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Harnessing programmed cell death as a therapeutic strategy in rheumatic diseases

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Abstract

Programmed Cell Death (PCD) is a key process regulating immune cell development and peripheral immune homeostasis. Caspase-dependent apoptosis as well as a number of alternative cell death mechanisms account for immune cell PCD induced by cell-intrinsic as well as extrinsic pathways. In animal models, compelling evidence has emerged that genetic defects in programmed cell death can result in autoimmune disease. Autoimmune disease can arise from single-gene mutations affecting PCD, and defective PCD has been observed in some tissues and cells from patients with rheumatic disease. Selectively inducing PCD in autoreactive B and T cells is very attractive as a therapeutic strategy because it offers the possibility of permanently eliminating these cells. In addition, the anti-inflammatory effects of apoptotic cells may add to the therapeutic benefit of inducing apoptosis. Immune cell subsets vary widely in their sensitivity to specific inducers of cell death, and understanding these differences is key to predicting the outcome of inducing apoptosis for therapeutic means. Here we review approaches that have been taken to induce programmed cell death in the therapy of autoimmune disease and prospects for bringing these experimental strategies into clinical practice.

Multicellular organisms use programmed cell death to eliminate excess cells during development and maintain tissue homeostasis. Programmed cell death (PCD) occurs through a number of different mechanisms, the best understood of which is caspase-dependent apoptosis that can be triggered by extrinsic or intrinsic stimuli. Apoptotic programmed cell death can eliminate autoreactive lymphocytes both during development and in the peripheral immune system, and in general apoptotic cells do not trigger inflammation or may actively suppress it. Utilizing this mechanism to eliminate autoreactive lymphocytes in autoimmune disease is an attractive strategy for immunotherapy because of the potentially long-lasting effects of the physical removal of pathogenic cells. Here we will review the major mechanisms of programmed cell death, their disruption in immunological and rheumatological diseases, and prospects for harnessing PCD for therapeutic purposes.

Mechanisms of Programmed Cell Death and the Immune System

Apoptosis can be triggered by extrinsic signals transduced through cell surface receptors, or cell-intrinsic pathways resulting from DNA damage or other cellular stresses. These pathways are integrated by mitochondria, and converge with the activation of caspases, cysteine proteases that cleave a multitude of cellular substrates that produce the cellular changes associated with apoptosis such as DNA fragmentation, dismantling of the

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cytoskeleton and nuclear envelope, and packaging of cellular contents into apoptotic blebs. Apoptotic cells also display specific surface markers such as phosphatidylserine that are recognized by phagocytic cells and usually rapidly removed from the circulation. In the immune system, the extrinsic cell death pathway is mediated principally by TNF family cytokines, in particular Fas Ligand and TRAIL (TNF-Related Apoptosis Inducing Ligand). Activated T cells can produce both TRAIL and Fas Ligand after stimulation through the TCR. FasL is also reported to be constitutively expressed in some tissues associated with immune privilege such as the eyes and testis, [1,2]. Myeloid cells can produce TRAIL, particularly after stimulation with type I interferons or viral infection [3]. Fas (also known as CD95 or TNFRSF6) and the two functional receptors for TRAIL, DR4 and DR5, can efficiently induce apoptosis due to a conserved domain called the 'death domain' found in the intracytoplasmic tail of these receptors. Fas and TRAIL receptor death domains interact with the death domain in the adapter protein FADD (Fas associated death domain). A related domain, termed the Death Effector Domain (DED) in FADD recruits the cysteine protease caspase-8 receptor signaling complex. Caspase-8 is part of the caspase subfamily of cysteine proteases that cleave substrate proteins at Aspartate residues. Caspases can be involved in both inflammatory and apoptotic signaling [4].

Caspase-8 is present in the cell as a pro-enzyme that requires aggregation and cleavage in the multimerized complex consisting of ligated receptor and FADD to become fully active. This multimerized complex is termed the death inducing signaling complex (DISC)[5l,6]. c-FLIP, an enzymatically inactive homologue of caspase-8, is required in low amounts for processing of caspase-8 in the DISC but can block caspase-8 activation when present in larger amounts [7–9]. The active fragments of caspase-8 assemble into a tetramer and dissociate from the receptor complex, enabling activation of downstream cytoplasmic "effector" caspases, caspase-3, -6 and -7 [4]. Effector caspases can cleave cellular substrates to carry out the apoptotic program. Tumor cell lines fall into two subtypes, depending on the ability of caspase-8 activated in the DISC to initiate caspase-3 cleavage directly [10,11]. In cells termed 'type I', large amounts of caspase-8 are produced by the receptor signaling complex that can directly cleave effector caspases, whereas in other cells, termed 'type II', the caspase-8 generated by the DISC requires amplification by the mitochondrial mechanisms shared with the intrinsic cell death pathway (Figure 1).

