



SARS-CoV-2 pathogenesis

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Abstract | The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a devastating pandemic. Although most people infected with SARS-CoV-2 develop a mild to moderate disease with virus replication restricted mainly to the upper airways, some progress to having a life-threatening pneumonia. In this Review, we explore recent clinical and experimental advances regarding SARS-CoV-2 pathophysiology and discuss potential mechanisms behind SARS-CoV-2-associated acute respiratory distress syndrome (ARDS), specifically focusing on new insights obtained using novel technologies such as single-cell omics, organoid infection models and CRISPR screens. We describe how SARS-CoV-2 may infect the lower respiratory tract and cause alveolar damage as a result of dysfunctional immune responses. We discuss how this may lead to the induction of a 'leaky state' of both the epithelium and the endothelium, promoting inflammation and coagulation, while an influx of immune cells leads to overexuberant inflammatory responses and immunopathology. Finally, we highlight how these findings may aid the development of new therapeutic interventions against COVID-19.

Acute respiratory distress syndrome

(ARDS). Life-threatening lung condition in which the lungs cannot provide enough oxygen to the body after acute lung injury.

Coronaviruses (family *Coronaviridae*) are common pathogens of humans and animals. Four coronaviruses are endemic in humans (human coronavirus NL63 (HCoV-NL63), HCoV-229E, HCoV-OC43 and HCoV-HKU1) and typically infect the upper respiratory tract, causing common-cold symptoms. In the past two decades, three zoonotic coronaviruses (severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2) have infected humans, after spilling over from animal reservoirs^{1–4}. SARS-CoV originated in China and caused an epidemic in 2003, whereas MERS-CoV is currently causing intermittent outbreaks in the Middle East. SARS-CoV-2, the causative agent of COVID-19, was first detected in Wuhan, China, in late 2019 in a cluster of patients with pneumonia⁵. These three viruses can replicate in the lower respiratory tract and may cause a potentially fatal acute respiratory distress syndrome (ARDS) (BOX 1).

SARS-CoV-2, which shares 79% sequence similarity with SARS-CoV, belongs to the genus *Sarbecovirus*⁶. This virus encodes a set of structural proteins (membrane protein, nucleocapsid protein, envelope protein and spike glycoprotein), non-structural proteins (of which most compose the viral replication and transcription complex) and accessory proteins. The structural proteins — together with a lipid bilayer derived from the host — form an enveloped virion (or virus particle) that delivers viral genomic RNA into the cell (FIG. 1a). The accessory proteins are dispensable for replication but often have immunoevasive activities^{7–9}. The main determinant of coronavirus tropism is the spike glycoprotein,

which forms trimers on the surface of virions¹⁰. The spike protein consists of two subunits: the S1 subunit, which binds to the host entry receptor angiotensin-converting enzyme 2 (ACE2)¹¹, and the S2 subunit, which mediates membrane fusion (FIG. 1b). These two subunits are separated by the S1–S2 site, which contains a furin cleavage motif and is cleaved in the virus-producing cell. After binding to ACE2 on the target cell, the spike protein is cleaved by the transmembrane serine protease TMPRSS2 at the S2' site^{12–14}. This cleavage activates the S2 subunit trimers to fuse viral and host lipid bilayers, releasing the viral ribonucleoprotein complex into the cell. Another entry route that may be used by the virus is the endosome, in which cathepsins can cleave the spike protein, but this route is not efficiently used in primary epithelial cells^{14–17}. Other co-receptors (for example, neuropilin 1) and proteases (for example, cathepsin L, TMPRSS11D and TMPRSS13) have been proposed to be involved in SARS-CoV-2 entry as well^{18–21}, but their respective contribution to SARS-CoV-2 pathogenesis remains unclear¹⁴.

The first cells targeted by SARS-CoV-2 during natural infection in humans are likely to be multiciliated cells in the nasopharynx or trachea, or sustentacular cells in the nasal olfactory mucosa^{22–24}. After entry, the positive-sense SARS-CoV-2 genome directly initiates the production of viral proteins, including the replicase proteins that form replication factories from endoplasmic reticulum membranes^{25,26}. These replication factories contain double-membrane vesicles in which transcription occurs, shielding the double-stranded RNA (dsRNA) transcription intermediates from

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Box 1 | Pathogenesis of other human coronavirus diseases

The coronavirus family contains a high diversity of viruses, many of which originate in bats. Severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 share 79% sequence similarity across the entire genome. Severe COVID-19 is highly similar to SARS, the term used to describe severe SARS-CoV disease. Both viruses cause similar symptoms and can lead to ARDS with diffuse alveolar damage as a typical histological pattern, infect ciliated and alveolar type 2 cells, and use angiotensin-converting enzyme 2 (ACE2) as their entry receptor^{13,14,193}. SARS-CoV causes immunopathology similar to that caused by SARS-CoV-2, with typical inflammatory macrophage infiltration and frequent pulmonary embolisms^{194,195}. However, as SARS-CoV caused only ~8,000 cases, there are relatively limited clinical data. From the available data it seems that SARS-CoV may have been more virulent (case fatality rate of ~10% for SARS-CoV versus ~1% for SARS-CoV-2 (REF.¹⁹⁶)) and may have more frequently caused diarrhoea than SARS-CoV-2 (16–73% for SARS-CoV¹⁹⁶ versus 7.4% for SARS-CoV-2 (REF.¹⁹⁷)). In contrast to those infected with SARS-CoV-2, people infected with SARS-CoV were not infectious before the onset of symptoms¹⁹⁶, which may indicate that SARS-CoV-2 replicates more quickly initially and may explain why SARS-CoV transmission could be effectively halted by public health interventions. Increasing age and male sex were risk factors for severe SARS¹⁹⁶.

Little is known on the pathology of Middle East respiratory syndrome coronavirus (MERS-CoV) infection owing to the scarcity of autopsies performed in the Middle East, but the case fatality rate associated with MERS-CoV is around 35%. Clinical and radiological observations indicate that the disease caused by MERS-CoV is similar to COVID-19 and SARS¹⁹⁸. Compared with SARS-CoV and SARS-CoV-2, MERS-CoV uses a different entry receptor, DPP4 (REF.¹⁹⁹), which in humans is expressed on alveolar type 1 cells, alveolar type 2 cells and macrophages in the alveoli²⁰⁰. Epithelial cells in the human upper respiratory tract in health do not seem to express DPP4 (with the exception of submucosal glands)²⁰⁰, which may be why there has not yet been sustained transmission of MERS-CoV in the human population. Secondary cases do occur, but they generally have a lower chance of death, indicating that MERS-CoV is severest in individuals with specific co-morbidities^{198,201}. As with SARS and COVID-19, the main risk factors for severe MERS are increasing age and male sex¹⁹⁸. When compared with SARS and COVID-19, predominantly type 2 diabetes and chronic kidney disease are important co-morbidities for MERS. The disease severity of MERS, SARS and COVID-19 increases with age.

The seasonal alphacoronavirus human coronavirus NL-63 (HCoV-NL63) is currently distributed globally²⁰², but likely jumped from bats to humans several hundred years ago^{203,204}. Although HCoV-NL63 is distantly related to SARS-CoV-2 and SARS-CoV, it uses ACE2 for cellular entry, but is not associated with severe lower respiratory tract infection. One potential reason for this difference may be that HCoV-NL63 uses a different protease for entry. Although there is some evidence supporting and contradicting the view that laboratory strains of HCoV-NL63 can use TMPRSS2 (REFS^{205,206}), it is currently unclear which protease is used by this virus in relevant cells. Recent findings showing that the SARS-CoV-2 Omicron variant does not efficiently use TMPRSS2, or infect alveolar type 2 cells³⁷, suggests that TMPRSS2 use may be an indicator for causing severe pulmonary disease. Further work is needed to address the mechanism behind this difference.

detection by cytoplasmic pattern recognition receptors (PRRs) (FIG. 1c). The main cytoplasmic PRR capable of detecting SARS-CoV-2 is thought to be MDA5 (REFS^{27,28}), which recognizes long dsRNAs and initiates a signalling cascade to promote the transcription of type I and type III interferons. Interferons and chemokines are also produced by bystander epithelial cells and local immune cells (for example, neutrophils and macrophages) in response to the detection of SARS-CoV-2 using endosomal Toll-like receptors (TLRs) or paracrine effects of locally produced interferons^{29–31}. Interferons signal in an autocrine and paracrine fashion to induce an antiviral cellular state through the production of interferon-stimulated genes, which may have direct or indirect (attraction of immune cells) antiviral functions. At the same time, the production of cytokines also promotes the development of adaptive B cell and T cell

responses that help clear the virus. If the virus is not cleared by innate or adaptive responses, it can spread to the lower respiratory tract by inhalation of virus particles from the upper respiratory tract or by gradual dissemination along the tracheobronchial tree. Alternatively, the initial site of infection can be the lower respiratory tract. This can ultimately lead to the infection of the alveoli, causing inflammation and limiting gas exchange. In the alveoli, SARS-CoV-2 has been shown to primarily infect alveolar type 2 (AT2) cells both in vivo and in vitro^{23,32–37}. Whereas alveolar type 1 (AT1) cells cover most of the alveolar surface and mediate gas exchange, AT2 cells secrete pulmonary surfactants necessary for lubricating the lung, which reduces surface tension in the alveoli during respiration. In addition, AT2 cells are the progenitor cells of AT1 cells in the adult human lung³⁸.

