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Can NLRP3 inhibitors improve on dexamethasone for the treatment of COVID-19?

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ABSTRACT

Dexamethasone, a corticosteroid, has been approved for use in the treatment of severe COVID-19, which is characterised by hyperinflammation and associated lung damage. However, dexamethasone shows no clinical benefit in the treatment of less severe disease, and prolonged treatment may lead to immunosuppression and an increased risk of opportunistic infections. Hence there is a need for more specific anti-inflammatory therapies which also prevent severe disease. The NLRP3 inflammasome is an intracellular signalling complex which is responsible for the cleavage and release of the cytokines IL-1β and IL-18 and has also been shown to be inhibited by dexamethasone. NLRP3 inflammasome activation is strongly correlated with COVID-19 severity and part of dexamethasone's clinical effect in COVID-19 may be via NLRP3 inhibition. Specific NLRP3 inhibitors are currently undergoing clinical trials for the treatment of COVID-19. In this review, we evaluate the evidence supporting the use of dexamethasone and speculate on the potential use of NLRP3 inhibitors to treat COVID-19 as a more specific approach that may not have the liabilities of dexamethasone.

1. COVID-19

COVID-19 (coronavirus disease 2019) was first identified in Wuhan, China in December 2019 (Lu et al., 2020). As of May 2021, there have been almost 160 million cases of COVID-19 worldwide, leading to over 3 million deaths. Common symptoms of COVID-19 are fever, cough, shortness of breath, breathing difficulties and anosmia, but complications may include pneumonia and acute respiratory distress syndrome (Yue et al., 2020).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded positive sense RNA coronavirus. Coronaviruses in general do not cause severe disease, with the exceptions of SARS-CoV, Middle East respiratory syndrome (MERS) and now SARS-CoV-2 (Fung and Liu, 2019). The nature of the immune response to SARS-CoV-2 is thought to be a factor in influencing disease severity. Patient studies have revealed that COVID-19 is generally characterised by increases in innate immune cells and markers, such as IL-6 and TNFα, which are accompanied by decreases in antigen-specific T cell responses (Huang et al., 2020; Mathew et al., 2020). Prolonged elevation of these innate immune cytokines, termed hyperinflammation or cytokine release syndrome (CRS), is correlated with increased disease severity (Lucas et al., 2020). The cytokines IL-1β and IL-18 are also elevated in severe patients compared to mild patients, and IL-18 was in fact shown to have the strongest association with risk of intensive care unit (ICU) admission and death of any biomarker (Lucas et al., 2020). These observations have raised awareness of the potential role that the NLRP3 inflammasome plays in COVID-19 pathogenesis, since NLRP3 is a key driver of IL-1β, IL-18 and an inflammatory type of cell death called pyroptosis in many inflammatory diseases (Mangan et al., 2018). Furthermore, increased NLRP3 expression has been detected in monocytes and lung tissue obtained from COVID-19 patients compared to healthy controls (Rodrigues et al., 2021).

Although mild cases of COVID-19 may be treated using over-the-counter medications such as paracetamol, there are few available treatment options which have been demonstrated to be effective in the treatment of severe COVID-19. Remdesivir, an antiviral medication, may reduce recovery time but does not significantly reduce mortality (Grein et al., 2020). However, in February 2021 the world's largest randomised controlled trial (RCT) of COVID-19 treatments, called the RECOVERY trial, found that the corticosteroid dexamethasone reduced mortality in patients receiving respiratory support (Group et al., 2021). As a cheap and widely available drug with an established safety profile, dexamethasone has become part of the standard-of-care given to severe COVID-19 patients. There are, however, potential disadvantages to the use of corticosteroids to treat COVID-19, which are mainly related to their

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prolonged immunosuppressive effects. Therefore, although much of the current research focus surrounds the development and rollout of preventive vaccines to SARS-CoV-2, there remains a need for more specific therapeutic options available to treat COVID-19.

2. Dexamethasone: mechanisms of action

Endogenous glucocorticoids are essential to restoring homeostasis following inflammation. They are synthesized in the adrenal cortex and are able to act on virtually all cells in the body due to the ubiquitous expression of the glucocorticoid receptor (GR) (Webster and Tonelli, 2002). Cortisol is the most important endogenous glucocorticoid and forms the basis for the structure of the synthetic glucocorticoid dexamethasone, which is a more potent and longer-acting glucocorticoid than endogenous cortisol (Nicolaides et al., 2000). Once bound to the cytosolic GR, the ligand-receptor complex translocates into the nucleus where it binds to DNA sequences called glucocorticoid response elements (GRE) in the promoter regions of target genes (Rhen and Cidlowski, 2005). The interactions which occur between the GR and these DNA elements may lead to the transcriptional activation (transactivation) of anti-inflammatory target genes, or the transcriptional repression (transrepression) of proinflammatory target genes. There is also evidence to suggest that the GR can induce epigenetic changes through interactions with histone acetyltransferases (HATs) and histone deacetylases (HDACs), which respectively acetylate or deacetylate DNA in promoter regions (Ito et al., 2000). Deacetylated DNA results in a closed chromatin structure and reduced accessibility of the DNA to proinflammatory transcription factors such as NF-κB. The reduced transcriptional activity of NF-κB is key to the anti-inflammatory effects of dexamethasone (De Bosscher et al., 2003), as expression of many of the pro-inflammatory cytokines implicated in COVID-19 is NF-κB-dependent, including IL-1, IL-18, IL-6, TNFα, and CXCL10 among others. Further transactivation targets of glucocorticoid signalling include annexin A1 (Goulding et al., 1990), which represses prostaglandin production, and MAPK phosphatase-1 (MPK-1) (Kassel et al., 2001), which inactivates all members of the proinflammatory MAPK family of proteins.

3. Dexamethasone in COVID-19

The RECOVERY trial examined 6 different therapeutic interventions for the treatment of COVID-19 including the antiviral drug cocktail lopinavir-ritonavir, the anti-malarial drug hydroxychloroquine, the antibiotic azithromycin, the anti-IL-6 monoclonal antibody tocilizumab and convalescent plasma from recovered COVID-19 patients, as well as dexamethasone. It is striking that dexamethasone and tocilizumab, two immunosuppressive drugs, were the only interventions to demonstrably reduce mortality in severe COVID-19. Although the reduction in mortality observed with tocilizumab was modest (4%), dexamethasone reduced mortality by one third in ventilated patients and one fifth in patients receiving oxygen. There was no benefit to patients who were not receiving respiratory support (Group et al., 2021). As the clinical benefit associated with dexamethasone use is only observed in the most severe patients, it is likely that the deterioration of COVID-19 patients is inextricably linked to immunopathological phenomena rather than viral load. This would perhaps also explain why antiviral medications reduce recovery time when given early, but do not reduce mortality in severe COVID-19 when hyperinflammation predominates (Grein et al., 2020). The aggressive inflammatory responses in COVID-19 result in damage to the airways, termed acute respiratory distress syndrome (ARDS), which may lead to respiratory failure- this is the cause of death in 70% of fatal COVID-19 cases (Zhang et al., 2020a). A similar triphasic disease course has been described for the other pathogenic coronaviruses SARS-CoV and MERS-CoV: viral replication followed by hyperinflammation and resultant immunopathological damage to the lungs (Lee et al., 2003; Zaki et al., 2012). Corticosteroid treatment reduced mortality during the first SARS epidemic (Yam et al., 2007), but subsequent meta-analyses have again highlighted the importance of timing when considering corticosteroid administration, as corticosteroid use was associated with increased viral load (Stockman et al., 2006). Furthermore, the initial stages of SARS-CoV-1 infection are characterized by a reduction of antigen-specific $CD4^+$ and $CD8^+$ T-cells (Stockman et al., 2006), as is also the case during SARS-CoV-2 infection. Early administration of dexamethasone would likely further suppress the mounting of adaptive immune responses, and also increase the risk of secondary bacterial or fungal infections (Chen et al., 2020). Corticosteroids were widely used in the treatment of MERS-CoV infection, but a large retrospective study of 309 adults with severe disease showed that corticosteroid treatment was not associated with reduced mortality, and in fact increased the need for mechanical ventilation. Again, corticosteroid treatment was associated with reduced viral clearance from the lungs (Arabi et al., 2018). Analysis of the use of corticosteroids in the treatment of other viral infections has led to similarly troubling results: a large meta-analysis showed increased mortality and increased length of stay in ICU for influenza patients given corticosteroid treatment (Ni et al., 2019), and corticosteroids are not recommended in the treatment of respiratory syncytial virus (RSV) in children due to a lack of clinical benefit (McGee and Hirschmann, 2008).