Fas and TRAIL receptors are both expressed on activated T cells. However, Fas-Fas Ligand interactions are principally responsible for eliminating chronically stimulated lymphocytes and maintenance of peripheral immunological tolerance [12–14]. More recently, Fas has been shown to trigger non-apoptotic responses such as increased migration, chiefly in myeloid cells [14, Chen, 2010 #9692,15]. TRAIL can induce rapid cell death in transformed tumor cells, but its physiological function is less clear. Experiments with TRAIL deficient and TRAIL-R deficient mice have revealed roles in tumor surveillance and elimination of $CD8^+$ T cells which have not received T cell help from $CD4^+$ T cells [16,17]. Two other members of the TNF superfamily, TNFR1 and DR3, interact with the adapter protein TRADD, which mediates assembly of a primary signaling complex containing TRAF (TNF-receptor associated factors), Inhibitor of Apoptosis (IAP) proteins 1 and 2 and the RIP1 (Receptor Interacting Protein 1). This protein complex triggers pro-inflammatory cellular responses through NF-kB and MAP-kinase signaling [18]. Not until hours after receptor internalization and dissociation of TRADD from the receptor are FADD and caspase-8 recruited to this signaling complex (termed complex II)[18], allowing apoptosis induction to occur. The regulatory protein c-FLIP is synthesized in response to NF-kB activation and is responsible for at least part of the protective functions of NF-kB. Thus, except in circumstances where NF-kB activation is blocked, stimulation of TNFR1 and DR3 usually result in cellular activation and synthesis of pro-inflammatory cytokines and do not induce PCD. Other members of the TNF-receptor family do not have a death domain and cannot directly induce

cell death. Instead they recruit TRAF proteins via peptide consensus sequences, triggering production of pro-inflammatory cytokines and cell survival. However, these receptors can trigger cell death indirectly, through modulating the availability of signaling proteins available to apoptosis-inducing receptors. For example, signaling by the TNF-family member TWEAK results in the recruitment and subsequent degradation of the TRAF2 and IAP-1. [19] Depletion of these proteins in the TNFR1 signaling complex enhances the apoptosis-inducing ability of TNFR1 [20]. Such cross talk might be harnessed for therapeutic purposes.

Cell-intrinsic stimuli can trigger apoptosis through activation of caspases independently of surface receptors (Figure 1). Intrinsic stimuli include withdrawal of cytokines that support cell survival, exposure to mutagens resulting in DNA damage, or hormones such as glucocorticoids [21,22]. Sensors of DNA damage or hormone receptors relay apoptotic signals through the Bcl-2 family of proteins comprising of pro-apoptotic and anti-apoptotic members characterized by the presence of the BH (Bcl-2 homology) domains. The anti-apoptotic proteins Bim, Bid, Noxa and Puma contain only BH3 domains, however, Bax, Bak are multi-BH domain members. Bid can be cleaved by active caspase-8 in the signaling complex of death receptors, resulting in a truncated protein (tBid). tBid translocates to the mitochondria where it induces conformational changes in other bcl-2 family member proteins, resulting a rapid opening of channels in the mitochondrial outer membrane, referred to as Mitochondrial Outer Membrane Permeability transition (MOMP). MOMP disrupts mitochondrial energy generation and releases cytochrome-c into the cytoplasm, which is a key activator of the ‘apoptosome’ a complex of caspase-9 and the APAF-1 protein. The anti-apoptotic members (Bcl-2, Bcl-XL, Mcl-1) contain multiple BH domains that play a critical role in directly inhibiting pro-apoptotic Bcl-2 members through a conformational interaction. A second layer of regulation is mediated by inhibitor of apoptosis (IAP) proteins such as XIAP (X-linked IAP), which bind to and inhibit the active form of effector caspases. Molecules such as SMAC/diablo that are also released from the mitochondria after MOMP can inactivate IAP proteins, providing an additional feed-forward loop that reinforces caspase activation. Together, these regulatory pathways result in a latent period in which upstream signals such as death receptor ligation produce small amounts of caspase activity and activate upstream BH3 proteins, followed by a very rapid (2–3 minute) onset of apoptosis with MOMP, release of cytochrome c, and effector caspase activation occurring almost simultaneously [23] (Figure 1).

