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Innate immunity during SARS-CoV-2: evasion strategies and activation trigger hypoxia and vascular damage

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Summary

Innate immune sensing of viral molecular patterns is essential for development of antiviral responses. Like many viruses, SARS-CoV-2 has evolved strategies to circumvent innate immune detection, including low cytosine–phosphate–guanosine (CpG) levels in the genome, glycosylation to shield essential elements including the receptor-binding domain, RNA shielding and generation of viral proteins that actively impede anti-viral interferon responses. Together these strategies allow widespread infection and increased viral load. Despite the efforts of immune subversion, SARS-CoV-2 infection activates innate immune pathways inducing a robust type I/III interferon response, production of proinflammatory cytokines and recruitment of neutrophils and myeloid cells. This may induce hyperinflammation or, alternatively, effectively recruit adaptive immune responses that help clear the infection and prevent reinfection. The dysregulation of the renin–angiotensin system due to down-regulation of angiotensin-converting enzyme 2, the receptor for SARS-CoV-2, together with the activation of type I/III interferon response, and inflammasome response converge to promote free radical production and oxidative stress. This exacerbates tissue damage in the respiratory system, but also leads to widespread activation of coagulation pathways leading to thrombosis. Here, we review the current knowledge of the role of the innate immune response following SARS-CoV-2 infection, much of which is based on the knowledge from SARS-CoV and other coronaviruses. Understanding how the virus subverts the initial immune response and how an aberrant innate immune response contributes to the respiratory and vascular damage in COVID-19 may help to explain factors that contribute to the variety of clinical manifestations and outcome of SARS-CoV-2 infection.

Keywords: COVID-19, endothelia, immunology, inflammation, SARS-CoV-2

Introduction

The emergence in Wuhan, China, of a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggered an epidemic of the coronavirus disease 2019 (COVID-19). As of 9 September 2020, the confirmed 27 761 748 cases, including 902–306 deaths, have been reported worldwide (worldometers.info/coronavirus). At the end of January 2020, the World Health Organization (WHO) declared COVID-19 a pandemic and a global health emergency.

The family *Coronaviridae* is subdivided into *Torovirinae* and *Coronavirinae*, that contains the genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*. The human coronaviruses (HCoV) belong to the αlpha-CoV (HCoV-229E and HCoV-NL63) and beta-CoV (Middle East respiratory syndrome coronavirus-MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) (Table 1 [1–11]). In comparison with most HCoVs that cause mild upper respiratory tract infections, SARS-CoV, MERS-CoV and SARS-CoV-2 induce severe pneumonia [12]. The clinical presentation of COVID-19 ranges from mild 'flu-like' symptoms to severe respiratory failure and death, although between 17·9 and 57% of SARS-CoV-2

Table 1. HCoVs – cell receptors and co-factors aiding viral entry

Virus	Date emergence and clinical manifestations	Primary cell receptor	Tissue expression of receptor	Receptors and co-factors augmenting viral entry	Refs.
HCoV-229E	1965 URTI, common cold	APN (CD13)	Renal and GI epithelia, synaptic membranes, pericytes, myeloid cells, fibroblast-like cells, neurones	TMPRSS2 TMPRSS11D	[1–3]
HCoV-OC43	1967 URTI, common cold	9-O-acetylated sialic acid	Human epithelial cells, neurones	IFIT2/IFIT3 HLA-1	[4,5]
SARS-CoV	2002 Severe acute respiratory syndrome (SARS)	ACE2	Respiratory, intestinal epithelial cells, endothelial cells, renal tubules, cerebral neurones, alveolar macrophages, DCs	Cathepsin L, TMPRSS2/11D DC-SIGN (CD206), DC-SIGNR	[6,7]
HCoV-NL63	2004 Bronchitis URI, common cold	ACE-2	Pneumocytes, intestinal epithelial cells, endothelial cells, renal tubules, cerebral neurones, alveolar macrophages, DCs	Heparan sulphate proteoglycans	[8]
HCoV-HKU1	2005 Pneumonia common in children	9-O-acetylated sialic acid	HUMAN alveolar type II cells	HLA-C	[9]
MERS-CoV	2012 SARS	DPP4 (CD26)	Intestinal, alveolar, renal, hepatic and prostate cells activated leucocytes	Furin	[10]
SARS-CoV-2	2019 SARS, severe disease associated with ageing and co-morbidities	ACE2	Respiratory, intestinal epithelial cells, endothelial cells, renal tubules, cerebral neurones, alveolar macrophages, DCs	Furin TMPRSS2	[11]

ACE 2 = angiotensin-converting enzyme 2; APN = aminopeptidase N; DCs = dendritic cells; DC-SIGN = dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (CD209); DC-SIGNR = DC-SIGN receptor; DPP4 = dipeptidyl peptidase 4; GI = gastrointestinal; HLA-C = human leucocyte antigen C; IFIT = interferon-induced proteins with tetratricopeptide repeats; TMPRSS = type II transmembrane serine proteases; URTI = upper respiratory tract infection.

infections are asymptomatic, depending on the population [13]. Common symptoms include fever, cough, fatigue, shortness of breath, headache and pneumonia. In addition, some patients develop gastrointestinal problems [14] and neurological manifestations, including headache, dizziness, hyposmia and hypogeusia. Age and co-morbidities, i.e. hypertension, chronic obstructive pulmonary disease, diabetes, obesity and cardiovascular disease, predispose to more severe manifestations, including severe respiratory failure, septic shock, coagulation dysfunction, strokes, cardiovascular problems [15] and neurological manifestations [16]. Although the origin and transmission of SARS-CoV-2 is unclear, genome sequencing reveals marked similarities with SARS-CoV [17]. However, in comparison, SARS-CoV-2 spreads more quickly than SARS-CoV, probably due to the 10–20%-fold higher in infectivity and transmissibility during the initial non-symptomatic period (4–5 days). In some cases, transmission has been reported after development of initial symptoms despite the presence of antibodies [18], indicating that both neutralizing antibodies and T cell responses are necessary to prevent reinfection and for protection [19]. This is further supported by studies showing that programmed cell death 1 $(PD\text{-}1)^{+}CD57^{+}$ T cell exhaustion, depletion or inactivation is associated with viral persistence in severe cases [20].

SARS-CoV-2 is a positive-sense RNA (29 903 nucleotides) enveloped virus of 60–140 nm diameter [21]. The envelope is studded with homotrimers spike proteins of 8–12 nm length that are heavily decorated with N-glycans (Fig. 1) [22,23]. Similar to other HCoVs, the SARS-CoV-2 genome encodes for four structural proteins: the spike (S), membrane (M), envelope (E) and the nucleocapsid

