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REVIEW

Biological Functions and Clinical Implications of CFLAR: From Cell Death Mechanisms to Therapeutic Targeting in Immune Regulation

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Abstract: Since its initial functional characterization in the late 1990s, CASP-8 and FADD-like apoptosis regulator (CFLAR) has been recognized as a crucial regulator of both apoptosis and immune responses. CFLAR inhibits caspase-8 activation by forming heterodimers with procaspase-8 at the death-inducing signaling complex (DISC), thereby preventing its proteolytic maturation. In addition to its role in cell death, CFLAR is integral to immune regulation, modulating NF-κB-dependent cytokine production (eg, IL-1β, TNF-α) and effector functions of T cells and macrophages. Recent studies underscore the pathological significance of dysregulated CFLAR expression in a variety of diseases, including cancers and inflammatory conditions. Within the tumor microenvironment, elevated CFLAR expression confers resistance to therapy, while in infectious and inflammatory diseases, its expression levels modulate the magnitude and direction of the immune response. This review provides an in-depth exploration of CFLAR's structural and functional properties, focusing on its involvement in apoptosis, autophagy, and immune modulation. Moreover, we examine its translational potential as a therapeutic target, evidenced by ongoing preclinical studies targeting CFLAR isoforms in cancer immunotherapy. By synthesizing recent advances in CFLAR's dual roles in cell death and immune surveillance, this review highlights actionable targets for overcoming therapy resistance and immune dysregulation.

Keywords: CFLAR, apoptosis, autophagy, immunomodulation, signal path

Introduction

Caspase-8 and FADD-like apoptosis regulator (CFLAR), commonly referred to as cellular FLICE-inhibitory protein (c-FLIP), has been recognized as a critical inhibitor of apoptosis since its discovery in 1997.[1] CFLAR exerts its anti-apoptotic effects by binding to Fas-associated death domain protein (FADD), thereby competitively inhibiting the interaction between caspase-8 and FADD. This prevents the assembly of the death-inducing signaling complex (DISC) and thereby impedes the transmission of extrinsic apoptotic signals. Beyond apoptosis regulation, CFLAR is also critical in immune responses and inflammation. It modulates cytokine secretion and immune cell activation, thus influencing the host's immune response to pathogens. These properties position CFLAR as a key molecule in the study of autophagy, immunity, and inflammation. For instance, CFLAR regulates key stages of autophagy, including autophagosome formation and maturation, which in turn affect the differentiation and migration of effector cells.

In recent years, the role of CFLAR in a range of diseases has gained significant attention. In cancer, upregulated CFLAR expression is strongly associated with immune evasion mechanisms, such as the inhibition of apoptosis and the modulation of immune checkpoint pathways, leading to enhanced tumor cell resistance to treatment. In the context of infection and inflammation, CFLAR expression directly influences immune cell function and the intensity of inflammatory responses. TThus, a more comprehensive understanding of the mechanisms by which CFLAR regulates autophagy

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and immune modulation may provide valuable insights into its potential as both a disease mediator and a therapeutic target. This review focuses on exploring the mechanistic involvement of CFLAR in these processes, offering a framework for future research into targeted therapeutic strategies.

Overview of the Structure and Function of CFLAR

In 1997, Thome et al first identified the viral FLICE-inhibitory protein (vFLIP), which contains two death effector domains (DEDs) and can inhibit apoptotic signaling by binding to death receptors.[1] Building upon this, Irmler et al subsequently identified a gene in the human genome homologous to vFLIP, which was named CFLAR.[2] Although CFLAR shares a high degree of homology with vFLIP, it differs significantly in terms of its functional roles and regulation of expression, particularly in how it influences apoptosis and immune signaling. The CFLAR gene is located on the human chromosome at region 2q33-34, adjacent to the genes encoding caspase-8 and caspase-10, a positioning that is likely related to its functional interactions with these key regulators of apoptosis. The CFLAR gene consists of 14 exons and generates multiple mRNA isoforms through alternative splicing. These isoforms encode different forms of the protein, collectively known as c-FLIP, which can vary in their ability to regulate apoptosis and immune responses.

In humans, CFLAR has three primary isoforms: c-FLIP_L, c-FLIP_S, and c-FLIP_R. These isoforms perform distinct functions in various immune and tumor cell types. c-FLIP_L is a 55 kDa protein that contains two N-terminal DEDs and a C-terminal caspase-like domain. Despite structural similarities to procaspase-8, the catalytic sites within the caspase-like domain are not conserved in c-FLIP_L, thus it lacks proteolytic activity. Despite the structural similarity of this domain to that of procaspase-8, the catalytic sites are not conserved in c-FLIP_L, and thus it lacks caspase-like proteolytic activity.[3–7] c-FLIP_S is a 27 kDa protein that contains only two DEDs and lacks a caspase-like domain. Unlike c-FLIP_L, c-FLIP_S is absent in mice, highlighting a potential species-specific difference in isoform expression.[8,9] c-FLIP_R is a 25 kDa protein that is specifically expressed in certain cell lines, such as Raji and SKW6.4, as well as in human primary T cells. Its expression pattern suggests a role in specific immune responses and cell signaling in these contexts.[10,11] In several signaling pathways, both c-FLIP_L and c-FLIP_S play crucial roles in promoting cell survival by activating or upregulating key pro-survival proteins, such as Akt, ERK, and nuclear factor-kappa B (NF-κB), often through their interaction with death receptors and modulation of caspase activity. All CFLAR isoforms are known to interact with caspase-8 via DED-DED interactions to form heterodimers. Moreover, caspase-8-mediated proteolysis at the Asp376 and Asp196 sites generates truncated c-FLIP fragments, known as p43-FLIP and p22-FLIP, respectively. These fragments are thought to have distinct functional roles, including modulating the apoptotic response and influencing immune signaling.[12] The biological functions of the major CFLAR isoforms in various immune and tumor cell types, including their roles in apoptosis and immune regulation, are summarized in Table 1.

CFLAR, as a multifunctional protein, plays a pivotal role in regulating caspase-8 activity, which in turn modulates key cellular processes such as apoptosis, necroptosis, and autophagy.[13] CFLAR can interact with FADD, caspase-8,

Table 1 Summary of CFLAR Subtypes and Their Biological Functions

CFLAR Subtype	Molecular Weight	Structural Features	Cell Expression	Main Functions	Signaling Pathways	Associated Proteins
c-FLIP L	55 kDa	Two N-terminal DEDs and one C-terminal caspase-like domain	Widely expressed in various immune and tumor cells	Inhibits caspase-8, regulates apoptosis and cell survival	Akt, ERK, NF-κB	p43-FLIP, p22-FLIP
c-FLIP S	27 kDa	Contains two DEDs, lacks caspase-like domain	Absent in mice	Promotes cell survival, enhances resistance to apoptotic signals	Akt, ERK	Unknown
c-FLIP R	25 kDa	Specific expression in Raji cell line and human primary T cells	Involved in immune response, regulates cytokine production	Unknown	Unknown	Unknown

Abbreviations: CFLAR, CASP-8 and FADD-Like Apoptosis Regulator; c-FLIP, cellular FLICE-inhibitory protein; DED, death effector domain.

caspase-10, and death receptor 5 (DR5), either in a ligand-dependent or independent manner, leading to the formation of an apoptosis inhibitory complex (AIC). This interaction prevents the assembly of the DISC and inhibits the activation of caspases. Furthermore, studies have demonstrated that caspase-8 and FADD can be recruited to specialized complexes at the endoplasmic reticulum (ER) and mitochondria, facilitating signal exchange between these organelles.[14–21] At the ER-mitochondria interface, several molecular platforms have been identified, which consist of membrane-bound proteins and cytosolic apoptosis regulators. These platforms are involved in regulating key processes such as membrane anchoring, lipid metabolism, Ca^{2+} signaling, and the coordination of apoptosis between the ER and mitochondria.[22,23]

Role of CFLAR in Cell Death Mechanisms

CFLAR is involved in both the classical death receptor-mediated extrinsic apoptotic pathway and the regulation of non-canonical, pattern recognition receptor (PRR)-dependent apoptotic pathways. Furthermore, CFLAR is crucial for the formation of the RIP1-dependent, non-death receptor apoptotic platform, termed the ripoptosome. Figure 1 illustrates the regulatory mechanisms of CFLAR in apoptosis and autophagy.