The COVID-19 pandemic continues to cause an immense global health crisis, with more than 3.5 million deaths. The overall case fatality rate of COVID-19 is ~1%, and around 3–20% of people with COVID-19 require hospitalization^{39,40}, of which a considerable subset (~10–30%) require intensive care^{41–43}, putting great strain on health systems. Currently, no specific therapies for COVID-19 have been developed, highlighting our limited understanding of the pathogenesis of COVID-19. In this Review, we explore recent clinical and experimental advances in understanding SARS-CoV-2 pathogenesis, interactions with host cells and the involvement of the immune system in the development of severe disease. Specifically, we focus on mechanisms underlying the development of COVID-19-associated ARDS.

COVID-19 clinical findings

SARS-CoV-2 is transmitted through respiratory droplets and aerosols, and the median incubation period is 4–5 days before symptom onset^{44–46}. Although in some cases the infection is asymptomatic, most patients present with mild to moderate respiratory disease, experiencing cough, fever, headache, myalgia and diarrhoea^{46–50}. Severe illness usually begins approximately 1 week after symptom onset. The most common symptom of severe disease is dyspnoea (shortness of breath), which is a result of hypoxaemia^{50,51}. Soon after the onset of dyspnoea and hypoxaemia, progressive respiratory failure develops in patients with severe COVID-19. These patients generally meet the criteria for ARDS^{47,52}, which is defined as severe hypoxaemia and bilateral radiographic opacities occurring within 7 days of exposure to known predisposing factors that is not fully explained by heart failure or fluid overload⁵³. ARDS is a form of lung injury that is characterized by inflammation, pulmonary vascular leakage and consequently a loss of aerated lung tissue. Patients with COVID-19 with hypoxic respiratory failure have evidence of systemic hyperinflammation, including release of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-8 and TNF, and elevated concentrations of inflammatory markers, including D-dimer, ferritin and C-reactive protein (CRP)^{49,54}. Serum levels of IL-6, IL-8 and TNF at the time of hospitalization are strong and independent predictors of patient survival⁵⁴. Severe COVID-19 may also lead to extrapulmonary disease, including

Alveoli

Tiny air sacs in the lungs where oxygen and carbon dioxide are exchanged.

Hypoxaemia

A below-normal level of oxygen in the blood.

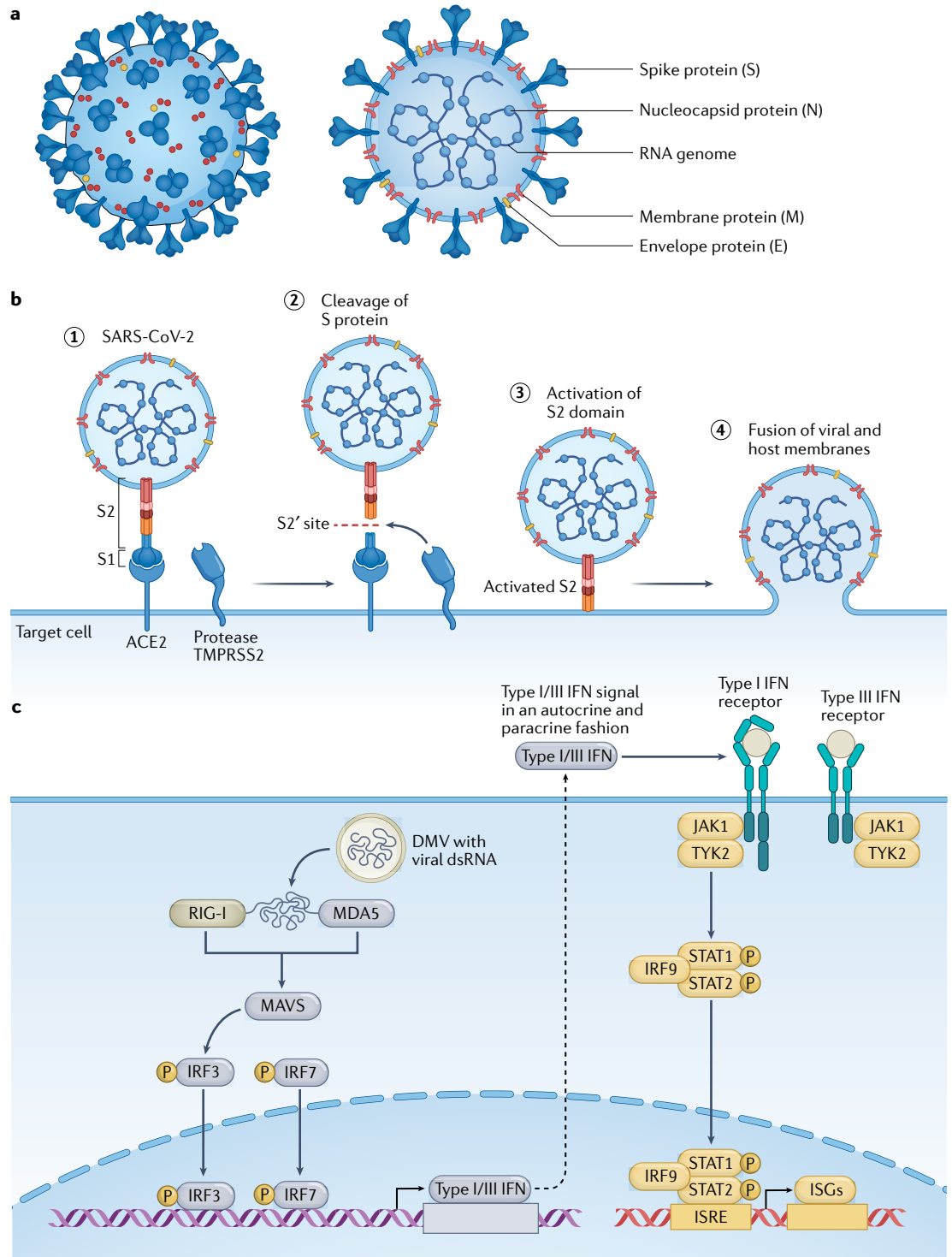


Fig. 1 | Molecular and cellular pathogenesis of SARS-CoV-2. **a** | The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virion consists of the following structural proteins: spike protein (S), nucleocapsid protein (N), membrane protein (M) and envelope protein (E). **b** | The S protein attaches to the receptor angiotensin-converting enzyme 2 (ACE2) on the host cell using the S1 domain (stage 1). This allows TMPRSS2 to cleave the S protein (stage 2), leading to activation of the S2 domain for fusion (stage 3). Activated S2 fuses viral and host lipid bilayers, leading to deposition of the viral positive-sense, single-stranded RNA genome into the host cell (stage 4). **c** | Viral replication creates double-stranded RNA (dsRNA) replication intermediates that can activate cytoplasmic innate immune sensing pathways through activation of MDA5 or RIG-I, initiating a signalling cascade through MAVS that eventually leads to the production of type I and type III interferons (IFNs). These interferons act in a paracrine and autocrine fashion via the plasma membrane receptors and a JAK–STAT1/2 signalling cascade and lead to the production of interferon-stimulated genes (ISGs) that have direct and indirect antiviral functions. DMV, double-membrane vesicle; ISRE, interferon-sensitive response element.

Rhabdomyolysis

A potentially life-threatening condition resulting from the breakdown of muscles with leakage of muscle contents into the circulation.

Coagulopathy

An imbalance in coagulation resulting in either excessive bleeding or clotting.

Anosmia

The inability to smell.

Pulmonary fibrosis

A condition in which the lungs are scarred, impairing the exchange of oxygen for carbon dioxide.

Hyaline membranes

Fibrin-rich exudates that seal the alveoli from fluid accumulation but also limit oxygen exchange.

Coagulation

Blood clotting.

Fibrinolysis

The process of the breakdown of blood clots.

gastrointestinal symptoms and acute cardiac, kidney and liver injury, in addition to cardiac arrhythmias, rhabdomyolysis, coagulopathy and shock⁵⁵. Although SARS-CoV-2 RNA has been detected in several organs at low levels^{56–59}, it is largely unknown to what extent these manifestations are the result of direct infection. Clearly, the intestine can also be infected by SARS-CoV-2, and gastrointestinal symptoms are relatively frequent, yet it is unknown how intestinal infection contributes to severe COVID-19. There is also evidence of the shedding of viral RNA in faeces^{60,61} and productive infection of gut enterocytes⁶², which express higher levels of ACE2 than respiratory cells. In addition, sustentacular cells are the main target of SARS-CoV-2 in the nasal olfactory mucosa, which may be the cause of COVID-19-related anosmia^{24,63}. Although increasing evidence suggests that severe COVID-19 is an inflammatory disease affecting many organs, the primary cause of COVID-19 is pulmonary viral replication, and therefore this Review focuses mainly on the pathophysiology of COVID-19-associated ARDS.

