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# Regulation of T cell apoptosis

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**Proliferative expansion of lymphoid cells is required for effective immune responses against invading microorganisms, but after the infection is controlled, the expanded effector cells must be eliminated to prevent non-adaptive accumulation of cells. Higher vertebrates have developed extensive networks of signal transduction pathways to ensure controlled activation and expansion of cells during immune responses and apoptotic deletion of lymphoid cells that are no longer needed at the end of immune responses. Extracellular signals received by cell surface receptors that trigger intracellular signaling cascades are essential elements that control both processes. These signal transduction pathways converge to regulate cell fate at both transcriptional and post-transcriptional levels. Here we review the role of pathways, especially those triggered by TNF receptor-related molecules, that determine the fate of T cells during development and activation. In addition, we introduce the possibility that these same pathways may be abnormally programmed and so lead to immune cell accumulation during inflammatory diseases such as asthma.**

*Keywords:* airway immunity and inflammation; Fas death receptor; T helper type 1 and 2 cells; TNFR-related molecules.

## Introduction

The immune system of higher vertebrates is a complex cellular defense network that provides targeted protection against microbial agents and degenerated or dysfunctional host cells. In that context, rapid proliferative expansion of specialized subsets of immune cells is critical for effectiveness of the immune system. While some of these immune cells persist following infections, a significant percentage must be removed through the process of apoptosis or programmed cell death (PCD). In contrast to accidental cell death by necrosis, PCD is precisely initiated and controlled by signaling cascades[1–5] that lead to the formation of apoptotic bodies. These structures can then be eliminated by neighboring phagocytic cells to avoid inflammation and secondary tissue damage. In this review, we will summarize the major pathways for regulating apoptosis in T cells and so lay the basis for defining how deficiencies in these pathways may contribute to inflammation.

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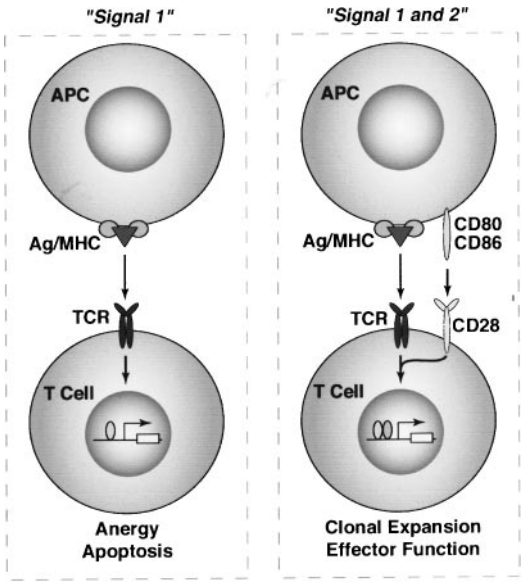
## Extracellular signals for apoptosis

The microenvironment in tissues provides cells with a complex matrix of growth factors, hormones, and cytokines. In turn, these ligands serve to engage cell surface receptors and trigger intracellular cascades that often inhibit cell death and support cell survival. In the absence of survival signals, at least some types of cells undergo PCD as a default mechanism.[6] In the case of T cells, T cell receptor (TCR) interaction with peptides in the context of major histocompatibility complexes (MHC) on antigen-presenting cells (APCs) can deliver a stimulatory cellular signal.[7] This "signal 1" appears to be insufficient to mediate full activation of T cells, so if the cells do not receive an additional co-stimulatory signal ("signal 2"), TCR signaling can result in a state of unresponsiveness (anergy) and trigger PCD (Figure 1). By contrast, when signal 2 is provided, e.g., by interaction of the co-stimulatory molecule CD28 with either of its ligands CD80 (B7.1) or CD86 (B7.2),[8,9] concomitant engagement of the TCR engagement causes activation of T cells. Subsequent production of pro-survival cytokines and up-regulation of anti-apoptotic cellular proteins lead to clonal expansion of T-cells and induction of effector functions.

