

Memory and death in the immune system

Apoptosis

Apoptosis refers to a form of death in which a cell initiates a suicide program resulting in characteristic morphological changes that accompany death. These changes include chromatin condensation, nucleolar disruption and cytoplasmic contraction..

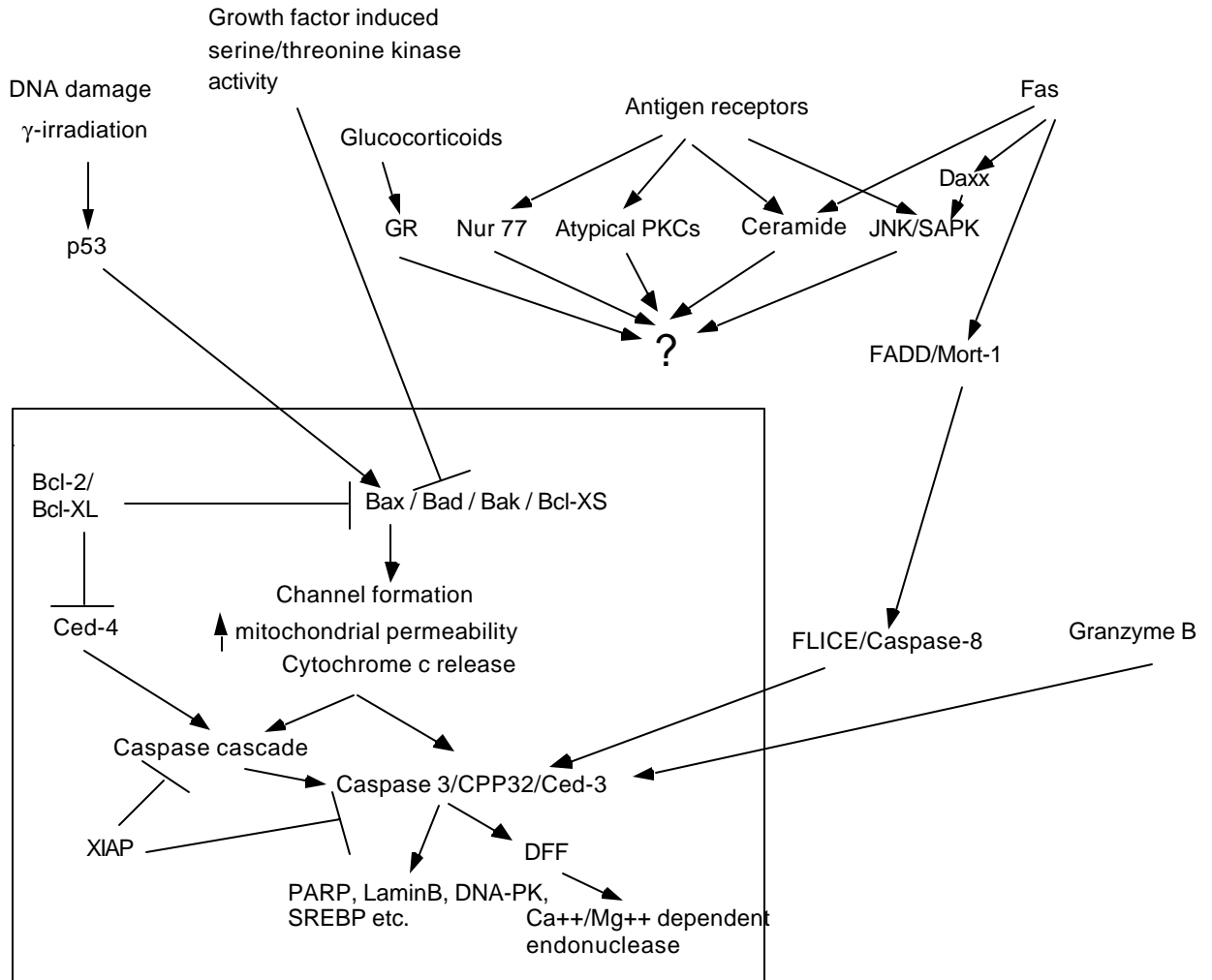


Figure 1. An overview of pathways leading to apoptosis. The "common death pathway" is highlighted in the box. GR refers to the glucocorticoid receptor. PKC refers to protein kinase C. Some atypical PKCs may prevent apoptosis while others may contribute to it. Similarly the JNK pathway may actually protect lymphocytes from apoptosis.

Apoptosis is often readily recognized by the cleavage of DNA into a "ladder" of oligonucleosome length fragments. This form of death is also referred to as "programmed cell death" or "physiological cell death".

Apoptosis is of critical importance in the development of virtually every multicellular organism. This form of death is initiated many times during lymphoid ontogeny - during the process of negative selection and whenever lymphocytes fail to be positively selected. The termination or down-regulation of immune responses is also in part achieved by the induction of the apoptotic death of activated T and B cells. Cytotoxic CD8+T, Natural killer cells, as well as CD4+ T lymphocytes express specific

molecules that are designed to assist in the execution of targets by apoptosis. The expression or activation of genes whose products (such as the Fas ligand, Perforin, and Granzyme B) are involved in the apoptotic elimination of other target cells is an important aspect of lymphocyte development.

The induction of DNA damage, the activation of a stress response, the withdrawal of growth factors, or the triggering of specific signaling receptors - all of the above may lead to the initiation of apoptosis. The cell type or state of differentiation of a cell can influence whether a particular receptor induces proliferation or apoptosis. Apoptosis may be kept at bay in certain cell types by the induction of anti-apoptotic signals. Death may occur by default in other cell types or stages of differentiation when proliferative signals are withdrawn, presumably because in these cells protective pathways are not turned on.

Some of the signals involved in the initiation of apoptosis in lymphocytes are outlined in Figure 1. The death of large pre-B and double negative T cells that fail to be selected by the pre-B and pre-T receptors may reflect the failure of these cells to receive cell survival or growth promoting signals. Signals from antigen receptors induce apoptotic death primarily in immature B and CD4+/CD8+ T cells as part of negative selection. The death by default of CD4+/CD8+ T cells may be mediated by endogenous glucocorticoids and the glucocorticoid receptor (GR). Apoptosis of activated T and B cells as well as of B cells that have been activated as bystanders (and not by specific antigen) depends on triggering of the Fas receptor. The Fas pathway may also be used by CD4+ T cells to kill infected targets. Cytotoxic T lymphocytes (CTLs) and Natural Killer (NK) cells both secrete proteins such as perforin and Granzyme B to induce apoptosis of their targets.