Increased age, obesity and male sex are well-established risk factors for the development of severe COVID-19 (REFS^{64–66}). Common co-morbidities include hypertension, heart failure, cardiac arrhythmia, diabetes, kidney failure and chronic pulmonary disease⁴². In addition, there are genetic predispositions to developing severe COVID-19, which can be highly informative in understanding SARS-CoV-2 pathophysiology. Genome-wide association studies have linked loci containing variants at *DPP9* and *FOXP4* (REFS^{67,68}), which have been linked to pulmonary fibrosis⁶⁹, as well as variants at the chemokine receptor genes *CXCR6* and *CCR9* (for which *CCR1* and *CCR2* are flanking genes) to severe COVID-19 (REFS^{67,68,70}). In addition, genetic predispositions for severe COVID-19 concern genes involved in TLR3-dependent and TLR7-dependent type I interferon induction and amplification^{71,72} and in type I interferon detection⁶⁸. These findings point towards an important role for interferon signalling in combatting SARS-CoV-2. This is underlined by studies that have found that neutralizing autoantibodies to interferon- α (IFN α) are associated with severe COVID-19 (REFS^{73,74}). These antibodies are present in ~4% of uninfected individuals older than 70 years and have been estimated to contribute to ~20% of COVID-19-related deaths⁷⁵.

Pathological findings

Diffuse alveolar damage and COVID-19-associated ARDS. Traditionally, pathologists have investigated the mechanisms behind diseases using histological and immunohistochemical methods. However, in recent years the development of single-cell omics has enabled us to combine what we see through the microscope with quantitative data on cell types and cell type-specific RNA expression patterns. In this section, we aim to integrate histological data on classical and COVID-19-associated ARDS with new insights from single-cell omics.

Histological examination of ARDS cases revealed a common histological lung injury pattern, known as diffuse alveolar damage (DAD)⁷⁶. The term ‘diffuse alveolar damage’ was coined by Katzenstein in 1976 to describe

a type of lung injury characterized by “endothelial and alveolar lining cell injury which leads to fluid and cellular exudation and in some cases progresses to extensive interstitial fibrosis”⁷⁷. DAD is typically characterized by an initial exudative phase with oedema, dying cells, hyaline membranes and inflammation. This is followed by a proliferative (or organizing) phase with AT2 cell hyperplasia in an attempt to regenerate the alveoli. In some cases, there can be a fibrotic phase with fibrosis mostly within the alveolar septa. The clinical syndrome ARDS can be caused by a wide range of predisposing factors, including viral infection.

Histological examination of lung tissues of deceased individuals with COVID-19 on autopsy show that DAD is the predominant pattern of lung injury⁷⁸. The DAD in lungs of deceased individuals with COVID-19 shows features of the exudative and proliferative phases with interstitial and intra-alveolar oedema, dying pneumocytes, hyaline membranes, microvascular thrombosis, capillary congestion and AT2 cell hyperplasia^{78,79}. The death of pneumocytes was confirmed by single-cell sequencing and immunostaining of COVID-19 lungs, which indeed showed a reduction of AT2 and AT1 cells compared with control lungs^{80–82}.

Alveolar epithelial damage and an imbalance in coagulation and fibrinolysis. Alveolar cell death or damage leads to a disruption of the alveolar epithelium, which sets off another key feature of the exudative phase of DAD seen in COVID-19: an imbalance between the activation of coagulation and the inhibition of fibrinolysis⁸³. This process results in the formation of hyaline membranes, which are fibrin-rich exudates that seal the alveoli from fluid accumulation, but also limit oxygen exchange⁸⁴. The same process is responsible for the formation of fibrin thrombi, which are found in the small arterial vessels (less than 1 mm in diameter) in most severe COVID-19 cases^{85–88}. Patients with fibrin thrombi present with elevated levels of D-dimers, fibrin degradation products that accumulate upon fibrinolysis⁸⁹, and high D-dimer levels are associated with fatal outcomes in COVID-19 (REFS^{51,89,90}). Low platelet count is associated with severe COVID-19, likely because platelets are used up for clotting⁹¹. Early initiation of prophylactic anticoagulation was shown to prevent severe disease and death of hospitalized patients with COVID-19, suggesting that coagulation plays a major role in SARS-CoV-2 pathophysiology⁹². The prothrombotic state seen in patients with COVID-19 is reminiscent of a process known as immunothrombosis, in which the immune and coagulation systems cooperate to block pathogens and limit their spread^{93,94}.

What triggers the imbalance in the coagulation system in COVID-19 is currently poorly understood, but it may start with the disruption of the alveolar epithelium. A wide variety of stimuli, such as hypoxia, cytokines, chemokines and damage-associated molecular patterns, can induce a leaky state in both the endothelium and the epithelium in which the bonds between cells can be disrupted^{95,96}. These stimuli trigger endothelial activation and may lead to endothelial cell death, which has been observed in COVID-19 cases⁹⁷. Virus particles

Tissue factor

A protein present inside endothelial cells or on the surface of many non-vascular cells, normally separated from the blood by the vascular endothelium, but which interacts with blood coagulation factors when the endothelium is disrupted, triggering the extrinsic coagulation pathway.

Neutrophil extracellular traps (NETs)

Large, extracellular, web-like structures composed of cytosolic and granule proteins that are assembled on a scaffold of decondensed chromatin and secreted by neutrophils.

were observed inside endothelial cells, but it is currently unclear whether these cells support viral replication *in vivo* and whether infection of these cells contributes to disease severity. In normal physiological conditions, tissue factor resides inside endothelial cells and in tissues, but when the endothelium is disrupted, vascular coagulation factors can interact with tissue factor, triggering the extrinsic coagulation pathway and ultimately leading to the cleavage of fibrinogen into fibrin, a major component of clots and hyaline membranes⁹⁸ (FIG. 2). Tissue damage can also trigger the intrinsic coagulation pathway through activation of factor XII by stimuli such as extracellular RNA, DNA and exposed collagen⁹⁹. At the same time, platelets seal the exposed subendothelial extracellular matrix to stop leakage and provide factors to sustain coagulation¹⁰⁰. Immune cells, attracted by cytokines and chemokines, also contribute to clotting⁹³. Monocytes and monocyte-derived microvesicles can present tissue factor on their surfaces, following the activation of PRRs^{93,101}. Neutrophils release neutrophil extracellular traps (NETs)^{102,103}, which can directly activate factor XII. In patients with severe COVID-19, neutrophils express high levels of tissue factor and release NETs coated with tissue factor, which may further promote clotting¹⁰³. NETs drive coagulation by recruiting platelets, which in turn can release pro-inflammatory cytokines in platelet-derived extracellular vesicles upon activation¹⁰⁴. In turn, activated platelets interact with neutrophils to stimulate NETosis (the formation of NETs)¹⁰⁵. Emerging evidence suggests that SARS-CoV-2 can trigger complement activation, in particular, the lectin pathway, leading to the generation of the cleavage fragment C5a, which increases

expression of tissue factor by neutrophils¹⁰⁶. Recently, a population of CD16-expressing T cells was identified in patients with severe COVID-19 (REF.¹⁰⁷). CD16, which was induced by complement activation, enabled immune complex-mediated degranulation and cytotoxicity. These cells promoted microvascular endothelial cell damage and release of the chemokines IL-8 and CCL2. Another factor that will likely promote clotting in the lungs of individuals with COVID-19 is the epithelial production of IL-6, which induces the transcription of clotting factors in the liver and tissue factor in the endothelium. Elevated levels of circulating IL-6 are predictive of severe COVID-19 (REFS^{54,108,109}). A recent study tested the effect of the IL-6 monoclonal antibody tocilizumab against severe COVID-19 and found that this therapy increased survival and the chance of discharge from hospital by 28 days in patients receiving corticosteroids¹¹⁰.

The formation of fibrin thrombi is counteracted by the fibrinolysis pathway. This pathway is inhibited by plasminogen activator inhibitor 1 (PAI1; also known as SERPINE1). PAI1 inhibits tissue plasminogen activator and urokinase, which both activate fibrinolysis by turning plasminogen into its active fibrin-degrading form, plasmin. PAI1 expression is highly increased in COVID-19, and high levels of PAI1 are associated with worse respiratory status^{111,112}, suggesting that this factor may contribute to the clotting imbalance observed in severe COVID-19. Increased PAI1 expression was also observed in mice infected with SARS-CoV¹¹³. Interestingly, in this mouse model, PAI1 protected mice from extensive lung haemorrhage, which may be a consequence of uncontrolled fibrinolysis in the absence of PAI1.