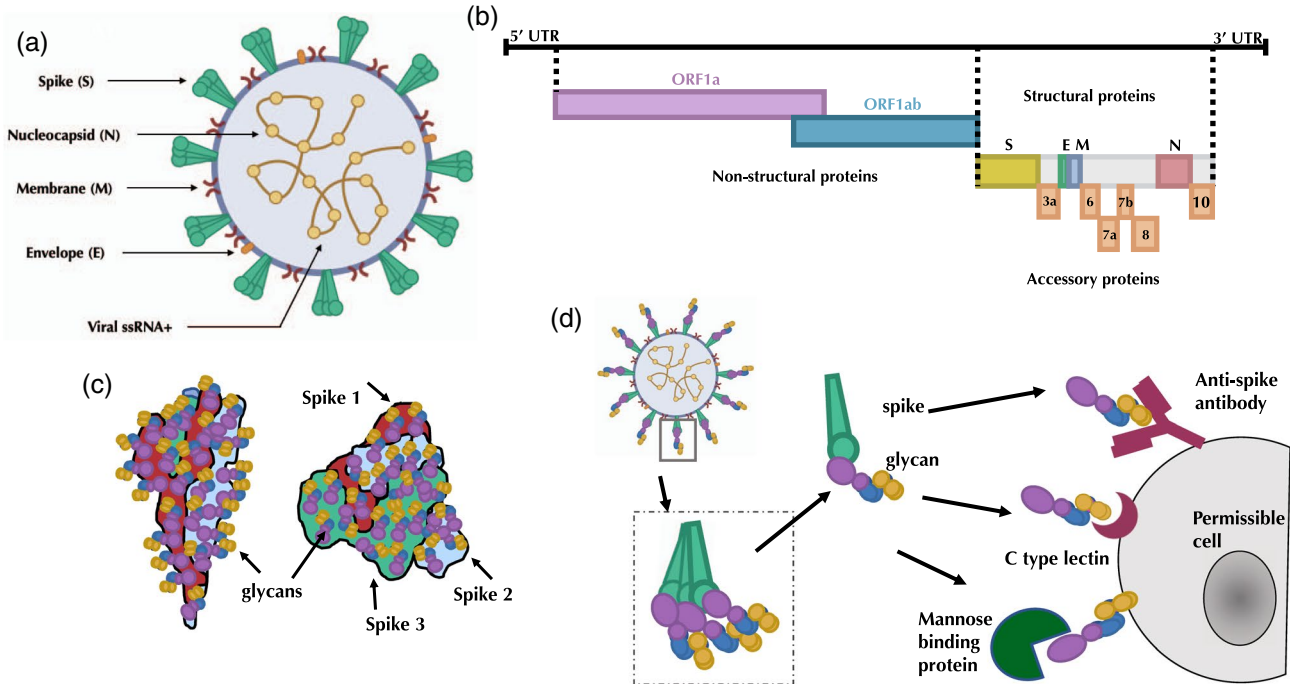

Fig. 1. SARS-CoV-2 structure and genome. (a) SARS-CoV-2 is a positive-sense RNA enveloped virus with the spike (S), membrane (M), envelope (E) proteins embedded in the lipid envelope, while the nucleocapsid (N) protein is associated with the RNA. (b) The 5′ end of the genome is comprised of open reading frame (ORF)a/ab encoding two large polyproteins, including the replicase protein crucial for self-generation of the non-structural proteins (nsp), while ORFs 2–10 encode the viral structural proteins (S, M, E and N) and accessory proteins. (c) The homotrimers spike proteins of 8–12 nm length are heavily decorated with N-glycans moieties that can be recognized by antibodies, C-type lectins and mannose-binding proteins that aid viral attachment to permissible cells, activate the complement system and may be recognised by macrophages and antibodies (d).

(N) protein. The 5′ end of the genome is comprised of open reading frame (ORFa/ab), encoding two large polyproteins including the replicase protein crucial for self-generation of the non-structural proteins (nsp), while ORFs 2–10 encode the viral structural proteins – spike, envelope, membrane and nucleocapsid, and the accessory proteins (Fig. 1b). Differences between the structural, non-structural and accessory proteins of SARS-CoV-2 and other coronaviruses help to explain the high infectivity rate and the range of pathologies observed [12,15,16]. While knowledge of SARS-CoV-2 is rapidly emerging, parallels with SARS-CoV, as well as ongoing sequencing data and antigenic typing, will be crucial to understand the dynamics of the pandemic. SARS-CoV-2 cell entry is similar to SARS-CoV, being mediated by the binding of the receptor-binding domain (RBD) of the S1 protein to the angiotensin-converting enzyme-2 (ACE-2), although other receptors, such as CD147 and CD-specific intercellular adhesion molecule-3-grabbing integrin (SIGN), have been reported (Table 1). Docking of the RBD to the receptor and the action of furin, a serine protease that separates the S1 and S2 proteins, exposes a second binding domain on S2 allowing membrane fusion. Binding of the S protein to ACE-2 requires priming by cell proteases, primarily transmembrane protease, serine 2 (TMPRSS2); however, TMPRSS2 is expressed by a subset of ACE2$^+$ cells supporting the notion that the virus probably uses other host enzymes such as TMPRSS4, lysosomal cathepsins and neuropilin-1 [24] to augment the impact of furin and expose the RDB, thus promoting SARS-CoV-2 entry [11]. The structural proteins M, E and N are crucial for stability of the viral genome and viral replication. The nsp and accessory proteins [25], encoded by 10 ORFs, have differing functions during viral replication (Table 2) [26–63] and many also act to deviate the innate immune response, thus augmenting viral replication and spread. The degree to which the innate immune system is suppressed and evaded clearly determines the viral load and the host's outcome to infection, the clinical symptoms and the severity of the disease.

Evading pattern recognition receptors

Following infection, viral RNA is sensed by several classes of pattern recognition receptors (PPRs). The retinoic acid-like receptors (RLRs) include retinoid inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5), Toll-like receptors (TLR) – classically 3, 7 and 8, that trigger IFN pathways – and cytokine

Table 2. Immune evasions strategies of genome and encoded proteins of SARS-CoV and (by inference) SARS-CoV-2

Gene	Protein	Function	Impact on immune response action	Comments	Refs.
Reduced CpG			Decreased activity of ZAP and APOBEC3G.	ZAP expressed in immune cells	[26]
ORF1a	nsp1	Mediates RNA replication and processing. Involved in RNA degradation	Modulates calcineurin/ NFAT pathway. Cleaves host RNA. Inhibits cyclophilins and immunophilins. Blocks ATF2/c-Jun, IRF3 and IRF7, NF-κB, decreases STAT-1 phosphorylation. Interferes with RIG-1 pathway	Associated with immune pathogenicity and long-term cytokine dysregulation. Promotes host RNA degradation	[27–29]
	nsp2	Replicase essential for proofreading replication	May bind to prohibitin 1 and 2 involved in apoptosis, mitochondrial biogenesis and intracellular signalling. Modulates host survival signalling	Associated with pathogenicity. Deletion attenuates viral growth and RNA synthesis	[30,31]
	nsp3	Papain like protease (PLpro). Processes pp1a and pp1ab	De-ubiquitinates and deISGylates host proteins. Blocks IFN-α, IFN-β, CXCL10 and CCL5. Inhibits TLR-7 signalling by removing Lys63-linked polyubiquitination of TRAF3 and 6.	Forms DMV and replication process evade innate immune recognition	[32–34]
		Complexes with nsp4 and nsp6	Antagonizes IRF3, stabilizes IκBα, thereby blocking NF-κB signalling		
			Interacts with STING-TRAF–TBK1 complex		
	nsp4	Complexes with nsp3 and nsp6 to form the DMV. May anchor RTC to ER	Helps replication process evade innate immune recognition		[33]
	nsp5	Chymotrypsin-like protease (3CLpro)	Induces apoptosis and growth arrest via caspase-3 and caspase-9		[35]
	nsp6	Complexes with nsp3 and nsp4 to form DMV	Helps replication process evade innate immune recognition. Activates autophagosome		[33]
	nsp7	Complexes with nsp8 and nsp12 for viral replication		Nsp7–nsp8 form the primase complex	[36]
	nsp8	Complexes with nsp7 and nsp 12 for viral replication		Nsp7–nsp8 form the primase complex	[36]
	nsp9	Involved in viral genomic RNA reproduction but exact role unclear		Interacts with nsp8	[37,38]
	nsp10	Complexes with nsp 1, 7 and 14. Multifunctional co-factor in replication	Interacts with the oxidoreductase system causing cytopathic effect	Activator of nsp14 function	[39–41]
			Aids RNA capping, thus evades RIG-1 and MDA-5 recognition	Forms a complex with nsp16	
	nsp11	Peptide resulting from cleavage of pp1a at nsp10/11 junction	Not known		
ORF1b (nsps in addition to 1–11)	nsp12	RNA-dependent	Targeting mitochondria limits host cellular responses		[36]
		RNA polymerase (RdRp)			
	nsp13	Helicase key for efficient replication of viral genome	Caps RNA, thus evades RIG-I and MDA-5 signalling	Failure to trigger IFIT1	[42]

