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The Role of Cell Death Mechanisms in SARS-CoV-2 and Respiratory RNA Viruses: From Pathogenesis to Potential Therapeutic Strategies

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THE ROLE OF CELL DEATH MECHANISMS IN SARS-COV-2 AND RESPIRATORY RNA
VIRUSES: FROM PATHOGENESIS TO POTENTIAL THERAPEUTIC STRATEGIES

by

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Abstract

Emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in the coronavirus disease-19 (COVID-19) pandemic that affected populations globally for several recent years. While effective vaccines have been developed as the major antiviral strategy against COVID-19, hospitalizations and severe long-term conditions of SARS-CoV-2 patients are still occurring. Cell death mechanisms are known to play a significant role in antiviral host defense mechanisms. However, recent research indicates that cell death may also be involved in the viral pathophysiology associated with coronavirus infection, and determining treatments based on mediators of dysregulated cell death can have advantageous applications for severe cases of COVID-19. Since current research on cell death mechanisms (pyroptosis, apoptosis, and necroptosis) does not extensively identify treatments related to the potentially harmful roles of cell death in COVID-19 infection, examining cell death mediators and existing treatments in previous respiratory RNA infections (such as influenza, respiratory syncytial virus, and human metapneumovirus) can elucidate potential therapeutic agents for SARS-CoV-2. This review serves to reveal the extent to which cell death mechanisms play a detrimental role in response to viral infections (specifically to SARS-CoV-2), which may highlight novel antiviral strategies to combat severe coronavirus infection.

Background

Coronaviruses describe a large class of enveloped, positive-sense, single-stranded RNA viruses that have been responsible for highly transmittable respiratory infections in recent decades. The general structure of coronaviruses is composed of four main structural proteins: spike protein (S), membrane protein (M), nucleocapsid protein (N), and the envelope protein (E) (Zawilska et al., 2021). The spike (S) protein serves as a transmembrane glycoprotein with two subunits (S1 and S2) that are used for host cell receptor recognition and binding (Figure 1). The membrane (M) protein interacts with the spike protein in order to mediate viral assembly in the endoplasmic reticulum and the Golgi apparatus, as well as membrane budding. The nucleocapsid (N) protein can be described as a helical structure within the viral envelope responsible for encapsulating the viral RNA genome. The envelope (E) protein, the smallest of the structural proteins, plays a crucial role in the escape of newly assembled viral particles by creating pores in the host cell's membrane. The presence of the envelope protein has also been implicated in the virulence of SARS-CoV-2.

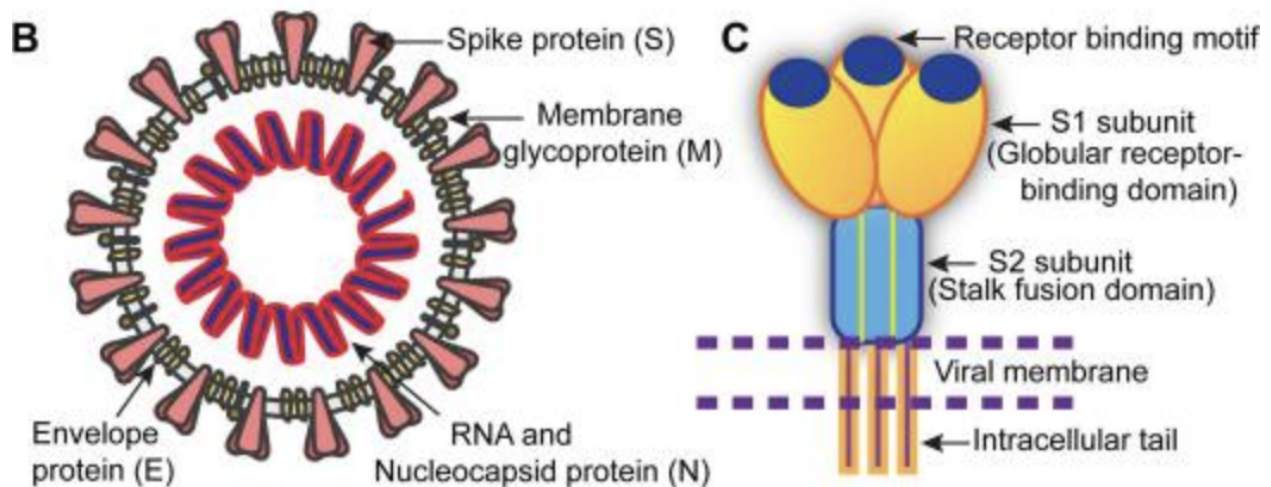


Figure 1. Components of SARS-CoV-2. (B) Schematic representation of the SARS-CoV-2 structure. (C) Schematic representation of the trimeric spike protein in SARS-CoV-2. Modified from (Mittal et al., 2020).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus belonging to the betacoronavirus genera, has emerged in recent years as a primary causative agent of potentially fatal human respiratory illnesses (Lu et al., 2020). The life-threatening COVID-19 pandemic, caused by the emergence of SARS-CoV-2, has highlighted the importance of understanding the underlying mechanisms of coronavirus pathogenesis. Coronaviruses have previously been known to activate various cell death pathways during the host immune response, such as pyroptosis, apoptosis, and necroptosis. Pyroptosis, a lytic cell death pathway which involves the activation of proinflammatory cytokines and gasdermin family members, has previously been implicated in organ dysfunction and severe respiratory symptoms resulting from SARS-CoV-2 (Kovacs & Miao, 2017). Necroptosis is also a lytic cell death pathway involving the interaction between Receptor Interacting Protein Kinase 3 (RIPK3) and Mixed Lineage Kinase Domain-Like (MLKL), which leads to the release of damage associated molecular patterns (DAMPs) and subsequent cytokine release (Pasparakis & Vandenabeele, 2015). Apoptosis differs from the aforementioned pathways in that its cell death mechanism is considered to be non-lytic and relies on the activation of multiple caspases, such as caspase-8 and caspase-9 (Kesavardhana et al., 2020). However, recent years have revealed mechanisms that support the existence of crosslinks and intricate interactions between these three major cell death mechanisms, collectively known as PANoptosis.

