The morphology of apoptotic cells is characterized by "membrane blebbing" with membrane-enclosed apoptotic bodies being released. These are engulfed by phagocytes, preventing an inflammatory reaction.

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Molecular Mechanisms of Death-Receptor-Mediated Apoptosis

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Apoptosis, also called "programmed cell death", can be induced by a variety of stimuli including activation of death receptors by the corresponding death ligands. Death receptors are a subgroup of the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily and are characterized by a death domain, which is required for signal transduction. Upon apoptosis induction, caspases, a family of aspartyl-specific cysteine proteases, are activated, which are the main executioners of apoptosis. Finally, specific death substrates are cleaved, resulting in the morphologic features of apoptosis. Depending on the cell type, activation of mitochondria is of central significance for apoptosis induction. This signaling pathway can be modulated by different pro- and anti-apoptotic proteins such as Bax and Bcl-2, which are localized at the mitochondria. Furthermore, apoptosis initiation can be prevented at the death receptor level by FLICE (caspase-8)-inhibitory proteins (FLIPs). Deregulation of apoptosis is associated with diseases like cancer, autoimmunity, and AIDS. Therefore, the elucidation of cell death pathways and the identification of modulators of apoptosis have many therapeutic implications.

KEYWORDS:

apoptosis · biological signal transduction · CD95 · receptors · tumor therapy

1. Introduction

Programmed cell death was discovered by C. Vogt in the middle of the nineteenth century[1] by observation of the morphology of dying cells during the metamorphosis of amphibians. Within more than one hundred years after the initial description, programmed cell death was rediscovered by several investigators. In the landmark paper by Kerr, Wyllie, and Currie[2] the name "apoptosis" for non-necrotic cell death was coined. Since then, apoptosis has become a major research area in biology and medicine.

Apoptosis is crucial for tissue homeostasis in multicellular organisms. It plays an important role in many physiological processes, especially in the immune system, in the nervous system, and in development.[3, 4] For example, apoptosis of cells in the interdigital spaces is involved in the development of fingers and toes out of the limb buds. Furthermore, many diseases are associated with either too much or too little apoptosis, such as AIDS, cancer, and autoimmunity.[3] Generally, two distinct ways of cell death are discriminated: necrosis and apoptosis. Necrotic cell death occurring upon tissue injury is a passive process. The damaged cell is enlarged, finally the plasma membrane disrupts, and cytosolic components are released into the extracellular space, causing an inflammatory reaction. In contrast, apoptosis is an active reaction. On the molecular level, the cell death program consists of three parts: initiation, execution, and termination of apoptosis. Apoptosis can be initiated by many different stimuli including growth factor withdrawal ("death by neglect"), UV- or γ-irradiation, chemotherapeutic agents, or by a family of transmembrane proteins called death receptors. In most cases, the execution phase is characterized by shrinkage of the cells, membrane inversion and exposure of phosphatidylserine, blebbing (zeiosis), fragmentation of the nucleus, chromatin condensation, and DNA degradation. In the termination phase, membrane-enclosed vesicles, the small remainders of the cell ("apoptotic bodies"), are engulfed by phagocytes,[3] which prevents an inflammatory reaction. This review focuses on the signaling by death receptors, particularly the CD95 pathway, since its molecular mechanisms have been almost completely elucidated.

2. Death receptors and death ligands

The growing subfamily of death receptors is part of the TNF-/NGF-receptor superfamily. This superfamily is characterized by two to five cysteine-rich extracellular domains. The death receptors are characterized by an intracellular death domain (DD), which is crucial for transduction of the apoptotic signal. Six members of this subfamily are known so far, namely TNF-R1 (CD120a), CD95 (APO-1 or Fas), DR3 (APO-3, LARD, TRAMP, WSL1), TRAIL-R1 (APO-2, DR4), TRAIL-R2 (DR5, KILLER, TRICK2), and DR6.[5] Among these, CD95 is the best characterized death receptor.[3] CD95 is a widely expressed glycosylated cell surface protein of approximately 45 to 52 kDa (335 amino acids).[6] It is a type I transmembrane receptor and can also occur in a soluble