(Continues)

Table 2. (Continued)

Gene	Protein	Function	Impact on immune response action	Comments	Refs.
	nsp14	Exons 3′–5′ exonuclease play crucial role in viral RNA synthesis and capping. Complexes with nsp10	Involved in the capping through its function as a guanine-N7 methyltransferase helping nsp16 evade RIG-1 and MDA-5 recognition		[43]
	nsp15	Uridylate-specific endoribonuclease (EndU)	Limits exposure of viral dsRNA to the sensors MDA-5, PKR and OAS/RNaseL. Inhibits poly U, thereby evading MDA-5 thus antagonizing IFN-a/β production		[44]
	nsp16	2′-O-ribose methyl transferase involved in RNA capping. Complexes with nsp10	Caps RNA, thus evades RIG-I and MDA-5 signalling	Failure to trigger IFIT1	[41,45,46]
ORF2	Spike	Heavily glycosylated with 22 glycans	Masks immunogenic protein epitopes		[22,23,47–49]
		ACE/ACE-2 interaction	Induced misbalanced in RAS that triggers inflammation		
		Requires priming to expose membrane fusion	Masks immunogenic protein epitopes		
ORF3a	ORF3a	Interact with SARS-CoV M, S, E and 7a proteins	Activates PERK pathway, triggers apoptosis through expression of ATF4 and CHOP. Down-regulates and degrades type 1 IFNR	Expressed on cell surface. Induces fibrinogen, stress pathways, necrotic cell death, activates inflammasome	[50–52]
		Forms viroporins			
ORF4	Envelope	Essential for viral assembly and budding. Forms viroporins	Induces ROS and activates inflammasome		[53,54]
ORF5	Membrane	Important for viral assembly	Inhibits type I interferon production by impeding the formation of TRAF3. TANK. TBK1/IKKε complex	Induces apoptosis	[55–57]
ORF6	ORF6	Plays a role in viral pathogenesis, interacts with ORF8. May aid viral virulence	Inhibits STAT-1 nuclear import	Promotes RNA polymerase activity	[51]
ORF7a	ORF7a	Interacts with S protein and p3a	Inhibits BST-2 glycosylation, leading to a loss of function of BST-2. SARS-CoV ORF7a induces caspase-dependent apoptosis	BST-2 restricts virion egress by tethering virions to plasma membrane. Interacts with LFA	[58,59]
		Not essential for replication			
ORF7b	ORF7b	Not essential for viral replication but structural component of the virion		It is an integral membrane protein located in the Golgi compartment	[60]
ORF8	ORF8	Differs from other HCoVs	Interact and down-regulates MHC-I	SARS-CoV encodes p8a and p8b that induce caspase-dependent apoptosis and activates UPR	[61]
ORF9	Nucleocapsid	Stabilizes viral RNA	Targets MAVS–RAF3–TRAF6 and antagonizes IFN-β		[51,62,63]
		Interacts with stress granules G3BP1			
ORF10	ORF10	Ubiquitin ligase	Unknown		

ACE 2 = angiotensin-converting enzyme 2; BST = bone marrow stromal antigen 2; CHOP = C/EBP homologous protein; DMV = double membrane vesicles; ER = endoplasmic reticulum; HCoV = human coronavirus; IFIT = interferon-induced proteins with tetratricopeptide repeats; IFN = interferon; IFNR = IFN receptor; LFA = lymphocyte function-associated antigen 1; MAV = mitochondrial anti-viral signalling protein; MDA = melanoma differentiation-associated; MHC = major histocompatibility complex antigen; ORF = open reading frame; PERK = PRKR-like endoplasmic reticulum kinase; RAS = renin–angiotensin system; RIG-1 = retinoic acid-inducible gene I; ROS = reactive oxygen species; RTC = replicase–transcriptase complex; STAT = signal transducer and activator of transcription; UPR = unfolded protein response; ZAP = zinc finger anti-viral protein.

production (Fig. 2). Once engaged these PPRs act downstream via the kinases TANK-binding kinase-1 (TBK1) and inhibitor-κB kinases (IKKs). Such triggering leads to the activation of the transcription factors interferon (IFN)-regulatory factor-3 (IRF3) and 7 (IRF7) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). These subsequently induce expression of type I IFNs (IFN-α/β) and IFN-stimulated genes (ISGs) (Fig. 2), many of which have potent anti-viral activities, as well as other proinflammatory mediators; for example, cytokines, chemokines and anti-microbial peptides that are essential to initiate the host innate and adaptive immune response. In addition, the absent in melanoma 2 (AIM2)-like receptors and NOD-like receptors (NLRs) trigger the inflammasome and IL-1β and IL-18 production, leading to pyroptosis (Fig. 2). Other PPRs and downstream factors relevant to SARS-CoV infection subversion of innate immune responses include C-type lectins and the stimulator of IFN genes (STING). While the cGas/STING pathway is commonly associated with sensing cytosolic DNA, it is also activated following binding of enveloped viruses to host cells and cytosolic viral RNA [64,65]. Similar to TLRs and RLR, STING engages TBK1 downstream to active IRF3 and/or NF-κB inducing type I IFN and/or proinflammatory cytokines (Fig. 2).