Although the administration of vaccines against SARS-CoV-2 has been largely effective in preventing the severe symptoms associated with coronavirus infection, variants of concern may still develop due to the rapid adaptability and mutation rates of coronavirus lineages. For example, until recently, the Omicron variant served as the predominant form of SARS-CoV-2 due to its increased immune evasion capabilities (Planas et al., 2021). Additionally, several long-

term complications of COVID-19 infection exist such as neurological, cardiac, and pulmonary conditions. Regulated cell death pathways including pyroptosis, apoptosis, and necroptosis likely play a dual role in both the antiviral host defense system and the increased pathophysiology of SARS-CoV-2.

While data regarding SARS-CoV-2 is continuously expanding, current data indicates that the cell death mechanisms of PANoptosis play a significant, complex role in the context of both antiviral defense mechanisms and viral pathophysiology. Although host cell death is an effective strategy for reducing viral replication, hyperactivity of inflammatory cell death processes results in cytokine storm and viral spread, which ultimately results in harmful disease progression of SARS-CoV-2 and even death. However, the discovery of various small molecules, inhibitors, agonists, and antagonists affecting the mediators of these cell death pathways in respiratory viral infections serves as the first step in identifying ways to decrease disease severity in COVID-19 patients with long-term tissue damage. Because other respiratory RNA viruses have commonly been associated with human infection, an examination of previous respiratory RNA viruses can illuminate potential therapeutic targets that may ameliorate long-term tissue damage associated with the aggressive infection induced by SARS-CoV-2. Therefore, reviewing previously studied respiratory RNA viruses can provide a means for identifying potential molecular targets that may disrupt the virulence of COVID-19.

Three significant respiratory RNA viruses that have been extensively studied prior to the emergence of SARS-CoV-2 include influenza, respiratory syncytial virus, and human metapneumovirus. Despite their distinct differences, the three viruses all induce common pathological symptoms in hosts based on their similar viral mechanisms as single-stranded, negative-sense, respiratory RNA viruses. These viruses display similarities to SARS-CoV-2 in

that they play a significant role in inflammation and respiratory distress, particularly among infants, elderly, and immunocompromised populations. Based on the complex nature of viral-host interactions and the cell death mechanisms involved in respiratory viral infections, investigating influenza, respiratory syncytial virus, and human metapneumovirus can provide valuable insights into broad-spectrum antiviral strategies that may have beneficial applications for COVID-19 as well. The present review aims to establish a comprehensive understanding of the significant cell death pathways involved in PANoptosis (pyroptosis, apoptosis, and necroptosis) in terms of their individual contributions to the pathogenesis of previously studied respiratory RNA viruses, and ultimately, to the pathogenesis of SARS-CoV-2. By combining the analysis of the cell death mechanisms involved in SARS-CoV-2 pathogenesis with the analysis of cell death mechanisms involved in other respiratory RNA viruses, potential therapeutic agents can be identified to target severe manifestations of coronavirus infection.

The General Mechanism of Pyroptosis

Pyroptosis is a vital cell death pathway that regulates the immune system's response against many pathogens. Immune cells, such as macrophages and monocytes, recognize external pathogens and subsequently initiate the assembly of the inflammasome complex (Liu et al., 2021). Initiation of the pathway is dependent on the activation of inflammasomes such as the NLRP3 inflammasome, which is one of the most extensively-studied inflammasomes. An inflammasome is characterized as a cellular protein complex that becomes activated after sensing microbial infections or harmful stimuli such as pathogen-associated molecular patterns (PAMPs) and DAMPs (Wang et al., 2019). After their activation is triggered by danger signals, inflammasomes recruit and activate procaspase-1 through auto-cleavage. Active caspase-1 then cleaves the pro-inflammatory cytokines pro-interleukin-1 β (pro-IL-1 β) and pro-IL-18 into their

bioactive forms. Caspase-1 further cleaves gasdermin D (GSDMD), which allows the N-terminal domain of GSDMD to form pores in the plasma membrane to subsequently release pro-inflammatory cytokines (Zhao et al., 2018). This process triggers the lytic, pro-inflammatory form of cell death known as pyroptosis, which is characterized by swelling of cells, release of intracellular contents, and ultimately recruitment of immune cells to the infection site.

Although pyroptosis plays a crucial role in host defense mechanisms, dysregulation of this pathway has been implicated in the increased pathogenesis of many diseases, including SARS-CoV-2. Irregular activation of the NLRP3 inflammasome triggers the excessive production of pro-inflammatory interleukins (IL-1 β and IL-18), which prolongs inflammatory effects within the host and causes tissue damage. The NLRP3 inflammasome has been implicated in the pathogenesis of viral infections such as mouse hepatitis virus (MHV), which was previously found to rapidly release reactive oxygen species (ROS) upon infection and subsequently trigger rapid formation of the NLRP3 inflammasome complex (Guo et al., 2015). Additionally, the excessive activation of mediators of pyroptosis by respiratory RNA viruses, such as influenza A virus (IAV), has previously been linked to the formation of cytokine storms within the body, which can result in disease progression and multiorgan failure in severe cases (Fujikura & Miyazaki, 2018).

Downstream players related to the pyroptosis pathway may also play an important role in pathogenesis. Along with caspase-1, other caspases have also been implicated in contributing to cell death by pyroptosis (Figure 2). The non-canonical inflammasome pathway induced by lipopolysaccharides in Gram-negative bacteria consists of caspase-4, caspase-5, and caspase-11, and this pathway provides an alternative mechanism for triggering pro-inflammatory cell death (Shi et al., 2014). More recently, a caspase-8-dependent cell death pathway was revealed as

being involved in the cleavage of both GSDMD and gasdermin E (GSDME) upon bacterial infection, leading to increased cell death (Sarhan et al., 2018). These findings highlight the complex processes through which regulation of pyroptosis may occur as a result of various infections. Detailed examination of the key mediators of this pathway in the context of coronaviruses may reveal potential targets for future therapeutic interventions of SARS-CoV-2.

