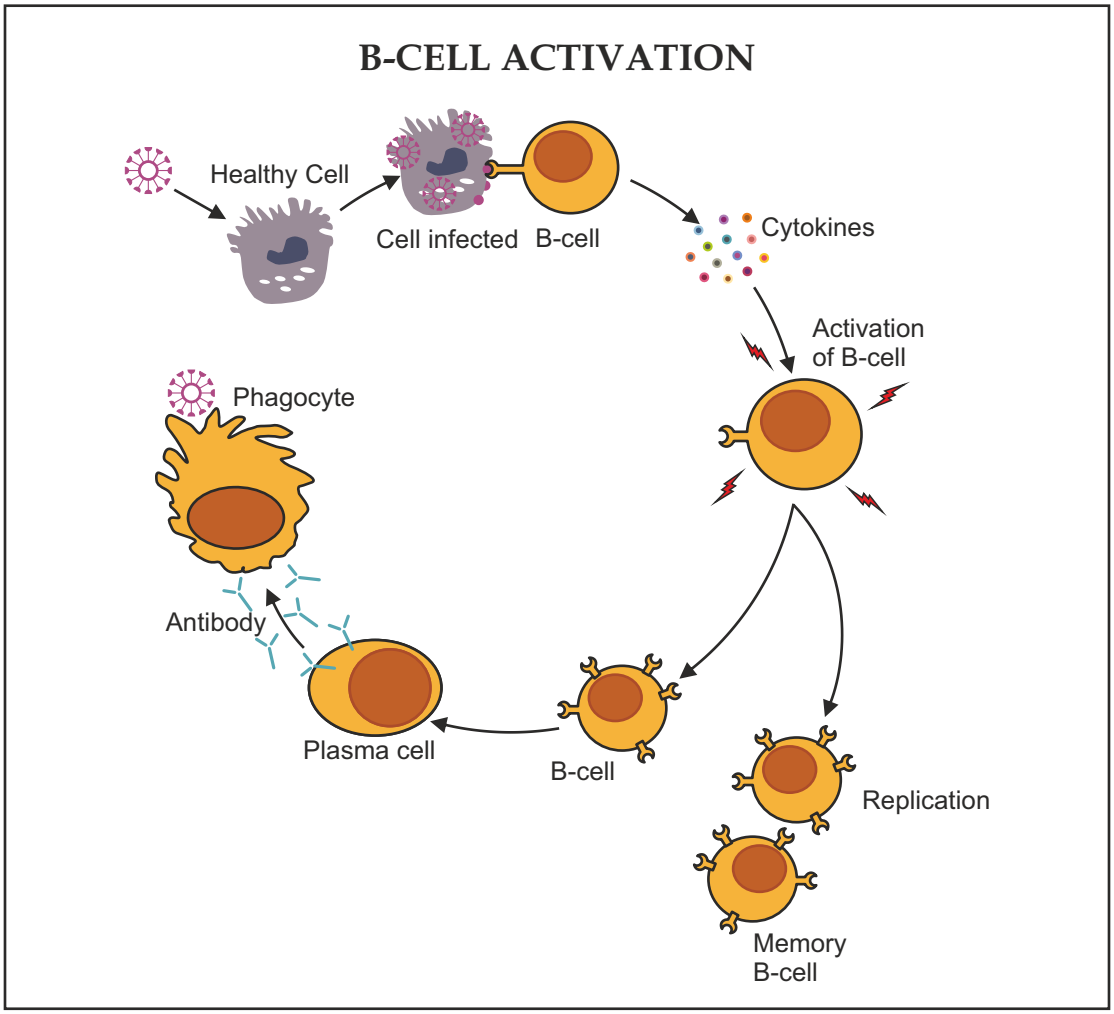

Figure 12: Activation of B-cells and their effector functions.

B cells, however, directly recognize the foreign particles and get activated. They generate antibodies: the IgM and IgG isotype. Within the first week following symptoms, the IgM primary virus-specific antibody response is observed. IgG

isotype antibodies follow the IgM response: this retains long-term memory.[126] B-cell maturation progresses through rearrangement of immunoglobulin heavy- and light-chain gene segments, culminating in the expression of IgM mature B-cell receptor (BCR) on the cell surface that can bind antigens[127] (Figure 12). The random rearrangement ensures that a vast repertoire of BCRs are generated that are essential for host defense, including autoreactive B cells (which are eliminated to avoid autoimmunity at various developmental checkpoints).[127] They also act as professional antigen presenting cells (like dendritic cells) and thus mediate CD4+ T-cells to mount an immune response.[128]

ANTIGEN-SPECIFIC MEMORY PERSISTS LONG-TERM

B- and T-cell epitopes are highly conserved between SARS-CoV and SARS-CoV-2. Structural proteins of SARS-CoV-2 are also homologous to SARS-CoV, but not to MERS-CoV.[129] This is important to study antibody memory in SARS infections.

Ni et al. reported that SARS-CoV-2-specific antibodies are detected in COVID-19 convalescent subjects and antibody titers correlate with the numbers of virus-specific T-cells.[130] In another study, three SARS-recovered individuals were analyzed for T-cell responses against 550 peptides from SARS-CoV structural proteins that may also cross-react with MERS-CoV. SARS-specific memory T-cells persisted at 9 and 11 years post infection in the absence of antigen. All memory T-cells detected were specific against SARS-CoV structural proteins, but lacked cross-reactivity to MERS.[131] Ju et al reported on RBD-specific monoclonal antibodies derived from single B cells of eight SARS-CoV-2-infected individuals demonstrating neutralizing activity unique to SARS-CoV-2 (compared to SARS-CoV or MERS-CoV).[132]

ENGAGING THE CELL-AUTONOMOUS IMMUNITY: IFN DYSREGULATION

Cell-autonomous immunity refers to the effector mechanisms induced by IFNs in non-immune cells.[133] IFN-induced antiviral mechanisms hinder at all stages of the viral life cycle: they block entry into host cells and uncoating of the virus, inhibit replication, prevent capsid assembly and budding and impede viral release.[107]

Although the immediate systemic IFN response in COVID-19 patients seems to

be suppressed[134], there is a robust upregulation of IFN Stimulating Genes (ISGs)[92] via the Janus activated kinase (JAK)-signal transducers and activators of transcription (STAT1) signaling pathways.[135] SARS-CoV-2 induces substantial but delayed IFN-β production and protein subsets of SARS-CoV-2 interfere with IFN-β activation, type I IFN production and downstream signaling[135] (Figure 13).

Type I and III IFNs disrupt the lung epithelial barrier upon viral recognition[136] and these are found in high levels in the bronchoalveolar lavage fluid of severe COVID-19 patients.[137] Further, type 1 IFNs trigger upregulation of ACE2 in upper respiratory nasal epithelium[91], worsening the SARS-CoV-2 infectivity.

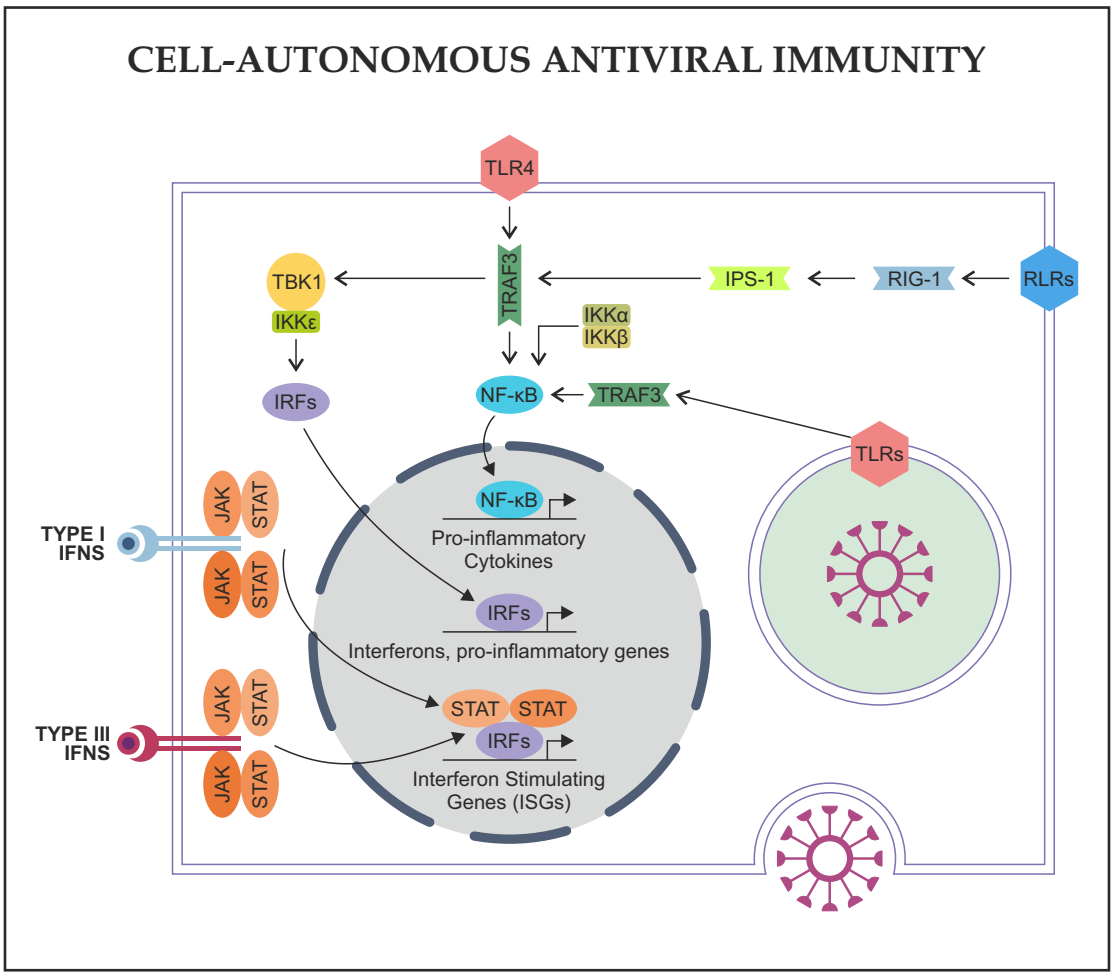

Figure 13: Cell-autonomous engagement of IFNs as antiviral responses.

CELLULAR DYSREGULATION FOLLOWING SARS-CoV-2 INDUCED CYTOKINE STORM

DYSREGULATION OF THE HOST ACE2 ENRICHED CELLS

SARS-CoV-2 invades the host cells via the Angiotensin Converting Enzyme (ACE)-2 receptor, a transmembrane protein abundantly found on Alveolar Type (AT)-2 cells.[86] Single-cell RNA sequencing of normal human donor AT2 cells reveals that these ACE-2 abundant cells highly express genes required for viral assembly and transmission.[61] Some of these, i.e. Caveolin (CAV)-2 [87] and those involved in the endosomal sorting complex required for transport (ESCRT) machinery[88], have been discussed in the 'Entry into Host Cells' subsection of the Structure of SARS-CoV-2 chapter.

Another enriched gene is Integrin Subunit Beta (ITGB)-6, responsible for inducing pulmonary fibrosis via TGFβ1[138], which is in turn activated by thrombin, a key enzyme in the coagulation process[139] and the platelet receptor protease-activated receptor (PAR)-1.[140] Thrombin hyperactivation has also been linked to acute lung injury[141] in addition to coagulopathies. ISG upregulation also upregulates the ACE2 receptor.[91] Thus, not only are the ACE-2 rich cells prime targets for SARS-CoV-2 entry and infection, but also form the basis for pulmonary fibrosis in COVID-19.

DYSREGULATION OF THE HOST IMMUNE CELLS

Coronaviruses activate mast cells, which release early inflammatory chemical compounds including histamines and proteases; while late activation provokes the generation of pro-inflammatory IL-1 family members including IL-1 and IL-33.[142] CD4+ T lymphocytes are rapidly activated to become pathogenic T helper (T_H) 1 cells and generate GM-CSF, etc. The cytokinetic environment induces inflammatory CD14+CD16+ monocytes with high expression of IL-6 and accelerates the inflammation. Further, type I IFNs proliferate and direct dendritic cells[143], which then regulate migration, localization, and induction of T_H2 cells.[144]

Given that large amount of inflammatory cells infiltrations have been observed in lungs from severe COVID-19 patients, these aberrant pathogenic T_H1 and T_H2 cells and inflammatory monocytes may enter the pulmonary circulation in huge

numbers and play an immune damaging role to cause lung functional disability and quick mortality. Coronaviruses are also known to activate intrinsic and extrinsic death pathways in T-cells[145], causing widespread cell death and worsening the onslaught of pro-inflammatory cytokines.

CYTOKINE PROFILE STUDIES OF COVID-19 PATIENTS

Indeed, Huang et al. show that COVID-19 patients have higher levels of proinflammatory cytokines, such as IL-1β, IL-2, IL-6 IL-7, IL-8, IL-9, IL-10, IL-18, TNFα, G-CSF, GM-CSF, IFN-γ-IP-10, fibroblast growth factor (FGF), macrophage inflammatory protein (MIP)-1α, compared to healthy individuals. Higher levels of IL-6, IL-10, and TNFα correlated with increased disease severity[146] and reduced total T-cell, CD4+, and CD8+ counts.[147] IL-6 may also suppress normal T-cell activation[148], contributing to lymphocytopenia observed in COVID-19 patients.[149] A previous Taiwanese study also showed that IFN-γ, IL-18, TGF-β, IL-6, IP-10, MCP-1, MIG, and IL-8 were highly elevated in the acute phase sera of SARS patients.[150] The role of a major player in the cytokine storm, IL-6, is discussed in the next chapter.

CHAPTER 5

ROLE OF IL-6

ACTIVATION OF IL-6

Immune system cells, such as monocytes, macrophages, B cells, T-cells, mast cells, dendritic cells, and many non-immune cells, such as fibroblasts, endothelial cells and almost all stromal cells produce IL-6.[151] Mainly, IL-1β and TNFα activate IL-6; but many other factors such as other cytokines, prostaglandins, adipokines, etc. can stimulate its release.[152]

IL-6 IN VIRAL INFECTION

IL-6 plays a crucial role in infectious diseases such as influenza; Il6-/- mice have an impaired memory CD4+ T-cell response.[153] Influenza virus persists in absence of IL-6 and causes extreme lung damage and death; whereas presence of IL-6 promotes neutrophil survival in the lung;[154] IL6-/- mice are also more susceptible to Herpes Simplex Virus-1 (HSV-1) infection.[155] Early depletion of IL-6 in Respiratory Syncytial Virus (RSV) infections in mice exacerbated the disease and caused an influx of cytotoxic CD8+ T-cells.[156]

EXCESSIVE IL-6 CAUSES PULMONARY DAMAGE

IL-6 mediates acute lung injury (ALI): in response to acid aspiration, IL-6−/− mice exhibited significant improvement of lung function, lung edema formation, and decreased lung pathologies. The authors also showed that SARS-CoV produces oxidized phospholipids (OxPL) which induce cytokine production and acute lung injury via Toll Like Receptor 4 TLR4.[157] TLR4 is associated not only with infections but also with tissue damage: TLR4-/- mice are highly resistant to influenza A infection.[158]

SARS-CoV PROMOTES IL-6 SECRETION

SARS-CoV can directly promote IL-6 secretion. The nucleocapsid protein (N) significantly activates IL-6 promoter in human airway epithelial cell cultures via the NF-κB pathway.[159] This protein is approximately 90.3% similar to SARS-CoV-2.[160] The N protein binds to the NF-kB regulatory element on the IL-6 promoter, and facilitates its translocation from cytosol to nucleus. The p65 subunit of NF-κB and the N protein then synergistically activate the expression of IL-6 gene, leading to IL-6 production.[159]

Since SARS-CoV-2 dismantles the initial anti-viral defense mechanism and induces a cytokine storm, it may lead to prolonged IL-6 secretion.[161] High viral RNA load also causes sustained IL-6 secretion[162] in critical patients, and is in turn correlated to ARDS severity.[163] Retrospective and meta-analysis studies show elevated IL-6 and C-reactive protein (CRP) correlate with mortality and severe disease in comparison to moderate disease.[164–168] Critical COVID-19 patients with severe respiratory have immune dysregulation driven by IL-6.[164]

IL-6 SIGNAL TRANSDUCTION

Binding of IL-6 to its alpha-receptor, Interleukin-6 Receptor (IL-6R), induces the signal transduction of IL-6. This binding complex activates a beta-receptor Glycoprotein (GP)-130 homodimer with high affinity, which then dimerizes and initiates intracellular signal transduction via the JAK/STAT and RAS/MAP kinase pathways.[169]