CFLAR and Autophagy

Autophagy is an intracellular degradation process in which cells sequester cytoplasmic proteins into autophagosomes, which are then transported to lysosomes for subsequent degradation.[24] This process is essential for maintaining cellular homeostasis by eliminating unnecessary proteins, dysfunctional complexes, and damaged organelles, thus preventing cellular stress and damage.[25] Under stress conditions, autophagy is activated to maintain cellular homeostasis. However, it is tightly regulated, and its dysregulation is strongly associated with various pathological conditions, including neurodegenerative diseases, cancer, and metabolic disorders. The formation of autophagosomes depends on a family of proteins known as autophagy-related gene (Atg), with Atg3 playing a crucial role in conjugating microtubule-associated protein light chain 3 (LC3) to the autophagosome membrane and facilitating its subsequent lipidation and

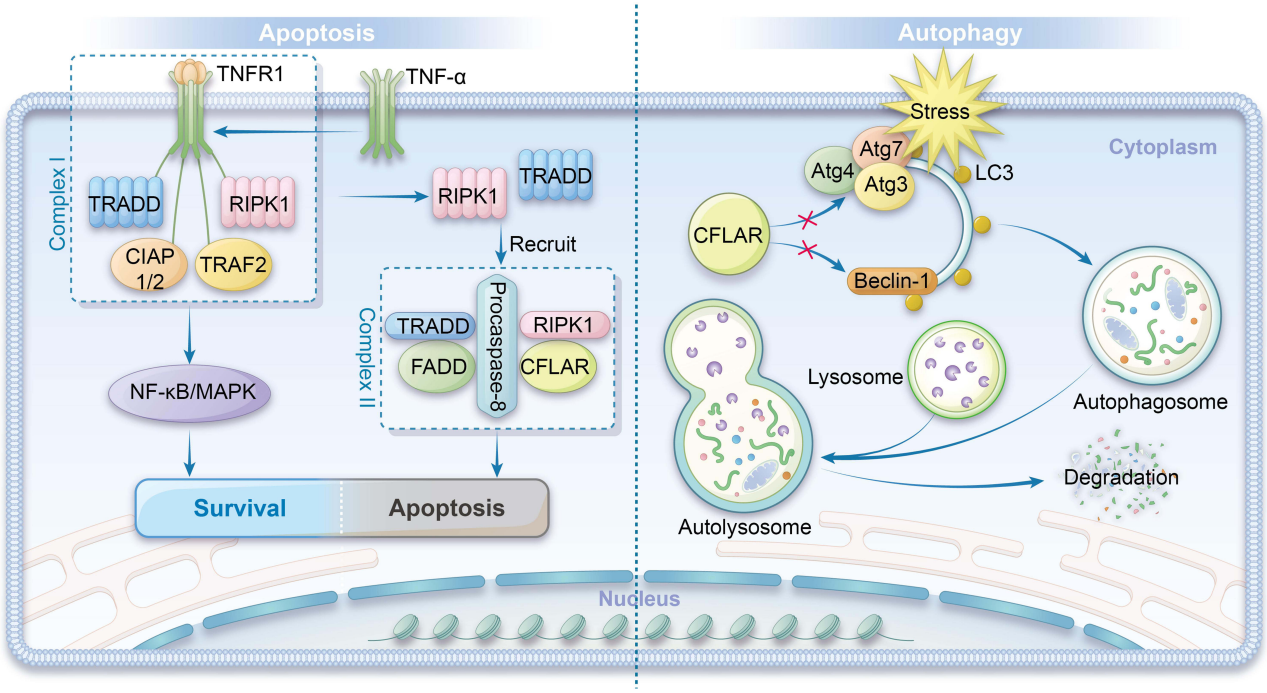

Figure 1 Regulatory Mechanisms of CFLAR in Apoptosis and Autophagy. Left panel: CFLAR modulates apoptosis and autophagy by interacting with key molecular complexes. TNF-α activates TNFR1, forming Complex I, which recruits TRADD, RIPK1, cIAP1/2, and TRAF2, thereby activating the NF-κB and MAPK signaling pathways. Additionally, Complex I can transition into a secondary Complex II, which associates with FADD, CFLAR, and procaspase-8 to further regulate cellular signaling events. Right panel: CFLAR inhibits autophagy by preventing the interaction between the Atg family proteins and LC3, and by suppressing Beclin-1 activity, thereby blocking autophagosome formation and protecting cells. Under stress conditions, autophagic membranes gradually form, eventually completing the autophagosome, which then fuses with lysosomes for the degradation of cellular components.

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processing. LC3, particularly its lipidated form LC3-II, is a critical component of the autophagosome membrane, where it plays a vital role in autophagosome maturation and cargo recognition.[26–28]

Studies have shown that CFLAR can directly bind to Atg3, inhibiting its interaction with LC3, thereby disrupting the lipidation process of LC3 and impairing the formation of autophagosomes.[29] In addition, CFLAR indirectly regulates autophagy by interacting with procaspase-10, potentially influencing its activation and subsequent downstream effects on autophagic signaling. Beclin-1, which is crucial for autophagosome formation, promotes the localization of Atg to the pre-autophagosomal membrane, a process facilitated by its interaction with the Vps34 complex. Beclin-1's activity is regulated by its release from the anti-apoptotic protein Bcl-2.[30] The interaction between Bcl-2 and Beclin-1 serves as a critical point of convergence between apoptosis and autophagy, as Bcl-2 prevents Beclin-1 from interacting with essential autophagy proteins. As an anti-apoptotic protein, Bcl-2 can suppress Beclin-1's autophagic function by interacting with it, potentially preventing autophagy-dependent cell death.

In multiple myeloma cells, the presence of c-FLIP_L inhibits the activation of Beclin-1, potentially through interference with the Beclin-1 activation complex or by preventing the dissociation of Bcl-2 from Beclin-1. The complex formed by the DED-mediated heterodimerization of procaspase-10 and c-FLIP_L exhibits proteolytic activity, which cleaves the Bcl-2 interacting protein BCLAF1. This cleavage prevents BCLAF1 from displacing Bcl-2, thereby blocking the activation of Beclin-1 and inhibiting Beclin-1-mediated autophagy.[31] Myeloma cells require basal levels of autophagy for survival; however, caspase-10 attenuates this autophagic response to prevent cell death, with c-FLIP_L promoting caspase-10 activation in multiple myeloma cells. This dysregulation of autophagy may contribute to the survival of tumor cells. Therefore, drugs that disrupt this balance may hold therapeutic potential for myeloma treatment.

It is important to note that the role of c-FLIP_L in these mechanisms is independent of caspase-8. However, in the context of procaspase-10 heterodimerization, caspase-10 acts as a classic pseudo-caspase, regulating the activity of its enzymatically active homologs and modulating apoptotic signaling.