Intrinsic and extrinsic apoptosis pathways come into play during different phases of the immune response. After the rapid proliferation of antigen-specific T cells that occurs during the first four to seven days of an acute immune response, the contraction of effector T cells is largely due to cytokine withdrawal via the intrinsic apoptotic pathway. Once the T cell pool expands beyond the availability of IL-7 and IL-15, levels of Bcl-2 fall, levels of the pro-apoptotic BH3 protein Bim rise, and apoptosis mediated by Bim ensues [24,25]. Fas comes into play in the elimination of T cells only under conditions of chronic infection or repetitive stimulation by autoantigens. [26–28]

Repeated T cell receptor (TCR) stimulation upregulates FasL, which induces apoptosis in Fas-bearing T cells through the process of restimulation induced cell death (RICD). RICD leads to clonal deletion of these cells without affecting bystander antigen non-specific T cells, even if they also express Fas [29]. This specificity occurs because TCR stimulation induces a ‘competency to die’ signal in addition to FasL synthesis. This TCR specific signal preferentially sensitizes restimulated T cells to die in comparison to bystander activated T cells. Not all aspects of TCR signaling are necessary to sensitize T cells to Fas-dependent apoptosis, but activation of Rac GTPases, members of the small G protein signaling family that regulate actin polymerization and cytoskeletal remodeling have been shown to be

necessary [30]. One of the results of Rac activation is translocation of Fas to lipid raft microdomains, which increase the efficiency of Fas-induced apoptosis [30,31]. How this type of cytoskeletal reorganization relates to changes that take place during formation of an immune synapse between a T cell and an antigen-presenting cell is not known.

Caspase-independent cell death mechanisms

Use of caspase inhibitors and caspase deficient mice have identified several mechanisms of caspase-independent cell death (CICD) [32]. Necrosis and autophagic cell death are two such caspase-independent mechanisms [33]. Necrosis, previously considered to be an uncontrolled catastrophic form of cell death, has been found resulting in some cases from regulated signaling events. Necrosis resulting from caspase inhibition during death receptor signaling is termed necroptosis, and is morphologically indistinguishable from necrosis. This 'backup' mechanism of cell death may constitute an immune defense mechanism against viruses, which often encode caspase inhibitors in their genome. Recent evidence has implicated RIP1 and RIP3, proteins also involved in TNF receptor induced NF-kB activation, in necroptosis [34]. Small molecule inhibitors of the RIP1 protein kinase termed necrostatins can block necroptosis in some cell types. [35]. Autophagy, on the other hand, is a physiological response to nutrient deprivation in which intracytoplasmic organelles are recycled in double walled membrane bound vacuoles termed autophagosomes. Autophagic cell death is characterized by accumulation of autophagic vacuoles in cells undergoing cell death, and can be blocked by inhibition of autophagy, suggesting that autophagy may be part of the mechanism of this type of cell death [36]. Recently, autophagic cell death has been suggested to underlie the cell death that occurs when T cells are activated in the presence of caspase inhibitors or genetic deficiency of FADD or Caspase-8 [37]. These findings suggest that caspases may play a critical role controlling lymphocyte survival at both ends of a spectrum: too little caspase activation triggers an autophagic death program, whereas high levels of caspase activation, such as after ligation of Fas, triggers apoptotic cell death.

Life after cell death – recognition and uptake of apoptotic cells

Apoptotic cells are usually rapidly phagocytosed by macrophages. The process of phagocyte recognition and engulfment depends on various recognition signals that are exhibited by the cell undergoing apoptosis. One of the classical recognition signals is the membrane lipid phophatidylserine (PS), which translocates from the inner to outer leaflet of the plasma membrane upon apoptosis. PS can be recognized by specific receptors on phagocytes, but the efficiency of apoptotic cell recognition is increased by many other intermediary molecules that aid in the recognition of dying cells [38].

