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Review

The CD95(APO-1/Fas) DISC and beyond

ME Peter*,[1] and PH Krammer[2]

[1] The Ben May Institute for Cancer Research, University of Chicago, 924 E. 57th Street, Chicago, IL 60637, USA
[2] German Cancer Research Center Heidelberg, Tumorimmunology Program, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany
* Corresponding author: ME Peter, The Ben May Institute for Cancer Research, University of Chicago, 924 E. 57th Street, Chicago, IL 60637, USA. Tel: 773 7024 728; Fax: 773 702 3701; E-mail: mpeter@uchicago.edu

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Abstract

CD95 (APO-1/Fas) is a prototype death receptor characterized by the presence of an 80 amino acid death domain in its cytoplasmic tail. This domain is essential for the recruitment of a number of signaling components upon activation by either agonistic anti-CD95 antibodies or cognate CD95 ligand that initiate apoptosis. The complex of proteins that forms upon triggering of CD95 is called the death-inducting signaling complex (DISC). The DISC consists of an adaptor protein and initiator caspases and is essential for induction of apoptosis. A number of proteins have been reported to regulate formation or activity of the DISC. This review discusses recent developments in this area of death receptor research.
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Abbreviations: AICD, antigen-induced cell death; ALPS, autoimmune lympho proliferative syndrome; DD, death domain; DED, death effector domain; DISC, death-inducing signaling complex; FLIP, FLICE-like inhibitory protein; IFN, interferon; TCR, T cell receptor; TRAF, TNF receptor-associated factor

Introduction

The phenomenon of apoptosis was first described by C Vogt in 1842[1] and rediscovered several times in the decades to follow on the basis of a characteristic morphology. During the last decade many of the molecular mechanisms of apoptosis signaling and morphology were examined and elucidated. Clearly, the discovery of diverse apoptosis pathways involving signals primarily via the death receptors (extrinsic pathway) or the mitochondria (intrinsic pathway) using caspases as effector molecules has dominated the field. Lately, a more sophisticated view of signaling pathways has arisen which leads to the observation that cell death can occur even in the absence of caspases. In this short review, we discuss the signaling pathways initiated by the CD95(APO-1/Fas) death receptor which has served as the paradigm for death receptor family signaling.

The CD95 receptor

CD95 is the best-characterized member of the tumor necrosis factor (TNF) superfamily of receptors. Its main and best-known function in signaling is the induction of apoptosis.[2] CD95 receptors are expressed on the surface of cells as preassociated homotrimers.[3,4] A similar association was described for several members of the TNF receptor superfamily, including the TNF receptor itself, CD40 and the TNF-related apoptosis-inducing ligand (TRAIL) receptor I.[5] These interactions were found to be mediated by a domain in the N-terminus, within the first of the cysteine-rich domains.[3,4] This association was described by binding of *in vitro* generated proteins to cells transfected with CD95, and by the use of chemical crosslinking reagents that allowed visualization of the associated homotrimers present on the cell surface.[3,4] Additionally, the use of the fluorescence resonance energy transfer technique, in which the emission wavelength of one spectral variant of the green fluorescent protein (GFP) is used to excite a second spectral variant of GFP when the two are in close proximity (approximately 100 Å), supported these observations. Using this method, Siegel *et al*.[4] demonstrated that a deletion or mutation in certain regions of the first cysteine rich domain of CD95 led to the disruption of these interactions. They termed this domain PLAD (for preligand binding assembly domain). Functionally, disruption of the association mediated by this domain led to reduced apoptotic potency of agonistic anti-CD95 antibodies or CD95L. Although the precise molecular role of this preassociation is not known, the physiological importance of the domain has been characterized. Mutations in this domain have been shown to result in the autoimmune disorder ALPS,[4] indicating the importance of preassociation for physiological signaling. This finding gains additional importance as heterotrimerization is required for efficient CD95 signaling. Heterozygous mutations in the CD95 gene, found in ALPS patients, could therefore act as dominant negative mutations, interrupting signaling, because CD95 receptors only function as trimers;[6] in fact, a single mutant protein could significantly disrupt signaling by CD95. It has been suggested that for efficient signaling superclustering of CD95 may be required,[7] a molecular configuration that would be especially susceptible to disruption by a single mutant protein.

The key components of the CD95 death-inducing signaling complex (DISC)

Preassociation of CD95 was observed to be independent of the expression of the intracellular domain of CD95.[4] The exact

molecular mechanism of the initiation of signaling through CD95 awaits further investigation; however, the general aspects of this initiation are well known. More than 6 years ago we reported formation of a complex of proteins that assembles at the activated CD95 receptor. This complex formed at the receptor in apoptosing cells and, thus, we termed this structure the DISC.[6] CD95 contains a protein–protein interaction domain in its cytoplasmic region termed the death domain (DD), a characteristic region of the death receptor subfamily of the TNF receptor superfamily which includes CD95, TNF receptor I (TNF-RI), DR3/APO-3/TRAMP, TRAIL receptor 1/DR4, TRAIL-R2/DR5, and DR6.[8] When the preassociated receptor is ligated, CD95 becomes competent to form the DISC. In the DISC, the adaptor molecule Fas-associated DD containing protein (FADD) is bound to CD95 through homotypic interaction of its DD with the DD of CD95.[6,9] In addition to its DD, FADD contains another protein–protein interaction domain at its N-terminus, termed the death effector domain (DED). This domain is required for the recruitment of caspases containing these DED domains to the DISC. Both the DD and DED enable proteins containing the same domains to interact with one another. FADD has been shown to interact with several proteins through its DED, including caspase-8, one of the two known DED-containing caspases.[10,11] Caspases are cysteine proteases which cleave after a loosely specific series of four amino acids and which absolutely require the presence of an aspartate in the P1 position of their substrate.[12] These proteases are responsible for performing the labor of apoptosis. Various caspases are involved in both the initiation of the apoptotic process and the execution of the final apoptotic program. The apoptotic caspases therefore perform different roles. The effector caspases, which include caspases 3, 7, and 6 are responsible for most of the cleavage of proteins characteristic of apoptosis and are responsible for the cleavage of the proteins which induce the major morphological changes observed during programmed cell death.[12] The initiator caspases transduce the first signals of apoptosis. Caspase-8, the main initiator caspase in CD95 signaling, is expressed as two isoforms, caspase-8/a and -8/b, which are both recruited to the activated CD95 receptor.[13] These two molecules, FADD and caspase-8 are the key components of the CD95 DISC (Figure 1). Caspase-8 resides primarily in the cytoplasm, or as recently observed, on the mitochondria,[14,15] as an inactive zymogen, and is recruited into the DISC upon binding of FADD. Once caspase-8 associates with FADD, the high local concentration of caspase-8 is believed to lead to its autoproteolytic cleavage and activation.[16,17] Aside from its two DED, caspase-8 contains a protease domain consisting of two subunits. Following the autoproteolytic cleavage of the enzyme, caspase-8 is released from the DISC as an active heterotetramer containing two p18 and two p10 subunits. So far, only DED-containing subunits of procaspase-8 have been reported to be part of the DISC. However, lately Lavrik *et al*. (Lavrik I, Krueger A, Schmitz I, Baumann S, Krammer PH, Kirchhoff S, submitted for publication) described that in human leukemia cell lines and in primary human T cells all cleavage products of procaspase-8 remain bound to the DISC, including the p10 and p18 subunits of caspase-8. This