CFLAR and Apoptosis

Necroptosis is a form of programmed cell death that is independent of caspase activation and distinct from the apoptosis pathway, which relies on caspases and TNFRSF signaling. It primarily involves the activation of receptor-interacting protein kinase 1 (RIPK1) and RIPK3, which subsequently activate mixed lineage kinase domain-like (MLKL), leading to cellular membrane rupture. Necroptosis can occur when apoptotic pathways are blocked.[13] Under specific conditions, such as in the absence of external death ligands or death receptor interactions, cells can autonomously activate caspase-8, a process that may involve intracellular signaling pathways or cellular stress responses.[3] RIPK1 was initially identified as a protein interacting with the tumor necrosis factor receptor 1 (TNF-R1) signaling complex.[32,33] It has since been shown to play a crucial role in regulating cell survival, apoptosis, and autophagy. RIPK1 mediates cell death through the formation of the necrosome complex, which involves its interaction with RIPK3 and MLKL, leading to necroptosis. Additionally, RIPK1's role in apoptosis is modulated through its kinase activity and interactions with other signaling molecules. The function of RIPK1 is modulated by phosphorylation and ubiquitination, which are critical for determining cell fate. Cellular inhibitor of apoptosis proteins (cIAPs), functioning as E3 ubiquitin ligases, can ubiquitinate RIPK1, thereby promoting its activation or preventing its degradation. This modification also extends to cIAPs themselves, influencing their own stability and interactions within cell death signaling complexes.[34]

To investigate the self-ubiquitination of cIAPs and its impact on cell death pathways, Tenev et al used the cytotoxic drug etoposide to induce genotoxic cell death.[35] Similarly, Feoktistova et al employed IAP antagonists (Smac mimetics) in combination with dsRNA poly I:C to induce immune-mediated cell death via Toll-like receptor 3 (TLR3) activation.[36] Both studies observed the spontaneous formation of the ripoptosome, a 2 MDa complex consisting of RIPK1, FADD, caspase-8, and CFLAR. This complex plays a pivotal role in mediating both caspase-dependent apoptosis and RIP kinase-dependent necroptosis, with its formation and function varying in a cell type-dependent manner. Both genotoxic stress and TLR3 stimulation require ripoptosome formation to induce cell death. According to the current model, procaspase-8 integrated into the ripoptosome is activated via homodimerization and processed into its active form, leading to the cleavage and inactivation of RIPK1, and disintegration of the ripoptosome. This process predominantly leads to caspase-8-mediated apoptosis.[37]

When c-FLIP_L is incorporated into the ripoptosome, it activates procaspase-8 through heterodimerization. However, procaspase-8 activated by c-FLIP_L cleaves only a limited subset of substrates, such as RIPK1, and upon disintegration of the ripoptosome, procaspase-8 is inactivated again. In this manner, c-FLIP_L effectively prevents apoptosis and promotes cell survival by controlling caspase-8 activation. In contrast, c-FLIP_S promotes the assembly of the ripoptosome but does not activate procaspase-8 to inactivate RIPK1. This prevents the execution of apoptosis while still facilitating the formation of the ripoptosome complex.[38] Furthermore, other studies suggest that the balance between the functional RIPK1-RIPK3-MLKL axis and CFLAR isoforms is a crucial determinant in the regulation of cell survival, apoptosis, and necroptosis. This balance influences the cellular decision to undergo either caspase-mediated apoptosis or RIP kinase-dependent necroptosis, depending on the specific cellular context.[39–41] Figure 2 illustrates the formation and mechanism of action of the ripoptosome.

Regulation of Other Death-Inducing Protein Complexes

CFLAR plays a significant role in the formation of several protein complexes associated with cell death. c-FLIP_L plays a significant role in regulating the signaling pathways of several death receptors, including Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptors (TRAIL), Fas/CD95, and TNF-R1. In particular, c-FLIP_L plays a critical role downstream of the TNF-R1 signaling pathway. TNF-R1, a member of the TNF receptor superfamily (TNFRSF), forms Complex I upon binding to its ligand TNF-α. This process recruits TNFR1-associated death domain (TRADD) protein, followed by RIPK1, TNF receptor-associated factor 2 (TRAF2), and cellular inhibitors of apoptosis (cIAP1/2). The assembly of this complex is driven by the formation of linear ubiquitin chains, a process dependent on RIPK1 ubiquitination. The resulting complex activates both the NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways.[42,43]

Following the formation of Complex I, RIPK1 undergoes deubiquitination by CYLD, a lysine deubiquitinase, which triggers the formation of the secondary TNFR1 Complex II. This complex consists of TRADD and RIPK1 dissociated from Complex I and can further recruit FADD, procaspase-8, and CFLAR. Ultimately, these molecules form a structure similar to the DISC.[44] In Complex II, the ratio of homodimers to heterodimers of c-FLIP_L and procaspase-8 plays a critical role in regulating signal transduction. Sufficient homodimerization of procaspase-8 is required to activate

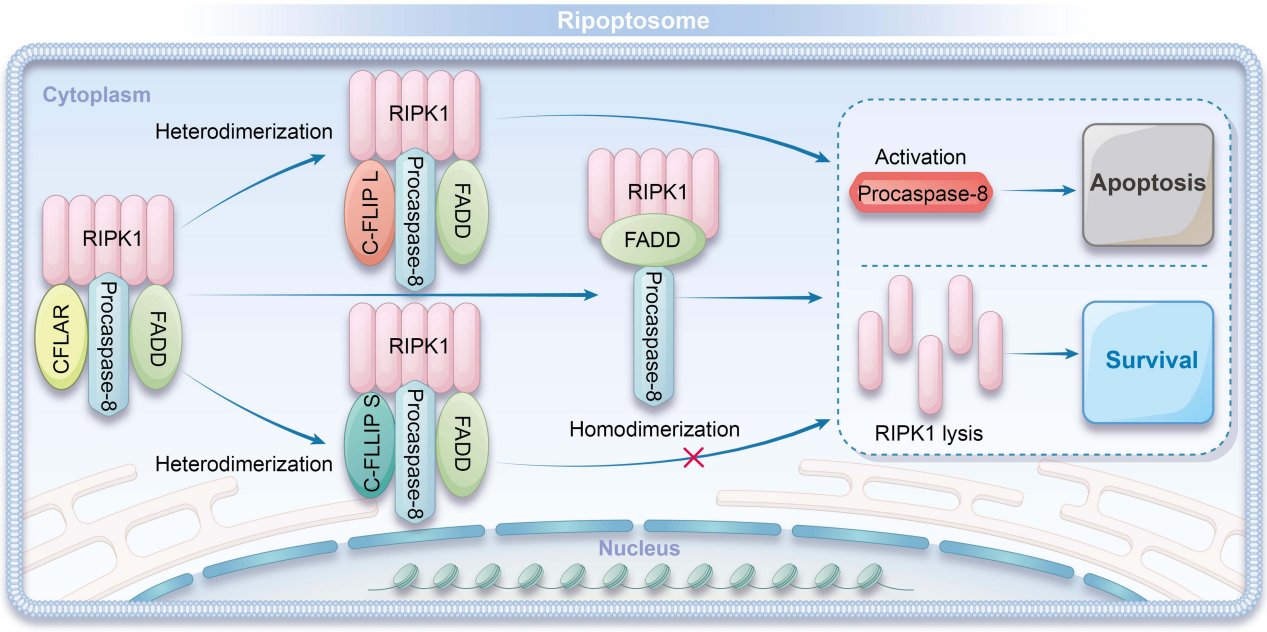

Figure 2 Formation and Mechanism of Ripoptosome. RIPK1 forms a complex with procaspase-8, FADD, and CFLAR isoforms to create the ripoptosome. c-FLIP L activates procaspase-8 through heterodimerization, leading to the inactivation of RIPK1 and subsequent disassembly of the ripoptosome, promoting apoptosis. In contrast, c-FLIP S facilitates ripoptosome assembly but inhibits the activation of procaspase-8 and prevents the inactivation of RIPK1, thereby modulating the apoptotic response.

