

Necroptosis in viral infections: a twilight among progeny dissemination and host defense

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ABSTRACT:

Decades ago, a unique cell death pathway was reported, namely 'Necroptosis', that exhibits both the characteristics of apoptosis, the programmed cell death mechanism, as well as the unprogrammed necrotic cell death pathway. Various reports show that virus infection sometimes upregulates and sometimes inhibits the necroptotic cell death pathway in favor of its progeny dissemination. Interestingly, sometimes host cells also induce necroptosis for eradication of viral load. In viruses like Influenza and Zika, necroptosis is mediated by the host protein ZBP1. Hepatitis B and E viruses also mediate necroptosis for the occurrence of disease pathology. Furthermore, Rotaviruses, Reoviruses, Respiratory Syncytial Viruses (RSV), Rhinoviruses, Human Cytomegaloviruses, Herpes Simplex Viruses (HSV), and many more mediate necroptosis either in favor of viral progeny dissemination and disease pathology or for virus eradication. Moreover, Oncolytic Adenoviruses are reported to induce necroptosis, which can be a potential mechanism in favor of cancer treatment. HIV, on the other hand, destroys CD4+ T lymphocytes through activating necroptosis. Hence, there are reported widespread roles of necroptosis in several viral infection scenarios. However, the detailed mechanisms and roles in specific virus infections are not yet dissected. Investigations regarding the roles of necroptosis in these viral infection scenarios can also wind up a way regarding virus limitation in the host through potent necroptotic targeting.

Keywords: Necroptosis, Viral infection, Progeny dissemination, Host defense, Necroptotic targeting.

INTRODUCTION

Programmed cell death is a potent host defense pathway upon various microbial infections. Mainly apoptosis and autophagy are regarded as programmed cell death. The molecular mechanisms related to cytolytic clearance of infected or damaged cells for eradication of pathogens and maintenance of health for metazoans have been widely studied in literature. On the other hand, necrosis which is regarded as "unprogrammed" because of its deregulated activity, is also a potential pathway for preventing infection. Decades ago, a unique cell death pathway that exhibited both the characteristics of apoptosis and necrosis was reported¹, also called "programmed necrosis", hence termed Necroptosis.

Necroptosis is described as the rupturing of cells by swelling caused by the rupture in the plasma membrane through phosphorylation of Mixed Linkage Kinase Domain Like proteins (MLKL). It is mediated by death receptors like Fas Ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), and TNF upon inhibition of Caspase-8, which is a potential player in the extrinsic apoptotic pathway, through the activation of Receptor interacting Serine/Threonine protein kinase 3 (RIPK3) and MLKL^{2,3}.

There are extensive studies^{3,5,9,10} that are focused on Tumor Necrosis Factor- α (TNF α), RIPK3, and Caspase-8 to understand the molecular mechanisms of necroptosis. Generally, the necroptosis pathway begins with the activation of TNF superfamily receptors



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(TNFR), Toll-like receptors (TLR3/TLR4), and interferon receptors. TNF α -mediated classical necroptosis begins with TNF complementary receptor binding to form a short-lived membrane signaling complex I that contains TNF-receptor associated death domain (TRADD), TNF-receptor associated factor 2/5 (TRAF2/TRAF5), cellular inhibitor of apoptosis (cIAP1/cIAP2) and RIPK1^{4,5}. TRADD, being it an adaptor molecule, recruits RIPK1 to complex I and it subsequently recruits cIAPs, TRAFs to complex I⁵. Complex I mediate NF- κ B and MAPK signaling, contributing to cell survival and other non-death pathways. Upon activation, cIAP1/2, and TRAF2/5 mediate the K63-linked ubiquitination of RIPK1⁶⁻⁸, which promote both the formation and activation of the transforming growth factor-activated kinase 1 (TAK1) binding protein (TAB) complex. The inhibitor of NF- κ B kinase (IKK) complex, consisting of NF- κ B essential modulator (NEMO), IKK α and IKK β , support the NF- κ B pathway activation, and ultimately leading to cell survival⁹.