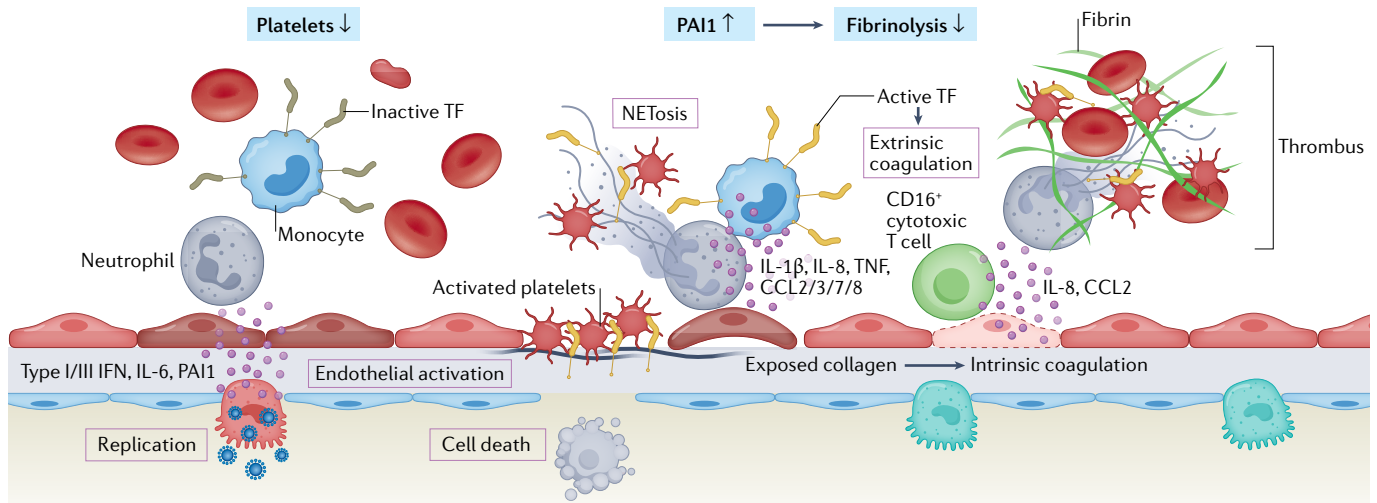


Fig. 2 | Immunothrombosis in severe COVID-19. Severe COVID-19 is characterized by an imbalance in coagulation and fibrinolysis, which may begin with the disruption of the alveolar epithelium. A wide variety of stimuli, such as hypoxia, cytokines, chemokines and damage-associated molecular patterns, can induce a leaky state in both the endothelium and the epithelium in which the bonds between cells can be disrupted. These stimuli can trigger endothelial activation, may lead to endothelial cell death and recruit immune cells (neutrophils and monocytes). CD16⁺ T cells can promote microvascular endothelial cell injury and release of chemokines. The exposed

extracellular matrix can trigger both extrinsic coagulation (via activated tissue factor (TF)) and intrinsic coagulation (for example, via collagen, RNA or DNA), leading to fibrin deposition. Activated platelets bind to the exposed extracellular matrix to seal the injury and stimulate neutrophils together with monocytes to release neutrophil extracellular traps (NETs). NETs contain TF and DNA, stimulating intrinsic and extrinsic coagulation. Ultimately, this immune system-driven process leads to the formation of fibrin thrombi and depletion of platelets. In the meantime, fibrinolysis may be reduced owing to high plasminogen activator inhibitor 1 (PAI1) levels. IFN, interferon.

PAI1 expression is induced by interferon and inhibits entry of influenza A virus by counteracting proteolytic activation of the haemagglutinin protein by TMPRSS2 (REF.¹¹⁴). As SARS-CoV-2 relies on TMPRSS2 as well for proteolytic activation^{12,14}, PAI1 may also exert antiviral effects against SARS-CoV-2. Altogether, the findings indicate that SARS-CoV-2 replication in the lower part of the lungs causes injury to the alveolar epithelium and endothelium, triggering an imbalance in coagulation and fibrinolysis involving fibrin, cytokines, chemokines, platelets, monocytes, neutrophils, NETs, complement activation and PAI1 (FIG. 2).

The roles and phenotypes of immune cells in COVID-19-associated ARDS. Epithelial damage and inflammation attract immune cells. Histology sections of COVID-19 lung show immune cell infiltrates that are largely composed of macrophages in the alveolar lumina and lymphocytes in the interstitium⁷⁸. Single-cell sequencing of post-mortem COVID-19 lung tissue indicates increased infiltration of monocytes and macrophages in comparison with control lungs, and it was noted that monocyte-derived macrophages and alveolar macrophages were aberrantly activated⁸⁰. In the blood, patients with mild COVID-19 had increased levels of inflammatory monocytes expressing high levels of HLA-DR, whereas patients with severe COVID-19 had monocytes expressing low levels of HLA-DR¹¹⁵, which are indicative of monocyte dysfunction¹¹⁶. Monocyte-derived macrophages differentially expressed two long non-coding RNAs (*NEAT1* and *MALAT1*) involved in aberrant macrophage activation and impaired T cell immunity¹¹⁷. Alveolar macrophages showed strongly decreased mRNA and protein expression of the receptor tyrosine kinase AXL, which is important for the clearance of apoptotic cells to reduce inflammation during tissue regeneration¹¹⁸. Monocyte-derived or macrophage-derived IL-1 β and epithelial cell-derived IL-6 have emerged as unique features of SARS-CoV-2 infection compared with other types of viral and bacterial pneumonia⁸⁰. Single-cell sequencing of the lungs from another cohort of individuals with fatal COVID-19 showed increased levels of dendritic cells (DCs), macrophages and natural killer (NK) cells⁸¹. Interestingly, no significant increases in the levels of T cells were detected in single-cell sequencing analyses of post-mortem lung tissues compared with controls, indicating that the lymphocytes seen in histology sections on autopsy may be predominantly NK cells. Elevated levels of circulating adaptive NK cells and an increase in the levels of ‘armed’ NK cells containing high levels of cytotoxic proteins have also been associated with severe disease¹¹⁹. Low levels of T cell infiltration could suggest that impaired T cell responses contribute to lethal outcomes in COVID-19. In a study that performed single-cell sequencing on bronchoalveolar lavage fluid obtained from patients with severe COVID-19 (within 48 h of intubation), an enrichment of monocytes and also CD8⁺ T cells was observed compared with non-pneumonia controls¹²⁰; however, of these patients, 75% survived the infection, perhaps pointing to a beneficial role for T cells in preventing fatal outcomes. In agreement, a study investigated

the difference in cell composition in bronchoalveolar lavage fluid between mild or moderate cases and severe cases and noted that in severe cases there were higher levels of pro-inflammatory macrophages but lower levels of myeloid DCs, plasmacytoid DCs and T cells¹²¹. Impaired B cell function in individuals with cancer who contracted COVID-19 was not associated with increased mortality, whereas a lack of adequate CD8⁺ T cell responses correlated with higher viral load and increased mortality¹²². Alveolar macrophages isolated from bronchoalveolar lavage fluid samples expressed the chemokines CCL7, CCL8 and CCL13, which can drive recruitment of T cells as well as monocytes via CCR2 (REF.¹²⁰). SARS-CoV-2 RNA, including the negative-strand replicative intermediate, was also found within inflammatory monocytes and macrophages, suggesting that they may become infected^{81,120}. Although this infection is likely abortive^{123,124}, it could further amplify the production of pro-inflammatory cytokines by triggering pyroptosis^{125,126}. The triggering of pyroptosis may be induced by the inflammasome activator NLRP1, which was recently shown to be directly triggered by dsRNA¹²⁷. Notably, NLRP1-mediated inflammasome activation is inhibited by DPP9, and an intronic variant in the gene encoding DPP9 was recently found to be associated with severe COVID-19 (REF.⁶⁸), and previously this gene was linked to pulmonary fibrosis⁶⁹. In addition, macrophages that have internalized virus particles may facilitate spreading of SARS-CoV-2 in the lungs¹²⁰. Alternatively, the SARS-CoV-2 RNA within these cells can be derived from phagocytosis of dead epithelial cells. Another single-cell sequencing study investigated nasopharyngeal responses and noted that inflammatory macrophages were enriched in patients with COVID-19 with critical disease and expressed *CCL2*, *CCL3*, *CCL20*, *CXCL1*, *CXCL3*, *CXCL10*, *IL8* (also known as *CXCL8*), *IL1B* and *TNF*¹²⁸. The induction of *CCL2* and *CCL3* corresponded to an induction of *CCR1* — the gene encoding the *CCL3* receptor — in neutrophils, cytotoxic T cells and macrophages, indicating that inflammatory macrophages may drive respiratory inflammation in response to SARS-CoV-2. Patients with critical COVID-19 also displayed a strong enrichment of neutrophils in the nasopharynx compared with patients with moderate COVID-19 and controls.