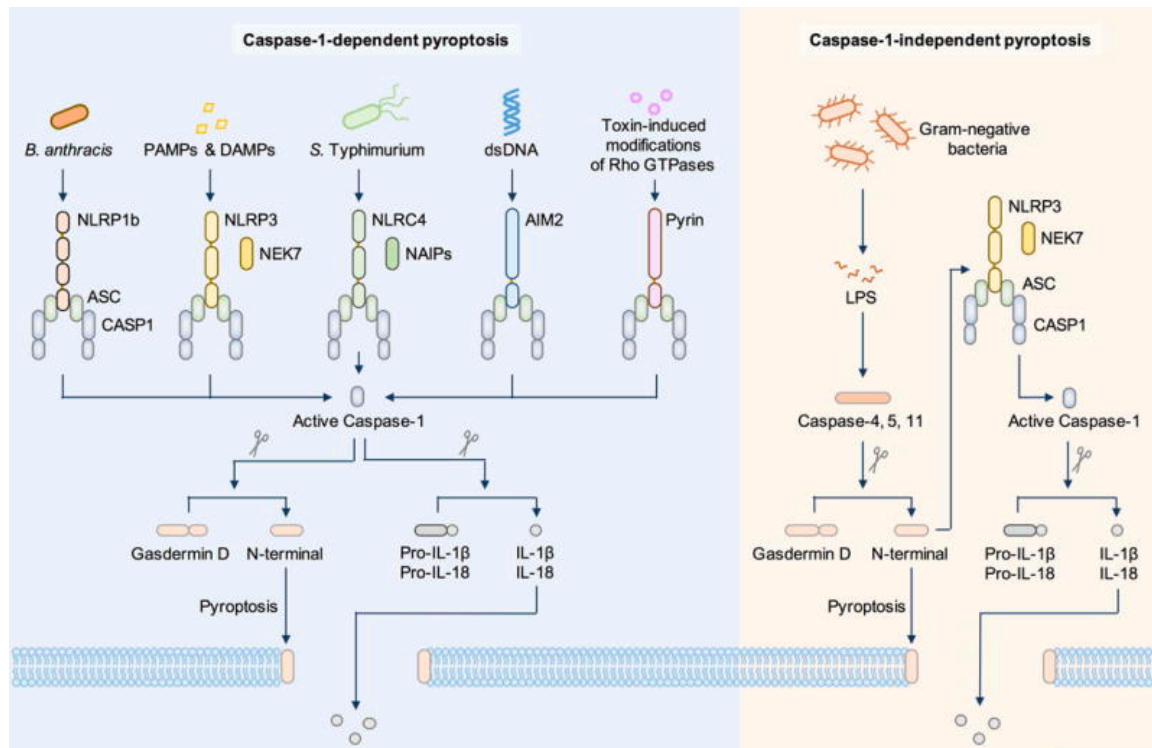


Figure 2. Mechanisms of Pyroptosis-Induced Inflammation. Pyroptosis can proceed via a caspase-1-dependent and a caspase-1-independent pathway. Both pathways involve the release of proinflammatory cytokines. Adapted from (Man et al., 2017).

The General Mechanism of Apoptosis

Apoptosis describes a form of programmed cell death involving cell shrinkage that can be induced by viral infections. Cells undergoing apoptosis are engulfed by phagocytes to prevent leakage of their contents. The initiation of apoptosis can proceed via the extrinsic or intrinsic pathway (Figure 3). The extrinsic pathway, which is activated through cytokines such as tumor necrosis factor alpha (TNF- α) and various death ligands, involves the activation of death

receptors belonging to the TNF receptor family (e.g., Fas/CD95, TNFR1, TRAIL-R1/DR4, and TRAIL-R2/DR5) (Zhou et al., 2017). Death receptors can also be activated through the release of perforin or granzyme by cytotoxic T cells and natural killer cells. Following the binding of ligands to the death receptors on the target cell, the death-inducing signaling complex (DISC) is assembled as a combination of the death receptors, the adaptor molecule FADD (Fas-associated protein with death domain), and procaspase-8 (Verburg et al., 2022). After the activation of caspase-8, further cleavage leads to the activation of downstream effector caspases such as caspase-6, which allows the cell to be committed to apoptosis. Regulation of the extrinsic pathway relies on various proteins such as c-FLIP (cellular FLICE-inhibitory protein), which serves as a competitive inhibitor of caspase-8 activation at the DISC (Verburg et al., 2022).

The intrinsic pathway of apoptosis occurs through mitochondrial outer membrane permeabilization (MOMP) when the cell senses internal stimuli such as oxidative stress or DNA damage. Following MOMP, pro-apoptotic proteins, such as cytochrome C, Smac/DIABLO, AIF-1, and Omi/HtrA2, are released from the intermembrane space of the mitochondria into the cytoplasm (Galluzzi et al., 2008). The release of cytochrome C allows the protein to interact with Apaf-1 and procaspase-9 in the cytoplasm, which leads to the formation of an apoptosome complex. The assembly of the apoptosome complex is crucial for the cleavage of procaspase-9 into active caspase-9, which triggers the activation of downstream effector caspases and commits the cell to apoptotic cell death (similarly to the extrinsic pathway) (Galluzzi et al., 2008). Regulation of the intrinsic pathway is primarily mediated through pro-apoptotic and anti-apoptotic proteins belonging to the Bcl-2 protein family, such as the Bax and Bak proteins responsible for stimulating MOMP formation via dimerization (Chipuk et al., 2004).