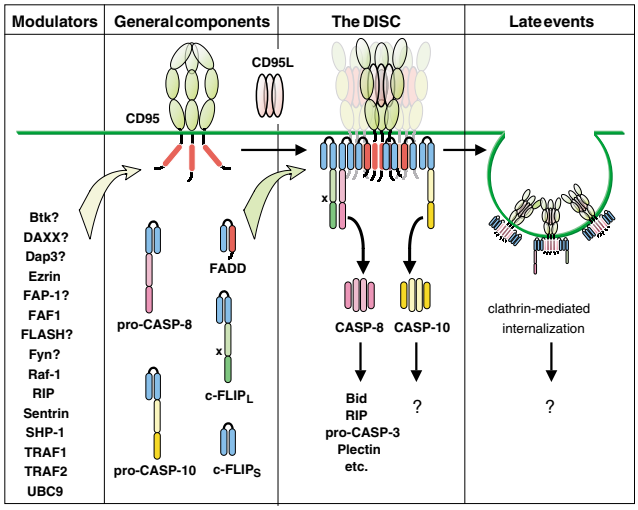

Figure 1 The DISC and its components. Upon binding of CD95L to CD95 the DISC assembles. FADD, procaspase-8/10 and c-FLIP have been shown to be key components of this structure. A number of signaling proteins have been reported to bind to the DISC or to CD95 directly. Btk, has been shown to interact with CD95 in DT-40 cells;[98] Daxx, was identified to bind to the DD of CD95;[43] the ERM protein ezrin could be coimmunoprecipitated with CD95.[119] FAP-1, binds to the three C-terminal amino acids of human CD95;[50] FLASH, was shown to enhance formation of the DISC;[54] Raf-1 and TRAF-1/2 were shown to bind to the DISC likely through binding to c-FLIP;[41] RIP, binds to CD95 directly likely involved in caspase-independent CD95-mediated necrosis;[57] Sentrin, UBC9, are two proteins of the ubiquitin pathway that were found to be associated with CD95;[120–122] SHP-1, was shown to bind to a phosphotyrosine containing motif in the DDs of death receptors.[100] For details see text.

implies that the active tetramer $p10_2$–$p18_2$ of caspase-8 is formed at the DISC. Thus, a modified model of initiation of CD95-mediated apoptosis is proposed, in which procaspase-8 activation and complete maturation into the caspase-8 heterotetramer occurs at the DISC. The active tetramer $p10_2$–$p18_2$ is only then released into the cytosol to propagate the apoptotic signal (see Figure 1).

Yet another component of the DISC observed in the first description of this complex[6] is Cap3. Sequence comparison revealed the complete identity of the Cap3 N-terminal DEDs with those of caspase-8.[10] Cap3 is thus a splice product of caspase-8 whose function is presently unknown, but which may serve to establish the correct conformation of the DISC.

Other components of the DISC

Caspase-10

A second caspase has recently been shown to associate with FADD through the homotypic association with its DED, and to be activated in the DISC. Caspase-10 was cloned several years ago as a homologue of caspase-8 and is the only other caspase known to contain tandem DED. Association with FADD has been demonstrated *in vitro*, as has the ability of caspase-10 to induce apoptosis when overexpressed. Recently, caspase-10 was shown to be associated with the DISC.[18–21] Kischkel *et al*. and Wang *et al*. demonstrated that caspase-10 is expressed, recruited to the DISC and

proteolytically activated. It is also processed by drug-induced dimerization *in vitro*, a feature identical to that of caspase-8.[21] The substrate specificity of caspase-10 may be different than caspase-8, however, making it attractive to speculate that the two caspases may play different roles in signaling through the CD95 receptor[19] (Figure 1). Another study has implicated the lack of functional caspase-10 in one form of the autoimmune disease ALPS type II.[22] This finding suggests that caspase-10 may indeed have a role in apoptosis signaling. The lack of a caspase-10 orthologue in the mouse has complicated the study of this caspase, however, though functional expression appears to be important in the human system.

c-FLIP

A viral cell death inhibitor v-FLIP (viral-FLICE-like inhibitory protein) was identified as a protein, expressed by γ-herpesviruses, capable of blocking CD95-mediated apoptosis through association with the receptor in the DISC.[23–25] This protein contains two tandem DED, which are highly homologous to the N-terminus of caspase-8. Two cellular homologues of the viral protein were subsequently identified,[26–33] termed c-FLIP$_S$ (short) and c-FLIP$_L$ (long). c-FLIP$_S$ contains only the tandem DEDs and is quite similar to v-FLIP (Figure 1). c-FLIP$_L$ contains not only the tandem DEDs, but also a protease-like domain, homologous to caspase-8, in which several amino acids important for protease activity are mutated, including the cysteine at the active site. This inactive homologue was found to be cleaved in the DISC by caspase-8,[34] and has been reported to be either an inhibitor of apoptosis signaling or an inducer of apoptosis (presumably by oligomerizing caspase-8 and inducing its autoproteolytic activation).[21] The inhibition of apoptosis was shown to be through the recruitment to and cleavage in the DISC, but because c-FLIP$_L$ is not an active protease, the cleavage is not reciprocated, resulting in the generation of two p10 subunits (one from caspase-8, the other from c-FLIP$_L$) followed by no further cleavage and, therefore, an inactive caspase-8 molecule.[35] c-FLIP$_L$ was shown to be downregulated in T cells following treatment with IL-2 and was suggested to be responsible for the CD95 apoptosis sensitivity of long-term-activated peripheral mouse T cells.[36,37] In contrast, we recently reported that CD95 sensitivity in peripheral human T cells is regulated by a mechanism that does not involve any changes in the expression levels of c-FLIP$_L$.[34]

A large number of studies concerning the function of c-FLIP have been published, implicating c-FLIP in numerous aspects of apoptosis. c-FLIP-deficient mice display a phenotype similar to that of caspase-8-deficient animals, and the mice are not viable past day E10.5.[38] This has complicated the study of this molecule *in vivo*, but does underscore its apparent importance, particularly in development. Recently, c-FLIP has been implicated in signaling alternative pathways, linking the CD95 receptor to the NF-κB, JNK, and MAPK pathways. Several studies have shown that c-FLIP is involved in the transmission of the NF-κB pathway, by association with TRAF2.[39,40] One study found that c-FLIP recruited not only TRAF1 and 2 but also RIP and Raf-1, which activated the extracellular signal regulated kinase (Erk) pathway.[41] These studies link the CD95-signaling pathway to survival pathways in addition to its well-known role in apoptosis. Several of these molecules are well-established mediators of TNF receptor signaling (reviewed in Wallach *et al*.[42]), though their role in CD95 signaling is still controversial. These studies, however, raise the interesting possibility that through c-FLIP, CD95 may be induced to signal in pathways that are distinct from those leading to the apoptosis of the cell. The physiological role of CD95 in these pathways is by no means clear, and requires further investigations to determine its significance.

Alternative CD95-associated molecules

Several other proteins have been shown to be recruited to the DISC by direct interaction with DISC proteins. The role of many of these proteins is presently unclear.

