Apoptosis and diseases

Regulation and clinical relevance

Faris Q. Alenzi, *PhD, FAMLI.*

ABSTRACT

It is increasingly clear that apoptosis plays a central role in the pathogenesis of several human diseases. For instance, an increase in apoptosis leads to cell loss accompanied by neurodegenerative diseases, whereas we know that genetically determined defects of apoptosis result in deregulated cell proliferation, typical of cancer. Hence, apoptosis may be relevant as therapeutic targets for many human diseases. This article reviews briefly the regulation and the clinical relevance of apoptotic mechanisms in several different human diseases.

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There are 2 major types of cell death, namely, necrosis and apoptosis. Unlike apoptosis, necrosis appears not to be involved in any developmental context, nor to require any protein expression. This type of cell death is mainly due to external factors including, hypoxia, toxins and extreme heat. The dying cells often release lysosomal and granule contents which result in an inflammatory process. In contrast to necrosis, apoptosis does not induce inflammation and mononuclear phagocytes engulfed apoptotic cells, and is largely found in a wide variety of physiological death settings where its role is to remove harmful, damaged or unwanted cells. Apoptosis is a complex, tightly regulated and active cellular process. Well-recognized morphological changes including: shrinkage of the nucleus and the collapses of cytoplasm into crescents along the nuclear envelope and blebbing of the plasma membrane accompanies the phases of apoptosis[1-6] (**Figure 1**). We commonly refer to the main pathways of apoptosis to as the intrinsic and extrinsic pathways. The intrinsic pathway centres on the mitochondria as initiators of cell death. Multiple signals converge on mitochondria, including DNA damage, hypoxia and oxidative stress, causing the release of cytochrome c (cyt c) from mitochondrial membrane and activation of other apoptogenic proteins in the cytosol. Upon release into the cytosol, cyt c binds to apoptosis activating factor (Apaf-1) triggering its oligomerization into a heptameric complex that binds pro-caspase-9, forming a multi-protein structure known as the "apoptosome". Physical binding of Apaf-1 to pro-caspase-9 is mediated by their caspase recruitment domains (CARDs), through homotypic CARD-CARD binding. Activation of apoptosome-associated cell death protease caspase-9 then initiates a proteolytic cascade, where activated caspase-9 cleaves and activates downstream effector proteases such as pro-caspase-3.[7-14] In contrast, the extrinsic apoptotic pathway relies on tumor necrosis factor (TNF) family death receptors for triggering apoptosis. A subgroup of the TNF family receptors contains a cytosolic death domain that enables their intracellular interaction with downstream adaptor

From the Department of Medical Laboratory Sciences, College of Applied Medical Sciences, King Faisal University, Dammam, *Kingdom of Saudi Arabia.*

Address correspondence and reprint request to: Dr. Faris Q. Alenzi, Department of Medical Laboratory Sciences, College of Applied Medical Sciences, King Faisal University, PO Box 2114, Dammam 31451, *Kingdom of Saudi Arabia*. Tel. +966 (3) 8571000 Ext. 2793. Fax. +966 (3) 8572872. E-mail: faris_alenzi@hotmail.com

proteins, linking this receptor to intracellular specific caspases. Upon ligand binding, TNF family receptor containing cytosolic death domain cluster in membranes, recruiting both a death domain and a death effector domain (DED). The DED of Fas associated death domain (FADD) binds to DED-containing procaspse-9 and -10, forming a death inducing signaling complex (DISC) and resulting in caspase activation.[15-21] A delicate balance between pro-apoptotic and anti-apoptotic regulators of apoptosis pathways is at play on a continual basis, ensuring the survival of long-lived cells and the turnover of short-lived cells in a variety of tissues, including the bone marrow and thymus. However, an imbalance in this delicate balance of pro- and anti-apoptotic proteins occurs in many disease scenarios including cancer where the imbalance is in favor of anti-apoptotic proteins, endowing cells with a selective survival advantage that promote neoplasia and malignancy.