A possible explanation for the lack of efficacy in corticosteroid treatment for viral infections is the role of type I IFN in these diseases. Type I IFN signals in an autocrine or paracrine manner to upregulate interferon-stimulated genes (ISGs), which are key to the induction of an antiviral state. Although the hyperinflammatory state which defines severe COVID-19 is characterized by increased levels of many proinflammatory cytokines, there are reports that type I IFN and ISGs may in fact be reduced in these cases (Blanco-Melo et al., 2020; Hadjadj et al., 2020). Furthermore, there are subsets of severe COVID-19 patients with autoantibodies against type I IFN and deficiencies in IFN signalling (Bastard et al., 2020; Zhang et al., 2020b), further emphasizing the importance of IFN signalling to the defence against SARS-CoV-2. Interestingly, robust IFN responses have also been observed in COVID-19 patients, and these responses are in fact associated with viral persistence and more severe disease (Wilk et al., 2020; Zhou et al., 2020b). Hence the role of type I IFN in COVID-19 may be highly time-dependent, whereby a strong early IFN response would aid viral clearance but a prolonged IFN response would contribute to immunopathological tissue damage (Park and Iwasaki, 2020). Perhaps the clinical benefit provided by dexamethasone administration in severe late-stage COVID-19 results from type I IFN suppression, and this is also why dexamethasone does not provide any clear benefit in the treatment of early-stage disease and in the treatment of other viral infections.

The conflicting evidence on corticosteroid use from previous coronavirus epidemics led to some dispute surrounding its use in treating COVID-19 prior to publication of the RECOVERY trial (Russell et al., 2020). However, the RECOVERY trial has shown that there is significant clinical benefit in the use of dexamethasone for the treatment of severe COVID-19. The hallmarks of severe COVID-19 resemble those of septic shock, where the use of corticosteroids has also been shown to reduce mortality (Annane et al., 2018). However, there remains a need for more specific anti-inflammatory therapeutics for COVID-19 which are not accompanied by the risk of secondary infections or prolonged viremia.

Recently, dexamethasone has been shown to inhibit the NLRP3 inflammasome in a model of allergic airway inflammation. Dexamethasone treatment reduced OVA-induced upregulation of NLRP3, caspase-1 and IL-1β in this model (Guan et al., 2020). Since NLRP3 has been implicated in COVID-19 pathogenesis (Rodrigues et al., 2021), this raises the possibility that part of the efficacy of dexamethasone treatment in COVID-19 might be via NLRP3 inhibition. This may also suggest that specific inhibition of NLRP3 might be a better option for the safe treatment of COVID-19.

4. NLRP3 inflammasome

NOD-like receptors (NLRs) are a sub-group of pattern-recognition

receptor (PRR) which are characterised structurally by the presence of a central nucleotide-binding and oligomerization (NACHT) domain flanked by a C-terminal leucine rich repeat (LRR) domain and an N-terminal caspase-recruitment (CARD) or pyrin (PYD) domain (Schroder and Tschopp, 2010). Some of these NLR family members form large intracellular protein complexes, called inflammasomes, which converge on and activate caspase-1 as an effector molecule to cleave IL-1β and IL-18 into their active forms prior to their release from the cell (Martinon et al., 2002). Caspase-1, in addition to cleaving these cytokines, also cleaves the protein gasdermin D into its active N-terminal subunit, which inserts into the cell membrane and initiates a programmed form of inflammatory cell death termed pyroptosis (Liu et al., 2016; Shi et al., 2015).

The NLRP3 inflammasome complex consists of the central NLRP3 protein, the adaptor protein ASC, the mitotic kinase NIMA-related kinase 7 (NEK7) and the effector protein pro-caspase-1. NLRP3, like all other NLRs, consists of a NACHT domain, an auto-inhibitory C-terminal LRR domain and an N-terminal PYD domain (Schroder and Tschopp, 2010). The NACHT domain provides ATPase activity which is essential for NLRP3 self-association (Duncan et al., 2007). Through PYD-PYD interactions, self-oligomerised NLRP3 interacts with and recruits ASC into the inflammasome complex (Cai et al., 2014; Lu et al., 2014). ASC in turn, through CARD-CARD interactions, recruits the effector protein pro-caspase-1, which autocatalytically cleaves to generate the intermediate subunits p10 and p33. Further processing is required to generate the active subunits p10 and p20 (Boucher et al., 2018). The processes leading up to caspase-1 cleavage are displayed in Fig. 1. The active caspase-1 subunits p10 and p20 carry out the effector functions of the inflammasome complex, namely cleaving the cytokines IL-1β and IL-18 into their mature forms and gasdermin D into its active N-terminal fragment. Gasdermin D, although described as 'the pyroptosis executioner' (Liu et al., 2016; Shi et al., 2015), is able to exert functions independent of its role in pyroptosis. For example, in dendritic cells it has been shown to regulate IL-1β release without initiating pyroptosis (Evavold et al., 2018). It may therefore be the case that gasdermin D is not the final protein in the pyroptosis pathway. Supporting this, a protein regulated downstream of gasdermin D, termed nerve injury-induced protein 1 (NINJ1), was recently identified as a regulator of membrane rupture following inflammasome activation (Kayagaki et al., 2020).

NLRP3 inflammasome activation is a two-step process. The first step is a priming signal (signal 1), provided by TLR stimulation by a pathogen- or damage-associated molecular pattern (PAMP or DAMP) leading to transcriptional upregulation of NLRP3 and pro-IL-1β. However, the second step (signal 2), which results in inflammasome assembly and activation, is less well-elucidated. The main issue concerning NLRP3 activation is that it may be triggered by a wide range of different stimuli, in contrast to most other inflammasomes which respond to more specific triggers. Therefore, there remains much debate about a unifying mechanism for NLRP3 inflammasome activation. K^+ efflux is a common mechanism for various triggers of inflammasome activation including ATP (Perregaux and Gabel, 1994; Surprenant et al., 1996), pore-forming toxins, and the phagocytosis of particulate matter (Halle et al., 2008; Hornung et al., 2008; Martinon et al., 2002; Munoz-Planillo et al., 2013). In addition to potassium efflux, additional triggers have also been described for NLRP3 inflammasome activation. These include but are not limited to mitochondrial dysfunction (Gross et al., 2016; Iyer et al., 2013; Nakahira et al., 2011; Zhong et al., 2016, 2018), *trans*-Golgi disassembly (Chen and Chen, 2018) and metabolic stimuli (Wen et al., 2011; Wolf et al., 2016; Youm et al., 2015).

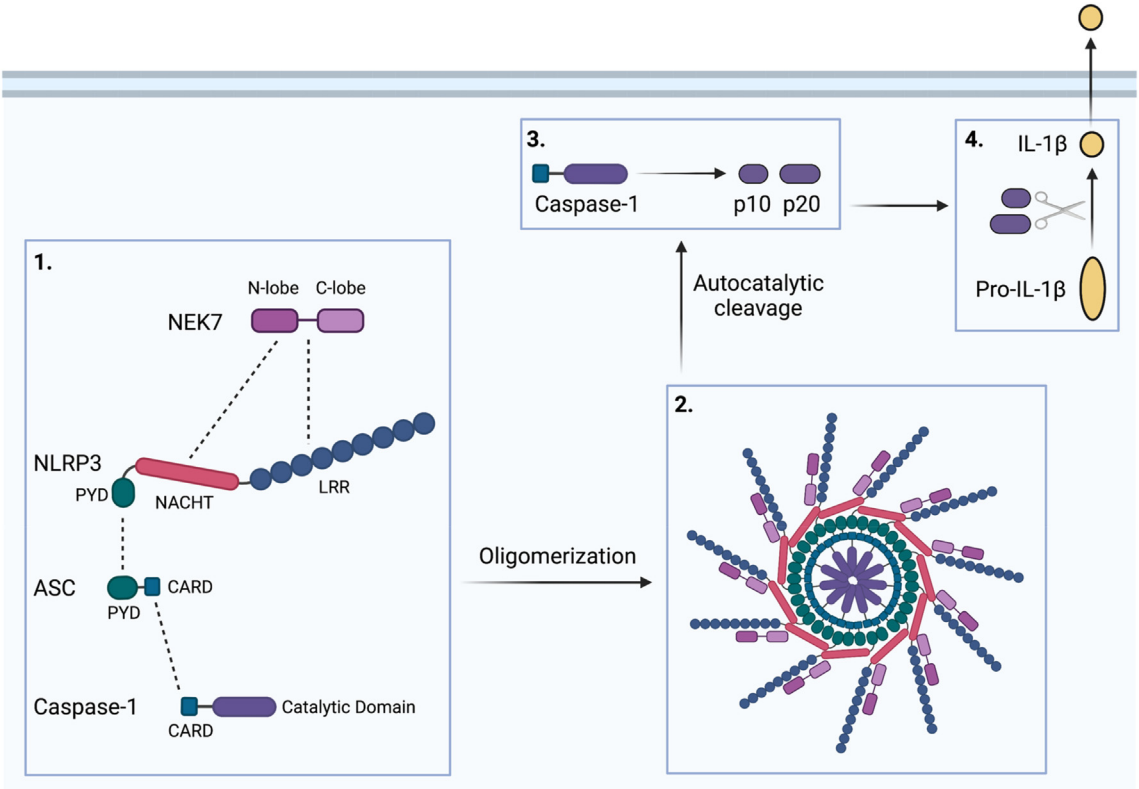

Fig. 1. Mechanics of NLRP3 inflammasome activation.
Upon inflammasome stimulation, the central protein NLRP3, consisting of its pyrin domain (PYD), NACHT domain and leucine-rich repeat (LRR) domain, associates with the adaptor protein ASC through PYD-PYD interactions. ASC, in turn, associates with caspase-1 through caspase activation and recruitment domain (CARD) interactions. NEK7 also binds NLRP3 at several surfaces, which aids oligomerization of NLRP3. These interactions lead to assembly of the inflammasome complex, which is followed by autocatalytic cleavage of the effector protein caspase-1 into the active subunits p10 and p20. These subunits carry out the effector functions of the inflammasome complex, including the cleavage of IL-1β into its active form.