Daxx

Daxx was identified as a CD95 binding protein which could link the receptor to the JNK-signaling pathway.[43] Overexpression of Daxx led to apoptosis, and Daxx was shown to interact with the C-terminal portion of CD95, containing the DD. It was suggested that Daxx induces CD95-mediated apoptosis through an alternate pathway which involves JNK activation followed by activation of an unknown caspase. The physiological role of Daxx is not known, as Daxx has not been shown to directly associate with the DISC. It is interesting that Daxx was recently also shown to be an integral part of nuclear PML bodies[44–46] and PML-deficient mice have a defect in CD95-mediated apoptosis.[47] At present, the molecular basis for this cytosolic/nuclear connection in the CD95 pathway is unknown. Furthermore, Daxx-deficient mice display a phenotype of extensive apoptosis and embryonic lethality rather than the expected hyperproliferative disorder, making this promiscuous protein even more enigmatic.[48,49]

FAP-1

FAP-1, or Fas-associated phosphatase-1, was identified in a yeast two-hybrid screen as a phosphatase that associates with the C-terminal end of the CD95 receptor.[50] Indeed, only the final three amino acids of the CD95 receptor were shown to be necessary for the binding of one of the FAP-1 domains. In this study, it was suggested that this phosphatase was a negative regulator of CD95 signaling. In addition, other data suggest that FAP-1-transfected cells are resistant to CD95-mediated apoptosis and that the activation of caspase-8, and thereby caspase-3, is inhibited in these cells.[51] Additionally, down-regulation of FAP-1 has been suggested to contribute to the IL-2-induced sensitivity of activated T cells since a decrease in FAP-1 mRNA in cells by competitive RT-PCR was found.[52] It is interesting to note that the three amino acids in human CD95 involved in binding to FAP-1 are not found in mouse CD95.[53] The physiological role of the binding of FAP-1 to human CD95 has therefore not been sufficiently established.

FLASH

The protein FLICE-associated huge protein (FLASH) was also suggested to associate with the CD95 receptor,[54]

and was shown to be recruited to the DISC of the activated receptor and actually required for the activation of caspase-8 at the DISC, but recent studies have cast serious doubt on the validity of these data and subsequent claims.[55]

RIP

RIP was described as a DD-containing protein which could interact with the CD95 receptor.[56] The role of this kinase, however, is not clear. A recent study implicates RIP in the initiation of a caspase-8-independent death-signaling pathway through the CD95 receptor[57] resulting in necrotic cell death. It was suggested that RIP binds to the CD95 receptor, phosphorylates an unknown protein and leads to CD95 mediated, necrotic death of the cell. This pathway was suggested to occur in primary human T cells treated with the caspase inhibitor zVAD-fmk, which in this study resulted in equal levels of cell death in comparison to untreated cells upon induction of AICD.

The role of all these molecules in cell death is suggestive, but they require much more investigation before their functions are fully characterized.

FAF1

Fas(CD95)-associated protein factor, FAF1, which specifically interacted with the cytoplasmic domain of wild-type Fas(CD95) but not the lpr^{cg}-mutated Fas(CD95) protein, was isolated again, in a yeast two-hybrid screen. When FAF1 was transiently expressed in L cells, CD95-induced apoptosis was potentiated.[58] Confirmation of these effects under native conditions are awaited.

Dap3

Dap3 was discovered in a special screen involving IFN-γ-induced cell death in HeLa cells. Inactivation of the Dap3 gene by antisense RNA protected the cells from this type of death. A dominant negative form of Dap3 also protected cells from CD95-induced apoptosis. Thus, Dap3 appears to act as a positive mediator of the CD95 death signal downstream of the DISC through an as yet unknown mechanism.[59] Recently, the function of Dap3 as a regulator of the DISC was challenged[60] since Dap3 is an established mitochondrial ribosomal protein.[61]

Regulation of activation of caspase-8 in the DISC

Effector caspases characterized by short prodomains are activated through cleavage by initiator caspases such as caspase-9 or caspase-8, both of which carry long prodomains. The mechanism of how initiator caspases are activated at the top of the caspase cascade is not well established. We showed that in most cells caspase-8 is activated by recruitment to the DISC, suggesting that this would bring the caspase molecules in close proximity triggering an autoproteolytic activity of the protease.[16] Forced dimerization using different systems then suggested activation of caspase-8 through 'induced proximity' involving two active enzymes.[62–64] Recently, it was demonstrated that procaspase-8 in the DISC gains enzymatic activity prior to its processing suggesting that dimerization induces a conformational change in the zymogen that results in the formation of the active sites.[21] It also became clear that c-$FLIP_L$, widely regarded as an inhibitor of the DISC, acts as an activator of caspase-8 by forming a heterodimer with procaspase-8 within the DISC.[21] To date the role of c-$FLIP_L$ in apoptosis remains controversial. In most reports, c-$FLIP_L$ has been described as antiapoptotic, largely because of its ability to inhibit apoptosis at high levels of ectopic expression.[26,28,30,32,33] However, in cell lines in which the level of endogenous c-$FLIP_L$ has been determined, it is approximately 1% of that of endogenous procaspase-8.[21,34] Since this ratio is so disproportionate it is unclear what role c-$FLIP_L$ plays in these cells. In addition, mice deficient in c-FLIP (lacking both c-$FLIP_L$ and c-$FLIP_S$) were recently generated. Embryonic fibroblasts (MEFs) derived from these mice (through an *in vitro* selection process for cell growth) were shown to be more sensitive to CD95-induced apoptosis than the wild-type MEFs. This observation has been widely accepted as a validation of the inhibitory role of c-$FLIP_L$ in apoptosis. However, inconsistent with these results, c-$FLIP^{-/-}$ mice showed developmental defects that strikingly resembled those of caspase-$8^{-/-}$ or $FADD^{-/-}$ mice.[38] These mice died between E10.5 and E11.5 with a failure in heart formation and hemorrhage suggesting a function of c-$FLIP_L$ that is similar to caspase-8 and FADD. Recent data suggest that c-$FLIP_L$ may play a more complex role in caspase activation and apoptosis than as a dedicated inhibitor. We have not found a single cell in which c-$FLIP_L$ is not part of the DISC regardless of the CD95 apoptosis sensitivity of the cells (Scaffidi *et al*.[34] and unpublished results). Furthermore, transient overexpression of c-$FLIP_L$ could induce as well as inhibit apoptosis, and this proapoptotic function required the c-$FLIP_L$ protease-like domain.[26,27,29–31] However, it remained undetermined whether c-$FLIP_L$ could promote apoptosis at endogenous expression levels and how this might be possible in the absence of a genuine protease activity. Using an *in vitro* caspase activation system based on controlled dimerization that closely resembles caspase activation in the DISC, it has recently become clear that the activation of procaspase-8 is potently enhanced by c-$FLIP_L$ upon procaspase-8/c-$FLIP_L$ heterodimerization. The c-$FLIP_L$ protease-like domain associates efficiently with the procaspase-8 protease domain and this interaction leads to induction of enzymatic activity of the caspase-8 zymogen. According to this model only one partner (procaspase-8) of this heterodimer forms an active site sufficient to start the caspase cascade (Chang DW, Peter ME and Yang X, submitted for publication). This scenario is highly reminiscent of the recently published structure of the procaspase-9 homodimer,[65] in which also only one active site was found. Interestingly, even though the cow pox serpin crmA is known to be a potent inhibitor of the mature caspase-8,[66] it could not block the processing of caspase-8 in the DISC,[16] nor could it inhibit the first cleavage event of procaspase-8 in the dimerization system.[62] The different effects of crmA on the activation of procaspase-8 *versus* the activity of mature caspase-8 provide further evidence that the caspase-8 activity within the c-$FLIP_L$:procaspase-8 intermediate is different from that of the mature enzyme.

c-FLIP$_L$, depending on its expression level, can therefore either activate caspase-8/10 in the DISC (at low concentrations) or block it (at high concentrations).[21] Thus, even an increase of c-FLIP$_L$ expression does not necessarily mean that cells are more resistant to CD95-mediated apoptosis. The situation is different for c-FLIP$_S$, which seems to have only antiapoptotic activity similar to the v-FLIP proteins. In this context, both restimulated and costimulated T cells which became CD95 apoptosis resistant were shown to specifically upregulate c-FLIP$_S$ and, thus, displayed strongly reduced caspase-8 activity.[67,68]