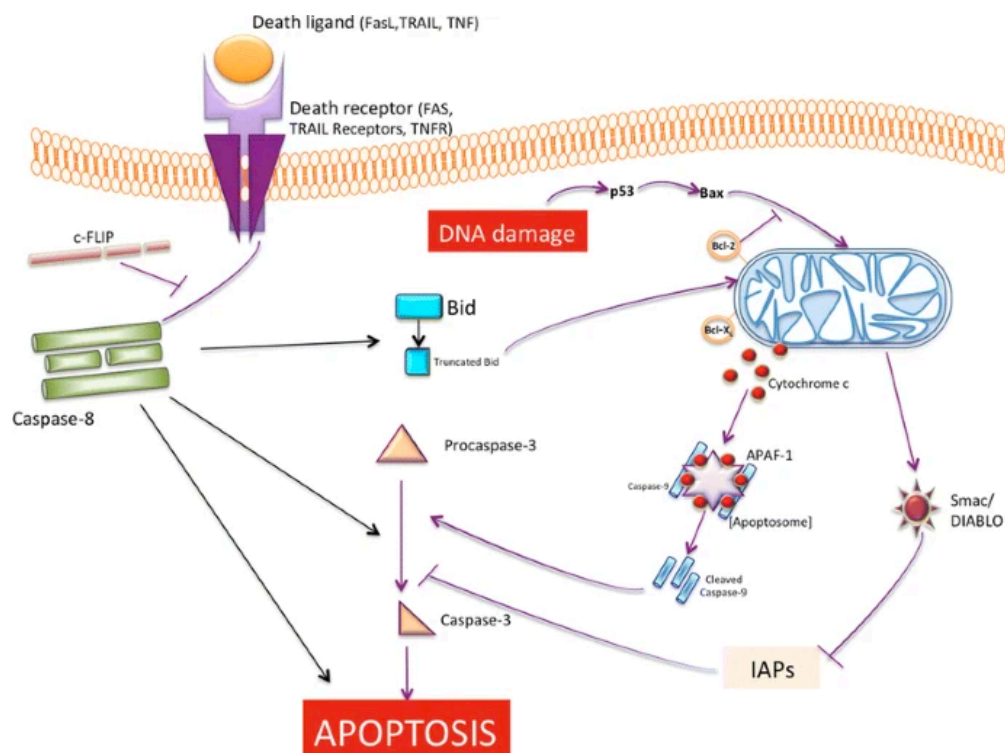


Figure 3. Extrinsic and Intrinsic Mechanisms of Apoptosis. The crosstalk between the intrinsic and extrinsic apoptotic pathways involves Bid, a pro-apoptotic protein of the Bcl-2 protein family. Regulation of the extrinsic pathway involves the activation of caspase-8 and inhibitors such as c-FLIP, while regulation of the intrinsic pathway involves mitochondrial activity and Bcl-2 proteins such as Bax. Adapted from (D'Arcy et al., 2019).

The General Mechanism of Necroptosis

Necroptosis, or programmed necrosis, primarily involves rupture of the cellular membrane, swelling of organelles, the release of DAMPs, and inflammation (Galluzzi et al., 2017). Similarly to apoptosis, initiation of necroptosis often begins with the activation of death receptors including TNFR1, TNFR2, and toll-like receptors (TLRs) (Rex et al., 2022). The binding of ligands (TNF- α) to death receptors (TNFR1) stimulates the formation of the necrosome complex, which consists of RIPK1, RIPK3, and MLKL. Necroptosis is executed by RIPK3-induced phosphorylation of MLKL and subsequent membrane translocation of MLKL to the cell membrane. This triggers an influx of sodium and an increase in cellular osmotic pressure, which ultimately causes rupture of the plasma membrane (Chen et al., 2014). However,

activation of TNFR1 can induce polyubiquitination of RIPK1 and the assembly of complex I in the plasma membrane via recruitment of TNF receptor-associated death domain (TRADD), cellular inhibitor of apoptosis 1 (cIAP1), cIAP2, TNF receptor-associated factor 2 (TRAF2), TRAF5 and the linear ubiquitin chain assembly complex (LUBAC) (Seifert & Miller, 2017). Complex I, the prosurvival complex, prevents cell death by activating the nuclear transcription factor-kappa B (NF- κ B) pathway to stimulate the release of prosurvival genes leading to inflammation, such as c-FLIP (Chen et al., 2019).

Cell death is triggered by the transition from complex I to complex II (IIa or IIb) formation (Figure 4). Stimulation of TNFR1 by TNF- α ultimately allows for deubiquitination of RIPK1 by enzymes such as deubiquitinase cylindromatosis (CYLD) or A20, which leads to the formation of complex IIa as TRADD and RIPK1 dissociate from TNFR1 (Amin et al., 2018). Furthermore, the localization of FADD and pro-caspase-8 to cytoplasmic complex IIa stimulates the activation of caspase-8, and subsequently, apoptosis. Further cell death is initiated by the suppression of activators of the NF- κ B pathway and the assembly of complex IIb, which consists of RIPK1, caspase-8, and FADD (Nailwal & Chan, 2019). When caspase-8 expression is suppressed in both complex IIa and IIb, RIPK1-induced apoptosis is paused while RIPK3-induced necroptosis is induced (via formation of the necrosome) (Pasparakis & Vandenabeele, 2015).

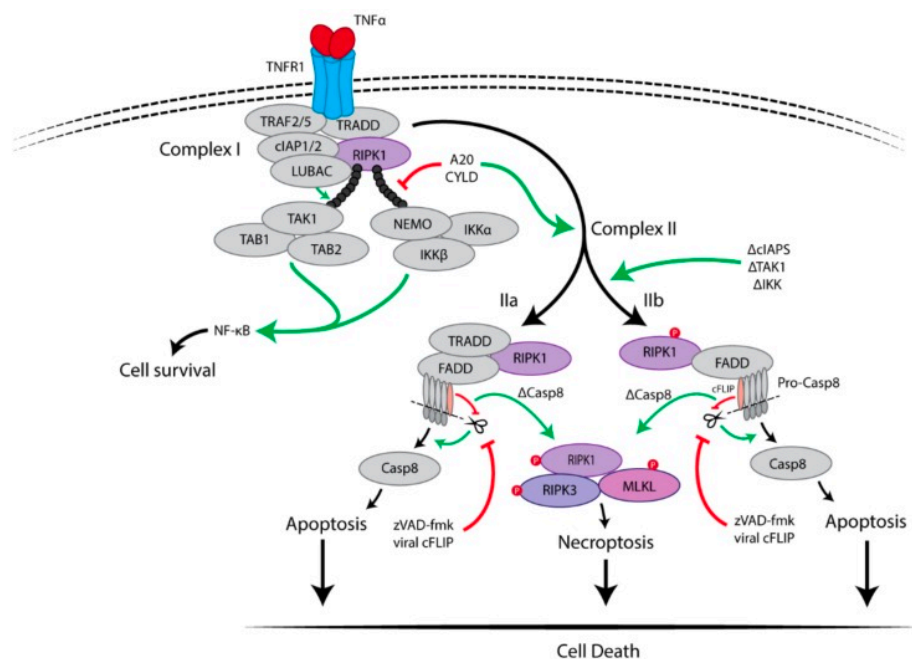


Figure 4. TNFR1-induced apoptosis and necroptosis pathways. Inhibition of caspase-8 and formation of complex II stimulates necroptosis. Adapted from (Chen et al., 2019).