5. NLRP3 activation in COVID-19

Due to the numerous and wide-ranging stimuli which have been described for NLRP3 activation, it is generally accepted that the NLRP3 inflammasome is a sensor for cellular stress or damage rather than a receptor for specific pathogens. Hence NLRP3 has been implicated in the host response to numerous pathogens, as well as various auto-inflammatory diseases where its activation is dysregulated.

However, monogenic NLRP3-driven diseases do exist. Gain-of-function mutations in NLRP3 cause a group of systemic auto-inflammatory diseases called cryopyrin-associated periodic syndromes (CAPS). CAPS include familial cold auto-inflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disorder (NOMID) (Agostini et al., 2004; Hoffman et al., 2001). The 3 diseases vary in severity, but they are generally characterised by blood neutrophilia, fever and localised inflammation caused by spontaneous inflammasome activation. Anti-IL-1 therapy is the current first-line treatment option for CAPS patients. In addition to the monogenic NLRP3-driven diseases, the NLRP3 inflammasome contributes to the pathogenesis of other sterile inflammatory diseases. Alzheimer's disease (Heneka et al., 2013), atherosclerosis (Duewell et al., 2010), asthma (Besnard et al., 2011), inflammatory bowel disease (IBD) (Bauer et al., 2010), multiple sclerosis (MS) (Gris et al., 2010), type I diabetes (Hu et al., 2015) and rheumatoid arthritis (Vande Walle et al., 2014) are all improved in *Nlrp3*$^{-/-}$ models, demonstrating the viability of NLRP3 as a therapeutic target. Although these disease models are models of sterile inflammation rather than pathogen-induced inflammation, they serve to reinforce the notion that NLRP3 can be a key driver of immunopathology.

The role of inflammasome signalling in host defence to infection was first made in 1995, prior to the identification of the inflammasome complex, with the observation that caspase-1-deficient mice were resistant to LPS-induced sepsis (Li et al., 1995). Although it was later clarified that this effect was due to inadvertent codeletion of caspase-11 (Kayagaki et al., 2011), other components of inflammasome signalling including gasdermin D were later also shown to be critical to LPS-induced lethality (Kayagaki et al., 2015). Inflammasome signaling has also been implicated in mediating inflammation in response to viral infection, particularly in severe infections accompanied by lung injury and ARDS. IL-1β levels are raised in the plasma and bronchoalveolar lavage (BAL) fluid of ARDS patients and seem to be associated with a worsened clinical outcome in these patients (Meduri et al., 1995a, 1995b). Furthermore, IL-1β levels are also increased in patients with respiratory viral infections including influenza (Beigel et al., 2005), MERS-CoV (Alosaimi et al., 2020) and SARS-CoV (He et al., 2006). Several studies have directly implicated inflammasome components in the immunopathology resulting from viral infection. Mice deficient in absent in melanoma 2 (AIM2), another caspase-1-activating inflammasome, and IL-1R1 were protected from influenza A virus (IAV)-induced lung injury (Schmitz et al., 2005; Zhang et al., 2017). IL-1R1-and MyD88-deficient mice were also protected from bleomycin-induced lung fibrosis (Gasse et al., 2007). Taken together, these studies provide strong evidence that inflammasome signalling and resultant IL-1β production are critical to the immunopathology driving acute lung injury. Although dysregulated inflammasome signalling may lead to tissue damage, it should be noted that these responses may also be important in restraining viral replication. For example, IL-1β signalling was shown to be critical in suppressing West Nile virus (WNV) replication in neurons (Ramos et al., 2012). Thus, it may be important to consider the balance between promoting pathogen replication and restraining immunopathology when considering the therapeutic targeting of NLRP3.

The link between inflammasome activation and disease severity made during previous coronavirus epidemics can also be extended to the current SARS-CoV-2 pandemic. As previously mentioned, IL-1β and IL-18 levels are correlated with mortality and disease severity in COVID-19 patients (Lucas et al., 2020). Cleaved caspase-1 was also shown to be increased in the sera COVID-19 patients, indicating the presence of active inflammasomes (Rodrigues et al., 2021). Furthermore, the release of lactate dehydrogenase (LDH) from dying cells is used as a measure of pyroptosis, also a consequence of inflammasome activation, and is considered a powerful predictive biomarker of lung damage and severe disease in COVID-19 (Rodrigues et al., 2021; Yan et al., 2020). Thus, it is likely that inflammasome signalling contributes to immunopathology in COVID-19. Further *in vitro* assays have indeed clarified that SARS-CoV-2 infection of healthy human monocytes can drive NLRP3 inflammasome-dependent IL-1β release (Rodrigues et al., 2021). Interestingly however, pyroptosis was not inhibited by the specific NLRP3 inhibitor MCC950 in the same assay, suggesting that virus-induced cell death may be dependent on multiple inflammasome signalling pathways (Rodrigues et al., 2021). As such it may be worth considering the role of other inflammasome complexes in the immune response to SARS-CoV-2 including the AIM2 inflammasome, which has been shown to play a role in IAV infection (Zhang et al., 2017), and the NLRP1 inflammasome, which was recently shown to bind dsRNA following infection with the positive-sense single-stranded RNA virus Semliki Forest Virus (SFV), ultimately driving pyroptotic cell death (Bauernfried et al., 2021).

The question of how the NLRP3 inflammasome in particular is being activated during SARS-CoV infection has been addressed by several studies. SARS-CoV encodes three proteins, also conserved in SARS-CoV-2, which are able to drive NLRP3 inflammasome activation, as can be seen in Fig. 2. Open reading frame (ORF) 3a protein does this through ubiquitination of ASC (Chen et al., 2019; Siu et al., 2019), ORF8b interacts directly with the LRR of NLRP3 (Shi et al., 2019) and the SARS-CoV envelope (E) protein also promotes IL-1β release in an unidentified manner (Nieto-Torres et al., 2014). MERS-CoV also drives NLRP3 inflammasome activation in human, but not bat, peripheral blood mononuclear cells (PBMCs) (Ahn et al., 2019). This was found to be due to various mechanisms, including reduced transcriptional upregulation and increased LRR-mediated autoinhibition of NLRP3 in bats (Ahn et al., 2019). Reduced NLRP3 activation is perhaps what enables bats to tolerate high viral loads without developing severe disease and supports the role of bats as reservoir hosts for zoonotic viruses such as MERS-CoV and SARS-CoV-2 (Li et al., 2005; Munster et al., 2016).

Lung injury and ARDS is often accompanied by the activation of coagulation pathways in severe COVID-19, leading to the development of microcoagulation, disseminated intravascular coagulation (DIC) and ultimately multiorgan failure (Levi et al., 2020). Levels of D-dimer, a marker of thrombosis, are increased in patients with severe COVID-19 and are associated with poor disease prognosis (Zhou et al., 2020a). The generation of thrombin, key to clot formation, is tightly regulated by an intricate procoagulant-anticoagulant balance which is disturbed during hyperinflammation (Jose and Manuel, 2020). This may be termed immunothrombosis. One such pathway of immunothrombosis is driven by the pyroptosis executioner gasdermin D. LPS-induced caspase-11 activation during bacterial endotoxemia drives gasdermin D-dependent phosphatidylserine exposure and tissue factor activation in macrophages, leading to DIC. As such, *Gsdmd*$^{-/-}$ and *Casp11*$^{-/-}$ mice are fully protected from LPS-induced sepsis (Deng et al., 2018; Yang et al., 2019). Although this pathway is predominantly focused on non-canonical inflammasome signalling, which is type I IFN-dependent (Yang et al., 2020), there is evidence to suggest that canonical NLRP3 inflammasome- and caspase-1-dependent cleavage of gasdermin D can also drive DIC (Wu et al., 2019). NLRP3 inhibitors, or perhaps more specifically gasdermin D inhibitors of which several have recently been described including the licensed therapeutic disulfiram (Hu et al., 2020; Humphries et al., 2020), may be effective in treating immunothrombosis associated with severe COVID-19. However, it should be noted that the studies elucidating this pathway were performed in the context of bacterial infection rather than viral infection. As such it is important to establish whether inflammasome signalling also drives DIC in COVID-19.