The common death pathway

Over the past few years a molecular understanding of a common apoptotic death pathway has emerged from a range of studies in a number of organisms and cell types. This pathway is summarized in the box in Figure 16.

The induction of death is generally linked to the activation of a set of cysteine proteases that cleave proteins immediately after aspartic acid residues. The first such enzyme to be identified was the mammalian homolog of the *C. elegans* cell death gene, Ced-3. This protein was identical to an enzyme previously described as the Interleukin-1 β Converting Enzyme (ICE) which was known to be

responsible for proteolytically converting pro-Interleukin-1 β to mature IL-1. It soon became apparent that the ICE protease was a member of a large family of related proteases most of which appear to be involved in cell death. These enzymes are now referred to as caspases. Apart from ICE itself (caspase 1) a critical mediator of cell death appears to be caspase-3 (also known as CPP32/ Yama/Apopain). Activation of the caspase cascade may depend on the induction in the cell of a reducing state which would favor the catalytic activation of these cysteine proteases (which contain a QACRG motif in which the cysteine forms a part of the active site).

A number of substrates of caspase-3 have been identified. These include Poly-ADP ribose polymerase (PARP), huntingtin (the protein product encoded by the gene that is mutated in Huntington's disease), lamin B, the sterol regulatory element binding proteins (SREBPs), D4-GDI, and the U1 associated 70 kD protein. The relevance of these substrates to the initiation or progression of apoptotic death is unclear. A particularly interesting substrate of caspase-3 is the DNA Fragmentation Factor or DFF. DFF is a cytosolic heterodimeric protein that consists of a 45kD subunit and a 40 kD subunit. DFF is activated by caspase-3 mediated cleavage of the 45 kD subunit at least two cleavage sites. The activated DFF in turn, by an unknown process, contributes to the activation of a Ca⁺⁺/Mg⁺⁺ dependent endonuclease which is responsible for the fragmentation of DNA that is characteristic of apoptosis.

Caspases are activated by proteolytic cleavage after specific aspartate residues. Cleavage of a given caspase may be mediated by another member of the family or by autoproteolysis. In the case of Fas signaling, a caspase molecule, caspase-8 is activated and targets caspase-3. Cytotoxic or NK cell mediated killing (also discussed later in the chapter) involves the introduction of a caspase-3 activating protease, Granzyme B, into the target cell's cytosol. A more general mechanism of caspas-3 activation may involve the induction of an increase in mitochondrial permeability during apoptosis and the release of activators of caspase-3 such as cytochrome c and apoptosis inducing factor (AIF) from the inter-membrane spaces surrounding mitochondria into the cytoplasm. The mitochondrial permeability transition involves the opening of large pores in the inner mitochondrial membrane which may contribute to the release of mitochondrial Ca⁺⁺ stores into the cytosol and possibly the generation of oxygen free radicals. An increased permeability of the outer mitochondrial membrane

be negatively or positively selected. In the B lineage, for instance, Bcl-2 levels are high in the earliest pro-B stages but late in pro-B development Bcl-2 levels drop and stay low through the pre-B and immature B stages. This drop presumably is permissive for the elimination/selection of lymphocytes at late pro-B, pre-B and immature B cell stages. Bcl-2 is expressed at relatively high levels in long-lived IgM⁺/IgD⁺ follicular B cells. In the germinal center, Bcl-2 levels drop once again in dark zone centroblasts and basal light zone centrocytes, presumably to permit selection processes to occur in the immediate aftermath of somatic mutation (Figure 2). Similarly in the T lineage Bcl-2 is expressed at relatively high levels in double negative (CD4⁻/CD8⁻) T cells, levels drop in the double positive stage (CD4⁺/CD8⁺) when positive and negative selection events occur, and high levels of this protein are expressed in peripheral CD4⁺ and CD8⁺ stages.

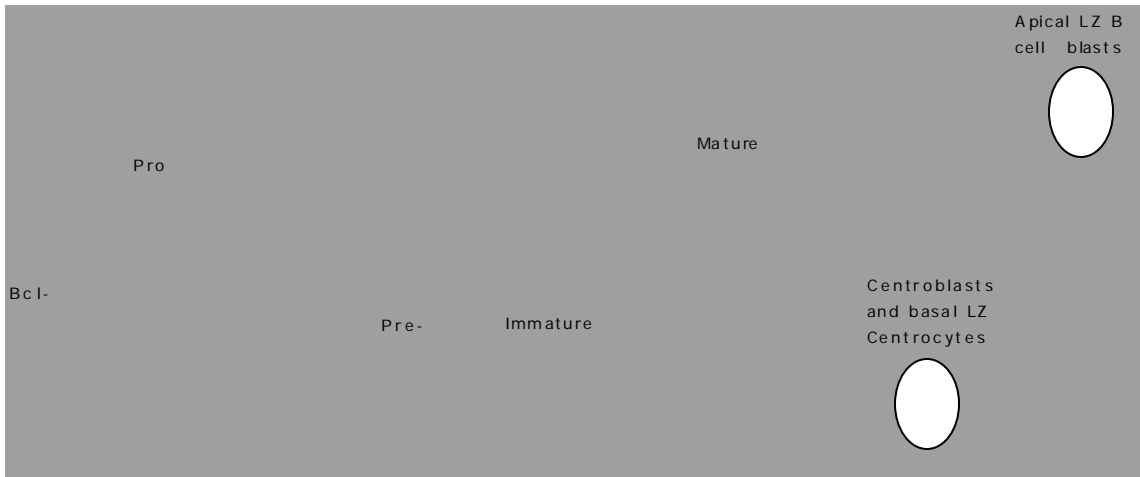


Figure 2. The levels of Bcl-2 are regulated during B cell differentiation. High levels of Bcl-2 are expressed in pro-B cells, at the IgM^{hi}/IgD^{hi} and IgM^{lo}/IgD^{hi} follicular B cell stages and in the apical light zone B cell blasts in germinal centers.

Bcl-X_L is structurally very similar to Bcl-2 (Figure 3) and is induced when B and T cells are activated. Bcl-X_L is expressed in pre-B cells at a time when signals are delivered via the pre-B receptor and it is probably induced as part of the positive selection process. Other members of the Bcl-2 family which are antagonists of apoptosis include A1 and Mcl-1.