After ingesting apoptotic cells, macrophages downregulate secretion of pro-inflammatory cytokines such as TNF, IL-8 and IL-1b and increase production of anti-inflammatory mediators such as TGF-b [39]. After ingestion of apoptotic cells dendritic cells produce TGF-b, and induce naïve T cells to differentiate into regulatory cells [40]. Because of these responses, uptake of apoptotic cells has been generally thought to suppress inflammation and promote adaptive immune tolerance.

The tolerogenic nature of apoptotic cell recognition and uptake is underscored by the autoimmunity that results from genetic lesions in the recognition and uptake of apoptotic cells. The classical complement component C1q is required for efficient apoptotic cell phagocytosis and binds to the C1q receptor on phagocytes. C1q knockout mice develop glomerulonephritis, accumulate apoptotic bodies and exhibit increased mortality [41] Humans with homozygous C1q deficiency also have a strong predisposition to develop SLE [42]. Mice lacking other components of complement, receptors in the axl/tyro3/mer family that indirectly recognize PS through intermediary bridge molecules, or Milk fat globule

epidermal growth factor-8 (MFG-E8), a bridging molecule in apoptotic cell recognition, also develop systemic autoimmunity [43–45]. Taken together these results suggest that coordinated recognition and uptake of apoptotic cells is an important part of immunological self-tolerance. The role of this process in controlling autoimmunity in the setting of spontaneous disease not associated with genetic deficiencies in complement or apoptotic uptake mechanisms remains to be elucidated.

Genetic diseases involving apoptosis and evidence for altered apoptosis in more common rheumatic diseases

The central role of the Fas-FasL system in maintaining peripheral tolerance is exemplified by the autoimmune syndromes that result from genetic defects in Fas or Fas Ligand. Mice harboring loss of function mutations in Fas (*lpr, lymphoproliferation*), or Fas Ligand (*gld, generalized lymphadenopathy*) develop massive lymphadenopathy, splenomegaly, anti-DNA antibodies and high levels of serum IgG and IgM. Mice on the MRL background succumb to lethal inflammatory arthritis and nephritis by 5 months of age [46]. One characteristic feature of impaired T cell apoptosis is the accumulation of large numbers of abnormal $\alpha\beta TCR^+$ $CD4^-CD8^-$ (double-negative or DN) T cells. Lineage specific deletion of Fas in T cells, B cells or antigen presenting cells all result in varying degrees of autoimmune manifestations in mice, indicating an essential role of Fas expression in all three cell types for maintaining peripheral immune tolerance [47]. In humans, dominant negative mutations in Fas or Fas Ligand cause the familial autosomal dominant Autoimmune Lymphoproliferative Syndrome (ALPS), which bears a striking resemblance to Fas deficiency in mice. ALPS typically presents in childhood with chronic lymphadenopathy, hepatosplenomegaly, and autoimmunity, most commonly manifested as autoimmune hemolytic anemia or idiopathic thrombocytopenia purpura. DN T cells accumulate in the peripheral blood and to an even greater extent in lymph nodes in ALPS [48–50]. Mutations throughout the Fas protein have been documented in ALPS patients, with the most common being mutations in the death domain that impair formation of the Fas signaling complex. Interestingly patients with caspase-8 deficiency present with immunodeficiency rather than autoimmunity and have defects in lymphocyte activation [51], perhaps due to the role of caspases in preventing autophagic cell death during lymphocyte activation as discussed above.

In the more common rheumatic diseases, multiple genetic susceptibility loci interact with the environment to produce disease. Although no single genetic locus controls disease, a number of candidate genetic variants may influence cellular susceptibility to apoptosis. Phenotypic studies have found alterations in apoptosis or apoptotic cell uptake in tissue samples from patients with rheumatic diseases. In Rheumatoid Arthritis (RA), the accumulation of inflammatory cells at sites of active disease could be due to increased cellular proliferation or reduced apoptosis. Experiments using a number of different detection techniques have yielded remarkably few apoptotic cells in rheumatoid synovium [52]. While peripheral monocytes express Fas/FasL and are highly sensitive to Fas apoptosis, synovial macrophages obtained from diseased joints have increased expression of c-FLIP and are resistant to apoptosis induced by Fas. Reduction in c-FLIP can restore Fas sensitivity, suggesting that reducing c-FLIP expression through RNA interference of blockade of NF-kB activity may be a therapeutic strategy to reduce macrophage numbers in RA [53]. Inhibition of NF-kB activity can also sensitize macrophages to undergo apoptosis in response to TNF [54], suggesting therapeutic NF-kB inhibition as another strategy to subvert the effects of TNF in RA.