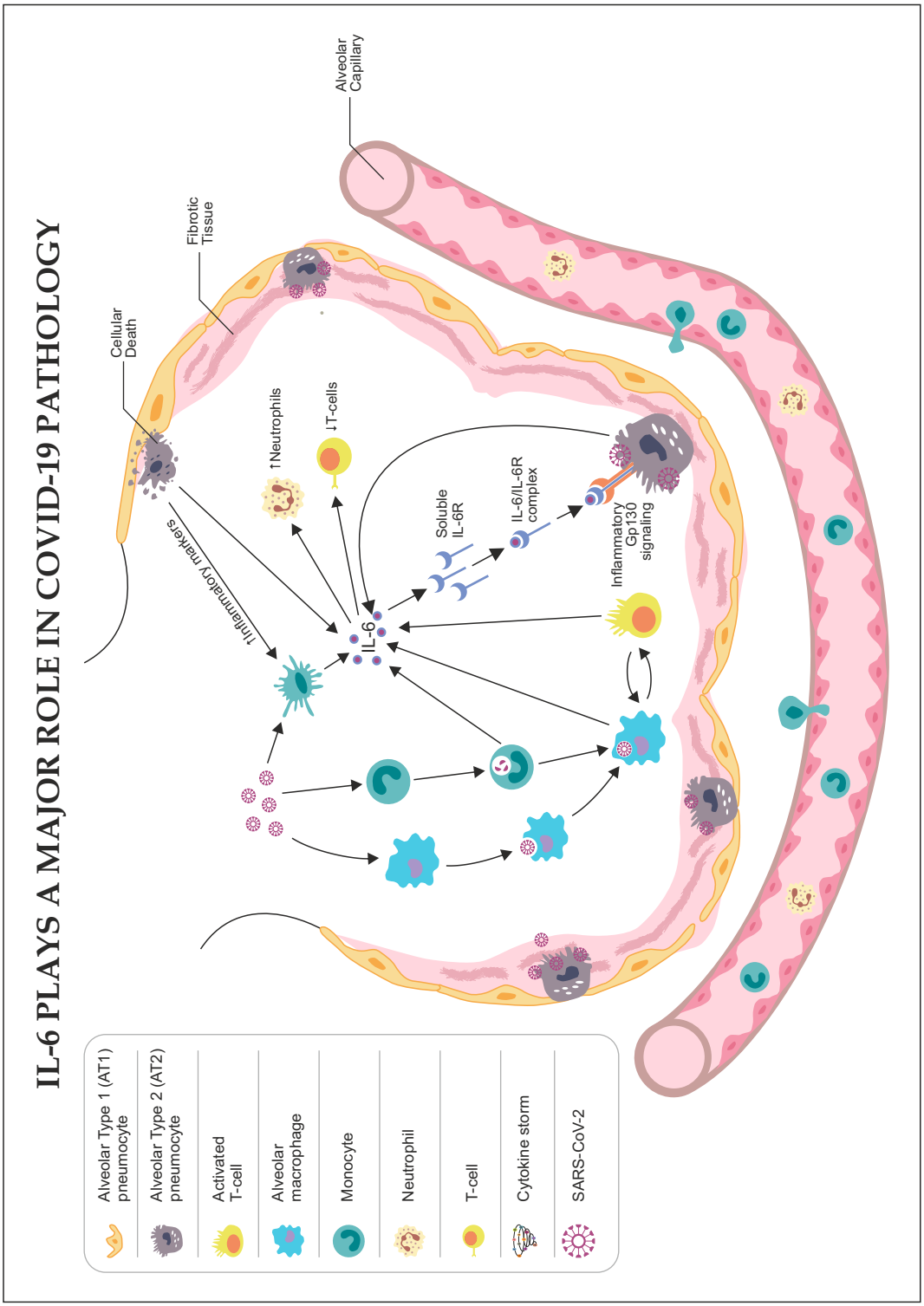

Figure 14: IL-6 is a critical player in the COVID-19 induced cytokine storm

CLASSIC AND TRANS IL-6 SIGNALING PATHWAY

IL-6 transduces signal by binding to IL-6R, which can either be membrane bound (classic pathway) or soluble (trans-signaling pathway).[170] IL-6R is cleaved proteolytically, generating a soluble IL-6R (sIL-6R). The sIL-6R binds IL-6; this complex binds GP130 even on cells that do not express IL-6R. GP130 is expressed on all cells of the human body; IL-6R is found on hepatocytes, leukocytes, and epithelial cells.[149] Theoretically, since all cells express GP130, all of them can transduce IL-6 signal via the trans pathway.[170]

Animal models show that membrane bound IL-6R (classic signaling) creates an anti-inflammatory environment.[171] Trans-signaling, however, may be involved in chronic inflammation.[172] Thus, the trans-signaling transduction might be pro-inflammatory, and the classic signaling transduction might be anti-inflammatory in nature.[173]

ACE2, RAS, AND IL-6

THE RENIN–ANGIOTENSIN SYSTEM (RAS)

ACE2 is a peptidase, and recently classified within the Renin–Angiotensin System (RAS). The RAS controls blood pressure in the mammalian system by controlling the vascular system. This family includes ACE1, which produces angiotensin II (Ang II), and ACE2, which converts Ang II to Angiotensin 1–7 (Ang1–7), Ang II receptors (Ang II receptor type 1 [AT1R] and Ang II receptor type 2 [AT2R]), and the Ang1–7 receptor. Ang1–7 is the endogenous agonist of the Mas receptor (MasR).[174–176]

ACE2 DOWNREGULATION MAY INDUCE IL-6 PRODUCTION IN ANGIOTENSIN II-DEPENDENT MANNER

ACE2 serves as the entry point for SARS-CoV-2. This disrupts the balance between the ACE2-Ang II–Ang I receptor axis and the ACE2-Angiotensin 1–7-MAS receptor axis.[177,178] Studies show that COVID-19 patients have increased Ang II compared to healthy individuals[179]; this signaling may be mediated by Ang I receptor axis.[180]

Ang II increases the expression of IL-6 significantly, in a dose-dependent manner[181] via NF-kB.[182] Plasma IL-6 concentrations also correlate with blood

pressure, plasma Ang II levels and vascular hypertrophy. Further, downstream activation of NADPH oxidase by Ang I receptor elevates oxidative stress, which directly promotes IL-6 gene expression.[183,184] IL-6 promotes cellular Ang I receptor expression; this paves the way for Ang II-dependent signaling, which may release cytokines from the activated cells, including IL-6, and promote inflammatory conditions,[185] oxidative stress and IL-6 expression. Notably, it has been shown that ACE2 prevents oxidative stress and ARDS in lethal avian influenza A H5N1 infection.[186] Moreover, it was recently reported that in COVID-19 patients with hypertension Ang receptor blockade attenuated the inflammatory response, possibly through IL-6 inhibition.[178] Therefore, IL-6 and Ang II may interact with each other via a feedback mechanism.[187] These data suggest that SARS-CoV-2-mediated ACE2 downregulation may cause Ang I receptor downstream activation of NADPH oxidase. This ACE2 downregulation may also drive Ang II accumulation. These processes lead to production of reactive oxygen species, and collectively promote IL-6 expression, worsening inflammation.[187]

RAS IN MACROPHAGES AND MICROGLIA PRODUCE IL-6

Activated macrophages exhibit either the pro-inflammatory (M1) or the anti-inflammatory (M2) phenotype.[188] M1 Macrophages, which also express RAS family members, release IL-6 when activated. RAS, particularly Ang II, has been implicated in pro-inflammatory and profibrotic pathways.[189] Generally, it is considered that inhibitors of ACE1 or antagonists of Ang II receptors could benefit patients with inflammatory diseases.[190]

Ang and Ang1–7 receptors are expressed in-as well as activate-microglia in the CNS. The neurological sequelae observed in COVID-19 patients could be mediated by the RAS system expressed in neurons and/or microglia.[191–194] The clinical presentation of the COVID-19-induced cytokine storm is discussed in detail in the next chapter.

CHAPTER 6

CLINICAL PRESENTATION

COVID-19 patients are mainly adult males. However, the entire spectrum has been affected, with confirmed pediatric cases worldwide.[195,196] Mortality is higher in adults above 65 years. Comorbidities include diabetes, pulmonary disorders, pre-existing cardiovascular diseases, cancer, endocrine disorders, diabetes, or compromised immunity.[167] Especially in comorbid patients, it may progress to interstitial pneumonia and ARDS.

SYMPTOMS AND SIGNS OF COVID-19

Severe COVID-19 patients have exorbitant serum levels of inflammatory markers like IL-6, C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), and TNFα; extremely high markers of thrombosis and injury, like procalcitonin and Fibrinogen/D-dimer levels respectively, disseminated intravascular coagulation (DIC), heavily impaired hepatic and renal function, and lymphocytopenia, and secondary hemophagocytic lymphohistiocytosis (sHLH).[146,149,197] These clinical and laboratory findings mimic those of SARS-CoV and MERS-CoV infections.[146,198] Transcriptome sequencing of the bronchoalveolar lavage fluid (BALF) cells of COVID-19 patients reveals excessive release of chemokines such as CXCL10 and CCL2[199], and indicates poor

prognosis.[200,201] Furthermore, ARDS and T-cell overactivation was evident in post mortem samples, due to an increase in T_H17 cells and CD8+ T-cells.[202] And, as discussed before, the acute-phase innate response along with the eventual adaptive response causes uncontrolled inflammation- leading to the cytokine storm.[203]

IN THE CLINIC

SARS-CoV-2 virus gains entry into the host cell by binding to the ACE-2 receptors, which are expressed abundantly on the surface of pulmonary epithelial, cells, cardiac, renal, intestinal and endothelial cells. Clinical presentation is consistent with involvement of the target organs, with predominant respiratory symptoms. Mild illness usually presents with flu-like symptoms: fever, dry cough, shortness of breath, myalgia, and headache. Other symptoms include nausea, sore throat, diarrhea, confusion, hemoptysis, loss of taste and/or smell, loss of appetite, and chest pain. Pneumonia usually occurs 1–2 weeks after the first symptom; signs include reduced oxygen saturation, abnormal arterial blood gas reports, and radiological abnormalities like multi-focal glass ground opacities, or patchy chest X-ray or CT. A majority of cases with mild and moderate illness improve with supportive treatment together with administration of antivirals. But, a subset of the population with COVID-19 disease progresses to severe illness and development of complications.

During infections, both innate and adaptive immunity are activated resulting in production of pro-inflammatory cytokines and chemokines. When in control, this serves to benefit the human body. But, in a subset of cases with impaired or failure of viral clearance (even with negative PCR test, presence of virus inclusion bodies in alveolar cells supports the possibility of impaired viral clearance) result in prolonged and exaggerated interactions between the innate and adaptive immune cells. This further may result in a cascade of events including increased macrophage activity and unrestrained secretion of pro-inflammatory cytokines resulting in cytokine storm, thrombotic tendency, ARDS, sepsis and septic shock, multi-organ failure, and even death.[146,204,205]

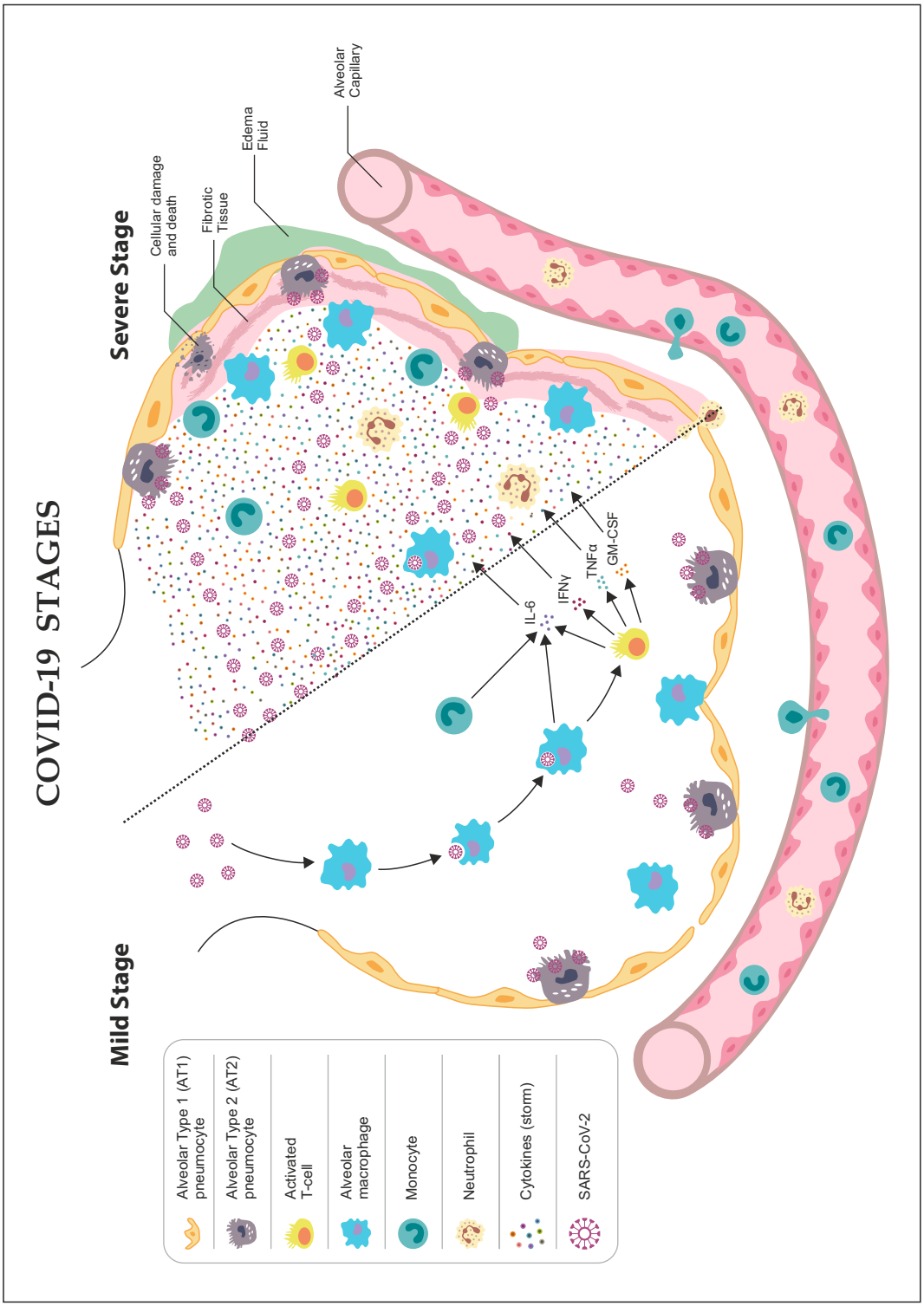

Figure 14: SARS-CoV-2 induced COVID-19 proceeds with cytokine storm, fibrosis, edema and finally death.

CLINICAL SIGNS ASSOCIATED WITH CYTOKINE STORM

1. Sustained fever;
2. Shortness of breath;
3. Chest pain and other symptoms of pneumonia;
4. Generalised weakness and extreme fatigue;
5. Confusion, and other neurological signs;
6. Signs of thromboembolism; DVT, pulmonary embolism, and stroke;
7. GI bleeding;
8. ARDS;
9. Myocardial infarction;
10. Splenomegaly;
11. Multi-organ failure-Liver failure, renal failure, cardiac failure;
12. Acute respiratory failure

The exaggerated inflammatory response results in infiltration of the lungs with inflammatory cells, mainly the monocytes, macrophages and multinucleated giant cells. This further causes alveolar edema and impaired oxygenation causing hypoxia, development of ARDS leading to pulmonary fibrosis and death.

SIGNS TO BE WATCHED IN PRE-ARDS STAGE

1. Severe lymphocytopenia. As the patient improves, it tends to normalize.
2. Increase in total leukocyte and neutrophil count
3. Increase in neutrophil/lymphocyte ratio (NLR). NLR may be used to assess treatment response as a follow-up parameter.
4. Elevated levels of pro-inflammatory cytokines including IL-6, IL-1, TNFα and IL-8.
5. Elevated D-dimer. Persistently elevated levels have been associated with a poor prognosis.

DEVELOPMENT OF DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

The infiltration of cytokines causes endothelial cell activation, vascular leakage, activation of complement and the coagulation cascade leading to DIC. This may lead to development of deep vein thrombosis (DVT) or even fatal pulmonary thromboembolism.

1. Prolongation of Prothrombin time (PT)
2. High fibrin degradation products
3. Severe thrombocytopenia

SEPSIS AND MULTI-ORGAN FAILURE

Severe cytokine storm may cause myocardial dysfunction leading to cardiomyopathy and also affection of other organs including kidneys, liver and intestine leading to multi-organ failure.

Signs of organ dysfunction include:

1. Altered mental status,
2. Difficult or fast breathing, low oxygen saturation,
3. Reduced urine output,
4. Fast heart rate/ weak pulse/ cold extremities/ low blood pressure,
5. Skin mottling, or laboratory evidence of coagulopathy, thrombocytopenia, acidosis, high lactate or hyperbilirubinemia.

SEPTIC SHOCK

Cases where hypotension is persistent despite volume resuscitation and requiring vasopressors to maintain a mean airway pressure (MAP) of ≥65 mmHg and serum lactate level of > 2 mmol/L.

In a Chinese retrospective, single-centre study, with 99 patients, 49 of whom had been exposed to the Huanan seafood market, most patients presented with flu-like symptoms on admission. Other symptoms included muscle ache, headache, confusion, chest pain, and diarrhoea. Many patients presented with organ function damage: 17% with ARDS, 8% with acute respiratory injury, 3% with acute renal injury, 4% with septic shock, and 1% with ventilator-associated pneumonia. Patients had clinical manifestations of fever (83%), cough (82%), shortness of breath (31%), muscle ache (11%), confusion (9%), headache (8%), sore throat (5%), rhinorrhoea (4%), chest pain (2%), diarrhoea (2%), and nausea and vomiting (1%). According to imaging examination, 75% patients showed bilateral pneumonia, 14% showed multiple mottling and ground-glass opacity, and one patient had pneumothorax. 17% patients developed ARDS, and among them, 11% patients worsened in a short period of time and died of multiple organ failure.[204]

Lai et al. summarized the clinical manifestations of 278 pooled patients with SARS-CoV-2 pneumonia. They report that fever was the most common symptom (92.8%; n=258), followed by cough (69.8%; n=194), dyspnoea (34.5%; n=96), myalgia (27.7%; n=77), headache (7.2%; n=20) and diarrhoea (6.1%; n=17). Rhinorrhoea, sore throat, and pharyngalgia were seen in only a few patients. 56.8% (n=158) of patients had leukopenia. Other symptoms included cough (46.3%), upper airway congestion (61.5%), myalgia (23.1%) and headache (23.1%).[205]

RADIOLOGICAL FINDINGS

COVID-19 pneumonia exhibits typical manifestations of pulmonary hyperinflammation.[206,207] On CT chest, it has a peripheral distribution, ground-glass opacity, fine reticular opacity, and vascular thickening; but less likely to have a central + peripheral distribution, pleural effusion, and lymphadenopathy.[206] Patients also show bilateral lung involvement with peripheral and subpleural lesions with diffuse distribution. Predominantly, ground-glass opacity, with ill-defined margins, air bronchograms, smooth or irregular interlobular or septal thickening, and thickening of the adjacent pleura have also been observed. Even in asymptomatic patients, COVID-19 pneumonia shows a rapid evolution from focal unilateral to diffuse bilateral ground-glass opacities that progress to or co-exist with consolidations, within 1-3 weeks.[207]

Radiological outcomes evolve as the disease progresses. Radiographs may appear normal initially. Sub-clinically, it may appear unilateral and multifocal with predominantly ground-glass opacities. Within the first week after symptom onset, lesions evolve to bilateral, diffuse disease, with pleural effusions, lymphadenopathy, a relative decrease in the frequency of ground-glass opacities and a transition to consolidation and mixed-pattern development. Throughout the second week after symptom onset, ground-glass opacities continue to decrease in frequency, while irregular interlobular/septal thickening and consolidation becomes common. Reticular patterns associated with bronchiectasis also increase progressively from the second week into the third. During resolution, fibrous stripes appear; these typically resolve after a month.[207–210]

CHAPTER 7

LABORATORY ABNORMALITIES

Inflammatory biomarkers, such as IL-6, C-reactive protein, ferritin, and lactate dehydrogenase; secondary infection markers like procalcitonin; and other measures of the cytokine storm, such as Cd3+, CD4+, and CD8+ T-cell counts, CD4/CD8 ratio, TNFα, neutrophil to lymphocyte ratio, and coagulopathy markers such as D-Dimer and Fibrinogen are discussed below.

INFLAMMATION BIOMARKERS

Inflammation causes aberrant release of various biomarkers. The most reliably tested inflammatory biomarkers include IL-6, C-reactive protein, ferritin, and lactate dehydrogenase.

