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REVIEW

Apoptosis signaling pathways and lymphocyte homeostasis

Guangwu Xu[1], Yufang Shi[1]

[1]*Department of Molecular Genetics, Microbiology and Immunology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 661 Hoes Lane, Piscataway, NJ 08854, USA*

It has been almost three decades since the term "apoptosis" was first coined to describe a unique form of cell death that involves orderly, gene-dependent cell disintegration. It is now well accepted that apoptosis is an essential life process for metazoan animals and is critical for the formation and function of tissues and organs. In the adult mammalian body, apoptosis is especially important for proper functioning of the immune system. In recent years, along with the rapid advancement of molecular and cellular biology, great progress has been made in understanding the mechanisms leading to apoptosis. It is generally accepted that there are two major pathways of apoptotic cell death induction: extrinsic signaling through death receptors that leads to the formation of the death-inducing signaling complex (DISC), and intrinsic signaling mainly through mitochondria which leads to the formation of the apoptosome. Formation of the DISC or apoptosome, respectively, activates initiator and common effector caspases that execute the apoptosis process. In the immune system, both pathways operate; however, it is not known whether they are sufficient to maintain lymphocyte homeostasis. Recently, new apoptotic mechanisms including caspase-independent pathways and granzyme-initiated pathways have been shown to exist in lymphocytes. This review will summarize our understanding of the mechanisms that control the homeostasis of various lymphocyte populations.

***Keywords*:** apoptosis, lymphocyte homeostasis, death-inducing signaling complex, apoptosome, signaling

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Apoptotic cell death

Apoptosis is a distinctive form of cell death [1] exhibiting specific morphological and biochemical characteristics, including cell membrane blebbing, chromatin condensation, genomic DNA fragmentation, and exposure of specific phagocytosis signaling molecules on the cell surface [2]. Cells undergoing apoptosis differ from those dying through necrosis. Necrotic cells are usually recognized by the immune system as a danger signal and, thus, resulting in inflammation; in contrast, apoptotic death is quiet and orderly. It is believed that most cell death in metazoan organisms occurs through apoptosis; apoptosis is critical in the formation of organs, limbs and other body structures, and in maintaining the function of most of the systems in an adult body. Therefore, dysregulation of the apoptotic signaling processes often leads to serious consequences, such as neurodegenerative diseases [3], cancer [4], or autoimmunity [5].

Our initial understanding of the molecular mechanisms that control apoptosis came from early studies using the nematode *Caenorhabditis elegans* [6-10]; these mechanisms were then extended to mammalian systems. A multitude of proteins and enzymes have now been identified that are involved in the initiation, amplification, or suppression of apoptosis. In most cells, apoptosis triggers usually lead to the activation of caspases, which ultimately mediate the autodestruction of the cell. Caspases normally exist as inactive precursors, called procaspases, which are cleaved to give rise to the active form. The preferred cleavage site for the known caspases is at the C-terminal side of a four

Correspondence: Yufang Shi
Tel: +1-732-235-4501; Fax: +1-732-325-4505
E-mail: shiyu@umdnj.edu

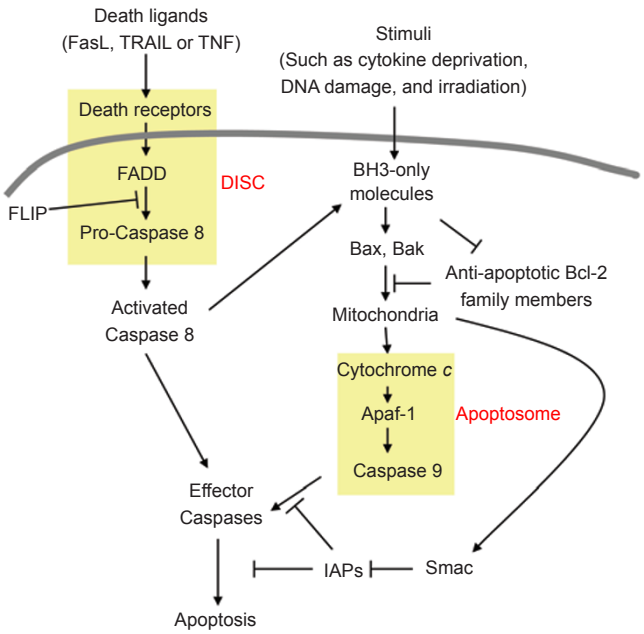

Figure 1 Schematic diagram of pathways to apoptosis. The left side shows the extrinsic apoptotic signaling pathway, and the right side shows the intrinsic apoptotic signaling pathway. These pathways converge at the activation of caspases. DISC and apoptosome are highlighted in yellow.

amino-acid motif, X-X-X-Asp (where X can be any amino acid). Activated caspases in turn cleave various intracellular and cytoplasmic membrane substrates [11], leading to cellular disintegration.