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caspase-3/7, while a higher proportion of procaspase-8/c-FLIP_L heterodimers inhibits this activation, thus impeding apoptosis. Furthermore, TNFR1 Complex I enhances the expression of CFLAR, which, in turn, modulates the signaling output from Complex II, influencing the balance between cell survival and apoptosis.[45,46]

Research by Day et al demonstrated that in MCF-7 breast cancer cells, removal of c-FLIP_L from the AIC, which includes DR5, FADD, and caspase-8, triggers spontaneous, ligand-independent cell death. The precise role of RIPK1 in this process remains unclear and requires further investigation to determine its involvement in regulating spontaneous cell death in the absence of ligands.[47] In addition, Estornes et al[48] identified an atypical death complex induced by double-stranded RNA, which includes TLR3, the TIR domain-containing adaptor protein (TRIF), and caspase-8. In contrast to the ripoptosome, the TLR3-dependent complex requires RIPK1, rather than FADD, for the activation of procaspase-8, highlighting a distinct mechanism of caspase-8 activation in response to RNA-induced stress. These findings suggest that various death-inducing stimuli can give rise to distinct apoptosis-inducing complexes, which share similarities with the ripoptosome, a key regulator of cell death in response to specific cellular stress signals.

Biochemical Properties of CFLAR

c-FLIP_L is considered a more potent activator of procaspase-8 compared to its isoform c-FLIP_S, particularly in terms of substrate specificity.[49] Forced dimerization experiments have demonstrated that c-FLIP_L can activate procaspase-8 in the absence of inter-domain cleavage, leading to changes in substrate specificity. This finding highlights an alternative mechanism by which c-FLIP_L modulates procaspase-8 activity.[50] Yu et al[51] found that the p43-FLIP fragment, generated by procaspase-8-mediated cleavage, displayed significantly higher affinity for heterodimerization with procaspase-8 compared to the uncleaved c-FLIP_L. This enhanced interaction suggests a potential regulatory mechanism for procaspase-8 activation. This finding suggests a potential mechanism by which p43-FLIP may enhance procaspase-8 activity, possibly through stabilization of the procaspase-8/p43-FLIP complex or by altering the conformational dynamics of procaspase-8. In related studies, Dickens[52] and Schleich[53] quantitatively analyzed the DISC initiated by TRAIL and Fas ligand (CD95L), respectively. They discovered that the number of procaspase-8 molecules in the DISC was significantly greater than the number of FADD molecules, while the content of CFLAR in the natural DISC was relatively low. These results indicate that multiple procaspase-8 molecules can simultaneously bind to a single FADD or c-FLIP_L molecule, suggesting a potential cooperative mechanism that enhances DISC stability and procaspase-8 activation.

Earlier studies have suggested that overexpression of procaspase-8, FADD, and CFLAR in cells leads to the formation of filamentous structures known as “death effector filaments”, which implies that procaspase-8 in the DISC may form multimeric complexes through DED-DED interactions.[54] However, quantitative Western blot analysis by Majkut et al revealed that the highest procaspase-8 to CFLAR ratio in the DISC induced by DR5 agonist antibodies was 2:1, a result that challenges the predictions of the DED-chain model.[55] Majkut et al further proposed a two-step DISC model based on site-directed mutagenesis and molecular modeling, in which the incorporation of c-FLIP_L facilitates heterodimerization with procaspase-8, thereby activating procaspase-8 without the need for inter-domain cleavage. However, their findings also indicated that the procaspase-8 to CFLAR ratio in the DISC did not exceed 2:1, which is inconsistent with the predictions made by the DED-chain model.[55]

Kallenberger et al used compartment-specific fluorescence probes to establish a mathematical model for procaspase-8 activation kinetics, concluding that procaspase-8 is initially cleaved at the pro-domain in a dimeric form within a single DISC complex.[56] However, these models were trained using cell population-level measurements, and may not fully capture the detailed dynamics at the single-cell resolution. Subsequently, the aggregation of multiple DISC complexes induces procaspase-8 cleavage at the catalytic domain through inter-dimer interactions, resulting in the release of fully processed caspase-8 into the cytoplasm. In contrast, the c-FLIP_S and c-FLIP_R isoforms within the DISC can bind to procaspase-8 but fail to induce its activation. Schleich’s study further showed that the N-terminal pro-domain of procaspase-8, generated upon full processing, structurally associates with c-FLIP_S/R within the DISC, forming a negative feedback loop that terminates procaspase-8 activation.[57]

Initially, these short isoforms were thought to primarily promote cell survival by inhibiting apoptosis. However, it is now recognized that they play a more complex role, not only in regulating cell survival but also in modulating multiple signaling pathways involved in both cell survival and cell death.

Role of CFLAR in Inflammatory Mediators and Signaling Pathways

Inflammatory Mediators and Signaling Pathways

The transcription of the CFLAR gene can be activated by various stimuli, including TNF ligands such as TNF-α, epidermal growth factor (EGF), interleukin-6 (IL-6), chemokines, and certain chemotherapy agents (eg, doxorubicin).[58] Several transcription factors, such as NF-κB, p63, and EGR1, are involved in regulating CFLAR gene expression during this process.[59] Notably, several transcription factors promote CFLAR transcription, including NF-κB, p63, and early growth response 1 (EGR1). Other factors, such as nuclear factor of activated T-cells cytoplasmic 2 (NFATc2), heterogeneous nuclear ribonucleoprotein K (hnRNP K), androgen receptor (AR), and specificity protein 1 (SP1), also contribute to its regulation. On the other hand, c-myc, forkhead box O3a (FOXO3a), c-Fos, interferon regulatory factor 5 (IRF5), and SP3 suppress CFLAR transcription.[58,59] Research suggests that p53 may upregulate CFLAR gene expression by modulating the levels of specific transcription factors, such as NF-κB, while simultaneously promoting CFLAR degradation through the proteasomal pathway. This indicates that p53 plays a critical role in balancing cell death and survival.[60] CFLAR participates in regulating cell survival, proliferation, and tumorigenesis by activating various cell-protective signaling pathways, such as NF-κB and ERK, which are crucial for preventing apoptosis and promoting cellular transformation.