Complex I maintain a crucial checkpoint between cell survival and necroptosis. Complex I internalize and transforms into a death-inducing complex II following Caspase-8 activation⁴. Usually, Caspase-8 induces exogenous apoptosis and simultaneously inhibits necroptosis by inhibiting the activity of RIPK1 and RIPK3. Upon inhibition of Caspase-8, RIPK1 and RIPK3 are combined *via* the RHIM domain and form complex IIc, also known as the “Necrosome”¹⁰. Complex IIc is a crucial cytoplasmic signaling complex, that does not appear in TNF-induced cell survival or apoptosis. Mitochondrial reactive oxygen species (ROS) oxidize RIPK1 at three

crucial cysteine residues (C257, C268, and C586), and also promote autophosphorylation of RIPK1 at Ser161¹¹. Autophosphorylation of RIPK1 is essential for the recruitment of RIPK3¹¹. In addition, the RHIM domain is also required for the RIPK1/RIPK3 complex formation. And after RIPK1 and RIPK3 are combined, RIPK1 gets phosphorylated by RIPK3. Intramolecular auto- and trans-phosphorylation of RIPK1 and RIPK3 promote the recruitment of another essential necroptotic-signaling protein, the mixed lineage kinase domain-like protein (MLKL). MLKL is then phosphorylated in Thr357/Ser358 (in human MLKL) residue by RIPK3 to initiate necroptosis¹². The phosphorylated MLKL is transferred from the cytosol to the plasma membranes. The oligomerization of MLKL causes membrane rupture, destroying membrane integrity and eventually leading to necroptotic cell death (Figure 1)¹³.

In addition to the above-described classical TNFR1-mediated necroptotic pathway, toll-like receptors (TLR) can also mediate necroptosis. The TLR signaling pathway is generally triggered by pathogen-associated molecular patterns (PAMPs) during viral or microbial infection¹⁴. TLR-mediated necroptosis typically destroys the infected cells, and the results benefit the host. The downstream MLKL signaling pathway of RIPK3 is indispensable for both TNFR1- and TLR-induced signaling, and Caspase-8 can block necroptosis directly initiated by the TIR domain-containing interferon- β (TRIF)/RIPK3/MLKL pathway¹⁵. TLR4 and TLR3 are respectively activated by lipopolysaccharide (LPS) and polyinosine-polycytidylic acid (I:C), a synthetic double-stranded RNA (dsRNA) mimic¹⁶. After that, TLR3