A number of Bcl-2 family members function as death agonists. These include Bax, Bad, Bak, Bik and Bcl-X_S. Bcl-X_S is an alternatively spliced version of Bcl-X_L which lacks

also occurs and this may be regulated by channels formed by the pro-apoptotic Bcl-2 family member, Bax (see section below). This latter event may be crucial for the activation of caspase-3 by cytochrome c and AIF.

An important activator of caspase activity, yet to be examined in a lymphoid context, is Ced-4. In *C. elegans* the Ced-4 gene is believed to play a role in the activation of Ced-3 (the worm equivalent of caspase-3). A mammalian Ced-4 homolog remains to be identified. Ced-4 dependent activation of Ced-3 is inhibited by members of the Bcl-2 family and this phenomenon will be discussed below.

Regulation of the death pathway by members of the Bcl-2 family

An important inhibitor of apoptotic death in lymphocytes was first identified in the course of the molecular characterization of a chromosomal translocation between chromosomes 14 and 18 seen in patients with follicular lymphoma. This translocation brings the immunoglobulin heavy chain locus on chromosome 14 in apposition with a gene on chromosome 18. The gene activated at this breakpoint, and which is thus inappropriately expressed, plays a role in the process of oncogenic transformation, and is known as Bcl-2. The Bcl-2 protein is a 26 kD molecule that is found in the outer mitochondrial membrane as well as in the ER membrane. Bcl-2 inhibits apoptosis in a number of cell types and this gene is the mammalian homolog of the *C.elegans* ced-9 gene (which antagonizes death induced by ced-4 and ced-3). Genetic analyses in the worm have revealed that ced-4 is an upstream activator of ced-3 (the worm caspase equivalent) and that ced-9 is upstream of ced-4 and can inhibit the activity of the latter.

In mammalian cells a large family of Bcl-2 like proteins has been identified. Some members of the family are, like Bcl-2, antagonists of cell death while others may induce apoptosis. In terms of lymphocyte development Bcl-2 and Bcl-XL (the long form of Bcl-X) are particularly important anti-apoptotic members of the Bcl-2 family. Bcl-X is a more readily induced member of this family and can be alternatively spliced to yield a long form (Bcl-XL) which is anti-apoptotic and a short form (Bcl-XS) which is pro-apoptotic. Bcl-2 may be viewed as a "maintenance factor for survival" while Bcl-XL is apparently induced by external signals when cells need to be instructed to survive. Accordingly the levels of Bcl-2 appear to be down regulated during periods of lymphocyte development when cells need to

the BH1 and BH2 regions which play an important role in Bcl-2 and Bcl-XL. Both Bcl-2 and Bcl-XL are type II membrane proteins, with C-terminal transmembrane anchor domains. Most members of the Bcl-2 family have very similar structures in certain regions which have been described as the BH1, BH2, BH3 and BH4 regions. Bcl-2 and Bcl-XL can form homodimers or heterodimerize with antagonists such as Bax. The BH1, BH2 and BH3 regions form a hydrophobic groove into which the BH3 domain of a partner might slide in during the formation of dimers. A relative excess of anti-apoptotic Bcl-2 members in a cell is assumed to promote survival while an excess of pro-apoptotic proteins such as Bax presumably leads to apoptosis. This "dueling dimer" model is still likely to be generally valid, although there are now two distinct models for the topology and function of the Bcl-2 family of proteins.

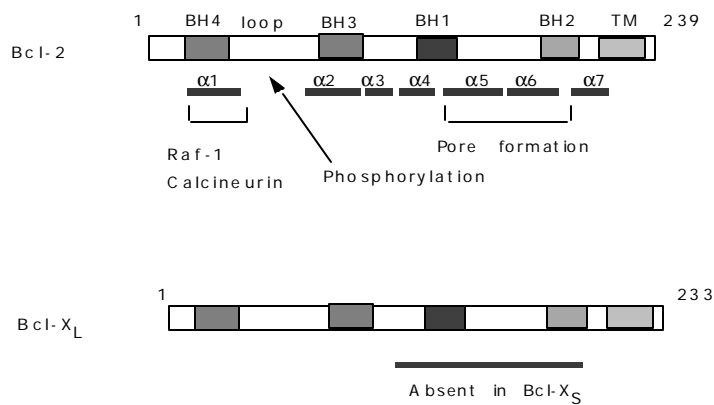


Figure 3. Structural features of Bcl-2, Bcl-XL and related proteins. The BH1, BH2, and BH3 regions form a hydrophobic groove into which the BH3 region of a partner may slide into. C-terminal hydrophobic anchors are present in most family members but not in Bad. The $\alpha 5$ and $\alpha 6$ helices are probably essential for the channel forming activities of Bcl-2, Bcl-XL, and Bax. The flexible loop region between the first two helices is a site for phosphorylation which contributes to negative regulation of function. The BH4 region may be essential for the association with Raf-1 and calcineurin.

inner mitochondrial membrane but Bax channels may play a role in the efflux of cytochrome C and AIF into the cytosol.

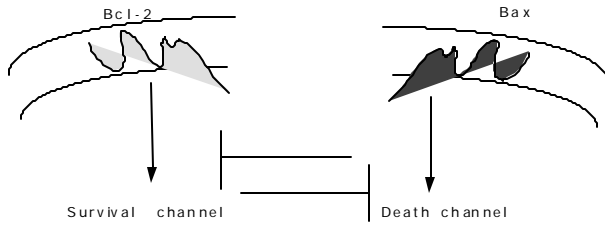
Bcl-XL has also been demonstrated to form channels in vitro. The $\alpha 4$ and $\alpha 5$ helices are very hydrophobic and may potentially span the membrane allowing the other 5

It is unclear whether Bcl-2 family members function primarily within intracellular membranes or whether they have extended cytoplasmic domains. There is growing evidence that both Bax and Bcl-2 can function as channels. Bax may function in the outer mitochondrial membrane to contribute to the mitochondrial permeability transition that accompanies apoptosis. Megapores formed during apoptosis are in the

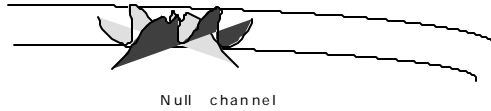
amphipathic helices to open out in an umbrella like fashion at the interface with the intermembranous space. While Bax channels might contribute to apoptosis, Bcl-2 and Bcl-XL might either form counter-channels that functionally antagonize pores formed by Bax, transporting cytochrome c back into the mitochondrion for instance, or contribute to occluding Bax channels when they heterodimerize (Figure 4).