INTERLEUKIN (IL)-6

Preclinical evidence suggests that IFNγ controls neutrophil trafficking in inflammatory conditions. It modulates IL-6 via the soluble receptor sIL-6R to regulate neutrophil recruitment as well as apoptosis and clearance.[211] This pathway is clinically significant: a meta-analysis by Lagunas-Rangel and Chávez-Valencia concludes that severe COVID-19 patients have a higher IL-

6/IFNγ ratio than moderate patients[212], and may be a relevant biomarker of disease severity.

Changes in IL-6 may drive the course of COVID-19. A study with sixty-nine severe COVID-19 patients indicated that significant increase in baseline IL-6 was positively correlated with the maximal body temperature during hospitalization and increased baseline of CRP, LDH, ferritin, and D-dimer. High baseline IL-6 was also associated with more progressed chest computed tomography (CT) findings. Significant decrease in IL-6 and improved CT assessment was found in patients during recovery, while IL-6 was further increased in exacerbated patients. These results suggest that the dynamic change in IL-6 can be used as a marker for disease monitoring in patients with severe COVID-19.[213]

The degree of inflammatory response in ARDS reliably dictates the outcome in acute lung injury. This degree can be measured by plasma IL-1 beta and IL-6 levels. Meduri et al. show that patients with higher plasma levels of IL-1 beta and IL-6 on day 1 of ARDS had persistent elevation of these inflammatory cytokines over time, and died. Survivors had lesser elevations on day 1 of ARDS and a rapid reduction over time.[214] A case series with 99 COVID-19 patients revealed a 52% increase in the IL-6 serum levels. Parallely, C-reactive protein (CRP) rose by 86%. Total number of neutrophils increased by about 38% while total lymphocytes decreased by 35%.[204] Concurrent to SARS-CoV-2 viral load, IL-6 levels were significantly elevated in critically ill patients-almost 10-folds higher in another study.[162] A third study showed that IL-6 levels were high enough to lose the dynamic range of measurement (i.e., two orders of magnitude increase or even higher).[215] A meta-analysis assessing 725 COVID-19 patient IL-6 reports showed that non-survivors had more significant increases in IL-6 compared to survivors.[216] Significantly greater increases were observed for IL-6 in non-survivors vs. survivors (weighted mean: 4.6 pg/mL) as compared to severe vs. non-severe form (weighted mean: 1.7 pg/mL). These elevations, along with elevated ferritin and CRP, point to development of a systemic inflammatory response syndrome (SIRS) in patients with severe COVID-19. Normal levels (for an Indian population) range from 0-7 pg/ml.

C- REACTIVE PROTEIN (CRP)

C-reactive protein (CRP) is an acute phase inflammatory protein that increases

up to 1,000-fold at infection or inflammation sites.[217] The CRP gene is transcriptionally induced in the liver in response to increased levels of inflammatory cytokines, especially interleukin-6 (IL-6).[218] It increases IL-8 and MCP-1 production.[217]

Wang et al. show that in the early stage of COVID-19, C-reactive protein levels can reflect the extent of lung lesions and disease severity.[219] A meta analysis by Sahu et al. with 1896 survivors and 849 non-survivors cases showed that CRP concentrations remained high in patients who died of COVID-19 infection.[220] A clinical trial (NCT04373798) is in progress to determine the potential of CRP as a triage assessment tool.[221] Normal levels (for an Indian population) range from 0-6 mg/L.

FERRITIN

Ferritin is an evolutionarily conserved iron storage protein, found in almost all cells. It has a protein shell that encloses a ferric iron core, to be used for haem synthesis.[222]

Liu et al. analyzed the peripheral blood of 69 patients with severe COVID-19, and found elevated levels of ferritin compared with patients with non-severe disease.[223] In a study with 20 COVID-19 patients, it was found that individuals with severe and critical COVID-19 exhibited increased serum ferritin level, and significant differences between the two stages.[224] In another case series with 99 patients, 63 had extremely high serum ferritin levels.[204] Another study revealed that ferritin levels were high in patients who died by COVID-19 from admission to death, with the median values of serum ferritin levels exceeding the upper detection limit.[167] Finally, elevated ferritin levels were found in autopsies of 12 COVID-19 patients.[225] Given the data above, it may be concluded that serum ferritin levels are closely related to the severity of COVID-19. Normal levels (for an Indian population) range from 30-220 ng/ml for males 20 -110 ng/ml for females.

LACTATE DEHYDROGENASE (LDH)

Lactate Dehydrogenase (LDH) is a housekeeping protein catalyzes the cyclical conversion of lactate to pyruvate.[226] LDH has high diagnostic value for early recognition of lung injury and severe COVID-19 cases, as it has maximum sensitivity (100.00%) and specificity (86.67%).[227]

Poggiali et al. correlated LDH levels with patients' respiratory function evaluated by the partial pressure of arterial oxygen to fraction of inspired oxygen ratio (PaO2/FiO2). The respiratory performance (PaO2/FiO2) showed a strong inverse correlation with LDH in 123 consecutive COVID-19 patients.[228] Wu et al. demonstrate that at diagnosis, significant differences exist in LDH levels between non-severe and severe COVID-19 patients. Increase or decrease of LDH was significantly associated with worsening or improvement on chest CT scans.[229] A pooled analysis of 1532 COVID-19 patients evaluated the association between elevated LDH levels measured at earliest time point in hospitalization and disease outcomes in COVID-19 patients. Elevated LDH levels increased the odds of developing severe disease ~6-fold and increased the mortality odds ~16-fold of in patients with COVID-19.[230] The normal range (for an Indian population) lies between 230-450 U/L.

SECONDARY INFECTION MARKERS

PROCALICITONIN (PCT)

Assicot et al. were the first to show that procalcitonin concentrations predict infectious complications, acute septic episodes, and severity of microbial invasion.[231] Procalcitonin is a 116 amino acid protein produced in thyroid C cells. In healthy subjects, it is further modified to calcitonin; PCT serum level in healthy subjects is very low (0.05 ng/mL). But, during inflammation PCT is released as is: induced by toxic metabolites from microbes such as lipopolysaccharides, or by various inflammatory mediators like IL-6, TNFα, etc. It is a reliable marker of sepsis.[232]

A study by Hu et al. showed that mean serum PCT levels were approximately four times higher in severe patients than in moderate patients, and approximately eight times higher in critical patients than in moderate patients. This severity-dependent PCT level elevation was similar to co-infection rate in patients with moderate disease severity (~10%). However, this linearity was lost in severe and critical patients: the co-infection rate was only 20% in severe patients, with 50% PCT elevation; and 50% in critical patients, with 80% PCT level elevation.[233] A meta-analysis by Lippi and Plebani shows that increased procalcitonin values are associated with almost 5-fold higher risk of disease severity (OR, 4.76; 95% CI, 2.74–8.29) with a modest 34% heterogeneity.[234] The normal range (for an Indian population) is <0.1 ng/ml.

OTHER MEASURES

Cd3+, CD4+, AND CD8+ T-CELL COUNTS

Cd3 is a biomarker of mature T lymphocytes, which activate the CD4+ and CD8+ T-cells[235] CD4+ and CD8+ T-cells are effector cells that regulate the stability of the immune system.[235] A study with 69 severe and 135 non-severe patients showed that the decrease of Cd3+, CD4+ and CD8+ T lymphocyte subsets significantly declined in severe patients compared to the non-severe group.[237] Another study with 154 COVID-19 patients divided into moderate group, severe group and critical group showed that CD3+, CD4+ and CD8+ T lymphocyte subsets were decreased in COVID-19-infected patients. Importantly, compared with the moderate group and the severe group, CD3+, CD4+ and CD8+ T-cells in the critical group were significantly lower.[238] Further, in a study with 103 COVID-19-infected patients and 22 non-COVID-19 pneumonia cases, the count for lymphocyte subsets-but not that for neutrophils and monocytes-exhibited a significant negative correlation with organ injury, specifically in the COVID-19 infected patients.[239] Finally, a multicenter retrospective cohort study analyzed data from the Early Risk Stratification of Novel Coronavirus Pneumonia (ERS-COVID-19) study (ChiCTR2000030494). The authors report that after adjusting for confounding factors, leukocyte count, neutrophil count, CD3+ T-cell count, CD4+ T-cell count, CD8+ T-cell percentage, and CD8+ T-cell count remain independent prognostic factors among others. The patient's leukocyte, neutrophil, lymphocyte counts, CD3+, CD4+, and CD8+ T-cell counts had strong predictive values for in-hospital mortality, organ injury, and severe pneumonia. Decreased lymphocyte subsets and increased neutrophils were independently associated with high risks of mortality and organ injury, respectively.[240]

CD4/8 RATIO

In one study, total lymphocytes, T-cells, B cells, and NK cells were significantly lower in COVID-19 patients as compared to healthy subjects, suggesting a correlation between lymphocyte subset alteration and the pathogenesis of SARS-CoV-2. CD4/8 ratio was also significantly higher in the ICU patients compared to non-ICU patients. The authors predict that significantly lower CD8+ T-cells and higher CD4/8 ratio seen in ICU patients indicate that CD8+ T-cells are reduced to a greater extent than CD4+ T-cells, and could be a predictor

of disease severity.[241] In another study assessing peripheral blood samples of 25 COVID-19 patients and 25 normal individuals, there was no significant difference in the ratio of CD4 to CD8 between two groups. However, the expression level of CD8 in COVID-19 patients was significantly higher than the normal individuals. They hypothesize that the cellular immune responses triggered by COVID-19 infection were developed through overexpression of CD8 and hyperactivation of cytotoxic T lymphocytes.[242]

TUMOR NECROSIS FACTOR (TNF)-α

TNFα, one of the most potent pro-inflammatory cytokines, was found to be elevated in several studies with COVID-19 patients.[146,243,244] Plasma concentrations of TNFα (as also IL-2, IL-7, IL-17, IL-10, MCP-1, and MIP-1A) in ICU patients are higher than non-ICU patients.[146] Moreover, the plasma levels of TNFα (and IL-2, IL-6, IL-8, and IL-10) observed in severe cases are prominently greater than those in non-severe cases.[149]

NEUTROPHIL TO LYMPHOCYTE RATIO (NLR)

Severe cases of COVID-19 tend to have higher neutrophil to lymphocyte ratio (NLR). NLR is calculated from a routine complete blood count: dividing the absolute neutrophil count by the absolute lymphocyte count yields the NLR. It indicates a patient's overall inflammatory status. Increasing NLR is a risk factor of mortality not only in infectious diseases but also in malignancy, acute coronary syndrome, intracerebral hemorrhage, polymyositis, and dermatomyostis.[245]

COAGULOPATHIES

In patients with COVID-19, DIC develops; it is characterized by elevated levels of fibrin degradation products, and severe thrombocytopenia, which may be life-threatening.[246]

COVID-19 patients have a propensity towards thrombosis. This could be due to several factors: damage due to viral invasion, endothelial cell activation, elevated plasma levels of cytokines and immunoglobulins, mechanical damage due to ventilation and vascular interventions etc. This may lead to higher blood viscosity, and may even lead to deep vein thrombosis or lethal pulmonary thromboembolism.[247] Further, severe COVID-19 patients have ischemic changes

in the fingers and toes mimicking vasculitis.[248] Monitoring coagulation thus is essential for COVID-19 patients. This is done by monitoring the levels of Fibrinogen and D-dimer, discussed below.

FIBRINOGEN

COVID-19 patients with ARDS show a procoagulant pattern. Fibrinogen, an acute phase protein, is synthesized in high quantity by the liver in response to IL-1 and IL-6 and is routinely used for monitoring DIC.[249] Han et al.[250] investigated the changes in blood coagulation of COVID-19 patients compared to healthy controls. The level of fibrinogen and its degradation products are significantly higher in COVID-19 patients compared to healthy controls. Additionally, the levels were higher in critical COVID-19 patients compared to mild or moderate cases.[250] However, in another study, fibrinogen was reported to be non-significant between surviving and non-surviving COVID-19 patients.[251] This suggests that fibrinogen levels are expected to be higher in hospitalized patients; however, it might not reliably predict mortality in COVID-19 patients. Fibrinogen and D-dimer levels should thus be considered together to evaluate DIC in COVID-19.[252]

D-DIMER

D-dimer is a product of fibrinolysis, a by-product of the conversion of fibrinogen to fibrin. Fibrin contains two D-fragments of joined by a cross-link (factor XIII). Breakdown of fibrin yields D-dimer in systemic circulation. D-dimer presence within the peripheral circulation correlates directly with the fibrin that undergoes lysis; however, it does not specify the site(s) of thrombus. Fibrinogen degradation products including D-dimer activate platelets[253,254], which contributes to DIC. Severe COVID-19 patients show consistent elevation of D-dimer levels (reviewed in great detail by Becker).[255] The normal range lies between 0-500 ng/ml (for an Indian population).

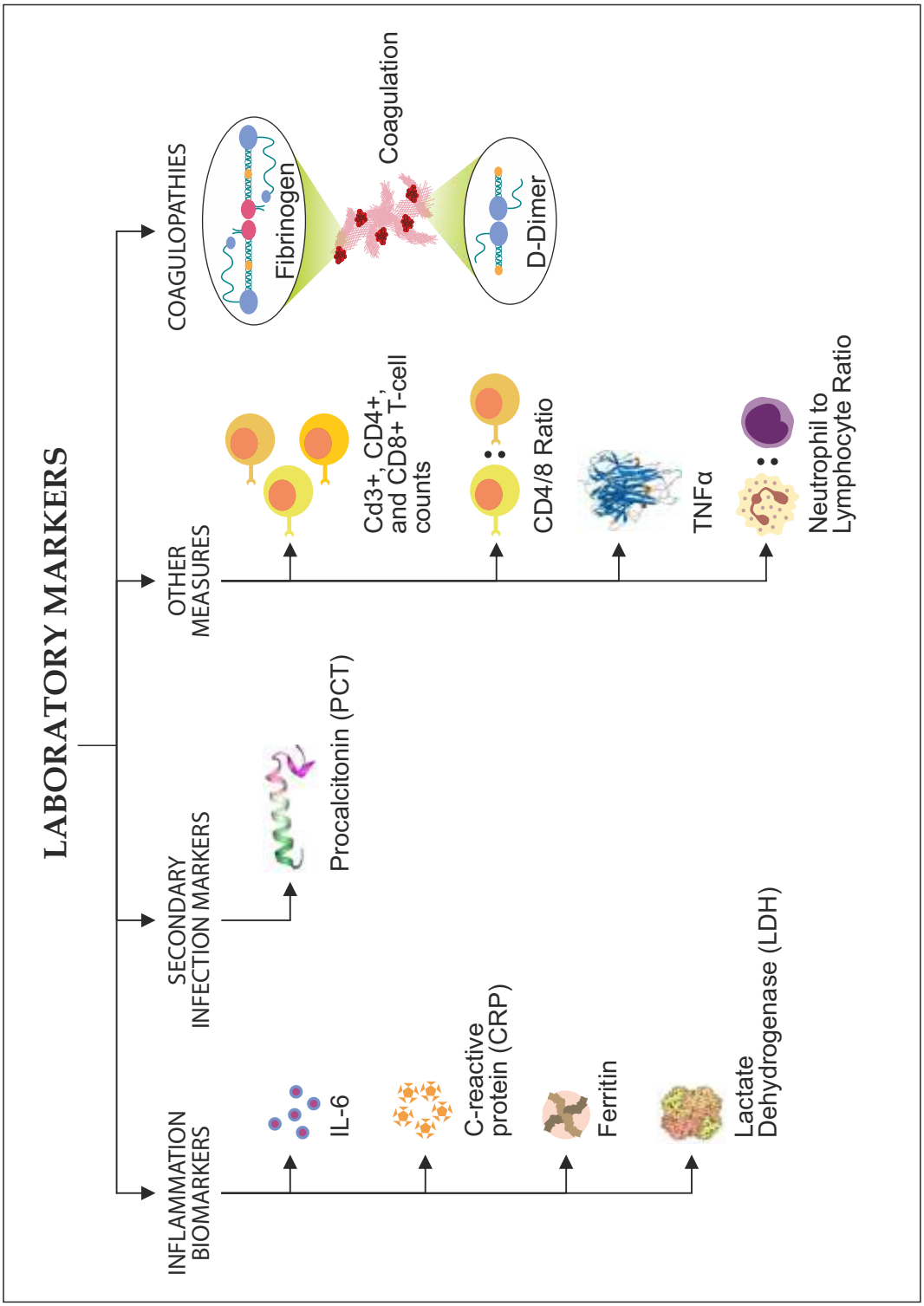

Figure 15: Various biomarkers for monitoring the COVID-19 cytokine storm.