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form.[6–8] The soluble form is generated by differential splicing with the transmembrane part being spliced out and may contribute to regulation of apoptosis.[9]

Death receptors are activated by their natural ligands which have co-evolved as a death ligand family, called the TNF family, corresponding to the death receptors (Figure 1). Except for LTα, the death ligands are type II transmembrane proteins, of which the respective soluble forms can be generated by the activity of metalloproteases. Several studies have reported that the soluble form of CD95L is capable of inducing apoptosis,[3] whereas others describe the membrane-bound form of CD95L as the only active form.[10, 11] A recent in vivo study has demonstrated that only membrane-bound CD95L induces apoptosis and elicits an inflammatory reaction whereas soluble CD95L even has suppressive effects.[12]

Closely related to the death receptors are the so-called decoy receptors. These comprise TRAIL-R3 (DcR-1, LIT, TRID),[13–15] TRAIL-R4 (DcR-2, TRUNDD),[16, 17] OPG,[18] and DcR-3 (TR6).[19] The latter binds to CD95L and another ligand called LIGHT,[19, 20] the others to TRAIL.[21] However, no correlation between the expression of TRAIL-R3 or TRAIL-R4 and resistance to TRAIL-induced apoptosis could be demonstrated so far.[22–24] Therefore, it remains to be elucidated whether these receptors actually function as decoys.

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3. Initiation of CD95-mediated apoptosis

3.1. Trimerization of CD95 by CD95 ligand

Triggering of CD95 by either agonistic antibodies or CD95L leads to oligomerization of CD95 receptors. Oligomerization of the death receptors by oligomerized ligands is essential for the transduction of the apoptotic signal since CD95 monomers and dimers do not induce apoptosis.[25] The most probable structure to transmit an apoptotic signal is a CD95 trimer since this structure corresponds to the predicted trimeric structure (based on results from X-ray crystallography) of members of the TNF-R superfamily, for example LTα complexed with TNF-R1[26] and TRAIL[27] complexed with TRAIL-R2.[28, 29]

3.2. The death domain

The intracellular part of CD95 does not contain any consensus sequences which would predict an enzymatic activity. A deletion of 15 amino acids of the C-terminus of CD95 has been shown to increase CD95-mediated apoptosis.[30] Further deletions inhibit the CD95 signal completely. By comparing the sequence of the intracellular part of CD95 with that of TNF-RI, a homologous region of 68 amino acids has been defined. Moreover, by deletion and point mutagenesis Tartaglia et al.[31] defined a region of TNF-RI essential for the cytotoxicity of the receptor. This stretch is 80 amino acids long and was later called "death domain" (DD). The DD also contains the valine residue (Val 238) mutated in *lpr*^{cg} mice (see Section 6.2), which abolishes signaling of apoptosis. The three-dimensional structure of the CD95 DD has been determined by NMR spectroscopy. It consists of six antiparallel, amphipathic α-helices arranged in a novel fold which is likely to be important for binding intracellular signaling molecules.[32] Further DD-containing proteins were found to be binding to the CD95 receptor, namely FADD (Mort1)[33, 34] and RIP.[35] In addition to its DD, FADD also contains a so-called death effector domain (DED), with both domains being important for homophilic interactions, for example with caspase-8 (see Section 3.3).