6. Therapeutic targeting of NLRP3

Excessive IL-1β production has been demonstrated to contribute to

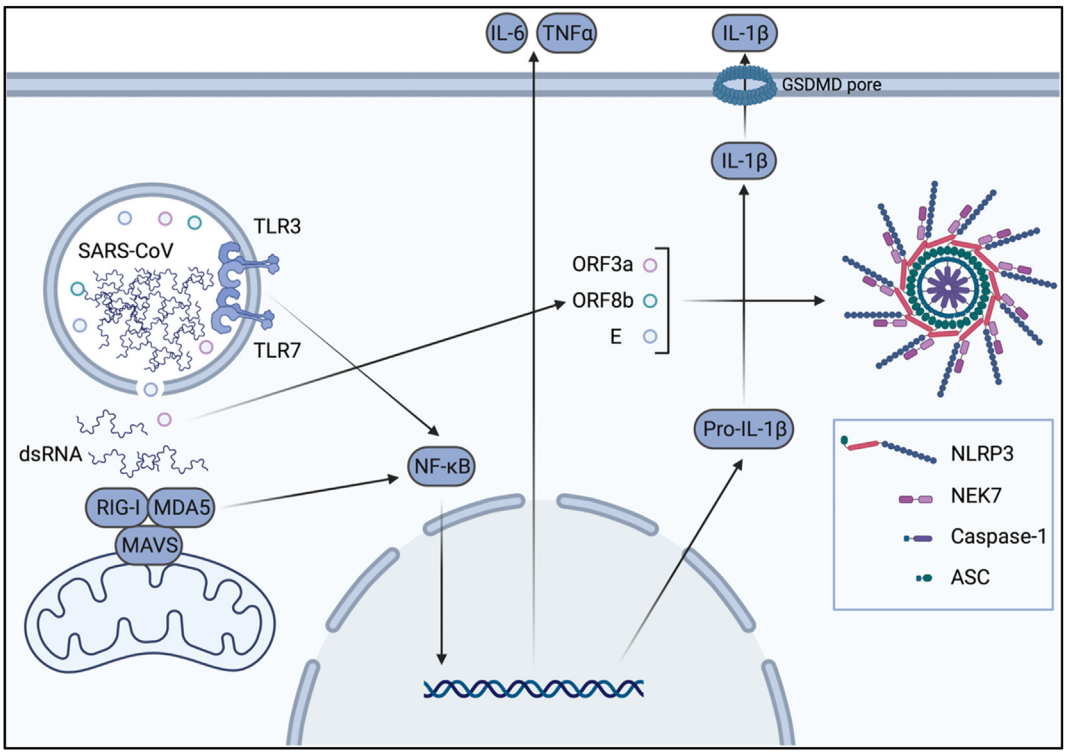

Fig. 2. SARS-CoV drives NLRP3 inflammasome activation.
Virus-derived dsRNA and ssRNA can be sensed by endosomal TLR3 and TLR7, as well as by the RIG-I-like receptors (RLRs) RIG-I and melanoma differentiation-associated protein 5 (MDA5), which signal through mitochondrial antiviral signaling protein (MAVS) to upregulate proinflammatory gene expression via NF-κB. Pro-IL-1β expression may be upregulated in this manner and is subsequently processed to mature IL-1β by the NLRP3 inflammasome complex. The NLRP3 inflammasome may be activated by specific viral peptides, including open reading frame (ORF) 3a, ORF8B and the envelope (E) protein. IL-1β is released from the cell through the gasdermin D (GSDMD) pore.

multiple autoinflammatory diseases including rheumatoid arthritis (Dinarello, 2000) and multiple sclerosis (Schrijver et al., 1999). Indeed anti-IL-1 therapies, in the form of a receptor antagonist (anakinra), soluble decoy receptor (rilonacept), or a neutralising monoclonal antibody (canakinumab) are licensed and effective in the treatment of both localised inflammatory disorders, such as gout, and systemic inflammatory disorders, such as the NLRP3-driven disorder cryopyrin-associated periodic syndrome (CAPS). Furthermore, results from the large scale RCT of canakinumab, called CANTOS, showed that the use of canakinumab significantly reduced the rate of cardiovascular events (Ridker et al., 2017a) and lung cancer incidence (Ridker et al., 2017b) when compared with placebo. However, the use of canakinumab was also associated with a higher incidence of fatal infection than placebo (Ridker et al., 2017a). Thus, it is generally accepted that the use of anti-IL-1 therapy is effective in treating inflammatory disorders, but may be associated with increased susceptibility to infection, roughly mirroring the properties of dexamethasone.

Previous reports of clinical benefit associated with the use of anakinra in systemic inflammatory disorders which share some of the hallmarks of severe COVID-19, including septic shock (Shakoory et al., 2016), haemophagocytic lymphohystiocytosis (Rajasekaran et al., 2014; Wohlfarth et al., 2019) and macrophage activation syndrome (MAS) (Lind-Holst et al., 2019) had raised hopes that anti-IL-1 therapy could improve outcomes for COVID-19 patients. Initial data from observational studies were promising. The first retrospective study performed in Italy found a 77% reduction in mortality in patients on non-invasive ventilation given anakinra (Cavalli et al., 2020), whereas a second prospective study in France also found a 50% reduction in mortality in patients requiring oxygen (Huet et al., 2020). However, the first multi-centre RCT (CORIMUNO-ANA-1) found that anakinra provided no clinical benefit over usual care in patients with mild-to-moderate COVID-19 pneumonia (group (2021)). In fact, the study was stopped early, and the frequency of serious adverse events was higher in the anakinra group than the usual care group (group, 2021). Although this particular RCT was not blinded and the sample size was relatively small, these results would clearly not support the use of anakinra in the treatment of COVID-19. However, a second RCT (CORIMUNO-ANA-2) within the same collaborative group to assess the impact of anakinra on ICU patients with more severe COVID-19 has been completed and is being analysed. The use of anakinra may have parallels with that of dexamethasone in COVID-19, where clinical benefit is only conferred to patients with the most severe forms of the disease.

The serious adverse events experienced by patients in the anakinra group of CORIMUNO-ANA-1 included bacterial and fungal infections (group, 2021), which may be associated with immunosuppression caused by anti-IL-1 therapy. Specific targeting of the NLRP3 inflammasome would allow for compensatory production of IL-1β by other inflammasomes during pathogen invasion, and perhaps result in a lower risk of infection than that associated with the use of immunosuppressive medications such as anakinra and dexamethasone. MCC950, also known as CRID3 or CP-456,773, is the most well-characterised inhibitor of NLRP3. It was first identified as being part of a group of sulfonylurea-containing compounds that inhibit IL-1β release (Perregaux et al., 2001), before subsequently being shown to specifically inhibit NLRP3 inflammasome activation by binding to the NLRP3 NACHT domain and blocking ATP hydrolysis (Coll et al., 2015, 2019; Tapia-Abellan et al., 2019). Importantly, MCC950 has shown efficacy in the majority of NLRP3-driven mouse models of disease (Mangan et al., 2018), and derivatives of the molecule are currently in clinical development for the treatment of some of these diseases. However, there are relatively few reports of the use of MCC950 in the treatment of infectious diseases. One such study compared the effect of early vs late administration of MCC950 in a murine model of lethal IAV infection and the results were striking regarding

the temporal differences in MCC950 administration. Early administration of MCC950 one day after IAV challenge increased mortality rates in infected mice, while administration of MCC950 starting on day 3 after IAV challenge, considered the peak of disease severity, delayed mortality (Tate et al., 2016). These results would be consistent with previous reports demonstrating that NLRP3 plays a protective role in the early stages of IAV infection, likely by restricting viral replication (Allen et al., 2009; Thomas et al., 2009). In later stages of the disease, NLRP3 contributes to hyperinflammation and cellular influx into the lungs. MCC950 was also shown to reduce inflammation in response to Chikungunya virus, an arthritogenic alphavirus, suggesting that NLRP3 inhibition may provide clinical benefit in the treatment of a wide range of viruses (Chen et al., 2017).

The pathophysiology of IAV infection bears many similarities to that of SARS-CoV-2 infection. Therefore, it may again be the case that NLRP3 inhibition would only be desirable in patients with severe COVID-19 where hyperinflammation and resulting lung damage predominate. *In vitro* mouse studies have demonstrated that MCC950 can inhibit SARS-CoV-2-induced caspase-1 activation and IL-1β release (Rodrigues et al., 2021), but as yet there are no data concerning the use of MCC950 in *in vivo* mouse models of SARS-CoV-2 infection. However, Novartis is currently analysing data from the RCT it completed in January 2021, testing its NLRP3 inhibitor DFV890 on 143 patients with SARS-CoV-2-associated pneumonia (NCT04382053). Interestingly, the company Olatec is pursuing a slightly different approach with its own NLRP3 inhibitor dapansutrile (NCT04540120). Rather than recruiting a cohort of ICU patients with severe COVID-19, Olatec has a target of 80 unhospitalized COVID-19 patients, with the hope of demonstrating that its NLRP3 inhibitor can prevent disease progression into severe COVID-19. It is possible therefore that we could soon have an answer as to whether NLRP3 inhibition is efficacious for the treatment of mild COVID-19, severe COVID-19 or both. The pharmacokinetics of these compounds may be an additional factor when considering the efficacy of targeting NLRP3 therapeutically. Small molecule inhibitors of NLRP3, such as MCC950, have been shown to have promising pharmacokinetics profiles (Mastrocola et al., 2016). For example, the oral bioavailability of MCC950 in mice was calculated to be 68%, which is comparable to the bioavailability of dexamethasone in humans (Coll et al., 2015; Duggan et al., 1975). One possible advantage that NLRP3 inflammasome inhibition has over the use of dexamethasone in the treatment of early COVID-19 concerns inhibition of type I IFN. As previously mentioned, it is thought that a robust early IFN response is required to limit viral replication (Park and Iwasaki, 2020). While dexamethasone and other immunosuppressive drugs block type I IFN signalling, NLRP3 inflammasome inhibition would leave type I IFN signalling intact, thereby allowing for increased viral clearance. Furthermore, type I IFN has previously been shown to repress NLRP3 inflammasome activation by reducing pro-IL-1β levels and IL-1β cleavage. To further support this, monocytes isolated from multiple sclerosis patients on IFN-β therapy released less IL-1β than monocytes from healthy donors (Guarda et al., 2011). High levels of type I IFN may therefore be beneficial not just in promoting an antiviral state, but also in restraining NLRP3 inflammasome activation and associated hyperinflammation.

