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Inhibitors of Apoptosis Proteins as Potential Research Targets for Decoding the Pathological Mechanisms of Autoimmune Diseases

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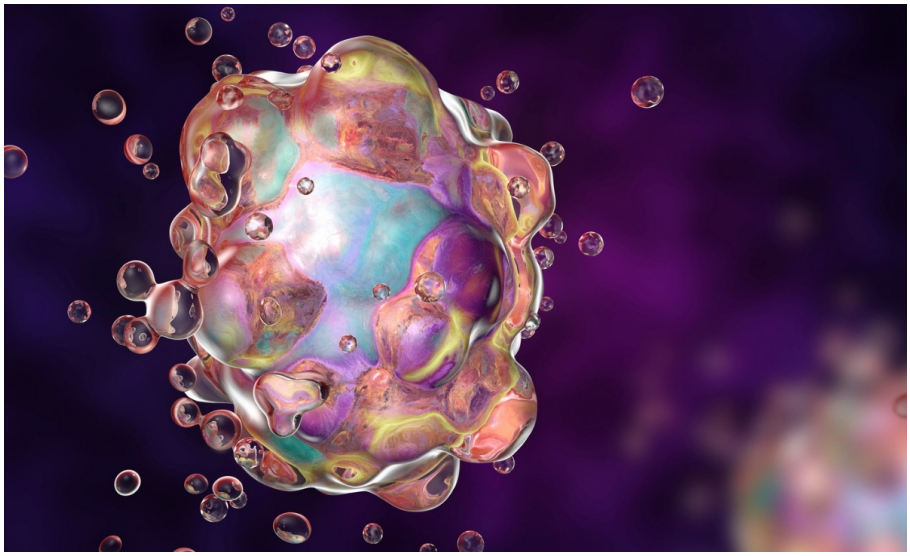

An artist's rendition of a cell undergoing apoptosis. Image Credits: Kateryna Kon / Shutterstock.com

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Abstract

Necroptosis, a type of pathological and inflammatory cell death, resembles necrosis, the termination of function of a bodily tissue, but adopts a unique molecular pathway that is not like apoptosis, resulting in vastly different immunological consequences. Until recently, necroptosis was believed to mainly function as a protective mechanism that counteracts the viral barrier of apoptosis. However, mouse model studies have indicated that deficiency in elements of the apoptosis machinery such as caspase-8 or FADD can result in embryonic lethality driven by necroptosis. Previous studies using conditional depletion of cellular inhibitors of apoptosis (cIAPs) revealed that the necroptosis pathway is triggered under certain stressor conditions. These data support a new approach of targeting molecules within the cell death pathways to identify the origin of autoimmune diseases. Hence, distinguishing between these two types of cell death may prove crucial during pathologic evaluations. This review provides a detailed insight into the emerging discussion on the various forms of cell death and the essential roles which certain molecules play in the development and progression of autoimmune diseases. Armed with this knowledge, greater efforts can be targeted towards devising more effective treatments for interception of pathological diseases, prior to their uncontrollable progression.

Introduction

Over the last decade, advances in cell death research have greatly contributed to our understanding of cell death. Dysregulation of cell death has been found to be critically involved in the onset of various human diseases, such as neurodegenerative diseases[1], autoimmune diseases[2], and cancer[3]. The three forms of cell death of interest – apoptosis[2,4,5,6], necrosis[7,8,9], and necroptosis[3,10] – have distinct features and activate unique signaling pathways. Apoptosis is a caspase-mediated programmed cell death that can be identified by chromosome condensation, nuclear fragmentation, and membrane blebbing[2]. Conversely, necrosis is an unregulated, accidental form of cell death, triggered by non-physiological stress inducers and characterized by the expansion of cellular organelles, plasma membrane rupture, and subsequent inflammatory responses caused by the release of the intracellular contents[7,8].