There are two major pathways leading to apoptosis in the mammalian system: an extrinsic pathway initiated by death receptors and an intrinsic pathway that occurs through the mitochondria (Figure 1). The extrinsic pathway depends on binding of appropriate exogenous mediators to death receptors at the cell surface. In contrast, the intrinsic pathway responds to signals from within the cell, such as damages caused by radiation and various chemotherapeutic agents, to induce apoptotic signaling via the release of mitochondrial factors.

Extrinsic apoptosis signaling pathways

The extrinsic pathway of apoptosis signaling is initiated when death receptors at the cell surface encounter specific cognate "death ligands," inducing a conformational change that is transmitted through the cell membrane. These receptors can activate caspases within seconds of ligand binding and lead to apoptotic cell death in a matter of hours. Three major specific cell death receptor/ligand pairs have been described, all members of the Tumor Necrosis Factor Receptor Superfamily (TNFRSF): (1) Fas and Fas ligand (FasL) (Fas is also called Apo-1, CD95 or TNFRSF6 [12]; FasL is also called CD178,CD95L or TNFSF6 [13]); (2) "death receptors" (DR4 and DR5) and TNF-related apoptosis inducing ligand (TRAIL, also called Apo2L or TNFSF10) [14, 15]; and (3) TNFα and the TNF receptor (TNF-R1). These death receptors, all members of the TNFRSF, are type I integral receptors with a conserved extracellular domain containing two to four cysteine-rich pseudo-repeats [16], a single transmembrane region, and a conserved intracellular "death domain" (DD) of about 80 amino acids that interacts with adaptor proteins [17].

Apoptosis-inducing receptors of the TNFR superfamily exist at the cell surface as pre-formed trimers. Ligation of the death receptors leads to apoptosis through a common transcription/translation-independent pathway: ligand binding induces the formation of the death-inducing signaling complex (DISC), which in turn cleaves and activates the initiator caspases (caspase 8 or 10). The initiator caspases in turn activate a second group of caspases, known as effector caspases, by proteolytic cleavage at specific sites. Upon activation, the effector caspases culminate the apoptotic process through the degradation of key intracellular substrates.

The Fas-FasL pathway

Among all the cell death receptors, Fas is the most extensively studied. The specific interaction of Fas with its membrane-bound cognate ligand, FasL, triggers the aggregation of Fas trimers. This enables the DD in the cytoplasmic tail of Fas to immediately recruit the DISC [18]. The DISC is composed of Fas, an accessory protein called Fas-associated death domain (FADD) [19] and pro-caspase 8. FADD is a universal adapter protein that mediates signaling by DD-containing members of the TNFRSF [20]. FADD harbors an N-terminal death effector domain (DED) along with a C-terminal DD. In response to receptor aggregation, FADD is recruited by Fas, an interaction that is coordinated through the highly conserved DD motif found in both proteins. The interaction of FADD and Fas through their C-terminal DDs unmasks the N-terminal DED of FADD, allowing it to recruit pro-caspase 8 to the Fas signaling complex [21, 22]. The presence of functional FADD protein is critical for Fas-mediated apoptosis as the expression of a dominant-negative form of FADD completely abrogates Fas-induced cell death [23]. Moreover, *in vivo* studies revealed a substantial attenuation of lymphocyte death in mice lacking FADD [24]. Formation of the DISC triggers the self-cleavage of pro-caspase 8 into active caspase 8. Activated caspase 8 (also called FADD-like IL-1 converting enzyme (FLICE)) then cleaves the

downstream pro-caspases 3, 6, and 7. Activated caspase 3 cleaves a variety of cellular substrates, including DNA repair enzymes, cellular and nuclear structural proteins, and endonuclease inhibitors. Moreover, caspase 3 has the ability to activate other caspases, such as caspases 6 and 7, normally found in their zymogenic forms, resulting in an amplification of cellular destruction that ensures full execution of Fas-induced apoptosis.

Molecules other than FADD and pro-caspase 8 may also be recruited in Fas-mediated apoptosis. Receptor-interacting protein (RIP) [25], RIP-associated ICH (ICE and ced-3 homolog)/CED-3-homologous protein with a DD (RAIDD) [26], and pro-caspase 2 [27] form another signaling cascade of the Fas-mediated death pathway. The RIP-RAIDD pathway might serve as a backup to the FADD-caspase 8 system. However, it does not normally represent a major Fas-mediated death pathway.