In SLE, alterations in the kinetics of cell death and uptake of apoptotic cells may alter the normal non-immunogenic nature of apoptosis. T lymphocytes in SLE have been observed to

undergo reduced apoptosis when stimulated through the TCR [55]. This may be due to alterations in TCR signaling proteins, including deficiency in the TCR zeta chain, which is known to be required for TCR-induced apoptosis [56]. However, T cells from patients with SLE have also been found to undergo accelerated spontaneous apoptosis [57]. However, some of these findings may be due to alterations in T cell subsets or activation status known to occur in SLE, rather than cell-intrinsic differences in apoptotic signaling. Another line of evidence has revealed altered uptake of apoptotic cells as a possible pathogenic mechanism in SLE. Macrophages derived from SLE patient monocytes are impaired in their ability to uptake apoptotic material in vitro [58]. In vivo, lymph node biopsies from SLE patients with active disease contained multiple non-phagocytosed apoptotic cells in the germinal centers, compared to none in control biopsies [58]. TUNEL assays have demonstrated attachment of apoptotic materials to follicular dendritic cells in SLE patients, allowing these antigens to ultimately be presented to T cells[58–60]. Accumulation of apoptotic cells in SLE may allow autoantigens that are normally eliminated through phagocytosis to become immunogenic, adding 'fuel to the fire' of autoimmunity in SLE and other autoimmune diseases [61].

Induction of programmed cell death by current and potential future therapies for rheumatic diseases

A number of therapeutic agents already in use for rheumatic disease induce apoptosis as all or part of their mode of action. Rituximab, a chimeric anti-CD20 B cell-specific monoclonal antibody that was originally developed to treat B cell malignancies, is approved for use in RA, and is used in other B-cell dependent autoimmune diseases, including pemphigous and SLE. Rituximab profoundly depletes circulating B cells through a number of mechanisms, including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and apoptosis [62,63]. Some studies have suggested that anti-TNF monoclonal antibodies (but not TNFR2-Fc fusion proteins such as etanercept) can acutely induce apoptosis in T cells and synovial macrophages that express surface TNF by similar mechanisms [64]. This may explain the superior efficacy of anti-TNF agents over etanercept in diseases such as inflammatory bowel disease. ADCC has been exploited in newer investigational agents to target specific populations of cells, such as an anti-lymphotoxin-β mAb that specifically depletes Th1 and Th17 T cells based on their increased expression of surface lymphotoxin [65].

Small molecule therapeutics currently used in the management of rheumatic diseases may also exert some of their therapeutic effects through apoptosis. High dose glucocorticoids induce apoptosis in eosinophils and lymphocytes. The mechanisms by which this occur are complex, likely involving repression of transcription factors that promote cellular survival such as NF-kB, and activation of caspase-dependent apoptosis [66]. Among other mechanisms, methotrexate, a mainstay of therapy for RA, may act through induction of programmed cell death. In vitro, methotrexate (MTX) can induce apoptosis in 20–30% of activated T cells [67,68] at concentrations comparable to peak plasma levels in patients on methotrexate for RA. Generation of reactive oxygen species and loss of mitochondrial membrane potential has been implicated in MTX-induced cell death, and MTX has also been reported to potentiate Fas-induced apoptosis [67,68]. Some support for the idea that MTX may deplete activated T cells came from a study in which MTX depleted a subset of activated $CD4^+CD28^+$ T cells that accumulate in RA patients [69]. Cyclophosphamide can also induce apoptosis in activated T cells [67], and some of its beneficial effects in SLE and vasculidites may stem from elimination of autoreactive lymphocytes.

Rational targeting of the intrinsic cell death pathway by manipulating the Bcl-2 family of proteins can induce apoptosis and may be useful in the treatment of rheumatic diseases. A phosphorothioate antisense oligonucleotide (Oblimersen sodium) targeting Bcl-2 has shown

modest effects on progression and survival in patients with chronic lymphocytic leukemia and malignant melanoma [70]. ABT-737 and other small molecule drugs have been developed that mimic the activity of BH3 peptides to bind and inactivate Bcl-2. These drugs can potently induce apoptosis in tumor cells, although phosphorylated Bcl-2 and other anti-apoptotic Bcl-2 family proteins such as Mcl-1 are resistant to the effects of ABT-737 [71–73]. ABT-737 was recently shown to be efficacious in mouse models of arthritis and immune complex nephritis, although significant depression of B and T cell responses were seen, suggesting that the use of this agent may result in some generalized immunosuppression [74].