Differences in the formation of the DISC defines two different CD95 apoptosis cell types

Following activation in the DISC caspase-8 can initiate the apoptotic program. We recently described two pathways of CD95 apoptosis signaling dependent on the quantity of production of caspase-8 at the DISC.[69] In Type I cells, a high production of caspase-8 at the DISC can process the effector caspase, caspase-3, directly, leading to its activation and to ultimate apoptosis of the cell. In Type II cells, however, only a small amount of caspase-8 is produced in the DISC. The DISC in these cells is formed quite poorly, little FADD is recruited and little active caspase-8 induced. Apoptosis in these cells is dependent, at least in part, on the cleavage of the BH3 domain containing Bcl-2 family member BID,[70,71] whose cleavage results in a proapoptotic fragment termed tBID (t for truncated). This fragment induces the proapoptotic functions of the mitochondria by causing aggregation of Bax or Bak (reviewed in Korsmeyer *et al*.[72]). This is followed by the loss of – among many other factors – cytochrome *c* from the mitochondrial intermembrane space. The adaptor APAF-1, cytochrome *c* and dATP then form a large protein complex, the apoptosome, a sort of submitochondrial DISC, where caspase-9 as initiator caspase is activated.[73] Caspase-9 then activates caspase-3 resulting in apoptosis of the cell. Another hallmark of CD95-mediated apoptosis in Type II cells is the effect of the expression of the antiapoptotic members of the Bcl-2 family. Expression of either Bcl-2 or Bcl-x$_L$ renders Type II cells resistant to CD95-mediated apoptosis.[69] Type I cells however, cannot overcome the production of the large amounts of caspase-8 produced at the DISC and are, therefore, not protected from CD95-mediated apoptosis even by the expression of very high levels of Bcl-2 or Bcl-x$_L$.[69] The physiological importance of the two-pathway model has recently been validated by the description of mice which forcibly express members of the apoptosis pathway, or which are deficient in various molecules involved in apoptosis. Mice deficient in BID have thymocytes, which are not protected from CD95-mediated apoptosis by the loss of this molecule, whereas their hepatocytes are protected from CD95-induced apoptosis.[74] Bax/Bak doubly deficient mice demonstrate resistance to CD95-mediated apoptosis in their hepatocytes as well, whereas their thymocytes and T cells are affected similarly to control mice.[75,76] The hepatocytes of mice expressing Bcl-2 as a transgene are also protected from CD95-induced apoptosis, whereas Bcl-2 transgenic T cells and thymocytes are not protected from apoptosis by CD95.[77–79] Thus, thymocytes and T cells are Type I and hepatocytes are Type II cells.

Inhibiting CD95 signaling

Several modes of inhibition of caspase signaling through CD95 have been characterized in detail. The cowpox virus expresses a serpin-like protease inhibitor, crmA. This caspase inhibitor has been shown to potently inhibit activity of caspase-1, primarily involved in inflammatory cytokine processing, as well as caspase-8.[80] The inhibition of caspase-8 by this viral inhibitor has been linked to the inhibition of the apoptosis of virally infected cells. Another mechanism of inhibition of CD95 signaling by viral infection is by forced internalization and/or degradation of the CD95 molecule from the cell surface. Removal of CD95 leads to the loss of apoptotic potency, and to the survival of the cell. This mode of receptor inhibition was described for the adenoviral protein RID (E3/10.4K–14.5K) causing internalization and degradation through a lysosomal pathway, inhibitable by bafilomycin A. This reagent blocks the transition of endosomes into lysosomes and, thereby prevents degradation of the receptor.[81,82]

Protein kinase C (PKC) was recently also shown to be a CD95 apoptosis regulator. PKC represents a family of serine/threonine kinases some of which can be activated by phorbol esters like PMA.[83] Activation of PKC by PMA inhibited apoptosis[84–86] while inhibition sensitized the cells to induction of apoptosis.[87,88] In addition, CD95 triggering inhibited PKC activity.[89] By testing different cell lines it was demonstrated that activation of PKC protected type II but not type I cells from CD95-mediated apoptosis because of reduced cleavage of Bid.[90] In addition, Bad was shown to be phosphorylated and inactivated in a PKC-dependent manner by p90^{RSK}.[91] Thus, PKC may exert its antiapoptotic function mainly by inactivation of proapoptotic Bcl-2 family members. However, it was also shown that PKC inhibited oligomerization of CD95.[92] Recently it was shown that activation of PKC reduced recruitment of FADD to the DISC. One study reported that this effect is specific for Type II cells[93] consistent with our data.[90] Another study, however, suggested reduced recruitment of FADD to the DISC in both Type I and Type II cells.[94] The reason for the discrepancy is currently unknown.

Finally, the role of tyrosine phosphorylation in CD95-mediated apoptosis is not clear. Although tyrosine phosphorylation has been described upon CD95 triggering[95] and CD95 has even been reported to bind the scr kinase fyn,[96] another study reported that CD95-mediated apoptosis is independent of src kinases.[97] However, Bruton's tyrosine kinase (Btk), a member of the Tec family of tyrosine kinases, has been shown to play a dual role in apoptosis. While enhancing radiation-induced apoptosis, it inhibits CD95-mediated death in B cells.[98] Subsequently, it was shown by the same group that Btk interacts with CD95, preventing recruitment of FADD and caspase-8.[99] The molecular targets of Btk at the DISC, however, remain unknown.

Recently, it was reported that DD containing receptors possess a conserved phosphotyrosine-containing motif within the DD that can mediate inhibitory function via binding of the src homology domain 2 (SH2) containing phosphatase

(SHP-1), SHP-2 and SH2-containing inositol phosphatase (SHIP).[100]

Ligand-independent DISC formation

Activation of CD95 receptor signaling has been described in the absence of deliberate CD95L-mediated stimulation. In these stimulations, for example, induced by the drug thymidine kinase/ganciclovir (TK/GCV)[101] or by exposure to ultraviolet light (UV),[102] CD95L-independent activation of the DISC and apoptosis of the cells occur. TK/GCV was demonstrated to lead to aggregation of the CD95 receptor and recruitment of FADD, forming an active DISC capable of recruiting and activating caspase-8. Additionally, this activity was inhibited by the expression of a dominant negative form of FADD and by the caspase-8 selective inhibitor zIETD-fmk, in a manner similar to ligand-induced apoptosis.[101] UV light was also shown to induce aggregation of the CD95 receptor capable of recruiting FADD, leading to the apoptosis of the cell.[102] Whether this is a physiologically relevant pathway in apoptosis of UV exposed cells is questionable, however, though expression of a dominant negative form of FADD did decrease apoptosis of cells, suggesting that these cells may utilize the CD95L independent CD95 pathway to apoptosis. However, expression of dominant negative FADD did not fully block apoptosis, suggesting that other apoptotic mechanisms may be functioning.[102] Clearly, the role of DNA damage in this system is critical, though CD95 aggregation and DISC formation were suggested to be a possible secondary, though not mutually exclusive pathway involved in UV-induced apoptosis. An additional case of CD95 DISC-mediated apoptosis in the apparent absence of demonstrable CD95L was observed in germinal center B cells. Also in this case, a mechanistic explanation is further awaited.[103]