Neutrophils are the first responders in many viral infections and play crucial roles in antiviral immunity in the airways¹²⁹, but as mentioned earlier herein, excessive NET formation can be detrimental. Whereas neutrophils are generally abundantly observed in ARDS caused by various agents (including infectious agents), only ~30% of patients with severe COVID-19 exhibited neutrophilia in bronchoalveolar lavage fluid^{120,130}, and neutrophils are not the dominant immune cell in COVID-19 lung histology sections^{78,131,132}. In peripheral blood, however, neutrophilia is commonly observed in patients with severe COVID-19 (REFS^{47,49,133–135}). One study suggested that neutrophils are massively enriched in asymptomatic individuals, and are mildly increased in critically ill patients, but that the phenotypes of these neutrophils are heterogeneous¹³⁶. In contrast to the neutrophils of asymptomatic individuals, the neutrophils of critically

Intubation

The placement of a flexible plastic tube through the throat into the trachea (windpipe) to facilitate breathing.

Pyroptosis

An inflammatory form of lytic programmed cell death.

ill patients expressed proteins involved in inflammatory pathways, neutrophil degranulation and NETs. Interestingly, a study detected a progenitor-like neutrophil population that expressed genes involved in degranulation specifically in patients with COVID-19 with ARDS¹³⁷. Another study also described neutrophil precursors, as well as dysfunctional neutrophils expressing programmed cell death 1 ligand 1 (PDL1)¹¹⁵ in the peripheral blood of patients with severe COVID-19. Altogether, these data indicate that the phenotypes of neutrophils in COVID-19 are heterogeneous and that neutrophils could be protective early and pathological later in the infection.

The proliferative phase of COVID-19-associated ARDS.

The proliferative phase of DAD is characterized by AT2 cell hyperplasia, which may reflect the proliferation of AT2 cells in an attempt to regenerate the damaged lung^{78,79}. AT2 cell hyperplasia is observed in COVID-19-associated ARDS. Single-cell sequencing revealed that AT2 and AT1 cells from patients with COVID-19 expressed lower levels of defining markers compared with controls. AT2 cells from patients with COVID-19 displayed decreased expression of *ETV5*, which encodes a transcription factor required for maintaining AT2 cell identity. Expression of this gene is also associated with differentiation towards AT1 cells¹³⁸. However, COVID-19 AT1 cells expressed lower levels of *CAVI*, a marker of late AT1 cell maturation¹³⁹. These data may suggest that the AT2 cells in COVID-19 lungs cannot effectively differentiate to AT1 cells^{80,81}. Recent studies have identified an AT2 cell state associated with lung injury (for example, idiopathic pulmonary fibrosis) and that is characterized by failure to fully differentiate into AT1 cells^{140–142}. This cell state has been termed ‘damage-associated transient progenitors’ (DATPs), ‘alveolar differentiation intermediate’ or ‘pre-AT1 transitional cell state’ (PATS). The relative amount of cells in this state is increased in COVID-19 lungs and they express genes associated with p53, TNF signalling and the hypoxia response via HIF1 α ⁸⁰. This state may be associated with prolonged interferon signalling as a study recently demonstrated that type I and type III interferons interfere with lung repair after influenza virus infection in mice, and that interferon-induced p53 directly reduces epithelial cell proliferation and differentiation¹⁴³. A subset of cells, distinct from KRT5⁺TP63⁺ airway basal cells, expressing genes associated with the PATS programme (*KRT8*, *CLDN4*, *CDKN1A* and *TP63*) were identified in COVID-19 lungs^{81,82}. These cells may resemble a cell type, termed ‘TP63⁺ intrapulmonary basal-like progenitor cells’, identified in mice in response to lung injury caused by H1N1 influenza A virus^{144–146}. It is currently unclear how these transitional cells contribute to the regeneration response, but typically they differentiate into tuft cells (also called ‘brush cells’ or ‘chemosensory cells’) and secretory cells (club cells and goblet cells) and rarely give rise to AT2 cells or AT1 cells^{144,145,147}. In agreement, a study noted an increase in the levels of ectopic tuft-like cells in COVID-19 lungs. The numbers of tuft cells were also increased threefold in the upper airways of patients with COVID-19 (REF.⁸⁰).

Notably, compared with controls, *Pou2f3*^{-/-} mice, which lack tuft cells, showed decreased infiltration of macrophages and decreased expression of chemokine genes (for example, *Ccl3* and *Ccl8*) in response to influenza A virus infection, indicating that these cells may contribute to the pathophysiology of COVID-19 (REF.⁸⁰).

The fibrotic phase of COVID-19-associated ARDS.

Patients with severe COVID-19-associated ARDS display clinical, radiographic, histopathological and ultrastructural hallmarks of pulmonary fibrosis¹⁴⁸. Studies have also noted the expansion of fibroblasts in COVID-19 lungs^{80,81}, and the degree of fibrosis correlated with the duration of the disease, indicating that fibrosis increases over time in COVID-19. A subset of fibroblasts expressed *CTHRC1*, a marker for pathological fibroblasts, which may contribute to the formation of pathological extracellular matrix and may drive lung scarring¹⁴⁹. A recent single-cell transcriptomic study revealed a population of CD163⁺ monocyte-derived macrophages that expressed a profibrotic gene set and displayed similarity to idiopathic pulmonary fibrosis-associated macrophages¹⁴⁸. Notably, human monocytes stimulated in vitro with SARS-CoV-2, but not influenza A virus or viral RNA analogues, displayed a similar transcriptional profile, indicating that SARS-CoV-2 directly triggers this response. These data indicate that a fibrotic phase occurs in COVID-19-associated DAD, which may impair regeneration, leading to chronic respiratory failure.

Mechanisms from in vitro and in vivo findings

In the previous sections, we saw how a tremendous number of descriptive studies have contributed to our understanding of severe COVID-19. However, the mechanisms underlying severe COVID-19 are still largely unknown and need to be assessed experimentally. Arguably, the main questions in understanding SARS-CoV-2 pathogenesis are as follows: what triggers the inflammatory cascade that leads to ARDS and at what stages does this cascade become a self-perpetuating positive feedback loop? COVID-19 animal (BOX 2) and in vitro (BOX 3) experimental model systems are pivotal to study this, and for assessing therapeutic interventions.

Mechanisms behind early upper respiratory tract infection and dissemination to the lungs.

Early events in infection may have a great influence on the development of severe disease. The first cells targeted by SARS-CoV-2 during natural infection in humans are likely to be the ACE2⁺TMPRSS2⁺ multiciliated airway cells in the nasopharynx or trachea^{22–24}. Nasal ciliated cells express high levels of ACE2 and TMPRSS2 on the apical membrane (despite low mRNA levels)^{22,23,150}. This had already been predicted on the basis of findings from studies of SARS-CoV^{151,152} and how readily these cells can be infected by SARS-CoV-2 in air–liquid interface differentiated 2D human airway cultures^{23,62}. In most COVID-19 cases, the infection is likely cleared at this stage through the induction of type I or type III interferon, and the induction of B and T cell responses; however, in some cases the virus can spread to the lower respiratory tract.

Box 2 | COVID-19 animal models

Animal models are important tools for investigating viral pathogenesis and testing intervention strategies (reviewed extensively in REF.²⁰⁷). For severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), several animal models have been established, but most develop a relatively mild disease compared with severe COVID-19 cases.

In ferrets, SARS-CoV-2 replicates mainly in the upper respiratory tract, with hardly any lung pathology^{208,209}. Cynomolgus macaques show some signs of lung pathology with diffuse alveolar damage, with focal exudation, fibrin deposition, inflammatory macrophages and fewer neutrophils and lymphocytes²¹⁰. Similar results were obtained in rhesus macaques, and a slightly more severe disease with coagulation abnormalities reminiscent of the coagulopathy observed in patients with COVID-19 (for example, thrombocytopenia and pulmonary microthrombi) is observed in African green monkeys^{211–213}. Syrian hamsters are very sensitive to wild-type SARS-CoV-2, shed high levels of infectious virus and develop upper and lower respiratory tract infection, but the disease is mild to moderate²¹⁴. Notably, aged and male hamsters seem to develop a more severe disease than young and female hamsters, respectively^{215,216}. Roborovski hamsters compared with Syrian hamsters develop a more severe disease with pulmonary microthrombi²¹⁷.

As wild-type SARS-CoV-2 does not efficiently infect mice owing to ineffective angiotensin-converting enzyme 2 (ACE2) binding²¹⁸, lethal mouse-adapted SARS-CoV-2 models have been set up that capture multiple aspects of severe COVID-19 (REFS^{219–221}). Mice infected with mouse-adapted strains develop diffuse alveolar damage, with focal exudation, sloughed epithelial cells, cellular debris, fibrin deposition and accumulation of inflammatory cells (neutrophils, macrophages and lymphocytes). More severe disease was observed in aged mice^{219–221}, which is in line with results obtained with SARS-CoV in aged cynomolgus macaques²²². A study also noted vessel and basement membrane damage with adherent inflammatory cells in aged mice, but coagulopathies are not typical for SARS-CoV-2 infection in mice. Transgenic mouse models expressing human ACE2 (for example, under the K18 or endogenous mouse *Ace2* promoter) have also been developed (reviewed extensively in REF.²⁰⁷).

