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Review

Apoptosis in the development of the immune system

JT Opferman*

Apoptosis is a conserved genetic program critical for the development and homeostasis of the immune system. During the early stages of lymphopoiesis, growth factor signaling is an essential regulator of homeostasis by regulating the survival of lymphocyte progenitors. During differentiation, apoptosis ensures that lymphocytes express functional antigen receptors and is essential for eliminating lymphocytes with dangerous self-reactive specificities. Many of these critical cell death checkpoints during immune development are regulated by the BCL-2 family of proteins, which is comprised of both pro- and antiapoptotic members, and members of the tumor necrosis factor death receptor family. Aberrations in the expression or function of these cell death modulators can result in pathological conditions including immune deficiency, autoimmunity, and cancer. This review will describe how apoptosis regulates these critical control points during immune development.
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Apoptosis

Apoptosis is an essential genetic program necessary for the proper development and homeostasis of metazoans. The cell death pathway responds to both normal and pathologic stimuli and aberrancies have been associated with human diseases including autoimmunity, cancer, immune deficiency, and neurodegenerative disorders. Apoptosis results in the activation of a family of aspartate-specific cysteine proteases known as caspases that exist as zymogens.[1] Caspases possess internal proteolytic sites that are themselves caspase consensus sites permitting the initiation of a catalytic cascade. Thus, activated caspases may activate other caspases as well as other enzymes that cleave a myriad of intracellular proteins to promote the orderly disassembly of the cell (Figure 1).[2] Most of these proteases are present in healthy cells as proenzymes that are only activated in the appropriate cellular context during development or upon cellular stress.

In mammals, cell demise downstream of death signals is regulated by two molecular programs, which both lead to caspase activation. In certain cell types, the two programs may be linked. The extrinsic pathway is initiated by the ligation of death receptors, such as Fas and other tumor necrosis factor (TNF) receptor family members, which recruit a death-inducing signaling complex (DISC) after ligand binding (Figure 1).[3–5] The DISC recruits and activates Caspase-8, causing the activation of other downstream effector caspases. Genetic deletion of the death adaptor *FADD* and *Caspase-8* in the T-cell lineage has demonstrated that these proteins are essential for death receptor-mediated apoptosis; however, such deficient cells exhibit normal sensitivity to a variety of intrinsic cell death stimuli including cytokine withdrawal and cytotoxic stress.[6–8] Death receptor signaling can be inhibited by FLICE-inhibitory proteins (FLIPs) that are recruited to the DISC blocking the activation and release of Caspase-8. In cells such as lymphocytes (known as type I cells), death receptor-mediated apoptosis is independent of the BCL-2 family as activation of Caspase-8 is sufficient to catalyze the activation of the downstream caspase cascade.[9] However, in other cells like hepatocytes (known as type II cells) the death receptor pathway is connected to the BCL-2 family by the Caspase-8-mediated activation of the proapoptotic molecule BID.[10]

The intrinsic pathway is marked by a requirement for the involvement of mitochondria.[11,12] Under the control of the BCL-2 family, mitochondria participate in apoptosis by releasing apoptogenic factors including cytochrome *c*, a component of the electron transport chain (Figure 2).[13] Upon release, cytochrome *c* associates with APAF-1 and Caspase-9 to form the 'apoptosome', which triggers the activation of downstream cascade of effector caspases.[14] These caspases coordinate the proteolytic cleavage of key cellular proteins leading to cellular demise. In addition to cytochrome *c*, other mitochondrial factors are released during this process. These apoptogenic factors can augment apoptosis by a variety of different mechanisms. For example, Smac/Diablo inhibits the inhibitors of caspase activation, apoptosis-inducing factor translocates to the nucleus and induces chromatin condensation, and endonuclease G assists in nucleosomal DNA fragmentation (reviewed by Saelens *et al.*[15]).

The BCL-2 Family

The BCL-2 family is made up of critical regulators of the apoptotic pathway residing upstream to irreversible commitment to cell death. Many BCL-2 family members reside largely

Department of Biochemistry, St. Jude Children's Research Hospital, 332 N Lauderdale Street D-4063C, Memphis, TN, USA
*Corresponding author: JT Opferman, Department of Biochemistry, St. Jude Children's Research Hospital, 332 N Lauderdale, Memphis, TN 38105, USA.
Tel: +901 495 5524; Fax: +901 525 8025; E-mail: joseph.opferman@stjude.org
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Abbreviations: BCR, B-cell receptor; BH, BCL-2 homology; DISC, death-inducing signaling complex; FLIPs, FLICE-inhibitory proteins; HEL, hen egg lysozyme; IL, interleukin; JAK, Janus kinases; MHC, major histocompatibility complex; TCR, T-cell receptor; TNF, tumor necrosis factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand
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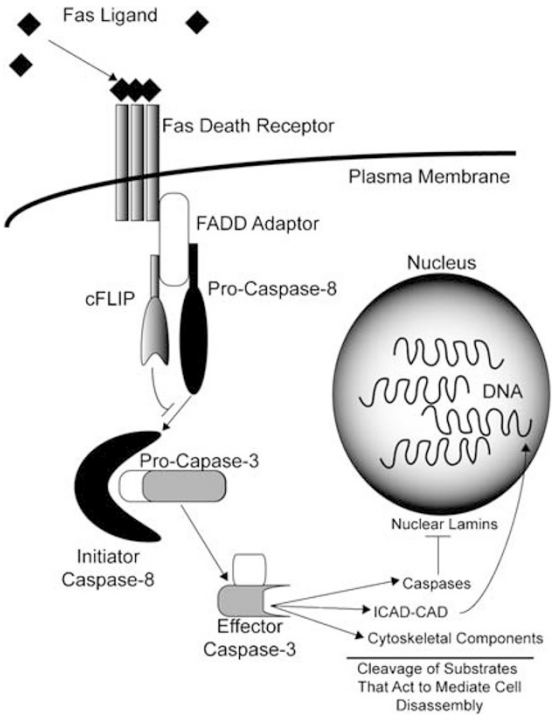