Regulation of apoptosis by intracellular signals. ***Tyrosine kinase.*** Many known survival factors directly activate receptors with intrinsic tyrosine kinase activity. Among these receptors are epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, and insulin growth factor. The downstream target that mediates anti-apoptotic effects of these survival factors have yet to be addressed. It also implicated phosphatidylinositol (PI-3) kinase in the suppression of apoptosis in rat pheochromocytoma (PC-12) cells. In serum-free medium PC-12 cells undergo apoptosis unless protected by survival factors such as epidermal growth factor and nerve growth factor. Two specific inhibitors of PI-3-kinase wortmannin and Ly294002 prevent this protection. While PI-3 kinase implicates the survival of PC-12 cells, it is likely that the signaling pathways involved in survival are the cell type and cytokine specific. The pathways mediating survival from many cytokines are presently unknown. Some survival factors, such as interlukin-3 (IL-3), activates receptors that possess no intrinsic kinase activity. Kinoshita et al[22] showed experimentally that IL-3 might suppress apoptosis via the activation of the Ras/Raf-1/MAP kinase pathways.[22] The notion that H-Ras may be involved in the suppression of apoptosis is supported by the observation that activated H-Ras inhibits apoptosis in myeloid leukemia cells.[23] Conversely, a dominant negative mutant of H-Ras induces apoptosis in a human chronic myeloid leukemia cell line.[24] Another non-receptor protein kinase which appears to be involved in the regulation of apoptosis in the protein tyrosine kinase Abl. Transfection of cells with a constitutively active form of protein tyrosine kinase Abl inhibits apoptosis induced by removal of survival factors or treatment with chemotherapeutic drugs. The requirement for protein kinase activities in the inhibition of apoptosis is supported by the observation that staurosporin kills all cells types so far tested. Staurosporin was originally isolated as a specific protein kinase inhibitor but also inhibits many other tyrosine kinase and serine kinases. It is therefore, possible that staurosporin interferes with the suppression of apoptosis mediated by distinct kinases in different cell types. Whilst protein kinase has been implicated in the inhibition of apoptosis, it appears that other kinases may trigger apoptosis. Activation of the protein tyrosine kinase p56lck is thought to be involved in the potentiation of T cell receptor-mediated apoptosis induced by Thy-1 antigen engagement of thymocytes. However, the effects of this kinase appears to be cell-type specific since p56lck suppresses apoptosis in hematopoietic cells upon factor deprivation.[25,26]

Calcium (Ca^{2+}). Calcium^{2+} receives the most attention in studies of apoptosis, which is one of the intracellular signals. Several experiments show that an increase in intracellular Ca^{2+} concentration may be involved in apoptosis. Additionally, treatment with agents that increase intracellular Ca^{2+} concentration (such as Ca^{2+} ionophores) trigger apoptosis in many cells.[27-29] However, an increase in Ca^{2+} concentration is not a universal requirement for apoptosis. Indeed, increasing intracellular Ca^{2+} concentrations by treatment with Ca^{2+} ionophores inhibits apoptosis in hemopoietic IL-3 dependent cells upon factor deprivation. In a similar fashion to the regulation by survival factors (namely cytokines), the regulation of apoptosis by intracellular Ca^{2+} also appears to be specific to certain cell types or apoptotic triggers.

Protein kinase C (PKC). Another signaling molecule implicated in the control of apoptosis is PKC. Activation of PKC by treatment with phorbol ester induces apoptosis in thymocytes.[30,31] In contrast, the activation of PKC is associated with the inhibition of apoptosis in thymocytes and leukemic B cells. Moreover, phorbol ester can block TNF-induced apoptosis and PKC antagonists can induce death.[31] The regulation of apoptosis by PKC appears to be cell type specific or dependent on the experimental conditions used.

Cyclic AMP (cAMP). Cyclic AMP is another signaling molecule implicated in apoptosis in certain situations. Similarly, the effects of cAMP appear to be cell type or context specific. Agents that elevate cAMP induce thymocyte apoptosis, whereas, cAMP analogues inhibit neuronal apoptosis upon nerve growth factor deprivation and result in the death of T cell hybridomas following T cell receptor-activation.[32,33]

Ceramide. Ceramide is a complex lipid produced upon hydrolysis of sphingomyelin by sphingomyelinase, and has also been proposed to regulate apoptosis. Tumor necrosis factor,[34] Fas,[31]

synthetic ceramide analogues and exogenous sphingomyelinase-mediated apoptosis are associated with a rapid increase in ceramide concentration.[35] The role of ceramide in other apoptotic systems has yet to be addressed.