Several studies have used the hamster model to assess differences in pathogenicity between SARS-CoV-2 variants. Some variants appear to be more or less pathogenic in this model^{223,224}, but these results must be interpreted with caution as they may be species specific. Some mutations may by chance increase hamster infectivity, such as N501Y, which increases binding to ACE2 (REF.²²⁵). In addition, it is difficult to standardize the infectious inoculum as some variants are attenuated on Vero cells, which are commonly used to grow and titrate virus stocks. In addition, SARS-CoV-2 rapidly adapts to Vero cells upon passaging^{16,26,226,227} (BOX 3), and different isolates may be more or less prone to culture adaptation. Cells containing an active TMPRSS2-mediated entry pathway (for example, TMPRSS2-expressing Vero E6 cells, Calu-3 cells or human airway organoids) can be used to prevent culture adaptation¹⁶. Before such comparative studies are performed, stocks should be grown on cells containing an active TMPRSS2-mediated entry pathway, characterized by deep sequencing, and care must be taken that different variants are similarly infectious on the cell line used for titrations.

SARS-CoV-2 may move deep into the lungs by inhalation of virus particles from the upper respiratory tract, gradually spread by infecting airway cells distally along the tracheobronchial tree or initially directly infect cells in the lower respiratory tract. From in vitro studies using 2D differentiated air–liquid interface organoids, it was concluded that ciliated cells in the lower airways are the main SARS-CoV-2 target, while club cells may be infected occasionally as well³⁶. In 3D distal airway organoids, club cells were identified as the main viral target cell, but these organoids are relatively poorly permissive to infection and consist mainly of progenitor cells, with few mature ciliated cells present³⁴. The infection of secretory airway cells appears to be rare in vivo²², at least in the upper respiratory tract. In general, the interferon response triggered in infected airway epithelial cells is relatively dampened compared with that triggered by infection with influenza A virus¹⁵³. Ciliated cell infection leads to the loss of ciliation in reconstituted human bronchial epithelial cells, which can disturb the

upward flow of mucus in the branching airways¹⁵⁴, possibly facilitating dissemination of virus into the alveoli. Once the virus reaches the alveoli, it seems that the AT2 cells there are susceptible to infection as they express ACE2 and TMPRSS2; they have been found to contain viral RNA on autopsy²³. However, the lack of available material from the early stages of the disease limits our understanding of the early stages of alveolar infection. Studies on AT2 cell organoids allow modelling of this phase^{32–34}. These studies showed that AT2 cells grown in vitro as organoids express ACE2 and TMPRSS2 on their apical membranes, but express little mRNA of the encoding genes, without requiring differentiation. SARS-CoV-2 infection in these cells led to the induction of a type I and type III interferon response. One study also noted that AT2 cells can lose their AT2 marker gene expression in response to the infection and may gain expression of basal cell marker genes, reminiscent of the transitional AT2 cell described in vivo^{80,81}. Besides interferon responses, apoptosis of infected AT2 cells and the induction of inflammatory responses were modelled as well³². Similar findings were obtained with use of a primary alveolosphere model¹⁵⁵. Another important aspect that is modelled in these systems is the decrease in surfactant gene expression in AT2 cells, which is also observed in vivo in late stages of the disease⁸¹. These data indicate that interferon induction, apoptosis, inflammation and loss of surfactant production and AT2 identity are direct effects of virus replication. Recent findings regarding the newly emerged Omicron variant support a central role for AT2 infection in SARS-CoV-2 pathogenesis. The Omicron variant appears to cause fewer hospitalizations and has lost the ability to efficiently replicate in AT2 cell organoids, whereas it efficiently replicates in airway organoids³⁷. Soon, alveolar models may be improved by mimicking the ratio of AT2 cells to AT1 cells found in human alveoli and growing the cells at air–liquid interfaces to allow modelling of leakage. Such systems may also include endothelial cells or specific immune cell subsets to investigate cell type-specific contributions to SARS-CoV-2 pathogenesis. Human organoids can also be used to investigate the roles of specific genes in SARS-CoV-2 pathogenesis using CRISPR systems (BOX 3).

The role of interferons in dissemination and their application in treatment. Dissemination of SARS-CoV-2 to the lower lung can be the result of poor or efficiently inhibited type I or type III interferon responses. Besides autoantibodies to interferon^{73–75} and inborn defects in interferon signalling^{71,72}, low induction of local and systemic interferon responses has indeed been observed in patients with severe COVID-19 (REFS^{156,157}), and this appears to be a general phenomenon associated with ageing¹⁵⁸, including a decrease in the amount of functional plasmacytoid DCs^{158,159}. One study additionally noted that expression of mTOR signalling proteins was decreased in plasmacytoid DCs, suggesting that they may have impaired type I interferon signalling^{160,161}. Although children can become infected with SARS-CoV-2 and shed levels of virus comparable to those shed by adults¹⁶², they rarely develop lethal disease⁶⁶.

Recently, a study characterized the single-cell transcriptional landscape in the upper airways of children and adults, and discovered that children display higher basal levels of relevant PRRs, RIG-I and MDA5, in upper airway epithelial cells, macrophages and DCs. In addition, children displayed stronger innate antiviral responses upon SARS-CoV-2 infection than adults. Notably, at the baseline, children had fewer nasal ciliated cells but more

goblet cells and neutrophils. Single-cell sequencing of nasal epithelial cells from patients with COVID-19 showed extensive induction of type I and type III interferon responses in patients with mild disease, whereas cells from patients with severe disease were essentially muted in their antiviral capacity despite higher local inflammatory myeloid cell populations and equivalent viral loads¹⁵⁶. In vitro studies have shown that SARS-CoV-2 is a poor inducer of interferon¹⁵³ owing to the way SARS-CoV-2 shields its RNA from detection by the host using membrane-enclosed replication factories and the expression of viral proteins that actively block key components of RIG-I-like receptor signalling^{163–165}. On the other hand, SARS-CoV-2 is more attenuated by type I interferon pretreatment than SARS-CoV¹⁶⁶. Compared with influenza A virus, SARS-CoV-2 appears to induce less type I interferon, but fails to counteract STAT1 phosphorylation upon type I interferon pretreatment, resulting in ablation of SARS-CoV-2 replication. Similarly, in a bronchoalveolar SARS-CoV-2 infection model, pretreatment with low concentrations of type III interferon significantly reduced SARS-CoV-2 replication³⁶. In this model, type III interferons were effective against SARS-CoV-2 replication even when added 24 h after infection.

From SARS-CoV studies in mice, we know that robust viral replication accompanied by delayed type I interferon responses leads to the induction of overexuberant inflammatory responses and consequent lung immunopathology¹⁶⁷. Work done on mouse coronaviruses showed that plasmacytoid DCs and plasmacytoid DC-derived type I interferon are crucial for controlling coronavirus infection¹⁶⁸. Plasmacytoid DCs are specialized for the rapid production of large amounts of type I interferon in response to viruses and are believed to be particularly important to control viral infections of the lungs¹⁶⁹. A recent in vitro study of SARS-CoV-2 showed that these cells are the predominant source of IFN α , which is released in response to cell–cell contact with infected epithelial cells (via the α L β 2 integrin–ICAM1 complex) in a TLR7-dependent manner¹⁷⁰. This study also demonstrated that plasmacytoid DC responsiveness correlated with disease severity, and is particularly impaired in patients with severe COVID-19. Moreover, patients with mild COVID-19 had high IFN α and IFN λ blood levels at early time points, whereas IL-6 levels were increased in individuals with severe infections. In SARS-CoV-infected mice, early administration of type I interferon ameliorated immunopathology¹⁶⁷, similar to results obtained with pegylated IFN α in SARS-CoV-infected macaques¹⁷¹ and in MERS-CoV-infected mice¹⁷². In SARS-CoV-infected mice, the delayed type I interferon response was associated with increased accumulation of inflammatory monocyte–macrophages, resulting in elevated inflammatory cytokine levels, vascular leakage and impaired virus-specific T cell responses¹⁶⁷, reminiscent of single-cell sequencing data from patients with COVID-19 (REFS^{80,81,120}). Depletion of inflammatory monocyte–macrophages using monoclonal antibody treatment reduced the levels of inflammatory cytokines (CCL2, TNF and IL-6) in the lung and resulted in protection from lethal disease¹⁶⁷, showing that these cells play a

Box 3 | Experimental systems to study SARS-CoV-2

Traditionally, in vitro model systems in virology are transformed or cancerous cell lines, which have drifted extensively from their natural in vivo counterparts and often have defects in cellular innate immunity, allowing unbridled viral replication. Therefore, findings from traditional in vitro systems should be interpreted with caution. Animal models are important for studying viral pathogenesis and the effect of interventions. However, animal models are labour-intensive and expensive, and often recapitulate only specific aspects of a particular human disease. This is also the case for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) animal models²⁰⁷. An issue with both cell lines and animal models is that the viral target cells in these systems are not representative of the target cells in humans in vivo. This is best exemplified by the rapid adaptation of SARS-CoV-2 to specific cell lines^{16,26,226} and animals^{209,220,221}, and indicates mismatches in virus–host interactions, which could result in the incorrect modelling of defining features of COVID-19 pathophysiology in humans.