Every step in Fas-mediated apoptosis is regulated. The FasL gene is transcriptionally inactive in most cells. The regulation of FasL expression controls FasL/Fas-mediated biological effects, such as the activation-induced cell death (AICD) of CD4+ T cells. The induction of Fas expression requires only TCR-activated PKC, while FasL expression requires the activation of both PKC and NFAT, the latter by Ca^{2+} mobilization [28]. Regulation of Fas expression also controls Fas responses, for example, in p53-induced apoptosis. Stimulation of Fas by membrane-bound FasL can be antagonized by the soluble decoy receptor DcR3 in humans [29], by various Fas isoforms lacking the transmembrane and/or DDs, and by soluble inactive FasL. The caspase 8-activating capacity of the Fas-DISC complex is regulated mainly by an inhibitory protein called FLICE-like inhibitory protein (FLIP) [26, 30-33]. FLIP exists in several isoforms that are structurally similar to caspase 8, but lacking in enzymatic activity [30]. Incorporation of FLIP into the DISC of death receptors disables DISC-mediated processing, thus preventing the activation caspase 8 [30]. In addition, Fas-mediated apoptosis is controlled by a host of regulators of the mitochondrial cell death pathway (see below), for example, by some Bcl-2 family members or inhibitor of apoptosis proteins (IAPs).

Fas-mediated cell death in T cells occurs not only by apoptosis but also by necrosis. Necrosis mediated by Fas requires FADD and RIP, whereas caspase 8 seems to be dispensable [34]. However, the molecular mechanisms linking Fas, FADD, and RIP in processing necrosis are not yet clear. Although Fas is recognized predominantly as a death inducer, it also triggers proliferative signals in T cells [35].

The TRAIL-DR pathway

Like other TNF family ligands, TRAIL exists as a type II membrane-bound protein, or it can be cleaved from the membrane by cysteine proteases to generate a soluble form. Both human and mice have five receptors for TRAIL: the death-inducing receptors DR4 [36] and DR5 [9, 30, 37-39], and the decoy receptors DcR1 [30, 36, 39, 40], DcR2 [40-42] and OPG (osteoprotegerin) [43]. Among these receptors, only DR4 and DR5 have functional DDs that mediate apoptosis induction; the decoy receptors do not. DcR1 lacks both a DD and a transmembrane domain. It is anchored to the membrane via a glycophosphatidyl inositol tail. DcR2 has a truncated and nonfunctional DD. OPG is secreted as a dimeric soluble form, which also binds to RANKL. These decoy receptors bind to TRAIL, but do not transduce a signal, thus protecting cells from TRAIL-induced apoptosis by interrupting the formation of functional death receptor trimers. DISC formation and Bid cleavage downstream of DR4 and DR5 is similar to that seen in Fas-induced apoptosis. Briefly, TRAIL triggers clustering of DR4 or DR5, which recruits FADD and pro-caspase 8 to form the DISC. After the activation of caspase 8, effector caspases are subsequently activated.

Both cell-bound TRAIL and genetically engineered soluble forms rapidly induced apoptosis in a wide variety of transformed cell lines of diverse origins [14, 41]. Since normal cells express decoy receptors, while tumor cells do not, it is believed that TRAIL may specifically kill tumor cells, but this remains controversial. There were reports suggesting that TRAIL therapy might cause hepatotoxicity [44, 45], and recombinant TRAIL was found to induce apoptosis in hepatocytes [45]. But a later study found that hepatotoxicity of recombinant TRAIL is due to the exogenous sequence tags [46].

TRAIL expression was significantly increased in epithelium, airway smooth muscle, vascular smooth muscle, and throughout the interstitial tissue of asthmatic patients compared to non-asthmatic subjects. Increased expression of the TRAIL decoy receptor DcR2 and decreased expression of the TRAIL receptors DR4 and DR5 were also observed in asthma patients. It has been suggested that modulation of TRAIL and TRAIL receptor interactions after antigen challenge may be crucial in promoting eosinophil survival in asthma [47].