Coronaviruses have evolved several strategies to escape such innate immune recognition, allowing widespread replication. Such evasion includes evolution of low genomic CpG, RNA shielding, masking of potential key antigenic epitopes as well as inhibition of steps in the IFN type I/III pathways. Generally, the zinc finger

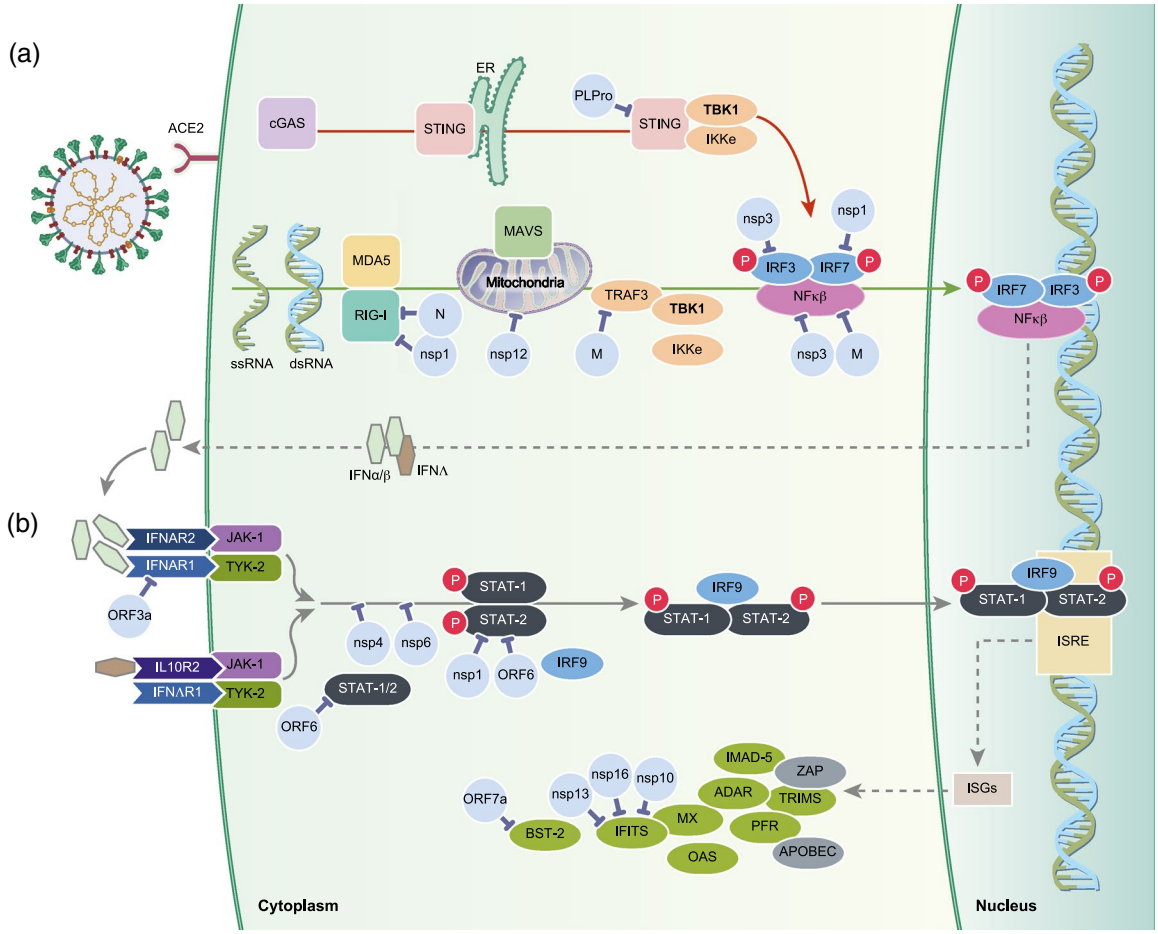

Fig. 2. SARS-CoV-2 subversion of interferon (IFN) pathways. SARS-CoV-2 infects permissible cells via the angiotensin-converting enzyme 2 (ACE2). Following infection (a) the virion or viral RNA is sensed by either the cGas/STING pathway where stimulator of interferon genes (STING) engages TBK1, or via retinoid inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5). These pathways lead to activation of IFN-regulatory factor (IRF3) and/or nuclear facror kappa B (NF-kB) inducing type I/III IFN that is recognized by IFN receptors (b) and subsequent induction of the IFN-stimulated genes (ISGs) and proteins, many of which have potent anti-viral activities. Based on the knowledge of other coronaviruses, especially SARS-CoV, and emerging data from SARS-CoV-2, many of the non-structural, structural and accessory protein subvert and inhibit numerous steps in these pathways, thereby inhibiting IFN production allowing increased viral replication.

anti-viral protein (ZAP) specifically binds to and degrades CpG motifs in genomes of RNA viruses. In comparison with other viruses, SARS-CoV-2 has evolved the most extreme cytosine–phosphate–guanosine (CpG) deficiency of all betacoronaviruses (Table 2) [26]], thereby evading ZAP action. This suggests that SARS-CoV-2 may have evolved under selective pressure in either a new host or tissues expressing high levels of ZAP [26]. Another strategy to protect mRNA used by the host and many viruses is the processing of capping the 5′ end. For both host and virus RNA, capping limits degradation and importantly blocks recognition by cytosolic PPRs. Like many RNA viruses, SARS-CoV-2 has exploited several mechanisms to protect the 5′ ends by a cap structure of RNA generated during replication. While some viruses snatch the caps from host RNA, SARS-CoV-2, like other coronaviruses, uses its own capping machinery composed of nsp10, nsp13 and the dedicated enzyme nsp16 to generate 2′-o-methyltransferase caps (Supporting information, Fig. S1 [41]). SARS-CoV-2 yields RNA caps indistinguishable from cellular mRNAs caps, thereby evading detection by MDA-5 and IFIT activity that target RNA for degradation (Fig. 2). The importance of such capping and viral replication is supported by studies of SARS-CoV in mice lacking 2′-O-MTase activity underscoring that MDA-5 and the IFN-induced proteins with tetratricopeptide repeats (IFIT) family are critical for IFN signalling [45] While counterintuitive, SARS-CoV uses its endoribonuclease (nsp15) to cleave its own viral RNA in the cytosol that would otherwise act as PAMPs, thus evading MDA-5, protein kinase R (PKR) and OAS/RNAse L [44,66] However, another strategy used by SARS-CoV-2 to protect the viral RNA and proteins generated during replication (Supporting information, Fig. S1) is the use of replicase–transcriptase complex (RTC) or replication organelle, formed of double membrane vesicles [33]. The RTCs link with the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) and Golgi apparatus shielding the virus during maturation (Supporting information, Fig. S1). Another immune evasion strategy utilized by coronaviruses is the use of glycans and probably other post-translational modifications to mask immunogenic viral protein epitopes (Fig. 1c,d). The envelope of SARS-CoV-2 is studded with glycoprotein spikes comprised of homotrimers spike proteins of 8–12 nm length that are heavily decorated with glycans. Each spike protein is comprised of two subunits (S1 and S2) that each bear 22 glycan groups [49]. Cell entry of the highly glycosylated S protein of SARS-CoV is promoted by DC-SIGN, possibly augmenting virus uptake or aiding capture and transmission of SARS-CoV by DCs and macrophages [6–8]. Similar to the spike protein, the other structural, non-structural and accessory proteins are also modified by glycosylation, palmitoylation, phosphorylation, SUMOylation and ADP-ribosylation [67]. Conversely, some viral proteins, for example nsp3, possess de-ubiquitinating (DUB) and deISGylation activity, thereby interfering with host functions targeting those that are critical for signalling transduction of innate immunity [34]. Insertion of the spike protein into cell membranes during replication is a key step for virus budding. While this takes place in the RTC (Supporting information, Fig. S1), receptor-bound spike proteins interact with TMPRSS2 expressed on the uninfected cell surface and mediates fusion between infected and uninfected cells promoting the formation of syncytia, allowing the virus to spread to adjacent uninfected cells while evading detection by the immune response [68].