Figure 1 Death receptor-mediated apoptosis. Death receptor ligands, such as Fas ligand, induce the oligomerization of the death receptor (Fas). This oligomerization recruits the DISC complex made up of the FADD adaptor protein and Pro-Caspase-8 (inactive). The inhibitor cFLIP is capable of outcompeting pro-caspase-8 for binding to FADD and thereby is capable of inhibition of initiation of the caspase cascade. Once Caspase-8 is activated, it is capable of activating downstream effector caspases such as Caspase-3 by proteolysis of the zymogen form. Activated effector caspases then mediate the proteolysis of a myriad of substrates including other caspases and cytoskeletal proteins (e.g. actin, nuclear lamins, etc.). One important target of effector caspases is the inhibitor of caspase-activated DNAse (ICAD), which releases CAD to initiate DNA degradation in the nucleus

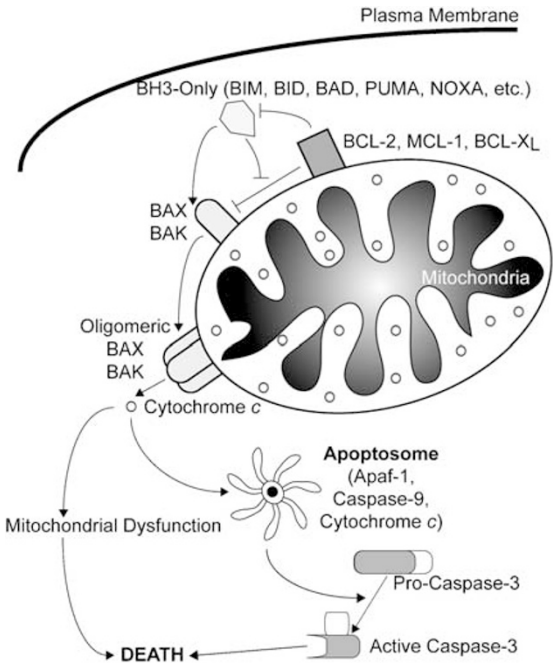

Figure 2 BCL-2 family-mediated apoptosis. The BCL-2 family integrates death signals from a variety of sources and regulates mitochondria-dependent apoptosis. BH3-only family members act as sentinels for many death stimuli and can be regulated by transcriptional and post-translational mechanisms allowing rapid response to changing cellular conditions. They can be directly sequestered by antiapoptotic BCL-2 family members (e.g. BCL-2, BCL-X_L, MCL-1, etc.). Upon sufficient activation, BH3-only family members can mediate the activation of the multidomain proapoptotics BAX and BAK either by a 'hit and run' interaction or by relieving antiapoptotic antagonism of BAX and BAK allowing oligomerization. Upon BAX and BAK oligomerization, the mitochondrial outer membrane is permeabilized releasing a variety of apoptogenic substrates from the mitochondrial intermembrane space such as cytochrome *c* into the cytosol. Released cytochrome *c* can complex with APAF1 and Pro-Caspase-9 to form the apoptosome, which catalyzes Caspase-9 activation. Activated Caspase-9 can then trigger the activation of a downstream caspase cascade leading to cell death

at subcellular membranes including the mitochondria outer membrane, endoplasmic reticulum, and nuclear membrane. The family consists of both death agonists and antagonists that share sequence homology within α-helical segments denoted as BCL-2 homology (BH) domains numbered BH1–4. Antiapoptotic family members (such as BCL-2, BCL-X_L, A1, and MCL-1) are highly conserved, possessing four BH domains. Structurally, the BH1–3 domains form a hydrophobic pocket capable of binding the BH3 domains of other family members. The proapoptotic members can be further subdivided according to the number of BH domains they possess. The multidomain proapoptotic members (BAX, BAK, and BOK) possess the BH1–3 domains and also form a hydrophobic pocket. In contrast, the 'BH3-only' subset of proapoptotics (BID, BAD, BIM, BIK, BMF, NOXA, and PUMA) possesses only the minimal BH3 domain. BH3-only proteins are attractive candidates in controlling apoptosis as they are dynamically regulated by several mechanisms including transcriptional control and post-translational control (reviewed by Willis *et al*.[16]).