7. Conclusion

Dexamethasone treatment is now part of the standard-of-care given to severe COVID-19 patients, and the clinical benefit provided by it is hard to dispute, despite unconvincing prior evidence regarding the use of dexamethasone in the treatment of respiratory viral infections. A clinical need still remains for treatments which (1) help to prevent ICU admission in the first place and; (2) are more specific and do not lead to the immunosuppressive side effects which may occur with dexamethasone administration. A case in point is the recent rise in cases of mucormycosis, or 'black fungus', in India, which has been linked to an excessive use of corticosteroids in the treatment of COVID-19 during the second wave of infections (Raut and Huy, 2021). NLRP3 inhibitors will likely address the issue of side effects, but question marks still remain over whether NLRP3 inhibition is desirable in early viral infection. Clinical trials which are currently underway with highly specific NLRP3 inhibitors will soon reveal the answers to these questions. It is perhaps premature to evaluate the potential utility of these compounds in treating COVID-19 when they are not yet FDA-approved. Furthermore, the side effect profile is unknown and could be problematic in a similar manner to dexamethasone. As such, their potential use in COVID-19 remains speculative, but promising nonetheless.

CRediT authorship contribution statement

Alexander Hooftman: Writing – original draft. **Luke A.J. O'Neill:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Agostini, L., Martinon, F., Burns, K., McDermott, M.F., Hawkins, P.N., Tschopp, J., 2004. NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20, 319–325. https://doi.org/10.1016/s1074-7613(04)00046-9.

Ahn, M., Anderson, D.E., Zhang, Q., Tan, C.W., Lim, B.L., Luko, K., Wen, M., Chia, W.N., Mani, S., Wang, L.C., et al., 2019. Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. Nat Microbiol 4, 789–799. https://doi.org/10.1038/s41564-019-0371-3.

Allen, I.C., Scull, M.A., Moore, C.B., Holl, E.K., McElvania-TeKippe, E., Taxman, D.J., Guthrie, E.H., Pickles, R.J., Ting, J.P., 2009. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity 30, 556–565. https://doi.org/10.1016/j.immuni.2009.02.005.

Alosaimi, B., Hamed, M.E., Naeem, A., Alsharef, A.A., AlQahtani, S.Y., AlDosari, K.M., Alamri, A.A., Al-Eisa, K., Khojah, T., Assiri, A.M., Enani, M.A., 2020. MERS-CoV infection is associated with downregulation of genes encoding Th1 and Th2 cytokines/chemokines and elevated inflammatory innate immune response in the lower respiratory tract. Cytokine 126, 154895. https://doi.org/10.1016/j.cyto.2019.154895.

Annane, D., Renault, A., Brun-Buisson, C., Megarbane, B., Quenot, J.P., Siami, S., Cariou, A., Forceville, X., Schwebel, C., Martin, C., et al., 2018. Hydrocortisone plus fludrocortisone for adults with septic shock. N. Engl. J. Med. 378, 809–818. https://doi.org/10.1056/NEJMoa1705716.

Arabi, Y.M., Mandourah, Y., Al-Hameed, F., Sindi, A.A., Almekhlafi, G.A., Hussein, M.A., Jose, J., Pinto, R., Al-Omari, A., Kharaba, A., et al., 2018. Corticosteroid therapy for critically ill patients with Middle East respiratory syndrome. Am. J. Respir. Crit. Care Med. 197, 757–767. https://doi.org/10.1164/rccm.201706-1172OC.

Bastard, P., Rosen, L.B., Zhang, Q., Michailidis, E., Hoffmann, H.-H., Zhang, Y., Dorgham, K., Philippot, Q., Rosain, J., Béziat, V., et al., 2020. Auto-antibodies against type I IFNs in patients with life-threatening COVID-19. Science eabd4585. https://doi.org/10.1126/science.abd4585.

Bauer, C., Duewell, P., Mayer, C., Lehr, H.A., Fitzgerald, K.A., Dauer, M., Tschopp, J., Endres, S., Latz, E., Schnurr, M., 2010. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut 59, 1192. https://doi.org/10.1136/gut.2009.197822.

Bauernfried, S., Scherr, M.J., Pichlmair, A., Duderstadt, K.E., Hornung, V., 2021. Human NLRP1 is a sensor for double-stranded RNA. Science 371. https://doi.org/10.1126/science.abd0811.

Beigel, J.H., Farrar, J., Han, A.M., Hayden, F.G., Hyer, R., de Jong, M.D., Lochindarat, S., Nguyen, T.K., Nguyen, T.H., Tran, T.H., et al., 2005. Avian influenza A (H5N1) infection in humans. N. Engl. J. Med. 353, 1374–1385. https://doi.org/10.1056/NEJMra052211.

Besnard, A.G., Guillou, N., Tschopp, J., Erard, F., Couillin, I., Iwakura, Y., Quesniaux, V., Ryffel, B., Togbe, D., 2011. NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. Allergy 66, 1047–1057. https://doi.org/10.1111/j.1398-9995.2011.02586.x.

Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.C., Uhl, S., Hoagland, D., Moller, R., Jordan, T.X., Oishi, K., Panis, M., Sachs, D., et al., 2020. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell 181, 1036–1045. https://doi.org/10.1016/j.cell.2020.04.026 e1039.

Boucher, D., Monteleone, M., Coll, R.C., Chen, K.W., Ross, C.M., Teo, J.L., Gomez, G.A., Holley, C.L., Bierschenk, D., Stacey, K.J., et al., 2018. Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. J. Exp. Med. 215, 827–840. https://doi.org/10.1084/jem.20172222.

Cai, X., Chen, J., Xu, H., Liu, S., Jiang, Q.X., Halfmann, R., Chen, Z.J., 2014. Prion-like polymerization underlies signal transduction in antiviral immune defense and