Regulation of Fas-mediated apoptosis by FLICE inhibitory proteins (FLIPs). Thome et al[36] identified certain herpes proteins and named viral-FLICE-inhibitory proteins (v-FLIPs), due to their ability to bind to the Fas/FADD complex and inhibit FADD-like ice (FLICE) activation. Shortly afterwards 2 human homologues to v-FLIPs were discovered by several groups and became known by various names: inhibitor of FLICE,[37] FADD-like anti-apoptotic molecule (FLAME)[38] caspase-8-related protein (Casper)[39] or caspase-like apoptosis regulatory protein (CLARP).[40] They show to bind it to the Fas and thereby inhibit activation of caspase-8.[36] They also found v-FLIPs inhibit apoptosis induced by several triggers other than Fas, such as tumor necrosis factor receptor-1 (TNFR1) and death receptor-3 (DR3), which uses a similar signaling pathway by these receptors.

Regulation of mitochondria-induced apoptosis. B cell lymphoma-2 (Bcl-2) family proteins regulate the mitochondrial apoptotic pathway. B cell lymphoma-2 is localized on the endoplasmic reticulum, nuclear membrane and outer mitochondrial membrane.[41-43] There are 2 theories concerning the relationship between the Bcl-2 family and mitochondrial transmembrane potential. The first is that Bcl-2 and Bcl-xL are able to inhibit mitochondrial dysfunction, including the mitochondrial membrane potential loss and permeability transition,[44] thereby inhibiting the release of apoptogenic proteins such as cyt c and apoptosis-induced factor,[45,46] which eventually blocks the activation of caspase-9. Therefore, it blocks the activation of the terminal effector caspase-3. The second suggests that the Bcl-2 family prevents both cytochrome-c release and Apaf-1 activation[47,48] because Bcl-xL binds to Apaf-1 inhibiting its association with cytochrome-c. B cell lymphoma-xL also binds to procaspase-9 inhibiting its association by Apaf-1 and resulting in eventual blockade of terminal caspase activation.[46,47] The first theory is the most likely to be correct since it localized Bcl-2 in the mitochondrial membranes, although the other theory should remain under consideration until unequivocally refuted (**Figure 2**).

Diseases associated with a decrease in apoptosis.

Autoimmunity. Recent data suggests that defective regulation of apoptosis in lymphoid cells may be a factor that contributes to the pathogenic mechanism of autoimmune diseases. Also, a decrease in the clearance of apoptotic cells might be a contributing factor in systemic autoimmunity.[49] Immunologically, a great deal of evidence suggests that during the immune repertoire, any lymphocytes that recognize our self cells will be deleted by apoptosis.[50,51] Defects in the deletion mechanism of auto-reactive T cells might play a critical event in the initiation and maintenance of autoimmune diseases. The lymphoproliferative (lpr) mice encoding a defective Fas gene show resistance in their lymphoid cells to apoptosis.[52,53] Like Fas, FasL-deficient mice develop generalized lymphoproliferative disease with autoimmunity identical to that in lpr/lpr.[54] These mice develop lymphadenopathy rapidly and systemic lupus erythromatosis-like autoimmune disease. Previous study demonstrates the different strains of lpr mice that develop various patterns of auto-antibodies which indicates the involvement of other unknown genetic factors.[55,56] A common feature characterized systemic lupus erythromatosis and rheumatoid arthritis, which are the imbalance between the production and loss of lymphocytes and synovial cells. Previous studies[57,58] noted that all potent inducers of apoptosis such as steroids and cyclophosphamide are among the identifiable therapies of autoimmune diseases. It is well established that any defect in the clearance mechanism of lymphoid cells and other cells dying by apoptosis might be crucial in the maintenance of autoimmune diseases. This concept has been developed by findings that unknown factors block phagocytic ingestion of apoptotic cells, so that the dead cells engulfed and cleared away whilst intact, but rather leak their contents, which include endonucleases. These circulating nucleosomes detect lupus.[59] Insulin dependent diabetes mellitus (IDDM) is likely to be associated with an inhibition of apoptosis. The resistance of T cells to apoptosis in non-obese diabetic (NOD) mice has recently been associated with upregulation of the anti-apoptotic Bcl-x protein in T cells. This finding in the NOD mouse may open a new research field for diabetes-susceptibility genes in human IDDM. It had been shown that both ß and T cells from subjects with IDDM and those at risk for the disease are highly defective in the surface expression of Fas. This led the authors to hypothesize that loss of tolerance in IDDM may be partly explained by a defective expression of Fas.[60] Cytotoxic T-cells destroyed the ß-cells using perforin or granzymes as effector molecules. Perforin causes lysis of the target cell, whereas granzymes A and B mainly cause apoptosis. Recent studies revealed that the ß-cell is one of the most susceptible cells for endoplasmic reticulum (ER) stress, and ER stress-mediated apoptosis in the ß-cells can be a cause of diabetes. A comprehensive understanding of the impact of the