CFLAR facilitates the transmission of survival signals by interacting with key proteins, particularly in the NF-κB and extracellular signal-regulated kinase (ERK) pathways, where it acts as a molecular scaffold to facilitate the assembly of signaling complexes. For example, c-FLIP_L interacts with TNF receptor-associated factor 1 (TRAF1), TRAF2, RIP1, and Raf-1 to facilitate NF-κB activation, promoting cell survival and inflammation.[12,61] TRAF1 and TRAF2 are crucial regulators in the TNF signaling pathway, modulating cell survival and apoptosis through their interactions with other molecules. Additionally, the N-terminal fragment of c-FLIP_L, the caspase-8 processed p43-CFLAR, recruits TRAF2 and RIP1 more effectively than the full-length c-FLIP_L, leading to stronger NF-κB activation.[12,62–64]

In viable cells, CFLAR forms a heterodimer with procaspase-8, resulting in the generation of a novel N-terminal fragment of CFLAR (p22-FLIP), which plays a pivotal role in regulating survival signaling by modulating key downstream effectors. The p22-FLIP fragment interacts with the IKK complex, facilitating its activation and thereby playing a critical role in the subsequent NF-κB activation.[65] In the c-Jun N-terminal kinase (JNK) signaling pathway, TNF-α-mediated JNK activation promotes CFLAR transcription by inducing NF-κB, which acts as a key mediator in this process.[66] This process is not directly driven by CFLAR phosphorylation but relies on JNK-mediated phosphorylation, which in turn activates the E3 ubiquitin ligase Itch, leading to the ubiquitination and degradation of CFLAR. E3 ubiquitin ligase Itch specifically ubiquitinates CFLAR, targeting it for proteasomal degradation, thus reducing its cellular levels. Therefore, JNK contributes to TNF-α signaling by promoting the proteasomal degradation of c-FLIP_L, thereby reducing its ability to inhibit caspase activation, which in turn enhances NF-κB signaling and promotes apoptosis. These findings underscore the intricate and context-dependent interplay between the JNK and NF-κB pathways, which together regulate cell survival and apoptosis in response to various stress signals.[66]

In the calcium/calmodulin-dependent signaling pathway, calcium/calmodulin-dependent protein kinase II (CaMKII) upregulates CFLAR expression by phosphorylating key transcription factors, thereby protecting cancer cells from TRAIL-induced apoptosis. Treatment with the CaMKII inhibitor KN-93 in resistant cells effectively suppresses CaMKII activity, leading to reduced CFLAR expression and phosphorylation, which in turn enhances the cells' sensitivity to Fas agonist antibody (CH-11) and promotes apoptosis.[67,68]

Inflammasomes

Inflammasomes are multiprotein complexes that primarily mediate the activation of caspases, particularly caspase-1, and are critical for recognizing damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns

(PAMPs), thereby initiating inflammatory immune responses, such as the production of pro-inflammatory cytokines. In inflammasomes, procaspase-1 assembles into a multi-protein complex, where it undergoes autocleavage at specific sites to activate caspase-1, which is a critical step in the inflammasome-mediated inflammatory response. The activated caspase-1 processes the inactive precursors of pro-inflammatory cytokines, such as pro-IL-1β and pro-IL-18, by cleaving them at specific sites, thereby generating their biologically active forms, IL-1β and IL-18.[69–71] Studies have shown that procaspase-8 plays a role in regulating inflammasome activity, but this regulation is primarily mediated through a specific splice variant of c-FLIP_L, rather than via the activation of caspase-8 itself. This underscores the non-canonical role of c-FLIP_L in modulating inflammasome function.[72] This finding underscores the significant role of c-FLIP_L as a "pseudocaspase" in mediating non-canonical functions, particularly its involvement in inflammasome regulation independent of caspase activation.

Interaction with the NLRP3 Inflammasome

c-FLIP_L directly interacts with NLR family pyrin domain-containing 3 (NLRP3) and procaspase-1, modulating the assembly and activation of the NLRP3 inflammasome. This interaction is essential for the efficient activation of caspase-1 and subsequent cytokine processing. This interaction promotes the processing and secretion of IL-1β, a critical mediator of inflammation, while the absence of c-FLIP_L impairs pyroptotic cell death, suggesting its essential role in regulating both inflammasome activation and cell death pathways. Upon the recognition of DAMPs and PAMPs, the NLRP3 inflammasome recruits procaspase-1 through the adapter protein ASC (apoptosis-associated speck-like protein containing a CARD), which facilitates the formation of the inflammasome complex and the activation of caspase-1. By interacting with the NLRP3 inflammasome, c-FLIP_L modulates the inflammatory response to infections and tissue damage, thus playing a crucial role in maintaining immune system homeostasis during acute and chronic inflammatory conditions, such as sepsis or autoimmune diseases.[72]

Interaction with the AIM2 Inflammasome

c-FLIP_L is known to influence the activity of the absent in melanoma 2 (AIM2) inflammasome. AIM2 inflammasome activation is triggered by the recognition of cytosolic DNA, either from host cells or pathogens, which serves as DAMPs or PAMPs. AIM2 contains an N-terminal pyrin domain (PYD), which mediates protein-protein interactions, and a C-terminal HIN domain, which is responsible for recognizing and binding to cytosolic DNA. Similar to NLRP3, AIM2 activation leads to the recruitment and activation of procaspase-1 via ASC, resulting in the secretion of IL-1β and IL-18, as well as the cleavage of gasdermin D, which is pivotal for initiating pyroptotic cell death. Studies have demonstrated that the caspase-like domain of c-FLIP_L directly interacts with the C-terminal HIN domain of AIM2, facilitating inflammasome assembly and ensuring the full activation of AIM2, which is crucial for effective immune responses.[73]

Wnt Signaling Pathway

c-FLIP_L is a key regulator of the Wnt signaling pathway, primarily by modulating the stability and transcriptional activity of β-catenin. Interestingly, this regulation occurs independently of its well-established role in procaspase-8 activation. Upon binding to the extracellular domains of Frizzled receptors, Wnt proteins initiate a cascade of signaling events involving the activation of dishevelled proteins and the stabilization of β-catenin, which subsequently translocates to the nucleus to regulate gene expression.[74,75]

Inhibition of β-Catenin Ubiquitination

c-FLIP_L inhibits the ubiquitination of β-catenin by interfering with the activity of E3 ubiquitin ligases, leading to a significant accumulation of β-catenin in the cytoplasm. This process prevents β-catenin degradation, allowing its translocation to the nucleus, where it interacts with TCF/LEF transcription factors to activate target genes associated with Wnt signaling. This c-FLIP_L-mediated regulation is closely linked to the development of various cancers, particularly colorectal cancer, by promoting the activation of Wnt/β-catenin signaling, which drives tumorigenesis and cancer cell proliferation.

Interaction with TIP49

Studies have shown that c-FLIP_L interacts with the nuclear protein TIP49 via its DED, modulating Wnt signaling by regulating the activity of the β-catenin-TCF complex. TIP49 is a regulatory factor that modulates the stability and transcriptional activity of the β-catenin-TCF complex in the Wnt pathway. cc-FLIP_L enhances the transcriptional activity of β-catenin by facilitating the accumulation of TIP49 at the promoter of immunoglobulin transcription factor 2 (ITF-2), thereby promoting the activation of downstream Wnt target genes.[76] This process is crucial for activating downstream Wnt target genes, which are involved in regulating cell proliferation, differentiation, and tumorigenesis.

Cellular Localization and Functional Regulation of c-FLIP_L

The C-terminal region of c-FLIP_L contains both a nuclear localization signal (NLS) and a nuclear export signal (NES), facilitating its shuttling between the cytoplasm and the nucleus in a highly regulated manner. The cellular localization of c-FLIP_L is critical for its regulatory role in the Wnt signaling pathway, as its subcellular distribution influences its interactions with key signaling components and transcription factors. If the NLS of c-FLIP_L is mutated, preventing its entry into the nucleus, its ability to modulate Wnt signaling is significantly impaired, including its influence on β-catenin stabilization and transcriptional regulation. This indicates that c-FLIP_L's role in Wnt signaling depends not only on its interactions with other proteins but also on its nuclear localization, where it enhances the transcriptional activity of key transcription factors involved in Wnt signaling.