Targeting Fas and other death receptors in rheumatic diseases

Because of its intrinsic ability to induce apoptosis and the heightened sensitivity of restimulated T cells to Fas-induced apoptosis, engagement of the TNF-receptor Fas could be an interesting therapeutic strategy to eliminate autoreactive T cells without knowing their antigenic specificity. Overexpression of the FADD adapter protein in cultured RA synoviocytes induced apoptosis in these cells, and in a xenograft mouse model of proliferating rheumatoid synovium, ectopic expression of FADD through retroviral transduction lead to a significant reduction of synoviocytes and mononuclear cells [75]. Reducing levels of c-FLIP, another molecule that regulates Fas signaling has been shown to sensitize fibroblast-like synoviocytes from RA patients to Fas-induced apoptosis and sensitized T cells to TCR-induced apoptosis [76,77]. Indirect targeting of Fas through its ligand is another plausible therapeutic mechanism. The forkhead transcription factor Foxo3a is an upstream inhibitor of FasL synthesis. Foxo3a deficient mice are very resistant to inflammatory arthritis in the K/BxN serum transfer model of rheumatoid arthritis due to increased neutrophil apoptosis as a result of upregulated FasL synthesis [78].

Directly inducing apoptosis through Fas is another strategy for elimination of potentially autoreactive cells. Use of Anti-Fas monoclonal antibodies in vivo has been limited by acute and fatal hepatic failure that was observed in mice due to Fas-induced hepatocyte apoptosis [79]. This effect is partially dependent on Fc-receptor mediated crosslinking of the anti-Fas mAb [80]. Using FasL rather than anti-Fas and targeting specific cell populations for Fas mediated apoptosis has avoided this problem in a number of experimental models. Although crosslinking of FasL with a secondary antibody reproduced the liver toxicity of anti-Fas mAb, an engineered hexameric version of Fas Ligand retains cytotoxic potential and does not induce fatal liver toxicity [81,82]. Human hexameric FasL (APO010, Topotarget) is currently in early stage human trials for cancer therapy. A fusion protein containing FasL and a fibroblast activation protein (FAP)-specific single chain antibody limited the induction of apoptosis to only cells expressing FAP. This FasL-anti-FAP fusion protein inhibited the growth of a FAP-expressing tumor without the lethality seen with nonspecific Fas targeting [83]. Focusing FasL on tumor cells through a fusion protein combining FasL with a high-affinity antibody against the T-cell leukemia-associated antigen CD7 lead to apoptosis of CD7-expressing cells, and moderate tumoricidal activity in CD7-expressing leukemic cells from T-ALL patients [84]. Delivery of FasL has had some success in treating animal models of arthritis. A T lymphoma cell line overexpressing FasL-induced apoptosis in cultured synoviocytes from RA patients and could deplete synoviocytes and mononuclear cells in the rheumatoid synovial xenografts [85]. Adenoviral vector-based delivery of FasL ameliorated joint pathology in CIA [86]. These studies suggest that treating inflammatory joint disease through sensitizing synovial cells to Fas-mediated apoptosis may be a viable therapeutic strategy provided that these effects can be spatially limited to affected joints.

Better understanding of the pathways that sensitize cells to apoptosis-inducing receptors has led to therapeutic strategies to activate these pathways and allow apoptosis to be triggered by endogenous ligands. The cytoplasmic apoptosis inhibitor XIAP restrains both extrinsic

and intrinsic apoptosis pathways by binding to and inhibiting active effector caspases 3 and 7. Mimetics of the N-terminal peptide in the protein SMAC, which is released from mitochondria during apoptosis, can displace XIAP from effector caspase. SMAC mimetics or reduction in XIAP levels sensitizes many cell lines to undergo apoptosis in response to TNF, TRAIL and Fas Ligand, [87,88]. Reduction of XIAP levels in mice through treatment with SMAC mimetics or genetic deficiency also sensitized hepatocytes to FasL mediated apoptosis. In some tumor cell lines, SMAC mimetics initiated an autocrine loop of TNF-TNFR1 mediated cell death through activating the alternative NF-kB pathway and TNF production, but this has not been observed in primary cells. SMAC mimetics are interesting candidates for therapies aimed at increasing sensitivity to apoptosis caused by endogenous or exogenous death receptor ligands.