inflammasome activation. Cell 156, 1207–1222. https://doi.org/10.1016/j.cell.2014.01.063.
Cavalli, G., De Luca, G., Campochiaro, C., Della-Torre, E., Ripa, M., Canetti, D., Oltolini, C., Castiglioni, B., Tassan Din, C., Boffini, N., et al., 2020. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol 2, e325–e331. https://doi.org/10.1016/S2665-9913(20)30127-2.
Chen, I.Y., Moriyama, M., Chang, M.F., Ichinohe, T., 2019. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. Front. Microbiol. 10, 50. https://doi.org/10.3389/fmicb.2019.00050.
Chen, J., Chen, Z.J., 2018. PtdIns4P on dispersed trans-Golgi network mediates NLRP3 inflammasome activation. Nature 564, 71–76. https://doi.org/10.1038/s41586-018-0761-3.
Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., et al., 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395, 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7.
Chen, W., Foo, S.S., Zaid, A., Teng, T.S., Herrero, L.J., Wolf, S., Tharmarajah, K., Vu, L.D., van Vreden, C., Taylor, A., et al., 2017. Specific inhibition of NLRP3 in chikungunya disease reveals a role for inflammasomes in alphavirus-induced inflammation. Nat Microbiol 2, 1435–1445. https://doi.org/10.1038/s41564-017-0015-4.
Coll, R.C., Hill, J.R., Day, C.J., Zamoshnikova, A., Boucher, D., Massey, N.L., Chitty, J.L., Fraser, J.A., Jennings, M.P., Robertson, A.A.B., Schroder, K., 2019. MCC950 directly targets the NLRP3 ATP-hydrolysis motif for inflammasome inhibition. Nat. Chem. Biol. 15, 556–559. https://doi.org/10.1038/s41589-019-0277-7.
Coll, R.C., Robertson, A.A., Chae, J.J., Higgins, S.C., Munoz-Planillo, R., Inserra, M.C., Vetter, I., Dungan, L.S., Monks, B.G., Stutz, A., et al., 2015. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat. Med. 21, 248–255. https://doi.org/10.1038/nm.3806.
De Bosscher, K., Vanden Berghe, W., Haegeman, G., 2003. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. Endocr. Rev. 24, 488–522. https://doi.org/10.1210/er.2002-0006.
Deng, M., Tang, Y., Li, W., Wang, X., Zhang, R., Zhang, X., Zhao, X., Liu, J., Tang, C., Liu, Z., et al., 2018. The endotoxin delivery protein HMGB1 mediates caspase-11-dependent lethality in sepsis. Immunity 49, 740–753. https://doi.org/10.1016/j.immuni.2018.08.016 e747.
Dinarello, C.A., 2000. The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. N. Engl. J. Med. 343, 732–734. https://doi.org/10.1056/NEJM200009073431011.
Duewell, P., Kono, H., Rayner, K.J., Sirois, C.M., Vladimer, G., Bauernfeind, F.G., Abela, G.S., Franchi, L., Nunez, G., Schnurr, M., et al., 2010. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature 464, 1357–1361. https://doi.org/10.1038/nature08938.
Duggan, D.E., Yeh, K.C., Matalia, N., Ditzler, C.A., McMahon, F.G., 1975. Bioavailability of oral dexamethasone. Clin. Pharmacol. Ther. 18, 205–209. https://doi.org/10.1002/cpt1975182205.
Duncan, J.A., Bergstralh, D.T., Wang, Y., Willingham, S.B., Ye, Z., Zimmermann, A.G., Ting, J.P., 2007. Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling. Proc. Natl. Acad. Sci. U. S. A. 104, 8041–8046. https://doi.org/10.1073/pnas.0611496104.
Evavold, C.L., Ruan, J., Tan, Y., Xia, S., Wu, H., Kagan, J.C., 2018. The pore-forming protein gasdermin D regulates interleukin-1 secretion from living macrophages. Immunity 48, 35–44. https://doi.org/10.1016/j.immuni.2017.11.013 e36.
Fung, T.S., Liu, D.X., 2019. Human coronavirus: host-pathogen interaction. Annu. Rev. Microbiol. 73, 529–557. https://doi.org/10.1146/annurev-micro-020518-115759.
Gasse, P., Mary, C., Guenon, I., Noulin, N., Charron, S., Schnyder-Candrian, S., Schnyder, B., Akira, S., Quesniaux, V.F., Lagente, V., et al., 2007. IL-1R1/MyD88 signaling and the inflammasome are essential in pulmonary inflammation and fibrosis in mice. J. Clin. Invest. 117, 3786–3799. https://doi.org/10.1172/JCI32285.
Goulding, N.J., Godolphin, J.L., Sharland, P.R., Peers, S.H., Sampson, M., Maddison, P.J., Flower, R.J., 1990. Anti-inflammatory lipocortin 1 production by peripheral blood leucocytes in response to hydrocortisone. Lancet 335, 1416–1418. https://doi.org/10.1016/0140-6736(90)91445-g.
Grein, J., Ohmagari, N., Shin, D., Diaz, G., Asperges, E., Castagna, A., Feldt, T., Green, G., Green, M.L., Lescure, F.-X., et al., 2020. Compassionate use of remdesivir for patients with severe covid-19. N. Engl. J. Med. 382, 2327–2336. https://doi.org/10.1056/NEJMoa2007016.
Gris, D., Ye, Z., Iocca, H.A., Wen, H., Craven, R.R., Gris, P., Huang, M., Schneider, M., Miller, S.D., Ting, J.P., 2010. NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. J. Immunol. 185, 974–981. https://doi.org/10.4049/jimmunol.0904145.
Gross, C.J., Mishra, R., Schneider, K.S., Medard, G., Wettmarshausen, J., Dittlein, D.C., Shi, H., Gorka, O., Koenig, P.A., Fromm, S., et al., 2016. K(+) efflux-independent NLRP3 inflammasome activation by small molecules targeting mitochondria. Immunity 45, 761–773. https://doi.org/10.1016/j.immuni.2016.08.010.
group, C.-C., 2021. Effect of anakinra versus usual care in adults in hospital with COVID-19 and mild-to-moderate pneumonia (CORIMUNO-ANA-1): a randomised controlled trial. Lancet Respir Med 9, 295–304. https://doi.org/10.1016/S2213-2600(20)30556-7.
Group, R.C., Horby, P., Lim, W.S., Emberson, J.R., Mafham, M., Bell, J.L., Linsell, L., Staplin, N., Brightling, C., Ustianowski, A., et al., 2021. Dexamethasone in hospitalized patients with covid-19. N. Engl. J. Med. 384, 693–704. https://doi.org/10.1056/NEJMoa2021436.
Guan, M., Ma, H., Fan, X., Chen, X., Miao, M., Wu, H., 2020. Dexamethasone alleviate allergic airway inflammation in mice by inhibiting the activation of NLRP3 inflammasome. Int. Immunopharm. 78, 106017. https://doi.org/10.1016/j.intimp.2019.106017.
Guarda, G., Braun, M., Staehli, F., Tardivel, A., Mattmann, C., Forster, I., Farlik, M., Decker, T., Du Pasquier, R.A., Romero, P., Tschopp, J., 2011. Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity 34, 213–223. https://doi.org/10.1016/j.immuni.2011.02.006.
Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., Péré, H., Charbit, B., Bondet, V., Chenevier-Gobeaux, C., et al., 2020. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 369, 718. https://doi.org/10.1126/science.abc6027.
Halle, A., Hornung, V., Petzold, G.C., Stewart, C.R., Monks, B.G., Reinheckel, T., Fitzgerald, K.A., Latz, E., Moore, K.J., Golenbock, D.T., 2008. The NALP3 inflammasome is involved in the innate immune response to amyloid-β. Nat. Immunol. 9, 857–865. https://doi.org/10.1038/ni.1636.
He, L., Ding, Y., Zhang, Q., Che, X., He, Y., Shen, H., Wang, H., Li, Z., Zhao, L., Geng, J., et al., 2006. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. J. Pathol. 210, 288–297. https://doi.org/10.1002/path.2067.
Heneka, M.T., Kummer, M.P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., Griep, A., Axt, D., Remus, A., Tzeng, T.-C., et al., 2013. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493, 674–678. https://doi.org/10.1038/nature11729.
Hoffman, H.M., Mueller, J.L., Broide, D.H., Wanderer, A.A., Kolodner, R.D., 2001. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. Nat. Genet. 29, 301–305. https://doi.org/10.1038/ng756.
Hornung, V., Bauernfeind, F., Halle, A., Samstad, E.O., Kono, H., Rock, K.L., Fitzgerald, K.A., Latz, E., 2008. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat. Immunol. 9, 847–856. https://doi.org/10.1038/ni.1631.
Hu, C., Ding, H., Li, Y., Pearson, J.A., Zhang, X., Flavell, R.A., Wong, F.S., Wen, L., 2015. NLRP3 deficiency protects from type 1 diabetes through the regulation of chemotaxis into the pancreatic islets. Proc. Natl. Acad. Sci. Unit. States Am. 112, 11318. https://doi.org/10.1073/pnas.1513509112.
Hu, J.J., Liu, X., Xia, S., Zhang, Z., Zhang, Y., Zhao, J., Ruan, J., Luo, X., Lou, X., Bai, Y., et al., 2020. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nat. Immunol. 21, 736–745. https://doi.org/10.1038/s41590-020-0669-6.
Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.
Huet, T., Beaussier, H., Voisin, O., Jouveshomme, S., Dauriat, G., Lazareth, I., Sacco, E., Naccache, J.M., Bezie, Y., Laplanche, S., et al., 2020. Anakinra for severe forms of COVID-19: a cohort study. Lancet Rheumatol 2, e393–e400. https://doi.org/10.1016/S2665-9913(20)30164-8.
Humphries, F., Shmuel-Galia, L., Ketelut-Carneiro, N., Li, S., Wang, B., Nemmara, V.V., Wilson, R., Jiang, Z., Khalighinejad, F., Muneeruddin, K., et al., 2020. Succination inactivates gasdermin D and blocks pyroptosis. Science. https://doi.org/10.1126/science.abb9818.
Ito, K., Barnes, P.J., Adcock, I.M., 2000. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. Mol. Cell Biol. 20, 6891–6903. https://doi.org/10.1128/mcb.20.18.6891-6903.2000.
Iyer, S.S., He, Q., Janczy, J.R., Elliott, E.I., Zhong, Z., Olivier, A.K., Sadler, J.J., Knepper-Adrian, V., Han, R., Qiao, L., et al., 2013. Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. Immunity 39, 311–323. https://doi.org/10.1016/j.immuni.2013.08.001.
Jose, R.J., Manuel, A., 2020. COVID-19 cytokine storm: the interplay between inflammation and coagulation. Lancet Respir Med 8, e46–e47. https://doi.org/10.1016/S2213-2600(20)30216-2.
Kassel, O., Sancono, A., Kratzschmar, J., Kreft, B., Stassen, M., Cato, A.C., 2001. Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. EMBO J. 20, 7108–7116. https://doi.org/10.1093/emboj/20.24.7108.
Kayagaki, N., Kornfeld, O., Lee, B., Stowe, I., O'Rourke, K., Li, Q., Sandoval, W., Yan, D., Xu, M., Ulas, G., et al., 2020. NINJ1 mediates plasma membrane rupture during lytic cell death. PREPRINT. https://doi.org/10.21203/rs.3.rs-62714/v1.
Kayagaki, N., Stowe, I.B., Lee, B.L., O'Rourke, K., Anderson, K., Warming, S., Cuellar, T., Haley, B., Roose-Girma, M., Phung, Q.T., et al., 2015. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature 526, 666–671. https://doi.org/10.1038/nature15541.
Kayagaki, N., Warming, S., Lamkanfi, M., Vande Walle, L., Louie, S., Dong, J., Newton, K., Qu, Y., Liu, J., Heldens, S., et al., 2011. Non-canonical inflammasome activation targets caspase-11. Nature 479, 117–121. https://doi.org/10.1038/nature10558.
Lee, N., Hui, D., Wu, A., Chan, P., Cameron, P., Joynt, G.M., Ahuja, A., Yung, M.Y., Leung, C.B., To, K.F., et al., 2003. A major outbreak of severe acute respiratory syndrome in Hong Kong. N. Engl. J. Med. 348, 1986–1994. https://doi.org/10.1056/NEJMoa030685.
Levi, M., Thachil, J., Iba, T., Levy, J.H., 2020. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol 7, e438–e440. https://doi.org/10.1016/S2352-3026(20)30145-9.
Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., Salfeld, J., et al., 1995. Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to