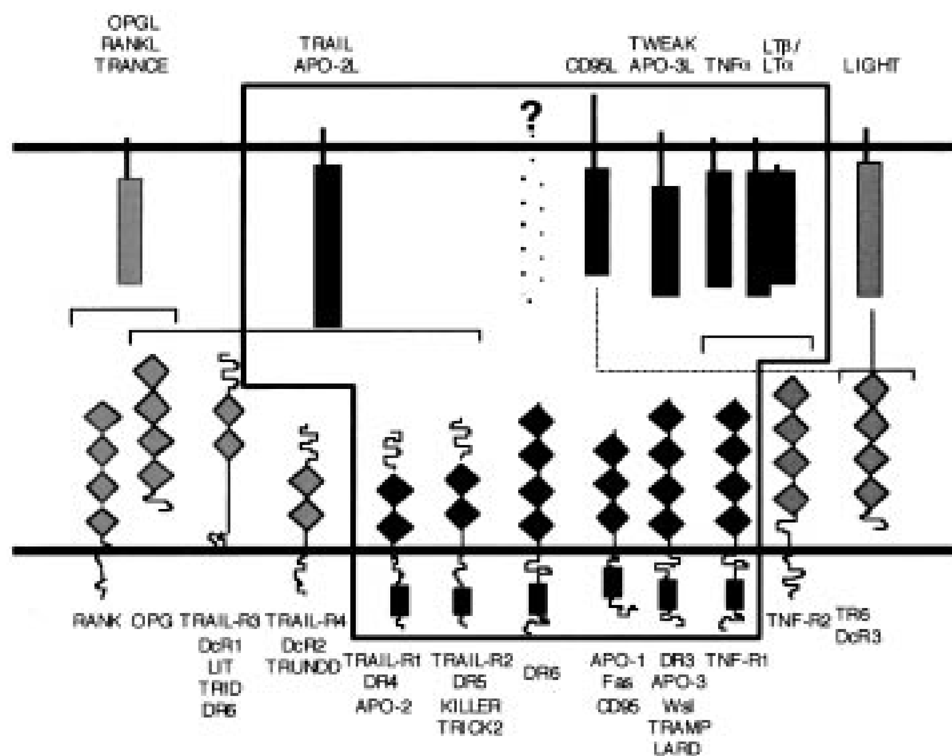

Figure 1. Death receptors and death ligands. Ligands are shown at the top, receptors at the bottom. Death receptors and death ligands are designated in black and grouped in a box. Homologues, including decoy receptors, are shown in gray. The plasma membranes are illustrated as horizontal lines, death ligands as rectangles. Cysteine-rich domains in the extracellular part of death receptors are depicted as diamonds. The black rectangles in the intracellular part are the death domains of the death receptors, which are essential for delivering the apoptotic signal.

3.3. The death-inducing signaling complex (DISC)

As CD95 does not contain an enzymatic activity, the death signal must be transmitted by signaling proteins which associate with the receptor upon stimulation. Such a complex of proteins was identified that associated only with stimulated CD95 (Figure 2).[36] Treatment of CD95-positive cells with the agonistic monoclonal antibody anti-APO-1 and subsequent immunoprecipitation of CD95 (APO-1 or Fas) resulted in the identification of four cytotoxicity-dependent APO-1-associated proteins (CAP1–4) that associated within seconds after CD95 triggering. These proteins were resolved on two-dimensional (2D) isoelectric focussing/SDS-polyacrylamide gels. CAP1–4 formed a complex with CD95 termed the death-inducing signaling complex (DISC). CAP1 and 2 were identified as two different serine-phosphorylated species of FADD binding to CD95 in a stimulation-dependent fashion in vivo. The phosphorylation of FADD, however, is independent of stimulation. This phosphorylation exclusively occurs at Ser 194 and was found to correlate with the cell cycle.[37]

When FADD dominant negative (DN; the C-terminal DD-containing part, without DED) was stably transfected into cells, the formation of the DISC was altered. FADD-DN was recruited to CD95 instead of the endogenous FADD, and analysis on 2D gels revealed that CAP3 and CAP4 were not part of the DISC anymore.[38] These proteins were therefore prime candidates for transducers of the CD95 death signal. Using nano-electrospray tandem mass spectrometry, sequence information for CAP4 was obtained, leading to the retrieval of a full-length clone from a cDNA data base that contained all sequenced peptides.[39] The deduced protein contains two DEDs at its N terminus, by which it binds to FADD. At its C terminus the protein shows the typical domain structure of an ICE-like protease and was therefore termed FLICE (FADD-like ICE). FLICE belongs to the aspartyl-specific cysteine proteases, which are now called caspases,[40] and was renamed caspase-8. Thus, identification of caspase-8 and its localization to the DISC upon CD95 triggering connected two levels in the apoptosis pathway, the CD95 receptor level with the intracellular level of the apoptosis executioners, the caspases (see Section 4.1).