SARS-CoV-2 subversion of innate immune responses

In addition to strategies to evade PPR recognition, SARS-CoV-2 has also evolved strategies to inhibit steps in the pathway leading to type I/III IFN production. This may be especially relevant in the lungs, where type IFN III (lambda) is considered to be more effective in controlling viral infections and critically affected in COVID-19. Knowledge arising from the study of other coronaviruses, especially SARS-CoV and MERS, has shown that many of the non-structural, structural and accessory proteins interfere with elements of the IFN pathway (Table 2, Fig. 2), essential for the development of effective immunity. IFN antagonism has been attributed to several of the structural, non-structural and accessory proteins that interfere with the STING–TRAF3–TBK1 complex, thereby blocking STING/TBK1/IKKε-induced type I IFN production, signal transducer and activator of transcription (STAT)-1/2 translocation to the nucleus, IRF3, NF-κB signalling as well as interfering with the actions of the ISG products, including IFITs (Table 2). As examples, nsp1, 4 and 6 and ORF6 interfere with STAT-1/2 signalling while nsp 10, 13 and 16 cap the viral RNA (Table 2), preventing recognition by RIG-I, MDA-5 and IFITs. Nsp3 also acts by DUB proteins, thereby preventing their activity such as RIG-I and other steps in the IFN pathways for which ubiquitination is essential. CoV PLPro (nsp 3) also interrupts the stimulator of IFN genes STING–TRAF3–TBK1 complex, thereby blocking STING/TBK1/IKKε-type I IFN production [32,34]. As well as subversion of the IFN pathway, SARS-CoV ORF7a (also present in SARS-CoV-2) blocks the activity of tetherin, also known as bone marrow stromal antigen 2 (BST-2) [58]. BST2 acts by tethering budding viruses to the cell membrane, thus preventing its release from the cells. ORF7a removes this inhibition aiding the release of mature virions.

In summary, emerging evidence from SARS-CoV-2, and comparison with other SARS-CoV and MERS, reveals

many strategies used to evade the innate immune response and subvert the IFN pathway. While this facilitates widespread viral replication, increasing the viral load also promotes the viral cytopathic effects leading to tissue damage described below, and probably leads to exacerbation and hyperinflammation of the innate immune response once triggered.

Triggering innate immunity

Despite immune evasion and subverting innate immune responses during early infection, SARS-CoV-2 effectively initiates immune signalling pathways. This is probably due to the increased viral load that exponentially produces viral RNA and viral proteins [pathogen-associated molecular patterns (PAMPS)], and also induces cell damage that release damage-associated molecular patterns (DAMPS), both of which trigger innate immune pathways.

Like SARS-CoV and NL63, SARS-CoV-2 uses the angiotensin (Ang)-converting enzyme-2 (ACE2) as a cell receptor (Table 1), expressed on epithelia in renal, cardiovascular and gastrointestinal tract tissues, testes and on pneumocytes and vascular endothelia [66]. ACE2 regulates the renin–angiotensin system (RAS) by balancing the conversion of angiotensin I to angiotensins 1–9 and angiotensin II to angiotensins 1–7. Binding of SARS-CoV-2 to ACE2 leads to endosome formation, reducing ACE2 expression on the cell surface (Figs. 3 and 4) and pushing the RAS system to a proinflammatory mode, triggering production of reactive oxygen species (ROS), fibrosis, collagen deposition and a proinflammatory environment, including IL-6 and IL-8 production, by macrophages and recruitment of

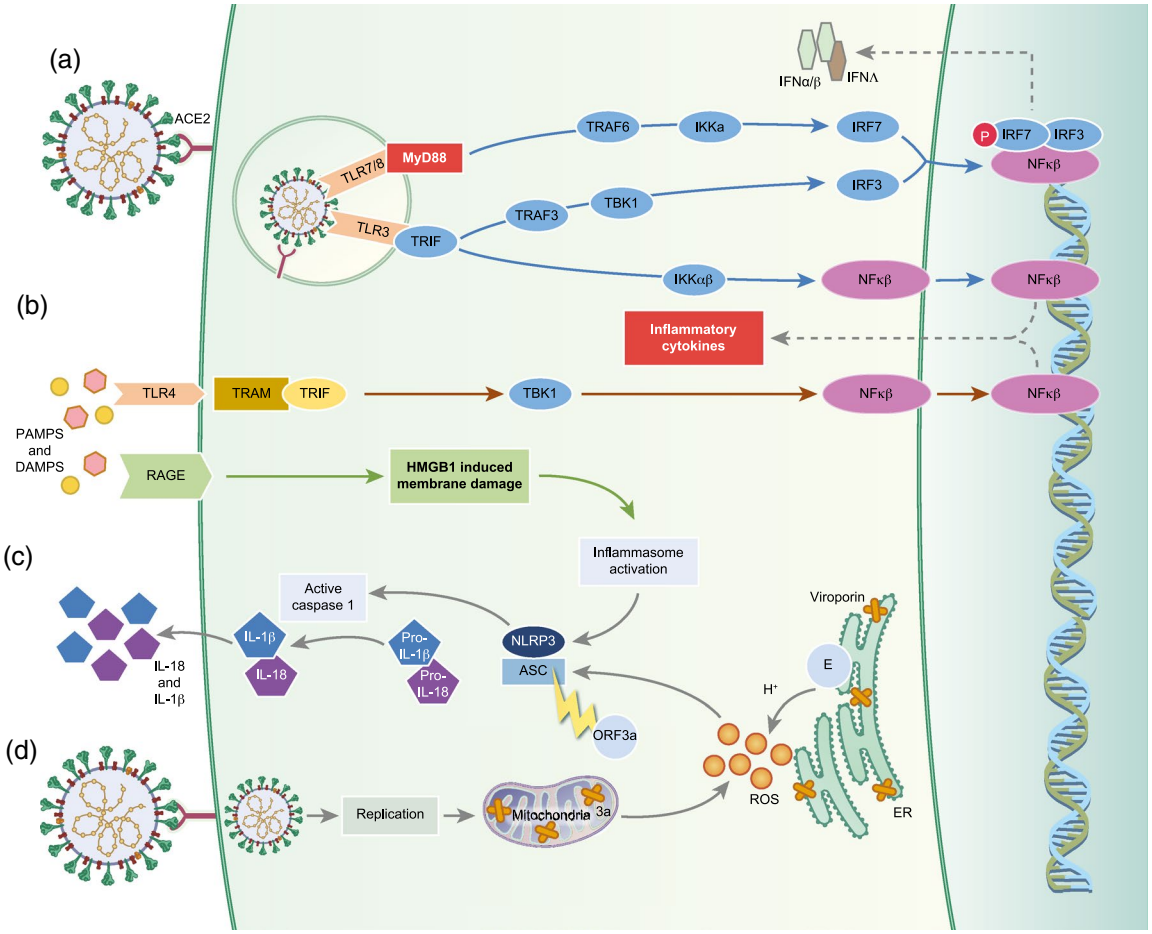

Fig. 3. SARS-CoV-2 activates innate immune pathways. SARS-CoV-2 infects permissible cells via the angiotensin-converting enzyme 2 (ACE2) and is taken by in the endosome where the virus is recognized by Toll-like receptors 7/9 triggering the myeloid differentiation primary response 88 (MyD88) pathway, or Toll-like receptor (TLR)-3 via the TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway (a). Pathogen-associated molecular patterns (PAMPS) and damage-associated molecular patterns (DAMPS) are also recognized by TLR-4 (b) or receptor for advanced glycation end (RAGE) (d) triggering high mobility group box 1 (HMGB1)-induced damage and NOD pyrin domain-containing 3 (NLRP3) inflammasome activation. During viral replication ORF3a and E proteins form viroporins that augment reactive oxygen species (ROS) production and inflammasome activation.