endotoxic shock. Cell 80, 401–411. https://doi.org/10.1016/0092-8674(95)90490-5.
Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J.H., Wang, H., Crameri, G., Hu, Z., Zhang, H., et al., 2005. Bats are natural reservoirs of SARS-like coronaviruses. Science 310, 676–679. https://doi.org/10.1126/science.1118391.
Lind-Holst, M., Hartling, U.B., Christensen, A.E., 2019. High-dose anakinra as treatment for macrophage activation syndrome caused by refractory Kawasaki disease in an infant. BMJ Case Rep. 12, e229708. https://doi.org/10.1136/bcr-2019-229708.
Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V.G., Wu, H., Lieberman, J., 2016. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature 535, 153–158. https://doi.org/10.1038/nature18629.
Lu, A., Magupalli, V.G., Ruan, J., Yin, Q., Atianand, M.K., Vos, M.R., Schroder, G.F., Fitzgerald, K.A., Wu, H., Egelman, E.H., 2014. Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. Cell 156, 1193–1206. https://doi.org/10.1016/j.cell.2014.02.008.
Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., et al., 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395, 565–574. https://doi.org/10.1016/S0140-6736(20)30251-8.
Lucas, C., Wong, P., Klein, J., Castro, T.B.R., Silva, J., Sundaram, M., Ellingson, M.K., Mao, T., Oh, J.E., Israelow, B., et al., 2020. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 584, 463–469. https://doi.org/10.1038/s41586-020-2588-y.
Mangan, M.S.J., Olhava, E.J., Roush, W.R., Seidel, H.M., Glick, G.D., Latz, E., 2018. Targeting the NLRP3 inflammasome in inflammatory diseases. Nat. Rev. Drug Discov. 17, 688. https://doi.org/10.1038/nrd.2018.149.
Martinon, F., Burns, K., Tschopp, J., 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol. Cell 10, 417–426. https://doi.org/10.1016/s1097-2765(02)00599-3.
Mastrocola, R., Penna, C., Tullio, F., Femmino, S., Nigro, D., Chiazza, F., Serpe, L., Collotta, D., Alloatti, G., Cocco, M., et al., 2016. Pharmacological inhibition of NLRP3 inflammasome attenuates myocardial ischemia/reperfusion injury by activation of RISK and mitochondrial pathways. Oxid Med Cell Longev 2016, 5271251. https://doi.org/10.1155/2016/5271251.
Mathew, D., Giles, J.R., Baxter, A.E., Oldridge, D.A., Greenplate, A.R., Wu, J.E., Alanio, C., Kuri-Cervantes, L., Pampena, M.B., D'Andrea, K., et al., 2020. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science 369. https://doi.org/10.1126/science.abc8511.
McGee, S., Hirschmann, J., 2008. Use of corticosteroids in treating infectious diseases. Arch. Intern. Med. 168, 1034–1046. https://doi.org/10.1001/archinte.168.10.1034.
Meduri, G.U., Headley, S., Kohler, G., Stentz, F., Tolley, E., Umberger, R., Leeper, K., 1995a. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. Chest 107, 1062–1073. https://doi.org/10.1378/chest.107.4.1062.
Meduri, G.U., Kohler, G., Headley, S., Tolley, E., Stentz, F., Postlethwaite, A., 1995b. Inflammatory cytokines in the BAL of patients with ARDS. Persistent elevation over time predicts poor outcome. Chest 108, 1303–1314. https://doi.org/10.1378/chest.108.5.1303.
Munoz-Planillo, R., Kuffa, P., Martinez-Colon, G., Smith, B.L., Rajendiran, T.M., Nunez, G., 2013. K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity 38, 1142–1153. https://doi.org/10.1016/j.immuni.2013.05.016.
Munster, V.J., Adney, D.R., van Doremalen, N., Brown, V.R., Miazgowicz, K.L., Milne-Price, S., Bushmaker, T., Rosenke, R., Scott, D., Hawkinson, A., et al., 2016. Replication and shedding of MERS-CoV in Jamaican fruit bats (Artibeus jamaicensis). Sci. Rep. 6, 21878. https://doi.org/10.1038/srep21878.
Nakahira, K., Haspel, J.A., Rathinam, V.A.K., Lee, S.-J., Dolinay, T., Lam, H.C., Englert, J.A., Rabinovitch, M., Cernadas, M., Kim, H.P., et al., 2011. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat. Immunol. 12, 222–230. https://doi.org/10.1038/ni.1980.
Ni, Y.N., Chen, G., Sun, J., Liang, B.M., Liang, Z.A., 2019. The effect of corticosteroids on mortality of patients with influenza pneumonia: a systematic review and meta-analysis. Crit. Care 23, 99. https://doi.org/10.1186/s13054-019-2395-8.
Nicolaides, N.C., Pavlaki, A.N., Maria Alexandra, M.A., Chrousos, G.P., 2000. Glucocorticoid therapy and adrenal suppression. In: Feingold, K.R., Anawalt, B., Boyce, A., Chrousos, G., de Herder, W.W., Dhatariya, K., Dungan, K., Grossman, A., Hershman, J.M., Hofland, J., et al. (Eds.), Endotext.
Nieto-Torres, J.L., DeDiego, M.L., Verdia-Baguena, C., Jimenez-Guardeno, J.M., Regla-Nava, J.A., Fernandez-Delgado, R., Castano-Rodriguez, C., Alcaraz, A., Torres, J., Aguilella, V.M., Enjuanes, L., 2014. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLoS Pathog. 10, e1004077. https://doi.org/10.1371/journal.ppat.1004077.
Park, A., Iwasaki, A., 2020. Type I and type III interferons - induction, signaling, evasion, and application to combat COVID-19. Cell Host Microbe 27, 870–878. https://doi.org/10.1016/j.chom.2020.05.008.
Perregaux, D., Gabel, C.A., 1994. Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. J. Biol. Chem. 269, 15195–15203.
Perregaux, D.G., McNiff, P., Laliberte, R., Hawryluk, N., Peurano, H., Stam, E., Eggler, J., Griffiths, R., Dombroski, M.A., Gabel, C.A., 2001. Identification and characterization of a novel class of interleukin-1 post-translational processing inhibitors. J. Pharmacol. Exp. Therapeut. 299, 187–197.
Rajasekaran, S., Kruse, K., Kovey, K., Davis, A.T., Hassan, N.E., Ndika, A.N., Zuiderveen, S., Birmingham, J., 2014. Therapeutic role of anakinra, an interleukin-1 receptor antagonist, in the management of secondary hemophagocytic lymphohistiocytosis/sepsis/multiple organ dysfunction/macrophage activating syndrome in critically ill children*. Pediatr. Crit. Care Med. 15.
Ramos, H.J., Lanteri, M.C., Blahnik, G., Negash, A., Suthar, M.S., Brassil, M.M., Sodhi, K., Treuting, P.M., Busch, M.P., Norris, P.J., Gale Jr., M., 2012. IL-1beta signaling promotes CNS-intrinsic immune control of West Nile virus infection. PLoS Pathog. 8, e1003039. https://doi.org/10.1371/journal.ppat.1003039.
Raut, A., Huy, N.T., 2021. Rising incidence of mucormycosis in patients with COVID-19: another challenge for India amidst the second wave? The Lancet Respiratory Medicine. https://doi.org/10.1016/S2213-2600(21)00265-4.
Rhen, T., Cidlowski, J.A., 2005. Antiinflammatory action of glucocorticoids–new mechanisms for old drugs. N. Engl. J. Med. 353, 1711–1723. https://doi.org/10.1056/NEJMra050541.
Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C., Fonseca, F., Nicolau, J., Koenig, W., Anker, S.D., et al., 2017a. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N. Engl. J. Med. 377, 1119–1131. https://doi.org/10.1056/NEJMoa1707914.
Ridker, P.M., MacFadyen, J.G., Thuren, T., Everett, B.M., Libby, P., Glynn, R.J., Group, C.T., 2017b. Effect of interleukin-1beta inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. Lancet 390, 1833–1842. https://doi.org/10.1016/S0140-6736(17)32247-X.
Rodrigues, T.S., de Sa, K.S.G., Ishimoto, A.Y., Becerra, A., Oliveira, S., Almeida, L., Goncalves, A.V., Perucello, D.B., Andrade, W.A., Castro, R., et al., 2021. Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. J. Exp. Med. 218. https://doi.org/10.1084/jem.20201707.
Russell, C.D., Millar, J.E., Baillie, J.K., 2020. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet 395, 473–475. https://doi.org/10.1016/S0140-6736(20)30317-2.
Schmitz, N., Kurrer, M., Bachmann, M.F., Kopf, M., 2005. Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. J. Virol. 79, 6441–6448. https://doi.org/10.1128/JVI.79.10.6441-6448.2005.
Schrijver, H.M., Crusius, J.B., Uitdehaag, B.M., Garcia Gonzalez, M.A., Kostense, P.J., Polman, C.H., Pena, A.S., 1999. Association of interleukin-1beta and interleukin-1 receptor antagonist genes with disease severity in MS. Neurology 52, 595–599. https://doi.org/10.1212/wnl.52.3.595.
Schroder, K., Tschopp, J., 2010. The inflammasomes. Cell 140, 821–832. https://doi.org/10.1016/j.cell.2010.01.040.
Shakoory, B., Carcillo, J.A., Chatham, W.W., Amdur, R.L., Zhao, H., Dinarello, C.A., Cron, R.Q., Opal, S.M., 2016. Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of macrophage activation syndrome: reanalysis of a prior phase III trial*. Crit. Care Med. 44.
Shi, C.S., Nabar, N.R., Huang, N.N., Kehrl, J.H., 2019. SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. Cell Death Dis. 5, 101. https://doi.org/10.1038/s41420-019-0181-7.
Shi, J., Zhao, Y., Wang, K., Shi, X., Wang, Y., Huang, H., Zhuang, Y., Cai, T., Wang, F., Shao, F., 2015. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526, 660–665. https://doi.org/10.1038/nature15514.
Siu, K.L., Yuen, K.S., Castano-Rodriguez, C., Ye, Z.W., Yeung, M.L., Fung, S.Y., Yuan, S., Chan, C.P., Yuen, K.Y., Enjuanes, L., Jin, D.Y., 2019. Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. Faseb. J. 33, 8865–8877. https://doi.org/10.1096/fj.201802418R.
Stockman, L.J., Bellamy, R., Garner, P., 2006. SARS: systematic review of treatment effects. PLoS Med. 3, e343. https://doi.org/10.1371/journal.pmed.0030343.
Surprenant, A., Rassendren, F., Kawashima, E., North, R.A., Buell, G., 1996. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). Science 272, 735. https://doi.org/10.1126/science.272.5262.735.
Tapia-Abellan, A., Angosto-Bazarra, D., Martinez-Banaclocha, H., de Torre-Minguela, C., Ceron-Carrasco, J.P., Perez-Sanchez, H., Arostegui, J.I., Pelegrin, P., 2019. MCC950 closes the active conformation of NLRP3 to an inactive state. Nat. Chem. Biol. 15, 560–564. https://doi.org/10.1038/s41589-019-0278-6.
Tate, M.D., Ong, J.D.H., Dowling, J.K., McAuley, J.L., Robertson, A.B., Latz, E., Drummond, G.R., Cooper, M.A., Hertzog, P.J., Mansell, A., 2016. Reassessing the role of the NLRP3 inflammasome during pathogenic influenza A virus infection via temporal inhibition. Sci. Rep. 6, 27912. https://doi.org/10.1038/srep27912.
Thomas, P.G., Dash, P., Aldridge Jr., J.R., Ellebedy, A.H., Reynolds, C., Funk, A.J., Martin, W.J., Lamkanfi, M., Webby, R.J., Boyd, K.L., et al., 2009. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity 30, 566–575. https://doi.org/10.1016/j.immuni.2009.02.006.
Vande Walle, L., Van Opdenbosch, N., Jacques, P., Fossoul, A., Verheugen, E., Vogel, P., Beyaert, R., Elewaut, D., Kanneganti, T.D., van Loo, G., Lamkanfi, M., 2014. Negative regulation of the NLRP3 inflammasome by A20 protects against arthritis. Nature 512, 69–73. https://doi.org/10.1038/nature13322.
Webster, J.I., Tonelli, L., 2002. Neuroendocrine regulation of immunity, 20, 125–163.
Wen, H., Gris, D., Lei, Y., Jha, S., Zhang, L., Huang, M.T., Brickey, W.J., Ting, J.P., 2011. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat. Immunol. 12, 408–415. https://doi.org/10.1038/ni.2022.
Wilk, A.J., Rustagi, A., Zhao, N.Q., Roque, J., Martinez-Colon, G.J., McKechnie, J.L., Ivison, G.T., Ranganath, T., Vergara, R., Hollis, T., et al., 2020. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. Nat. Med. 26, 1070–1076. https://doi.org/10.1038/s41591-020-0944-y.