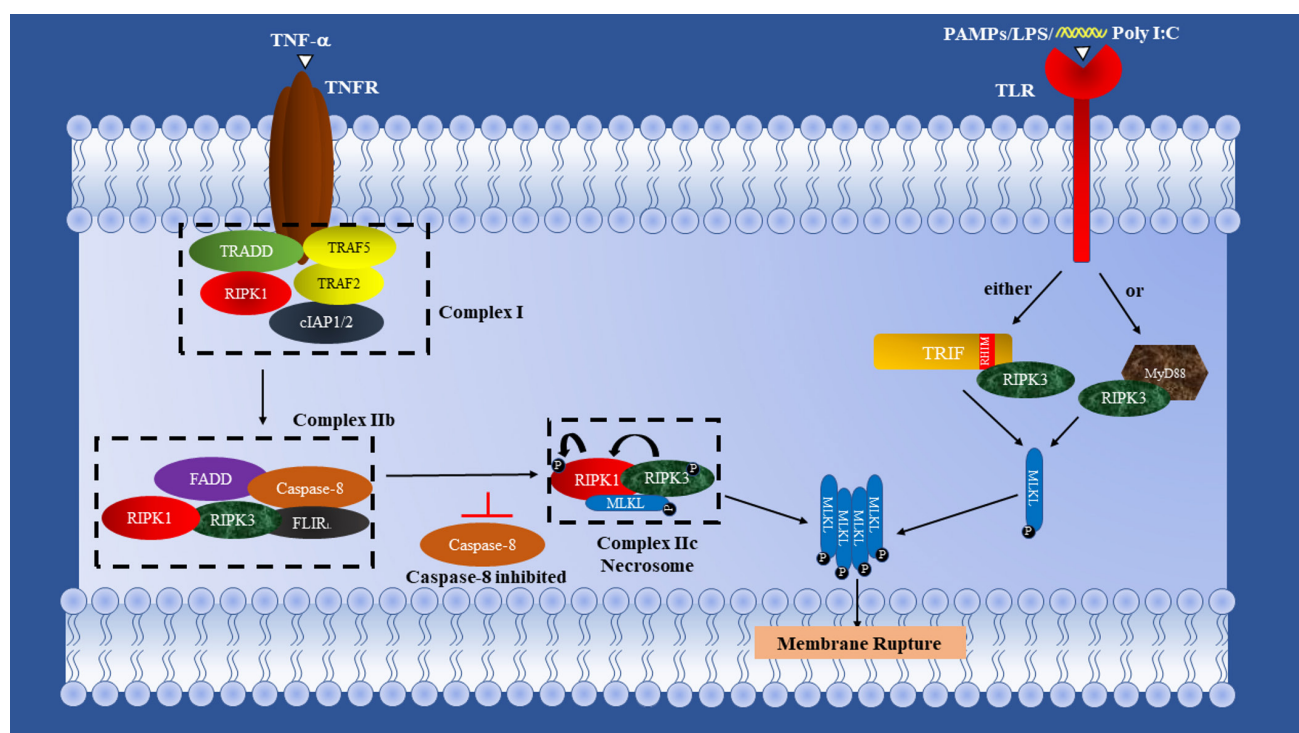


Figure 1. Molecular mechanism of RIPK1-dependent (mediated by TNFR) and RIPK1-independent (mediated by PAMPs/LPS/Poly I:C) necroptosis.

and TLR4 activate RIPK3 and exert necroptosis *via* TRIF and/or MyD88¹⁷. The C-terminal RHIM motif of TRIF/MyD88 is required for RIPK3 to interact with them. The RIPK3/TRIF signaling complex recruits and eventually phosphorylates MLKL, inducing ROS accumulation and mediating TLR3- and TLR4-induced necroptosis (Figure 1)¹⁵.

A wide range of necroptotic stimuli has been identified and divided into two groups, namely RIPK1-dependent and RIPK1-independent. RIPK1-dependent stimuli include TNF- α , Fas, TRAIL, interferon (IFN)- α , and IFN- β . The primary death-inducing signaling complex (DISC) is assembled by stimulation of Fas or TRAILR at the plasma membrane, activating Caspase-8 and triggering apoptosis independently of RIPK³. cIAP deficiency promotes the recruitment of RIPK1 and Fas when Caspase-8 is blocked and enhances the formation of the cytosolic ripoptosome complex which induces necroptosis¹⁸. RIPK1-independent stimuli generally refer to LPS, dsRNA, and viruses¹⁸. For example, a DNA-dependent activator of IFN (DAI) can identify viral dsRNA, which promotes the recruitment of RIPK3 to form necrosomes without RIPK1, and subsequently induce RIPK3-dependent necroptosis¹⁹. Manifesting the molecular mechanisms involved in necroptosis will, further unravel the molecular biology underlying the pathology. There is a role of many viral proteins, as well as their genomic material also, that affects necroptosis and somehow uses this mechanism in favor of propagating viral progeny and/or as a host defense mechanism. In this review, the current knowledge on the role of various viruses in necroptosis induction and/or inhibition is summarized.

ZBP1 Mediated Necroptosis

The necroptotic pathway in such viruses that produce Z-RNAs during virus infection, interacts with host protein ZBP1 to activate it. ZBP1 contains two Z α domains that selectively bind with left-handed double helical 'Z form' RNA structures *in vitro*. ZBP1, also called DAI (DNA activator of interferons) initiates the cell death pathway necroptosis. Upon activation, ZBP1 recruits RIPK3 which phosphorylates and activates MLKL in the host cell nucleus. Then, following the same necroptotic pathway, pMLKL triggers disruption of the nuclear envelope that promotes leakage of cellular DNA into the cytosol, as well as disrupts the plasma membrane to mediate cell death by necroptosis. In 2020, it was reported²⁰ that orthomyxoviruses (Influenza A and B virus) produce Z-RNAs, which activate ZBP1 in infected nuclei and trigger necroptosis by RIPK3-MLKL pathway (Figure 2). One year later, the reported upregulation of ZBP1 upon Zika virus infection in human astrocytes, which may activate RIPK3 and simultaneously induce RIPK3-dependent necroptotic cell death because it could be suppressed by GSK'872, an inhibitor of RIPK3²¹.