In addition to the action of the TCR and costimulatory molecules, T cell fate is also determined by cytokine-dependent pathways. Lymphocytes originate from pluripotent hematopoietic progenitor cells where a series of soluble factors and cell-cell contacts determine if cells differentiate into T cells, B cells, or Natural Killer (NK) cells. For example, engagement of the interleukin- (IL-) 7 receptor (IL-7R) by its ligand is crucial for early steps of T and B cell development.[10,11] In this setting, IL-7 mediates recombination of antigen receptors and promotes cell-cycle entry and proliferation of immature lymphocytes.[12–18] In addition, IL-7 exhibits anti-apoptotic effects by increasing

**Figure 1**. Schematic diagram for the two-signal model of T cell activation. In the first case (left box), T cell activation results from the engagement of the TCR by antigen (Ag) bound to self MHC (signal 1), but the absence of additional costimulatory signals leads to anergic or apoptosis. In the second case (right box), signal 1 is received coordinately with a costimulatory signal (signal 2, e.g., CD28 engagement by CD80 (B7.1) or CD86 (B7.2), so T cell activation leads to clonal expansion and differentiation into an effector cell.




expression of bcl-2 and decreasing expression of the pro-apoptotic bcl-2 family member bax in thymocytes.[15,19] Animals defective in signaling through the IL-7R partially overcome the block in lymphocyte differentiation when a bcl-2 transgene is constitutively expressed in the lymphoid compartment.[20–22] This finding argues for the importance of IL-7 for prolonged survival during lymphocyte development but also suggests additional effects regulated by IL-7 signaling pathways.

## TNFR-related proteins as cellular regulators of apoptosis

Despite the anti-apoptotic nature of signals received by cell surface receptors, repeated stimulation of mature lymphocytes can still lead to PCD or activation-induced cell death (AICD). An overall scheme for how effectors of apoptosis are engaged in response to extracellular events is still under construction, but it appears that distinct members of the TNFR (TNF receptor) superfamily regulate this process in many cell types, including T cells. In particular, activation of lymphocytes normally results in the up-regulation of death domain-containing members of the tumor necrosis factor (TNF) receptor (TNFR) superfamily and/or their ligands. Members of the TNFR superfamily are characterized by several conserved cysteine-rich repeats in their extracellular domain, a single transmembrane region, and a cytoplasmic tail with little sequence conservation.[23] This superfamily can be divided into two subfamilies based on presence or absence of a death domain (DD) in the cytoplasmic tail (Table 1). TNFR-I (TNFR p55, TNFR p60), Fas (CD95), DR3 (AIR, Apo-3, LARD, TR3, TRAMP, Wsl-1), DR4 (TRAIL-R1), DR5 (TRAIL-R2, Trick2, KILLER, Apo2), and DR6 contain a death domain of approximately 80 amino acids in length. Multimerization of these receptors triggers interaction of the death domain with intracellular proteins that contain similar death domains.[24]

Most ligands of TNFR-related proteins are homotrimeric cytokines that belong to the TNF family.[25] Structural studies of TNFR-I and one of its ligands, lymphotoxin $\beta$, as well as resonance structure analysis of ligated CD95 suggest the clustering of intracellular death domains by self-association.[26–28] The recently identified SODD (silencer of death domains) that can interact with the DDs of TNFR-I and DR3 is thought to be the first member of a class of proteins that inhibits spontaneous self-aggregation of receptor DDs.[29] Crosslinking of receptors results in release of SODD and recruitment of intracellular adapter molecules like TRADD (TNFR-associated death domain containing protein) and FADD/Mort-1 (Fas-associated death domain containing protein/Mediator of receptor-induced toxicity). Recruitment of these proteins to the cytoplasmic domains of the receptors leads to formation of multi-protein complexes that result in activation of caspases and consequent apoptosis[30–36] (Figure 2, left box).

TNFR-related molecules that lack DDs include TNFR-II (TNFR p75, TNFR p80), CD27, CD30 (Ki-1), CD40, Ox40 (CD134, ACT35) and 4-1BB (CD137, ILA). These receptors initiate signaling events that lead to cellular survival, proliferation, and cytokine production[37–44] (Figure 2, right box). Ligand engagement and multimerization of these receptors results in recruitment of TNFR-associated factors (TRAFs).[45] TRAFs are intracellular adapter proteins that initiate formation of multiprotein complexes and trigger activation of MAPK/ERK (mitogen-activated protein kinase/mitogen and extracellular signal-regulated kinase) cascades. These events can result in activation of transcription factors of the AP-1 and rel families. Both AP-1 and NF-kB are involved in transcription of survival genes and therefore may have mainly anti-apoptotic function.[46,47] In some cases, this cascade may also be triggered by TNFR-I receptors. For example, TNFR-I may also recruit TRAF-2 via a TRADD intermediate and so generate a cell survival signal.