T cell subsets vary in their sensitivity to Fas-induced apoptosis [89, Riou, 2007 #3747] and signaling through the TCR sensitizes cells to Fas-induced apoptosis as well as inducing transcription and secretion of Fas Ligand [90] Understanding the signaling pathways through which the TCR induces apoptosis may allow therapeutic manipulation to promote apoptosis of autoreactive lymphocytes. Sensitivity to Fas-induced apoptosis can be induced by low affinity peptides for the TCR that do not induce Fas Ligand or cytokine induction, suggesting that this is mediated through distinct signaling pathways [91]. TCR-mediated sensitization to Fas-mediated apoptosis requires translocation of Fas to lipid raft microdomains in a manner dependent on the Rac Family of GTPases [30,31]. T cells lacking the Wiskott-Aldrich Syndrome protein (WASp), which mediates actin remodeling downstream of the TCR, have defective TCR-induced apoptosis associated with reduced secretion of Fas Ligand [92]. This defect may be one factor predisposing patients with the Wiskott Aldrich Syndrome and WASp deficient mice to develop systemic autoimmunity at high frequencies in addition to the immunodeficiency associated with the Wiskott-Aldrich Syndrome. Alternative ligands that activate these signaling pathways may be one strategy to sensitize autoreactive to undergo TCR-mediated apoptosis even when the autoantigen is unknown, as is the case with many human diseases.

These findings suggest a number of therapeutic targets that may specifically sensitize T cells to undergo apoptosis when they are chronically stimulated. Agents already used in the treatment of rheumatic disease, such as cyclophosphamide, glucocorticoids or anti-TNF mAb may also exert part of their effects through inducing apoptosis. Although in diseases such as SLE apoptotic cells may become abnormally immunogenic, conventional therapies that likely induce apoptosis in vivo are more beneficial then harmful. Blockade of Interleukin-6 with tocilizumab, a mAb against IL-6R recently approved for the treatment of RA in the U.S., is one example, as interleukin-6 can protect against TCR and Fas-mediated apoptosis in a number of settings [93–95]. Development of these therapeutic strategies has the potential to fulfill one of the long sought goals in the therapy of autoimmune disease: eliminating autoreactive cells with minimal generalized immunosuppression.

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Key Points

- Apoptosis or programmed cell death is mediated by extrinsic ‘death receptors’ or intrinsic signals triggered by responses to cellular stress. Apoptosis is essential in maintenance of immune cell numbers
- The caspase family of intracellular proteases are the main effectors of apoptosis, but caspases also have non-apoptotic functions, and caspase-independent necrotic or autophagic cell death also occurs
- Uptake of apoptotic cells by antigen-presenting cells promotes immunological tolerance through suppressing the ability of these cells to activate the immune system, while phagocytosis of necrotic or infected cells activates immune responses
- Genetic mutations or variants in regulators of cell death or dead cell clearance can predispose to autoimmunity
- A number of current therapies for autoimmune disease lead to immune cell depletion via programmed cell death
- Better understanding of the mechanisms regulating apoptosis sensitivity of immune cells suggests new targets for therapies designed to specifically deplete autoreactive lymphocytes

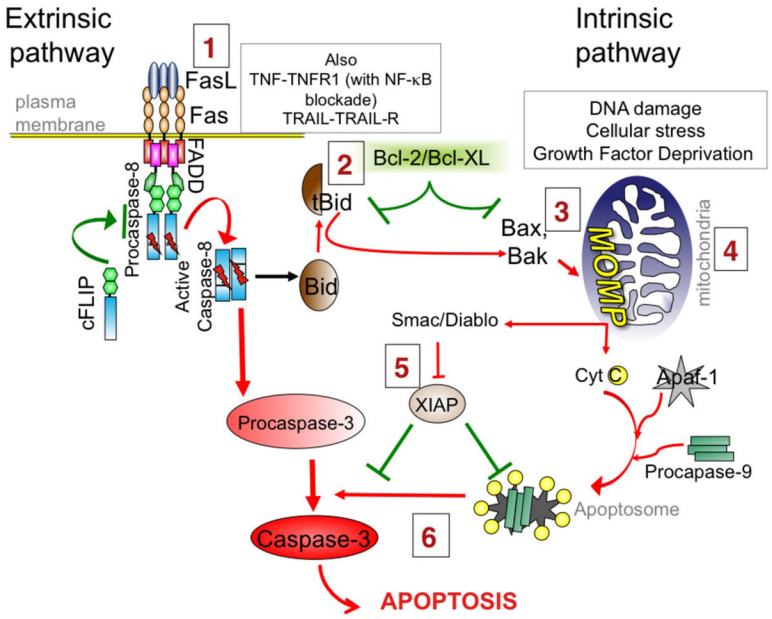

Figure 1.