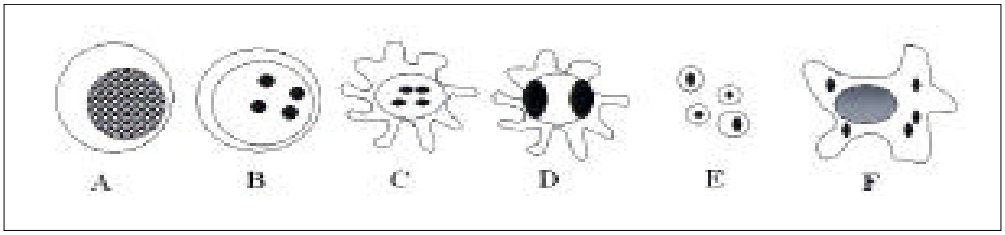

Figure 1 - Phases of apoptosis **a)** normal resting cell **b)** cell volume is lost and chromatin clumped **c)** blebbing process **d)** chromatin collapsed into the margins of the nuclear envelope **e)** apoptotic cell breaks down into apoptotic bodies **f)** apoptotic bodies ingested by macrophage.

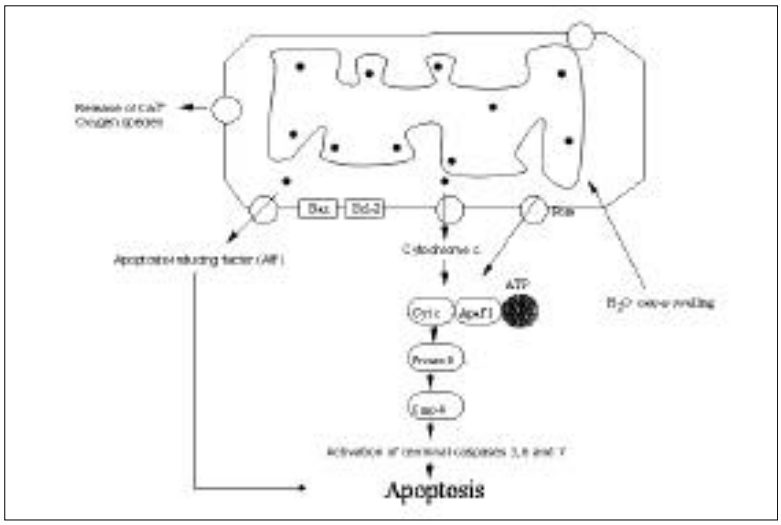

Figure 2 - Relationship between Bcl-2, cytochrome-c release and caspase-3 activation. Cyt c - cytochrome c, Apaf 1 - apoptosis activating factor, ATP - adenosine triphosphatase.

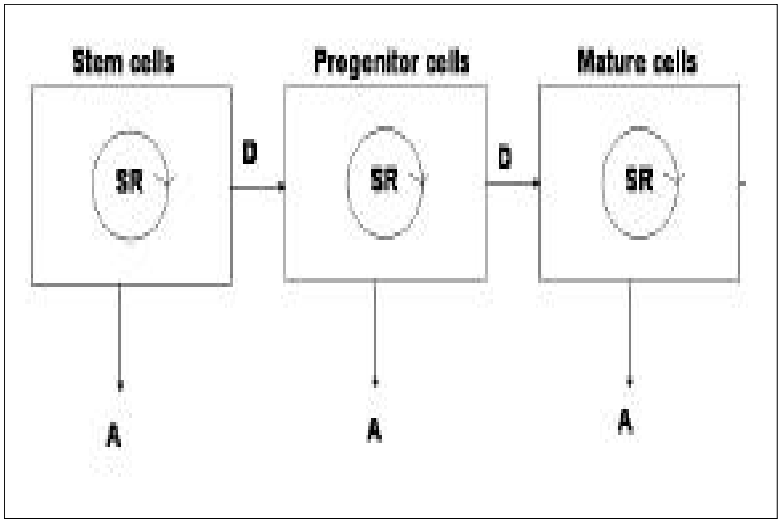

Figure 3 - Kinetic determinants of progenitor cell population size. SR - Self-renewal, D - Differentiation, A - Apoptosis.