All channel models assume that Bcl-2 and related proteins must be membrane anchored. Although most members of this family are type II membrane proteins, in some experiments removal of the transmembrane anchor at the C-terminus did not significantly compromise Bcl-2 function. One of the alternative models for Bcl-2 function suggests that membrane anchored Bcl-2 may sequester Ced-4 associated with Caspase-3/Ced-3 and prevent Caspase mediated cleavage of death targets. When Bcl-2 associates with a death inducing partner such as Bax, it can no longer hold back the Ced-4 - Caspase complex and death ensues. Although the mammalian homolog of Ced-4 remains to be identified, worm Ced-4 has been shown to interact with both Ced-9 and mammalian Bcl-XL and to form a physical bridge between Ced-9 and Ced-3.

CHANNEL MODEL 1



CHANNEL MODEL 2



CYTOSOLIC SEQUESTRATION MODEL

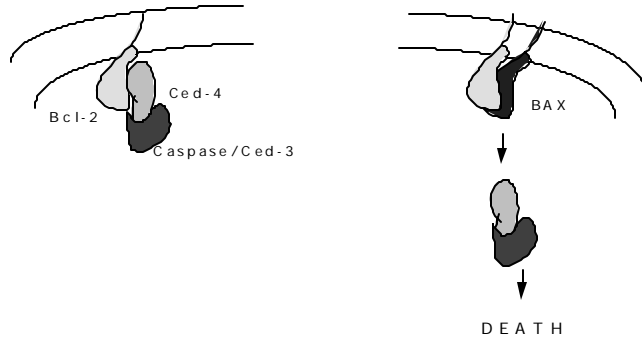


Figure 5. Mechanisms of anti- and pro-apoptotic activity of Bcl-2 family members. Regulation by phosphorylation is not considered in this figure. Bax may form channels permitting the efflux of apoptogenic proteins into the cytosol. Bcl-2 may form channels that antagonize Bax channels or may heterodimerize with Bax to neutralize bax channel activity. Alternatively Bcl-2 might, in a channel-independent manner, sequester Ced-4-caspase complexes preventing death.

irradiation), an important event appears to be the transcriptional induction of Bax. The bax promoter can be activated by p53 and contains a p53 binding site.

Growth factor receptors may sustain cell survival by inducing the serine kinase dependent phosphorylation of pro-apoptotic members of the Bcl-2 family such as Bad. Bad lacks a C-terminal hydrophobic domain. In its active non-phosphorylated form it can associate with Bcl-2 or Bcl-XL and neutralize their anti-apoptotic activity. However when Bad is phosphorylated on a critical serine residue in the

How do signals from the cell surface influence the activity or function of Bcl-2 family members? The expression of Bcl-2 can be induced in germinal center B cells by signals that promote the selection and survival of B cells. The molecular basis for the up-regulation of Bcl-2 expression by antigen receptor signaling and by CD40 is poorly understood. A Bcl-2 homolog, A1, which is not known to be expressed in lymphocytes, is expressed in myeloid cells which have been induced by granulocyte-macrophage colony stimulating factor. Another Bcl-2 homolog that is expressed in myeloid cells is Mcl-1. The mcl-1 gene can be induced in these cells by phorbol esters. In the case of apoptosis that is induced in a p53 dependent manner (in response to DNA damage or γ -

flexible loop region between the first two α -helices, presumably by protein kinase A, it preferentially associates with 14-3-3 proteins in the cytosol and can no longer efficiently inhibit Bcl-2 or Bcl-XL.

The N-terminal BH4 domain of Bcl-2 and Bcl-XL is essential for the association of these survival factors with c-Raf as well as calcineurin. This BH4 structure is found in anti-apoptotic members of the Bcl-2 family and not in pro-apoptotic relatives. It has therefore been argued that the recruitment of c-Raf and calcineurin may help Bcl-2 and Bcl-XL mediate survival functions. c-Raf is a serine/threonine kinase and may phosphorylate Bad thus inhibiting the ability of the latter to induce apoptosis. How calcineurin recruitment to intracellular membranes facilitates survival is less clear. It has been suggested that cytosolic calcineurin, a Ca^{++} regulated phosphatase, may promote apoptosis by dephosphorylating Bad.

Bcl-2 itself can be inactivated by serine phosphorylation of residues in the loop region. How exactly this event is regulated by extracellular signals remains to be established.

Bcl-2 was originally discovered as an oncogene, as a survival protein which was constitutively expressed in the B lineage because its expression was driven by the immunoglobulin heavy chain enhancer. It is of interest to note that during evolution a number of viruses have co-opted Bcl-2 like proteins and these proteins permit the transformation of mammalian cells by these viruses. Notable examples of Bcl-2 like proteins include the adenovirus E1B 19K protein, the Epstein-Barr virus BHRF-1 protein. Both of these proteins functionally mimic Bcl-2. The African swine fever virus also has a Bcl-2 homolog, LMMW5-HL, which has not been extensively studied.

Antigen receptor mediated apoptosis during negative selection

Both in the T lineage as well as in the B lineage self-reactive lymphocytes may be eliminated when they are stimulated very strongly during a susceptible period of development. In B cells this period is presumed to be the immature B stage in the bone marrow. There is increasing evidence that immature B cells that receive strong crosslinking, potentially life-threatening, signals most often evade death by the process of receptor editing.

The process of negative selection was once believed not to require the Fas-FasL pathway. This view has been challenged and it is likely that negative selection involves

signal transduction via the antigen receptor as well as triggering of the Fas molecule.

Our current understanding of negative selection is as follows. Early in B and T cell development before the CD4/CD8 double positive T cell stage and the IgM+/IgD- immature B cell stages the levels of Bcl-2 in these cells drop quite dramatically, as a preparatory step to permit positive and negative selection to operate. At these equivalent stages of development in the B and T lineages death ensues if lymphocytes are triggered by "strong" stimuli - high affinity self-peptide bound to MHC molecules in the case of T cells and multivalent membrane bound antigens for B cells. One critical feature is that these cells have low basal Bcl-2 levels and can be easily triggered to die. These cells also express relatively high levels of Fas. Activation of these cells via the antigen induces the Fas ligand (FasL) and this leads to the triggering of the Fas pathway as well. The molecular mechanisms involved in Fas mediated death are beginning to be understood. The induction of FasL in T cells depends on the activation of NFATp (inhibited by cyclosporinA) and also probably involves a transcription factor known as Nur77. Nur 77 is an orphan steroid hormone receptor which is induced by TCR crosslinking and may be involved in regulating FasL expression.