In addition to distinct receptor complex formations, the action of TNFRs is also regulated by their pattern of expression and the distribution of the corresponding ligand. Thus, some members of the family like TNFR-I

**Table 1**. Characteristics of the TNFR superfamily summarized as two subfamilies based on the presence (A) or absence (B) of a death domain in the cytoplasmic tail of the receptor

| Receptor | Site of expression | Recruited proteins | Reference |
|---|---|---|---|
| (A) Receptors containing a death domain | | | |
| TNFR-I (CD120a, TNFR p55, TNFR p60) | Ubiquitous | TRADD | (156–161) |
| p75 neurotrophin receptor | | TRAF6 | (162) |
| CD95 (Fas, Apo-1) | Ubiquitous | FADD, FAP | (163, 164) |
| DR3 (AIR, Apo-3, LARD, TR3, TRAMP, Wsl-1) | Spleen, thymus, PBL | TRADD | (48–52) |
| DR4 (TRAIL-R1, Apo-2) | Most tissues | TRADD (?), FADD (?), RIP (?) | (165, 166) |
| DR5 (TRAIL-R2, Trick2, KILLER) | Ubiquitous | TRADD, FADD, RIP | (166–171) |
| DR6 | Ubiquitous | TRADD | (172) |
| (B) Receptors lacking a death domain | | | |
| TNFR-II (CD120b, TNFR p75,TNFR p80) | Myeloid, activated T and B | TRAF1, 2 | (23, 157, 173–176) |
| LT-$\beta$R (CD18, TNFR-III) | Leukocytes | TRAF3, 5 | (177, 178) |
| CD27 | B, T, medullary TC | Siva, TRAF2, 3, 5 | (179, 180) |
| CD30 (Ki-1) | NK, M, activ. T and B | TRAF1–3, 5 | (181) |
| CD40 | B, MØ, DC, basal epithelial cells | TRAF2, 3, 5, 6 | (182) |
| Ox40 (CD134, ACT35) | Activated T | TRAF1–3, 5 | (183–185) |
| 4-1BB (CD137, ILA) | Activated T | TRAF1-3 | (186–188) |
| HVEM (ATAR, TR2) | Lung, spleen, thymus | TRAF1–3, 5 | (189–191) |
| TACI | Lymphoid cells | CAML | (192) |
| GITR (AITR) | Activated T | ? | (193) |
| OPG (FDCR-1) | LN, FL, BM, spleen, thymus, B, DC | ? | (194–196) |
| RANK | DC | TRAF1–3, 5 | (197) |
| DcR1 (TRAIL-R3, TRID) | Ubiquitous | – | (167, 168, 198, 199) |
| DcR2 (TRAIL-R4, TRUNDD) | Ubiquitous | ? | (200–202) |
| DcR3 | FL, fetal brain and lung; adult spleen, colon, lung | – | (137) |

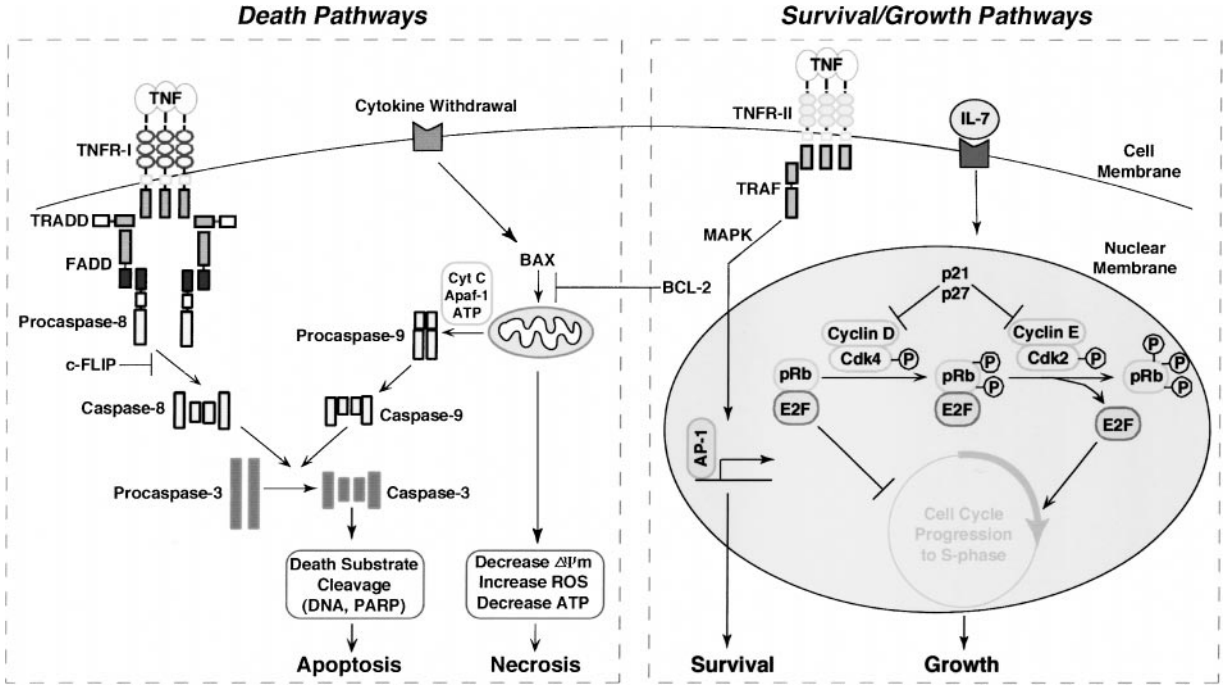
Abbreviations: AIR, apoptosis-inducing receptor; AITR, activation-induced TNFR family related protein precursor; ATAR, another TRAF-associated receptor; DcR, decoy receptor; DR, death receptor; FDCR, follicular dendritic cell-derived receptor; GITR, glucocorticoid-induced TNFR family-related protein precursor; HVEM, Herpes virus entry mediator; ILA, receptor induced by lymphocyte activation; LARD, lymphocyte-associated receptor of death; LT-$\beta$R, lymphotoxin $\beta$ receptor; OPG, osteoprotegerin; RANK, receptor activator of NF-$\kappa$B; TACI, transmembrane activator and CAML-interactor; TRAIL, TNF-related apoptosis-inducing ligand; TRAMP, TNF receptor-related apoptosis-mediating protein; TRICK, TRAIL receptor inducer of cell killing; TRID, TRAIL receptor without an intracellular domain; TRUNDD, TRAIL receptor with a truncated death domain; B cells, BM bone marrow; DC, dendritic cells; FL, fetal liver; LN, lymph nodes; MØ, macrophages; CAML, calcium-modulator and cyclophilin ligand; FADD, Fas-associated death domain-containing protein; FAP, Fas-associated phosphatase; RIP, receptor-interacting protein; TRADD, TNFR-associated death domain-containing protein; TRAF, TNFR-associated factor.