Influenza-Induced Activation of Cell Death Pathways

IAV is a single-stranded, negative-sense RNA virus belonging to one of three influenza genera (A, B, and C) and the *Orthomyxoviridae* family (Yamauchi, 2020). Although IAV is largely preventable through proper vaccination, influenza is still responsible for 250,000-650,000 annual deaths worldwide (Doyon-Plourde et al., 2019). As an acute respiratory illness, IAV is capable of causing severe respiratory illnesses by primarily causing cell death of airway epithelial cells and subsequent inflammation due to the mobilization of macrophages and neutrophils. Previous research has revealed that influenza has promoted the expression of proinflammatory cytokines and the increased production of ROS, which indicates that multiple cell death pathways may be induced by IAV infection (Julkunen, 2000). By exploring the cell death pathways that play a dual role in the host defense systems against influenza viruses as well as in the increased pathogenesis of influenza viruses, a better understanding of similar respiratory illnesses (such as SARS-CoV-2) can be developed.

Previous data has indicated that both FADD-mediated apoptosis and MLKL-dependent necroptosis are activated by IAV-induced stimulation of RIPK1 and RIPK3 in airway epithelial cells, which indicates the importance of these pathways in antiviral host mechanisms (Nogusa et al., 2016). MLKL-induced rupture of the cell membrane recruits neutrophils to the site of infection, which exacerbates the inflammatory damage seen at the infection sites via necroptosis. Mice deficient in MLKL previously displayed lower recruitment of neutrophils into lung epithelial cells and increased survival against a lethal dose of IAV, indicating that key players of necroptosis may contribute to the increased virulence of influenza in severe cases (Zhang et al., 2020). Further support for the influence of cell death pathways in IAV respiratory symptoms is provided by the ability of the influenza virus matrix 1 (M1) protein to stimulate toll-like receptor 4 (TLR4)-dependent proinflammatory cytokine release and lung pathology, which indicates that initiation of cell death by TLR4 can lead to lung tissue damage (via proinflammatory cytokines such as IL-6, CXCL1, and CXCL10) associated with increased IAV severity (Kim et al., 2023). Additionally, apoptosis may also be implicated in the enhanced virulence of influenza, since the M1 protein of IAV was also implicated in elevated cytokine production and ROS production in lung immune cells, along with increased expression of apoptotic markers such as TNF, Fas, and Bax during IAV infection (Kim et al., 2023). In line with this, the knockout of Bax, a pro-apoptotic Bcl-2 family protein, from mouse embryonic fibroblasts was found to prevent viral propagation of IAV, which aligns with the notion that inhibition of Bax-mediated apoptosis decreases the viral spread and pathogenicity of IAV by restricting IAV nucleoprotein transport from nuclear to cytoplasmic viral components (McLean et al., 2009). Collectively, current research indicates that apoptosis and necroptosis are involved in harmful effects associated with IAV infection, which is suggested by the association of apoptotic markers (such as Bax and Fas)

and necroptotic markers (such as MLKL) with ROS-mediated cell death and neutrophil-mediated inflammatory damage, respectively.

Respiratory Syncytial Virus (RSV)-Induced Activation of Cell Death Pathways

Respiratory Syncytial Virus (RSV) has been a leading cause of acute respiratory infection in infants for several years. Similarly to the effects of SARS-CoV-2, increased severity of the RSV infection can lead to bronchiolitis or pneumonia through lower respiratory tract disease (LRTD) in older adults as well (Harker & Snelgrove, 2020). Airway epithelial cell death occurs at elevated rates during RSV infection, indicating the involvement of at least one cell death pathway in its viral mechanism. Based on its similarity to SARS-CoV-2 as a single-stranded RNA virus responsible for causing respiratory disease, investigation of RSV-induced cell death can highlight cell death pathways that may be of interest to understanding the virulence and severity of SARS-CoV-2 infection.

RSV pathogenesis has been found to predominantly rely on activators of necroptosis including MLKL, RIPK1, RIPK3, and TNF. Recent research has indicated that inhibition of TNFR1 (a death receptor responsible for stimulating necroptosis) improves RSV disease severity and bronchoconstriction in mice, while also preventing necroptosis-dependent inflammatory damage by reducing the number of cytokines and chemokines such as IL-1 (Morris et al., 2020). Additionally, the depletion of TNF- α and macrophages prevented the aggravation of asthma symptoms in mice exposed to RSV, which indicates that necroptosis-dependent rupture of the cell membrane recruits macrophages to the site of infection and exacerbates RSV-induced asthma (Nguyen et al., 2016). While alveolar macrophages seem to be beneficial for early antiviral mechanisms against RSV infection (Goritzka et al., 2015), they have also been implicated in increased airway obstruction and weight loss in response to other respiratory

viruses including pulmonary coronavirus (Hartwig et al., 2014). Accordingly, alveolar macrophages in pulmonary tissue were found to upregulate RSV-induced expression of key stimulators of necroptosis, RIPK3 and MLKL, leading to tissue destruction due to the activation of these necroptotic mediators involved in causing inflammatory damage (Santos et al., 2021). Conclusively, necroptosis and its key mediators, MLKL, RIPK3, TNF- α , TNFR1, play a role in the inflammatory damage associated with the progression of RSV infection.

Human Metapneumovirus (hMPV)-Induced Activation of Cell Death Pathways

Human metapneumovirus (hMPV) is a single-stranded RNA virus responsible for causing acute respiratory tract infection in immunocompromised, elderly, and children populations. In children under 5 years, hMPV maintains a global prevalence of up to 86% (Divarathna et al., 2020). The virus usually manifests as upper and lower respiratory tract infections, and the symptoms of hMPV-induced infection include bronchiolitis, pneumonia, and exacerbations of asthma (Uddin & Thomas, 2023). Exploring the inflammatory responses induced by hMPV infection may provide insights into the cell death mechanisms involved in hMPV pathogenesis, since previous studies have indicated that vital cell death pathways may be involved in the increased virulence of hMPV while also conferring a protective advantage to the host. Since both SARS-CoV-2 and hMPV display many of the same respiratory symptoms and share a similar viral structure, elucidating the mechanisms involved in hMPV-induced inflammatory cell death can also highlight potential implications for the virulence of SARS-CoV-2.