LABORATORY MARKERS AS PREDICTORS OF THE CYTOKINE STORM

In mice models infected with SARS-CoV, robust viral replication delayed IFN-I signaling. Delayed IFN-I signaling causes furious accumulation of pathogenic inflammatory monocytes and macrophages, thus worsening the cytokine storm, promoting vascular leakage, and impairing virus-specific T-cell responses. This exacerbates inflammatory responses and lung pathology, and diminishes survival. Early IFN-I administration ameliorates immunopathology, even though IFN-I persists after peak viral titres. Genetic ablation of the endogenous IFN-I receptor Interferon-α/β receptor (IFNAR) or depletion of macrophages and monocytes protects mice from lethal infection, without affecting viral load. Thus, IFN-I as well as monocytes and macrophages promote lethal SARS-CoV infection and thus may potentially be used as early indicators to predict the COVID-19 cytokine storm.[256]

Further, changes in IL-6 may drive the course of COVID-19 as discussed before. Significant increase in baseline IL-6 is positively correlated with an increased baseline of CRP, LDH, ferritin, and D-dimer.[213] Severe COVID-19 patients also have a higher IL-6/IFNγ ratio than moderate patients.[212] Additionally, in the early stage of COVID-19, CRP levels can reflect the extent of lung lesions and disease severity.[219] Serum ferritin levels are also closely related to the severity of COVID-19.[167,204,223–225] LDH recognizes lung injury and disease severity early in COVID-19 cases, as it has maximum sensitivity (100.00%) and specificity (86.67%).[227] These markers can thus be used as predictors for the cytokine storm, and advice the course of future treatment. However, although excessively generated, PCT is not reliably associated with disease severity and cannot be used as a predictor of cytokine storm.[233]

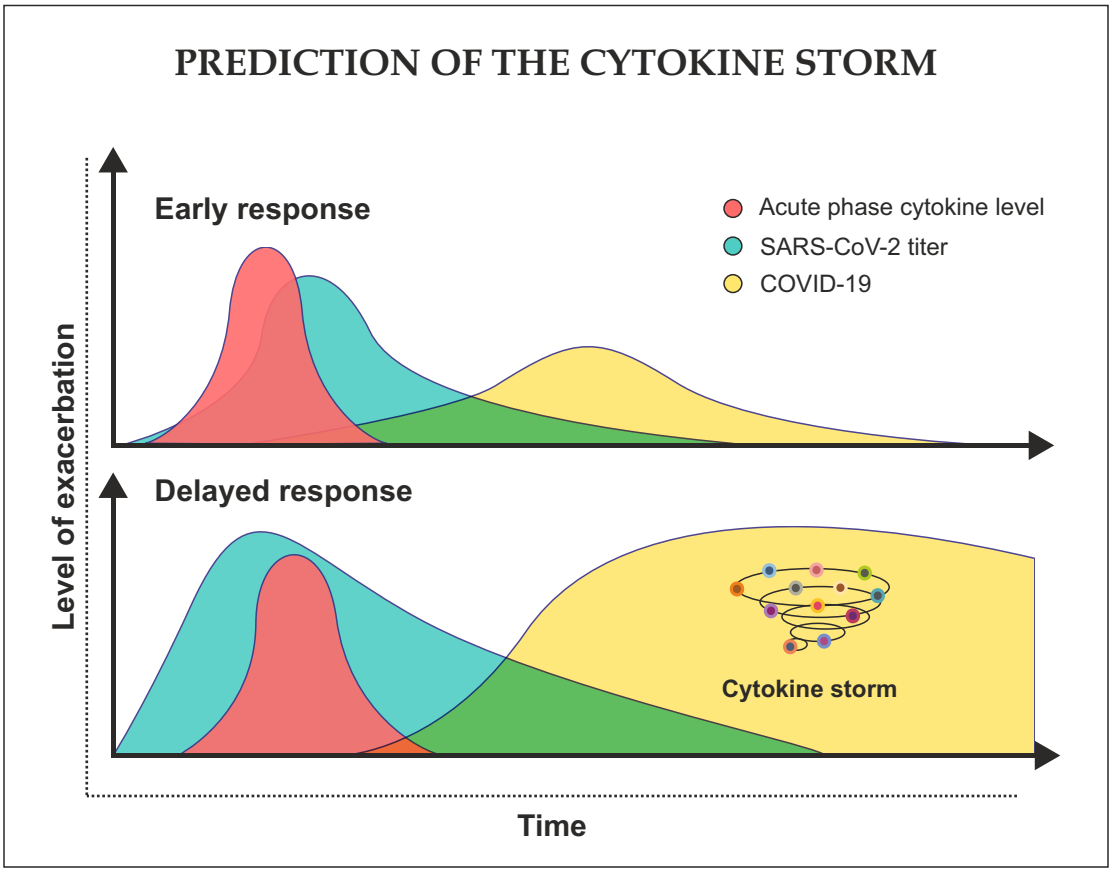

Figure 16: Acute phase cytokines like IFN-I may be strong predictors of the cytokine storm.

CHAPTER 8

MANAGEMENT OF THE CYTOKINE STORM

Treatment of immunological sequelae like including cytokine storm is essential during COVID-19.[256] Post viral infection, a normal antiviral immune response triggered through activation of the inflammatory pathways is required. But, dysregulated or an exaggerated immune response can cause severe disease if it remains uncontrolled. Early recognition of cytokine storm and prompt treatment can mitigate some of the complications and lead to a better outcome. In addition to the antivirals and standard supportive management, immune modulation is thus necessary in severe cases of the disease. Currently, no specific cure exists for COVID-19. The possible agents that can be used to target the hyper inflammation are corticosteroids like methylprednisolone and dexamethasone, IL-6R antagonists like tocilizumab (TCZ) and itolizumab, IL-1 antagonists like anakinra, TNF inhibitors, Janus kinase inhibitors like baricitinib, and CCR5 inhibitors.

CORTICOSTEROIDS

Corticosteroids like methylprednisolone and dexamethasone are anti-inflammatory agents and can effectively suppress the hyper inflammatory responses. Corticosteroids inhibit the action of most cytokines through an in

vivo feedback system that is aimed at achieving a balance between the host defense and the anti-inflammatory systems of the body. The anti-inflammatory action of the corticosteroids is mediated through the glucocorticoid receptor complex. Corticosteroids bind to the receptor complex and inhibit the activity of key pro-inflammatory transcription factors such as Activator Protein (AP)-1 and NF-κB, which inhibit IL-2 and IL-6 transcription.[257–259] Administration of corticosteroids leads to a shortened life of the mRNAs of IL-6, GM-CSF and IFNβ. Cytokines work in cascades and hence, administration of corticosteroids can block production of the next cytokines.

Timely use of corticosteroids can avoid the need for invasive mechanical ventilation and improve the outcomes of critically ill COVID-19 patients. The EVMS medical group recommends administration of corticosteroids in cases with progressive respiratory symptoms, or where patients demonstrate hypoxia requiring oxygen support of ≥ 4 L/min.

A study including 26 patients with severe illness showed that the use of methylprednisolone correlated with shorter oxygen use duration and improvement in radiographic signs.[260] Use of corticosteroids in ARDS has shown to reduce mortality and mechanical ventilation.[261] It has also been associated with faster resolution of shock.[262]

CORTICOSTEROIDS IN USE

1. DEXAMETHASONE

Indicated in moderate to severe COVID 19 cases.

DOSE

Inj. Dexamethasone 0.1 – 0.2 mg/kg for 3-5 Days in moderate COVID-19 cases
Inj. Dexamethasone 0.2 – 0.4 mg /kg for 5-7 Days in severe COVID-19 cases

CONTRAINDICATION

i. Allergy to the drug,
ii. Severe Hyperglycemia

2. METHYLPREDNISOLONE

Indicated in COVID-19 patients with moderate or severe pneumonia (indication of raised inflammatory markers)

DOSE

Inj. Methylprednisolone 0.5 -1 mg/kg in moderate cases.
Inj. Methylprednisolone 1-2 mg/kg for 5-7 days in severe cases.

CONTRAINDICATION

i. Liver abnormalities,
ii. Kidney abnormalities,
iii. High uncontrolled diabetes,
iv. Pressure Glaucoma,
v. Insufficiency of the hypothalamus and pituitary gland,
vi. High cholesterol,
vii. Low amount of potassium in the blood,
viii. A reduction in the body's resistance to infection.

SIDE EFFECTS

i. Fragile skin,
ii. Dry scaly skin,
iii. Rashes,
iv. Increased appetite,
v. Loss of muscle mass,
vi. Hyperglycaemia
vii. Secondary bacterial and fungal infections

IL-6 ANTAGONISTS

As discussed in the Chapter "Role of IL-6", the cytokine storm in COVID-19 infections is precipitated by IL-6. IL-6 is thus a prime candidate for managing the deleterious effects of the cytokine storm.

1. TOCILIZUMAB (TCZ)

Tocilizumab (TCZ) is an anti-inflammatory humanized anti-IL-6 receptor antibody that inhibits IL-6. It is currently used for a variety of inflammatory diseases: rheumatoid arthritis, temporal arteritis, and many other autoimmune rheumatic diseases[263] as well as the cytokine storm.[264] Several studies report improved laboratory biomarkers and disease outcomes with TCZ treatment.[265–267] This has been our experience as well.

Several clinical trials are ongoing to assess blockage of various IL-6 pathway components in treating COVID-19. The CORIMUNO-TOCI (NCT04331808) trial is assessing the effects of tocilizumab versus placebo in patients with severe COVID-19 pneumonia not requiring mechanical ventilation.[268] A phase 2, randomized, double-blind clinical trial (NCT04380961) is evaluating the effect of sirukumab, another antibody targeting IL-6 in severe or critical COVID-19, administered as a single intravenous dose compared to placebo in addition to standard care.[269] An IL-6 receptor inhibitor sarilumab is also being assessed for efficacy in adult patients with severe or critical COVID-19 (NCT04327388) in another ongoing trial. Anakinra (IL-1 blocker), tocilizumab, and siltuximab (both IL-6 blockers) in different combinations or alone is being assessed for improved clinical outcomes in a large clinical trial (NCT04330638).

However, Canziani et al. report that intravenous TCZ is not effective in treating COVID-19 in a multicenter retrospective case-control survival analysis of 128 patients. Sixty-four adult patients with COVID-19 on respiratory support, with elevated C-reactive protein, received intravenous TCZ in addition to standard care. Sixty-four control patients did not receive TCZ, and were matched for sex, age and respiratory support. Intravenous TCZ was not associated with reduced 30-day mortality in severe COVID-19 patients; although, the need for invasive ventilation was less compared to the control group.[270] As mentioned before, we have positive experience with TCZ, consistent with all the other studies discussed.

INDICATIONS AND SPECIAL CONSIDERATIONS BEFORE USE

i. Presence of raised inflammatory markers (e.g., CRP>20 mg/L, Ferritin>300 ng/ml, IL-6>20 pg/ml)
ii. Check PCT level, which should be less than 0.5 ng/ml.
iii. Active infections and Tuberculosis should be ruled out before use.

iv. Patients should be carefully monitored post TCZ for secondary infections, fungal infections, persistent fever 101F, neutropenia and leukocytosis.
v. As TCZ is an IL-6 receptor inhibitor, repeat IL-6 levels within 24 hours are expected to rise.

CONTRAINDICATIONS

i. Liver problems,
ii. Abnormal liver function tests,
iii. Tuberculosis,
iv. Bacterial infection,
v. Opportunistic fungal infection,
vi. Pneumonia with a fungus called Pneumocystis jirovecii,
vii. Cancer or malignancy,
viii. Demyelination

DOSE

8mg/kg (maximum 400 mg at one time) given slowly in 100 ml NS over 1 hour; dose can be repeated once after 12 to 24 hrs.

SIDE EFFECTS

The most common adverse effects of TCZ in clinical studies were:

i. Respiratory tract infections,
ii. Headaches,
iii. Hypertension,
iv. Liver injury,
v. Injection site reactions (rash, redness, swelling, itching),
vi. Fungal infections.

2. ITOLIZUMAB

Itolizumab is a humanized anti-CD6 monoclonal antibody of immunoglobulin G1 (IgG1) isotype. It binds to domain 1 of CD6 found on T-cells. CD6 is a glycosylated membrane protein that plays a role in T-lymphocyte proliferation,

adhesion, differentiation and survival. Itolizumab down regulates the phosphorylation of intracellular proteins involved in the activation of CD6 and reduces IFN-γ, IL-6 and TNFα production.[271] A multi-centric, open label, randomized, controlled trial of itolizumab in COVID-19 patients with moderate to severe ARDS in India has demonstrated safety and efficacy of the drug in prevention of cytokine storm (BIOCON).

INDICATIONS

i. Patients with moderate to severe ARDS, or more than 25% deterioration;
ii. Baseline serum ferritin level ≥400ng/mL or IL-6 levels, in patients, greater than 4 times the upper limit of normal (ULN)

CONTRAINDICATIONS

i. Patients having a history of severe allergy or known hypersensitivity reaction to any component of Itolizumab or any murine proteins,
ii. Active infections or sepsis,
iii. The studies in paediatric population <18 years old has not been done to study the safety and efficacy of Itolizumab,
iv. Patients having hepatic and renal impairment,
v. Nursing mothers & pregnancy,
vi. Severe allergic reactions to mAbs,
vii. Active tuberculosis (TB) infection or a history of inadequately treated or latent tuberculosis,
viii. Oral intake of anti-rejection or immune-suppressive drugs in the past 6 months,
ix. A known history of Hepatitis B, Hepatitis C or HIV,
x. Absolute neutrophils count (ANC) <1000/mm3, platelet count <50,000/mm3 and absolute lymphocyte count (ALC) <500/mm3

DOSE

Administration of First IV infusion 1.6 mg/kg in 250 mL of 0.9% normal saline at 50 ml/hour; subsequent infusions at 0.8 mg/kg dose weekly could be completed over 3–4 hours (max 4 doses) only if the first infusion is well tolerated.

INTERLEUKIN (IL)-1 INHIBITORS

As discussed before, IL-1 plays a major role in the SARS-CoV-2-induced cytokine storm. Anakinra, a recombinant IL-1R antagonist (rHIL-1Ra), blocks the binding of IL-1α as well as IL-1β to IL-1R.[272] In a phase 3 randomized clinical trial, it has already been found to be beneficial in severe sepsis without significant adverse effects.[273] In a study with 29 COVID-19 patients with ARDS on non-ICU non-invasive mechanical ventilation treated with high-dose anakinra showed clinical improvement in 72% of the patients and a robust safety profile.[274] In another study by Huet et al., subcutaneous anakinra reduced the need for invasive mechanical ventilation in the ICU as well as mortality in severe COVID-19 patients without side effects.[275]

Another IL-1 blocker, canakinumab, is a high affinity, fully humanized, monoclonal anti-IL-1β antibody with IgG1/κ isotype.[272] In a small retrospective analysis, 10 severe COVID-19 patients with hyperinflammation were treated with canakinumab. Patients showed rapid improvement of the inflammatory response and resolution of the hypoxemia.[276] A phase 3 clinical trial (NCT04362813) is currently assessing the efficacy of canakinumab in severe COVID-19.

TNFα INHIBITORS

TNFα is a major proinflammatory cytokine as discussed previously. Anti-TNF agents are commonly used for inflammatory conditions, including rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis. High TNFα levels serum levels have been observed in patients with COVID-19 infection, with a positive correlation with disease severity.[256,277] A clinical trial evaluating the effects of the TNFα blocker adalimumab in COVID-19 infection is currently ongoing (ChiCTR2000030089).

JANUS KINASE INHIBITORS

A major pathway that exacerbates proinflammatory cytokine production and systemic inflammation in COVID-19 is the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway.[278] The adaptor-associated protein kinase 1 (AAK1), a member of the Janus kinase (JAK) family, plays a vital role in viral endocytosis. Barcitinib, a high affinity AAK1 inhibitor, has been the first to be considered due to its relative safety.[279] However, inhibition of IFN production as a result of concomitant JAK-STAT pathway blockage may cause impairment of anti-viral immunity.[280] Two clinical trials (ChiCTR2000029580 and ChiCTR2000030170) are investigating the role of JAK

and AAK1 inhibition in COVID-19 patients.

C-C CHEMOKINE RECEPTOR TYPE (CCR)-5 INHIBITION

C-C chemokine receptor type (CCR)-5 is expressed on the surface of CD4+ T-cells. It modulates migration of macrophages to the site of inflammation and induces release of inflammatory cytokines. A phase 2 trial is currently evaluating a CCR5-inhibitor leronlimab in severe COVID-19 for reducing mortality (NCT04347239).[281] Leronlimab is a monoclonal antibody and is also being evaluated for treatment of HIV (CytoDyn).

Table 1 summarizes all the agents to manage the COVID-19 induced cytokine storm, their targets, and their dosage discussed in this chapter.

Table 1: Summary of the agents that may help manage the COVID-19 induced cytokine storm.

Sr. No.	Name	Target	Dose
1	**CORTICOSTEROIDS**		
	Dexamethasone	Pro-inflammatory transcription factors such as Activator Protein (AP)-1 and NF-κB	Inj. Dexamethasone 0.1 – 0.2 mg/kg for 3-5 Days in moderate COVID-19 cases Inj. Dexamethasone 0.2 – 0.4 mg/kg for 5-7 Days in severe COVID-19 cases
	Methylprednisolone	Pro-inflammatory transcription factors such as Activator Protein (AP)-1 and NF-κB	Inj. Methylprednisolone 0.5 - 1 mg/kg in moderate cases. Inj. Methylprednisolone 1-2 mg/kg for 5-7 days in severe cases.

Sr. No.	Name	Target	Dose
2	**IL-6 ANTAGONISTS**		
	Tocilizumab (TCZ; anti-inflammatory humanized anti-IL-6 receptor antibody)	Inhibits IL-6	8mg/kg (maximum 400 mg at one time) given slowly in 100 ml NS over 1 hour; dose can be repeated once after 12 to 24 hrs.
	Sirukumab (monoclonal antibody)	Inhibits IL-6	NCT04380961 is evaluating the safety and efficacy of this antibody for treating COVID-19
	Sarilumab (monoclonal antibody)	IL-6 receptor inhibitor	NCT04327388 is evaluating the safety and efficacy of this antibody for treating COVID-19
	Itolizumab (humanized anti-CD6 monoclonal antibody)	Downregulates the phosphorylation of intracellular proteins involved in the activation of CD6 and reduces IFN-γ, IL-6 and TNFα production	Administration of First IV infusion 1.6 mg/kg in 250 mL of 0.9% normal saline at 50 ml/hour; subsequent infusions at 0.8 mg/kg dose weekly could be completed over 3–4 hours (max 4 doses) only if the first infusion is well tolerated.

Sr. No.	Name	Target	Dose
3	**INTERLEUKIN (IL)-1 INHIBITORS**		
	Anakinra (recombinant IL-1R antagonist [rHIL-1Ra])	Blocks the binding of IL-1α as well as IL-1β to IL-1R	Under investigation by multiple trials (refs 273-275)
	Canakinumab (a high affinity, fully humanized, monoclonal anti-IL-1β antibody)		A phase 3 clinical trial (NCT04362813)is currently assessing the efficacy of canakinumab in severe COVID-19
4	**TNFα BLOCKERS**		
	Adalimumab (monoclonal antibody)	Blocks TNFα	ChiCTR2000030089 is evaluating the safety and efficacy of this antibody for treating COVID-19
5	**JANUS KINASE INHIBITORS**		
	Barcitinib (high affinity AAK1 inhibitor)	Adaptor-associated protein kinase 1 (AAK1), a member of the Janus kinase (JAK) family	ChiCTR2000029580 and ChiCTR2000030170 are investigating the role of JAK and AAK1 inhibition in COVID-19 patients
6	**C-C CHEMOKINE RECEPTOR TYPE (CCR)-5 INHIBITORS**		
	Leronlimab (monoclonal antibody)	C-C chemokine receptor type (CCR)-5 is expressed on the surface of CD4+ T-cells	A phase 2 trial is currently evaluating leronlimab in severe COVID-19 for reducing mortality (NCT04347239).

CHAPTER 9

NOVEL APPROACHES TO CURB THE CYTOKINE STORM

Several novel approaches are being investigated to curb the cytokine storm in COVID-19. The most promising therapies include ozone therapy and stem cell therapy. The following sections discuss what these approaches are, their mechanism of action to counteract the cytokine storm and ongoing clinical trials investigating their use in COVID-19.

OZONE THERAPY

WHAT IS OZONE?

Ozone is a naturally occurring triatomic molecule. Medical Ozone has long been used for treating various chronic diseases like infected wounds, circulatory disorders, geriatric conditions, macular degeneration, viral diseases, rheumatism/ arthritis, cancer, severe acute respiratory syndrome and AIDS.[282-285]

Many preclinical studies have shown the efficacy of ozone therapy in improving lung and peripheral tissue oxygenation and exchange of gases. Peripheral vasodilatation mediated by nitric oxide modulation and enhanced glycolysis in

erythrocytes produce more ATP and secondary higher 2,3 Diphosphoglyceric Acid levels (2,3-DPG) improves oxygenation via the Bohr effect. 2,3-DPG is a critical mediator of oxygen transfer from hemoglobin to tissues.