are expressed ubiquitously, but other members exhibit a more restricted expression pattern (Table 1). For example, DR3 shows structural and functional homology to TNFR-I, but DR3 expression is restricted to cells in spleen, thymus, and peripheral blood and can be up-regulated upon T-cell activation.[48–52] In contrast, DR3 ligand (DR3L) is found in many tissues and cell types while TNF is mainly expressed in activated T-cells and macrophages.[53–55]

## Intracellular signal transduction pathways for apoptosis

After cell surface receptors for mediating death or survival are activated, the signal must be transmitted to the nucleus. Phosphorylation and dephosphorylation of intracellular substrates by receptors with kinase or phosphatase domains in their cytoplasmic tails is widely used

**Figure 2**. Schematic diagram for representative pathways that mediate cell death (left box) or survival and growth (right box). The death box features pathway initiation by TNFR superfamily members (e.g., TNFR-I) that contain a death domain. This domain mediates recruitment of death domain-containing adapter proteins (e.g., TRADD) and FADD that serve to activate the caspases. Caspase activation then mediates degradation of essential structural proteins leading to programmed cell death. Other extracellular signals (e.g., cytokine withdrawal) may trigger pro-apoptotic BCL-2 family members (e.g., BAX) that leads to mitochondrial damage and necrosis or apoptosis (via apoptosome formation). This pathway is also subject to inhibition by anti-apoptotic members of the BCL-2 protein family (e.g., BCL-2). The survival/proliferation box features TNFR superfamily members (e.g., TNFR-II) that lack a death domain and can trigger signal transduction pathways that support cell survival. In this case, recruitment of TRAF3 leads to activation of a MAP kinase cascade that activates transcription factors (e.g., AP-1) with subsequent transcription of genes that support cell survival. In addition, cytokine stimulation (e.g., by IL-7) may lead to receptor activation and nuclear signals that inactivate retinoblastoma protein (pRb) and so allow for cell cycle progression to S-phase. The death and survival/growth pathways must be tightly balanced to maintain homeostasis of a multi-cellular organism.




as a mechanism for the integration of signals from the environment.[56] Since TNF receptors lack an enzymatic domain in their cytoplasmic tail, they depend on recruitment of cytoplasmic proteins to initiate cellular responses. Many of these proteins are enzymes, (e.g. kinases, phosphatases, and proteases, while others are simple adapter molecules that function as scaffolds for the formation of multi-protein complexes or as chaperones for enzymes that fulfill downstream effector functions.[57] These signaling events are linked in complex networks of signal transduction that are necessary to determine the fate of a cell in response to the variety of signals that are received. Downstream effects of these signaling cascades can initiate transcription of new genes that lead to changes in cellular programs and result in activation, proliferation, and/or differentiation of cells. Alternatively, signals that are received at the cell surface can be initiators of apoptotic pathways that lead to the elimination of a particular cell without the consequences of necrotic cell death, e.g. inflammation or autoimmune disease.