The multidomain proapoptotic molecules BAX and BAK constitute a requisite gateway to the mitochondrial pathway of apoptosis in that cells doubly-deficient for both of these proteins are dramatically resistant to a wide array of death signals.[17,18] Upon receipt of death signals, BAX and BAK are activated and oligomerize at the mitochondria resulting in permeabilization of the outer membrane and release of cytochrome *c* (Figure 2).[19] BH3-only molecules require BAX and BAK to mediate death and operate upstream of the mitochondria connecting the proximal death and survival signals to the core intrinsic pathway. Antiapoptotic family members function by sequestering BH3-only molecules in stable complexes, preventing the activation of BAX and/or BAK or by directly antagonizing BAX and BAK.[20–22]

Cell Death and Immune Development

Although apoptosis participates in the development of virtually all cell lineages, it plays a very essential role in the immune system. This fact is best illustrated by the fact that aberrations in a myriad of pro- or antiapoptotic genes have been implicated in the initiation of diseases involving lymphocytes including immunodeficiency, autoimmunity, and cancer.

During development, the survival of lymphocytes is mediated by both active signaling and passive processes that regulate survival. These processes are extremely selective resulting in the elimination of the majority of developing lymphocytes.[23] Both T- and B-lymphocytes undergo developmental stages and appear to share many regulatory mechanisms. For example, the early survival of lymphocyte precursors is mediated primarily by cytokines, which both regulate the numbers of progenitors and play critical roles in initiating the rearrangement of the antigen receptor genes.[24] Developing lymphocytes must create unique antigen receptors by rearrangement to generate the incredible diversity characteristic of an adaptive immune response.[25] A consequence of the stochastic nature of this process is that only 1/3 of rearrangements are joined appropriately and give rise to a functional antigen receptor.[25] Although several mechanisms (i.e. use of alternative antigen receptor gene loci and receptor editing) exist to allow further opportunities for successful rearrangement, the majority of lymphocytes fail to generate functional antigen receptors and are thus eliminated by programmed cell death.[26,27] Following successful antigen receptor rearrangement, signals from the pre-T-cell receptors (pre-TCRs) or pre-B-cell receptors (pre-BCRs) signal the lymphocytes to promote the survival of the progenitors and induce their further differentiation.[28,29]

T-lymphocyte progenitors that express a functional receptor are further subjected to both positive and negative selection to ensure that a functional receptor exists while eliminating those cells with self-reactive antigen receptors, which could be dangerous due to the potential for autoimmunity (Figure 3).[30] When the avidity of interaction of the TCR and endogenous major histocompatibility complex (MHC) molecules is low, T-cell progenitors fail to be positively selected and undergo apoptosis. Conversely, in self-reactive T-cells, the avidity between the TCR and MHC is too high; such T-cells are eliminated by negative selection. During B-cell development, B-cell progenitors with self-reactive surface Ig BCR also face negative selection as a result of the antigen-mediated signaling (Figure 4).[29] Although the exact apoptotic mechanisms used to mediate these processes are complex, recent work has demonstrated the primary roles of several apoptotic players.