Further, ozone improves respiratory functions in disorders like asthma[286] and COPD.[287] Ozone therapy attenuates viral infections like HPV-16[288], HBV[289–292], HIV[293], and HCV infections.[294] There are multiple ongoing trials exclusively to test the efficacy of ozone therapy as an adjuvant in novel corona virus, discussed ahead.

HOW IT WORKS AGAINST THE CYTOKINE STORM

ANTI-INFLAMMATORY ACTION OF OZONE

Ozone increases the production of anti-inflammatory cytokines like TGFβ, IGF-1, IL-4, IL-10, and IL-13 and the anti-inflammatory pathway of IL-6 (classical signaling). It also reduces the pro-inflammatory profiles of TNFα, IL-1, IFN-β, IFN-γ[295], IL-8 and IL-12.[296]

Further, Bocci et al. show that ozone therapy activates the anti-inflammatory pathways of classical pro-inflammatory cytokines, including Interleukins (IL-1β, IL-2, IL-6), TNFα, Interferons (IFN-β and IFN-γ), and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF).[297] Interferons and Interleukins have important antiviral, antiproliferative, and immunomodulatory activities,[298,299] especially in the context of respiratory diseases.[300] An anti-inflammatory cytokinetic balance is maintained by TNFα and IL-1β in ARDS by dampening of the inflammatory response, despite an initial pro-inflammatory surge in the acute phase cytokines.[301] These also induce the classical pathway of IL-6, a potent anti-inflammatory mechanism, which in turn downregulates inflammatory responses.[302] GM-CSF renders dendritic cells 'tolerogenic', i.e. induces the inherent tolerance mechanisms of the dendritic cells instead of rendering them immunogenic. This further facilitates T-cell-mediated tolerance.[303]

GERMICIDAL EFFECTS OF OZONE:

Ozone is an effective adjuvant to standard care for COVID-19 patients, as it increases the overall oxygen levels of the body, in addition to its antioxidant and germicidal activity, reviewed extensively by Martínez-Sánchez et al.[304]

It has been shown to inhibit viral life cycles in lipid-enveloped viruses by peroxidation of viral lipoproteins and glycoproteins. This interferes in host-virus contact, mitigating virulence.[305,306] It may also oxidize free viral components, reducing viral load.[307] Ozone therapy has shown attenuation of viral infections like herpes by shortening the prescription course while relieving pain in hepatitis B[289–292] and HIV[293] infections, and reducing viral load dramatically in HCV infections.[294]

ANTIOXIDANT EFFECTS OF OZONE

Low-dose ozone generates a mild, transient eustress that induces the potent antioxidant activator nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 acts in multiple ways to combat oxidative microenvironments: a) it prevents RNA polymerase II from engaging in pro-inflammatory cytokines IL-6 and IL1β transcription[308]; b) it induces heme oxygenase-1 (HO-1), a protective enzyme, together with heat-shock proteins like HSP60, HSP70 and HSP90[309]; c) It engages in bidirectional crosstalk with the NF-κB pathway, cross-regulating in positive and negative feedback mechanisms[310]; and, d) it modulates over 200 Antioxidant Responsive Element (ARE) genes[311] like glutathione peroxidase (GPx), glutathione-s-transferase (GSTr), NADPH-quinone-oxidoreductase (NQO-1), and superoxide dismutase (SOD). These yield free radical scavengers that increase in concentration in response to the transient stress induced by ozone and rapidly nullify unstable reactive oxidative species and equilibrate the cellular oxidation states.[312]

ANTICOAGULANT EFFECTS OF OZONE

Ozone also reduces the coagulopathic sequelae of COVID-19 infection. In a study with 50 patients of COVID-19, D-dimer levels were found to be significantly reduced post ozone therapy, along with respiratory indices, inflammatory markers and clinical symptom resolution. This indicates that ozone is capable of mitigating thromboembolic tendencies in COVID-19 patients.[313] Figure 17 describes all the effects of medical ozone discussed above.

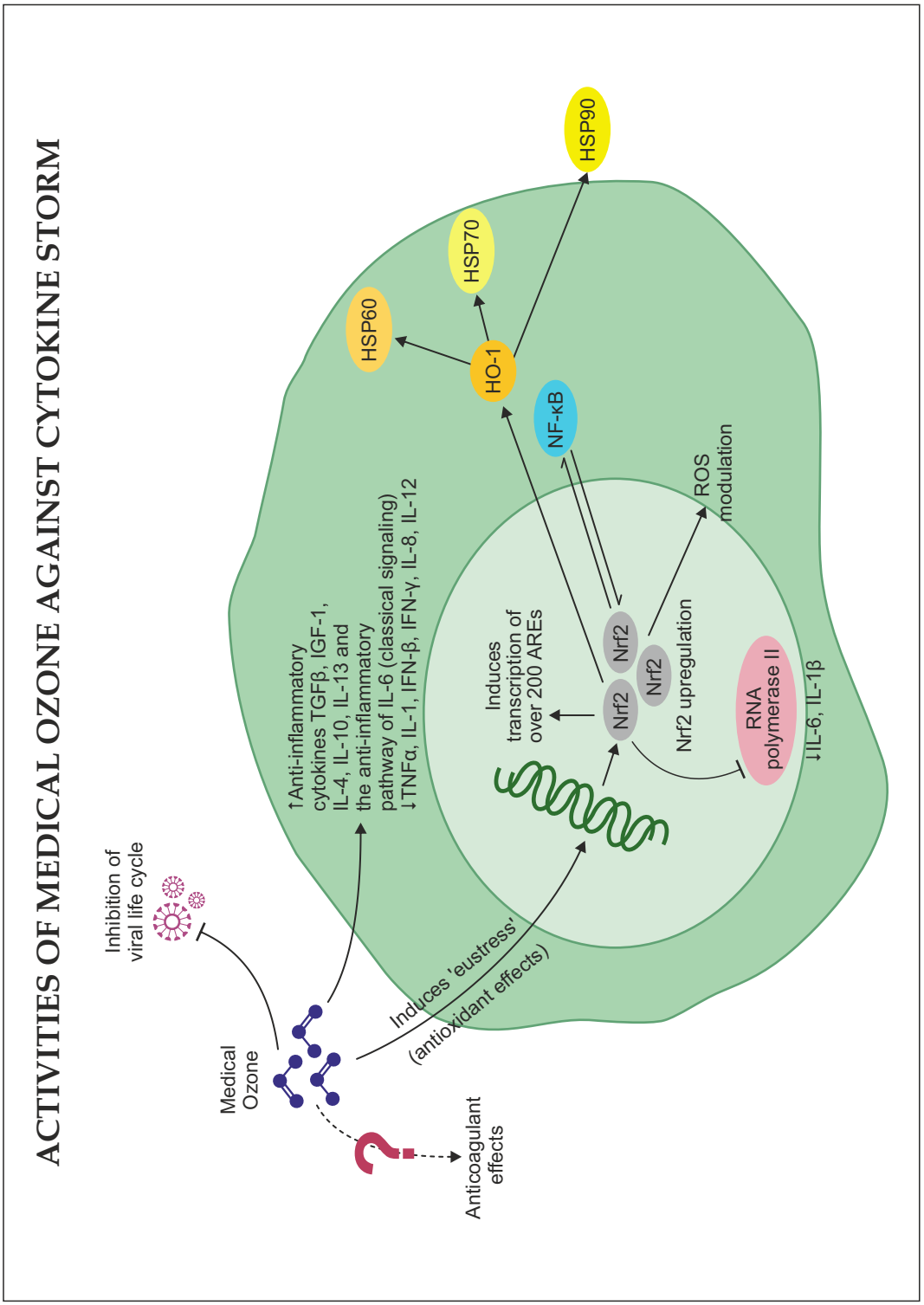

Figure 17: Ozone and its various actions to counteract the COVID-19 induced cytokine storm.

ONGOING CLINICAL TRIALS

WORLDWIDE RESEARCH ON OZONE

There are a total of 14 ongoing clinical trials worldwide that are investigating the use of ozone in COVID-19. 3 are from China, (ChiCTR2000030165, ChiCTR2000030102, ChiCTR2000030006), 2 are from Spain (NCT04370223, NCT04359303), 1 is from Italy (NCT04366089), 1 from Turkey (NCT04400006), 2 from India (CTRI/2020/07/026354, CTRI/2020/07/026671), 4 are from Iran (IRCT20191125045492N2, IRCT20190618043923N4, IRCT20200616047792N1, IRCT20200730048253N1), and 1 is from Cuba (RPCEC00000320).

10 of these studies are investigating the role of ozone autohemotherapy, 2 are investigating ozonized saline (O3SS) (CTRI/2020/07/026671, IRCT20200730048253N1), one is investigating a combination of autohemotherapy and rectal insufflation, and one is investigating rectal insufflation of ozone (RPCEC00000320). The results of their safety and efficacy are awaited.

INITIAL REPORTS OF SUCCESSFUL OZONE THERAPY

A preliminary Chinese study reports successful treatment of severe COVID-19 with ozone therapy with no adverse events. 2 COVID-19 patients, 53-year-old and 66-year-old males, were treated with major autohemotherapy for 7 consecutive days along with standard treatment. The 53-year-old patient presented with mild fever and dyspnea for 7 days, accompanied by headache, runny nose, fatigue and loss of appetite. On admission, chest CT revealed multiple small patchy shadows, linear interstitial changes and consolidation in both lungs. Lymphopenia, elevated CRP and IL-6, and mild hypoxemia were noted in laboratory tests. After treatment, his clinical symptoms resolved, laboratory parameters normalized, and follow-up serial CT scans showed gradually absorbed bilateral lung lesions.[314]

The 66-year-old patient had a history of chronic respiratory disease. He presented with fever, cough and sore throat. Chest CT scan showed "viral pneumonia-like changes". Antiviral therapy and antibiotics did not improve his condition; cough, hemoptysis and dyspnea occurred. Repeated chest CT scan revealed lesion progression, with multiple ground-glass opacities and interstitial changes in both lungs. Post ozone therapy, he recovered rapidly,

with normalization of laboratory indicators. Follow-up CT scan showed obviously absorbed bilateral lung lesions.[314]

An Italian study included 50 hospitalized COVID-19 subjects suffering from ARDS, aged more than 60 years, all males and undergoing non-invasive mechanical ventilation in ICUs. Authors report that following ozone therapy, inflammation and oxygenation indexes improved significantly and rapidly within the first 9 days after the treatment. Inflammatory and thromboembolic markers (CRP, IL-6, D-dimer) reduced significantly with amelioration in the major respiratory indexes, such as respiratory and gas exchange markers (SatO2%, PaO2/FiO2 ratio).[313]

POTENTIAL OF OZONISED SALINE AS A TREATMENT FOR COVID-19

Schwartz et al. published a study with 25 mild to severe COVID-19 patients and concluded that patients who received intravenous O3SS as a complementary therapy demonstrated improved clinical symptoms, improved laboratory markers, and a reduction in mortality with no side effects.[315]

Sharma et al. (at NeuroGen BSI Dedicated COVID Hospital) also conducted a study to assess the safety and efficacy profile of ozonized saline as an adjuvant to standard therapy for treating moderate COVID-19 patients (CTRI/2020/07/026671). In this pilot study, 10 moderately affected COVID-19 patients received 8 daily sessions of intravenous ozonised saline as adjuvant therapy to standard care. Clinical symptoms resolved, inflammatory and injury biomarkers (CRP, D-Dimer, PCT, IL-6, Ferritin, and LDH) reduced, and no procedure or treatment related adverse events occurred (unpublished data).

STEM CELLS

WHAT ARE STEM CELLS

Stem cells are specialized cells that have the unique capabilities of developing into different types of tissue systems. The defining characteristics[316] of a stem cell are the unique capabilities of clonogenicity, multilineage differentiation and tissue regeneration. These are explained in detail below. This modality has tremendous potential to combat the cytokine storm as stem cells function primarily via paracrine mechanisms to optimize cellular microenvironments.[317]

CLONOGENICITY

Stem cells undergo symmetric division to produce identical daughter cells, and thereby maintain the stem cell pool in the organism.

MULTILINEAGE DIFFERENTIATION

Stem cells also have the capacity to differentiate and divide asymmetrically. This yields an identical daughter (stem) cell and a non-identical, specialized daughter cell that acquires the properties of a cell type specific to a tissue.

TISSUE REGENERATION

Stem cells have the capacity to renew the tissues that they populate. The body contains stem cell "niches", i.e. specific regulatory microenvironments conducive to the maintenance, proliferation and differentiation of stem cells.[318]

TYPES OF STEM CELLS

Stem cells are classified based on

(a) Their source,
(b) Their potency,
(c) Their recipient.

Depending on the source, stem cells are classified as embryonic stem cells (ESCs), fetal stem cells (FSCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs).[319] On the basis of their potential to differentiate into cells of various tissue types, stem cells are classified as totipotent, pluripotent, multipotent, oligopotent or unipotent.[320] Based on their recipient, they are classified as being autologous or allogeneic.[321]

HOW DO THEY WORK AGAINST THE CYTOKINE STORM

PARACRINE EFFECTS

Depending on the cellular microenvironment, stem cells secrete and regulate a plethora of essential trophic factors, like Nerve growth factor-β (NGF-β)[322]; Ciliary neurotrophic factor (CNTF)[323]; Brain-derived neurotrophic factor (BDNF)[322]; Glial cell–derived neurotrophic factor (GDNF)[324]; and Ang I.[325]

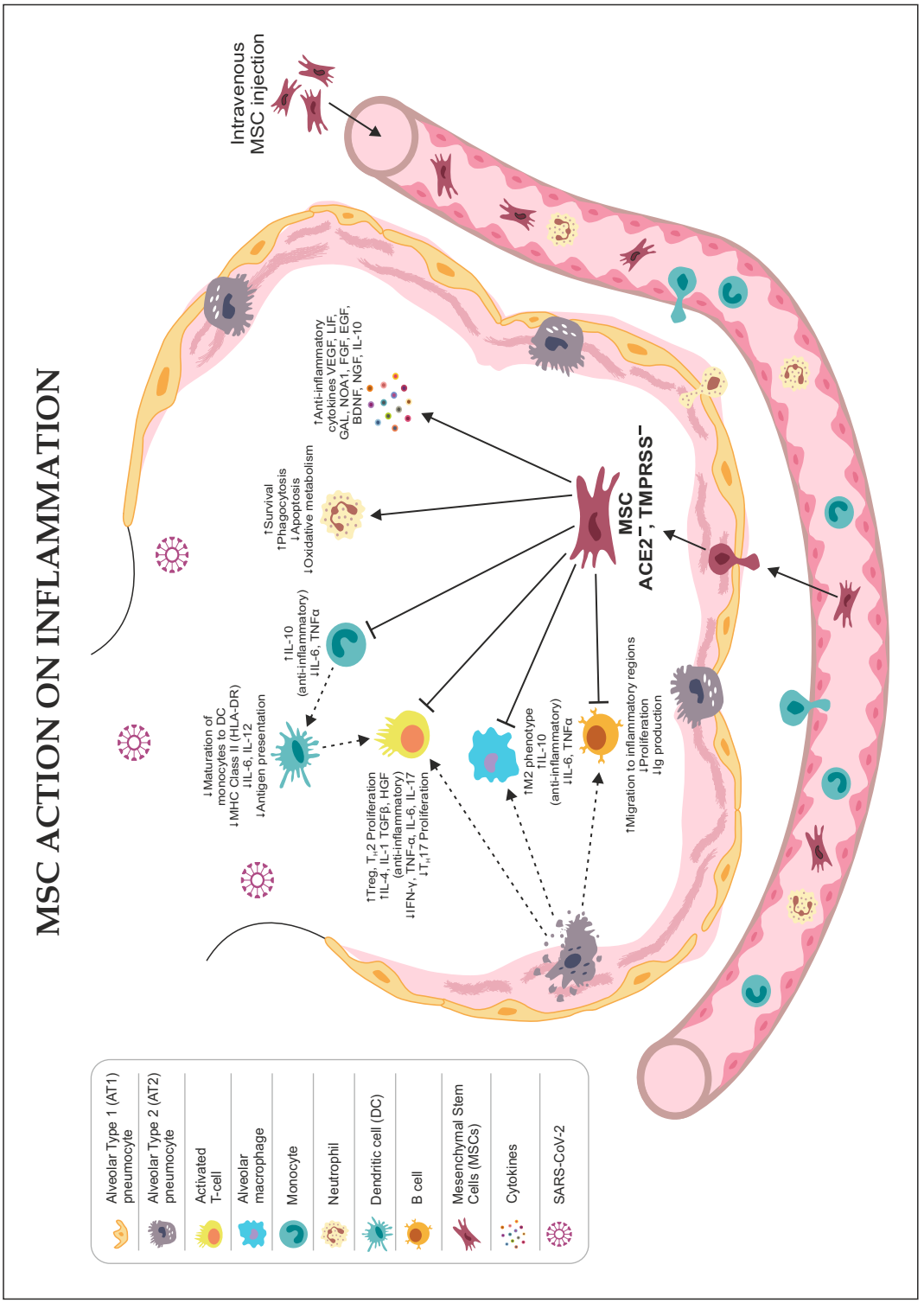

Figure 18: MSCs effectively combat the cytokine storm by exerting anti-inflammatory effects on immune cells.

IMMUNOMODULATION

These cells exude various beneficial immunomodulatory effects. Various secreted factors like connective tissue growth factor (CTGF), fibroblast growth factor (FGF) 2 and 7 and various interleukins (ILs) are responsible for cell proliferation and cytoprotection. Stem cells regulate innate and adaptive immune cells through release of soluble factors such as tumor growth factor (TGF)-ß and elevation of regulatory T-cells (Tregs) and T_H2cells.[326]

Reduced levels of TNFα, IL-1ß, IL-1a, IL-6 and increased levels of IL-10 lead to an anti-inflammatory effect on the microenvironment.[327–330] Stem cells also produce vascular endothelial growth factor (VEGF), hepatic growth factor (HGF) and Insulin growth factor (IGF)-1.[331] Secretion of growth factors like VEGF, fibroblast growth factor (FGF), brain fibroblast growth factor (bFGF) leads to neoangiogenesis and upregulation of hormones like erythropoietin.[330] The cascade of events triggered due to these leads to formation of new vessels as well as improved blood circulation, and may retrieve lost pulmonary functions.