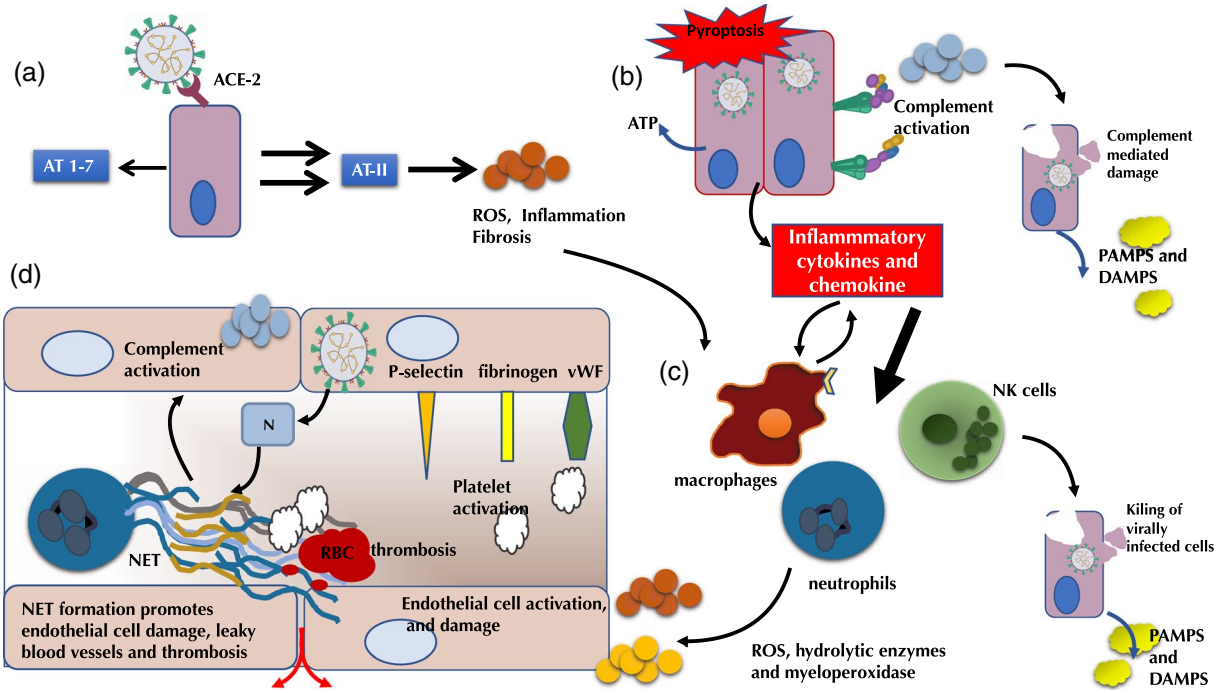

Fig. 4. SARS-CoV-2 is a vascular and coagulation disease. (a) Binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2) blocks ACE2-induced formation of anti-oxidant angiotensin, facilitating oxygen free-radical formation. Infection in some people also triggers pyroptosis, complement activation (b) and hyperinflammation with influx of macrophages, natural killer (NK) cells and neutrophils (c). This self-augmenting cycle triggers further cell damage and damage-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPS) release, as well as reactive oxygen species (ROS) production. (d) Activation of neutrophils induces neutrophil extracellular traps (NET) aided by the N protein and generated in response to ROS-induced endothelial cell damage. Disruption of the vascular barrier and endothelial cell exposure to proinflammatory cytokine and ROS increases expression of P-selectin, von Willebrand factor (vWF) and fibrinogen that attract platelets triggering expression of tissue factor. Together, this sequence activates the complement system, one of many pathways that crucially activates the coagulation cascade leading to thrombi formation.

neutrophils (Fig. 4). Thus, binding and entry of SARS-CoV-2 via the ACE2 is likely to be the first step in a line of augmented and detrimental immune responses in COVID-19 that involves complement activation, innate immune activation via PAMPS and DAMPS, inflammasome activation and pyroptosis, natural killer (NK) cell activation, hyperactivation of macrophages, neutrophils and innate T cells and induction of a cytokine storm, as discussed below.

Complement

SARS-CoV-2 is heavily decorated with glycans (Fig. 1) that are recognized by DC-SIGN and other lectins that facilitate viral uptake by dendritic cells (DCs). Glycans also activate the lectin complement pathway following binding of mannose-binding protein (MBP) to SARS-CoV-2 viral proteins expressed on infected cells (Figs. 1 and 2). Pathology studies and transcriptional profiles of tissues from COVID-19 cases reveal robust activation of the complement system with the deposition of MBL, C4d, C3 and C5b-9, forming the membrane attack complex (MAC) in alveolar and epithelial cells [68,69] (Fig. 4). In addition, C4d and C5b-9 deposits in lung and skin microvasculature co-localized with spike glycoproteins indicates systemic complement activation, supporting the role of complement in tissue damage [68]. Importantly, activation of the lectin, as well as the classical pathway following antibody binding to viral proteins, probably contributes to cell damage (Fig. 4) [70] by either direct complement-mediated lysis or via antibody-dependent cell-mediated cytotoxicity. Of relevance to the coagulation dysfunction, thrombosis and vascular damage observed following SARS-CoV-2 infection is that complement components induce secretion of von Willebrand factor [71], but also promotes monocyte and neutrophil recruitment as well as stimulating NET formation [72] that, in turn, perpetuates complement activation (Fig. 4). Complement may thus contribute to widespread tissue damage in SARS-CoV-2-infected cases. The pathogenic role of complement in disease is supported by findings in mice. For example, mice deficient in C3 had similar viral load as wild-type mice, but lacked the overt pathology

with fewer neutrophils and macrophages in the lung [73]. Thus, while complement activation is not required for control of virus infection it probably plays a key role in the tissue damage.

PAMPS and DAMPS mediate innate immune signalling

Infected pneumocytes and other permissible cells undergo cell damage and cell death releasing virally associated molecules, so-called PAMPS. In addition, intracellular components released due to damage, so-called DAMPS, include ATP, oxidized lipids, heat shock proteins and other components associated with regulated cell death programmes including apoptosis, autophagy, necroptosis and pyroptosis (Figs. 3 and 4). Thus, both DAMPS and PAMPS contribute to innate immune activation in COVID-19.