Through its inhibition of β-catenin degradation, interaction with TIP49, and nuclear localization, c-FLIP_L exerts a multifaceted regulatory function in the Wnt signaling pathway, influencing both cytoplasmic and nuclear processes. It not only stabilizes β-catenin, promoting its translocation to the nucleus and activation of target genes, but also directly interacts with transcription factor complexes, such as TCF/LEF, to further amplify Wnt signaling at the transcriptional level. These mechanisms demonstrate that c-FLIP_L is not merely a passive participant in the Wnt pathway but actively regulates multiple levels of signaling, influencing processes such as cell proliferation, differentiation, and tumorigenesis.

Role of CFLAR in Immune Cells

CFLAR plays a complex and critical role in immune cells, including T and B cells, by regulating cellular processes such as apoptosis and immune responses through modulation of multiple signaling pathways.

Role of CFLAR in T Cells

CFLAR, particularly its long form c-FLIP_L, plays a crucial role in regulating T cell survival, proliferation, and activation, primarily by modulating key signaling pathways involved in these processes.[64,77] During T cell activation, c-FLIP_L not only acts as an initiator of caspase-8 activation but also serves as a critical regulator of downstream signaling events essential for T cell activation. The p43FLIP complex, formed by c-FLIP_L and caspase-8, stabilizes caspase-8 activity and facilitates the activation of signaling pathways that promote T cell proliferation.[77] Studies have shown that T cells lacking CFLAR exhibit significantly reduced proliferation and activation in response to T cell receptor (TCR) stimulation, accompanied by impaired activation of the ERK signaling pathway and other key downstream effectors.[78,79] CFLAR protects mature T cells from exogenous apoptotic signals by inhibiting TCR-mediated apoptosis, thereby promoting T cell survival under stress conditions.[80] In resting T cells, CFLAR effectively inhibits endogenous apoptosis by preventing the release of cytochrome c from the mitochondria, thus blocking the initiation of the intrinsic apoptotic pathway.[81] In the context of T cell apoptosis, CFLAR plays a key negative regulatory role during T cell maturation, primarily by suppressing intrinsic apoptotic pathways through inhibition of pro-apoptotic molecules such as Bim.[82] Resting T cells deficient in CFLAR are more susceptible to apoptosis mediated by Bcl-2 interacting mediator (Bim), accompanied by a decrease in reactive oxygen species (ROS) levels and a weakened inflammatory response, which further supports CFLAR's role as a negative regulator of endogenous apoptosis during immune responses.[82] Additionally, studies have shown that in the context of bacterial infections, c-FLIP_L protects T cells from apoptosis induced by inflammatory mediators, whereas c-FLIP_S promotes T cell death and inhibits their proliferation, potentially through modulation of caspase activation and inflammatory cytokine signaling.[83,84]

CFLAR plays a central role in regulatory T cells, regulating their survival, proliferation, and immunosuppressive function through modulation of key signaling pathways. Research has found that Tregs with low CFLAR expression exhibit a heightened inflammatory state. Mice deficient in CFLAR develop fatal autoimmune responses, marked by a dramatic reduction in peripheral Treg cell numbers, hyperactivation of effector T cells, and extensive multi-organ infiltration of immune cells. This phenotype is associated with impaired Treg-mediated suppression of effector T cell responses.[85] These findings underscore the crucial role of CFLAR in preserving Treg function and preventing autoimmune pathology, likely through its regulation of Treg survival, differentiation, and suppression of effector T cell activation.

Role of CFLAR in B Cells

In CD40L-activated naïve B cells, CFLAR is recruited to the signaling complex, significantly delaying the onset of apoptosis by inhibiting caspase activation, thus preventing premature cell death.[86] In contrast, CFLAR-deficient B cells exhibit increased susceptibility to Fas-induced apoptosis and altered responses to proliferation signals from TLRs and B cell receptors (BCRs), suggesting a disruption in key signaling pathways. These studies underscore the critical regulatory role of CFLAR in B cell immune function, including its involvement in immune tolerance and antibody production.[87] Abnormal activation of the p38 MAPK and JNK pathways in CFLAR-deficient B cells further suggests that CFLAR plays a pivotal role in regulating these critical signaling networks. The absence of CFLAR leads to a reduction in peripheral B cell numbers, impaired proliferative responses, and reduced recruitment to sites of immune activation and inflammation.[88] However, CFLAR deletion does not impact the key developmental processes of B cells, such as differentiation and class switching, but significantly impairs their function in activated immune responses, including antigen presentation and antibody production. These findings emphasize the importance of CFLAR in regulating B cell function.

CFLAR in Other Immune Cells

In myeloid cells, Gordy et al[89] generated a myeloid-specific CFLAR knockout mouse model by expressing Cre recombinase under the control of the lysozyme 2 (Lyz2) promoter in a c-FLIP F/F background, aiming to investigate the specific role of CFLAR in myeloid cell differentiation and immune responses. These mice exhibited increased circulating neutrophils and spleen enlargement, which were directly associated with macrophage differentiation defects and impaired neutrophil clearance, indicating the key regulatory role of CFLAR in immune cell development. In addition, Huang et al independently developed CFLAR-floxed mice and generated myeloid-specific CFLAR knockout mice using the same strategy, further confirming the critical role of CFLAR in myeloid cells.[90]

In dendritic cells (DCs), Huang et al specifically deleted CFLAR by expressing Cre recombinase under the control of the integrin alpha X (Itgax) promoter.[91] These mice exhibited spontaneous inflammatory arthritis, accompanied by an increase in autoreactive CD4+ T cells and a decrease in Tregs, further confirming CFLAR's pivotal role in immune regulation and inflammatory responses. The roles of CFLAR in immune cells are summarized in Table 2.

CFLAR and Cancer

CFLAR plays a critical role in cancer biology, particularly in regulating tumor cell survival and anti-apoptotic mechanisms, which are essential for cancer cell immune evasion and resistance to therapy.[92–94] CFLAR inhibits apoptosis by binding to the DISC and suppressing the activation of caspase-8, thus blocking downstream signaling pathways mediated by death receptors (eg, DR5). This mechanism is crucial for cancer cells to evade immune surveillance and contributes to the resistance of cancer cells to apoptotic signals, highlighting CFLAR as a potential target for developing caspase-8-based targeted therapies. Elevated CFLAR expression is often observed in various cancers, including breast, colorectal, and lung cancer, and is associated with tumor initiation, metastasis, and poor prognosis by enabling cancer cells to resist apoptotic signals and chemotherapy-induced cell death. CFLAR blocks the apoptosis process by binding to caspase-8, preventing its activation and subsequent execution of apoptotic signaling, thus enhancing tumor cell survival. Due to its structural similarity to caspase-8, selective inhibitors targeting CFLAR must be carefully designed to avoid off-target effects that could inadvertently inhibit caspase-8, potentially leading to further suppression of apoptosis and resistance to