CD95 clustering and internalization

Recently, stimulation-induced clustering of CD95 was described.[104–106] Ligation of CD95 with soluble ligand or agonistic antibody resulted in aggregation of the receptor followed by its internalization into an endosomal pathway (Figure 1).[106] This occurred in a variety of cell lines including one that did not subsequently undergo apoptosis because of the protective expression of Bcl-x_L.[15] Clustering and internalization were dependent on the actin cytoskeleton as treatment of cells with latrunculin A, an inhibitor of the formation and stability of actin filaments, led to inhibition of this phenomenon. Further, treatment with this drug led to inhibition of CD95-mediated apoptosis, implicating the actin filament network in induction of CD95-mediated apoptosis. Additionally, clustering and internalization of CD95 were dependent on activation of caspases, specifically, of caspase-8.[106] This is the first study to implicate the involvement of caspases in the internalization of surface receptors. Other studies have implicated the involvement of ceramide released by acid sphingomyelinase, enriched in lipid rafts in the clustering of CD95 and subsequent apoptosis.[105] It was also shown that ceramide is essential for the efficient signaling of CD95 and that signaling is preceded by capping of CD95.[104] Recently, another study suggested that CD95 acts in lipid rafts,[107] a finding which could not be confirmed by others.[108] The reason for these differences is currently unknown and requires further studies. In the study by Algeciras-Schimnich *et al*., the clustering and internalization of CD95 were also independent of lipid rafts suggesting that these structures are not required for signaling in certain cells.[106] The physiological implications of CD95 internalization are not yet known, but it was suggested that this phenomenon could play a role in the protection of cells from receiving an apoptotic signal from neighboring cells which have a CD95 receptor that has bound soluble CD95L, and may play a role in the evasion of some tumor cells from CD95-mediated apoptosis. Alternatively, it could also be involved in signaling by promoting formation of the DISC.

CD95 and costimulation

The CD95 receptor has recently been implicated in the stimulation of T cells. It has been known for some time that the CD95 receptor can transduce activation signals to T cells.[109] This study has gained support, recently, with reports on the requirement for active caspases in T cell proliferation. Some of the first indications for this possibility arose with the observations that T cells from mice deficient in or expressing a transgene for the dominant negative form of FADD had a defect in proliferation.[110,111] Additionally, mice expressing a Bcl-2 transgene had a deficiency in proliferation.[112] Two recent studies have suggested that activation of caspases occurs following stimulation through the T cell receptor and that treating cells with caspase inhibitors leads to a loss of proliferative capacity that was not because of apoptosis.[113,114] Furthermore, one study determined that T cells stimulated with suboptimal levels of anti-CD3 and CD95L responded with more proliferation than cells stimulated with anti-CD3 alone, and that treatment of cells with a CD95-Fc fusion which blocked CD95 signaling led to a decrease in the proliferative response of T cells.[114] These studies implicate CD95 in the costimulation of T cells and suggest that there may be a role for caspases in other nonapoptotic pathways. Perhaps in cells in which the balance between proliferation and apoptosis is tipped toward proliferation, caspases are important in aiding this proliferative pathway by cleavage of certain substrates that may inhibit progression through the cell cycle. Indeed, one study implicating activation of caspases in proliferation described the cleavage of a protein that inhibits cell cycle progression, Wee1, an inhibitor of the Cdc2 cyclin-dependent kinase which is required for the G_2/M transition.[115] Thus, caspase activity induced by stimulation through the CD95 receptor under certain circumstances may also play a critical, nonapoptotic, and, indeed, proliferative role. The exact pathway that is initiated in CD95 costimulated T cells requires further study. Additionally, the stimulation of memory T cells results in significantly less apoptosis than in naïve T cells.[116] In this study, TCR transgenic mice were used to produce memory cells which were much less susceptible to CD95-mediated apoptosis than activated naïve T cells. This resistance was found to be because of the increase in c-FLIP expression in these restimulated cells, at least in part. This study suggests that CD95 signaling is regulated differently in restimulated T cells compared to naïve T cells. Additionally, it was demonstrated that the regulation of c-FLIP was

dependent on the cell cycle status of the T cells.[37] In T cells stimulated through the TCR, this study found that T cells in the G_1 phase expressed high levels of c-FLIP, whereas in S phase c-FLIP levels were lower. These results may help to explain the observation that T cells are more susceptible to TCR-mediated apoptosis in the S phase of the cell cycle.[117] Another study using T cell clones and the cell line Jurkat found that in these cells no difference in apoptosis susceptibility dependent on the cell cycle could be observed.[118] The differences in these studies are likely because of the model system used.

Conclusion

For over 100 years, programmed cell death, apoptosis, was defined by the morphology of the dying cell. This has dramatically changed since the discovery of molecules, which determine the complex death-signaling pathways. Thus, it became apparent that apoptosis is a general biological phenomenon like cell proliferation and activation. In fact, it is clear now that death is an essential feature of a regulated process of cellular life. Finally, the multitude of molecules discovered that are essential for the apoptotic process may make it possible to apply molecular intervention strategies wherever death is dysregulated.

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References

1. Vogt C (1842) Untersuchungen über die Entwicklungsgeschichte der Geburtshelferkröte (Alytes obstetricans). Jent & Gassmann, Solothurn
2. Schulze-Osthoff K, Ferrari D, Los M, Wesselborg S and Peter ME (1998) Apoptosis signaling by death receptors. Eur. J. Biochem. 254: 439–459
3. Papoff G, Hausler P, Eramo A, Pagano MG, Di Leve G, Signore A and Ruberti G (1999) Identification and characterization of a ligand-independent oligomerization domain in the extracellular region of the CD95 death receptor. J. Biol. Chem. 274: 38241–38250
4. Siegel RM, Frederiksen JK, Zacharias DA, Chan FK, Johnson M, Lynch D, Tsien RY and Lenardo MJ (2000) Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. Science 288: 2354–2357
5. Chan FK, Chun HJ, Zheng L, Siegel RM, Bui KL and Lenardo MJ (2000) A domain in TNF receptors that mediates ligand independent receptor assembly and signaling. Science. 288: 2351–2354
6. Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH and Peter ME (1995) Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J. 14: 5579–5588
7. Siegel RM, Chan FK, Chun HJ and Lenardo MJ (2000) The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. Nat. Immunol. 1: 469–474
8. Peter ME, Scaffidi C, Medema JP, Kischkel F and Krammer PH (1999) The death receptors. Results Probl. Cell. Differ. 23: 25–63
9. Chinnaiyan AM, Tepper C, Lou L, O'Rourke K, Kischkel FC, Hellbardt S, Krammer PH, Peter ME and Dixit VM (1996) FADD/MORT1 is a common mediator of Fas/APO-1- and tumor necrosis factor-induced apoptosis. J. Biol. Chem. 271: 4961–4965
10. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME and Dixit VM (1996) FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell 85: 817–827
11. Boldin MP, Goncharov TM, Goltsev YV and Wallach D (1996) Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85: 803–815
12. Earnshaw WC, Martins LM and Kaufmann SH (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu. Rev. Biochem. 68: 383–424
13. Scaffidi C, Medema JP, Krammer PH and Peter ME (1997) FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b. J. Biol. Chem. 272: 26953–26958
14. Stegh AH, Herrmann H, Lampel S, Weisenberger D, Andra K, Seper M, Wiche G, Krammer PH and Peter ME (2000) Identification of the cytolinker plectin as a major early *in vivo* substrate for caspase-8 during CD95 and TNF-receptor mediated apoptosis. Mol. Cell. Biol. 20: 5665–5679
15. Stegh AH, Barnhart B, Volkland J, Algeciras-Schimnich A, Ke N, Reed JC and Peter ME (2002) Inactivation of caspase-8 on mitochondria of Bcl-x_L expressing MCF7-Fas cells: role for the BAR protein. J. Biol. Chem. 277: 4351–4360
16. Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH and Peter ME (1997) FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J. 16: 2794–2804
17. Salvesen GS and Dixit VM (1999) Caspase activation: the induced-proximity model. Proc. Natl. Acad. Sci. USA 96: 10964–10967
18. Kischkel FC, Lawrence DA, Tinel A, LeBlanc H, Virmani A, Schow P, Gazdar A, Blenis J, Arnott D and Ashkenazi A (2001) Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. J. Biol. Chem. 276: 46639–46646
19. Wang J, Chun HJ, Wong W, Spencer DM and Lenardo MJ (2001) Caspase-10 is an initiator caspase in death receptor signaling. Proc. Natl. Acad. Sci. USA. 98: 13884–13888
20. Sprick MR, Rieser E, Stahl H, Grosse-Wilde A, Weigand MA and Walczak H (2002). Caspase-10 is recruited to and activated at the native TRAIL and CD95 death-inducing signalling complexes in a FADD-dependent manner but cannot functionally substitute caspase-8. EMBO J. 21: 4520–4530
21. Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, Peter ME and Yang X (2002) c-$FLIP_L$ is a dual function regulator for caspase-8 activation and CD95 (APO-1/Fas-mediated apoptosis. EMBO J. 21: 3704–3714
22. Wang J, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, Yao X, Puck JM, Straus SE and Lenardo MJ (1999) Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell 98: 47–58
23. Hu S, Vincenz C, Buller M and Dixit VM (1997) A novel family of viral death effector domain-containing molecules that inhibit both CD-95- and tumor necrosis factor receptor-1-induced apoptosis. J. Biol. Chem. 272: 9621–9624
24. Bertin J, Armstrong RC, Ottilie S, Martin DA, Wang Y, Banks S, Wang GH, Senkevich TG Alnemri ES, Moss B, Lenardo MJ, Tomaselli KJ and Cohen JI (1997). Death effector domain-containing herpesvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. Proc. Natl. Acad. Sci. USA 94: 1172–1176
25. Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, Neipel F, Mattmann C, Burns K, Bodmer JL, Schroter M, Scaffidi C, Krammer PH, Peter ME and Tschopp J (1997) Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature 386: 517–521
26. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE and Tschopp J (1997) Inhibition of death receptor signals by cellular FLIP. Nature 388: 190–195