The finding that caspase-8 is part of the CD95 DISC in vivo suggested its activation at the DISC level. It was shown recently that the entire cytoplasmic caspase-8 pool is converted into active caspase-8 subunits at the DISC.[41] After stimulation, FADD and pro-caspase-8 are recruited to CD95 within seconds after receptor engagement. According to the "induced proximity model", oligomerization of the recruited pro-caspase-8 is sufficient for its autoproteolytic activation, upon which the active subunits of caspase-8 are released into the cytoplasm.[42–44] This leads to a cascade of caspase activation events induced by caspase-8. The order of caspases in this cascade has been partially elucidated.[45, 46] In a similar fashion as in CD95 signaling, caspase-8 is involved in the signal transduction of the other death receptors as revealed by the study of caspase-8 knock-out mice[47] and analysis of the TRAIL-R2 DISC.[48–50]

4. Execution of apoptosis

4.1. Caspases

A number of recent studies with knock-out mice have demonstrated that caspases play an important role in apoptosis and development.[51, 52] Caspases are a growing family of aspartyl-specific cysteine proteases.[40] They are not only essential for death-receptor-proximal events but also for execution of apoptosis, since inhibition of caspases by a broad-spectrum

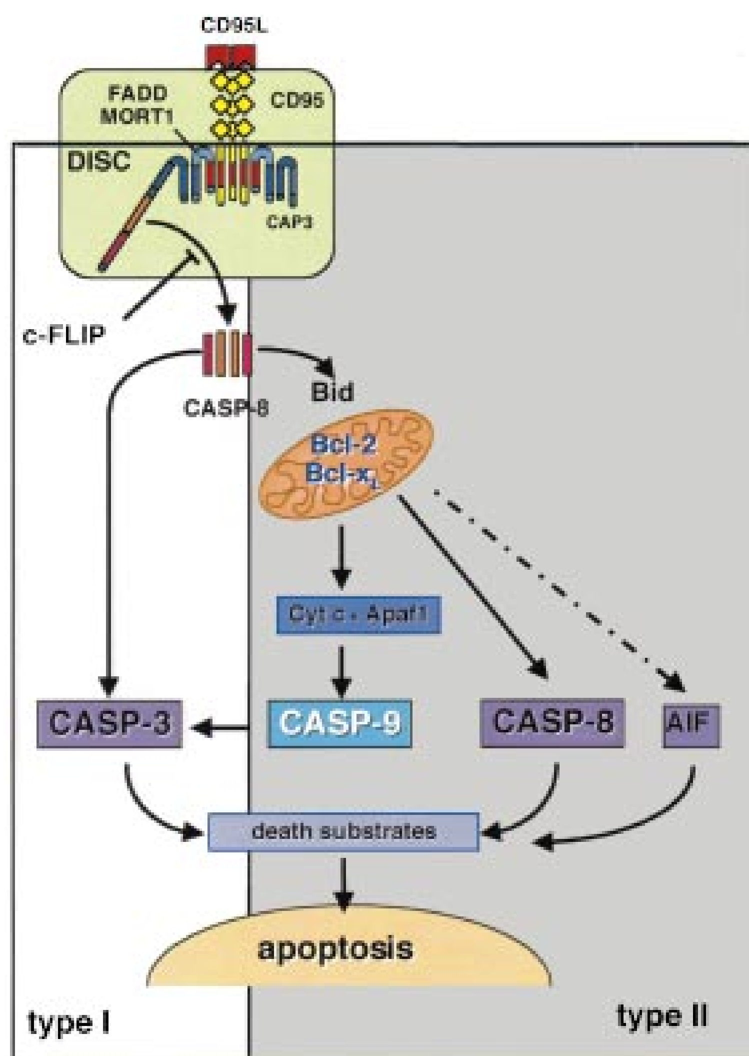

Figure 2. CD95 signaling pathways. Ligation of CD95 results in DISC assembly and release of the active caspase-8 subunits. c-FLIP blocks activation of caspase-8 directly at the DISC. In type I cells, CD95 triggering causes strong caspase-8 activation at the DISC, directly leading to the activation of other caspases like caspase-3 and subsequently to apoptosis. In type II cells, only a small amount of DISC is formed resulting in mitochondrial activation and the release of apoptogenic factors such as cytochrome c and AIF. In the cytosol, the active apoptosome is formed, initiating a caspase cascade downstream of mitochondria. Pink box = death domain; light blue box = death effector domain.