ER stress pathway in the ß-cells and how it relates to the development of diabetes may contribute to provide new targets for the prevention and treatment of this disease.[61-66] Two important factors in the etiology and pathogenesis of diabetes are: (1) Apoptosis in the regulation of the ontogenic development of pancreatic islets; (2) Apoptosis in the ontogenetic development and function of the immune system. The death of ß-cells in autoimmune diabetes, results from the cytolysis of antigen-specific CD8 T cells. Wong et al[60] shows to be possible in vitro where certain CD8 clone can transfer diabetes in the complete absence of CD4 T cells in lymphocytes-deficient recipient.[67] Fas normally, not expressed in ß-cells, was upregulated by transfer of diabetogenic cells. This observation indicates that Fas-mediated ß-cells apoptosis, triggered by diabetogenic helper T cells, is an important mechanism of ß-cell killing in the NOD mice model. Fas ligation using its agonistic monoclonal antibody induces a rapid tyrosine phosphorylation of Jun-amino terminal kinase (Juk) and formation of activator protein-1 corresponding to apoptosis of rheumatoid arthritis (RA).[68] Additionally, over expression of DAXX, a novel Fas death domain associated protein, induces activation of Juk and potentiated Fas-mediated apoptosis.[69] Fas ligation induces activation of caspase-3 and the cleavage of poly adenosine deoxy phosphatase (ADP) ribose polymerase (PARP) in RA synoviocyte.[70] Therefore, the FADD/caspase-8/ caspase-3/PARP pathway appears to be the key signal for Fas-mediated apoptosis in RA synoviocytes, suggesting signal transduction via the Fas molecule regulated the Fas-mediated apoptosis in synoviocytes.

Hematological malignancies. Aberrant inhibition of apoptosis prevents normal homeostasis and promotes tissue tumorigenesis (**Figure 3**). In addition, acquisition of the ability to evade cellular suicide or apoptosis is one of the master switches that contributes to cellular transformation and ultimately invasive cancer. Also, the responsiveness of tumors to chemotherapy in part derives from the capability of cells to undergo apoptosis.[71] One of the best characterized apoptotic signaling cascades follows the engagement of Fas/Apo-1/CD95 with its ligand FasL/CD95L. An increase in Fas/CD95 expression has been found on myeloid progenitor cells from patients with chronic myeloid leukemia (CML), myelodysplasia and aplastic anemia compared to normal marrow progenitors.[72-74] Roth et al[75] and Leithauser et al,[76] found elevated serum levels of Fas in beta-chronic lymphocytic leukemia (CLL) patients. Previous studies found that Bcl-2 expression correlates with a poor response to chemotherapy in acute myeloid leukemia.[77-79] Treatment with Bcl-2 antisense oligonucleotides sensitizes cells to undergo apoptosis.[80] Webb et al[81] showed that Bcl-2 antisense oligonucleotides reduced tumor mass in 2 patients with non-Hodgkin's lymphoma. They showed a reduction in Bcl-2 protein levels after treatment.[81] It has been found that Bcl-2 is highly expressed among CML patients in blast crisis phase (BC), but not in chronic phase.[82] Preisler's group[83] shows a significant difference in the Bcl-2 expression among accelerated phase-CML and BC-CML patients compared both to treated and untreated chronic phase-CML patients. An antisense Bcl-2[84] molecule abolished the protective function of break point cluster region-Ableson (BCR-ABL) following IL-3 withdrawal from cell lines; however, there is still controversy about whether Bcl-2 and Bcl-xL is involved in the inhibition of apoptosis of leukemic cells. It shows that transfection of HL-60 with BCR-ABL protein, leads to up-regulation of Bcl-xL.[85,86] In a CLL study, Kitada et al[87] demonstrates the presence of high levels of Mcl-1 (anti-apoptotic protein) correlated significantly with poor remission.[87] Interestingly, the expression of Bcl-2 is down regulated after treatment with a signal transduction inhibitor (Imatinib, STI571 Novartis), showing involvement of this protein in the pathogenesis of CML.[88,89] Wild type p53 is a tumor suppressor gene,[90-92] which is inactivated either by deletion or mutation in many human cancers.[93] Deletions or mutations of p53 have been noted in many hematological disorders including: CML[94] and CLL.[95-97] Wild type p53 induces cell death following DNA damage[93] and treatment with inhibitory negative cytokines.[98] Hemopoietic myeloid progenitor cells from p53 knockout mice are resistant to apoptosis induced by heat shock and γ-irradiation.[99] Additionally, thymocytes derived from p53 knockout mice were found to be resistant to apoptosis induced by γ-irradiation.[100,101] Inactivation of the tumor suppressor gene p53 was found in 20-40% of BC-CML patients. Like other tumors, mutation of p53 in CML is associated with disease progression. Bis et al[102] and Lanza et al[103] show that p53 synergizes with p210 BCR-ABL protein when a p53 mutant was transfected into CD34+ cells from blast crisis CML samples, thus promoting the survival and proliferation of CML progenitor cells. Apoptosis induces transduction of the wild type p53 into a Burkett's lymphoma cell line containing mutant p53.[104] The chromosomal translocation t(9;22) is the hallmark of CML, and results in the fusion of 5'end of BCR gene 22q11 with 3'end of ABL gene on 9q11 known as Philadelphia (Ph+) chromosome. This translocation is seen in 20% of adult acute lymphocyte leukemia (ALL), 5% of pediatric ALL's and rarely in acute myeloid leukemia.[105-107] Anti-apoptotic effects, high proliferation and defects in the adhesion mechanism between primitive progenitor cells and stromal cells are considered to result from BCR-ABL fusion protein. These defects