Table 2 Overview of CFLAR Functions in Immune Cells

Cell Type	CFLAR Function	Mechanisms and Effects	Clinical Significance
T cells	Regulates survival, proliferation, and activation	c-FLIP L acts as an initiator for caspase-8 activation, promoting T cell activation	Maintains T cell function, prevents autoimmunity
	Protects mature T cells from apoptosis	Inhibits endogenous apoptosis by blocking cytochrome c release	CFLAR deficiency increases T cell sensitivity to Bim-mediated apoptosis
	Protects T cells during bacterial infections	c-FLIP R prevents apoptosis induced by inflammatory mediators, while c-FLIP S accelerates T cell death	Essential for immune response to infections
B cells	Delays rapid apoptosis of CD40L-activated B cells	CFLAR regulates p38 and JNK signaling pathways to prevent premature B cell death	CFLAR deficiency impairs B cell function and affects immune activation
	Increases sensitivity of B cells to Fas-induced apoptosis	Reduced peripheral B cell numbers and decreased proliferation capacity	Does not affect B cell development but significantly weakens activation-dependent immune responses
Myeloid Cells	Regulates development and differentiation of myeloid cells	CFLAR-deficient mice exhibit neutrophilia and impaired macrophage differentiation	Key regulatory factor with significant impact on immune cell development
DCs	Plays a critical role in immune modulation and inflammatory response	CFLAR deficiency leads to spontaneous inflammatory arthritis and reduced Treg cell populations	Influences the onset and progression of autoimmune diseases

Abbreviation: DCs, dendritic cells.

treatment. RRecent studies have identified small molecules and RNA-based therapeutics that downregulate CFLAR expression, thereby enhancing tumor cell sensitivity to targeted therapies and improving the efficacy of chemotherapeutic agents.[95,96] For instance, agonist antibodies targeting DR5 promote tumor cell apoptosis and simultaneously downregulate CFLAR expression through caspase-dependent signaling, thus increasing the sensitivity of tumor cells to chemotherapy-induced apoptosis.[97] Furthermore, specific CFLAR variants hinder the activation of JNK and p38 MAPK by blocking late-phase death signals, which underscores its critical inhibitory role in regulating cell death and inflammation pathways.[98] Recent studies have further revealed that CFLAR regulates the expression of forkhead box M1 (FoxM1), a key transcription factor that plays a pivotal role in enhancing the resistance of non-small cell lung cancer (NSCLC) to chemotherapy and promoting tumor progression.[99] This finding suggests that CFLAR not only regulates apoptosis but also modulates additional survival pathways, including cell cycle progression and DNA repair mechanisms, which contribute to tumor cell resistance to therapy. Consequently, CFLAR's multifaceted roles—such as inhibiting apoptosis, enhancing resistance to cancer therapies, and promoting tumor cell survival—make it a complex and vital therapeutic target in cancer treatment.

Potential Implications of CFLAR in Sepsis Immune Regulation and Treatment

Following our discussion of the role of CFLAR in cancer and its involvement in regulating cell death pathways, it is equally important to explore CFLAR's functions in other pathological contexts, such as sepsis. Sepsis represents a complex inflammatory condition where immune dysregulation plays a crucial role. Future research should aim to elucidate the specific role of CFLAR in immune regulation within the context of sepsis, particularly its contribution to immune dysregulation. A deeper understanding of CFLAR's regulatory effects across different immune cell subsets (eg, T cells, B cells, and myeloid cells) and its involvement in sepsis pathophysiology will be pivotal in identifying targeted therapeutic strategies for the personalized treatment of sepsis patients.[100–102] Moreover, further investigation is needed to explore the dynamic expression patterns of CFLAR and its regulatory mechanisms under various pathological conditions,

particularly in the context of sepsis-induced inflammation and immune dysregulation. This research could provide the necessary foundation for developing precise intervention strategies targeting CFLAR, offering the potential for more effective treatments for sepsis patients.[103]

In drug development, targeting CFLAR with specific inhibitors, such as small molecules, gene-editing technologies like Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 (CRISPR-Cas9), and RNA interference approaches, should be explored. These strategies must be designed to selectively modulate CFLAR without inducing off-target effects on caspase-8 activity, which could lead to unwanted apoptosis inhibition.[104,105] Targeting CFLAR in sepsis may help restore immune homeostasis by modulating immune cell activation and tolerance, thereby reducing excessive inflammatory responses and pathological inflammation. Combining CFLAR-targeted therapies with immune checkpoint inhibitors, TRAIL agonists, or ER stress inducers should also be investigated. Such combination treatments may synergistically enhance immune response and apoptotic sensitivity in sepsis, improving therapeutic outcomes. These approaches could enhance the apoptotic sensitivity of immune cells by restoring CFLAR-mediated regulation, as well as modulating cytokine release to mitigate the hyperinflammatory response that characterizes sepsis.[106,107] CFLAR not only serves as a promising target for anti-cancer drug development but also holds significant therapeutic potential in treating acute inflammatory diseases such as sepsis, particularly in cases unresponsive to conventional therapies. Continued research into the basic biology of CFLAR and its clinical translational potential in the context of sepsis may open new therapeutic avenues, ultimately improving patient outcomes in this challenging disease. Figure 3 provides a brief overview of the potential applications of CFLAR in immunotherapy for sepsis.

CFLAR in Autoimmune Diseases and Chronic Inflammatory Conditions

CFLAR may also play a crucial regulatory role in the onset and progression of autoimmune and chronic inflammatory diseases. In chronic inflammation, the expression of Fas, FasL, and FLIP is upregulated, and these molecules are crucial in inflammatory cells. Studies indicate that apoptotic inflammatory cells accumulate in inflamed tissues, and the upregulation of FLIP in these cells may inhibit Fas-mediated apoptosis, contributing to chronic inflammation persistence. During the resolution of granulomatous experimental autoimmune thyroiditis (G-EAT), the increased expression of FLIP in thyroid cells may suppress their apoptosis, promoting cell survival. Conversely, the expression of FasL in thyroid cells may induce apoptosis in inflammatory cells, aiding in the resolution of inflammation.[108]

Maria Feoktistova et al demonstrated that c-FLIP negatively regulates TNF-dependent apoptosis and localized epidermal inflammation. In c-FLIP-deficient mice, the epidermis exhibits excessive proliferation, impaired differentiation in epidermal cells, and increased apoptosis. Interestingly, the absence of TNF delays the progression of chronic

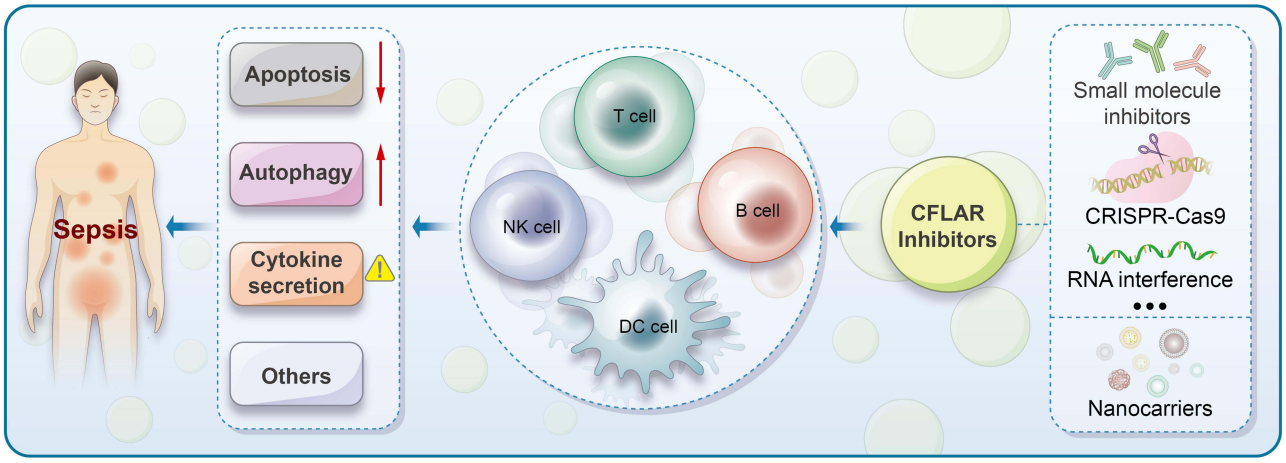

Figure 3 Potential Applications of CFLAR in Sepsis Immunotherapy. Sepsis is characterized by dysregulated inflammation, involving complex immune responses that include alterations in apoptosis, autophagy, and cytokine secretion. Immune cells, including T cells, B cells, NK cells, and DCs, play critical roles in the progression of sepsis. CFLAR inhibitors represent a promising therapeutic strategy, and through the application of CRISPR-Cas9, RNA interference, and nanoparticle-based delivery systems, these inhibitors can be engineered to restore immune homeostasis by targeting key processes such as apoptosis, autophagy, and cytokine secretion.