RNA viruses trigger several Toll-like receptors (TLRs), including TLR-7/8 and TLR-3, and elegant molecular *in-silico* docking studies show that the spike protein of SARS-CoV-2 can bind to TLR-1, TLR-4 and TLR-6 [74] (Fig. 3), whereas *in vitro* the SARS-CoV spike protein triggers NF-κB activation and IL-8 production via TLR-2 signalling in human peripheral blood mononuclear cells (PBMCs) [75]. In mice in which specific points in the TLR pathway were deleted; that is, TLR-3$^{-/-}$, TLR-4$^{-/-}$ and TRIF-related adaptor molecule (TRAM)$^{-/-}$ animals, were more susceptible to SARS-CoV infection, although the clinical severity of disease was dramatically reduced. This was in direct contrast to deficiency in TIR-domain-containing adapter-inducing IFN-β (TRIF, the TLR adaptor protein (Fig. 3) in which TRIF$^{-/-}$ mice developed severe disease, exacerbated influx of macrophages and neutrophils and lung pathology indicative of COVID-19 pathology. Thus, a balanced response to infection via the TLR-3 pathway is essential to trigger a protective response to SARS-CoV [76]. This study also supports the idea that in addition, PAMPS, immune pathways triggered by DAMPS such as oxidized phospholipids, high mobility group box 1 (HMGB1), histones, heat shock proteins and adenosine triphosphate released by damaged cells may contribute to COVID-19 outcome (Figs. 3 and 4). In addition to RIG-I, MDA-5 and MAVS, RNA viruses are also sensed by the STING that is activated by cGAMP when enveloped RNA viruses interact with the host membranes [64]. Downstream, STING engages TBK1 that actives IRF3 and/or NF-κB inducing type 1 IFN and/or proinflammatory cytokines. That hyperactivation of STING contributes to severe COVID-19, as has been hypothesized by Berthelot and Lioté [77]. These authors present several lines of evidence, the strongest being that gain-of-function mutations of STING associated with hyperactivation of type I IFN induces the disease SAVI (STING-associated vasculopathy with onset in infancy). Affected children with SAVI present with pulmonary inflammation, vasculitis and endothelial-cell dysfunction that mimics many aspects of COVID-19 [78]. Furthermore, STING polymorphisms are associated with ageing-related diseases such as obesity and cardiovascular disease, possibility explaining the impact of co-morbidities and development of severe COVID-19 [78]. Also, in bats, in which SARS-CoV-2 may have arisen, STING activation, and thus consequently IFN-β, is blunted [79], probably aiding viral replication and spread, as observed in early SARS-CoV-2 infection in humans. That DAMPS released due to viral cytotoxicity may contribute to severe COVID-19, which is best exemplified by HMBG1 released by damaged and dying cells, as well as activated innate immune cells, especially in sepsis [80]. Depending on its conformation, HMGB1 triggers TLR-2, TLR-4 and TLR-9, the receptor for advanced glycation end-products (RAGE) and triggering receptor expressed in myeloid cells 1 (TREM-1) (Fig. 3). In mice, intratracheal administration of HMGB1 activates mitogen-activated protein kinase (MAPK) and NF-κB, inducing proinflammatory cytokines, activating the endothelium and recruiting neutrophils in the lung: key pathological features of severe COVID-19 [80,81]. HMGB1, and especially the platelet-derived source, may play a crucial role in SARS-CoV-2 vascular damage as HMGB1$^{-/-}$ mice display delayed coagulation, reduced thrombus formation and platelet aggregation [82]. Furthermore, blocking HMGB1 is beneficial in experimental lung injury and sepsis, suggesting that therapies targeting HMGB1 might also be beneficial in severe COVID-19 [83,84].

Inflammasome activation and pyroptosis

Studies of peripheral blood and post-mortem tissues from severe COVID-19 cases reveal high levels of IL-1β and IL-6 and increased numbers of CD14^{+}IL-1β monocytes, suggesting activation of the NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome pathway [85]. Activation of the NLRP3 inflammasome, essential for effective anti-viral immune responses, is elicited by several factors associated with SARS-CoV infection, including RAS disbalance, engagement of PPR, TNFR and IFNAR, mitochondrial ROS production and complement components including MAC, as well as SARS-CoV viral proteins such as ORF3a, N and E [86] (Fig. 3, Table 2). As a consequence, NLRP3 interaction with adaptor apoptosis speck-like protein (ASC) recruits and activates procaspase-1, processing pro-IL-1β and pro-IL-18 to the activated forms (Fig. 3). This drives the propyroptotic factor gasdermin D (GSDMD) formation of pores in the cell membrane; that is, pyroptosis that facilitates the release of proinflammatory cytokines. The pores also aid the release of cellular DAMPS such as HMGB1 and viral PAMPS that further exacerbate

inflammation, suggesting that targeting the NLRP3 pathway might be beneficial in severe COVID-19 cases.

Hyperinflammation and severe COVID-19

The delayed IFN response, increased viral load and virus dissemination, coupled with the release of DAMPS and PAMPs, lead to activation of several innate immune pathways. Following infection, pneumocytes, epithelial and alveolar cells and infiltrating monocyte–macrophages and neutrophils probably produce the first wave of tumour necrosis factor (TNF)-α, IL-6, IFN-γ-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α and regulated on activation, normal T-expressed and secreted (RANTES) production [87,88]. Hyperinflammation is probably promoted by co-morbidities due to increased ACE2 expression, concurrent bacterial infections and ageing as well as a direct effect of SARS-CoV-2 replication, as virus–host interactome studies reveal that SARS-CoV-2 nsp10 regulates the NF-κB repressor factor NKRF, facilitating IL-8 production [89]. This is followed by a second wave of cell recruitment, including NK cells that produce IFN-γ and further recruitment of (alternatively activated) monocytes/macrophages and neutrophils (Fig. 4), as observed in bronchial lavages, post-mortem tissues and peripheral blood studies [88,89]. NK cells are key players in disease outcome of infection, critically balancing the direct response to the virus by eliminating infected cells while also augmenting tissue damage (Fig. 4). Probably aided by IFN-γ induction by NK cells, hyperinflammation in severe COVID-19 is also characterized by recruitment of immature and mature human monocyte-derived DCs that harbour SARS-CoV infection; however, infection is abortive and mature virions are not released. During infection, DCs express only low levels of cytokines probably due to innate immune subversion strategies. The sustained activation of infiltrating monocytes and monocyte-derived macrophages [90] observed in severe COVID-19 cases is probably driven by a number of factors, including oxidative stress, anti-SARS-CoV-2 antibody–antigen complexes, NLRP3 inflammasome activation and complement activation that converge to sustain an aberrant hyperinflammatory response, or cytokine storm [91]. Following SARS-CoV-2 infection, one of the first innate immune cells to infiltrate into the tissues are neutrophils, probably recruited by chemokine (C-X-C motif) ligand (CXCL)2 and CXCL8 generated by infected cells [92]. While neutrophils do not clear viral particles, they phagocytose apoptotic bodies containing virus and debris, releasing proteolytic enzymes, anti-microbial peptides, matrix metalloproteinases and high levels of ROS to inactivate viruses. A key function of neutrophils relevant to the pathology of SARS-CoV-2 is the production of neutrophil extracellular traps (NETs) generated in response to endothelial damage, ROS production, IL-1β production and virus replication (Fig. 4, reviewed in [93]). The formation of NETs by neutrophils are aided by activated platelets associated with damaged endothelial cells that further activate the complement, fuelling the coagulation cascade and thrombi formation. While the NETs act to prevent further spread of the virus, they trigger platelet activation and bind erythrocytes thereby promoting (micro)thrombi formation (Fig. 4).