27. Shu HB, Halpin DR and Goeddel DV (1997) Casper is a FADD- and caspase-related inducer of apoptosis. Immunity 6: 751–763
28. Srinivasula SM, Ahmad M, Ottilie S, Bullrich F, Banks S, Wang Y, Fernandes-Alnemri T, Croce CM, Litwack G, Tomaselli KJ, Armstrong RC and Alnemri ES (1997) FLAME-1, a novel FADD-like anti-apoptotic molecule that regulates Fas/TNFR1-induced apoptosis. J. Biol. Chem. 272: 18542–18545
29. Inohara N, Koseki T, Hu Y, Chen S and Nunez G (1997) CLARP, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis. Proc. Natl. Acad. Sci. USA 94: 10717–10722
30. Goltsev YV, Kovalenko AV, Arnold E, Varfolomeev EE, Brodianskii VM and Wallach D (1997) CASH, a novel caspase homologue with death effector domains. J. Biol. Chem. 272: 19641–19644
31. Han DK, Chaudhary PM, Wright ME, Friedman C, Trask BJ, Riedel RT, Baskin DG, Schwartz SM and Hood L (1997) MRIT, a novel death-effector domain-containing protein, interacts with caspases and BclXL and initiates cell death. Proc. Natl. Acad. Sci. USA 94: 11333–11338
32. Hu S, Vincenz C, Ni J, Gentz R and Dixit VM (1997) I-FLICE, a novel inhibitor of tumor necrosis factor receptor-1- and CD-95-induced apoptosis. J. Biol. Chem. 272: 17255–17257
33. Rasper DM, Vaillancourt JP, Hadano S, Houtzager VM, Seiden I, Keen SL, Tawa P, Xanthoudakis S, Nasir J, Martindale D, Koop BF, Peterson EP, Thornberry NA, Huang J, MacPherson DP, Black SC, Hornung F, Lenardo MJ, Hayden MR, Roy S and Nicholson DW (1998) Cell death attenuation by 'Usurpin', a mammalian DED-caspase homologue that precludes caspase-8 recruitment and activation by the CD-95 (Fas, APO-1) receptor complex. Cell Death Differ. 5: 271–288
34. Scaffidi C, Schmitz I, Krammer PH and Peter ME (1999) The role of c-FLIP in modulation of CD95-induced apoptosis. J. Biol. Chem. 274: 1541–1548
35. Krueger A, Schmitz I, Baumann S, Krammer PH and Kirchhoff S (2001) Cellular FLICE-inhibitory protein splice variants inhibit different steps of caspase-8 activation at the CD95 death-inducing signaling complex. J. Biol. Chem. 276: 20633–20640
36. Refaeli Y, Van Parijs L, London CA, Tschopp J and Abbas AK (1998) Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. Immunity 8: 615–623
37. Algeciras-Schimnich A, Griffith TS, Lynch DH and Paya CV (1999) Cell cycle-dependent regulation of FLIP levels and susceptibility to Fas-mediated apoptosis. J. Immunol. 162: 5205–5211
38. Yeh WC, Itie A, Elia AJ, Ng M, Shu HB, Wakeham A, Mirtsos C, Suzuki N, Bonnard M, Goeddel DV and Mak TW (2000) Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. Immunity 12: 633–642
39. Hu WH, Johnson H and Shu HB (2000) Activation of NF-kappaB by FADD, Casper, and caspase-8. J. Biol. Chem. 275, 10838–10844
40. Chaudhary PM, Jasmin A, Eby MT and Hood L (1999) Modulation of the NF-kappa B pathway by virally encoded death effector domains-containing proteins. Oncogene 18, 5738–5746
41. Kataoka T, Budd RC, Holler N, Thome M, Martinon F, Irmler M, Burns K, Hahne M, Kennedy N, Kovacsovics M and Tschopp J (2000) The caspase-8 inhibitor FLIP promotes activation of NF-kappaB and Erk signaling pathways. Curr. Biol. 10: 640–648
42. Wallach D, Varfolomeev EE, Malinin NL, Goltsev YV, Kovalenko AV and Boldin MP (1999) Tumor necrosis factor receptor and Fas signaling mechanisms. Annu. Rev. Immunol. 17: 331–367
43. Yang X, Khosravi-Far R, Chang HY and Baltimore D (1997) Daxx, a novel Fas-binding protein that activates JNK and apoptosis. Cell 89, 1067–1076
44. Torii S, Egan DA, Evans RA and Reed JC (1999) Human Daxx regulates Fas-induced apoptosis from nuclear PML oncogenic domains (PODs). EMBO J. 18: 6037–6049
45. Li H, Leo C, Zhu J, Wu X, O'Neil J, Park EJ and Chen JD (2000) Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. Mol. Cell. Biol. 20: 1784–1796
46. Zhong S, Salomoni P, Ronchetti S, Guo A, Ruggero D and Pandolfi PP (2000) Promyelocytic leukemia protein (PML) and Daxx participate in a novel nuclear pathway for apoptosis. J. Exp. Med. 191: 631–640
47. Wang ZG, Ruggero D, Ronchetti S, Zhong S, Gaboli M, Rivi R and Pandolfi PP (1998) PML is essential for multiple apoptotic pathways. Nat. Genet. 20: 266–272
48. Michaelson JS, Bader D, Kuo F, Kozak C and Leder P (1999) Loss of Daxx, a promiscuously interacting protein, results in extensive apoptosis in early mouse development. Genes Dev. 13: 1918–1923
49. Michaelson JS (2000) The Daxx engima. Apoptosis 5: 217–220
50. Sato T, Irie S, Kitada S and Reed JC (1995) FAP-1: a protein tyrosine phosphatase that associates with Fas. Science 268: 411–415
51. Li Y, Kanki H, Hachiya T, Ohyama T, Irie S, Tang G, Mukai J and Sato T (2000) Negative regulation of Fas-mediated apoptosis by FAP-1 in human cancer cells. Int. J. Cancer. 87: 473–479
52. Zhou YW, Komada Y, Inaba H, Azuma E and Sakurai M (1998) Down-regulation of Fas-associated phosphatase-1 (FAP-1) in interleukin-2-activated T cells. Cell. Immunol. 186: 103–110
53. Yanagisawa J, Takahashi M, Kanki H, Yano-Yanagisawa H, Tazunoki T, Sawa E, Nishitoba T, Kamishohara M, Kobayashi E, Kataoka S and Sato T (1997) The molecular interaction of Fas and FAP-1. A tripeptide blocker of human Fas interaction with FAP-1 promotes Fas-induced apoptosis. J. Biol. Chem. 272: 8539–8545
54. Imai Y, Kimura T, Murakami A, Yajima N, Sakamaki K and Yonehara S (1999) The CED-4-homologous protein FLASH is involved in Fas-mediated activation of caspase-8 during apoptosis. Nature 398: 777–785
55. Koonin EV, Aravind L, Hofmann K, Tschopp J and Dixit VM (1999) Apoptosis. Searching for FLASH domains. Nature 401: 662–663
56. Stanger BZ, Leder P, Lee TH, Kim E and Seed B (1995) RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 81: 513–523
57. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B and Tschopp J (2000) Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat. Immunol. 1: 489–495
58. Chu K, Niu X and Williams, LT (1995) A Fa-associated protein factor, FAF1, potentiates Fas-mediated apoptosis. Proc. Natl. Acad. Sci. 92: 11894–11898
59. Kissil JL, Cohen O, Raveh T and Kimchi A (1999) Structure–function analysis of an evolutionary conserved protein, DAP3, which mediates TNF-alpha- and Fas-induced cell death. EMBO J. 18: 353–362
60. Berger T and Kretzler M (2002) Interaction of DAP3 and FADD only after cellular disruption. Nat. Immunol. 3: 3–5
61. Suzuki T, Terasaki M, Takemoto-Hori C, Hanada T, Ueda T, Wada A and Watanabe K (2001). Proteomic analysis of the mammalian mitochondrial ribosome. Identification of protein components in the 28 S small subunit. J. Biol. Chem. 276: 33181–33195
62. Yang X, Chang HY and Baltimore D (1998) Autoproteolytic activation of pro-caspases by oligomerization. Mol. Cell. 1: 319–325
63. Muzio M, Stockwell BR, Stennicke HR, Salvesen GS and Dixit VM (1998) An induced proximity model for caspase-8 activation. J. Biol. Chem. 273: 2926–2930
64. Martin DA, Siegel RM, Zheng L and Lenardo MJ (1998) Membrane oligomerization and cleavage activates the caspase-8 (FLICE/MACHalpha1) death signal. J. Biol. Chem. 273: 4345–4349
65. Renatus M, Stennicke HR, Scott FL, Liddington RC and Salvesen GS (2001) Dimer formation drives the activation of the cell death protease caspase 9. Proc. Natl. Acad. Sci. USA 98: 14250–14255
66. Garcia-Calvo M, Peterson EP, Leiting B, Ruel R, Nicholson DW and Thornberry NA (1998) Inhibition of human caspases by peptide-based and macromolecular inhibitors. J. Biol. Chem. 273: 32608–32613
67. Kirchhoff S, Muller WW, Krueger A, Schmitz I and Krammer PH (2000) TCR-mediated up-regulation of c-FLIPshort correlates with resistance toward CD95-mediated apoptosis by blocking death-inducing signaling complex activity. J. Immunol. 165: 6293–6300
68. Kirchhoff S, Muller WW, Li-Weber M and Krammer PH (2000) Up-regulation of c-FLIPshort and reduction of activation-induced cell death in CD28-costimulated human T cells. Eur. J. Immunol. 30: 2765–2774
69. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH and Peter ME (1998) Two CD95 (APO-1/Fas) signaling pathways. EMBO J. 17: 1675–1687
70. Li H, Zhu H, Xu CJ and Yuan J (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94: 491–501
71. Luo X, Budihardjo I, Zou H, Slaughter C and Wang X (1998) Bid, a Bcl2 interacting protein, mediates cytochrome *c* release from