One significant cell death pathway activated by hMPV appears to be pyroptosis, as indicated by the upregulation of crucial markers of pyroptosis. Infection of epithelial cells by hMPV results in enhanced production of proinflammatory cytokines (such as IL-18) as well as

important inflammasome components such as IL-1 β and NLRP3 (Malmo et al., 2016). Although scientific support has not been established for the direct involvement of pyroptotic markers such as GSDMD in hMPV pathogenesis, previous research found that the NLRP3 inflammasome exhibited a detrimental effect on host survival via activation of IL-1 β during hMPV infection. Aligning with this finding, research indicates that hMPV-infected mice without caspase-1 had better survival rates, reduced weight loss, and reduced inflammation as opposed to hMPV-infected mice containing caspase-1 (Lê et al., 2019). Collectively, these findings indicate that the cleavage of GSDMD and IL-1 β by caspase-1 during hMPV infection may result in the formation of pores in cell membranes and the subsequent release of pro-inflammatory cytokines responsible for tissue damage via inflammatory cell death mechanisms. Therefore, while research on cell death markers involved in hMPV pathogenesis is limited, preliminary research seems to indicate that pyroptosis plays an important role in the pathogenesis of this respiratory RNA virus. Interestingly, TNF- α and IL-6 protein levels were also found to be elevated in blood serum levels of patients with hMPV, which may suggest the involvement of apoptosis and/or necroptosis during the pathogenesis of hMPV (Malmo et al., 2016).

Therapeutic Treatments for General Respiratory RNA Viral Infections (IAV, RSV, hMPV)

Existing treatments for IAV, RSV, and hMPV have been effective against several strains of these viruses. However, the synthesis of new therapeutic agents provides a way to combat the current emergence of viral strains that are resistant to existing treatments. For instance, IAV is most commonly treated with neuraminidase inhibitors (NAIs) such as oseltamivir, zanamivir, and peramivir. These drugs have been highly effective in preventing serious IAV infection, since NAIs function by binding to the active site of the viral neuraminidase enzyme and preventing its activity (Laborda et al., 2016). However, oseltamivir-resistant IAV strains have previously been

discovered (Hurt et al., 2011). The emergence of resistant strains of IAV in recent decades underscores the advantage of developing new drugs that target IAV in a mechanism different from that of NAIs.

An example of a potential therapeutic agent for IAV infection is FP7, which is a recently developed TLR4 antagonist. FP7 has been shown to prevent excessive TLR4 activity and protect mice receiving a lethal dose of IAV by decreasing the expression of genes within the lungs of proinflammatory cytokines such as IL-6 and IL-8 (Perrin-Cocon et al., 2017). FP7 was hypothesized to inhibit the cytokine storm activated by TLR4 activity and DAMPs. Another anti-inflammatory drug that has been examined as a potential therapeutic target for influenza treatment is etanercept. Although etanercept has traditionally been used to treat inflammatory conditions such as rheumatoid arthritis, the drug has been able to serve as a TNF-receptor decoy, ultimately reducing the production of proinflammatory cytokines (such as IL-6) and downregulating NF- κ B signaling post-infection with a subtype of IAV (Shi et al., 2013). While TNF- α has been implicated in providing antiviral properties to hosts during early infection with IAV (DeBerge et al., 2014), anti-TNF therapy in murine models of IAV have conversely been effective in reducing the severity of IAV-induced respiratory disease and preventing lung damage due to excessive inflammation (Szretter et al., 2007). However, further research is needed to understand whether anti-inflammatory drugs such as etanercept can mediate between the protective and pathogenic effects of TNF (specifically TNF- α) activity.

While IAV has multiple existing treatments proven to be effective, RSV is more limited in its available treatment options. Currently, there are only two vaccines available for RSV: Arexvy and Pfizer Abrysvo. The development of these vaccines occurred over several decades due to the complicated mechanism of RSV infection. While the prevalence of RSV decreased

during the onset of the SARS-CoV-2 pandemic due to mandatory isolation procedures, the virus joined influenza and SARS-CoV-2 in spiking rates of hospitalization and respiratory disease in 2022 (Adams et al., 2023). The continued presence of RSV highlights the importance of investigating potential therapeutic agents that may mitigate the severity of RSV-induced infection.

Existing research on potential treatments for RSV is limited. However, preliminary data suggests that MLKL-inhibitors, such as necrosulfonamide, may be an avenue for decreasing necroptosis-based cell death and inflammation. Necrosulfonamide allows for phosphorylation of MLKL by RIPK3, but it does not allow MLKL to localize to the cell membrane and cause rupture of the cell membrane. Necrosulfonamide treatment of RSV-infected macrophages resulted in a significant reduction in lytic cell death (Bedient et al., 2020). Another potential treatment for RSV is GSK-872, a RIPK3 necroptosis inhibitor. Treatment of RSV-infected macrophages with GSK-872 exhibited decreased lytic cell death (Bedient et al., 2020). Based on these preliminary results, inhibition of key necroptosis markers may result in decreased pathogenesis of RSV.

As compared to IAV, hMPV has a very limited number of treatments available for current use. Common symptoms of hMPV include fever, coughing, and wheezing, but at-risk populations of young children or elderly adults can experience more serious respiratory illness via conditions such as bronchiolitis, pneumonia, or asthma. Despite this, there are currently no existing vaccines for hMPV. Additionally, there are also no existing antiviral medications designed to treat hMPV specifically. Examination of pyroptosis-mediated cell death in hMPV pathogenesis can highlight potential therapeutic agents of interest for the treatment of this virus.

The NLRP3 inflammasome and other markers of pyroptosis including pro-inflammatory cytokines were previously implicated in the increased pathogenesis of hMPV. While there is very limited existing research on potential treatments, reducing the pyroptosis-mediated cell death involved in the increased disease severity associated with serious hMPV infections can serve as a potential starting point for developing antiviral treatments against hMPV. For instance, β -hydroxybutyrate (BHB) was previously discovered to lower NLRP3-induced production of IL-1 β and IL-18 in human monocytes (Youm et al., 2015). Additionally, MCC950 was developed as a small-molecule inhibitor specific to NLRP3. Aligning with this, administration of MCC950 previously indicated lower IL-1 β secretion and improvement of disease severity associated with another inflammatory disease, multiple sclerosis (Coll et al., 2015). Modifying inhibitors, such as MCC950 and BHB, of the NLRP3 inflammasome for treatment of hMPV may mediate the severity of the inflammatory respiratory diseases associated with this viral infection.

SARS-CoV-2-Induced Pyroptotic Cell Death

Viral invasion by SARS-CoV-2 can lead to hyperinflammatory responses and cytokine storms, which can ultimately result in severe respiratory distress (Zafer et al., 2021; Henderson et al., 2020). The heightened cell damage invoked by SARS-CoV-2 seems to depend on pyroptosis markers for increased pathogenesis as indicated by the increased presence of the NLRC5 inflammasome, caspase-1, caspase-4, GSDMD, IL-1 β and IL-18 in monocytes of severely affected SARS-CoV-2 patients as compared to patients with moderate symptoms (Xu et al., 2022). The NLRP3 inflammasome was also implicated in inflammasome-mediated pyroptosis in the endothelium, which contributes to the severe damage of respiratory endothelial cells often associated with SARS-CoV-2 (Paul et al., 2021). Fluorescence staining of coronavirus-infected

monocytes and lung macrophages circulating within SARS-CoV-2 patients confirmed the activation of NLRP3 inflammasome-dependent pyroptosis (Junqueira et al., 2021).