Necroptosis in Hepatitis Viruses and Cytomegaloviruses

Recently, in 2022, Gong et al²² published a report about an increase in the necroptotic cell death pathway in Hepatitis B virus-induced acute liver failure. In HBV-associated hepatocellular carcinoma, the mRNA level of RIPK3 in

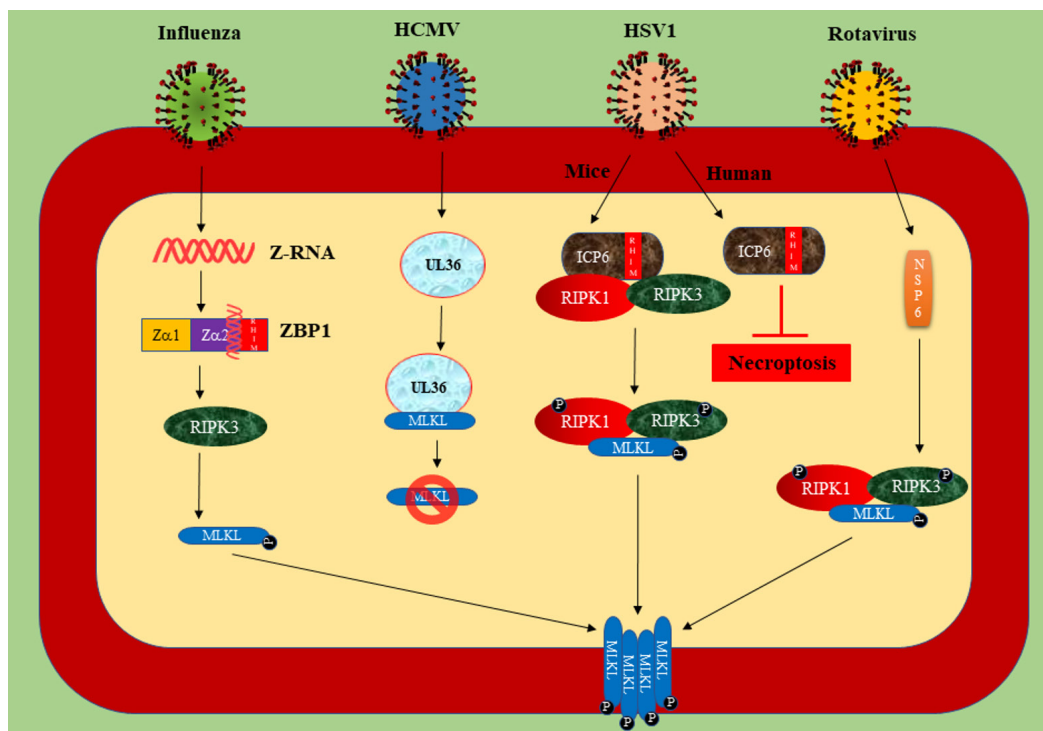


Figure 2. The participation of necroptotic cell death in different viral infection conditions either to facilitate the viral progeny dissemination or for host defense response.

peripheral blood mononuclear cells (PBMCs) is elevated in patients with HBV-associated HCC in comparison to patients with chronic hepatitis B (CHB) and patients with HBV-related liver cirrhosis (LC)²³. This can be an implication of the role of necroptosis in HBV-associated HCC and its clinical significance. The circulating serum RIPK3 level is also significantly increased in HBV-related Acute Chronic Liver Failure (ACLF), which also implies the effect of necroptosis in hepatitis B virus infections²⁴.