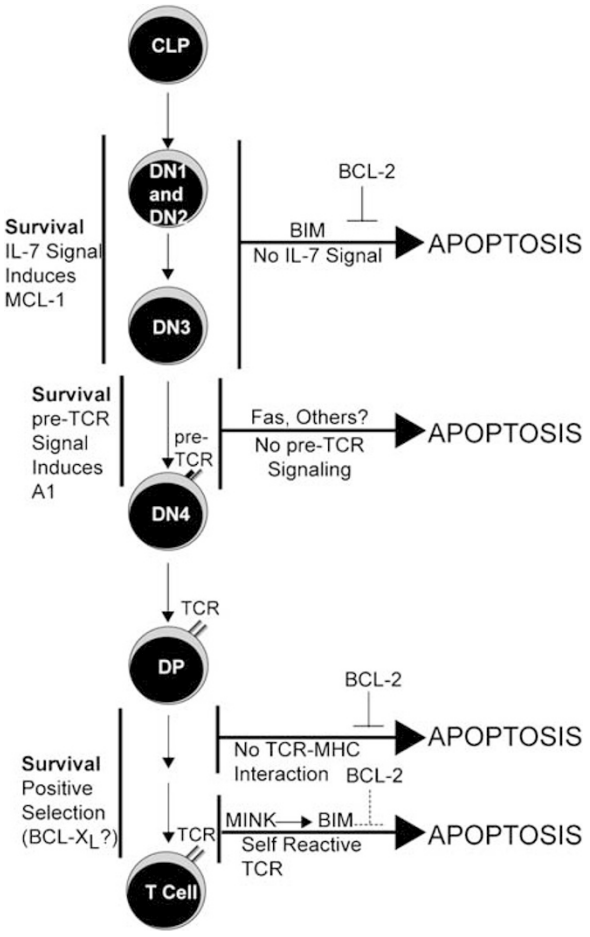

Figure 3 Apoptosis and survival during thymic development. Common lymphoid progenitor (CLP) cells migrate from the bone marrow to the thymus, there developing T-cell progenitors undergo development. This process is tightly regulated and several critical apoptotic checkpoints exist to maintain homeostasis and prevent the generation of potentially dangerous autoreactive T-lymphocytes. Early development (double negative (DN)1 and DN2 stages) are dependent on cytokine signaling to mediate the survival and differentiation. Pre-TCR signaling promotes survival and differentiation to the double positive (DP) stage in which the functional TCR is tested for recognition of self-MHC molecules in a process known as positive selection. Those DP cells that express TCRs with specificities that react too strongly to self-MHC are induced to die by negative selection. Those that have been positively selected downregulate either CD4 or CD8 and become mature single positive (SP) T-cells that are competent to leave the thymus and enter the periphery

Cytokines Regulate Survival in Early Lymphocyte Development

During both early T- and B-cell development, interleukin-7 (IL-7) has been demonstrated to be a critical cytokine required for both progenitor maturation and survival. IL-7 is a member of the common γ chain (γc) cytokine receptor superfamily that includes other critical factors (IL-2, IL-4, IL-9, IL-15, and IL-21) involved at various stages of lymphoid development and mature homeostasis (Figures 3 and 4).[24] IL-7 receptor is a heterodimer composed of the high-affinity IL-7Rα and the γc chains. Downstream of the receptor, IL-7 activates several signaling cascades including the Janus kinases (JAK)-1 and -3 that activates signal transducer and activator of transcription-5, phosphoinoside-3 kinase, Ras, and mitogen-activated protein kinase/extracellular signal-related kinase.

Mice targeted for the deletion of the *IL-7 receptor*, *IL-7* cytokine, *γc*, or *JAK-3* exhibit dramatic blocks in lymphoid development receptors. Such mice exhibit a severe combined immunodeficiency, lacking mature cells in both lymphoid lineages, in part due to a failure to promote the rearrangement of the antigen receptors (reviewed by Milne and Paige,[31] and Lee and Surh[32]). In addition to promoting maturation, these cytokines function to promote survival by regulating members of the BCL-2 family.[33–36] This is best illustrated by the ability of BCL-2 overexpression to facilitate the development of mature T-cells (but strikingly not B-cells) in mice deficient for the *IL-7 receptor* or the γc.[37–39]

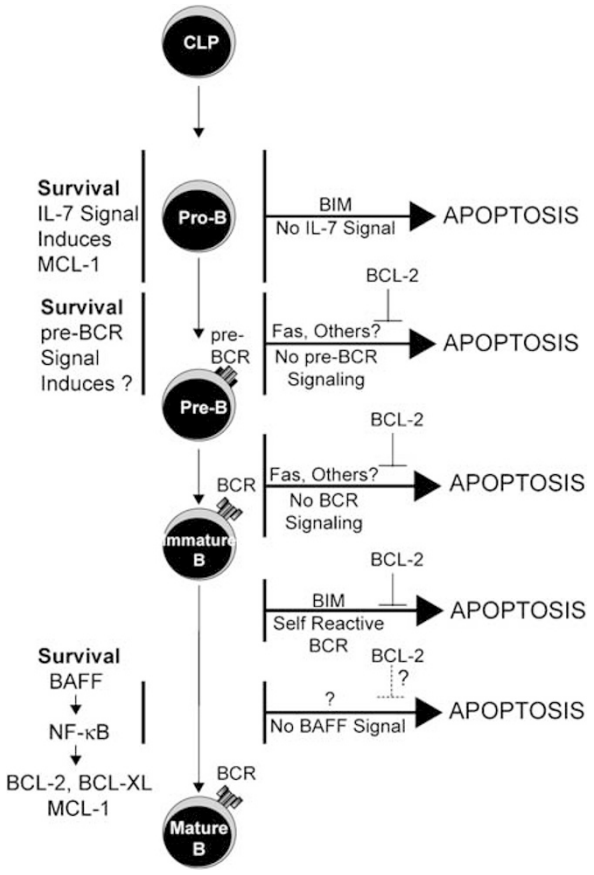

Figure 4 Apoptosis and survival during B-lymphocyte development. Common lymphoid progenitors (CLP) undergo development within the bone marrow into B-cell progenitors. Early pro-B-cells are dependent on IL-7 cytokine signaling to mediate their survival. Pre-B-cells express a pre-BCR complex that is tested for successful rearrangement of the immunoglobulin (Ig) heavy chain locus and those cells that do not express a receptor are deleted. Pre-BCR expressing cells undergo rearrangement of the Ig light chain and express a surface-bound BCR (IgM). In the bone marrow, immature B-cells expressing BCR with autoreactive specificities are deleted to prevent generation of self-reactive mature B-cells. Additionally, signaling mediated by the TNF family members BAFF are needed to promote the maturation of immature B-cells to the mature B-cell stage. Upon this maturation step, the B-lymphocytes are competent to enter the periphery