The cytokine storm initiated by SARS-CoV-2 plays an important role in the disease severity. Elevated levels of pro-inflammatory cytokines and chemokines, such as IL-1 β , IL-8, IL-6, IL-10, and TNF- α , were previously associated with an increased severity of SARS-CoV-2 viral symptoms in COVID-19 patients (Del Valle et al., 2020). Pro-inflammatory cytokines mediated by pyroptosis are involved in the recruitment of neutrophils, macrophages, or other immune cells involved in inflammation. Excessive activity of inflammatory cells ultimately results in dysfunction of the respiratory system of the SARS-CoV-2 host. This process triggers a pro-inflammatory cascade that can travel through the lymphatic system to other areas of the body as well, such as the cardiovascular system (Tschöpe et al., 2021).

SARS-CoV-2-Induced Apoptotic Cell Death

There is increasing evidence that mechanisms of apoptosis contribute to the pathophysiology of SARS-CoV-2. Previous studies have discovered that the expression of Fas (CD95), a major apoptotic marker, on T-cells in COVID-19 patients occurs at significantly higher rates than non-COVID-19 patients (Bellesi et al., 2020). Apoptosis-mediated T-cell activation is associated with lymphopenia and increased severity of SARS-CoV-2 infection (Cizmecioglu et al., 2021). Furthermore, caspase-6, a downstream effector of the apoptotic cascade, has been implicated in facilitating SARS-CoV-2 viral replication activating an N protein that can serve as an IFN antagonist. Inhibition of caspase-6 in hamsters infected with SARS-CoV-2 displayed reduced lung pathophysiological symptoms and increased survival (Chu et al., 2022).

The decreased presence of dendritic cells is also associated with heightened SARS-CoV-2 disease severity. Apoptosis mediated by caspase-3 was previously indicated in monocyte-derived dendritic cells, along with monocyte-derived macrophages, of COVID-19 patients (Zheng et al., 2021). Apoptosis of THP-1 macrophages were also found to be induced by the SARS-CoV-2 S protein via an elevated production of caspase-3, caspase-6, and ROS (Barhoumi et al., 2021). This aligns with findings that indicate that the SARS-CoV-2 S protein plays a vital role in the promotion of ROS, the suppression of Bcl-2 protein, and the activation of the intrinsic apoptotic pathway (Li et al., 2021). These studies indicate that downstream effectors of the apoptosis cascade are mediated by the structural components of SARS-CoV-2.

SARS-CoV-2-Induced Necroptotic Cell Death

The role of necroptosis-mediated cell death induced by SARS-CoV-2 infection has yet to be fully understood. However, increasing evidence is being discovered for the involvement of necroptosis within the SARS-CoV-2 pathogenic mechanism. Examination of postmortem lung sections of fatal SARS-CoV-2 patients indicated activation of both apoptosis and necroptosis mechanisms and increased necrotic cell death (Li et al., 2020). Another study found that RIPK3- and MLKL-dependent necroptosis of monocytes occurred in response to SARS-CoV-2 immune complexes (Santos et al., 2023). Furthermore, elevated levels of serum RIPK3 levels were previously found in COVID-19 patients with increased disease severity as compared to those with mild disease symptoms, which may indicate that necroptosis plays a role in the transition from SARS-CoV-2-induced pneumonia to acute respiratory distress syndrome in severe cases (Nakamura et al., 2020).

Necroptosis has been known to confer a protective advantage against SARS-CoV-2 viral infection. Necroptosis of infected cells allows harmful viral components to be limited in their

replication throughout the host, since infected cells are eliminated from the body (Nguyen & Kanneganti, 2022). Through crosslinking mechanisms with apoptosis, the activation of necroptosis can inhibit c-FLIP, an anti-apoptotic protein which can allow for SARS-CoV-2 replication (He & He, 2013). However, dysregulated necroptosis can trigger the release of PAMPs, DAMPs, and inflammatory cytokines from epithelial cells. This can lead to an upregulation of the pro-inflammatory cascade and a cytokine storm with the release of pro-inflammatory mediators such as IL-2, IL-6, and TNF into the lungs, which ultimately results in major lung tissue dysfunction (Perico et al., 2021).

Current Treatments to Target SARS-CoV-2 Infections

Currently, vaccines with a high efficacy against severe COVID-19 infection exist. These vaccines include Pfizer-BioNTech, Moderna, or Novavax. The 2023-2024 versions of the vaccines protect against the Omicron variant that was prevalent until recently, XBB.1.5, as well as the current dominant variant that has been circulating since December 2023, JN.1 (Link-Gelles et al., 2024). However, although mostly mild cases of COVID-19 have been circulating recently, existing long-term complications of SARS-CoV-2 infection can encompass cardiac, pulmonary, and neurological conditions post-infection. Many of these conditions are tied to unregulated cell death mechanisms that cause endothelial injury and multiorgan dysfunction (Desai et al., 2022). Identifying potential therapeutic targets based on the contributions of pyroptosis, apoptosis, and necroptosis to the propagation of SARS-CoV-2 infection can reveal future treatments that may allow the long-term complications of COVID-19 to be treated more effectively.

Pyroptosis has been heavily implicated in SARS-CoV-2 pathogenesis, mainly as an activator of the cytokine storm that leads to tissue dysfunction. Previous treatment strategies

have included controlling the hyperactivity of cytokines directly through targets such as IL-6 (siltuximab), IL-1 β (canakinumab), IL-6 receptor (tocilizumab), and IL-1 receptor (anakinra) (Gordon et al., 2021; Falasca et al., 2021; Xiong et al., 2021). However, targeting upstream regulators in the pyroptosis pathway may be more effective in combating hyperinflammatory processes associated with SARS-CoV-2 infection. Treatment with MCC950, an NLRP3 inflammasome inhibitor that was previously seen to improve disease outcomes of other inflammatory infections, effectively inhibited SARS-CoV-2 acute lung injury and cytokine production induced by N protein (Pan et al., 2021). Another inhibitor of the NLRP3 inflammasome, tranilast, was previously observed to increase the O₂ saturation and reduce length of hospitalization in a sample of COVID-19 patients compared to a control group of patients (Saeedi-Boroujeni et al., 2022).