Other events (apart from the induction of FasL) may be involved in antigen receptor mediated apoptosis. Studies in B cell lines susceptible to antigen receptor mediated apoptosis have suggested that the ceramide pathway may be induced and may contribute to apoptosis. It is possible that atypical PKC family members such as PKC δ may also be activated and lead to the induction of apoptotic death.

Glucocorticoids and thymocyte apoptosis

It has long been observed that immature double positive thymocytes are exquisitely susceptible to the induction of apoptosis by high doses of exogenous glucocorticoids. At times of systemic stress, high levels of endogenous steroids may induce thymic atrophy. However lower levels of steroid may serve an anti-apoptotic function. Endogenously produced steroids in the thymus, may at their physiological low levels, antagonize TCR signaling in double positive thymocytes induced by moderate to low avidity ligands and thus facilitate positive selection. TCR signaling induced by high avidity ligands may be too strong to be abrogated by glucocorticoids, and it has been suggested that endogenous glucocorticoids may thus help set thresholds for TCR

signaling to mediate deletion versus positive selection. It has been suggested that "death by neglect" in the thymus of T cells that cannot recognize self MHC with any affinity, might depend on T cells being triggered by endogenous steroids.

Signals that abrogate default apoptosis

There are a number of pathways that are believed to be anti-apoptotic. Many of these pathways culminate in the activation of an anti-apoptotic member of the Bcl-2 family or the inactivation of a pro-apoptotic member of the same family. Early in T cell development activation of the IL-7 receptor provide survival signals possibly by influencing the levels of Bcl-2. Antigen and pre-antigen receptor signals can presumably contribute to the up-regulation of Bcl-X_L. As noted in the section above, NFκB activation has been linked to the prevention of apoptosis. It is possible that NFκB contributes to the induction of anti-apoptotic Bcl-2 family members but this remains to be formally established. NFκB could help abrogate apoptosis via the induction of c-myc as discussed above, a phenomenon that has so far only been shown to be relevant in a B cell line. It has been suggested that in some cells NF-κB activated downstream of TNFRI might inhibit apoptosis by the transcriptional activation of c-IAP1 and c-IAP2. A few growth factor receptors including the IL-3 receptor, have been shown to prevent the induction of apoptosis by negatively regulating the function of pro-apoptotic proteins such as Bad and Caspase-9 via a PI3K dependent survival pathway, which may also contribute to the activation of NFκB and forkhead transcription factors as outlined in Figure 6.

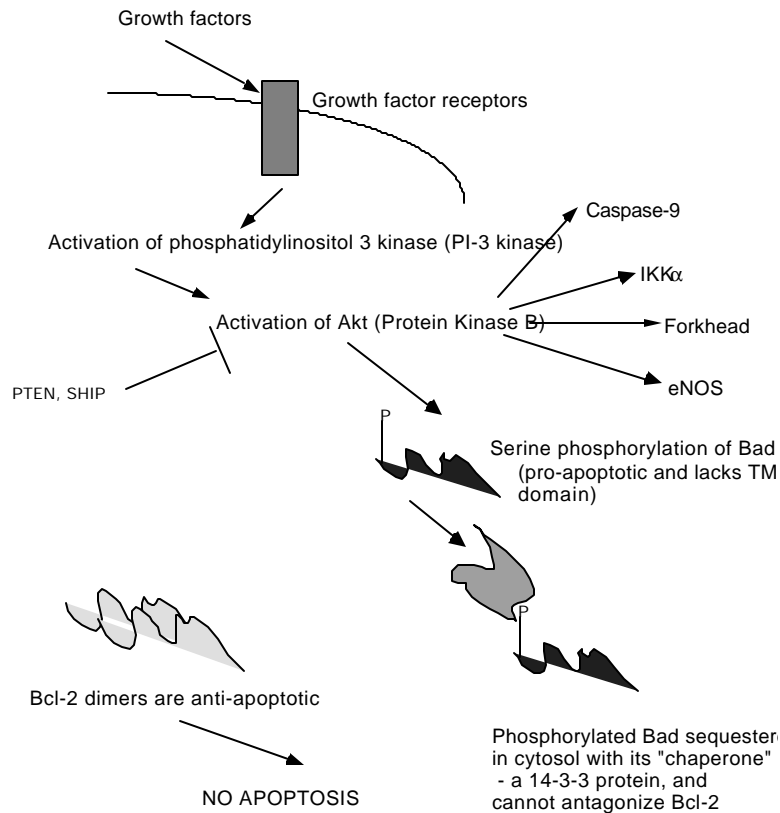


Figure 6. Abrogating default death by interfering with dueling dimers and other apoptotic mechanisms. Growth factor receptors may activate PI-3 kinase. PIP3 generation may be opposed by SHIP and by PTEN.. Activation of Akt may contribute to the abrogation of death signals in a number of ways. Potential consequences of Akt activation include the inactivation of Bad, the activation of NF κ B, the inactivation of procaspase 9, the reduced synthesis of FasL (as a consequence of forkhead phosphorylation), and the activation of eNOS or endothelial NO synthase.

Growth factors such as IL-3 activate the PI-3 kinase pathway. Activation of PI-3 kinase may occur by a number of means including recruitment of the regulatory p85 subunit to phosphotyrosinylated proteins or via Ras activation. These events have been discussed in Chapter Four. A target of PI-3 kinase is the serine-threonine kinase known as Akt (also known as protein kinase B or RAC α). Akt can phosphorylate a pro-apoptotic Bcl-2 family member known as BAD on a critical serine residue. BAD lacks a transmembrane domain and can interfere with the function of Bcl-2 or Bcl-XL. When BAD is phosphorylated on serine 112 or serine-136 by Akt or by other serine-kinases it is inactivated. Inactivation involves the sequestering of BAD by a protein known as 14-3-

3 which prevents BAD from associating with and inhibiting the function of Bcl-2.