In some cases, it appears that the same receptor can lead to cell death or survival depending on the context. Thus, Fas mediates deletion of activated mature T cells at the end of an immune response, death of virus-infected or cancerous target cells by cytotoxic T cells and NK cells, and elimination of immune effector cells at immune-privileged sites.[2,58] The importance of Fas in this process is underscored by the observation that inactivating mutations of Fas or FasL cause autoimmune disease in mice and humans.[59–62] By contrast, IL-2 ordinarily promotes cell survival, yet mice lacking IL-2 or functional IL-2 receptor also develop severe lymphoproliferation and autoimmune disease.[63–68] In this case, it appears that stimulation of cells through the IL-2 receptor leads to increased cell surface expression of FasL and decreased levels of FLIP (FLICE-like inhibitor protein) an intracellular inhibitor of Fas-induced PCD.[69] Thus, signaling pathways that support cell survival and cascades that culminate in apoptosis are tightly linked during the immune response.

## Protein phosphorylation and regulation of apoptosis

As noted above, regulation of protein activity by phosphorylation is a common mechanism used for a variety of signal transduction pathways.[70] Co-stimulatory signals of T and B cells result in activation of protein kinase C (PKC) that is tightly associated with the regulation of intracellular calcium levels.[7,9] A second network of kinase signaling is regulated by members of the MAP kinase family.[71] The most upstream kinases of MAP kinase cascades are MAP kinase kinase kinases (MAPKKKs). Signaling through small guanine binding molecules like Ras results in activation of the MAPKKK Raf-1 that can activate MAPKKs which results in phosphorylation of effector MAPKs, e.g. JNK and p38. Both of these members of the MAPK family appear to be critical regulators of cell survival.[72] Several kinases, e.g. PAK2, distinct PKC isoforms, and MEKK-1, are activated after proteolytic cleavage by caspases.[73–78] Mutants of these enzymes that lack the caspase recognition sequence delay the induction of PCD. Overexpression of truncated forms of these kinases can trigger apoptosis that can not be blocked with caspase inhibitors. Taken together these findings are consistent with putative function of these kinases downstream of caspases. The substrates of kinases that can induce PCD upon phosphorylation by kinases are yet to be determined.

## Mitochondria as relay stations for survival pathways

Mitochondria are critical components of signaling pathways that lead to apoptotic cell death.[79] A change in mitochondrial transmembrane potential ($\Delta\Psi_m$) is one of the first irreversible steps of PCD in many cell types. This change along with increased calcium concentration, generation of reactive oxygen species (ROS), activation of caspases, and depletion of ADP and ATP, two physiological inhibitors of a pore complex known as mitochondrial megachannel or permeability transition (PT) pore, can result in depolarization of the inner mitochondria membrane.[80] This event or damage of the outer mitochondrial membrane can result in release of cytochrome c, a soluble component of the respiratory chain that is normally retained in the space between outer and inner mitochondrial membrane. Complexes of cytochrome c and Apaf-1 (apoptotic protease-activating factor 1), the cellular homologue of the *C. elegans* protein Ced-4, that are formed in the cytoplasm trigger activation of pro-caspase 9 in structures known as apoptosomes[81,82] (Figure 2). Subsequent activation of effector caspase 3 triggers endonuclease activity of DFF (DNA fragmentation factor) /CAD (caspase-activated DNase).[83–85] This event appears to be an irreversible step in apoptosis. Alternatively, the apoptosis-inducing factor (AIF) may directly induce DNA degradation and activate caspase-3 independent of cytochrome c.[86–89] Degradation of Bcl-2 by caspases favors mitochondrial depolarization and/or disintegration of the outer mitochondrial membrane and results in same sequence of events. Alternatively, pro-apoptotic Bcl-2 family members like Bid and Bad can function as sensors of cytosolic death stimuli. Upon post-translational modifications, both proteins can bind to Bcl-2 or Bcl-$x_L$ in the outer mitochondria membrane and thereby result in cytochrome c release from mitochondria.[90–93]