The TNFα-TNFR1 pathway

TNFa is a multifunctional proinflammatory cytokine. It exists as a homotrimer of three 157-amino-acid peptide chains. TNFα exerts its function via two receptors: TNFR1 (also known as p55), which contains a DD [48-50], and TNFR2 (also known as p75), which lacks a DD [51, 52]. The initial step in TNFR1 signaling involves the binding of the TNF trimer to the extracellular domain of TNF-R1 and the release of the inhibitory protein, silencer of death

domains, from TNF-R1's intracellular domain. The resulting aggregated TNF-R1 intracellular domain is recognized by the adaptor protein TNF receptor-associated death domain (TRADD), which recruits RIP, TNF-R-associated factor 2 (TRAF2), and FADD. These latter proteins recruit key enzymes to TNF-R1 that are responsible for initiating signaling events. Caspase 8 is recruited by FADD to the TNF-R1 complex, where it becomes activated by self-cleavage, and initiates a protease cascade leading to apoptosis. TRAF2 recruits cellular inhibitor of apoptosis protein-1 (cIAP-1) and cIAP-2. TRAF2 is also thought to activate MAPKKKs, such as extracellular signal-regulated kinase kinase kinase 1 (MEKK1) or ASK1, in a complex at or near the receptor, thereby activating a cascade of kinases resulting in the activation of JNK. The protein kinase RIP is critical to the functional activation of the transcription factor NF-κB.

TNFR1-induced apoptosis involves two sequential signaling complexes. First, the binding of TNF to TNFR1 recruits TRADD, RIP1, and TRAF2 to form a plasma membrane bound complex (complex I consisting of TNFR1, TRADD, RIP1 and TRAF2), and rapidly signals activation of NF-κB. After forming complex I, TRADD and RIP1 become modified and dissociate from TNFR1, then TRADD (and or RIP1) binds to FADD resulting in recruitment of caspase 8/10, forming a cytoplasmic complex (complex II consisting of TRADD, RIP1, FADD, and caspase 8/10) and leading to apoptosis. The FLIP levels determine if the latter complex forms. When NF-κB is successfully activated by complex I, cellular FLIP levels are sufficiently elevated to inhibit the formation of complex II and block apoptosis. If the initial signal by complex I fails to activate NF-κB, complex II signals for apoptosis [53].

The biological outcome of TNFα ligation is determined by the balance between NF-κB and JNK signaling: NF-κB promotes survival, whereas JNK enhances cell death. TNFα-mediated JNK activation accelerates turnover of the NF-κB-induced anti-apoptotic protein c-FLIP. JNK mediates phosphorylation and activation of the E3 ubiquitin ligase Itch, which specifically ubiquitinates c-FLIP and induces its proteasomal degradation. JNK1 or Itch deficiency or treatment with a JNK inhibitor makes mice resistant in three distinct models of TNFα-induced acute liver failure, and cells from these mice do not display inducible c-FLIP ubiquitination and degradation [54].

Induction of apoptosis may not be the major physiological role of TNFα. TNFα mediates the inflammatory response and regulates immune function through activation of transcription factors, NF-κB and c-Jun. Inappropriate production of TNFα or sustained activation of TNFR signaling has been implicated in the pathogenesis of a wide spectrum of human diseases, including autoimmune diseases such as multiple sclerosis [55, 56] and rheumatoid arthritis [57]. In fact, anti-TNFα therapy has become an effective treatment for these autoimmune disorders.

Intrinsic signaling pathways

The mitochondrial pathway of apoptosis in mammalian cells centers on a key event: mitochondrial outer membrane permeabilization (MOMP), considered the point-of-no-return in apoptosis induction. Release of certain proteins from the mitochondrial intermembrane space due to MOMP triggers a cascade of caspase activation that results in irreversible events culminating in apoptosis.

MOMP is normally prevented by anti-apoptotic members of the Bcl-2 family, which is composed of both anti-apoptotic and pro-apoptotic proteins. Bcl-2 was first described as a unique oncogene in B cell lymphomas that acted to inhibit cell death [58-60]. Since the identification of Bcl2, numerous family members have been found. These proteins share up to four Bcl-2 homology domains (BH1 to BH4). Bcl-2 family is generally divided into three subgroups based on their roles in apoptosis and the BH regions they shared: one anti-apoptotic group and two pro-apoptotic groups. The anti-apoptotic group contains Bcl-2, Bcl-x_L [61], Bcl-w [62], Bcl-B [63], A1 [64] and Mcl-1 [65], which share three or four BH regions. One group of the pro-apoptotic Bcl-2 family members, including Bax [66], Bak [67, 68], Bcl-x_s [61] Bok [69], and Bcl-G_L [70], have two or three BH domains. The other group contains the BH3-only proteins, including Bad [71], Bid [72], Bim [73], Bik [74], Noxa [75], Puma [76], Bcl-Gs [70], Blk [77], Bmf [78] and Hrk [79], which share only the BH3 domain.