Although overexpression of antiapoptotic BCL-2 was capable of rescuing thymic development in mice lacking IL-7 signaling, genetic models have demonstrated that neither BCL-2 nor BCL-X_L are required for early lymphoid development (reviewed by Ranger *et al*.[40]). However, mice lacking antiapoptotic *MCL-1* during early lymphoid development exhibited an arrest in lymphoid development and an increase in apoptosis before antigen-receptor rearrangement strikingly similar to the phenotype of the *IL-7*- or *IL-7R*-deficient mice.[41] This finding implicated antiapoptotic MCL-1 as the target of IL-7 signaling essential for promoting the survival of developing lymphocytes in both the B- and T-cell lineages (Figures 3 and 4). MCL-1 protein is unique among antiapoptotic BCL-2 family members in that it has a short half-life and is the target of the ubiquitin/proteasome-dependent degradation pathway.[42] This degradation may be regulated by cytokines as growth factor withdrawal resulted in MCL-1 phosphorylation by glycogen synthase kinase-3β and enhanced ubiquitinylation.[43] These data suggest that during lymphocyte development, MCL-1 phosphorylation may play a critical role in regulating its protective function and expression, but this hypothesis still needs to be tested *in vivo*.

Various proapoptotic BH3-only family members have been implicated in inducing death of developing thymocytes upon growth factors withdrawal. Although in certain situations proapoptotic BAD may influence death in response to growth factor withdrawal, *BAD*-deficient animals do not exhibit any dramatic developmental abnormalities, nor are developing lymphocytes from these mice resistant to growth factor withdrawal suggesting that BAD is not a critical regulator of lymphoid development.[44] In contrast, *BIM*-deficient developing lymphocytes are resistant to growth factor withdrawal.[45] Indeed, when *BIM*-deficient animals were bred to *IL-7R*-deficient mice, there was a partial rescue of thymocytes development and a near restoration of mature T-cells in the periphery.[46] These data imply that while BIM is not the sole mediator of apoptosis in developing T-lymphocytes, it plays a substantial role in mediating death downstream of growth factor withdrawal. It has been demonstrated that loss of *PUMA* in cultured myeloid cells rendered these cells resistant to growth factor withdrawal.[47] Thymocytes from mice lacking both *BIM* and *PUMA* demonstrate an increased resistance to death by neglect, indicating that both BIM and PUMA act to mediate death owing to growth factor withdrawal during T-cell development.[48] However, in developing B-cells loss of *BIM* and *PUMA* is no different than loss of *BIM* alone, suggesting another BH3-only may act with BIM to regulate early B-cell development. These data suggest that PUMA and BIM may act in concert to regulate cell death downstream of growth factor withdrawal in some cell types.

The loss of proapoptotic *BAX* can partially compensate for genetic deletion of the *IL-7 receptor* during T-cell development in that it restored the cellularity of cytokine receptor mutant mice.[49] However, similar to *BCL-2* overexpression, loss of *BAX* was not able to overcome the defect in B-cell development in *IL-7R*-deficient mice. Thus, the death signals mediated by deficiencies in growth factor signaling are likely mediated primarily by the BH3-only family member BIM inducing the activation of proapoptotic BAX.

It is clear that the death induced by growth factor withdrawal is mediated by BH3-only family members as mice deficient in both critical multidomain proapoptotic molecules (*BAX* and *BAK*) are extremely resistant to growth factor withdrawal in both the T-cell and B-cell lineages.[50,51] This dramatic resistance appears to be more dramatic than the resistance observed in mice deficient in *BIM* demonstrating the critical role of BAX and BAK in integrating apoptotic signaling.

Antiapoptotic A1 in Pre-TCR Selection

The pre-TCR consists of a productively rearranged TCR-β chain, the invariant pre-TCR-α, and the CD3 signaling complex.[28] This complex is absolutely required for thymocyte development as mice lacking any of the components of the pre-TCR are blocked from further differentiation and undergo cell death (Figure 3).[52] Thus, the pre-TCR complex is essential not only for stimulating cell proliferation and further

development, but for sustaining the survival of thymic progenitors.

Signals transmitted through the pre-TCR primarily utilize the NF-κB signaling cascade to mediate the survival of developing T-lymphocytes.[53,54] Although several antiapoptotic BCL-2 family members have been implicated to be induced by the NF-κB pathway, only the antiapoptotic BCL-2 family member A1 was specifically induced in response to signals transmitted through the pre-TCR.[52] Ectopic expression of the *A1* gene in *recombination activation gene-1*-deficient thymocytes protected and promoted their further differentiation despite lacking a pre-TCR complex. Conversely, mRNA knockdown of *A1* impaired the survival of cultured pre-T-cell lines despite the continued expression of BCL-2 and BCL-X_L demonstrating the selective requirement for A1 in pre-TCR-mediated survival. These data demonstrate that antiapoptotic A1 is required to mediate survival during pre-TCR selection, but it is still unclear which proapoptotic BCL-2 family members are being antagonized by A1. Further studies will be necessary to identify such proapoptotic players.