## Caspases serve as killer proteins

The first evidence for the involvement of caspases in apoptosis came from studies in *C. elegans*.[94] Later, apoptotic gene products of this nematode were shown to have structural and functional counterparts in mammalian cells. Members of the caspase family contain a conserved pentapeptide (QACXG) with a central cysteine at their active site.[95] Caspases exist as inactive precursor molecules (zymogens) in the cytoplasm. Proteolysis by upstream caspases or auto-catalytic activity induced by dimerization of zymogen isoforms leads to cleavage of the zymogens.[96] The crystal structures of active caspase-1 and caspase-3 revealed the existence of two independent catalytic sites within tetrameric complexes.[97–99]

Similar to the complement system, caspases are organized in cascades that amplify the initial death signal (Figure 2). Activation of downstream effector caspases results in cleavage of a number of cellular proteins and initiation of pathways that induce PCD.[96] The most prominent caspase targets are involved directly or indirectly in cellular ultrastructure. Thus, proteolysis of lamins leads to the breakdown of the nuclear lamina,[100,101] and degradation of gelsolin, focal adhesion kinase (FAK), and p21-activated kinase 2 (PAK2) effects cytoskeletal structure.[78,102,103] In addition, inactivation of proteins essential for DNA repair, mRNA splicing, and DNA replication may also facilitate PCD,[104–107] while degradation of substrates like Bcl-2, Bcl-$x_L$, and I-CAD can inactivate proteins vital for cell survival.[83,85,108,109]

In contrast to other posttranslational modifications, proteolytic cleavage is irreversible and therefore must be tightly controlled. Specificity of caspases for their targets is achieved by four residues N-terminal of the cleavage site and can be used to subdivide the protease family into three groups recognizing either a WEXD, a DEXD, or an (L/V)EXD sequence motif. Interestingly, not all proteins containing recognition sites of caspases are degraded, suggesting an important role of the three-dimensional structure of the substrates. The high efficiency of caspase activity combined with the restricted subset of proteins that are

cleaved by caspases emphasizes the regulated mechanism that leads to disassembly of apoptotic cells.

## Bcl-2 family members balance pro- and anti-apoptotic pathways

Bcl-2 was the initial member of a protein family that now contains members with pro- and anti-apoptotic activities.[110] Bcl-2 expression can interfere with cell death induced by CD3 crosslinking, growth factor withdrawal, or treatment with glucocorticoids, phorbol esters, ionophores, or $\gamma$-irradiation.[111,112] Unlike classical oncogenes (or inactivated tumor suppressor genes) that promote cell cycle progression and cellular proliferation (Figure 2), Bcl-2 and its closest homologue Bcl-$x_L$ prevent PCD and maintain cells in the $G_0$ phase of the cell cycle.[113–116] Thus, anti-apoptotic members of the Bcl-2 family appear to inhibit cell cycle progression,[117,118] whereas the absence of these proteins or expression of pro-apoptotic family members like Bax results in an accelerated cell cycle.[119,120]

The precise mechanism for how Bcl-2 family members achieve pro- or anti-apoptotic function remains uncertain. Despite their capability of homo- and heterodimerization, members of both subfamilies seem to mediate protective or death-promoting effects independent of the interaction between distinct family members.[121] These findings have been emphasized by studies of the death machinery in the nematode *C. elegans*.[122] Gene elimination studies in the worm have demonstrated that the Bcl-2 homologue Ced-9 functions as an inhibitor of the protease Ced-3.[123] Similarly, Bcl-2 expression in mammalian cells functions to prevent proteolytic activation of the downstream effector caspase-3.[124–127] Bcl-$x_L$ can block activation of the effector caspase after Fas-induced activation of caspase 8 by maintaining mitochondrial integrity.[128] However, overexpression of Bax is sufficient to induce PCD even in the presence of the peptide inhibitors of caspases.[129]

Proteins of the Bcl-2 family are targeted to the membranes of distinct organelles including mitochondria,[130] and both pro- and anti-apoptotic members of the Bcl-2 family mediate their effects at least in part by regulating mitochondrial morphology and/or function. The pro-apoptotic Bax protein may form channels in the outer mitochondrial membrane, whereas Bcl-2 interferes with that mechanism and maintains the integrity of mitochondria.[131] Downstream effects like Bax-induced loss of mitochondrial transmembrane potential, increased generation of ROS, and plasma membrane permeability were not inhibited by decreased caspase activity.[129] Furthermore, cytochrome c release from the intermembrane space of mitochondria induced by various apoptotic stimuli is significantly decreased in the presence of Bcl-2 or Bcl-$x_L$.[132–134] These observations and the intracellular localization of Bcl-2, Bcl-$x_L$, and Bax at the mitochondrial membranes suggest a role of the organelle in the susceptibility of cells to die in response to extracellular signaling events.