Another way in which Akt contributes to cell survival is by the induction of pro-caspase-9 phosphorylation. Phosphorylation of a critical serine residue (serine-196), on pro-caspase-9, prevents the activation of this pro-caspase in a cytochrome c dependent manner. Akt may also contribute to the induction of anti-apoptotic transcriptional regulators, particularly members of the winged-helix/forkhead family. Phosphorylated forkhead proteins may be retained in the cytosol and thus the transcription of specific pro-apoptotic genes including the gene encoding the Fas ligand may be transcriptionally inactivated. Akt can also phosphorylate and activate IKK α thus contributing to the activation of NF κ B. A less well understood mechanism of protecting cells from apoptosis may depend on the Akt dependent activation of endothelial nitric oxide synthase, and the increased production of endothelial NO.

Ending T cell responses and Activation Induced Cell Death

By using artificially generated reagents such as labeled MHC class I-peptide tetramers, it has been possible to follow the fate of antigen specific CD8⁺T cells in infected mice and in human patients. It is now recognized that during an active immune response a very large number of the T cells in the host represent the clonal outgrowth of a few antigen specific CD4⁺ or CD8⁺ T cells. Examination of these clones has revealed that a large proportion of activated T cells are in the process of undergoing apoptosis. While large numbers of T cells do get activated by antigen most of these cells probably die because the amount of antigen is limiting and these cells no longer receive survival signals. Another major way in which activated T cells may be rendered quiescent is by the induction of inhibitory signaling via CTLA-4. Some cells which are restimulated by antigen also receive signals to die.

AICD is probably as important for the elimination of activated CD8⁺ cells as it is for the elimination of CD4⁺ cells. However it has been examined mainly in the context of CD4⁺ cells. Evidence from *lpr* and *gld* mice, from human lymphoproliferative syndromes involving Fas mutations, and from IL-2 and IL-2R α chain knockout mice has all come together to suggest the following scenario. When both Signal One and Signal Two combine to generate maximal T cell activation, transcriptional induction of both the IL-2 gene

and of the IL-2R is achieved. This form of complete activation leads to signal transduction via the IL-2R and the maximal induction of both CD40L and FasL. During the course of these events the T cell makes cytokines, and may activate specific B cells and professional APCs via CD40L-CD40 interactions as well as by triggering specific cytokine receptors.

The majority of properly activated T cells commit suicide. They do so because the FasL induced on individual T cells triggers Fas receptors expressed by the same cell and thus induces death. We have considered the FasL-Fas pathway in Chapter Five. A potentially important aspect of this pathway is the destruction by caspases of Bcl-2 and other Bcl-2 family survival factors. Signaling via the IL-2 R is critical for the induction of AICD, probably because signals delivered via this cytokine receptor are required for the maximal induction of FasL. In addition IL-2 signaling may contribute to an inhibition of the cellular levels of FLIP. FLIP is a protein that is structurally similar to Caspase-8 but which lacks proteolytic activity. It can prevent the recruitment of Caspase-8 to FADD and thus protect a cell from Fas induced apoptosis. The reduction of FLIP levels by IL-2 may be key in allowing the suicide process to go through successfully.

In CD8⁺T cells AICD might require signals to be delivered both by IL-2 as well as by TNF- α . Given that "proper" activation of a T cell leads to death, it is pertinent to ask how memory T cells are ever generated.

Making the choice between activation and memory: memory T cells

The existence of "true" memory lymphocytes has often been questioned and while their existence remains controversial, there is a growing acceptance of the view that such cells do exist. The persistence of antigen, either preserved by a low-grade viral infection or as part of long-lived immune complexes sequestered by follicular dendritic cells, could potentially contribute to the identification of recently restimulated cells as "memory" cells. The best evidence for the existence of "true" memory cells has come from studies on the transfer of CD8⁺ cells in the apparent absence of antigen which can result in the transfer of immune responsiveness for extended time periods. Although many of the central issues regarding memory remain controversial, there is some evidence to suggest that memory CD8⁺ cells can be maintained by being "tickled" via their TCRs even by non-specific, MHC class I-peptide complexes.

Antigen, even if it is only required in a cross-reactive non-specific form, may well be required for the maintenance of what may well be "true" memory T cells. These cells may be best described as long-lived antigen specific T cells that emerge after activation by specific antigen but which do not require specific antigen for their extended *in vivo* survival.

The following general features characterize memory T lymphocytes (whether or not they pass the litmus test of antigen-independent survival):

1. Memory cells apparently survive for a long time *in vivo* in the apparent absence of specific antigen. They may survive as long as the lifespan of some small vertebrates.

2. Memory lymphocytes generally present with an activated phenotype, but can be distinguished from effector cells on the basis of size and function. They are smaller than effector cells. Effector CD4⁺ cells may secrete large amounts of cytokines whereas memory CD4⁺ cells may need to be triggered in order to do so. Effector CD8⁺ cells may be able to kill *ex vivo* targets directly while memory cells need to be triggered in order to be able to kill.

3. Memory cells do not require antigen for survival

4. Memory T cells can be activated by signals which may be below the threshold for the activation of naive T cells. This may in part be due to the high levels of adhesion molecules expressed on memory T cells.

5. Most memory cells probably live for a long time because they have been programmed by antigen exposure to express high levels of survival factors which include but may not be restricted to members of the Bcl-2 family.

In humans, memory T cells may be categorized, into "central" memory cells which express CCR7 and traffic like naïve T cells to lymph nodes, and into "effector" memory cells which do not express CCR7 and return to tissue sites. Memory cells as defined in the mouse may be generally considered to be of the "central" variety" whereas cells generally referred to as effector T cells in the mouse may be synonymous with human "effector" memory cells.

The vast majority of effector T cells that are generated during an immune response are eliminated by AICD. A number of models have been proposed to explain how memory cells are generated. The most likely scenario is as follows: Following T cell activation a very large number of effector T cells are generated. As antigen is cleared, most of the vigorously activated T cells either die because they are no

longer being stimulated or undergo AICD. A small number of these cells, perhaps those that were not activated as well because they arrived late on the scene, or those which were activated by distinct APCs in a slightly less vehement manner, fail to induce the suicide pathway and may go on to become memory cells. For CD8+ cells it has been established that effector cells go on to become memory cells. It is also possible that certain effectors are induced to "rest" and become memory cells, or that distinct activated cells give rise to effector cells and memory cells (Figure 7).