Thymocyte Negative Selection

Thymocytes expressing TCRs that bind with high avidity to MHC molecules undergo apoptosis. It is clear that the death of such autoreactive thymocytes requires caspase activation as mice doubly-deficient for both *Caspase-3* and *Caspase-7* are dramatically resistant to TCR-mediated deletion despite being sensitive to death receptor ligation.[55] However, the mechanisms by which the effector caspases are activated are less certain. For example, mice in which their immune system have been reconstituted with *APAF1*- or *Caspase-9*-deficient fetal liver cells, both demonstrated no abnormalities in negative selection.[56,57] Furthermore, even lymphocytes lacking both *Caspase-2* and *Caspase-9* underwent normal apoptosis.[58] Therefore, how the effector Caspase-3 and Caspase-7 are being activated without these critical components of the apoptosome is still unclear.

One way of activating caspases independently of the apoptosome is by triggering death receptor signaling (Figure 1). However, the role of death receptor signaling during thymocytes negative selection is somewhat controversial. Negative selection is intact in mice lacking *Fas*, the death receptor adaptor *FADD*, or *Caspase-8* suggesting that these pathways are not required to clear autoreactive thymocytes.[8,59,60] It was proposed that the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required in thymocyte apoptosis.[61] However, an *in vivo* model of thymocytes negative selection found that negative selection occurred normally despite the loss of *TRAIL*.[62] These data support the previous findings that overexpression of dominant negative *FADD* in thymocytes, which would block both TRAIL and Fas-mediated apoptosis, has no effect on thymocyte negative selection.

Although death receptor-mediated apoptosis does not appear to be responsible for the enforcement of thymic central tolerance, loss of members of the BCL-2 family have profound effects on mediating apoptosis during thymocyte negative selection. Although genetic disruption of the BH3-only family members *BAD*, *BID*, *PUMA*, and *NOXA* have exhibited little or no effect on early lymphoid apoptosis, loss of the proapoptotic BH3-only family member *BIM* or the multi-domain proapoptotics *BAX* and *BAK* profoundly perturb thymocytes selection.[40,63] Initial studies in mice deficient for *BIM* demonstrated a massive expansion of the lymphocyte compartment and a predisposition of these animals to autoimmune pathology.[45] These animals exhibited a failure to delete autoreactive thymocytes (Figure 3).[64] Signals through the TCR may regulate BIM via the action of the serine-threonine kinase misshapen-Nck-interacting kinase, which mediates thymic negative selection by inducing BIM expression.[65] Therefore, BIM appears to be the critical mediator of cell death induced by negative selection in the thymus. Mice lacking both *BIM* and *PUMA* exhibit an even more dramatic increase in the number of peripheral lymphocytes than mice solely lacking *BIM*.[48] Thymocytes from these mice are also much more resistant to a variety of cell death stimuli than mice lacking either *BIM* or *PUMA* alone.[48]

The multidomain proapoptotic members, BAX and BAK, have been demonstrated to be the critical mediators of apoptotic signaling downstream of the action of BH3-only family members. Embryos and mice doubly-deficient for both *BAX* and *BAK* possess multiple abnormalities in cellular homeostasis, including in the immune system.[17] These developmental defects are quite severe, often resulting in embryonic lethality, but the few surviving mice display increased numbers of hematopoietic progenitors, lymphocytes, and exhibit immune cell infiltration of tissues including liver and kidney.[17,51] Thymocytes from these animals are also extremely resistant to a variety of death stimuli demonstrating the critical role of BAX and BAK for modulating death from a variety of intrinsic and extrinsic stimuli.[17] When *BAX* and *BAK* doubly-deficient mice were tested to identify the extent of negative selection, they were found to be completely resistant to death induced by endogenous self-antigens.[50] These data are consistent with BIM signaling requiring BAX and BAK to modulate the cell death stimuli at the mitochondria during the elimination of autoreactive thymocytes.

Interestingly, while loss of proapoptotic BCL-2 family members led to striking resistance to apoptosis induced by thymic negative selection, the overexpression of antiapoptotic BCL-2 family members BCL-2 or BCL-X_L has varying effects on negative selection. In response to various models of thymocytes death *in vitro*, overexpression of antiapoptotics can render thymocytes resistant to apoptosis (Figure 3).[66,67] However, overexpression of antiapoptotics does not protect thymocytes from negative selection to the same extent as *BIM* deficiency.[64,68–70]

The orphan nuclear hormone receptors Nur77 and Nor-1 are rapidly induced in thymocytes responding to TCR stimulation during negative selection.[71–73] Indeed, the constitutive expression of either Nur77 or Nor-1 in thymocytes induces massive apoptosis leading to a dramatic reduction in mature T-lymphocytes.[73,74] However, genetic ablation of either gene alone does not lead to any overt thymocyte selection defects. Transgenic expression of a dominant negative Nur77 protein, which can inhibit both Nur77 and Nor-1, does antagonize negative selection.[75,76] Therefore, it has been hypothesized that there may be redundancies between the family members. How Nur77 can induce

apoptosis is still unclear, but one proposed mechanism is that upon TCR stimulation Nur77 is translocated from the nucleus to the mitochondria where it interacts with BCL-2 inducing a conformational change that converts BCL-2 into a proapoptotic molecule.[77] Further experiments are necessary to clarify the mechanism behind these death-inducing molecules.