A. **Pathways of Apoptosis -** Induction of apoptosis by the extrinsic pathway depends on the FADD/Caspase-8 DISC formed by the death receptor (Fas/TRAIL/TNFR1). Active caspase-8 generated from an efficient DISC, which can be inhibited by high levels of c-FLIP, is sometimes sufficient to induce apoptosis via caspase-3 activation. However, crosstalk between extrinsic and intrinsic pathway can occur through Bid cleavage leading to activation of the pro-apoptotic BH-3 family proteins, Bak and Bax, and mitochondrial MOMP. The intrinsic apoptosis pathway depends on MOMP and apoptosome formation resulting in caspase-3 activation and apoptosis. Intrinsic cell death is more closely regulated by the balance between the anti-apoptotic and pro-apoptotic members of the BH3 family of proteins including Bcl-2, Bax, Bak and IAP activity (see text for details). Irrespective of the initiating apoptosis stimuli, activation of caspase-3 indicates an irreversible final stage in the process of apoptosis. Red arrows indicate pro-apoptotic events, and green arrows anti-apoptotic regulation. Abbreviations: FADD (Fas-activated death domain protein), c-FLIP (cellular FLICE-inhibitory protein), Bcl-2 (B-cell lymphoma 2), Bcl-xL (Bcl-2-related gene, long isoform), Bak (Bcl-2 antagonist killer 1), Bax (Bcl-2 associated × protein), BID (Bcl-2-interacting domain death agonist), SMAC (second mitochondria-derived activator of caspases).

B. **Therapeutic Targeting of Apoptosis.** Many emerging and conventional therapies are thought to mediate their effects via induction of apoptosis through the extrinsic and/or intrinsic apoptosis pathways. (1) Increased FasL (Methotrexate, Cyclophosphamide), (2) Downregulation of Bcl-2 (Oblimersen sodium, a Bcl-2 antisense oligonucleotide injection), (3) Activation of Bax/Bak via inhibition of anti-apoptotic Bcl-2 family members (ABT-737, a BH3 mimetic), (4) Generation of mitochondrial reactive oxygen species (Methotrexate), (5) Displacement and degradation of IAPs (SMAC mimetics, glucocorticoids), (6) Activation of caspase 3 and caspase 9 (Rituximab, glucocorticoids)

Table 1

Therapies Targeting Apoptosis

Numbering from Figure 1	Apoptosis-inducing therapeutic strategies	Mechanism of Action	Therapies
1	Caspase activation	Activation of caspase-9, caspase-3	Rituximab (anti-CD20), glucocorticoids
2	Targeting Bcl-2	Bcl-2 antisense BH3 mimetics	Oblimersen sodium ABT-737.
3	Induction of intrinsic apoptosis pathway	Generation of reactive oxygen species	Methotrexate
4	Sensitization to Fas/FasL extrinsic pathway	Increased FasL	Methotrexate, Cyclophosphamide
5	Blocking X-linked inhibitor of apoptosis	Displacement of XIAP from effector caspases, degradation by proteasomes	SMAC mimetics, glucocorticoids

Table 2

Potential therapies targeting the Fas/FasL apoptosis pathway

Therapeutic Strategy	Mechanism/Evidence
FADD Overexpression	Induction of apoptosis in cultured RA synoviocytes [75] Efficacy in RA synovium xenograft model [75]
c-FLIP Reduction	c-FLIP antisense oligonucleotide increased apoptosis of CD8+ T cells and reduced contact hypersensitivity in mouse models [77], sensitized RA fibroblast like synoviocytes to Fas-mediated apoptosis [76]
Hexameric FasL	Induces Fas-dependent apoptosis in cell lines [82]
Targeted FasL fusion protein	Induce apoptosis restricted to cell types expressing target antigen [83,84]