mitochondria in response to activation of cell surface death receptors. Cell 94: 481–490
72. Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ and Schlesinger PH (2000) Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome *c*. Cell Death Differ. 7: 1166–1173
73. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wang X (1997) Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91: 479–489
74. Yin X-M, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, Roth KA and Korsmeyer SJ (1999) Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. Nature 400: 886–891
75. Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, Shiels HA, Ulrich E, Waymire KG, Mahar P, Frauwirth K, Chen Y, Wei M, Eng VM, Adelman DM, Simon MC, Ma A, Golden JA, Evan G, Korsmeyer SJ, MacGregor GR and Thompson CB (2000) The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. Mol. Cell 6: 1389–1399.
76. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB and Korsmeyer SJ (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292: 727–730
77. Lacronique V, Mignon A, Fabre M, Viollet B, Rouquet N, Molina T, Porteu A, Henrion A, Bouscary D, Varlet P, Joulin V and Kahn A (1996) Bcl-2 protects from lethal hepatic apoptosis induced by an anti-Fas antibody in mice. Nat. Med. 2: 80–86
78. Rodriguez I, Matsuura K, Khatib K, Reed JC, Nagata S and Vassalli P (1996) A bcl-2 transgene expressed in hepatocytes protects mice from fulminant liver destruction but not from rapid death induced by anti-Fas antibody injection. J. Exp. Med. 83: 1031–1036
79. Strasser A, Harris AW, Huang DC, Krammer PH and Cory S (1995) Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. EMBO J. 14: 6136–6147
80. Zhou Q, Snipas S, Orth K, Muzio M, Dixit VM and Salvesen GS (1997) Target protease specificity of the viral serpin CrmA. Analysis of five caspases. J. Biol. Chem. 272: 7797–7800
81. Tollefson AE, Hermiston TW, Lichtenstein DL, Colle CF, Tripp RA, Dimitrov T, Toth K, Wells CE, Doherty PC and Wold WS (1998) Forced degradation of Fas inhibits apoptosis in adenovirus-infected cells. Nature 392: 726–730
82. Elsing A and Burgert HG (1998) The adenovirus E3/10.4K-14.5K proteins down-modulate the apoptosis receptor Fas/Apo-1 by inducing its internalization. Proc. Natl. Acad. Sci. USA 95: 10072–10077
83. Toker A (1998) Signaling through protein kinase C. Front. Biosci. 3: D1134–D1147
84. Copeland KF, Haaksma AG, Goudsmit J, Krammer PH and Heeney JL (1994) Inhibition of apoptosis in T cells expressing human T cell leukemia virus type I Tax. AIDS Res. Hum. Retroviruses 10: 1259–1268
85. Cuvillier O, Rosenthal DS, Smulson ME and Spiegel S (1998) Sphingosine 1-phosphate inhibits activation of caspases that cleave poly(ADP-ribose) polymerase and lamins during Fas- and ceramide-mediated apoptosis in Jurkat T lymphocytes. J. Biol. Chem. 273: 2910–2916
86. Rudert F, Wang Y, Lindridge E and Watson J (1997) Apoptosis through CD95(Fas/APO-1), but not a CD40/CD95 chimeric receptor, is inhibited by phorbol-12-myristate-13-acetate. DNA Cell Biol. 16: 197–205
87. Drew L, Kumar D, Bandyopadhyay D and Gupta S (1998) Inhibition of the protein kinase C pathway promotes anti-CD95-induced apoptosis in Jurkat T cells. Int. Immunol. 10: 877–889
88. Zhou T, Song L, Yang P, Wang Z, Lui D and Jope RS (1999) Bisindolylmalmeide VIII facilitates Fa-mediated apoptosis and inhibits T cell-mediated autoimmune diseases. Nat. Med. 5: 42–48
89. Chen CY and Faller DV (1999) Selective inhibition of protein kinase C isozymes by Fas ligation. J. Biol. Chem. 274: 15320–15328
90. Scaffidi C, Schmitz I, Zha J, Korsmeyer SJ, Krammer PH and Peter ME (1999) Differential modulation of apoptosis sensitivity in CD95 type I and type II cells. J. Biol. Chem. 274: 22532–22538
91. Tan Y, Ruan H, Demeter MR and Comb MJ (1999) p90(RSK) blocks bad-mediated cell death via a protein kinase C-dependent pathway. J. Biol. Chem. 274: 34859–34867
92. Ruiz-Ruiz C, Robledo G, Font J, Izquierdo M and Lopez-Rivas A (1999) Protein kinase C inhibits CD95 (Fas/APO-1)-mediated apoptosis by at least two different mechanisms in Jurkat T cells. J. Immunol. 163: 4737–4746
93. Meng XW, Heldebrant MP and Kaufmann SH (2002) Phorbol 12-myristate 13-acetate inhibits death receptor-mediated apoptosis in Jurkat cells by disrupting recruitment of Fas-associated polypeptide with death domain. J. Biol. Chem. 277: 3776–3783
94. Gomez-Angelats M and Cidlowski JA (2001) Protein kinase C regulates FADD recruitment and death-inducing signaling complex formation in Fas/CD95-induced apoptosis. J. Biol. Chem. 276: 44944–44952