The recent identification of necroptosis has transformed our understanding of regulated cell death. It has become increasingly evident that although the different types of cell death have distinctive characteristics, they are ultimately interconnected. Thus, the activation or inhibition of a particular signaling molecular pathway under certain conditions determines the regulation or dysregulation of another associated cell death mechanism[11,12,13,14]. In this review, we focus on the specific mechanisms involved in each particular type of cell death and the connections between them. By highlighting the pathophysiological relevance of necroptosis and the key roles of certain cell mediators and signaling molecules, we are proposing a new perspective for consequent medical research that investigates the pathogenesis of various diseases across the body, including neurological[13,15], cardiovascular[16], pulmonary, gastrointestinal, infectious, and autoimmune[3,17,18] conditions, all of which have been linked to necroptosis.

Cell Death and Autoimmune Disorders

Programmed cell death is an intricate biological element that plays a vital role in several physiological processes, including homeostasis, regulation of the immune system, and disease pathogenesis[1]. It involves multiple pathways and is regulated by various intrinsic cell death programs. Over the past two decades, extensive research has transformed the understanding of programmed cell death and the different mechanisms that control it.

Initially, apoptosis was believed to be the only form of programmed cell death, whereas necrosis was considered unregulated and resulting from a damaged environmental stress response[3]. However, emerging experimental evidence reveals a regulated form of necrosis, termed necroptosis, a form of cell death controlled by specific intrinsic programs. Autoimmune disorders, such as rheumatoid arthritis, can result from defects in multiple stages of these forms of cell death, ranging from mutant death receptors and ligands to specific biochemical changes in death-inducing signaling molecules[2]. Closely examining these complex mechanisms is very essential in gaining a better understanding of the pathogenesis of rheumatoid arthritis and several other autoimmune disorders.

Apoptosis	Necrosis	Necroptosis
Regulated	Unregulated (accidental)	Regulated
Mediated by caspases	Triggered by non-physiological stress and infections	Mediated by caspases
Chromosome condensation	--	--
Nuclear fragmentation	--	--
Plasma membrane blebbing	Plasma membrane rupture	Plasma membrane rupture
Apoptotic bodies formation	Swelling of organelles	Swelling of organelles
Cell shrinkage	Cell swelling	Cell swelling
--	Release of intracellular contents	Release of intracellular contents

***Table 1**: Comparison between morphological features of apoptosis, necrosis, and necroptosis*

Apoptosis

Apoptosis is an intracellular process that occurs during cellular development, providing a homeostatic mechanism to remove damaged cells[3,4,5,6]. Cells undergoing apoptosis display morphological characteristics such as plasma membrane blebbing, chromosome condensation, nuclear fragmentation, formation of apoptotic bodies, and cell shrinkage. In the early stages of the disease, they also exhibit biochemical changes, such as the exposure of phosphatidyl-l-serine on the outer plasma membrane[19,20,21]. The mechanism of apoptosis involves certain molecules that play an important role in mediating cell death.

Apoptosis involves an energy-dependent cascade of molecular events that results from three primary pathways: the extrinsic (cell death) receptor

pathway, the intrinsic (mitochondrial) pathway, and the stress-induced pathway in the endoplasmic reticulum[22]. Despite having different initiator caspases (caspase-8, caspase-9, and caspase-10), all of these pathways are linked via caspase-3 activation of downstream molecules[1]. The activation of the death receptor-mediated apoptosis pathway occurs when the Fas ligand, TNF-α (tumor necrosis factor α), binds to the corresponding death receptors[11,12]. The adaptor protein FADD[11,23], and the procaspase-8 protein form a complex, the death-inducing signaling complex (DISC), in which procaspase-8 is activated by autohydrolysis[24,25]. The activated caspase-8 then transduces the apoptosis signal through, either the activation of caspase-3, or the cleavage of BID to truncated BID (tBID). tBID translocates to the mitochondria, resulting in conformational changes in Bax and Bak, as well as their oligomerization for pore formation in the outer mitochondrial membrane[25,26.]

Stress inducers such as DNA damage, growth factor withdrawal, and oxidative stress activate the mitochondrial-dependent pathway[27,28]. This intrinsic pathway is controlled by the Bcl-2 family of proteins, which regulates the permeability of the outer mitochondrial membrane[29,30,31]. Upon release into the cytoplasm from the mitochondria, cytochrome c combines with Apaf-1 to promote caspase-9 activation, which, in turn, activates effector caspases[32,33] to trigger a cascade of proteolytic events.