Recently, Leng et al. showed that stem cells highly express anti-inflammatory and trophic factors like TGF-ß, HGF, VEGF, Leukaemia Inhibiting Factor (LIF), Galanin (GAL), Nitric Oxide Associated protein 1 (NOA1), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), BDNF, and NGF, further bolstering their immunomodulatory role.[332]

ONGOING CLINICAL TRIALS

There are over 100 clinical trials that are studying the safety and efficacy of stem cells in COVID-19 (see Upcoming Medicines: Clinical Trials section). Leng et al. conducted a study on 10 patients with COVID-19 pneumonia.[332] They administered MSCs in 7 patients intravenously and assessed them for 14 days, while 3 patients served as controls. It was observed that MSCs were safe, effective and could cure or significantly improve the functional outcomes of COVID-19 patients with no adverse effects. Significant improvements were noted in pulmonary function and symptoms in all patients within 2 days after MSC transplantation. After treatment, an increase in the peripheral lymphocytes levels was noted whereas the C-reactive protein was decreased. The overactivated cytokine-secreting immune cells CXCR3+CD4+ T-cells, CXCR3+CD8+ T-cells, and CXCR3+ NK cells disappeared in 3-6 days. In

addition, a group of CD14+CD11c+CD11bmid regulatory dendritic cell population dramatically increased. As compared to the placebo control group, the level of pro-inflammatory TNFα was significantly decreased in treatment group. Anti-inflammatory IL-10 levels were increased in MSC treatment group. Importantly, the MSCs were ACE2 and TMPRSS2 negative; this may indicate that these transplanted cells are not vulnerable to COVID-19 infection.[332]

Sharma et al. (at NeuroGen BSI Dedicated COVID Hospital) have registered a randomized, placebo-controlled, phase I and II study using intravenous administration of mixture of umbilical cord and placenta mesenchymal stem cells (MSCs) for COVID-19 patients (CTRI/2020/08/027043) (unpublished data, results awaited).

MSCs have shown to reduce mortality and inflammatory cytokine storm seen in ARDS. They alleviate acute lung injury by transferring mitochondria to injured epithelial cells.[333] MSCs have been studied in various pulmonary conditions in addition to ARDS, including COPD, idiopathic pulmonary fibrosis and and obstructive bronchiolitis.[334] Recently, Leng et al. have shown the potential of MSCs for the treatment of COVID-19.[332]

MSCs have the critical ability to modulate immune function via multiple paracrine effects. MSCs have also been shown to be ACE2- and TMPRSS2-negative, which may indicate reduced susceptibility to SARS-CoV-2 infection. Further, they express lung surface proteins, which is suggestive of their ability to differentiate into various types of alveolar cells.[332]

ANTIVIRAL ACTIVITY OF MESENCHYMAL STEM CELLS

MSCs regulate multiple antiviral pathways in a variety of conditions, not just pulmonary – including herpes viral infections[335], Japanese encephalitis[336], and latent HIV-1.[337] KEGG pathway analysis shows enrichment of pathways involved in the regulation of Epstein-Barr, Hepatitis B, viral carcinogenesis and human T-cell leukaemia virus 1 infection.[332] They may also inhibit proliferation of virus-specific CD8+ T-cells[332], curtailing excessive immune responses. Chan et al. show that these cells suppress influenza A/H5N1 infection in vitro and in vivo, promoting alveolar fluid clearance.[338] Transplantation of these cells has also lead to reduced mortality in H7N9 infections.[339] MSCs synergistically inhibit viral replication in hepatitis B virus-replicating cells via tight regulation of IFN-

γ and TNFα.[340] Laan et al. have filed a patent for an MSC exudate that causes novel attenuation of chronic viruses like HCV and HBV.[341]

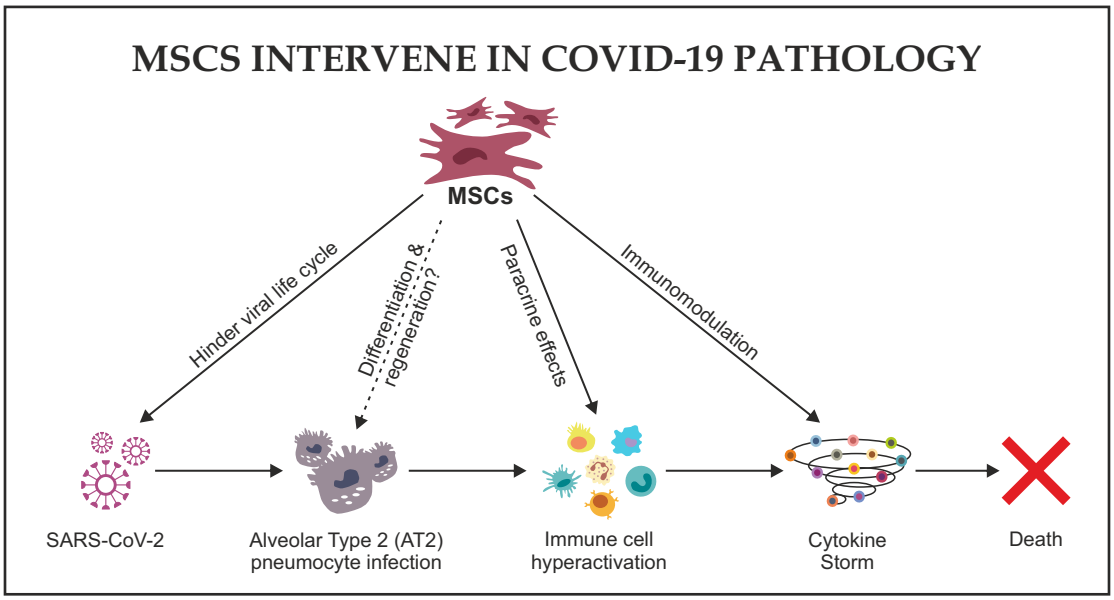

Figure 19: MSCs intervene at various stages of the disease to mitigate COVID-19 infection and its deleterious effects.

UPCOMING MEDICINES: CLINICAL TRIALS

Table 2 (P.T.O.) lists all the clinical trials of upcoming modalities to combat the COVID-19 cytokine storm, compiled from the WHO International Clinical Trials Registry Platform (ICTRP) as of September 3, 2020.

Trial ID	Scientific title
Ozone Therapy	
ChiCTR2000030165	Clinical study for ozonated autohemotherapy in the treatment of Novel Coronavirus Pneumonia (COVID-19)
ChiCTR2000030102	A multicenter randomized controlled trial for ozone autohemotherapy in the treatment of novel coronavirus pneumonia (COVID-19)
ChiCTR2000030006	A randomized controlled trial for the efficacy of ozonated autohemotherapy in the treatment of Novel Coronavirus Pneumonia (COVID-19)
NCT04366089	Oxygen-Ozone as Adjuvant Treatment in Early Control of Disease Progression in Patients With COVID-19 Associated With Modulation of the Gut Microbial Flora
IRCT20191125045492N2	Comparison of the effectiveness of Ozone therapy with conventional therapy in improving patient outcomes in Covid-19 patients
IRCT20190618043923N4	Investigation the effects medical Ozone Autohemotherapy on clinical and para clinical features of patients with Covid19
NCT04400006	The Effectiveness of Ozone Therapy in the Prevention of COVID-19 Infection
NCT04370223	A Trial of Ozone Auto-hemotherapy in Adults Hospitalized With Covid-19 Pneumonia

NCT04359303	Randomized Clinical Trial to Evaluate Efficacy and Safety of Systemic Indirect Endovenous Ozone Therapy (SIEVOT) as Adjuvant Treatment in COVID19 Non-intubated Patients
CTRI/2020/07/026354	Phase I/II randomized controlled clinical trial to assess safety and efficacy of ozone therapy via rectal insufflation and minor auto haemotherapy as an adjuvant in mild to moderate Covid-19 subjects. - Nil
CTRI/2020/07/026671	A pilot study for treatment of COVID-19 patients in moderate stage using intravenous administration of ozonized saline as an adjuvant treatment
IRCT20200616047792N1	Study of intra-venous ozonated auto-hemotherapy on severe cases of COVID-19: A randomized clinical trial
IRCT20200730048253N1	Evaluating the effectiveness of intravenous ozonized saline in treatment of severe COVID-19 disease: A randomized Control trial
RPCEC00000320	Exploratory study of the therapeutic effect and safety of rectal ozone therapy in patients positive to SARS-CoV 2 with mild to moderate symptoms (COVID-19) - orc
Stem Cell Therapy	
ChiCTR2000029606	Clinical Study for Human Menstrual Blood-derived Stem Cells in the Treatment of Acute Novel Coronavirus Pneumonia (COVID-19)
ChiCTR2000029572	Safety and efficacy of umbilical cord blood mononuclear cells in the treatment of severe and critically novel coronavirus pneumonia(COVID-19): a randomized controlled clinical trial

ChiCTR2000029569	Safety and efficacy of umbilical cord blood mononuclear cells conditioned medium in the treatment of severe and critically novel coronavirus pneumonia (COVID-19): a randomized controlled trial
ChiCTR2000030173	Key techniques of umbilical cord mesenchymal stem cells for the treatment of novel coronavirus pneumonia (COVID-19) and clinical application demonstration
ChiCTR2000030138	Clinical Trial for Human Mesenchymal Stem Cells in the Treatment of Severe Novel Coronavirus Pneumonia (COVID-19)
ChiCTR2000030116	Safety and effectiveness of human umbilical cord mesenchymal stem cells in the treatment of acute respiratory distress syndrome of severe novel coronavirus pneumonia (COVID-19)
ChiCTR2000030088	Umbilical cord Wharton's Jelly derived mesenchymal stem cells in the treatment of severe novel coronavirus pneumonia (COVID-19)
ChiCTR2000030020	The clinical application and basic research related to mesenchymal stem cells to treat novel coronavirus pneumonia (COVID-19)
ChiCTR2000029990	Clinical trials of mesenchymal stem cells for the treatment of pneumonitis caused by novel coronavirus (COVID-19)
ChiCTR2000030261	A study for the key technology of mesenchymal stem cells exosomes atomization in the treatment of novel coronavirus pneumonia (COVID-19)
NCT04276987	A Pilot Clinical Study on Aerosol Inhalation of the Exosomes Derived From Allogenic Adipose Mesenchymal Stem Cells in the Treatment of Severe Patients With Novel Coronavirus Pneumonia

ChiCTR2000029580	Severe novel coronavirus pneumonia (COVID-19) patients treated with ruxolitinib in combination with mesenchymal stem cells: a prospective, single blind, randomized controlled clinical trial
ChiCTR2000030866	Open-label, observational study of human umbilical cord derived mesenchymal stem cells in the treatment of severe and critical COVID-1
ChiCTR2000030835	Clinical study on the efficacy of Mesenchymal stem cells (MSC) in the treatment of severe novel coronavirus pneumonia (COVID-19)
NCT04302519	Clinical Study of Novel Coronavirus Induced Severe Pneumonia Treated by Dental Pulp Mesenchymal Stem Cells
ChiCTR2000031139	Safety and Effectiveness of Human embryonic stem cell-derived M cells (CAStem) for Pulmonary Fibrosis Correlated with novel coronavirus pneumonia(COVID-19)
ChiCTR2000030944	An open, multi-center, control, exploratory clinical study of human NK cells and UC-MSCs transplantation for severe novel coronavirus pneumonia
NCT04293692	Human Umbilical Cord Mesenchymal Stem Cells Treatment for Pneumonia Patients Infected by 2019 Novel Coronavirus
NCT04313322	Treatment of COVID-19 Patients Using Wharton's Jelly-Mesenchymal Stem Cells
ChiCTR2000031319	Safety and Efficacy Study of Allogeneic Human Dental Pulp Mesenchymal Stem Cells to Treat Severe Pneumonia of COVID-19:a Single-center, Prospective, Randomised Clinical Trial

ChiCTR2000031494	Clinical study for stem cells in the treatment of severe novel coronavirus pneumonia (COVID-19)
ChiCTR2000031430	Evaluation of the safety and efficacy for human umbilical cord mesenchymal stem cells in COVID-19 induced pulmonary fibrosis
NCT04331613	Safety and Efficacy Study of Human Embryonic Stem Cells Derived M Cells (CAStem) for the Treatment of Severe COVID-19 Associated With or Without Acute Respiratory Distress Syndrome (ARDS)
NCT04336254	Safety and Efficacy Study of Allogeneic Human Dental Pulp Mesenchymal Stem Cells to Treat Severe Pneumonia of COVID-19:a Single-center, Prospective, Randomised Clinical Trial
NCT04339660	Clinical Research of Human Mesenchymal Stem Cells in the Treatment of COVID-19 Pneumonia
EUCTR2019-002688-89-ES	Phase 1/2 clinical study to assess the feasibility, safety, tolerability and preliminary efficacy of the administration of HCR040, a drug whose active substance is HC016, allogeneic adipose-derived adult mesenchymal stem cells expanded and pulsed with H2O2, in patients with acute respiratory distress syndrome. (included patients COVID-19)
EUCTR2020-001682-36-ES	Double-blind, placebo-controlled phase I/II clinical trial to evaluate the safety and efficacy of allogeneic mesenchymal stem cells (MSVå¨-allo) in acute respiratory failure in patients with COVID-19 pneumonia. - Treatment of COVID.19 with allogeneic mesenchymal cells (MSVå¨).

EUCTR2020-001266-11-ES	Two-center, randomized, controlled clinical trial with two treatment arms to evaluate the safety and efficacy of intravenous administration of expanded allogeneic adipose tissue adult mesenchymal cells in critically ill patients COVID-19.
IRCT20140528017891N8	Evaluation of the efficacy and safety of cord-derived mesenchymal stem cell transplantation in the treatment of COVID-19
IRCT20140911019125N6	Study the effect of intravenous injection of dental pulp mesenchymal stem cells in treatment of patients with COVID-19 pneumonia
IRCT20200325046860N2	Mesenchymal stem cell utilization in reducing complications and enhancing pneumonia healing in patients infected with 2019-nCoV (phase I clinical trial)
NCT03042143	Repair of Acute Respiratory Distress Syndrome by Stromal Cell Administration (REALIST): An Open Label Dose Escalation Phase 1 Trial Followed by a Randomized, Double-blind, Placebo-controlled Phase 2 Trial (COVID-19)
NCT04252118	Safety and Efficiency of Mesenchymal Stem Cell in Treating Pneumonia Patients Infected With COVID-19
NCT04273646	Clinical Study of Human Umbilical Cord Mesenchymal Stem Cells in the Treatment of Severe COVID-19
NCT04299152	Clinical Application of Stem Cell Educator Therapy for the Treatment of Viral Inflammation Caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

NCT04346368	Safety and Efficacy of Intravenous Infusion of Bone Marrow-Derived Mesenchymal Stem Cells in Severe Patients With Coronavirus Disease 2019 (COVID-19): A Phase 1/2 Randomized Controlled Trial
NCT04348461	Two-treatment,Randomized, Controlled, Multicenter Clinical Trial to Assess the Safety and Efficacy of Intravenous Administration of Expanded Allogeneic Adipose Tissue Adult Mesenchymal Stromal Cells in Critically Ill Patients COVID-19
NCT04349540	A Prospective Non Interventional Study to Evaluate the Role of Immune and Inflammatory Response in Recipients of Allogeneic Haematopoietic Stem Cell Transplantation (SCT) Affected by Severe COVID19 Infection
NCT04352803	IV Infusion of Autologous Adipose Derived Mesenchymal Cells for Abatement of Respiratory Compromise in SARS-CoV-2 Pandemic (COVID-19)
NCT04366830	Intermediate-size Expanded Access of Remestemcel-L, Ex-vivo Cultured Adult Human Mesenchymal Stromal Cells for Acute Respiratory Distress Syndrome Due to COVID-19 Infection
EUCTR2020-001364-29-ES	Phase I/II clinical trial to evaluate the safety and efficacy of Allogenic Adipose Tissue-Derived Mesenchymal Stem Cells Expanded in patients with severe COVID-19 pneumonia

EUCTR2020-001505-22-ES	Double-blind, randomized, parallel, placebo-controlled pilot clinical trial, nested in a prospective cohort observational study, for the evaluation of the efficacy and safetyof two doses of WJ-MSC in patients with acute respiratory distress syndrome secondary to infection by COVID-19 - COVIDMES
NCT04361942	Double Blind, Placebo-controlled, Phase II Trial to Evaluate Safety and Efficacy of Allogenic Mesenchymal Stromal Cells MSV_allo for Treatment of Acute Respiratory Failure in Patients With COVID-19 Pneumonia (COVID_MSV)
NCT04366063	Mesenchymal Stem Cell Therapy for Acute Respiratory Distress Syndrome in Coronavirus Infection: A Phase 2-3 Clinical Trial
NCT04371601	Safety and Effectiveness of Mesenchymal Stem Cells in the Treatment of Pneumonia of Coronavirus Disease 2019
IRCT20200217046526N2	Mesenchymal Stem Cell Therapy for Acute Respiratory Distress Syndrome in Coronavirus Infection: A Phase 2-3 Clinical Trial
IRCT20190717044241N2	Cell therapy in patients with coronavirus19 using mesenchymal stem cells
IRCT20200413047063N1	Placental Mesenchymal Stem cells for treatment of ARDS in Coronavirus infection, Phase 1 and 2 Clinical Trials
IRCT20200418047121N2	Investigation the adipose and placenta-derived mesenchymal stem cells effect on the respiratory distress syndrome in patients with COVID-19: a pilot study

NCT04366271	Phase II Clinical Trial to Explore the Efficacy of Allogeneic Mesenchymal Cells From Umbilical Cord Tissue in Patients With Severe Pulmonary Involvement by COVID-19
NCT04390139	A Prospective, Double-blind, Randomized, Parallel, Placebo-controlled Pilot Clinical Trial for the Evaluation of the Efficacy and Safety of Two Doses of WJ-MSC in Patients With Acute Respiratory Distress Syndrome Secondary to Infection by COVID-19
NCT04392778	What is the Effect of Mesenchymal Stem Cell Therapy on Seriously Ill Patients With Covid 19 in Intensive Care? (Prospective Double Controlled Study)
NCT04397471	A Study to Collect Bone Marrow for Process Development and Production of Bone Marrow Mesenchymal Stromal Cells to Treat Severe COVID19 Pneumonitis
NCT04341610	Allogeneic Adipose Tissue Derived Mesenchymal Stromal Cell Therapy for Treating Patients With Severe Respiratory COVID-19. A Danish, Double-blind, Randomized Placebo-controlled Study
NCT04382547	Treatment of Covid-19 Associated Pneumonia With Allogenic Pooled Olfactory Mucosa-derived Mesenchymal Stem Cells
NCT04416139	Mesenchymal Stem Cells for the Treatment of Severe Acute Respiratory Distress Syndrome Due to COVID-19. Pilot Study
IRCT20200217046526N1	Mesenchymal Stem Cell Therapy for Acute Respiratory Distress Syndrome in Coronavirus Infection : A Phase 1 and 2 clinical trial