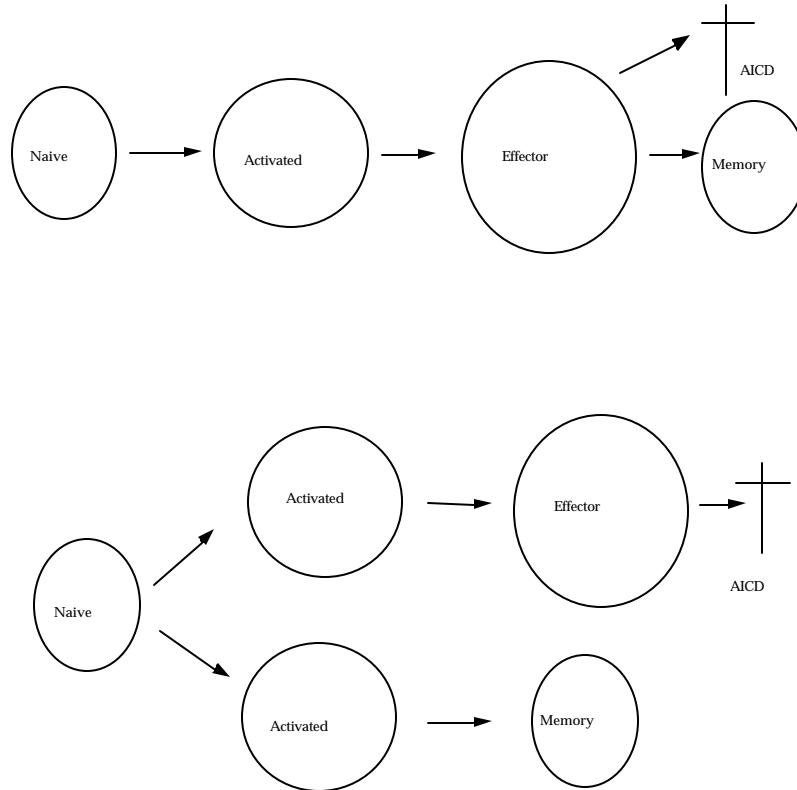


Figure 7 Models for memory T cell generation. Memory cells share many features of effector cells. However effector cells tend to be larger than memory cells. In a linear model for memory cell generation (which has experimental support in CD8+ cells) most effectors are lost due to AICD but a few are "chosen" to survive or come to "rest" as memory cells. In an alternative model, separate activated cells may either become effectors or memory cells.

The exact molecular pathways that are responsible for memory cell generation remain to be identified. It is very likely that anti-apoptotic Bcl-2 family members such as Bcl-2 and Bcl-XL are induced by antigen receptor and

costimulatory signals. Costimulation through CD28 may be required for the induction of Bcl-XL. The induction of cytosolic kinases such as Akt might contribute to the phosphorylation and inactivation of pro-apoptotic Bcl-2 family members such as Bad.

In Figure 8 a totally speculative scenario is presented in an attempt to explain how an activated T cell may receive distinct sets of signals that might favor memory versus death or vice versa. If one assumes that memory cells may be presented the same antigen by different APCs, one set of which express high levels of costimulatory ligands and another which do not, it could be predicted that excessive activation might lead to AICD while more circumscribed activation either later in an infection or in a distinct site could potentially lead to the generation of memory. There is absolutely no evidence for any such model but it is presented as one of many potential and distinct models that could be conceived of to explain how activated T cells might make these decisions.

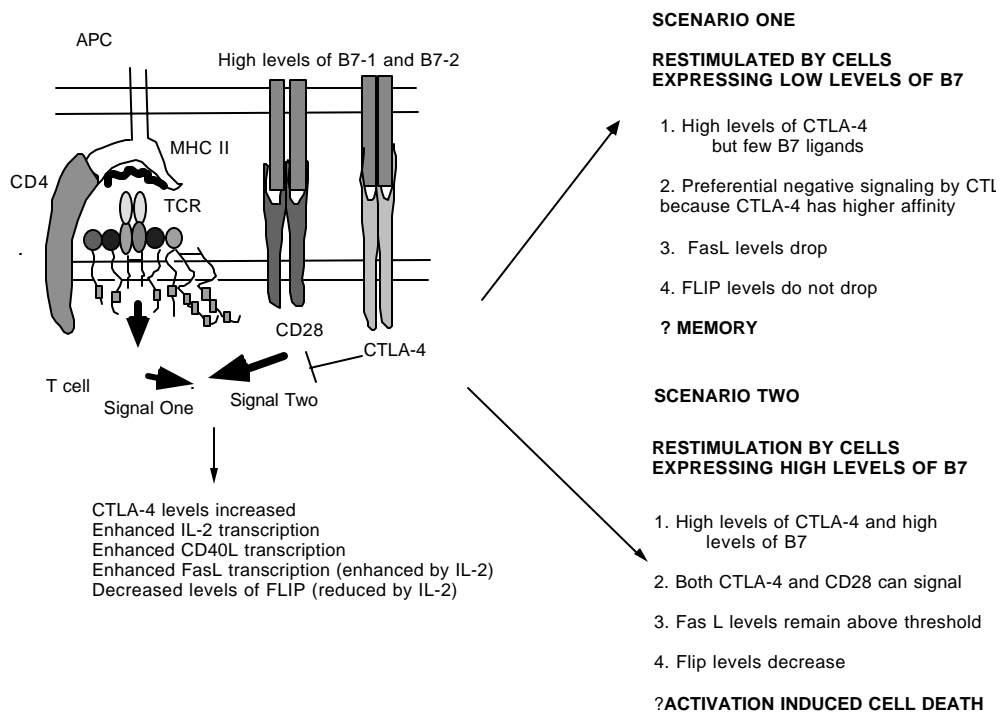


Figure 8. A totally speculative model for how a T cell might be led to choose between memory and AICD. Spatially or temporally determined differences in costimulatory ligand levels may lead to two different fates. In this model, repeated signaling with potent costimulation might lead to the induction of AICD, while a second triggering with little costimulation might contribute to memory T cell generation.

It is possible that much of the memory phenotype may be explained by two critical sets of biochemical changes:

1. Memory cells can be much more easily triggered through the TCR. This might be because memory cells express higher levels of adhesion molecules than naive cells or because of alterations in intracellular signaling pathways. This may explain their ability to continually receive survival signals when tickled even by "non-specific" MHC-peptide complexes. Genes downstream of TCR signaling may be in an "open chromatin" configuration in memory cells thus allowing low-intensity signals to trigger these cells, or to dramatically shorten the G1 period in cell cycle progression following a mitogenic stimulus.