Among the pro-apoptotic proteins, Bax and Bak appear to be requisite for MOMP and are likely to directly mediate MOMP by forming size-indeterminate openings in the outer mitochondrial membrane [80]. Cells deficient in both Bax and Bak are resistant to many apoptotic stimuli that induce cell death through mitochondrial disruption [81, 82]. Bax and Bak are present in most cells in inactive forms, and their activation is triggered directly or indirectly by other proteins, such as the BH3-only proteins. Once activated, Bax and Bak permeabilize the outer membrane of mitochondria, resulting in release of pro-apoptotic factors such as cytochrome *c*.

This activation of Bax and Bak is inhibited by the anti-apoptotic Bcl-2 family proteins which either inhibit the apoptosis-activating BH3-only proteins or sequester Bax and Bak to prevent their activation. This restraint of Bax, Bak by anti-apoptotic Bcl-2 proteins can be reversed by the BH3-only proteins, as well as by protein modifications (such as phosphorylation or deamidation) of the anti-apoptotic proteins.

BH3-only proteins serve as sensors for apoptotic stimuli [83]. Upon stimulation such as deprivation of cytokines, BH3-only proteins are activated. It is still a hotly debated issue whether BH3-only proteins activate Bax and Bak directly [84, 85] or whether they do so indirectly by their binding to anti-apoptotic Bcl-2 proteins that normally sequester Bax and Bak [86, 87].

Once MOMP occurs, proteins in the intermembrane space are released to the cytosol. One such protein, cytochrome *c* [88], binds to cytosolic, monomeric apoptotic protease activating factor-1 (APAF-1) at its WD40 domain. This interaction with cytochrome *c* induces a conformational change in APAF-1, promoting APAF-1 oligomerization to initiate apoptosome formation [26, 89, 90]. The apoptosome then binds to the proform of caspase 9, the initiator caspase of the intrinsic pathway, via the caspase recruitment domains in both APAF-1 and pro-caspase 9. The oligomerization of pro-caspase 9 on the apoptosome triggers auto-cleavage, possibly by simply bringing pro-caspase 9 molecules into proximity, giving rise to active caspase 9. Interestingly, recently it was found that acetylcholinesterase is important in apoptosis [91], as it plays a pivotal role in the formation of apoptosome [92, 93]. Active caspase 9 then cleaves the effector caspases, such as caspase 3 and caspase 7, resulting in their activation. Recently, it was found that intracellular nucleotides can bind to cytochrome *c* and prevent the formation of apoptosome. To undergo apoptosis, cells might need to break this nucleotide shield. It is not known how this is achieved [94]. However, it is also possible that cells might undergo apoptosis without breaking down the nucleotide shield. It was found that although APAF-1 is required for mitochondrial apoptosis [95], cytochrome *c* and the apoptosome are not always required for caspase 9 activation, since caspase 9 can also be activated in mice bearing the cytochrome *c* that is deficient in the apoptosis inducing function [96].

The process of apoptosis downstream of MOMP is regulated at the level of the apoptosome and at each of the subsequently activated caspases. Caspase activity can be modulated by caspase-binding proteins of the inhibitor of apoptosis proteins (IAPs) family [97, 98]. For example, human X-linked IAP (XIAP) directly inhibits at minimum, caspases 3 and 7 [99]. Overexpression of these pro-survival molecules, including XIAP [98] and surviving [100], is observed in many human tumors. Additionally, these caspase inhibitors can be antagonized by the pro-apoptotic protein, second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI (SMAC/DIABLO) [101, 102]. SMAC/DIABLO can displace XIAP from its interaction with activated caspase 3, allowing cell death to proceed. Thus, both the activation and function of caspases can be regulated through multiple binding proteins.

Another mithochondrial component with a regulatory role in apoptosis is the apoptosis inducing factor (AIF) [103]. AIF has homology to bacterial oxidoreductases and is normally localized in the mitochondria, but translocates to the cytoplasm during apoptosis. Apoptosis induced by AIF cannot be inhibited by the caspase inhibitors, and can result in nuclear condensation and fragmentation independent of other pro-apoptotic factors. Interestingly, the redox-active enzymatic region of AIF is anti-apoptotic, giving the molecule a dual role in cell survival and cell death. The detailed pathways of caspase-independent apoptosis are poorly understood.

The intrinsic and extrinsic pathways are used for the sake of description. Actually, there is cross-talk between these pathways. Depending on the cell types and stimuli and other environmental factors, different apoptotic pathways play different roles. For example, in cells where there is no sufficient amount of activated caspase 8 allowing direct cleavage and activation of effector caspases, cleavage of the BH3-only protein Bid by caspase 8 might become a major pathway (Figure 1). This cleavage of Bid produces the pro-apoptotic tBID fragment that induces cytochrome *c* release from mitochondria and caspase 9 activation [104, 105]. Caspase 9 can then cleave caspase 8, thus forming a positive feedback loop that amplifies the original signal from caspase 8.