IRCT20200421047150N1	Assessment of safety, efficacy and effective dose determination of human umbilical cord Wharton's jelly mesenchymal stem cell transplantation on treatment of COVID-19 (coronavirus) pneumonia and complications in humans
IRCT20160809029275N1	Evolution of Allogenic Mesenchymal stem cell-derived Umbilical cord transplantation for ARDS patients infected with COVID19.
NCT04366323	Phase I / II Clinical Trial, Multicenter, Randomized and Controlled, to Assess the Safety and Efficacy of Intravenous Administration of Allogeneic Adult Mesenchymal Stem Cells of Expanded Adipose Tissue in Patients With Severe Pneumonia Due to COVID-19
NCT04315987	Exploratory Clinical Study to Assess the Efficacy of NestaCellå¨ Mesenchymal Stem Cell to Treat Patients With Severe COVID-19 Pneumonia
NCT04429763	Safety and Efficacy of Mesenchymal Stem Cells in the Management of Severe COVID-19
ACTRN12620000676910	Allogeneic Amniotic Epithelial Cells for the Treatment of COVID-19 related respiratory failure, a pilot feasibility randomised controlled trial
ISRCTN33578935	Rationale and investigational study for the treatment of COVID-19 with severe viral pneumonia with isolated, placental, mesenchymal stem cell exosomes
NCT04437823	Efficacy of Intravenous Infusions of Stem Cells in the Treatment of COVID-19 Patients

NCT04399889	Pilot Study of Safety and Efficacy of Cord Tissue Derived Mesenchymal Stromal Cells (hCT-MSC) in COVID-19 Related Acute Respiratory Distress Syndrome (ARDS)
NCT04445402	Retrospective and Prospective Database of COVID-19 Prevalence and Clinical Course in Pediatric and Young Adult Hematology/ Oncology/Stem Cell Therapy Patients in the New York Tri-State Area.
NCT04445454	Mesenchymal Stromal Cell Therapy for Severe Covid-19 Infection
NCT04377334	Prospective Phase II Study: MSCs in Inflammation-Resolution Programs of SARS-CoV-2 Induced ARDS
NCT04444271	Prospective, Randomized Phase 2 Clinical Trial of Mesenchymal Stem Cells(MSCs) for the Treatment of Coronavirus Disease 2019(COVID-19)
NCT04269525	Clinical Research Regarding the Availability and Safety of UC-MSCs Treatment for Serious Pneumonia and Critical Pneumonia Caused by the 2019-nCOV Infection
NCT04456439	Intermediate-size Expanded Access of Remestemcel-L, Human Mesenchymal Stromal Cells, for Multisystem Inflammatory Syndrome in Children (MIS-C) Associated With Coronavirus Disease (COVID-19)
ACTRN126200006 12910	A pilot, open-label, randomised controlled clinical trial to investigate early efficacy of CYP-001 in adults admitted to intensive care with COVID-19

IRCT20200426047206N2	Clinical trial of efficacy and safety of mesenchymal stem cell transplantation in patients with COVID-19 pneumonia
NCT04457609	Application of Umbilical Cord Mesenchymal Stem Cells as Adjuvant Therapy for Critically-Ill COVID-19 Patients
NCT04348435	A Randomized, Double-Blind, Single Center, Efficacy and Safety Study of Allogeneic HB-adMSCs to Provide Immune Support Against COVID-19
NCT04349631	A Phase II, Open Label, Single-Center, Clinical Trial to Assess Efficacy of HB-adMSCs to Provide Immune Support Against Coronavirus Disease
NCT04390152	Safety and Efficacy of Intravenous Infusion of Wharton's Jelly Derived Mesenchymal Stem Cell Plus Standard Therapy for the Treatment of Patients With Acute Respiratory Distress Syndrome Diagnosis Due to COVID 19: A Randomized Controlled Trial
NCT04461925	Treatment of Coronavirus COVID-19 Pneumonia (Pathogen SARS-CoV-2) With Cryopreserved Allogeneic Multipotent Mesenchymal Stem Cells of the Placenta and Umbilical Cord
NCT04467047	Safety and Feasibility of Allogenic Mesenchymal Stromal Cells in the Treatment of COVID-19
EUCTR2020-002193-27-ES	Double-blind, randomized, controlled, clinical trial to assess the efficacy of allogenic mesenchymal stromal cells in patients with acute respiratory distress syndrome due to COVID-19

NCT04428801	Clinical Study for the Prophylactic Efficacy of Autologous Adipose Tissue-Derived Mesenchymal Stem Cells (AdMSCs) Against Coronavirus 2019 (COVID-19)
NCT04473170	Adaptive Open-label Study Evaluating the Safety and Efficacy of Autologous Non-Hematopoietic Peripheral Blood Stem Cells Therapy in COVID-19 Outbreak in Abu Dhabi, 2020 (SENTAD-COVID Study)
NCT04486001	COVID-19 Stem Cell Therapy: A Phase I Study of Intravenous Administration of Allogeneic Adipose Stem Cells
NCT04345601	Single Donor Banked Bone Marrow Mesenchymal Stromal Cells for the Treatment of COVID19-Induced ARDS: A Randomized, Controlled Study
EUCTR2020-002102-58-BE	Mesenchymal stromal cell therapy for severe COVID-19 infection - MSC therapy for severe COVID-19 infection
NCT04397796	Phase 1b Randomized, Double-Blind, Placebo-Controlled Study Of The Safety Of Therapeutic Treatment With Immunomodulatory Mesenchymal Stem Cells In Adults With COVID-19 Infection Requiring Mechanical Ventilation
NCT04451291	DSC-COVID-19: An Open-label Study on the Safety and Efficacy of Decidual Stromal Cells in Respiratory Failure Induced by COVID-19
NCT04456361	A Study of Mesenchymal Stem Cells as a Treatment in Patients With Acute Respiratory Distress Syndrome Caused by COVID-19

CTRI/2020/08/027043	A Phase 1 clinical trial of intravenous administration of mesenchymal stem cells derived from umbilical cord and placenta in patients with novel COVID-19 virus pneumonia.
RPCEC00000322	Treatment of lung lesions with autologous stem cells in patients recovered from COVID-19 (COVID-19)
NCT04355728	Umbilical Cord-derived Mesenchymal Stem Cells for COVID-19 Patients With Acute Respiratory Distress Syndrome (ARDS)
NCT04371393	Mesenchymal Stem Cells for the Treatment of Moderate to Severe COVID-19 Acute Respiratory Distress Syndrome
NCT04445220	A Multi-center, Randomized, Case Controlled, Double-blind, Ascending-dose Study of Extracorporeal Mesenchymal Stromal Cell Therapy (SBI-101 Therapy) in COVID-19 Subjects With Acute Kidney Injury Receiving Renal Replacement Therapy
NCT04466098	Multi-center, Randomized, Placebo Controlled, Interventional Phase 2A Clinical Trial Evaluating the Safety and Potential Efficacy of Multiple Dosing of Mesenchymal Stromal Cells in Patients With Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2)
NCT04494386	Phase 1/2a Study of Umbilical Cord Lining Stem Cells (ULSC) in Patients With ARDS Due to COVID-19
NCT04288102	A Phase II, Multicenter, Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Efficacy and Safety of Human Umbilical Cord-derived Mesenchymal Stem Cells in the Treatment of Severe COVID-19 Patients

NCT04365101	A Phase I/II Study of Human Placental Hematopoietic Stem Cell Derived Natural Killer Cells (CYNK-001) for the Treatment of Adults With COVID-19
NCT04482699	Phase I/Phase II Trial of Off-the-Shelf Allogeneic Hybrid TREG/Th2 Cell (RAPA-501-ALLO) Therapy for Severe, Post-Intubation Stage 3 COVID-19 Disease
NCT04490486	Phase I, Randomized, Double Blinded, Placebo Control Study to Evaluate the Safety and Potential Efficacy of Intravenous Infusion of Umbilical Cord Tissue (UC) Derived Mesenchymal Stem Cells (MSCs) Versus Placebo to Treat Acute Pulmonary Inflammation Due to COVID-19 With Moderate to Severe Symptoms
NCT04522817	CLBS119 (Autologous Peripheral Blood Derived CD34+ Cells) for Repair of COVID-19 Induced Pulmonary Damage
NCT04525378	Mesenchymal Stromal Cell-based Therapy for COVID-19-associated Acute Respiratory Distress Syndrome: a Pilot Clinical Study
NCT04527224	A Phase I/Ⅱa Trial to Explore the Safety and Efficacy of Allogenic Adipose Tissue-derived Mesenchymal Stem Cell (AstroStem-V) Therapy in Patients With COVID-19 Pneumonia
ACTRN12620000840987	Phase I trial on safety and tolerability of bone-marrow derived mesenchymal stromal cells (MSC) for deteriorating COVID-19 pneumonia
Corticosteroids	
ChiCTR2000029656	A randomized, open-label study to evaluate the efficacy and safety of low-dose corticosteroids in hospitalized patients with novel coronavirus pneumonia (COVID-19)

ChiCTR2000030481	The clinical value of corticosteroid therapy timing in the treatment of novel coronavirus pneumonia (COVID-19): a prospective randomized controlled trial
CTRI/2020/07/026608	Randomized Study Of the Effect of Dexamethasone and Methylprednisolone on levels of IL-6 and clinical outcome in severe COVID-19 - REDMIC
EUCTR2020-001307-16-ES	Efficacy and Safety of corticoids in patients with adult respiratory distress syndrome (ARDS) secondary to COVID-19. - Steroids In Coronavirus (SIC)
EUCTR2020-001333-13-FR	Dexamethasone associated with hydroxychloroquine vs. hydroxychloroquine alone for the early treatment of severe ARDS caused by COVID-19 : a randomized controlled trial - DHYSCO
EUCTR2020-001413-20-ES	Phase 2, randomized, open-label study to compare the efficacy and safety of siltuximab vs. corticosteroids in hospitalized patients with COVID19 pneumonia
EUCTR2020-001445-39-ES	Pragmatic, controlled, open, single center, randomized, phase ii clinical trial to evaluate methylprednisolone pulses and tacrolimus in hospitalized patients with severe pneumonia secondary to COVID-19 (TACROVID)
EUCTR2020-001457-43-FR	Dexamethasone and oxygen support strategies in ICU patients with Covid-19 pneumonia - COVIDICUS
EUCTR2020-001553-48-FR	Corticoïdes au cours de la pneumonie virale Covid-19 liée à l'infection par le SARS-Cov-2 - CORTI-Covid

EUCTR2020-001622-64-ES	Outpatient treatment of COVID-19 with early pulmonary corticosteroids as an opportunity to modify the course of the disease
EUCTR2020-001707-16-ES	Phase III randomized, unicentric, open, controlled clinical trial to demonstrate the effectiveness of Tocilizumab against systemic corticotherapy in patients entered by COVID-19 with bilateral pneumonia and bad evolution - TOCICOVID
EUCTR2020-001827-15-ES	Early treatment of pneumonia COVID-19 with glucocorticoids. Randomized controlled clinical trial - CORTIVID
EUCTR2020-001889-10-GB	Use of inhaled corticosteroids as treatment of early COVID-19 infection to prevent clinical deterioration and hospitalisation - The STOIC Study (STerOids in COVID)
EUCTR2020-001934-37-ES	Use of corticosteroids in patients with SARS-CoV-2 coronavirus infection (GLUCOCOVID). Pragmatic trial inserted in real practice during a pandemic COVID-19 - use of corticosteroids in patients with SARS-CoV-2 coronavirus infection
EUCTR2020-002186-34-ES	Efficacy of the early use of corticotherapy in CoV-2 infection to prevent the progression of acute respiratory distress syndrome (ARDS) in COVID-19
EUCTR2020-003363-25-DK	Higher vs. Lower Doses of Dexamethasone in Patients with COVID-19 and Severe Hypoxia: the COVID STEROID 2 trial - COVID STEROID 2

IRCT20080901001165N52	Investigating the efficacy of high dose of glucocorticoid in patients with moderate to severe pneumonia related to COVID-19
IRCT20120215009014N354	Evaluating the effect of intravenous hydrocortisone, methylprednisolone, and dexamethasone in treatment of patients with moderate to severe acute respiratory distress syndrome caused by COVID-19: A double blind randomized clinical trial
IRCT20120225009124N4	Efficacy of different methods of administeration of combination regiemn including dexamethasone, IV-IG and Interferone beta for treatment of patients with severe COVID-19: a randomized controlled trial
IRCT20151227025726N17	Evolution of the efficacy and safety of Dexamethasone administration in patients with mild to moderate COVID-19 acute respiratory disease syndrome
IRCT20200204046369N1	Evaluation of Methylprednisolone Administration as a Therapeutic Option in the 2019 Novel Coronavirus (COVID-19): A Non-Randomized Controlled Study
IRCT20200406046963N1	Evaluation of the efficacy and safety of methylprednisolone pulse therapy in treatment of Covid-19 patients with ARDS
IRCT20200522047542N1	Effect of inhaled corticosteroids in the treatment of anosmia in patients with COVID-19
JPRN-jRCTs041200025	Japanese, multicenter, phase II trial of combination therapy with favipiravir and corticosteroids for coronavirus disease 2019 (COVID-19) patients with mild respiratory failure - J-CRITICAL trial
JPRN-UMIN000040211	A Multicenter, Retrospective Study to Evaluate the Efficacy of Systemic Gluco-corticoid Against COVID-19 - DEFEAT COVID-19

NCT04244591	Glucocorticoid Therapy for Critically Ill Patients With Severe Acute Respiratory Infections Caused by COVID-19: a Prospective, Randomized Controlled Trial
NCT04273321	Efficacy and Safety of Corticosteroids in COVID-19: A Prospective Randomized Controlled Trails
NCT04323592	Prolonged Low Doses of Methylprednisolone for Patients With COVID-19 Severe Acute Respiratory Syndrome
NCT04325061	Efficacy of Dexamethasone Treatment for Patients With ARDS Caused by COVID-19
NCT04327401	COVID-19-associated ARDS Treated With DEXamethasone: an Open-label, Randomized, Controlled Trial: CoDEX (Alliance Covid-19 Brasil III)
NCT04329650	Phase 2, Randomized, Open-label Study to Compare Efficacy and Safety of Siltuximab vs. Corticosteroids in Hospitalized Patients With COVID19 Pneumonia
NCT04341038	Open Randomized Single Centre Clinical Trial to Evaluate Methylprednisolone Pulses and Tacrolimus in Patients With Severe Lung Injury Secondary to COVID-19
NCT04343729	Efficacy of Injectable Methylprednisolone Sodium Succinate in the Treatment of Patients With Signs of Severe Acute Respiratory Syndrome Under the New Coronavirus (SARS-CoV2): a Phase IIb, Randomized, Double-blind, Placebo-controlled, Clinical Trial.
NCT04344288	Corticosteroids During Covid-19 Viral Pneumonia Related to SARS-Cov-2 Infection
NCT04344730	Dexamethasone and Oxygen Support Strategies in ICU Patients With Covid-19 pneumonia_COVIDICUS

NCT04345445	An Open-label, Randomized, Cross-over Interventional Study to Evaluate the Efficacy and Safety of Tocilizumab Versus Corticosteroids in Hospitalised COVID-19 Patients With High Risk of Progression
NCT04347980	Dexamethasone Combined With Hydroxychloroquine Compared to Hydroxychloroquine Alone for Treatment of Severe Acute Respiratory Distress Syndrome Induced by Coronavirus Disease 19 (COVID-19): a Multicentre, Randomised Controlled Trial
NCT04355247	Prophylactic Corticosteroid to Prevent COVID-19 Cytokine Storm
NCT04355637	Treatment With Inhaled Corticosteroids in Patients Hospitalized Because of COVID19 Pneumonia
NCT04359511	Efficacy and Safety of Corticosteroids in Oxygen-dependent Patients With COVID-19 Pneumonia in Grand Ouest Interregion France
NCT04374071	Early Short Course Corticosteroids in Hospitalized Patients With COVID-19
NCT04377503	Comparison of the Efficacy and Safety of Tocilizumab Versus Methylprednisolone in the Cytokine Release Syndrome of Patients With COVID-19. A Prospective Randomized Controlled Phase II Trial
NCT04395105	High Versus Low Dose Dexamethasone for the Treatment of COVID-19 Related ARDS: a Multicenter and Randomized Open-label Clinical Trial
NCT04416399	Use of High Dose Inhaled Corticosteroids as Treatment of Early COVID-19 Infection to Prevent Clinical Deterioration and Hospitalisation

NCT04425863	Evaluation of Ivermectin Aspirin Dexametasone and Enoxaparin as Treatment of COVID19
NCT04438980	Treatment of COVID-19 Pneumonia With Glucocorticoids. A Randomized Controlled Trial
NCT04445506	The Effect of the Short-term Use of Systemic Corticosteroids in COVID-19 Patients in Regard to Hospital Length of Stay, Morbidly and/or Mortality.
NCT04451174	Early Use of Corticosteroids in Hospitalized Patients With Moderate COVID19 Pneumonia (PREDCOVID)
NCT04451239	Combined Topical Corticosteroid and Topical Cyclosporine-A for Management of COVID-19 Keratoconjunctivitis; a Pilot Study
NCT04452565	Randomized Controlled Phase 2/3 Clinical Trial of NA-831 Alone or With Atazanavir, or NA-831 With Dexamethasone, or Atazanavir With Dexamethasone in the Treatment of COVID-19 Infection
NCT04476979	Comparison of Tocilizumab Plus Dexamethasone vs. Dexamethasone for Patients With COVID-19
NCT04484493	Role of Corticosteroid Nasal Spray in Recovery of Smell Sensation in COVID-19 Patients
NCT04485429	Efficacy Assessment of Methylprednisolone and Heparin in Patients With COVID-19 Pneumonia: A Randomized, Controlled, 2x2 Factorial Study

NCT04492358	Phase 2/3, Randomized, Open Study to Compare the Efficacy and Safety of Colchicine and Glucocorticoids Compared With the Standard of Treatment for Moderate/Severe COVID-19 in a Fragile and Vulnerable Population, Admitted to a Geriatric Hospital Unit or in a Transicional Care Center
NCT04499313	The Outcome of Dexamethasone and Methylprednisolone Treatment for Patients With ARDS Caused by COVID-19
NCT04509973	Higher vs. Lower Doses of Dexamethasone in Patients With COVID-19 and Severe Hypoxia
NCT04513184	Randomized Clinical Trial of Intranasal Dexamethasone as an Adjuvant in Patients With COVID-19
NCT04519385	Toclizumam Versus Dexamethasone in Severe Covid-19 Cases
NCT04528329	Time to Recover of Anosmia and / or Ageusia and Early Corticosteroid Use
NCT04530409	Timing of Corticosteroids in COVID-19
Tocilizumab	
ChiCTR2000029765	A multicenter, randomized controlled trial for the efficacy and safety of tocilizumab in the treatment of new coronavirus pneumonia (COVID-19)
ChiCTR2000030442	The therapeutic efficacy of combination of Tocilizumab, IVIG and CRRT in sever patients with novel coronavirus pneumonia (COVID-19)
ChiCTR2000030894	Favipiravir Combined With Tocilizumab in the Treatment of novel coronavirus pneumonia (COVID-19) - A Multicenter, Randomized, Controlled Trial