Human cytomegalovirus (HCMV) protein UL36 inhibits necroptosis by binding to MLKL protein and induces its degradation. It interacts with both human and murine MLKL, having a higher affinity to human MLKL (Figure 2). While the recombinant murine cytomegalovirus (MCMV) UL36 causes a reduction of murine MLKL level though not reducing necroptosis in murine cells, suggesting that UL36 inhibits necroptosis in a species-specific manner, similar to ICP6 of HSV-1²⁵. MCMV protein M45 which contains the RHIM domain suppresses necroptotic cell death of infected cells²⁶.

Antagonistic Role of HSV R1 Protein in Necroptosis for Human and Mice

The R1 protein of HSV1 and HSV2, ICP6, and ICP10 respectively limit HSV infection in mice by triggering necroptosis. ICP6 has RIP Homotypic Interaction Motif (RHIM) that interacts with RIPK1/RIPK3 and forms an oligomer by its C terminal R1 domain^{27,28}. This binding induces RIPK1-RIPK3 heterodimer or probably RIPK3 homodimer formation that triggers its autophosphorylation and transphosphorylation. Activation of RIPK3 induces phosphorylation of MLKL to drive toward necroptosis. However, sparkling data showed that HSV1 and HSV2 ribonuclease reductase (RR) large subunit R1 protein, ICP6 and ICP10 respectively, prevent necroptosis in human cells by inhibiting the interaction between RIPK1 and RIPK3 that is essential for TNF α mediated necroptosis (Figure 2). Another study demonstrated that both RHIM and RR domains are essential for the suppression of necroptotic pathway²⁹.

Oncolytic Adenovirus-Induced Necroptosis Can Be Beneficial for Cancer Treatment

A 2018 study by Weigert et al³⁰ reported a somewhat different kind of necroptotic cell death triggered in human malignant cells upon induction of oncolytic adenovirus type 5 including E1A CR2 deletion mutant *dI922-947*. This suggests a similar morphology induction as TSZ treatment that is seen by electron microscope, though is independent of TNF α signaling, as well as RIPK1 and MLKL. Whereas this induced morphology is RIPK3 dependent is proved by the inhibition of Caspase-8³⁰. This phenomenon induces RIPK3-dependent necroptosis and significantly increases *dI922-947* cytotoxicity³⁰. Oncolytic adenovirus carrying IFN β (ZD55-IFN β) induces the necroptotic cell death pathway in cancer cells,

leading to the destruction of malignant cells, which can be a potential method in cancer therapy³¹.

Human Papillomavirus (HPV) Suppresses IFN γ /TNF α Mediated Necroptosis

High-risk human papillomavirus (hrHPV) infection suppresses necroptosis by downregulating RIPK3 expression. hrHPV infection downregulates RIPK3 expression, leading to the impaired induction of TNF α / IFN γ mediated necroptosis³². A study by Ma et al³² with undifferentiated keratinocytes (KCs) showed that stimulation by TNF α and/or IFN γ did not lead to the activation of Caspase-8, thereby not inducing apoptosis signaling in undifferentiated KCs, though the stimulation with both TNF α and IFN γ leads to the activation of RIPK3 dependent necroptosis in undifferentiated uninfected KCs. Also, it was observed in hrHPV-infected cervical cancer patients that HPV infection resists tumor-associated macrophage (TAM) necroptosis. Moreover, RIPK3 inhibits TNF α expression which indicates TNF α macrophage necroptosis might form negative feedback in the tumor microenvironment³³. Therefore, the significance of HPV infection and its relationship with necroptosis is not at all clear and opens a wide area of research that may be in all the way helpful and revolutionary in hrHPV-infected cancer prognosis.

HIV-1 Infection Leads to Necroptotic Cell Death of CD4+T Lymphocytes

HIV-1 infection is characterized by the progressive loss of CD4+ T lymphocytes resulting in the dysfunction of the immune response to certain infections. The loss of CD4+ cells is known to take place *via* the apoptosis pathway, which is well documented in literature. In another pathway, necroptosis is also induced *via* the infection of HIV-1 and is an alternative pathway to the virus for cell death in the absence of apoptosis³⁴. Also, the HIV-induced syncytia formation in CD4+ T cell lines is partially dependent upon the necroptosis-related process. HIV envelop and Tat protein augmented TNF α mediated necroptosis³⁴, though the detailed mechanism is not clear till now. The Simian immunodeficiency virus (SIV) infection leads to the induction of massive necroptosis, mediated by RIPK3, in perivascular macrophages of neonatal macaque brain³⁵. These findings prove a potential role and interaction of retroviral proteins concerning the necroptotic cell death pathway that can also be a potential target in its prevention.