Other apoptosis pathways

The granzymes form a family of serine proteases, different from the caspases, that also induce cell death. T cells and natural killer cells utilize a granule-exocytosis pathway to eliminate virus-infected cells. Cytotoxic granules deliver perforin and granzymes to the target cells through yet unidentified pathways. The apoptotic signaling processes triggered by each granzyme species are relatively distinct. Granzyme B triggers apoptosis via caspase-dependent and -independent mechanisms [106] that involve direct cleavage of downstream caspase substrates. Granzyme B has been shown to cleave caspase 3, caspase 8 [18] and other substrates, including Bid and ICAD (inhibitor of caspase-activated deoxyribonuclease), which results in activation of the CAD endonuclease. Granzyme A induces cell death via a caspase-independent pathway by inducing single-strand DNA nicks. It targets the SET complex (a 270 to 420-kDa endoplasmic reticulum-associated complex) resulting in the degradation of selected components, freeing the NM23-H1 DNase and resulting in single-stranded DNA nicks [107]. The granzymes were recently shown to induce apoptosis in the cells harboring them [108].

Another family of apoptosis-mediating cysteine proteases – the calpains (calcium-activated neutral proteases)

– are non-lysosomal intracellular cysteine proteases. The mammalian calpains include two ubiquitous proteins, CAPN1 and CAPN2, two stomach-specific proteins, and CAPN3, which is muscle-specific. Calpains also share some common substrates with caspases [109]. Furthermore, purified calpain enzymes cleaved Bax in a calcium-dependent manner [110]. It has been shown that neutrophils require calpains for BAX activation and apoptosis [111, 112]. Recently, it has been shown that there is cross-talk between the caspases and the calpains [113, 114]. Further elucidation of protease-induced cell death should uncover caspase-independent programs.

Apoptosis in lymphocytes

Apoptosis in T cells plays a key role in their development, differentiation, and homeostasis. Several mechanisms are involved in controlling apoptosis of different subpopulations of T cells; even the same subpopulation can use different mechanisms under different circumstances.

Intrathymic selection

It has long been shown that apoptosis plays a crucial role in intrathymic selection of T cells during their development. It is generally believed that immature T cells undergo apoptosis once they recognize self-antigens presented by self-MHC molecules, a process referred to as negative selection [115]. Although negative selection has been the focus of many prominent investigations, the molecular mechanisms controlling negative selection are still largely unknown.

The role of death receptors has been established in various cellular systems, but it remains controversial whether death receptors have a role in the negative selection of T cells in the thymus. TRAIL-/- mice showed increased numbers of immature thymocytes and decreased apoptosis of activated T cells induced by restimulation [116], while there was also evidence showing no defects in lymphoid or myeloid cell homeostasis or T cell function in TRAIL-/- mice [117, 118]. In addition, anti-CD3 stimulation induced cell death of thymocytes *in vitro* has been shown to be TRAIL-independent, since it could not be blocked by soluble DR5-Fc [119]. Although it has been shown that TNFR1 [120, 121], TNFR2 [122] or TNFR1 and TNFR2 double [123] deficient mice and Fas/FasL deficient mice [124-128] exhibited normal negative selection, there was also evidence supporting a role of TNF and Fas/FasL in negative selection in some models [129, 130]. For example, overexpression of TNF in mice resulted in a small thymus with decreased populations of double positive thymocytes [131]. Nevertheless, negative selection is intact in mice lacking FADD or caspase 8 [132, 133], major signaling molecules in death receptor signal transduction, which suggests that death receptors are not important in negative selection. However, further systemic investigations are still needed to clarify the role of death receptors in negative selection and the models should be standardized as the use of different negative selection models sometimes led to discrepant conclusions [123].

Activation-induced cell death

The homeostasis of peripheral T cells, especially after activation by specific antigens, is maintained by AICD [134-136]. AICD plays a key role in controlling the vigorousness and persistence of immune responses. AICD has been shown to be mediated mainly by the extrinsic Fas/FasL pathway [137-140], since interference with the Fas/FasL interaction by soluble Fas-Fc fusion protein or neutralizing anti-FasL inhibited AICD, and Fas and FasL are upregulated shortly after TCR ligation. Some T cells do undergo apoptosis even with the blockade of Fas/FasL signaling, however, suggesting that Fas-independent pathways are also involved in the AICD of T cells [138]. AICD in T cells from AIDS patients was dependent on TRAIL, as it was blocked not by antibodies to Fas or FasL [141] but by the neutralizing antibody to TRAIL [142]. However, there were also conflicting data showing that there was upregulation of TRAIL receptors in peripheral T cells upon activation, but these cells remained resistant to TRAIL [119]. TNF has also been suggested by some studies to play a role in AICD [126, 143, 144]. It was found that TCR stimulation can induce the expression of TNF [145] and antibody to TNF can partially inhibit antigen induced cell death of T cells *in vivo* [126]. In addition, microinjection of antibody to TNF inhibited AICD induced by anti-CD3 in CD8 cells [143]. However, it has also been reported that TNFR:Fc fusion protein did not inhibit AICD of effector T cells [146].