## Dysregulation of programmed cell death linked to disease

During development and after maturity, cell death is necessary to maintain homeostasis and to eliminate cells that are no longer needed or may be potentially harmful to the organism. Both uncontrolled proliferation of cells or the lack of controlled cell death would disrupt the integrity of the organism. In fact, several diseases, e.g. AIDS, neurodegenerative disorders, cancer and autoimmune disorders may result from an imbalance between cell survival and PCD.[135]

The balance between cellular life and death that is normally regulated by a network of proto-oncogenes and tumor-suppressor genes needs to be strictly controlled to avoid uncontrolled proliferation of cell clones. Both hyperproliferation and the inability to die give rise to benign tumors that may eventually result in malignancies after acquiring additional mutations. One hallmark of many tumor cells is either the loss of genes that are involved in PCD or increased expression of decoy proteins that interfere with the crosslinking of death receptors by their natural ligand.[136,137] Many of these cells therefore escape the defense mechanisms of the immune system and/or treatments that are designed to eliminate malignant cells by inducing PCD.

Studies of transgenic mice overexpressing Bcl-2 or Bcl-$x_L$ reveal that the development of the lymphoid compartment is a strictly regulated process dependent on controlled cell death and interference in PCD can give rise to tumors.[138–140] Overexpression of Bcl-2 as a transgene in T cells or B cells leads to increased cell numbers in the particular lymphoid compartment and subsequently, in the event of additional mutations, to malignant transformation and tumor formation.[138,141,142] Increased Bcl-2 levels due to a chromosomal translocation event interfere with PCD. This event allows the accumulation of additional mutations that result in a malignant phenotype of cells and the formation of follicular lymphomas.

Abnormalities in apoptosis may also be critical in host defense. Infection of $CD4^+$ T-lymphocytes with the human immunodeficiency virus (HIV) results in depletion of these lymphocytes due to PCD.[143] The lack of helper T-cells subsequently effects other lymphoid lineages, e.g. B-cells and $CD8^+$ T-cells, which depend on cytokines produced by $CD4^+$ cells for their survival and effector functions and results in clinical manifestation of immunodeficiency. In addition, deregulation of the immune system can also lead to proliferation of B-cells, hypergammaglobulinemia, and increased auto-antibody production.

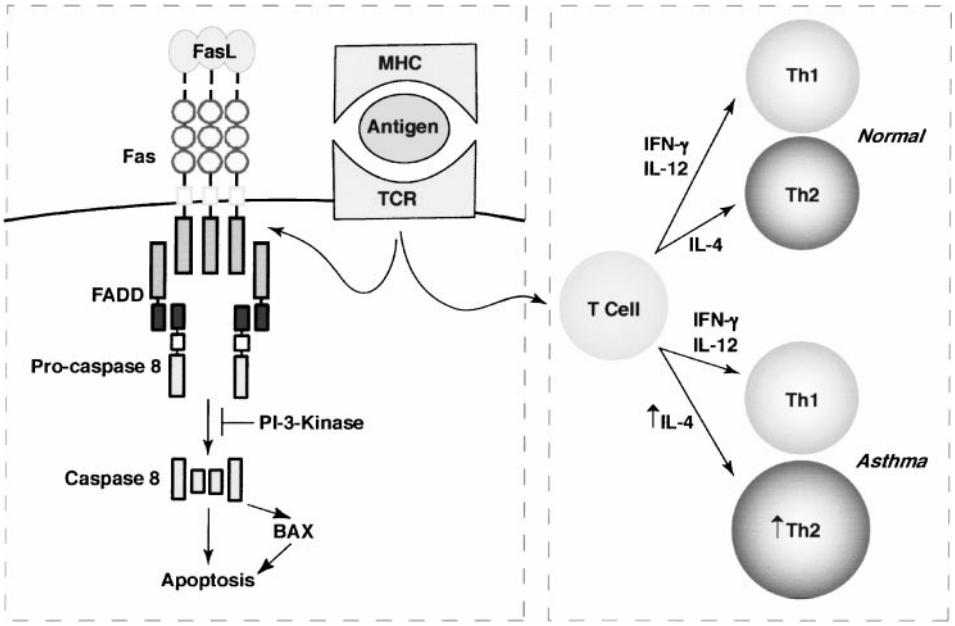
## Abnormal T cell death in airway inflammation