Rotavirus Activates Necroptosis to Spread Viral Progeny

More recently, in 2021, it was reported³⁶ in Rotavirus group A (RVA) infection that RVA infection facilitates necroptosis as a mean to spread out viral progeny in host cells

simultaneously with apoptosis. The NSP4 protein of RVA is reported to induce necroptosis through RIPK1/RIPK3/MLKL pathway (Figure 2). Also, it is reported to have an opposite role of RVA-induced necroptosis and apoptosis as they function as proviral and antiviral respectively, and counterbalance each other in RVA-infected cells³⁷.

Reovirus Activates Necroptotic Cell Death Pathway

In 2013, Berger and Danthi³⁸ reported the novel phenomenon of activating an alternative caspase-independent regulated cellular death pathway necroptosis in the reovirus strain T3D infection in the L929 cell line. They hypothesized that host cells may detect different stages of viral infections and attempt to limit the viral replication by switching the cell towards cellular suicidal mechanisms. Being a programmed cell death mechanism, necroptosis can be a potent way for host cells heading that³⁸. After 3 years, the same investigators reported that genomic dsRNA of incoming mammalian reovirus is required to be sensed by cytoplasmic RNA sensors of host cells to produce type I interferons (IFN), which eventually leads to the activation of necroptosis^{39,40}. Reovirus outer capsid protein m1 limits the accumulation of viral gene products that lead the cells towards necroptosis and thereby m1 limit necroptosis⁴¹. Furthermore, the investigations^{39,40} revealed that reovirus outer capsid protein $\sigma 3$ activates $\mu 1$ protein and also limits the induction of necroptosis by preventing excessive production of IFNs following infection⁴².

CONCLUSIONS

Necroptosis includes a programmed regulatory network that ultimately resembles the characteristics of necrotic cell death through the loss of cell membrane integrity and release of a group of protein products called Damage-associated molecular patterns (DAMPs) in the extracellular milieu. There are such viruses that mediate its infectivity and progeny dissemination through activating necroptosis, like Influenza A, Zika viruses, Rotaviruses, and/or Human immunodeficiency viruses. Respiratory syncytial viruses (RSV) are also reported to induce lytic cell death mechanisms through the RIPK3 - MLKL-dependent necroptotic pathway in macrophages⁴³. In rhinovirus infection, viral particles exit the cell after replication through necroptosis. Rhinovirus infection produces several cytokines and chemokines like IL-1, IL-6, IL-8, GM-CSF, and eotaxins and this inflammation triggers necroptosis within cells⁴⁴. Pancreatitis in Hepatitis E Virus (HEV) infection in miniature pigs is also seen⁴⁵ to be influenced by RIPK3-MLKL mediated necroptosis. Alternatively, shreds of evidence of such viruses also exist that downregulates the necroptotic pathway, that is used by the host cells to eradicate the infection load like Human papillomaviruses and/or Human cytomegaloviruses.

Information and research regarding the detailed mechanism of necroptotic cell death in different circumstances and also the role, as well as controlling mechanism of necroptosis in various virus infection scenarios, is yet to be deciphered. In a maximum case, there is scattered information regarding the reported participation of necroptosis upon virus infection, but how it occurs is not clear. Hence, this review provides comprehensive knowledge regarding most of the reported cases of major viruses in which the infection is somehow linked with necroptosis. As a recent and newly deciphered mechanism and of late reported role in host-viral infection scenarios, necroptosis can also be seen as a potential target in many folds to limit the virus infection.

ACKNOWLEDGEMENTS:

The author is grateful to the Department of Life Science and Biotechnology, Jadavpur University for intellectual support, and to Pritam Chandra, Senior Research Fellow in ICMR-NICED, for providing the initial interest to pursue this study.

CONFLICT OF INTEREST:

The author declares that he has no conflict of interest to declare.

FUNDING:

No funding was received.

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