The ER-dependent pathway is mediated by an ER-resistant caspase (caspase-12). It is activated under the presence of ER stresses: the disturbance of calcium homeostasis, excessively unfolded or misfolded protein accumulation, nutrient deprivation, and hypoxia[22]. Activated caspase-12 directly cleaves caspase-9 after its translocation from the ER into the cytosol, followed by the activation of caspase-3[34]. Similarly, forming a complex with the inositol requiring enzyme-1α-TNF receptor-associated factor 2 (TRAF2), or by calpains, a family of Ca2+-dependent intracellular cysteine proteases, activates the caspase-12 during ER stress.[7,35].

Together, these pathways result in phagocytosis of the apoptotic bodies by macrophages, neoplastic cells, or neighboring parenchymal cells[24]. Interestingly, caspase-3 is the common factor linking these 3 pathways together, initiating the apoptosis execution pathway upon activation[8]. This marks caspase-3 as the potential target for studying the divergence of

each pathway, allowing for the manipulation of apoptosis. Understanding the morphological and cellular characteristics of apoptotic mechanisms, as well as identifying the key molecules involved in mediating cell death, is integral in discerning their roles in autoimmune diseases.

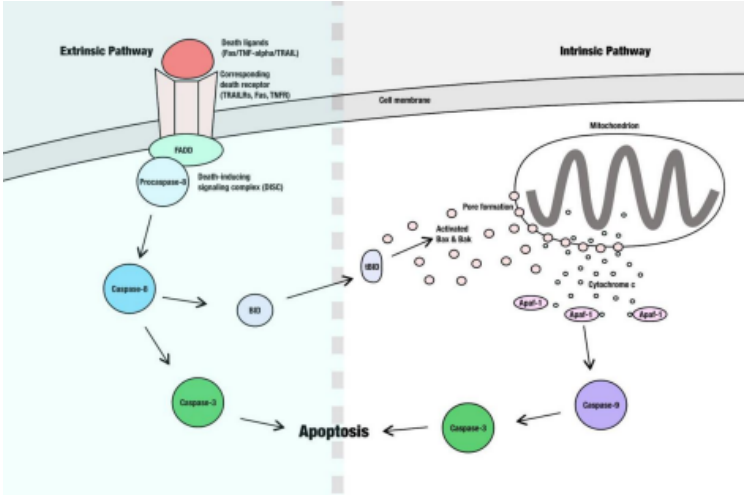

Figure 1: *Mechanisms of Apoptosis*

Apoptotic Pathway	Abbreviation	Protein Name
Extrinsic pathway	Fas ligand	Fatty acid synthetase ligand
	TNF-α	Tumor necrosis factor alpha
	TRAIL	TNF-related apoptosis-inducing ligand
	FADD	Fas-associated death domain
	caspase-8	Cysteine-aspartic acid protease 8
	DISC	Death-inducing signaling complex
	caspase-3	Cysteine-aspartic acid protease 3
Intrinsic pathway	BID	BH3 interacting domain death agonist
	tBID	Truncated BH3 interacting domain death agonist
	Bax	BCL2 associated X protein
	Bak	BCL2 antagonist killer 1
	Bcl-2	B-cell lymphoma protein 2
	Apaf-1	Apoptotic protease activating factor
	caspase-9	Cysteine-aspartic acid protease 9
ER-dependent pathway	caspase-12	Cysteine-aspartic acid protease 12
	TRAF2	TNF Receptor Associated Factor 2

Table 2: *Names and abbreviations of proteins in the extrinsic, intrinsic, and ER-dependent pathways of apoptosis.*

Apoptosis and Disease

The repression of apoptosis increases the possibility of malignancy, as it inhibits tumor cell deaths. On the other hand, uncontrolled apoptosis is associated with various degenerative diseases including acquired immunodeficiency syndrome[36], cancer[2], Parkinson's disease[37] and Alzheimer's disease[38]. Additionally, apoptosis has been correlated with HIV[39], Type 1 diabetes[41,42], autoimmune thyroid diseases, systemic lupus erythematosus, rheumatoid arthritis, and Sjogren's syndrome.