are therefore likely to be responsible for myeloid expansion, and these include defects in adhesion,[108,109] self-renewal,[110,111] insensitivity to negative regulators[112-114] and defects in apoptosis. In CML, proliferation increases and proposed resistance to apoptosis as a mechanism accounting for myeloid cell expansion. For this reason, it needs further research in order to identify specific protein substrates for BCR-ABL and their role in apoptosis. When BCR-ABL transduces into hemopoietic cell lines, they become growth factor independent. A reduction of apoptosis might result in the expansion of progenitor cells in CML. Several studies show that cell lines transfected with BCR-ABL are protected from apoptosis.[115-120] BCR-ABL protects growth factor-dependent murine cell lines from apoptosis caused by growth factor deprivation, irradiation, and exposure to chemotherapeutic agents. This suggests that BCR-ABL p210 may increase the survival of CML cells and rescue them from undergoing apoptosis. Inhibition of the BCR-ABL kinase reverses the anti-apoptotic effects of BCR-ABL and is associated with downregulation of Bcl-x.[121] However, the exact mechanism underlying the inhibition of apoptosis in the cell lines by BCR-ABL remains to be elucidated. Bedi et al[122] noted that expression of the BCR-ABL chimeric gene produced by a balanced translocation in chronic myeloid leukemia, conferred resistance to multiple genotoxic anticancer agents, and that BCR-ABL expression inhibited the apoptotic response to DNA damage without altering either G1 arrest or DNA repair. They felt that the inherent resistance of human cancers to genotoxic agents may result not only from the loss or inactivation of the wild-type p53 gene, but also from the genetic alterations such as BCR-ABL that can delay G2/M transition after DNA damage. It is relevant to point out that BCR-ABL activates Ras, which leads to an increase in the transcription of c-Myc, and protection of cells from apoptosis.[123] Increased survival of progenitors, precursors and mature Ph+ cells, could lead to abnormal accumulation of these cells in blood and marrow. Amarante et al[124] demonstrates p185 BCR-ABL inhibits apoptosis in response to many stimuli in HL-60 and K562 cell lines by blocking the cyt c release from mitochondria, and thereby inhibiting caspase-3 activation.