inflammatory skin diseases.[109] Death receptor-mediated apoptosis plays a critical role in controlling immune responses, and dysregulation of this pathway may lead to the onset of autoimmune diseases. F. Ewald et al further revealed that transgenic mice with forced expression of c-FLIP R (a variant of c-FLIP) develop a systemic lupus erythematosus (SLE)-like phenotype with aging, indicating that c-FLIP R is a significant regulator of apoptosis.[110]

In active ulcerative colitis (UC), signaling through the Fas receptor mediating apoptosis in intestinal epithelial cells (IECs) is impaired. Moreover, the expression of c-FLIP S is elevated in IECs of patients with active UC. The MEK-ERK pathway is recognized as a key signaling pathway that regulates IEC apoptosis during UC flare-ups and plays a critical role in the induction of c-FLIP S.[111] In c-FLIP L transgenic mice, activated $CD4^+$ T cells exhibit enhanced secretion of Th2 cytokines, and the production of Th1 cytokines is reduced. Furthermore, the Th2 bias in these c-FLIP L transgenic $CD4^+$ T cells is associated with impaired NF-κB activity and upregulation of GATA-3, leading to a decrease in IFN-γ levels and an increase in Th2 cytokines. This Th2 skewing significantly enhances the susceptibility of these mice to the OVA-induced asthma model.[112] Overall, c-FLIP plays a crucial role in regulating apoptosis and immune regulation in chronic inflammation and autoimmune diseases. Its involvement in disease progression and immune homeostasis highlights its potential as a therapeutic target in these conditions.

Challenges and Limitations of CFLAR-Targeted Therapies

CFLAR-targeted therapies offer significant potential for modulating cell death and immune responses in cancer and inflammatory diseases, but several challenges must be addressed before their clinical application. One major concern is the risk of off-target effects due to CFLAR's involvement in multiple signaling pathways, including apoptosis, necroptosis, and NF-κB activation. Non-specific targeting of CFLAR could lead to unintended immune dysregulation, excessive activation, or suppression, which may ultimately jeopardize therapeutic safety.

The context-dependent duality of CFLAR further complicates its therapeutic targeting. CFLAR can exert both protective and pro-inflammatory effects, depending on disease type, cellular context, and microenvironment. For example, inhibiting CFLAR may sensitize certain cancers to apoptosis while exacerbating inflammatory responses in conditions like sepsis or autoimmune diseases[113,114.] This duality underscores the need for targeted strategies that consider disease-specific mechanisms.

Resistance mechanisms significantly challenge the efficacy of CFLAR-targeted therapies in cancer treatment. Cancer cells frequently exhibit phenotypic plasticity through the activation of alternative survival pathways that enable them to evade CFLAR inhibition. A study by Yongping Shao et al demonstrated that c-FLIP is crucial for TNFα-induced protection against PLX4720, a selective inhibitor which is used to study vemurafenib. Overexpression of c-FLIP confers protection to melanoma cells against PLX4720-induced apoptosis. Thus, c-FLIP may play a role in RAF inhibitor resistance by preventing the activation of caspase-8, a key mediator of apoptosis, in response to RAF inhibitors.[115] This suggests that monotherapies targeting CFLAR are unlikely to achieve long-term efficacy due to the development of resistance, which highlights the need for combination strategies with other apoptotic regulators or immune modulators.

Moreover, the development of selective CFLAR modulators with optimal pharmacokinetics and bioavailability remains challenging. Only a few small-molecule inhibitors or peptide-based agents can specifically target CFLAR activity without affecting other components of cell death pathways. Ongoing research is crucial to improve drug design (eg, isoform-specific modulators) and enhance the specificity and efficacy of CFLAR-based therapies.

Given these complexities, future studies should focus on elucidating the molecular mechanisms that regulate CFLAR in diverse disease contexts and establishing patient-specific biomarkers to predict therapeutic responses. Such efforts will be vital for developing safer, more effective treatment strategies.

Conclusion

CFLAR, a critical anti-apoptotic regulator, plays a pivotal role in immune cell survival, immune regulation, and tumorigenesis by modulating apoptosis pathways and influencing cellular responses to stress. Future research should focus on developing targeted inhibitors of CFLAR, utilizing advanced molecular techniques such as CRISPR-Cas9, RNA interference, and small molecule screening. These approaches could unlock CFLAR's therapeutic potential in clinical immunotherapy, providing novel strategies for the treatment of sepsis, autoimmune diseases, and cancer. As our

understanding of the underlying biological processes deepens, several promising research directions are emerging, including the precision targeting of apoptosis pathways, personalized medicine strategies, the exploration of combination therapies, the identification of new biomarkers, the investigation of immune evasion mechanisms, and the integration of systems biology approaches. Ultimately, these advancements could facilitate the effective translation of research findings into clinical applications, thereby enhancing the therapeutic landscape.

Abbreviations

CFLAR, CASP-8 and FADD-like apoptosis regulator; FADD, Fas-associated death domain; c-FLIP, cellular FLICE-inhibitory protein; DISC, death-inducing signaling complex; vFLIP, viral FLICE-inhibitory protein; DED, death effector domain; NF-κB, nuclear factor-kappa B; DR5, death receptor 5; ER, endoplasmic reticulum; AIC, apoptosis inhibitory complex; PRR, pattern recognition receptor; Atg, autophagy-related gene; LC3, light chain 3; RIPK1, receptor-interacting protein kinase 1; MLKL, mixed lineage kinase domain-like; TNF-R1, tumor necrosis factor receptor 1; cIAPs, cellular inhibitor of apoptosis proteins; TLR3, Toll-like receptor 3; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand receptors; TNFRSF, TNF receptor superfamily; TRADD, TNFR1-associated death domain; TRAF2, TNF receptor-associated factor 2; MAPK, mitogen-activated protein kinase; TRIF, TIR domain-containing adaptor protein; EGF, epidermal growth factor; EGR1, early growth response 1; SP1, specificity protein 1; NFATc2, nuclear factor of activated T-cells cytoplasmic 2; FOXO3a, forkhead box O3a; IRF5, interferon regulatory factor 5; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; CaMKII, calcium/calmodulin-dependent protein kinase II; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; NLRP3, NLR family pyrin domain-containing 3; AIM2, absent in melanoma 2; PYD, pyrin domain; ITF-2, immunoglobulin transcription factor 2; NLS, nuclear localization signal; NES, nuclear export signal; TCR, T cell receptor; ROS, reactive oxygen species; Lyz2, lysozyme 2; DCs, dendritic cells; Itgax, integrin alpha X; FoxM1, forkhead box M1; NSCLC, non-small cell lung cancer; CRISPR-Cas9, CRISPR-associated protein 9; G-EAT, granulomatous experimental autoimmune thyroiditis; SLE, systemic lupus erythematosus; UC, ulcerative colitis; IECs, intestinal epithelial cells.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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