caspase inhibitor does not prevent death but results in necrosis rather than apoptosis.[53, 54] So far, fourteen mammalian caspases have been identified, which can be subdivided into three families based on their sequence homology and substrate specificity[55] (Table 1).

Caspases are synthesized as proenzymes (zymogens) that are activated by proteolytic cleavage. The active enzyme is a heterotetrameric complex of two large subunits containing the active site and two small subunits, as deduced from the crystal structures of caspase-1, caspase-3, and caspase-8.[56–60] Activation of caspases has been reported for various apoptotic stimuli.[3] A variety of splice variants of several caspases have been reported.[61] They function either as promoters or inhibitors of caspase activation.[62, 63] Thus, differential splicing may be one of the mechanisms regulating apoptosis. Most of the known splice variants have been described at the mRNA level. The number of isoforms expressed as proteins, however, seems to be more limited.[64]

4.2. Death substrates

The activity of caspases characterizes the execution phase of apoptosis. Therefore, the search for substrates cleaved by caspases during apoptosis should provide insight into the more downstream events involved in apoptosis signaling. Several of these so-called "death substrates" have been identified so far. Among these are molecules involved in DNA repair, ribonucleoproteins, signaling molecules, structural proteins of the cell, and oncoproteins (for review see [55]). An example for signaling molecules is the DNA fragmentation factor (DFF)[65] also called caspase-activated DNase (CAD).[66, 67] Signaling molecules can either be activated or inactivated by cleavage depending on their mode of action. Cleavage of structural proteins may account for some of the massive morphological changes, such as membrane blebbing, nuclear fragmentation, and the formation of apoptotic bodies during apoptosis. Therefore, different features of apoptosis might be analyzed at the level of caspase targets. However, caspase activation does not necessarily lead to apoptosis but can occur even without inducing cell death.[68, 69]

The number of identified caspase substrates will be increasing in the future. Further studies are necessary to unravel the caspase cascades induced by the different death receptors and to identify specific targets for caspases that establish the link between caspase activation and more downstream events in apoptosis.

Table 1. The caspase family. All known mammalian caspases are listed by numbers. Synonyms, references, and specificity group are indicated.

Name	Alternative Name	Reference	Group[a]
Caspase-1	ICE	[134, 135]	I
Caspase-2	ICH-1, Nedd-2	[136, 137]	II
Caspase-3	CPP-32, Yama, Apopain	[138 – 140]	II
Caspase-4	ICH-2, TX, ICE-rel-II	[141 – 143]	I
Caspase-5	ICE-rel-III, TY	[143, 144]	I
Caspase-6	Mch2	[145]	III
Caspase-7	Mch3, ICE-LAP3, CMH-1	[146 – 148]	II
Caspase-8	FLICE, MACH, Mch5	[39, 149, 150]	III
Caspase-9	Mch6, ICE-LAP6	[151, 152]	III
Caspase-10	Mch4, FLICE2	[150, 153]	III
mCaspase-11	mICH-3, mCASP-11	[154, 155]	n.d.
mCaspase-12	mCASP-12	[155]	n.d.
Caspase-13	ERICE	[156]	I
Caspase-14		[100, 157, 158]	n.d.

[a] n.d. = not determined.

5. Regulation of apoptosis

Apoptosis can be modulated directly at the death receptor level. For instance, the glycosylation status of CD95 has been shown to modify CD95-mediated apoptosis.[70, 71] Inside the cells apoptosis can be regulated at different levels. For example, inhibitor of apoptosis proteins (IAPs) inhibit caspases directly.[72] Here, we will focus on the modulation of apoptosis initiation at the DISC level and on the regulation of the mitochondrial pathway.