Controlled apoptosis regulates normal T-cell selection and function. In turn, type 1 diabetes is caused by the insulin-secreting β-cells of the pancreas being attacked by T-cells. The FOXP3+CD4+CD25high T-cells (Tregs) represent one of the best characterized sub populations of regulatory T-cells that actively suppress effector T-cells. The increasing evidence of Tregs deficiency in various autoimmune diseases[3], as well as in Type 1 diabetes[41,42], suggests a correlation between greater levels of Tregs apoptosis and a decline in suppressive potential of these cells. However, the data collected are not always consistent in these studies since the investigations are conducted in different phases of the diseases and with various ongoing immunosuppressive therapies. Most of these studies also have limitations in terms of distinguishing nTreg cells from activated Teff cells and characterizing their suppressive function. Current studies fail to elucidate the pathways and genes that make Tregs sensitive to apoptosis during the progression of the disease.

Recent evidence supports the involvement of the Fas and TRAIL mediated apoptotic pathways in the autoimmune diseases of the thyroid[17,18]. One of the earliest breakthroughs occurred when Giordano et al. (1997) used immunohistochemistry, flow cytometry, and RT-PCR to discover the constitutive expression of FasL (Fas ligand) on normal and Hashimoto's thyroiditis (HT) thyrocytes[43,44]. Although thyrocytes are known to express the death receptor Fas, not much is known about how the expression is modulated. Upregulation of Fas was also found in the thyrocytes of patients with Graves' disease. Discoveries like these strongly support the theory that apoptosis and the proliferation of thyrocytes may be abnormally accelerated in patients with thyroid disease, although the proliferation of thyrocytes may exceed their apoptosis, which would result in hyperplasia.

Another autoimmune disease, systemic lupus erythematosus (SLE), is linked to apoptosis through an increased number of apoptotic lymphocytes and macrophages observed in patients with SLE[45]. Characterized by the declining tolerance of self-antigens, this disease causes the production of antibodies, reactive with multiple self proteins[46]. Apoptosis is crucial for regulating the duration of immune responses and maintaining the diversity of the lymphoid armamentarium. Based on considerable statistical data, studies have identified that the deficiency of central molecules involved in lymphocyte apoptosis causes lymphoproliferative and autoimmune diseases in mice and humans[47,48]. In rheumatoid arthritis (RA) tissues in vitro, synoviocytes, synovial T cells and macrophages have been found to express high levels of Fas and/or FasL and are highly susceptible to Fas/FasL-induced apoptosis. Conversely, abnormalities in Fas/FasL expression and susceptibility to Fas-induced apoptosis are generally not observed in osteoarthritis[49]. In some studies, invading T cells have been observed to be defective in FasL expression, which could explain the ineffective clearance of activated (Fas-expressing) cells. Additionally, rheumatoid synovial fluid contains high levels of caspase-3 inhibiting nitric oxide. Therefore, though these studies indicate that Fas induced apoptosis is impaired in RA joints, they do not explain whether these phenomena are a direct result of the initial inflammatory pathways of RA or whether they underlie the disease etiology.

Apoptosis evidently plays a valuable role in the pathogenesis of several of the autoimmune diseases. The extent of apoptotic regulation dictates the pathological manifestations of these diseases. Considerably repressed apoptosis increases the likelihood of malignancy, whereas unregulated apoptosis can directly lead to the initiation and progressions of several autoimmune diseases. The exact molecules and cellular targets determine the type of degenerative disease. Thus, studies discovering the functions and mechanisms of cell mediators involved in cell death are crucial to elucidating the pathogenesis of autoimmune diseases.

Necrosis

Necrosis is an unregulated or accidental cell death due to internal or external stresses, resulting from the disruption of membrane homeostasis It leads to water imbalance between the extracellular and intracellular environments[50,51,52]. Recently it has been suggested that a programmed

form of necrosis, commonly known as necroptosis, is a critical pathway that is involved in a number of autoimmune diseases, as well as heart diseases. The discovery of necroptosis signifies the need to differentiate between the mechanisms that induce passive necrosis, and those that stimulate necroptosis, to better understand disease progression[8,9]. Although necrosis and necroptosis share nearly identical characteristics, the implication of necroptosis in several diseases raises a topic of interest in research studying autoimmune diseases.