2. Possibly as a result of being constantly "tickled", memory T cells express higher levels of anti-apoptotic proteins, or appropriately inactivate pro-apoptotic proteins.

Summary:

- On a molecular level, two major pathways for apoptosis are known. Both pathways include caspases, which are cytosolic proteins activated by proteolytic cleavage after specific aspartate residues. The first involves signaling at the surface with death receptors (Fas being a key example) that induces activation of caspase-8 via a multi-step process. Caspase-8 then activates caspase-3, which executes apoptosis via an unclear mechanism.
- The second molecular pathway may be induced by many noxious agents or by growth factor withdrawal etc. and involves mitochondrial leakiness, which may be regulated by the Bcl-2 family of proteins. During apoptosis, mitochondrial permeability increases resulting in the release of cytochrome c and apoptosis inducing factor (AIF) into the cytoplasm. Cytochrome c binds to APAF-1 and activates caspase-9 which then activates downstream caspases.
- The Bcl-2 family contains both anti-apoptotic (Bcl-2, Bcl-XL) and pro-apoptotic (Bax, Bad, Bak) factors that form homodimers and heterodimers. These proteins are principally anchored in the mitochondrial membrane. Bcl-2 is known to be down regulated during stages of lymphocyte development in which selection occurs, presumably to allow for the elimination of cells. Two models exist for

Bcl-2 family function: (1) the channel model suggests that the Bcl-2 homodimer acts as a survival channel whereas the Bax homodimer is a death channel, both of which regulate mitochondrial permeability. The Bax channel may help form pores for cytochrome c/AIF release whereas the Bcl-2 channel may inhibit these pores or may act as counter channels. (2) The cytosolic sequestration model suggests that Bcl-2 sequesters caspases and prevent the death pathway. When Bax binds to Bcl-2, sequestered caspase is released and death ensues.

- Apoptosis in the immune system occurs via three possible overarching mechanisms: (1) Direct activation of death (i.e., by granzymes released by CTLs and NK cells or via Fas signaling). (2) Indirect activation due to gene induction (i.e., ionizing radiation, DNA damage). (3) Removal of survival signals (i.e., lack of growth factors or of MHC binding).
- CTLs and NK cells release granzyme B into the cytosol of target cells (presumably via perforin channels). Granzyme B is a serine protease that activates the caspase cascade of cell death.
- Growth factors can work to inhibit the apoptotic pathways. For example, some growth factors activate PI3 kinase, which activates Akt. The activated protein Akt inactivates procaspase-9, inhibits FasL synthesis, and inhibits Bad.
- In an immune response, activated T cells express both CD40L and FasL on the cell surface. For B cells making Ig's specific for antigen, BCR signaling as well as the CD40/CD40L interaction provides the stimulus for activation. Fas/FasL interactions are negated by BCR signaling. However, bystander B cells do not have BCR signaling and the activated T cell will induce death of these B cells via the Fas/FasL interaction (despite the CD40/CD40L interaction). In this way, only B cells specific for antigen will proliferate and become activated.
- Memory cells behave like immortalized cells, with no requirement for antigen signaling to survive. These cells can respond more rapidly to antigen. These characteristics of memory cells are potentially due to an altered chromatin state of cytokine genes, a higher expression of adhesion factors which helps to lower the threshold needed for signaling, and a higher expression level of anti-apoptotic factors such as Bcl-2.

Selected Reviews

Adams, J. M., and Cory, S. (1998). The Bcl-2 protein family: arbiters of cell survival. *Science* 281, 1322-1326.

Ashkenazi, A., and Dixit, V. M. (1998). Death receptors: signaling and modulation. *Science* 281, 1305-1308.

Crispe, I. N. (1994). Fatal interactions: Fas induced apoptosis of mature T cells. *Immunity* 1, 347-349.

Ellis, R. E., Yuan, J., and Horvitz, H. R. (1991). Mechanisms and functions of cell death. *Annu. Rev. Cell. Biol.* 7, 663-698.

Evan, G., and Littlewood, T. (1998). A matter of life and cell death. *Science* 281.

Green, D. R. (1998). Apoptotic pathways: the roads to ruin. *Cell* 94, 695-698.

Green, D. R., and Reed, J. C. (1998). Mitochondria and apoptosis. *Science* 281.

Hawley, S. R., and Friend, S. (1996). Strange bedfellows in even stranger places: the role of ATM in meiotic cells, lymphocytes, tumors, and in functional links to p53. *Genes and Development* 10, 2383-2388.

Henkart, P. A. (1996). ICE family proteases: mediators of all apoptotic cell death. *Immunity* 4, 195-201.

Kastan, M. (1997). On the TRAIL from p53 to apoptosis. *Nature Genetics* 17, 130-131.

Nagata, S. (1997). Apoptosis by death factor. *Cell* 88, 355-365.

Nagata, S., and Golstein, P. (1995). The Fas death factor. *Science* 267, 1449-1456.

Oltvai, Z. N., and Korsmeyer, S. J. (1994). Checkpoints of dueling dimers foil death wishes. *Cell* 79, 189-192.

Reed, J. C. (1997). Cytochrome c: Can't live with it-Can't live without it. *Cell* 91, 559-562.

Salvesen, G. S., and Dixit, V. M. (1997). Caspases: intracellular signaling by proteases. *Cell* 91, 443-446.

Theophilopoulos, A. N., and Dixon, F. J. (1968). Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37, 269-305.

Thornberry, N. A., and Labeznik, Y. (1998). Caspases: enemies within. *Science* 281, 1312-1316.

Vaux, D. L., and Strasser, A. (1996). The molecular biology of apoptosis. *Proc. Natl. Acad. Sci. USA* 93, 2239-2244

White, E. (1996). Life, death, and the pursuit of apoptosis. *Genes and Development* 10, 1-15.

Yang, E., and Korsmeyer, S. J. (1996). Molecular thanaptosis: a discourse on the Bcl-2 family and cell death. *Blood* 88, 386-401

Wyllie, A. H., Kerr, J. F. R., and Currie, A. R. (1980). Cell death: the significance of apoptosis. *Int. Rev. Cytol.* 68, 251-306.

Yuan, J. (1997). Transducing signals of life and death. *Current Opinions in Cell Biology* 9, 247-251.