5.1. Modulation of apoptosis initiation by c-FLIP

Recently, a family of proteins containing death effector domains (DED) was found by searching the data bases. Some of these proteins are components of the class of γ-herpes viruses such as herpes virus Saimiri (HVS), human herpes virus 8 (HHV 8), a virus associated with Kaposi sarcoma, and moluscum contagiosum virus. These proteins were called viral FLICE-inhibitory proteins (v-FLIPs). v-FLIPs contain two DEDs, by means of which they bind to the CD95 DISC and thus inhibit the activation of caspase-8. v-FLIPs capable of inhibiting apoptosis mediated by several death receptors (CD95, TNF-R1, DR3, and TRAIL-R1), suggesting the use of similar signaling pathways by these receptors.[73–75]

A human homologue of v-FLIP was identified by several laboratories and termed c-FLIP/FLAME-1/I-FLICE/Casper/CASH/MRIT/CLARP/Usurpin.[76–83] At the protein level, c-FLIP exists in two splice variants, a short one and a long one.[84] The short form structurally resembles v-FLIP whereas the long form has a similar domain structure as caspase-8 but an inactive enzymatic site. It interferes with the generation of active caspase-8 subunits at the receptor level in both CD95 type I and type II cells (see Section 5.2.2) (Figure 2).[84, 85]

It was reported that in melanoma cell lines resistance to TRAIL-induced apoptosis correlated with the expression of c-FLIP.[22] However, this observation could not be confirmed by others.[23] In contrast to death-receptor-mediated apoptosis, cell death induced by γ-irradiation, chemotherapeutic agents, and perforin/granzyme B cannot be inhibited by c-FLIP.[86]

5.2. Regulation of signal transduction

5.2.1. The Bcl-2 family

Bcl-2 was discovered first in the development of human follicular lymphoma, a B-cell malignancy.[87] The Bcl-2 family is characterized by several homologous α-helical amino acid stretches, called Bcl-2 homology domains, BH1 – 4. According to their function, Bcl-2 family members can be differentiated into anti-apoptotic (e.g. Bcl-2, Bcl-x_L) and pro-apoptotic proteins (e.g. Bax, Bak). The BH4 domain is specific for the anti-apoptotic group, whereas the BH3 domain is required for apoptosis induction. This is illustrated by a subgroup of the pro-apoptotic Bcl-2 family members, the BH3-domain-only proteins (e.g. Bid, Bad, Bim).[88, 89]

Although the impact of Bcl-2 family members on apoptosis is quite obvious, the biochemical mechanism of their function has not yet been elucidated completely. Several models have been established to explain apoptosis promoting and inhibiting functions. The first model was based on the heterodimerization properties of the family members, suggesting that the ratio between pro- and anti-apoptotic family members determines cell fate.[88] However, the scenario became more complex when mitochondria joined the apoptotic play. Upon apoptosis induction by a variety of stimuli, the mitochondrial transmembrane potential ($\Delta\Psi_m$) is rapidly lost, and apoptogenic factors such as cytochrome *c* are released from the mitochondrial intermembrane space into the cytosol (see Section 5.2.2).

Some family members (e.g. Bcl-2, Bcl-x_L, Bak, Bax) have a transmembrane domain in common, targeting these proteins to intracellular membranes like the endoplasmic reticulum, the nuclear envelope, and the mitochondrium. Research has concentrated on the latter. It has been shown that Bcl-2 family members can regulate the opening of the so-called permeability transition pore (PT pore), a multiprotein ensemble containing proteins from both mitochondrial membranes. Indeed, Bax and Bcl-2 interact with proteins of the PT pore complex, namely adenine nucleotide translocator and voltage-dependent anion channel.[90–92] Moreover, Bcl-x_L and Bid are structurally similar to pore-forming bacterial toxins,[93–97] and Bcl-2, Bcl-x_L, Bid, and Bax are capable of forming pores in artificial membranes.[98, 99] However, the connection between the PT pore, loss of $\Delta\Psi_m$, and the release of apoptogenic factors is not clear so far.