Wohlfarth, P., Agis, H., Gualdoni, G.A., Weber, J., Staudinger, T., Schellongowski, P., Robak, O., 2019. Interleukin 1 receptor antagonist anakinra, intravenous immunoglobulin, and corticosteroids in the management of critically ill adult patients with hemophagocytic lymphohistiocytosis. J. Intensive Care Med. 34, 723–731. https://doi.org/10.1177/0885066617711386.

Wolf, A.J., Reyes, C.N., Liang, W., Becker, C., Shimada, K., Wheeler, M.L., Cho, H.C., Popescu, N.I., Coggeshall, K.M., Arditi, M., Underhill, D.M., 2016. Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. Cell 166, 624–636. https://doi.org/10.1016/j.cell.2016.05.076.

Wu, C., Lu, W., Zhang, Y., Zhang, G., Shi, X., Hisada, Y., Grover, S.P., Zhang, X., Li, L., Xiang, B., et al., 2019. Inflammasome activation triggers blood clotting and host death through pyroptosis. Immunity 50, 1401–1411. https://doi.org/10.1016/j.immuni.2019.04.003 e1404.

Yam, L.Y., Lau, A.C., Lai, F.Y., Shung, E., Chan, J., Wong, V., Hong Kong Hospital Authority, S.C.G., 2007. Corticosteroid treatment of severe acute respiratory syndrome in Hong Kong. J. Infect. 54, 28–39. https://doi.org/10.1016/j.jinf.2006.01.005.

Yan, L., Zhang, H.-T., Goncalves, J., Xiao, Y., Wang, M., Guo, Y., Sun, C., Tang, X., Jing, L., Zhang, M., et al., 2020. An interpretable mortality prediction model for COVID-19 patients. Nature Machine Intelligence 2, 283–288. https://doi.org/10.1038/s42256-020-0180-7.

Yang, X., Cheng, X., Tang, Y., Qiu, X., Wang, Y., Kang, H., Wu, J., Wang, Z., Liu, Y., Chen, F., et al., 2019. Bacterial endotoxin activates the coagulation cascade through gasdermin D-dependent phosphatidylserine exposure. Immunity 51, 983–996. https://doi.org/10.1016/j.immuni.2019.11.005 e986.

Yang, X., Cheng, X., Tang, Y., Qiu, X., Wang, Z., Fu, G., Wu, J., Kang, H., Wang, J., Wang, H., et al., 2020. The role of type 1 interferons in coagulation induced by gram-negative bacteria. Blood 135, 1087–1100. https://doi.org/10.1182/blood.2019002282.

Youm, Y.H., Nguyen, K.Y., Grant, R.W., Goldberg, E.L., Bodogai, M., Kim, D., D'Agostino, D., Planavsky, N., Lupfer, C., Kanneganti, T.D., et al., 2015. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat. Med. 21, 263–269. https://doi.org/10.1038/nm.3804.

Yue, H., Bai, X., Wang, J., Yu, Q., Liu, W., Pu, J., Wang, X., Hu, J., Xu, D., Li, X., et al., 2020. Clinical characteristics of coronavirus disease 2019 in Gansu province, China. Ann. Palliat. Med. 9, 1404–1412. https://doi.org/10.21037/apm-20-887.

Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D., Fouchier, R.A., 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N. Engl. J. Med. 367, 1814–1820. https://doi.org/10.1056/NEJMoa1211721.

Zhang, B., Zhou, X., Qiu, Y., Song, Y., Feng, F., Feng, J., Song, Q., Jia, Q., Wang, J., 2020a. Clinical characteristics of 82 cases of death from COVID-19. PloS One 15, e0235458. https://doi.org/10.1371/journal.pone.0235458.

Zhang, H., Luo, J., Alcorn, J.F., Chen, K., Fan, S., Pilewski, J., Liu, A., Chen, W., Kolls, J.K., Wang, J., 2017. AIM2 inflammasome is critical for influenza-induced lung injury and mortality. J. Immunol. 198, 4383–4393. https://doi.org/10.4049/jimmunol.1600714.

Zhang, Q., Bastard, P., Liu, Z., Le Pen, J., Moncada-Velez, M., Chen, J., Ogishi, M., Sabli, I.K.D., Hodeib, S., Korol, C., et al., 2020b. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science eabd4570. https://doi.org/10.1126/science.abd4570.

Zhong, Z., Liang, S., Sanchez-Lopez, E., He, F., Shalapour, S., Lin, X.J., Wong, J., Ding, S., Seki, E., Schnabl, B., et al., 2018. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. Nature 560, 198–203. https://doi.org/10.1038/s41586-018-0372-z.

Zhong, Z., Umemura, A., Sanchez-Lopez, E., Liang, S., Shalapour, S., Wong, J., He, F., Boassa, D., Perkins, G., Ali, S.R., et al., 2016. NF-kappaB restricts inflammasome activation via elimination of damaged mitochondria. Cell 164, 896–910. https://doi.org/10.1016/j.cell.2015.12.057.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., et al., 2020a. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395, 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3.

Zhou, Z., Ren, L., Zhang, L., Zhong, J., Xiao, Y., Jia, Z., Guo, L., Yang, J., Wang, C., Jiang, S., et al., 2020b. Heightened innate immune responses in the respiratory tract of COVID-19 patients. Cell Host Microbe 27, 883–890. https://doi.org/10.1016/j.chom.2020.04.017 e882.