Human primary cell models offer an attractive alternative to cell lines and animal models. Primary bronchial or tracheal airway epithelial cultures have been used extensively in virology and accurately model the human airway, but not all cells can be cultured in vitro while maintaining their in vivo phenotypes. For example, human primary alveolar type 2 cells rapidly differentiate to an alveolar type 1-like cell in 2D culture²²⁸. Such primary alveolar cultures are poorly susceptible to SARS-CoV-2 (REF²³). In addition, traditional primary cell cultures are generally not amenable to genetic engineering.

In the past few years, human organoids have emerged as important tools for studying viruses^{62,229–233}. Although human airway organoids had been established several years before the onset of the COVID-19 pandemic²³⁴, methods to grow human adult-derived alveolar organoids were not published until the autumn of 2020, when three groups published their alveolar type 2 cell organoid models and data on SARS-CoV-2 infection modelling^{32–34}. The intestinal epithelium was also shown to support productive viral infection with use of intestinal organoids^{92,233}. A major advantage of intestinal organoids is that they can be efficiently genetically modified using CRISPR tools.

Recent studies have used CRISPR–Cas9 knockout screens to identify factors essential for SARS-CoV-2 replication in cell lines^{21,235,236}. However, these results should be validated in more physiologically relevant infection models²³⁷, such as organoids, to identify which genes or pathways are important enough for the virus to be realistic druggable targets. A recent study generated a validated clonal biobank of organoids containing deletions of genes involved in coronavirus replication, including (putative) (co-)receptors, spike-activating proteases, attachment factors and several genes involved in coronavirus replication¹⁴. This study validated that angiotensin-converting enzyme 2 (ACE2) is indispensable for SARS-CoV-2 replication in gut organoids. Deletion of the proposed co-receptor NRP1 did not impact virus replication^{18,19}. This study also showed that cathepsin L and cathepsin B are not essential for SARS-CoV-2 replication, whereas TMPRSS2 is. Although in vitro several other transmembrane serine proteases (for example, TMPRSS4, TMPRSS11D and TMPRSS13) can prime SARS-CoV-2 (REF²⁰), deletion of the encoding genes in gut organoids did not impact SARS-CoV-2 replication, demonstrating the relevance of genetic knockout experiments in physiologically relevant models. Another study used inhibitors to show that SARS-CoV-2 uses serine proteases (likely TMPRSS2), but not cathepsins, for entry into human airway organoids, suggesting that intestinal and pulmonary epithelial cells possess a similar SARS-CoV-2 entry route¹⁵. These findings may also explain why hydroxychloroquine was not effective against COVID-19 as the virus enters physiologically relevant cells using TMPRSS2 before reaching an acidified endosome with active cathepsins. Drugs targeting TMPRSS2 specifically may be efficient in blocking SARS-CoV-2 entry and dissemination.

A big advantage of 3D organoid technology is that co-cultures with all kinds of cell types (for example, immune cells and endothelial cells) are possible. Organoid studies can greatly advance our mechanistic understanding of severe COVID-19, especially when combined with novel techniques, such as CRISPR gene editing and single-cell omics.

major role in coronavirus-induced pathogenesis. In contrast, a recent study of SARS-CoV-2-infected mice showed that mice lacking the receptor CCR2, which mediates monocyte chemotaxis, had higher viral loads in the lungs, increased lung viral dissemination and elevated inflammatory cytokine responses¹⁷³. This indicates that monocytes could be protective early and pathological later during infection resolution in COVID-19, similarly to neutrophils. These data are consistent with findings in SARS-CoV-2-infected mice, which were protected from death and cytokine shock after TNF and IFN γ had been neutralized¹⁷⁴. This study also found that the combination of these two cytokines induced inflammatory cell death in immune cells, known as PANoptosis.

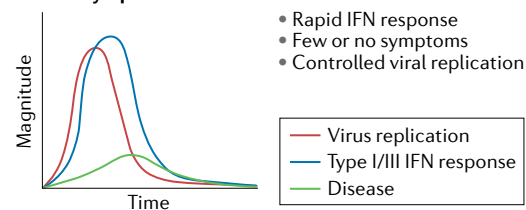
Overall, it seems that type I and type III interferons protect against severe coronavirus-induced pneumonia when their expression or administration is timed correctly (FIG. 3). Given that SARS-CoV-2 effectively inhibits early type I and type III interferon responses, inflammatory responses induced in certain cell types could underlie severe disease. Cross-regulation between type I or type III interferon and IL-1 β signalling systems could potentially further unleash inflammatory responses in the absence of appropriate interferon responses¹⁷⁵. IL-1 β secretion is triggered by inflammasome activation in myeloid cells, and inflammasomes may be triggered by SARS-CoV-2. dsRNA can activate NLRP1 (REF.¹²⁷), whereas several viral proteins, complement activation, reactive oxygen species and cell debris containing double-stranded DNA can activate NLRP3 (REF.¹⁷⁵). IL-1 β is a pleiotropic pro-inflammatory cytokine stimulating inflammatory responses. In support of this, IL-6 and TNF secretion is completely abolished in vitro in SARS-CoV-2-infected primary monocytes treated with exogenous IL-1 receptor antagonist (IL-1RA)¹²⁵. Thus, simultaneous inhibition of type I or type III interferon signalling and inflammasome activation could trigger hyperinflammation, resulting in lung damage and reduced lung regeneration¹⁴³.

Conclusions and perspectives

Most people infected with SARS-CoV-2 do not develop severe disease, and the infection is likely limited to ciliated and sustentacular cells in the upper conducting airways. However, in some people, infection with the virus leads to a severe pneumonia dominated by immunopathology likely set off by infection of the lower respiratory tract. Individuals with severe COVID-19 often have predispositions that lead to poor or mistimed immune responses, in particular type I or type III interferon responses. Alveolar damage may be a direct effect of the infection of AT2 cells or an indirect effect caused by local inflammatory responses. A 'leaky state' of both the epithelium and the endothelium is induced, promoting inflammation and coagulation, with key roles for monocytes or macrophages and neutrophils, which further amplify pro-inflammatory and/or profibrotic responses. Uncontrolled inflammation ultimately leads to severe immunopathology characteristic of COVID-19 (FIG. 4).

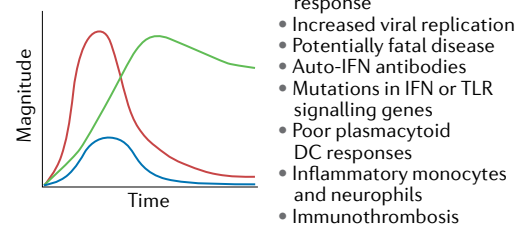
The initial treatment strategy for patients with severe COVID-19 was oxygen therapy using a high-flow oxygen nasal cannula or orotracheal intubation and mechanical

Mild or symptomatic case



- Rapid IFN response
- Few or no symptoms
- Controlled viral replication

Severe COVID-19



- Delayed or poor IFN response
- Increased viral replication
- Potentially fatal disease
- Auto-IFN antibodies
- Mutations in IFN or TLR signalling genes
- Poor plasmacytoid DC responses
- Inflammatory monocytes and neutrophils
- Immunothrombosis

Fig. 3 | Delayed or poor type I and type III interferon responses increase COVID-19 severity. A rapid interferon (IFN) response is associated with controlled viral replication and mild disease (top graph), whereas a poor or delayed interferon response is associated with increased viral replication and severe disease (bottom graph). DC, dendritic cell; TLR, Toll-like receptor.

ventilation. Clinical management of COVID-19, however, has improved during the pandemic. In the first wave in Germany, ~30% of hospitalized patients required intensive care, whereas this dropped to ~14% in the second wave^{42,43}. This drop was associated with several changes in the management of patients with COVID-19, including fine-tuning of ventilation procedures, measures to prevent thromboembolisms^{43,176} and administration of the corticosteroid dexamethasone^{177,178}. Corticosteroids are frequently used general inhibitors of inflammation. Administration of dexamethasone was shown to reduce mortality associated with patients with severe COVID-19 by 50%^{177,178}. Additionally, the inhaled corticosteroid budesonide reduced the likelihood of needing urgent medical care¹⁷⁹. A neutralizing monoclonal antibody to IL-6 (tocilizumab) and a JAK1/2 inhibitor (baricitinib) were also shown to increase survival in hospitalized patients^{110,180}. Tocilizumab treatment restored decreased HLA-DR expression on monocytes in vitro¹³⁴, indicating that the downregulation of HLA-DR on monocytes may be driven by IL-6.