Another hypothesis for the anti-apoptotic function of Bcl-2 and Bcl-x_L proposes an interaction with the so-called apoptosome, the formation of which leads to apoptosis. The active apoptosome consists of the adapter protein Apaf1, cytochrome *c*, and caspase-9. Apaf1 was reported to bind to Bcl-x_L in over-expression systems.[100, 101] With endogenous proteins, however, this could not be confirmed.[102]

The effect of Bcl-2 on CD95-mediated apoptosis is subject of a controversial discussion. This controversy has recently been resolved by experiments described by Scaffidi et al.[103] and will be discussed in the next section.

5.2.2. Two CD95 signaling pathways

By comparing different cell lines with respect to CD95-mediated apoptosis signaling pathways, two cell types, termed type I and type II, were identified.[103, 104] In type I cells, induction of apoptosis is accompanied by activation of large amounts of caspase-8 at the DISC. This is followed by rapid cleavage of caspase-3, the main executioner caspase, prior to loss of $\Delta\Psi_m$, suggesting that a caspase cascade independent of mitochondria is activated (Figure 2).

In contrast, in type II cells activation of the mitochondrial pathway is required. This pathway is important for many apoptotic stimuli, for example chemotherapeutic drugs.[88, 105] In these cells, DISC formation is significantly reduced and activation of caspases occurs mainly subsequent to the loss of $\Delta\Psi_m$.[103] Therefore, in type II cells mitochondria play a role as "amplifiers" to initiate the executionary apoptosis caspase cascade. Activation of mitochondria is mediated by the pro-apoptotic BH3-only Bcl-2 family member Bid. Bid is cleaved by caspase-8 which is activated in low amounts at the DISC of type II cells. Truncated Bid translocates to the mitochondria and

induces loss of $\Delta\Psi_m$ and release of apoptogenic factors like cytochrome *c*, several pro-caspases, and apoptosis-inducing factor (AIF).[106–109] In the cytosol, cytochrome *c* binds to Apaf1, which recruits dATP and pro-caspase-9 after oligomerization.[110, 111] At this complex, termed the apoptosome, pro-caspase-9 is autocatalytically processed to the mature enzyme and initiates a caspase cascade downstream of the mitochondrium (Figure 2).[112, 113]

It should be noted that in both type I and type II cells mitochondria are equally activated upon CD95 triggering. In both cell types apoptogenic activities of mitochondria are blocked by Bcl-2 overexpression. However, only in type II but not in type I cells Bcl-2 overexpression blocks caspase-8 and caspase-3 activation as well as apoptosis. Therefore, in type II cells CD95-mediated apoptosis is dependent and in type I cells independent of mitochondrial activity.

By the analysis of specific knock-out mice, it can be dissected which apoptotic pathway is utilized by specific cell types. For example, thymocytes from caspase-9- and Apaf1-deficient mice are sensitive to CD95-mediated apoptosis, suggesting thymocytes to be type I cells.[114–116] In contrast, hepatocytes from Bid-deficient mice and embryonic fibroblasts from Apaf1-deficient mice are resistant to CD95-induced killing, identifying them as type II cells.[117, 118]

6. Physiological and pathological relevance of apoptosis

6.1. Involvement of apoptosis in the lymphoid system

In the normal lymphoid system, apoptosis occurs in primary lymphoid organs such as the bone marrow, liver, and thymus. Its function is to eliminate non-functional precursor cells with non-rearranged or aberrantly rearranged non-functional antigen receptors. In addition, apoptosis is essential for deletion of autoreactive T cells in the thymus, which is the basis of central self-tolerance. Peripheral deletion by apoptosis is a second line of establishing self-tolerance and downregulation of an excessive immune response, for example in lymph nodes and in the spleen. Only lymphocytes that survive this process determine immunological memory. The CD95/CD95L system contributes substantially to the elimination of peripheral lymphocytes after an immune response, mediated by the so-called "activation-induced cell death" (AICD).[119–122]