Although there are some morphological and mechanistic differences between apoptosis and necrosis, these two processes overlap in some ways. Evidence indicates that necrosis and apoptosis share a biochemical network described as the "apoptosis-necrosis continuum."[53] For example, a decrease in the availability of caspases, or intracellular ATP, can convert an ongoing apoptotic process into a necrotic process[54,55]. The tissue type, the nature of the cell death signal, the physiological make up, and the developmental stage of the tissue all determine whether a cell dies by necrosis, or apoptosis[53,56]. Using conventional histology to distinguish between apoptosis and necrosis proves difficult, as they can occur simultaneously depending on factors such as the availability of caspases, the intensity and duration of the stimulus, and the extent of ATP depletion[53].

One of the primary differences is that necrosis is an unregulated, passive process that usually impacts a large range of cells. Apoptosis is contained, energy-dependent, and can affect clusters or individual cells. Necrosis is initiated by two main mechanisms: interference with the energy supply of the cell and direct damage to cell membranes. On the other hand, there is virtually no inflammatory reaction in apoptotic cells, because they do not release their cellular constituents into the surrounding interstitial tissue and are quickly phagocytosed by macrophages or adjacent normal cells[57,58].

Necroptosis

Necroptosis differs from apoptosis in several ways. Cellls undergoing apoptosis maintain the integrity of their cell membrane, whereas necroptosis disrupts the cell membrane. Although apoptosis and necroptosis share certain triggers, the intracellular signaling pathways that ultimately lead to each cascade differ[16]. Apoptosis is known to be regulated by key mediators called caspases, whereas the main mediators of

necroptosis are receptor-interacting protein kinases (RIPKs). Additionally, apoptosis and necroptosis intersect at several points during the signal transduction process. One of the most well-researched convergence points between the two processes is the role of caspase-8 in inhibiting necroptosis by cleaving necroptosis mediators[59,60].

In simple terms, necroptosis is a cellular response to environmental stress, triggered by mechanical or chemical injury, inflammation, or infection. The existing knowledge of necroptosis revolves around a key signaling molecular pathway: the TNF-α receptor system — a pleiotropic molecule capable of inducing a survival, apoptotic, or necroptotic response based upon the assembly of various sequential cell death complexes[61,62]. Under some cellular conditions, the binding of the ligand TNF-α to the receptor TNF-RI triggers the formation of complex I (a prosurvival complex that signals through NF-κB). However, in cases where RIPK1 is de ubiquitinated, the complex has become an apoptotic complex IIa[63]. Furthermore, the absence of caspase 8, in addition to elevated levels of Receptor-interacting serine/threonine-protein kinase 3 (RIPK3), alter the complex to IIb (also called the necrosome). This necrosome contains RIPK1, RIPK3 and the Fas-associated protein with death domain, allowing the cell to undergo necroptosis via direct phosphorylation of mixed lineage kinase domain-like protein (MLKL) by RIPK3[64,65]. Phosphorylation of MLKL results in a pore-forming oligomer that punctures the plasma membrane and causes subsequent cell death[66].