Viral infections. Viral infection may also cause apoptosis through direct viral cytotoxicity, induction of tumor necrosis factor, or conflicting signals controlling cell growth. Apoptosis was found to be part of the viral pathogenesis in case of adenovirus and influenza viruses, while other viruses can inhibit apoptosis namely baculovirus gene p35. Both p35 gene and inhibitor of apoptosis (IAP) found in baculovirus are able to inhibit apoptosis in a response to large number of triggers.[125,126] Like baculovirus, Pox virus seems to abolish apoptosis by producing an inhibition for IL-1 converting enzyme (ICE), similar to cowpox gene ceramide-A (crm-A) in which it acts as specific inhibitor for ICE.[127] Additionally, crm-A appears to be involved in the inhibition of inflammatory response development against viral infection and thereby contributing to the viral pathogenesis.[127,128] Viral latency is an important event especially during Epstein-Barr virus (EBV) infection, the viral gene latent membrane protein-1 (LMP-1) produced during latency, upregulates Bcl-2 expression, thus providing a proper survival environment to latency infected cells.[129] Transfection of LMP-1 into apoptosis-sensitive ß-cell lines can render them resistant to cell death.[130] Exceptionally, apoptosis is a general phenomenon in severe acute respiratory syndrome (SARS) and the invasive cells in the pathological tissues are primarily monocytes, suggesting that apoptosis and invasion of monocytes play important roles in the progression of SARS. The cell apoptosis and decreased number of T cell and ß-cells in the lungs and CD4+CD8+ T cells and CD20+/CD45RA+ ß-cells in the spleen and lymph nodes indicate that the SARS virus may exercise an immune cell-killing effect to some extent during its pathogenesis.[131]

Diseases associated with an increase in apoptosis.

Acquired immune deficiency syndrome (AIDS). Inappropriate induction of CD4+ T cell apoptosis by the human immunodeficiency virus (HIV) may be relevant to the pathogenesis of AIDS.[132] A viral transcription gene-Tat- was demonstrated to affect mRNA transcription of some genes which seems to be involved in the cell survival. Moreover, the Tat gene was identified to upregulate the expression of Bcl-2 oncogenic protein, thereby, protecting the cell from apoptosis.[133] The early observational studies in apoptosis of AIDS pathogenesis have shown that peripheral blood T cells from HIV-infected individuals were highly sensitive to in vitro-induced cell death. In fact, the incubation of T cell from HIV patients alone in medium will trigger apoptosis shortly after short term culture.[134-136] In addition, following activation with a wide variety of inducers including mitogens, super-antigens will increase significantly the percentage of apoptotic cells.[134,137] Amendola et al[138] highlighted that T cells from lymph nodes and peripheral blood of HIV patients are expressing tissue transglutaminase (tTG) and a Ca^{2+} - independent enzyme. These 2 factors seem to be involved in the pre-apoptotic process.[138] It is becoming more evident that, not only CD4 subset is primed for apoptosis in HIV infection, but also CD8 subset.[139] Interestingly, only activated T lymphocytes expressing CD45RO, HLA-DR, CD38 have shown to be more prone to apoptosis compared with

controls.[140,141] Histopathological studies from lymph nodes and thymus of HIV-infected individuals show that apoptosis occurs in the neighboring cells not only in the infected cells.[142] This observation is supported by ex-vivo experiments which show that approximately 50% of peripheral blood lymphocytes from HIV-infected individuals undergo apoptosis.[143] The underlying mechanism that lead to CD8 T cells anergy and apoptosis are not yet well understood. It has been suggested that CD8 T cells from HIV-infected individuals behave in a similar manner as if they are deprived of cytokines.[144]