The mediators involved in apoptosis-induced cell death have also been extensively studied in the viral mechanism of SARS-CoV-2. The early stages of viral infection proceed by inhibiting apoptotic functions within the host, thereby allowing viral replication and viral spread. Induction of the apoptosis cascade may be advantageous as a potential therapeutic treatment in the early stages of infection. Histone deacetylase inhibitors, such as LBH589, can combat the early stages of COVID-19 infection by blocking the apoptosis suppressor c-FLIP (Scuto et al., 2008). The apoptotic pathway can also be triggered by small molecules targeting c-FLIP, which enhance caspase-8 activity after assembly of the DISC complex (Hillert et al., 2020).

Conversely, apoptosis is induced in a pathogenic manner by SARS-CoV-2 structural proteins at later stages of infection. This causes significant tissue damage and organ dysfunction, which contributes to the increasing severity of disease often associated with COVID-19. Therefore, a promising strategy to attenuate disease severity in later stages of SARS-CoV-2

infection is to block the apoptosis cascade. Infliximab functions as a monoclonal antibody blocking TNF- α , an activator of the apoptotic pathway. Results of a previous one-arm phase II clinical trial indicated that Infliximab treatment reduced pathological levels of pro-inflammatory cytokines in critical COVID-19 patients, which suggests that inhibition of TNF- α may ameliorate disease progression in later stages of infection (NCT04425538). The elevated caspase levels associated with apoptosis are also a potential therapeutic target. Emricasan, a pan-caspase inhibitor, suppressed the excessive activation of caspase-3 in blood cells of COVID-19 patients (Plassmeyer et al., 2022).

While the roles of pyroptosis and apoptosis have been identified in playing significant roles in progression of respiratory damage associated with SARS-CoV-2, the role of necroptosis has yet to be fully elucidated. Similarly to apoptosis, necroptosis has been known to play a dual role during coronavirus infection. While necroptosis-induced cell death can suppress viral replication, necroptotic mechanisms can conversely also allow for viral spread by releasing internal cellular contents after rupture of the cell membrane. This signifies that regulation of markers of necroptosis may support anti-SARS-CoV-2 strategies. Current targets of necroptosis in the context of COVID-19 treatment include MLKL, RIPK1, and RIPK3. However, therapeutic agents against RIPK1 have mainly been identified thus far.

Necrostatin-1 is a known RIPK1 inhibitor. Accordingly, administration of Necrostatin-1 or its analogs have been previously hypothesized to limit inflammation and the cytokine storm initiated by SARS-CoV-2 (Cao & Mu, 2021). The FDA-approved drug primidone may serve as a beneficial treatment for COVID-19 patients, since previous research has suggested that primidone is capable of decreasing TNF- α inflammation in vivo and RIPK1-dependent necroptosis in vitro (Riebeling et al., 2021). Although drugs targeting other necroptotic markers

such as RIPK3 (GSK872) and MLKL (necrosulfonamide) have not been investigated in the context of coronavirus infection, they have shown a promising effect in preventing dysregulated cell death in other respiratory RNA viruses such as RSV. The existing data on necroptosis-based antiviral treatments for preventing severe SARS-CoV-2 infection must be expanded upon for a more comprehensive understanding of the role of necroptosis within the viral pathogenesis of coronaviruses.

Mouse Hepatitis Virus (MHV) and Future Perspectives

Mouse models have served as excellent models for in-depth examination of viral mechanisms in the past. Therefore, future research on SARS-CoV-2 treatments and cell death pathways can be conducted by utilizing mouse hepatitis virus (MHV). MHV, the murine coronavirus, has been a pivotal starting point in studying coronavirus-induced (including SARS-CoV-2) inflammation and virulence. MHV models provide an effective way to understand coronavirus-induced cell death, since MHV infection triggers a similar inflammatory immune response as SARS-CoV-2 infection through various common key processes, such as activation of the NLRP3 inflammasome (Zheng et al., 2020). Previous research has indicated that MHV successfully induces COVID-19-related symptoms such as weight loss, multiorgan dysfunction, neurological complications, and various other long-term effects of SARS-CoV-2 infection (Masciarella et al., 2023). Utilizing MHV rather than SARS-CoV-2 eliminates the necessity for costly transgenic mice and provides a more accessible means of investigating the underlying mechanisms of SARS-CoV-2 pathogenesis.

MHV has been used extensively as a model for understanding SARS-CoV-2 and coronavirus immune responses (Sariol & Perlman, 2020). Previous research has revealed that bone marrow-derived macrophages (BMDMs) of mice infected with MHV displayed cleavage of

caspase-1 and GSDMD fragments (which indicate the involvement of pyroptosis in coronavirus infection), phosphorylated MLKL (which indicates the involvement of necroptosis in coronavirus infection), and cleavage of caspase-8, -7, and -3 (which indicate the involvement of apoptosis in coronavirus infection). Collectively, this data suggests that MHV (and coronavirus) infection can induce PANoptosis (Zheng et al., 2020). Furthermore, the presence of pro-inflammatory cytokines IL-1 β , IL-18, IL-7 and TNF in BMDMs infected by MHV has been linked to inflammatory responses initiated by coronavirus infection (Zheng et al., 2020).

Future research on coronavirus-induced immune responses should continue to use MHV as a model. When developing new antiviral molecules against SARS-CoV-2, an emphasis should also be placed on researching the involvement of key markers of necroptosis in MHV infection. The complex role of necroptosis within COVID-19 pathogenesis has yet to be fully understood, but preliminary data suggests that necroptosis can serve a role in both antiviral protection and viral propagation. More intricate observations are needed to fully illuminate the exact contexts in which necroptosis plays a beneficial role rather than a harmful role during coronavirus infection. Previous research on other respiratory RNA viruses indicates that inhibitors of MLKL and RIPK3 may be helpful in reducing inflammation associated with respiratory viral infections, which suggests that these inhibitors may also have implications for COVID-19 pathogenesis at different stages of infection. Investigating this area of coronavirus pathogenesis with MHV models can have important implications for developing better treatments targeting dysregulated cell death in COVID-19 infections.

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