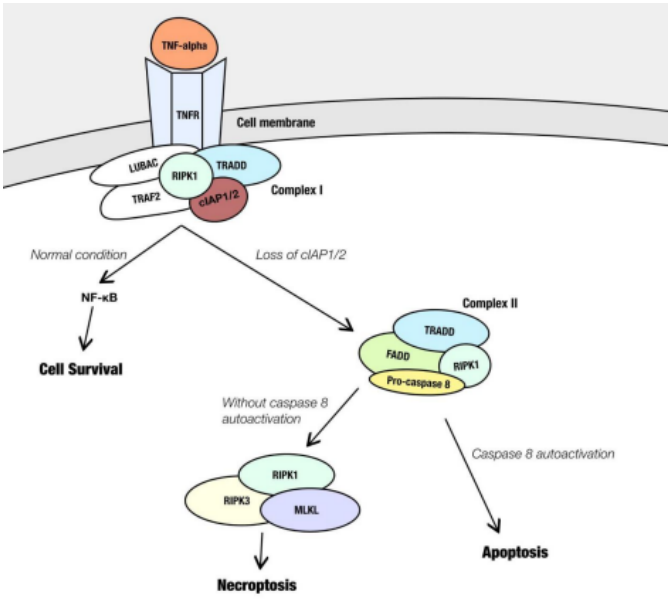

Figure 2: *TNFR1-mediated cell death and survival pathways*

A number of proinflammatory cytokines, such as the key mediators of necroptosis, TNF-α and IL-1β, as well as inflammatory cells, can cause cell death[67]. However, the pathological manifestation of inflammation varies according to the type of cell death. Apoptosis generates a milder inflammatory response because it maintains the integrity of the cell membrane and avoids the overspill of intracellular contents[68]. Conversely, necroptosis directly activates and regulates inflammatory responses by releasing intracellular contents through the ruptured plasma membrane. The strong connection found between necroptosis and inflammation has been thought to be the primary component of the pathogenesis of necroptosis associated diseases. Moreover, RIPK1 and RIPK3 have been found to incite an inflammatory response regardless of cell death[69,70]. These distinguishable characteristics of necroptosis draw interest to their implication in the progression of autoimmune diseases.

Necroptosis in Autoimmune Diseases

The examination of certain neurodegenerative diseases, such as Alzheimer's disease (AD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS), have contributed to the study of the implications of necroptosis[71]. Alzheimer's disease (AD) is a degenerative brain disease, which is characterized by the damage and loss of neurons. A study examining human AD brains and mouse model AD brains[72] confirmed the activation of necroptosis[73] after observing a major increase in the level of necroptosis markers — RIPK1, MLKL, necrosome complex and MLKL oligomers — in AD brains compared to normal. Subsequently treating mice with AD brains with the necroptosis inhibitor, Cl-O-necrostatin, significantly suppressed necroptosis and prevented neuronal loss[73]. This suggests that inhibiting the function of necrosome components interferes with the activation of necroptosis, highlighting a promising strategy in the treatment of AD. In some reports, apoptotic morphology was not directly observed in any sections of the brain. Instead, the cells showed swollen morphologies and were positive for DNA fragmentation, implying that AD pathogenesis may not involve apoptosis. Others have argued that the apoptosis theory and the clinical manifestations contradict each other. Cells directed to apoptosis have shown to die within days, and with great levels of caspase-3 activity, suggesting an acute and massive neuronal loss according to the apoptosis theory. In such cases, the clinical symptoms of AD patients should be observed in the early phase of the disease rather than

assessing the progression of the disease over a decade.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of motor neurons. Compared to healthy control spinal cords, the ALS spinal cords showed an increase in necrosome components, including RIPK1, RIPK3 and MLKL, in a mouse model of ALS[15]. Furthermore, inhibiting RIPK1 with Nec-1, or knocking-out RIPK3, protected against demyelination and prevented the progression of the disease[15]. Thus, through promoting inflammation and cell death, RIPK1 and RIPK3 induce axonal degradation and act as mediators of ALS and PD. This further indicates that, since necroptosis is activated in ALS, inhibition of necroptosis can be a potential therapeutic target for this disease. Additionally, a study observing a Parkinson's disease (PD) model found that upon inhibition of RIPK1 by Nec-1[13] neuronal degradation was reduced by a half, compared to untreated cells. The protective effect of Nec-1 indicates the therapeutic potential of this drug in ALS and PD. The study used a human iPSC based model to effectively capture both early pathological events in mutant neural cells and the beneficial effects of blocking necroptosis, thereby, strengthening the validity of the results.

Multiple Sclerosis (MS) is a chronic neurodegenerative disease characterized by the loss of oligodendrocytes and demyelination. High concentrations of necroptosis components, including phosphorylation of RIPK1, RIPK3 and MLKL, were detected in pathological samples from MS patients, as well as a prominent increase of MLKL oligomers in MS pathological samples compared to the control[40]. This indicates that necroptosis is also involved in the pathogenesis of MS[40]. In the study, oral administration of RIPK1 inhibitor 7-Cl-O Nec-1 diminished oligodendrocyte degeneration and reduced the disease severity in a mouse model of M_S[40]. These findings reveal that inhibiting RIPK1 specifically could be of potential therapeutic value in the treatment of MS. Although the study does not take into consideration the potential metabolic consequences of orally administering the drug, their results and conclusion are strongly supported by significant amounts of experimental data. Rheumatoid arthritis (RA) is one of the most common chronic inflammatory diseases that is characterized by joint inflammation and osteoclastogenesis. The key regulators of necroptosis, RIPK1, RIPK3 and MLKL were detected in significantly higher amounts in the synovium of a collagen-induced arthritis mouse model displayed significantly higher