Direct antiviral strategies are therefore expected to be effective only when they are administered very early. Indeed, the antiviral ribonucleoside analogue molnupiravir was shown to be effective against hospitalization and death in outpatients¹⁸¹ but ineffective in patients hospitalized with COVID-19 (REF.¹⁸²). Similarly, an interim analysis of a clinical trial testing a combination of the viral protease inhibitors PF-07321332 and ritonavir suggests that this therapy is effective when administered early in non-hospitalized adults with COVID-19 (REF.¹⁸³). The use of the broadly acting nucleoside analogue remdesivir showed no clinical benefit in patients with severe COVID-19 (REF.¹⁸⁴). An inhibitor of virus entry through the endosomal route, hydroxychloroquine, was also not effective¹⁸⁵, perhaps partially

Thromboembolism

Obstruction of a blood vessel by a blood clot that has become dislodged from another location in the circulation.

because SARS-CoV-2 does not use this entry route in physiologically relevant cells^{14,15} (BOX 3). Another way to directly block virus replication by using monoclonal antibodies or convalescent plasma did not increase survival in patients hospitalized with COVID-19 (REFS¹⁸⁶⁻¹⁸⁸) but did reduce the chance of COVID-19-related hospitalization and death in outpatients¹⁸⁹. These studies confirm that direct antiviral approaches are unlikely to have large effects on mortality when patients are hospitalized,

as the virus has already disseminated deep into the lungs and triggered immunopathology.

Intervention strategies to effectively treat coronavirus-associated ARDS are needed. Clinical observations confirm that the critical stage of severe COVID-19 is dominated by immunopathology, with virus replication playing a secondary role. The beneficial effects of corticosteroids and tocilizumab in patients with severe COVID-19 suggests that better, and perhaps

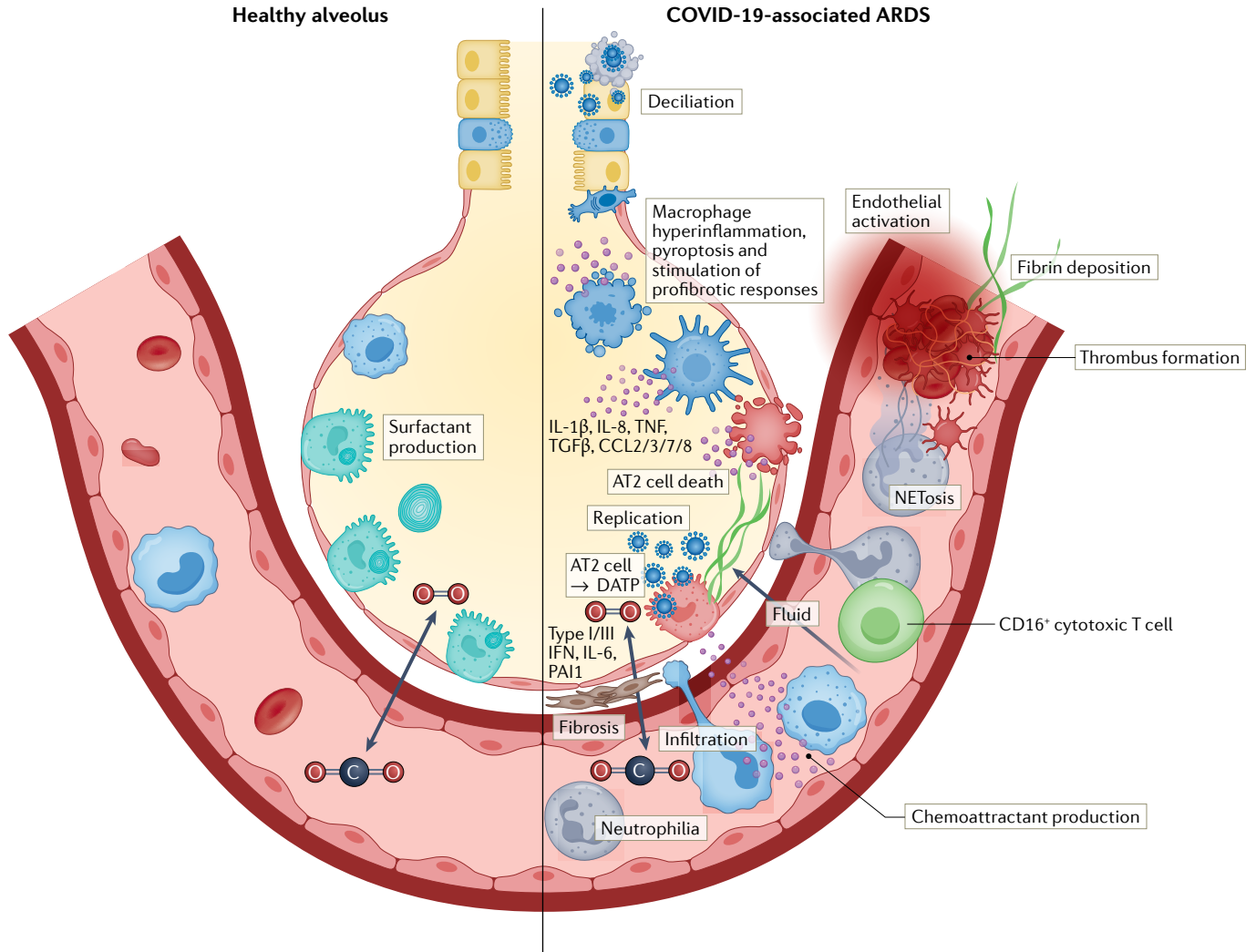


Fig. 4 | A model for COVID-19-associated acute respiratory distress syndrome development. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection starts with the infection of ciliated cells in the upper conducting airways, from where the virus can spread down the bronchiotracheal tree to the alveoli, likely as a result of poor or mistimed immune responses, in particular type I and type III interferon (IFN) responses. Alveolar damage may be a direct effect of the infection of alveolar type 2 (AT2) cells or an indirect effect caused by local inflammatory responses, which can result in endothelial activation. AT2 cells adopt a damage-associated transient progenitor (DATP) phenotype, an AT2 cell state associated with lung injury and that is characterized by failure to fully differentiate into AT2 cells. The disrupted epithelium and endothelium allow fluid to leak into the alveoli. The exposed subendothelial extracellular matrix attracts and activates platelets and initiates the coagulation cascade, leading to fibrin deposition. At the same time, immune cells, such as monocytes and neutrophils, are attracted, and these have dysfunctional

phenotypes and can further promote inflammation and coagulation. Immature neutrophil populations are increased in severe COVID-19. Neutrophils, activated by platelets, release neutrophil extracellular traps (NETs) containing tissue factor, promoting the formation of microthrombi. The upregulation of plasminogen activator inhibitor 1 (PAI1) may further promote microthrombus formation by inhibiting fibrinolysis. Eventually, platelets may be used up, leading to thrombocytopenia. Macrophages in the alveoli may adopt a pro-inflammatory profibrotic phenotype and when infected may go into pyroptosis, while hyperinflammation may promote PANoptosis of T cells. CD16⁺ T cells are induced by complement activation and promote microvascular endothelial cell injury and the release of chemokines. 'Armed' natural killer cells expressing high levels of cytotoxic proteins are also associated with severe disease. The end result is a focal pattern of highly inflamed and flooded lung tissue, impairing oxygen exchange and leading to hypoxaemia. ARDS, acute respiratory distress syndrome.

more specific, immunomodulatory agents may further improve clinical outcomes. Recent studies suggest that reducing inflammation by counteracting immune cell infiltration (for example, by blocking chemokine receptor CCR1, CCR2 or CCR5 (REF.¹²⁸)), inflammasome activation, NETosis or complement activation may be a worthwhile strategy. Ideally these interventions are combined with effective antivirals that are administered early to non-hospitalized patients at risk of severe disease.

The roll-out of highly efficacious vaccines has tremendously decreased the incidence of COVID-19 in developed countries, and a global effort to distribute these vaccines equally is the only way out of this pandemic at the moment¹⁹⁰. Although vaccination has been shown to be the best way to prevent infection and severe disease, another interesting way of decreasing

susceptibility or disease severity may be preactivation of the innate immune system to a state reminiscent of the airways of children¹⁹¹, a concept referred to as ‘trained immunity’¹⁹².

Tragically, the planet’s current changes in climate, wildlife trade, ecosystem health, land use, urbanization and global connectivity guarantee that humans will face new zoonotic coronaviruses, or other zoonotic viruses capable of causing severe pneumonia, in the next few decades. An enormous amount of work has been done to try to understand how SARS-CoV-2 causes COVID-19, but the lack of an effective treatment for COVID-19-associated ARDS shows that there is still a lot to be learned before we are prepared for future zoonotic coronavirus pandemics.

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Author contributions

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