NCT04306705	A Retrospective Study of Evaluating Safety and Efficacy of Tocilizumab Compared to Continuous Renal Replacement Therapy in Controlling CRS Triggered by COVID-19
EUCTR2020-001386-37-IT	Uno studio randomizzato multicentrico in aperto per valutare l'efficacia della somministrazione precoce del Tocilizumab (TCZ) in pazienti affetti da polmonite da COVID-19 (RCT-TCZ-COVID-19)
EUCTR2020-001373-70-FR	IMMUNONCOVID-20 : A prospective, controlled, randomized, multicenter study to compare the efficacy of a chloroquine analog (GNS561), anti PD-1 (nivolumab) and anti-interleukine-6 receptor (tocilizumab) versus standard of care in advanced or metastatic cancer patients with SARS-CoV-2 (COVID-19) infection - IMMUNONCOVID-20
EUCTR2020-001442-19-ES	Pilot, randomized, multicenter, open-label clinical trial of combined use of hydroxychloroquine, azithromycin, and tocilizumab for the treatment of SARS-CoV-2 infection (COVID-19) - TCOVID
NCT04310228	Favipiravir Combined With Tocilizumab in the Treatment of Corona Virus Disease 2019-A Multicenter, Randomized and Controlled Clinical Trial Study
NCT04315480	Tocilizumab (RoActemra) as Early Treatment of Patients Affected by SARS-CoV2 (COVID-19) Infection With Severe Multifocal Interstitial Pneumonia
NCT04332094	Pilot, Randomized, Multicenter, Open-label Clinical Trial of Combined Use of Hydroxychloroquine, Azithromycin, and Tocilizumab for the Treatment of SARS-CoV-2 Infection (COVID-19)

NCT04332913	Efficacy and Safety of Tocilizumab in the Treatment of Patients With Respiratory Distress Syndrome and Cytokine Release Syndrome Secondary to COVID-19: a Proof of Concept Study
EUCTR2020-001160-28-ES	A Randomized, Controlled, Open-Label, Phase II Trial to Evaluate the Efficacy and Safety of Tocilizumab Combined with Pembrolizumab (MK-3475) in Patients with Coronavirus Disease 2019 (COVID-19)-Pneumonia Who Are Unresponsive to Standard Care - Tocilizumab plus Pembrolizumab in COVID-19 (COPERNICO Study)
IRCT20151227025726N13	Evaluation the Efficacy and Safety of Tocilizumab in Patients with Novel Coronavirus (COVID-19)
NCT04345445	An Open-label, Randomized, Cross-over Interventional Study to Evaluate the Efficacy and Safety of Tocilizumab Versus Corticosteroids in Hospitalised COVID-19 Patients With High Risk of Progression
NCT04331808	Cohort Multiple Randomized Controlled Trials Open-label of Immune Modulatory Drugs and Other Treatments in COVID-19 Patients - Tocilizumab Trial - CORIMUNO-19 - TOCI (CORIMUNO-TOCI)
NCT04335071	CORON-ACT - a Multicenter, Double-blind, Randomized Controlled Phase II Trial on the Efficacy and Safety of Tocilizumab in the Treatment of Coronavirus Induced Disease (COVID-19)

EUCTR2020-002032-69-ES	Single-center, randomized, open-label clinical trial on the efficacy of tocilizumab in modifying the inflammatory parameters of patients with COVID-19
NCT04377503	Comparison of the Efficacy and Safety of Tocilizumab Versus Methylprednisolone in the Cytokine Release Syndrome of Patients With COVID-19. A Prospective Randomized Controlled Phase II Trial
NCT04377750	The Use of Tocilizumab in the Management of Patients Who Have Severe COVID-19 With Suspected Pulmonary Hyperinflammation
IRCT20150303021315N17	Evaluation of the safety and efficacy of tocilizumab (AryoGen Pharmed Co., Iran) in patients with severe COVID-19
IRCT20200406046968N1	The effect of IL-6 inhibitor (Tocilizumab) on the prognosis of covid-19 patients with acute respiratory failure
ACTRN12620000580976	Tocilizumab for the treatment of COVID-19 in intensive care patients: effect on days free of ventilatory support.
EUCTR2020-001995-13-ES	A multicentre, open-label clinical trial to evaluate the effectiveness and safety of intravenous tocilizumab for treating patients with COVID-19 pneumonia: the BREATH-19 Study
EUCTR2020-001754-21-FR	An open prospective randomized therapeutic trial using ANAKINRA or TOCILIZUMAB alone or in combination with RUXOLITINIB in severe stage 2b and 3 COVID-19 disease - INFLAMMACOV

NCT04412772	A Randomized, Controlled Clinical Trial of the Safety and Efficacy of Tocilizumab for the Treatment of Severe COVID-19
ChiCTR2000033705	Preliminary efficacy of Tocilizumab in the treatment of the patients with novel coronavirus pneumonia (COVID-19)
IRCT20200510047383N1	Evaluation of the effect of Tocilizumab on outcomes of the severe COVID-19, determining indications within the paradigm of host-directed therapy
JPRN-JapicCTI-205270	A Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients With Severe COVID-19 Pneumonia in Japan
JPRN-UMIN000040614	A retrospective study on the therapeutic effect of tocilizumab on COVID-19 - J-COVID-RT
NCT04359667	Prognostic Value of Serum Interleukin-6 (IL-6) and Soluble Interleukin-6 Receptor (sIL-6R) in Severe Coronavirus Disease (COVID-19) Pneumonia Treated With Tocilizumab - a Prospective Single Center Study (UHID-COVID19)
NCT04361552	Tociluzumab for Cytokine Release Syndrome With SARS-CoV-2: An Open-Labeled, Randomized Phase 3 Trial
NCT04363853	Treatment of Serious and Critical Patients With COVID-19 With Tocilizumab
NCT04435717	Unicenter, Randomized, Open-label Clinical Trial on the Efficacy of Tocilizumab in Modifying the Inflammatory Parameters of Patients With COVID-19

EUCTR2020-001767-86-IE	An open-label, multi-centre, randomised trial comparing different doses of single-dose tocilizumab in adults with severe, non-critical, PCR-confirmed COVID-19 infection with evidence of progressive decline in respiratory function and evolving systemic inflammation on time to intubation, non-invasive ventilation and/or all-cause mortality
NCT04346355	An Open-label Randomized Multicenter Study to Evaluate the Efficacy of Early Administration of Tocilizumab (TCZ) in Patients With COVID-19 Pneumonia
NCT04424056	An Open Randomized Therapeutic Trial Using ANAKINRA, TOCILIZUMAB Alone or in Association With RUXOLITINIB in Severe Stage 2b and 3 of COVID19-associated Disease
NCT04412291	A Single-center, Randomized, Open-label Study in Patients With COVID-19 and Respiratory Distress Not Requiring Mechanical Ventilation, to Compare Standard-of-care With Anakinra and Tocilizumab Treatment The Immunomodulation-CoV Assessment (ImmCoVA) Study
NCT04445272	A Multicentre, Open-label Clinical Trial to Evaluate the Effectiveness and Safety of Intravenous Tocilizumab for Treating Patients With COVID-19 Pneumonia: the BREATH-19 Study
EUCTR2020-001375-32-NL	Pre-emptive tocilizumab in hypoxic COVID-19 patients, a prospective randomized trial - PreToVid

NCT04363736	A Phase-II, Open-Label, Randomized, Multicenter Study to Investigate the Pharmacodynamics, Pharmacokinetics, Safety, and Efficacy of 8 mg/kg or 4mg/kg Intravenous Tocilizumab in Patients With Moderate to Severe COVID-19 Pneumonia
IRCT20081027001411N4	Study of Tocilizumab effect on treatment and clinical symptoms and laboratory signs of Iranian COVID-19 patients: a ?crinical trial study
NCT04317092	Multicenter Study on the Efficacy and Tolerability of Tocilizumab in the Treatment of Patients With COVID-19 Pneumonia
EUCTR2020-001903-17-ES	A randomized clinical trial (IIIb) of eficacy of a single dose of Tocilizumab or a combination of Tocilizumab plus Vitamin D (single i.m. dose) for the treatment of the COVID-19 hyperimmune complication. Assessment of IL-6. - Tocilizumab vs. its combination with Vitamin D. Variation in IL-6
NCT04479358	COVIDOSE-2: A Multi-center, Randomized, Controlled Phase 2 Trial Comparing Early Administration of Low-dose Tocilizumab to Standard of Care in Hospitalized Patients With COVID-19 Pneumonitis Not Requiring Invasive Ventilation
EUCTR2020-001707-16-ES	Phase III randomized, unicentric, open, controlled clinical trial to demonstrate the effectiveness of Tocilizumab against systemic corticotherapy in patients entered by COVID-19 with bilateral pneumonia and bad evolution - TOCICOVID

NCT04423042	A Nested Interventional Cohort Study to Assess the Efficacy and Safety of Adjunctive Humanized Monoclonal Interleukin-6 Receptor Blocker Tocilizumab (TCZ) Therapy to Standard of Care for the Reduction of Hyperinflammation Related Mortality in SARS-CoV-2 Positive Patients
NCT04320615	A Randomized, Double-Blind, Placebo-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients With Severe COVID-19 Pneumonia
NCT04331795	Early Institution of Tocilizumab Titration in Non-Critical Hospitalized COVID-19 Pneumonitis
EUCTR2020-001770-30-BE	COVID 19: Experimental use of tocilizumab (Roactemraå¨) in severe SARS-CoV-2 related pneumonia.
IRCT20200525047570N1	A comparative study of the effects of Tocilizumab, interferon-gamma and vitamin C on the recovery of critically ill COVID-19 patients and cytokine storm
RBR-3zdynp	Clinical Characterisation Protocol for Severe Emerging Infections ISARIC/WHO: COVID-19
PER-027-20	A randomized, double-blind, placebo-controlled, multicenter study to evaluate the efficacy and safety of tocilizumab in hospitalized patients with COVID-19 pneumonia
EUCTR2020-002039-31-FI	COVIDSTORM - study protocol COVID-19: Slavage TOcilizumab as a Rescue Measure - COVIDSTORM

NCT04370834	Tocilizumab in Hospitalized Cancer Patients With Coronavirus 2019 (SARS-CoV-2) and Severe Complications of Coronavirus Disease 19 (COVID-19)
NCT04409262	A Phase III, Randomized, Double-Blind, Multicenter Study to Evaluate the Efficacy and Safety of Remdesivir Plus Tocilizumab Compared With Remdesivir Plus Placebo in Hospitalized Patients With Severe COVID-19 Pneumonia
NCT04356937	Tocilizumab to Prevent the Progression of Hypoxemic Respiratory Failure in Hospitalized Non-Critically Ill Patients With COVID-19
NCT04361032	Assessment of Efficacy and Safety of Tocilizumab Compared to DefeROxamine, Associated With Standards Treatments in COVID-19 (+) Patients Hospitalized In Intensive Care in Tunisia. Multicentric, Comparative, Randomized Study
NCT04372186	A Randomized, Double-Blind, Placebo-Controlled, Multicenter Study to Evaluate the Efficacy and Safety of Tocilizumab in Hospitalized Patients With COVID-19 Pneumonia
NCT04377659	A Phase II Study of IL-6 Receptor Antagonist Tocilizumab to Prevent Respiratory Failure and Death in Patients With Severe COVID-19 Infection
NCT04403685	Safety and Efficacy of Tocilizumab in Moderate to Severe COVID-19 and Increased Inflammatory Markers: a Phase III Randomized Clinical Trial (COVID-19 Coalition Brazil VI) (TOCIBRAS)

NCT04476979	Comparison of Tocilizumab Plus Dexamethasone vs. Dexamethasone for Patients With Covid-19
EUCTR2020-001408-41-DE	A prospective, randomized, double blinded placebo-controlled trial to evaluate the efficacy and safety of tocilizumab in patients with severe COVID-19 pneumonia - TOC-COVID
NL8504	Pre-emptive tocilizumab in hypoxic COVID-19 patients, a prospective randomized trial
Itolizumab	
NCT04475588	A Multi-Centre, Open Label, Two Arm Randomized, Pivotal Phase 2 Trial to Study the Efficacy and Safety of Itolizumab in COVID-19 Complications
CTRI/2020/05/024959	A Multi-Centre, Open label, Two Arm Randomized, Pivotal Phase 2 Trial to Study the Efficacy and Safety of Itolizumab in COVID-19 Complications
RPCEC00000311	Treatment of patients with severe SARS-CoV-2 pneumonia with the anti-CD6 monoclonal antibody itolizumab. (COVID-19) - VICTORIA
Anakinra	
NCT04341584	CORIMUNO-ANA: Trial Evaluating Efficacy Of Anakinra In Patients With Covid-19 Infection, Nested In The CORIMUNO-19
NCT04357366	suPAR-guided Anakinra Treatment for Validation of the Risk and Early Management of Severe Respiratory Failure by COVID-19: The SAVE Open-label, Non-randomized Single-arm Trial

EUCTR2020-001825-29-ES	Clinical trial of the use of Anakinra (ANTI IL-1) in cytokine storm syndrome (CSS) secondary to COVID-19 - ANA-COVID-GEAS
NCT04364009	Efficacy and Safety of Anakinra During Adult " COVID-19" With Aggravating Respiratory Symptoms: a Multicenter Open-label Controlled Randomized Trial
EUCTR2020-001754-21-FR	An open prospective randomized therapeutic trial using ANAKINRA or TOCILIZUMAB alone or in combination with RUXOLITINIB in severe stage 2b and 3 COVID-19 disease - INFLAMMACOV
EUCTR2020-001963-10-FR	Interleukin-1 (IL-1) and Interferon gamma (IFNg) inhibition during COVID 19 inflammation: Randomized, controlled study assessing efficacy and safety of Anakinra and Ruxolitinib - JAKINCOV
EUCTR2020-001734-36-FR	Efficacy and safety of ANAkinra during Adult COVID-19 with Aggravating respiratory symptoms: a multicenter open-label controlled randomized trial - ANACONDA-COVID-19
IRCT20120703010178N20	Evaluation of Safety and efficacy of Anakinra utilization in COVID-19, a randomized controlled clinical trial
NCT04366232	Interleukin-1 (IL-1) and Interferon Gamma (IFNγ) Inhibition During COVID 19 Inflammation: Randomized, Controlled Study Assessing Efficacy and Safety of Anakinra and Ruxolitinib

EUCTR2020-001636-95-GB	Subcutaneous and Intravenous anakinra in COVID-19 Infection - Feasibility & Pharmacokinetics/ Pharmacodynamics study - SCIL-COV19 PK/PD trial
NCT04424056	An Open Randomized Therapeutic Trial Using ANAKINRA, TOCILIZUMAB Alone or in Association With RUXOLITINIB in Severe Stage 2b and 3 of COVID-19 associated Disease
NCT04443881	Clinical Trial of the Use of Anakinra in Cytokine Storm Syndrome Secondary to Covid-19. A Phase 2/3, Randomized, Open-label, Parallel Group, 2-arm, Multicenter Study Investigating the Efficacy and Safety of Intravenous Administrations of Anakinra, an Interleukin-1(IL-1) Receptor Antagonist, Added to Standard of Care, Versus Standard of Care, in Reducing Hyper-inflammation and Respiratory Distress in Patients With SARS- CoV-2 Infection
NCT04412291	A Single-center, Randomized, Open-label Study in Patients With COVID-19 and Respiratory Distress Not Requiring Mechanical Ventilation, to Compare Standard-of-care With Anakinra and Tocilizumab Treatment The Immunomodulation-CoV Assessment (ImmCoVA) Study
NCT04324021	A Phase 2/3, Randomized, Open-label, Parallel Group, 3-arm, Multicenter Study Investigating the Efficacy and Safety of Intravenous Administrations of Emapalumab, an Anti-interferon Gamma (Anti-IFNγ) Monoclonal Antibody, and Anakinra, an Interleukin-1(IL-1) Receptor Antagonist, Versus Standard of Care, in Reducing Hyper-inflammation and Respiratory Distress in Patients With SARS-CoV-2 Infection.

ISRCTN74727214	Anti-interleukin IL-1 receptor antagonist (anakinra) for the treatment of severe COVID-19 pneumonia and hyperinflammatory syndrome in patients admitted at Sultan Qaboos University Hospital
NCT04148430	A Phase II Study of IL-1 Receptor Antagonist Anakinra to Prevent Severe Neurotoxicity and Cytokine Release Syndrome in Patients Receiving CD19-Specific Chimeric Antigen Receptor (CAR) T Cells And to Treat Systemic Inflammation Associated With COVID-19
Canakinumab	
NCT04348448	Observational Study on the Use of Canakinumab Administered Subcutaneously in the Treatment of Patients With COVID-19 Pneumonia
EUCTR2020-001370-30-DE	Phase 3 multicenter, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of canakinumab on cytokine release syndrome in patients with COVID-19-induced pneumonia (CAN-COVID).
EUCTR2020-001370-30-GB	Phase 3 multicenter, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of canakinumab on cytokine release syndrome in patients with COVID-19-induced pneumonia (CAN-COVID).
NCT04365153	Canakinumab to Reduce Deterioration of Cardiac and Respiratory Function in SARSCoV2 Associated Acute Myocardial Injury With Heightened Inflammation
NCT04476706	Managed Access Program (MAP) to Provide Access to Canakinumab Treatment of Cytokine Release Syndrome (CRS) in Patients With COVID-19-induced Pneumonia

NCT04362813	Phase 3 Multicenter, Randomized, Double-blind, Placebo-controlled Study to Assess the Efficacy and Safety of Canakinumab on Cytokine Release Syndrome in Patients With COVID-19-induced Pneumonia (CAN-COVID)
NCT04510493	Canakinumab in Patients With COVID-19 and Type 2 Diabetes - CanCovDia Trial
TNF alpha inhibition	
IRCT20200312046749N1	Evaluation the efficacy of Tumor Necrosis Factor alpha inhibitor in COVID-19 outcomes: a prospective clinical trial study.
Janus Kinase Inhibition	
ChiCTR2000030170	Study for safety and efficacy of Jakotinib hydrochloride tablets in the treatment severe and acute exacerbation patients of novel coronavirus pneumonia (COVID-19)
ChiCTR2000029580	Severe novel coronavirus pneumonia (COVID-19) patients treated with ruxolitinib in combination with mesenchymal stem cells: a prospective, single blind, randomized controlled clinical trial
Leronlimab	
NCT04343651	Study to Evaluate the Efficacy and Safety of Leronlimab for Mild to Moderate COVID-19
NCT04347239	Study to Evaluate the Efficacy and Safety of Leronlimab for Patients With Severe or Critical Coronavirus Disease 2019 (COVID-19)

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• Dementia • Other Neurological Disorders

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