The AICD we mentioned above was studied mainly in either bulk lymphocytes (unpurified lymphocytes, as opposed to purified subgroups of lymphocytes) or pure subgroup of lymphocytes from pathogen or antigen primed animals. It is conceivable that AICD induced in different types of lymphocytes and under different situations are mediated through different pathways. In the last two decades, there have been a huge amount of studies on AICD of lymphocytes. There are some discrepancies about the pathways involved in AICD of lymphocytes, which are largely due to the different models of AICD used. For example, the antigen or antibody, the duration and the doses of the stimulation and restimulation differed from experiments to experiments. The precise mechanisms involved in AICD of lymphocytes need further investigation. In the future, some standardized models might be used to dissect the apoptotic pathways involved in AICD of lymphocytes

under normal or disease situations. In the following paragraphs, we will discuss AICD in some differentiated effector T-cell subgroups. These cells were differentiated under relatively standardized specific cytokine milieu. Pathways employed in AICD of these effector cells are sometimes different from those seen in AICD of bulk lymphocytes. Also, AICD in distinct effector cells are executed through different mechanisms (Table 1).

CD4+ T cells differentiate into either type I (Th1) and type II (Th2) helper T cells, depending on the cytokine environment during activation [147, 148]. Th1 cells evolved to clear intracellular pathogens and are defined on the basis of their production of interferon-γ (IFN-γ). On the other hand, Th2 cells are critical for the control of certain parasitic infections and are defined by their production of cytokines IL-4, IL-5, and IL-13. While it is not clear whether the Fas/FasL interaction is involved in negative selection, it is quite certain that Fas/FasL contributes to the polarization of Th1 [149, 150]. Th1 cells are susceptible to AICD. They undergo rapid apoptosis upon the antigen stimulation. AICD in Th1 cells is mainly mediated by Fas/FasL. Th1 cells generated from *gld* or *lpr* mice did not show rapid AICD compared to the Th1 cells from normal mice. Soluble Fas could block AICD in Th1 cells. Th2 cells also undergo AICD when stimulated with antigen. However, they died much slower and soluble Fas had limited effect on AICD in Th2 cells. Unlike Th1 cells, Th2 cells are resistant to Fas-mediated apoptosis [151, 152]; however, the underlying mechanism is controversial. Some studies indicated that Th2 cells express less Fas/FasL [150, 153]. On the other hand, there was also evidence showing no difference in expression of Fas/FasL between Th1 and Th2 cells [154]. Furthermore, it was found that Th2 cells express high levels of FAP-1, an Fas-associated phosphatase that may act to inhibit Fas signaling [154]. Still, there are other data showing that selective upregulation of phosphatidylinositol 3-kinase activity in Th2 cells induced by TCR activation inhibited caspase 8 cleavage, which is believed to be a mechanism for Th2 resistance to Fas-mediated apoptosis [155, 156].

AICD in Th2 cells are less well studied compared to Th1 cells. For a long time, it has not been known what molecule(s) is responsible for AICD in Th2 cells. Recently, granzyme B was found to play a critical role [157]. Death receptors are not involved in AICD of Th2 cells since blocking their cognate ligands had no effect on apoptosis of activated Th2 cells. However, inhibition of granzyme B activity abolished AICD in Th2 cells. Furthermore, Th2 cells derived from granzyme B-deficient mice were resistant to AICD. As a consequence, granzyme B deficiency or inhibition of granzyme B activity enhanced the production of Th2 cytokines and increased susceptibility to allergen-induced asthma.

Th17 (also called Thi), a new subgroup of effector T cells secreting IL-17, has been recently described [158, 159] and has generated significant interest. Th17 cells have been shown to play important roles in the pathogenesis of some immune-related diseases. Therefore, it would be interesting to examine how this critical T-cell population undergoes AICD. Recently, we found that neutralizing antibody to Fas largely blocked the AICD induced by anti-CD3 in Th17 cells, while blocking TRAIL and TNF did not show any effects (Yingyu Zhang and Guangwu Xu *et al*., paper in preparation). These results suggest that Fas/FasL might be the major pathway involved in the AICD of Th17 cells.