amounts of the key regulators of necroptosis, RIPK1, RIPK3 and MLKL, which emphasized the involvement of necroptosis in the pathogenesis of RA[14]. In the mouse model, RIPK1 inhibitor Nec-1 greatly suppressed the expression of these key regulators and the main inflammatory cytokines, IL-17, IL-1β, IL-6 and TNFα[74]. Therefore, similarly to the previous conclusions, this study supports the potential of inhibiting RIPK1 as a novel therapeutic approach for the treatment of RA.

Although these studies provide compelling evidence for targeting RIPK1 and MLKL molecules for subsequent therapeutic research, a close examination of another class of proteins that play an important role in necroptosis may shed light on another new approach. Inhibitors of apoptosis proteins (IAPs) form a family of genetically conserved proteins characterized by the presence of 1-3 baculovirus IAP repeat (BIR) motifs[75]. Previous studies have identified three family members (termed XIAP, cIAP1, and cIAP2) as potent suppressors of cell death. XIAP, the most-studied IAP, inhibits apoptosis by binding and inhibiting caspases, and it has been broadly assumed that cIAP1 and cIAP2 block apoptosis through a similar mechanism. However, recent structure function analyses have indicated that these IAPs are not direct caspase inhibitors, suggesting that their anti-apoptotic function must involve alternative mechanisms[75].

Cellular Inhibitors of Apoptosis

Inhibitors of apoptosis (IAP) are proteins that belong to the family of antiapoptotic proteins that prevent cell death, direct cell growth, and participate in cellular signal transduction[76]. The mechanism of IAPs in inhibiting apoptosis involves both the intrinsic and extrinsic apoptotic pathways[1]. Among these IAPs, cellular IAP1 and 2 (cIAP1 and cIAP2), the key molecules of the tumor necrosis factor α (TNFα) signaling pathway, are recruited upon TNF receptor (TNF1) activation, along with the other adaptor proteins such as TNF receptor-associated factor (TRAF), TNFR-associated death domain protein (TRADD), receptor-interacting protein kinase 1 (RIPK1) and linear ubiquitin chain assembly complex (LUBAC). These then go on to form the signaling complex I, which activates the nuclear factor kappa B (NF-κB) signaling pathway and promotes cell survival[77]. The overexpression of the IAP protein family has been reported to be associated with cancer development, with X-linked inhibitor of apoptosis protein (XIAP) classified as the most potent IAP family member[78]. The XIAP, cIAP1, and cIAP2 contain three baculovirus

IAP repeat (BIR) domains, a ubiquitin-associated (UBA) domain, and a newly discovered gene (RING) finger motif, which exhibits ubiquitin E3 ligase activity[79]. The IAPs prevent apoptosis by inhibiting downstream caspases, which are essential proteins of the apoptotic pathways. The cleaving of upstream caspases, such as caspase 8 and caspase 9, leads to the activation of downstream effector caspases, such as caspase 3, eventually resulting in programmed cell death[76].

The most recently discovered IAP is the mammalian IAP ML-IAP, which is detectable in embryonic tissue, certain adult tissues and several cancer cell lines[80]. Although ML-IAP has only one BIR domain, it is reported to inhibit both the initiator caspase 9 and effector caspases 3 and 7. Thus, it inhibits cell death induced through death receptors by overexpression of the cell death pathway proteins FADD, Bax, RIP, RIP3 and DR3[81]. In fact, the most compelling evidence for the regulation of developmental cell death by IAPs originates from studies in *Drosophila,* in which loss of DIAP1 resulted in extensive early embryonic cell death and a corresponding increase in caspase activity[82]. However, these studies cannot be extrapolated to human models due to obvious anatomical and physiological differences. An equivalent study in mammals is necessary to establish the role of these proteins in mammalian developmental cell death. Recent studies have greatly advanced our understanding of IAPs and their part in inhibiting cell death. Structurally, it is now evident how IAPs interact with caspases, and how this interaction can be regulated by IAP antagonists such as DIABLO. Though, further research is still required to define the various roles for different mammalian IAP proteins in the presence of cell-death stimuli. Indeed, there could potentially be some redundancy between family members. As a result, it is necessary to generate mice deficient for more than one IAP, to establish the role of IAPs in mammals. So far, researchers have only found one mammalian IAP antagonist, DIABLO. However, considering that there are three such proteins in *Drosophila,* other mammalian IAP antagonists must certainly exist. Future research investigating the direct interactions of BIR domains from these proteins with other cellular proteins are likely to illustrate the roles of IAP proteins in regulating cell death.