Neurodegenerative diseases. Acute and chronic neurodegenerative diseases are illnesses associated with high morbidity and mortality, and few or no effective options are available for their treatment. A characteristic of many neurodegenerative diseases which include stroke, brain trauma, spinal cord injury, amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, and Parkinson's disease is neuronal cell death. Given that central nervous system tissue has very limited regenerative capacity, it is of utmost importance to limit the damage caused by neuronal death. There are 2 essential factors; firstly, Bcl-2 over-expression might decline the neurotoxicity of the potential inducers. Secondly, neurotrophic growth factor and extracellular matrix also affect the cell death of neurons.[145,146] Caspases have a pivotal role in the progression of a variety of neurological disorders. In acute neurological diseases, both necrosis and caspase-mediated apoptotic cell death occur.[147] By contrast, in chronic neurodegenerative diseases, caspase-mediated apoptotic pathways have the dominant role in mediating cell dysfunction and cell death.[148] A primary difference between acute and chronic neurological diseases is the magnitude of the stimulus causing cell death. The greater stimulus in acute diseases results in both necrotic and apoptotic cell death, whereas the milder insults in chronic diseases initiates apoptotic cell death.[149] Intervention in upstream events of apoptosis by anti-apoptotic therapy provides morphological and functional rescue. In contrast, inhibition of the propagation and execution phase of apoptosis, namely by inhibition of caspases, blocks or delays cell death but may not recover neuronal function.[150] At this stage, the combination of an anti-apoptotic together with a neuro-restorative therapy may be promising.[151] Activation apoptotic pathways are a feature of a broad range of neurological diseases that makes them important and attractive therapeutic targets. Pharmaceutical companies are actively searching for compounds that inhibit these pathways. The first clinical trials of an inhibitor of apoptosis (minocycline) for neurodegenerative disorders (Huntington's disease and ALS) are in progress. It is likely that in the next several years, additional inhibitors of apoptosis will become part of the everyday armamentarium of clinicians who are treating neurological diseases that involve caspase-mediated cell dysfunction and cell death.[152] Alzheimer's disease is associated with the continuous accumulation of beta-amyloid peptide. In addition, mutation of beta-amyloid precursor proteins are mainly connected with Alzheimer's disease. Just recently identified, several investigators demonstrates that beta-amyloid proteins induce the neurons to undergo apoptosis.[153,154] Ultrastructural analysis of neurons exposed to amyloid ß revealed the morphological changes of apoptosis.[155] Exposure of cultured neurons against amyloid ß results in caspase activation and caspase inhibition protect these neurons against amyloid ß-induced apoptosis, consistent with the involvement of apoptosis cascade in the neurodegenerative action of amyloid ß.[156] There is evidence that mitochondrial reducing potential and cellular adenosine triphosphatase levels decrease following exposure of cultured cells to amyloid ß.[157] Additionally and more importantly, the transcriptional factor nuclear factor-kß is increasingly implicated in the prevention of neuronal apoptosis.[158] Spinal muscular atrophies are group of recessive neurodegenerative origin diseases characterized by gradual spinal cord motor loss. Neuronal apoptosis inhibitory protein is one of candidate genes, which is homolog to baculovirus inhibitor of apoptosis.[159] As it has been reported previously that baculovirus inhibitor of apoptosis is able to prevent apoptosis in many cell types. Berry and Boulton[160] augmented that any mutation in neuronal apoptosis inhibitory protein gene might render motor neurons more sensitive to cell death.

Myocardial infarction. In the context of myocardial infarction, apoptosis contributes to the total amount of cell death.[161] In myocardial infarction in humans, apoptosis has been observed in 3 different regions: (a) in the core of the ischemic myocardial area, (b) in the border zone of the infarction, and (c) in the viable myocardium, remote from the ischemic area. It was shown in an animal model that the increased apoptosis in remote areas after myocardial infarction is associated with an increase in the expression of pro-apoptotic proteins p53, Bax and of caspase-3.[162] In attempting to answer the question of whether apoptosis is a clinically important phenomenon in the context of myocardial infarction, one should study the effect of apoptosis inhibition. Several publications described the decrease in the number of terminal deoxynucleotide transferase (TdT) deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL)-positive cells in response to pharmacological interventions. Using the non-specific caspase inhibitor (benzyloxycarbonyl-Val-Ala-Asp [ZVAD]), the number of TUNEL-positive cells and the infarct size reduces in a rat model of ischemia-reperfusion.

In contrast, with specific inhibitors of caspase-1 and -3, only the amount of TUNEL-positive cells reduces without affecting the size of infraction. Consequently, future studies will have to be focused not only on the reduction of infarct size but also on the improvement of functional parameters at different time points after infarction.[163]

In conclusion, there have been huge advances in apoptosis research. Our current understanding of apoptotic signaling and resulting new insights into therapeutic possibilities will continue to improve. Improved understanding of apoptosis genes has opened a whole new treatment modality for treating many disorders in patients, also for designing better therapeutic models for these diseases. With important practical problems to be solved, apoptosis will continue to occupy a central position in biology and medicine research over the coming years.

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