As noted above, the cell surface receptor Fas is upregulated during activation of T cells through the antigen receptor,[144] and the coordinated activation of Fas in this setting transduces an apoptotic signal that may dampen the response of $CD4^+$ T helper (Th) cells.[145,146] Overactivation of $CD4^+$ T cells in the peripheral blood and airway tissues is an invariant feature of asthma,[147] so we reasoned that a potent mechanism for augmenting the numbers of activated T cells in this disease would be resistance to the normally programmed pathway for cell death. We expected that T cell apoptosis in asthma was mediated via antigen activation of the TCR, so we concentrated on a Fas-dependent pathway for T cell death that is linked to TCR-dependent apoptosis and elimination of activated T cells after they respond to foreign antigens (deletional tolerance).[148]

In our initial study, we found that mitogen-stimulated peripheral blood T cells of asthmatic subjects expressed cell surface Fas but failed to undergo the normal degree of apoptosis following Fas receptor ligation, thereby providing initial evidence for a defect in programmed cell death in the pathogenesis of asthma.[149] In that context, the findings suggest that the increased level of activated T cells that mediate this (and other) inflammatory disease may be due to decreased elimination of activated T cells as well as previously cited increases in T cell recruitment and activation.[150] Further investigation of the mechanism(s) underlying defective T cell apoptosis in asthma indicates decreased efficacy of antigen-driven T cell activation, an event that is required for Fas-dependent apoptosis.[148] The observed abnormality and the consequent T cell phenotype in asthma is therefore distinct from inherited defects in the Fas gene that lead to autoimmune disease.[151,152] T cells from asthmatics exhibited normal apoptotic responses to $\gamma$-irradiation (dependent on ICE-family proteases), ceramide, and mitogen challenge, suggesting functional integrity of the apoptotic pathway. Furthermore, the defect in Fas-dependent apoptosis is overcome by pre-stimulation with allogeneic accessory cells (instead of mitogen). Taken together, the findings suggest that selective resistance to Fas-dependent apoptosis reflects altered antigen-driven, accessory cell-dependent signaling and that ineffective activation of Fas signal transduction may contribute to T cell-dependent immunoinflammation in asthma (Figure 3).

Studies of T cell clones have indicated that Th2 effector cells may be more resistant to Fas-induced apoptosis.[153,154] Accordingly, we recently analyzed whether the induction of Fas sensitivity is associated with the differentiation of functionally distinct T cell subsets in normal control and asthmatic subjects. In cultures from both types of subjects, allogeneic antigen stimulated the generation of two IFN-$\gamma$-producing T cell subsets, one that

**Figure 3**. Scheme for regulation and dysregulation of T cell apoptosis in asthma. The left box depicts a molecular cascade leading from TCR activation to upregulation of Fas and FasL and consequent Fas-mediated apoptosis. The right box presents a cellular scheme for TCR- and concomitant cytokine-dependent generation of T helper (Th) effector cells. In normal subjects, IFN-$\gamma$/IL-12 and IL-4 promote balanced Th1 and Th2 cell differentiation (upper scheme). However, in asthma, antigen-dependent downregulation of IFN-$\gamma$ and upregulation of IL-4 may result in a T cell population with decreased Th1 and increased Th2 effector cells, respectively. This imbalance in T cell phenotypes may be manifest as decreased sensitivity to Fas-mediated cell death as described in the text.



generated IL-2 plus IFN-$\gamma$ (Th1-like) and another distinct one that generated IFN-$\gamma$ alone.[155] However, mitogen stimulated only the development of T cells producing IFN-$\gamma$ alone, and these cells were found only in cultures from normal control but not in asthmatic subjects. As observed earlier, allogeneic antigens but not with mitogen rendered T cells from asthmatic subject sensitive to Fas-mediated apoptosis, whereas both stimuli resulted in T cells from control subjects with similar sensitivities to Fas-mediated apoptosis. These results suggest that induction of Fas sensitivity in T cells may be linked to IFN-$\gamma$ production that is compromised in polyclonally activated T cells in asthma.

## Conclusion

Controlled elimination of cells that are no longer needed or may be potentially dangerous is critical for proper homeostasis, and this process is especially critical for proper T cell function in immunity. In that context, T cells have developed complex signaling cascades that allow tight control of cellular survival, proliferation, and differentiation. The intricacies of this network as well as mechanisms for crosstalk between pathways that trigger cell death versus survival serve to complicate the development of therapeutic approaches that may interfere with cell fate. However, recent data indicates a primary role for death pathway dysfunction in the pathogenesis of autoimmune and inflammatory diseases. Defining the molecular basis for T cell death versus survival and growth will therefore provide a more rationale basis for maintaining normal immune responses and correcting destructive inflammatory responses.

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