The Fas/Fas ligand system is involved in AICD of CD4+ T cells, but it seems not essential for CD8+ T cell death [160]. Instead, apoptotic signaling through TNFR is likely engaged in AICD of CD8+ cells [143, 161]. A marked expansion and prolonged persistence of functional activated cytotoxic T cells was observed in mice lacking TNFR p55 [161]. In the study aimed at understanding the role of CD4+ T cell help in CD8+ T-cell memory, memory CD8+ T cells, without the appropriate CD4+ T-cell help, underwent AICD upon secondary stimulation. These unhelped CD8+ T cells synthesize TRAIL on antigen restimulation which mediated AICD [162]. However, it should be pointed out that memory CD8+ T cells are differentiated CD8+ T cells. AICD in these cells might be different from that in the naïve CD8+ T cells or *in vitro* differentiated Tc cells. Recently, it was reported that depletion of granzyme B by RNA interference resulted in the suppression of procaspase 3 processing and tBid production and enhanced the survival of CD3-stimulated CD8+ T cells. Furthermore, this effect is independent of perforin status [163]. Taken together, the question of what constitutes the major pathway mediating AICD of CD8+ T cells needs further investigation.

Similar to their CD4+ counterparts, the existence of distinct subsets of CD8+ T cells has been established in mouse [164-166], rat [167, 168], and human [169-171].

Table 1 AICD in main effector T-cell subgroups

T-cell subgroup	Pathway
Bulk activated T cells	Fas/FasL
Th1	Fas/FasL
Th2	Granzyme B
Th17	Fas/FasL?
Tc1	Fas/FasL/Granzyme B
Tc2	?
Treg, γδT cells, NK, NKT	?

They were termed Tc1 and Tc2. Tc1 cells secret IFNγ, while Tc2 cells secret IL-4, IL-5, IL-6, and IL-10 [164]. Limited data are available on AICD in Tc1 and Tc2. Like Th1 cells, Tc1 cells were also more susceptible to AICD than their type 2 counterparts and Fas/FasL plays important roles in AICD of Tc1 cells, as the neutralizing antibody to FasL partially abolished AICD in these cells. However, it was found that Granzyme B inhibitor (z-AAD-CMK) also inhibited AICD in Tc1 cells, indicating the involvement of the granule exocytosis mechanism in Tc1 AICD [146]. Further experiments in Granzyme B deficient mice will be helpful in understanding the role of granzyme B in Tc1 AICD. Though Tc2 are relatively resistant to Fas/FasL-induced apoptosis, cell-surface expression of Fas and FasL was found at similar levels on both subsets [146]. It is still unknown why Tc2 are relatively resistant to Fas/FasL-induced apoptosis and how AICD is regulated in Tc2 cells.

The apoptotic mechanisms employed by other subgroups of T cells, such as NKT cells and CD4+CD25+ regulatory T cells, are largely unknown; these cells, however, seem to be relatively resistant to apoptosis [172, 173]. Freshly isolated regulatory T cells are highly sensitive to CD95-mediated apoptosis, but upon TCR restimulation, regulatory T cells displayed a reduced sensitivity toward AICD compared with CD4+CD25– T cells [174]. Since these cells are important in innate immunity and immune regulation, studying their AICD will provide novel information for the understanding of the immune system.

Compared to what we know about AICD in T cells, much less is known about B cell apoptosis. Like in T cells, cell death induced by activation via the B cell receptor has also been referred to as AICD [175, 176]. Unlike in T cells, however, AICD in B cells is almost independent of death receptors, and is instead dependent on the intrinsic pathway [177]. Blocking the Fas/FasL system or preventing FADD signaling by inhibitors like FADD/MORT1 or CrmA did not block AICD in B cells. Furthermore, Fas deficient *lpr* mice showed BCR ligation-induced B cell death that was similar to their wild-type counterparts [178-180]. BCR crosslinking leads to morphological changes in mitochondria, including extensive disruption of the mitochondria membrane and swelling of mitochondria during BCR-induced apoptosis [181]; it also results in depolarization of mitochondrial membrane potential [182-184]. Furthermore, mitochondrial inhibitors, which can stabilize mitochondrial membrane potential, are able to protect B cells from AICD [181, 185]. Still, many details of the regulation of mitochondria signaling in B cell AICD remain unknown.

The mechanisms that control apoptosis in various lymphocyte populations are clearly different. These differences are beginning to be recognized and have attracted the attention of immunologists. Further studies in this exciting area will undoubtedly reveal novel information that will advance our understanding of the immune system and its regulatory mechanisms.

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