Conclusion

Apoptosis and necrosis are two of the most known types of cell death that play an essential role in cell development. The strictly distinct

morphological features of apoptosis include chromosome condensation, nuclear fragmentation, and membrane blebbing. Necrosis, on the other hand, is characterized by the expansion of cellular organelles, plasma membrane rupture, and inflammatory responses resulting from the release of the intracellular contents. The apoptotic pathway — an energy-dependent cascade of molecular events — is initiated by 3 primary pathways: the extrinsic (cell-death) receptor pathway, the intrinsic (mitochondrial) pathway, and the stress-induced pathway in the endoplasmic reticulum. Although these pathways utilize different initiator caspases (caspase-8, caspase-9, and caspase-10, respectively), they activate the same downstream mediator molecule, caspase-3. Meanwhile, necrosis is classified as accidental cell death caused by internal or external stresses. In necrosis, the disruption of membrane homeostasis results in water imbalance between the extracellular and intracellular environments. Whether cells undergo apoptosis or necrosis depends on the availability of caspases, and intracellular ATP that determine the cell-death signal, signaling duration, tissue type, and the developmental stage of the tissue. In summary, necrosis is an unregulated and passive process that usually affects a larger region of cells, whereas apoptosis is a contained and energy-dependent process that affects an individual or smaller clusters of cells.

Studies have demonstrated that the repression of apoptosis, or the inhibition of programmed cell death, increases the possibility of malignancy. In contrast, uncontrolled apoptosis has been found to be associated with degenerative diseases such as acquired immunodeficiency syndrome (AIDS), cancer, Parkinson's disease, and Alzheimer's disease. Recently, a programmed form of necrosis, necroptosis, has been proposed as an important pathway involved in many diseases. The primary function of necroptosis was believed to be infection control and protection against virus-induced apoptosis. However, like apoptosis, necroptosis has also been found to be associated with certain neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis, and amyotrophic lateral sclerosis. Due to the emerging crosstalk between the differing forms of cell death, these processes are now considered interconnected. Thus, differentiating between apoptotic, necrotic, and necroptotic cellular mechanisms is critical to understanding disease progression. The recent discovery of a family of genetically conserved proteins, known as IAPs, provided new insights into the molecules that play a critical role in mediating these cell death pathways. Inhibitors of apoptosis proteins (IAP) are a type of

antiapoptotic protein involved in both, the intrinsic and extrinsic apoptotic pathways. Cellular IAP1 and 2 (cIAP1 and cIAP2) in particular, are the key molecules of the tumor necrosis factor α (TNFα) signaling pathway, which activates adaptor proteins such as TNF receptor-associated factor (TRAF), TNFR-associated death domain protein (TRADD), Receptor-interacting serine/threonine-protein kinase 1 (RIPK1), and linear ubiquitin chain assembly complex (LUBAC) to induce the NF-κB signaling pathway and promote cell survival. In short, the IAPs prevent apoptosis by inhibiting the normal functions of downstream caspases, including caspase 8, caspase 9, and caspase 3. The impaired function of IAP could instruct the cells to undergo necroptosis or apoptosis instead of the NF-κB cell survival pathway. From this step of the cell death process, autoactivation of caspase 8 would result in apoptosis whereas the absence of caspase-8 autoactivation would result in necroptosis.

Studies have demonstrated how the IAPs inhibit programmed cell death. However, further investigation is necessary to clarify the roles of different mammalian IAP proteins in the presence of various cell-death stimuli. Considering their newfound functions, continued research into the characteristics and mechanisms of IAP molecules will likely prove an effective and viable approach to diagnosing or treating several elusive diseases.

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