Deletion of Autoreactive B Cells in the Bone Marrow

The process of BCR rearrangement can lead to the development of cells with autoreactive specificities (Figure 4). Elimination of B-cell bearing self-reactive antigen receptors is initiated by the binding of self-antigens to surface Ig on immature B-cells within the bone marrow. The requirement for Ig engagement in triggering the elimination of autoreactive B-lymphocytes has been largely established by the generation of mice in which the B-lymphocytes express a single, transgene-encoded rearranged Ig gene specific for an antigen such as hen egg lysozyme (HEL) or MHC antigens.[78,79] In these animals, antigen-specific B-cells develop normally and populate peripheral lymphoid tissues, but when the transgene is expressed in mice that also express a membrane-bound form of the antigen on the surface of cells within the bone marrow, the HEL-specific B-cells are eliminated or induced to undergo receptor editing.[78,79]

The elimination of autoreactive B-cells is not Fas-mediated deletion. When *lpr* mice (which lack Fas expression) were bred to mice expressing BCR that recognize membrane-bound autoantigen these autoreactive B-cells underwent elimination as efficiently as B-cells bearing the Fas gene.[80] Overexpression of antiapoptotic *BCL-2* was capable of preventing the apoptosis induced by recognition of membrane-bound self-antigens, but could not prevent the immature B-cells developmental arrest.[81] Furthermore, in an immature B-cell line, inhibition of death receptor-mediated apoptosis by expression of a dominant negative FADD or the cowpox virus serpin CrmA were unable to block cell death in antigen-receptor ligation-induced apoptosis.[82] These studies suggested that elimination of autoreactive B-lymphocytes was mediated by the BCL-2 family.

Mice lacking the proapoptotic *BIM* exhibit an extensive autoimmune pathology.[45] Although this dramatic phenotype can be based in part on a failure to efficiently delete autoreactive T-cells, *BIM*-deficient mice also fail to eliminate autoreactive B-cells (Figure 4). To test the role of BIM in B-cell deletion, *BIM*-deficient mice were bred to doubly transgenic animals expressing both the anti-HEL Ig and membrane-bound HEL as a self-antigen. Loss of *BIM* results in the substantial rescue of autoreactive B-cells in the periphery of these mice.[83] Genetic deletion of both *BIM* and *PUMA* does not appear to lead to an increase in B-cell progenitor numbers when compared to loss of *BIM* alone.[48]

A conditional *BAX* allele was generated and bred to *BAK*-deficient mice to analyze the effect of *BAX* and *BAK* loss in the B-cell lineage.[51] Deletion of *BAX* and *BAK* in B-cells results in the accumulation of developing B-cells. Most dramatically both pro-B-cells and immature B-cells were increased.[51] These developing B-cells were also very resistant to apoptosis induced by BCR crosslinking. The resistance of the *BAX* and *BAK* double-deficient cells is somewhat more dramatic than those observed in *BIM*-deficient B-cells, suggesting that there may the contribution of other BH3-only member(s).

TNF Family Members in B-Cell Development

Members of the TNF family play critical roles in regulating immune homeostasis. The B-cell activating factors APRIL and BAFF (also widely known as BLyS) are TNF-related ligands that have been implicated in B-cell survival and costimulation.[84] Although *APRIL*-deficient mice have normal immune development, BAFF plays an important role in B-cell development.[85] Transgenic *BAFF* expression causes the accumulation of immature and mature B-cells, increased serum Ig levels, and a systemic lupus erythematosus-like syndrome presumably due to the survival of autoreactive B-cells in the bone marrow.[86] Conversely, ablation of *BAFF* results in profound block in B-cell development with normal numbers of pro-B-, pre-B-, and immature B-cells, but a complete lack of mature B-lymphocytes. This indicates that BAFF plays a critical role in mediating the transition through the BCR checkpoint and for the transition to long-lived B-cells (Figure 4).[87,88]