6.2. Gene defects in the CD95/CD95L system

Several mouse mutations have been identified that cause similar, complex disorders of the immune system, manifested as lymphadenopathy (enlargement of the lymph nodes) and autoimmunity. In *lpr* (lymphoproliferation) mice a retroviral insertion causes a splicing defect of the CD95 gene, premature termination, and greatly reduced expression of CD95 mRNA. In lpr^{cg} mice a point mutation (I225N or V238N) in the intracellular "death domain" of CD95 abolishes the transmission of the apoptotic signal. In *gld* (generalized lymphoproliferative disease) mice a point mutation in the C terminus of CD95L impairs its ability to interact successfully with CD95 to initiate apoptosis. Thus, a failure of apoptosis accounts for the complex immune disorders in *lpr* and *gld* mutant mice (for review see refs. [123, 124]). The finding that *lpr* and *gld* mutations are defective in the CD95/CD95L system has greatly helped to determine the physiological role of CD95-mediated apoptosis in the immune system.

A human disease phenotypically resembling *lpr* and *gld* mutant mice is the autoimmune lymphoproliferative syndrome (ALPS). It is characterized by mutations in CD95 (type Ia), CD95L (type Ib), and presumably caspase-10 (type II), which impair deletion of T lymphocytes by apoptosis.[125]

6.3. Clinical implications

Apoptosis has come a long way from the description of its morphological features to a molecular understanding of its signaling pathways and its physiological and pathological consequences. It adds a new chapter to the understanding of the pathogenesis of many diseases. Generally, there are diseases with too little apoptosis like cancer and autoimmunity and diseases with too much apoptosis like AIDS. Cancer could be regarded as a disease with too little apoptosis where the net increase of the tumor burden is the sum of an increased growth rate and a decreased apoptotic rate. The induction of apoptosis by chemotherapeutic agents, for example cisplatin, has general implications for the clinical situation (for review see refs. [126, 127]).

Recent studies reveal that FLIPs might be involved in tumorigenesis. In B cells transformed with Epstein–Barr virus, resistance and sensitivity to CD95-mediated apoptosis correlates with the ratio between c-FLIP and caspase-8.[128] Furthermore, tumor cells transfected either with v-FLIP encoded by human herpes virus 8 or with murine c-FLIP have a growth advantage in vivo.[129, 130] A dysregulation of c-FLIP expression also disturbes the homeostasis of normal cells. Indeed, retrovirus-mediated expression of c-FLIP in lymphocytes results in autoimmunity due to an accumulation of activated B and T cells.[131] In contrast, AIDS is characterized by too much apoptosis in cells of the lymphoid and non-lymphoid compartment, particularly in CD4-positive T cells.[132, 133]

7. Summary and outlook

During the last decade, the pathways resulting in apoptosis were partially elucidated at the molecular level. It is now important to increase our understanding of the molecular events that determine the particular steps of apoptosis and to develop methods of targeting apoptosis-regulatory molecules to specific cells of different tissues. Particularly, through using such methods the modulation of apoptosis will become an established therapeutic tool for the treatment of diseases. In addition, therapeutic windows for apoptosis modifiers have to be determined which only affect aberrant cells while leaving normal cells intact. By understanding the signaling pathways of apoptosis, it might be possible to reconstitute the normal level of apoptosis.

Abbreviations

$\Delta\Psi_m$	mitochondrial transmembrane potential
AIF	apoptosis-inducing factor
Apaf1	apoptosis-activating factor 1
Bak	Bcl-2-homologous antagonist/killer
Bax	Bcl-2-associated X protein
BH3	Bcl-2 homology domain 3
Bid	BH3-interacting domain death agonist
c-FLIP	cellular FLICE-inhibitory protein
DD	death domain
DED	death effector domain
DISC	death-inducing signaling complex
FADD	Fas-associated death domain
FLICE	FADD-like ICE
IAP	inhibitor of apoptosis
ICE	IL1β-converting enzyme
IL1β	interleukin-1β
TNF	tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
v-FLIP	viral FLICE-inhibitory protein

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