SARS-CoV-2 is a vascular and coagulation disease

While respiratory damage and complications are the major clinical signs of severe COVID-19 many tissues and organs are affected often prior to, or independently of lung pathology, for example Kawasaki-like vascular disease in children [94]. Clinical, post-mortem studies and experimental animal models of SARS-CoV reveal infection of endothelial cells and the widespread damage of endothelial cells, vascular dysfunction and thrombosis [94,95] that are emerging as a common pathological feature of SARS-CoV-2 infection. The link between SARS-CoV-2 infection, vascular damage and thrombosis is evidenced by high levels of D-dimers in 20–40% critically ill patients probably produced in an attempt to dissolve thrombotic clots. The endothelial cell damage is supported by the finding that endothelial cells express ACE2 and are thus permissible to SARS-CoV-2 infection [94]. Thus, infection not only leads to reduced ACE2 in endothelial cells, but also direct viral cytopathic damage and increased vascular permeability (Fig. 4), although more recent data challenge this view, suggesting that pericytes and not endothelial cells are permissible to infection and viral-induced damage [95,96]. Damage of endothelial cells and pericytes leads to vascular permeability in severe COVID-19 that is probably amplified by activation of complement components widely expressed in post-mortem tissues of COVID-19 cases [68,69]. Disruption of the vascular barrier and endothelial cell exposure to IL-1β, TNF-α and ROS increase expression of P-selectin, von Willebrand factor (vWF) and fibrinogen, and attracting platelets that trigger expression of tissue factor (Fig. 4). Together, this sequence triggers the coagulation cascade and explains the finding of increased D-dimer and fibrin, abnormal clotting times in severe COVID-19 cases and widespread disseminated thrombi in post-mortem tissues.

Disease severity, co-morbidities and innate immunity

SARS-CoV-2 exploits many strategies to subvert innate immune responses allowing the virus to replicate and disseminate within the host. The extent to which the virus

Table 3. Impact of innate immune suppression on disease outcome

	Asymptomatic or mild	Moderate	Severe
Type I/III IFN	Increased and prolonged production	Moderate suppression	High and prolonged suppression
Viral replication	Limited	Mild or chronic	High and sustained
Immune response	Strong and rapid induction of adaptive immunity, viral clearance	NK cell and complement mediated clearance of infected cells, reduced adaptive immune responses	Hyperinflammation, cytokine storm, for example, IL-6, IL-8, TNF-α, delayed or ineffective adaptive immune response, innate T cell activity
Pathology	None, subclinical	Tissue damage due to inflammatory response	Viral-induced cytotoxicity, ADCC, complement-mediated damage, pyroptosis, necroptosis, neutrophil-driven NETosis
Vascular damage and thrombosis	No	Not likely or mild	Highly likely and contributes to clinical disease and tissue damage, hence the finding of high levels of D-dimer produced to counteract thrombi formation

ADCC = antibody-mediated cell cytotoxicity; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor; NK = natural killer.

Table 4. Factors and co-morbidities, aberrant innate immune responses and COVID-19

Factor or co-morbidity	Impact on COVID-19	Proposed impact on innate immune responses	Refs.
Age	Increased CFR > 80–14·8% vf 2·3% 70–79 to 8·0%	Increased oxidative stress, decreased IFN responses Elevated proinflammatory cytokines	[97–99,101]
Blood groups	Higher risk in blood group A and protective effect in blood group O in a cohort of 1610 cases	Neutralizing antibodies against protein-linked N-glycans on SARS-CoV-2, or stabilization of vWF	[102–103]
Cardiovascular disease	Increased CFR 10·5% vf 2·3%	Infection of cardiomyocytes, Increased myocarditis, impact of drugs on RAS. Increase levels of vWF	[97,104]
Cancer	4·7%, 5·6%	unknown	[97,105]
Diabetes mellitus	7·3%	Reduced ACE2 levels in diabetes already predispose to a proinflammatory environment. Increased IL-6 levels. Increased levels of vWF	[97,106]
Gender	Increased CFR for males across all ages	Differential expression levels of ACE2, hormonal regulation of immune reposes, IL-6 higher in men	[107,108]
Ethnicity	Higher risk in some ethnic groups not due to socio-economic conditions	Difference in TLR expression, levels of IL-6 and TNF-α Reduced levels of VitD	[109,110]
Obesity	BMI > 25 or 30 increased risk of severe pneumonia by 86% and 140%	Dysregulated NK cells, increased numbers of myeloid cells in adipose tissues and expression of ACE2 by adipocytes	[111,112]

ACE 2 = angiotensin-converting enzyme 2; BMI = body mass index; CFR = case fatality ratio; NK cells = natural killer cells; TLR = Toll-like receptors; vWF = von Willebrand factor.

replicates within the host, and the efficacy of the host innate immune response to eradicate the infection and trigger effective adaptive immune responses, but not hyper-responsiveness of innate immunity, strongly determines the disease outcome (Table 3). The severity of infection has been linked to age, smoking, co-morbidities such as cancer, immune suppression, autoimmune diseases, inflammatory disease, neurodegenerative diseases, obesity, gender and race [97–106]. For example, in a large cohort of 72 314 cases the case fatality ratio for more than 80 years was 14·8 *versus* 2·3% in the total cohort [97]. This is probably higher due to inflamm-ageing, an aberrant innate immune response such as lower production of IFN-β [98], increased oxidative stress [99] and sensence of macrophages that become less effective in their reparative functions with age [100]. Similarly, viral load, obesity, gender, race, blood groups and co-morbidities have all been reported to influence the response to SARS-CoV-2 infection (Table 4) [101–112], although few studies have fully examined the extent to which subversion and activation of innate

immune components contribute to susceptibility in these cases.

Future perspectives

Understanding the innate immune factors that exacerbate the vascular complications will be crucial to control severe disease following SARS-CoV-2 infection. Rapidly emerging studies reveal the extent to which therapeutic approaches for other viral infections and inflammatory diseases can be repurposed to target innate immunity to treat COVID-19 patients [113,114]. Similarly, novel approaches have been put forward to target the susceptible ageing population or those with co-morbidities. One approach under investigation is to re-establish the youthful function of macrophages and repair mechanisms using metformin, a drug used in type II diabetes that has been shown to attenuate hallmarks of ageing [115]. In a retrospective study of 25 326 subjects tested for COVID-19, while diabetes was reported to be an independent risk factor for COVID-19-related mortality [116], the risk in subjects taking metformin was significantly reduced (odds ratio = 0·33; 95% confidence interval = 0·13–0·84), suggesting that metformin might be protective in high-risk populations, especially as metformin has also been reported to suppress neutrophil-induced NETosis *in vitro* and reduce circulating NETosis biomarkers in *vivo* [117]. Thus, metformin and other drugs such as niacin [118],that rejuvenate the innate immune system, may be useful in COVID-19.

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Author contributions

S. A. wrote the paper, L. F. B. produced Figs. 1 and 2 and the Supplementary figure and made the first draft of the paper. D. B. made the second draft. All authors read and approved the final draft.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site:

Fig. S1. Replication Cycle of SARS-CoV-2. Based on knowledge emerging from the SARS-CoV-2 infection and other coronaviruses, the cycle comprises of viral binding to the host cell via ACE-2 (1) and virion uptake into the endosome. (2) The positive stranded RNA allows direct translation of the genome (which is capped by nsp – see text for details) generating the replicase polyprotein and subsequent viral proteins some of which are necessary to form the double membrane vesicles (DMV) (3). The replicase polyprotein enzyme synthesises the negative strands to transcribe the small subgenomic positive RNAs (4). These are used to produce the other viral proteins (N, S, M and E and accessory proteins) and the positive RNA strands for the new virions. The nucleocapsid protein binds to the RNA. The S, M and E proteins are incorporated in the lipid envelope in the ER. The new virons assemble in the ER-Golgi intermediate compartment (ERGIC) and exocytosed in the Golgi complex (6). Finally, mature SARS-CoV-2 virions are r eleased from the host cell (7).