The receptors for BAFF and APRIL are expressed on the surface of B-lymphocytes. Three such receptors have been identified and genetic analyses have revealed their roles in lymphocyte development. For example, B-cell maturation protein (BCMA) and transmembrane activator and calcium modulator and cyclophillin ligand (TACI) were originally identified to interact with both BAFF and APRIL.[89,90] Deletion of *BCMA* did not result in any abnormalities in B-cell development or survival suggesting that TACI must be a redundant family member.[91] Transgenic expression of a soluble TACI-Ig, which can antagonize BAFF signaling, gave rise to a phenotype that mirrored the *BAFF*-deficient mouse; therefore, it was expected that a *TACI*-deficient mouse would have a similar phenotype. Unexpectedly, *TACI*-deficient mice exhibited hyperplasia in the B-cell lineage that closely resembled the effect of BAFF overexpression, suggesting that TACI may actually be an inhibitory receptor that antagonizes BAFF and APRIL signaling.[86,92,93] The inhibitory function of TACI and the lack of a phenotype in the *BCMA*-deficient mice implied that there must be another receptor that can mediate the survival functions of BAFF. Such a receptor known as BAFF-R or BR3 (BLyS receptor 3) was indeed identified by an expression-cloning strategy.[94,95] Unlike TACI and BCMA, BR3 is specific only for BAFF. *BAFF-R* has also been shown to be specifically disrupted by a spontaneously insertional event in the A/WySnJ mouse strain, which lacks peripheral B-cells, that results in the truncation of the receptor and the deletion of the last eight amino acids of the intracellular domain of the receptor.[94,95] It should be noted that A/WySnJ mice have more B-cells than mice deficient for *BAFF*, indicating that the *BAFF-R* allele in the A/WySnJ mice may represent a partial loss of function of the receptor. These data suggest that BAFF-R is the critical mediator of B-cell survival by BAFF.

BAFF signaling induced NF-κB activation and the upregulation of the antiapoptotic BCL-2 family members BCL-2, BCL-X, and MCL-1.[96,97] Indeed, constitutive activation of the

NF-κB pathway results in B-cell hyperplasia and obviated the BAFF-R-dependence of B-cell development. It also blocked the nuclear translocation of protein kinase C-δ, which had been shown to induce the death of B-cells by phosphorylating histone H2B upon growth factor withdrawal.[98,99] It remains to be tested whether bypassing the need for NF-κB signaling by constitutive expression of antiapoptotic BCL-2 family members can rescue the B-cell developmental defects due to *BAFF* deletion.

A Nonapoptotic Role for Caspases During Immune Development

While caspases are perhaps best known to be activated downstream of the apoptotic pathway, mice deficient in components of the death-receptor signaling pathway and associated caspases have revealed unanticipated defects during immune cell development and differentiation. Mice lacking *Caspase-8*, the death domain adaptor *FADD*, or the *Caspase-8-like inhibitory protein*, *cFLIP*, die during embryogenesis displaying impaired cardiac development.[40] A conditional allele of *Caspase-8* has yielded instructive in assessing the role of Caspase-8 in lymphocyte development and homeostasis.[7,8] Unexpectedly, inducible deletion of *Caspase-8* during bone marrow development caused hematopoietic progenitor cells to lose their ability to differentiate into lymphoid and myeloid lineages and to repopulate lethally irradiated recipients.[7] However, T-cell lineage-specific deletion of *Caspase-8* demonstrated that thymocyte development occurs normally in these mice. These cells were markedly resistant to cell death induced by anti-Fas treatment but exhibit normal sensitivity to death mediated by signaling through the TCR.[8]

Lack of FADD expression in thymocytes or the expression of a dominant negative FADD during thymocyte development also unexpectedly perturbed T-cell development. In these mice, early thymocytes development was retarded at the double-negative stage and mature peripheral T-cells were severely reduced.[60,100,101] Blockade of FADD in *recombination activating gene-1*-deficient thymocytes, which cannot rearrange their TCR alleles, allowed some cells to progress to the DP stage illustrating a role for FADD in promoting the death of thymocytes lacking the pre-TCR.[102] Furthermore, transgenic expression of cFLIP has been shown to increase TCR-induced proliferation.[103] Although germline deletion of *cFLIP* is embryonic lethal, a T-cell-specific loss of *cFLIP* generated T-cells in which proliferation and cellularity were reduced and survival was impaired.[104–106]

Components of the death receptor apparatus may be involved in the proliferation and differentiation of T-lymphocytes and may link TCR signaling to the NF-κB pathway.[107] However, other groups have demonstrated that T-cell lacking *FADD* or *Caspase-8* undergo normal NF-κB activation when stimulated through the TCR.[8,108] Therefore, it is still unclear as to the roles that FADD and Caspase-8 play during T-cell activation. Further dissection of death receptor-mediated apoptosis in lymphocyte development and activation promises to provide additional insights into its participation in autoimmunity and cancer.

Conclusion

A core programmed cell death process whose basic tenets are evolutionarily conserved is essential for the proper development of the immune system. Both extrinsic and intrinsic activation of the apoptotic program are responsible for ensuring homeostasis in the immune system. Cell death regulation safeguards the fidelity of lymphocyte responsiveness and serves to avoid the aberrations of immunodeficiency, autoimmunity, and cancer. Some control points in the pathway are well defined and rather absolute, such as the need for multidomain BAX and BAK to effect intrinsic pathway deaths. Yet the integration between specific proximal signals unique to lymphocytes at each specific stage of development is less clear-cut. BH3-only members are a natural control point for regulating cell fate as they are responsive to transcriptional or post-translational modification in response to distinct signals. The TNF family clearly regulate some aspects of the BCL-2 family, but also link the apoptotic program to direct caspase activation in both apoptotic and nonapoptotic roles. Understanding the key steps that confer specificity throughout the myriad of regulatory pathways may be essential for selectively intervening in the aberrations of immunodeficiency, autoimmunity, and cancer.

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