95. Eischen CM, Dick CJ and Leibson PJ (1994) Tyrosine kinase activation provides an early and requisite signal for Fas-induced apoptosis. J. Immunol. 152: 1947–1954
96. Atkinson EA, Ostergaard H, Kane K, Pinkoski MJ, Caputo A, Olszowy MW and Bleackley RC (1996). A physical interaction between the cell death protein Fas and the tyrosine kinase p59fynT. J. Biol. Chem. 271: 5968–5671
97. Schraven B and Peter ME (1995) APO-1(CD95)-mediated apoptosis in Jurkat cells does not involve src kinases or CD45. FEBS Lett. 368: 491–494
98. Uckun FM (1998) Bruton's tyrosine kinase (BTK) as a dual-function regulator of apoptosis. Biochem. Pharmacol. 56: 683–691
99. Vassilev A, Ozer Z, Navara C, Mahajan S and Uckun FM (1999) Bruton's tyrosíne kinase as an inhibitor of the Fas/CD95 death-inducing signaling complex. J. Biol. Chem. 274: 1646–1656
100. Daigle I, Yousefi S, Colonna M, Green DR and Simon HU (2002) Death receptors bind SHP-1 and block cytokine-induced anti-apoptotic sgnaling in neutrophils. Nat. Med. 8: 61–67
101. Beltinger C, Fulda S, Kammertoens T, Meyer E, Uckert W and Debatin KM (1999) Herpes simplex virus thymidine kinase/ganciclovir-induced apoptosis involves ligand-independent death receptor aggregation and activation of caspases. Proc. Natl. Acad. Sci. USA 96: 8699–8704
102. Aragane Y, Kulms D, Metze D, Wilkes G, Poppelmann B, Luger TA and Schwarz T (1998) Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/APO-1) independently of its ligand CD95L. J. Cell. Biol. 140: 171–182
103. Hennino A, Bérard M, Krammer PH and Defrance T (2001) FLICE-inhibitory protein is a key regulator of germinal center B cell apoptosis. J. Exp. Med. 193: 447–458
104. Cremesti A, Paris F, Grassme H, Holler N, Tschopp J, Fuks Z, Gulbins E and Kolesnick R (2001) Ceramide enables fas to cap and kill. J. Biol. Chem. 276: 23954–23961
105. Grassme H, Jekle A, Riehle Schwarz H, Berger J, Sandhoff K, Kolesnick R and Gulbins E (2001) CD95 signaling via ceramide-rich membrane rafts. J. Biol. Chem. 276: 20589–20596
106. Algeciras-Schimnich A, Shen L, Barnhart BC, Murmann AE, Burkhardt JK and Peter ME (2002) Molecular ordering of the initial signaling events of CD95. Mol. Cell. Biol. 22: 207–220
107. Hueber AO, Bernard AM, Herincs Z, Couzinet A and He HT (2002) An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes. EMBO Rep. 3: 190–196
108. Legembre P, Moreau P, Daburon S, Moreau JF and Taupin JL (2002) Potentiation of Fas-mediated apoptosis by an engineered glycosylphosphatidylinositol-linked Fas. Cell Death Differ. 9: 329–339
109. Alderson MR, Armitage RJ, Maraskovsky E, Tough TW, Roux E, Schooley K, Ramsdell F and Lynch DH (1993) Fas transduces activation signals in normal human T lymphocytes. J. Exp. Med. 178: 2231–2235
110. Newton K, Harris AW, Bath ML, Smith KG and Strasser A (1998) A dominant interfering mutant of FADD/MORT1 enhances deletion of autoreactive thymocytes and inhibits proliferation of mature T lymphocytes. EMBO J. 17: 706–718
111. Zhang J, Cado D, Chen A, Kabra NH and Winoto A (1998) Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. Nature 392: 296–300
112. O'Reilly LA, Huang DC and Strasser A (1996) The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. EMBO J. 15: 6979–6990
113. Kennedy NJ, Kataoka T, Tschopp J and Budd RC (1999) Caspase activation is required for T cell proliferation. J. Exp. Med. 190: 1891–1896

114. Alam A, Cohen LY, Aouad S and Sekaly RP (1999) Early activation of caspases during T lymphocyte stimulation results in selective substrate cleavage in nonapoptotic cells. J. Exp. Med. 190: 1879–1890
115. Zhou BB, Li H, Yuan J and Kirschner MW (1998) Caspase-dependent activation of cyclin-dependent kinases during Fas-induced apoptosis in Jurkat cells. Proc. Natl. Acad. Sci. USA 95: 6785–6790
116. Inaba M, Kurasawa K, Mamura M, Kumano K, Saito Y and Iwamoto I (1999) Primed T cells are more resistant to Fas-mediated activation-induced cell death than naive T cells. J. Immunol. 163: 1315–1320
117. Dao T, Huleatt JW and Hingorani R (1997) Specific resistance of T cells to CD95-induced apoptosis during S phase of the cell cycle. J. Immunol. 159: 4261–4267
118. Karas M, Zaks TZ, Liu JL and LeRoith D (1999) T cell receptor-induced activation and apoptosis in cycling human T cells occur throughout the cell cycle. Mol. Biol. Cell. 10: 4441–4450
119. Parlato S, Giammarioli AM, Logozzi M, Lozupone F, Matarrese P, Luciani F, Falchi M, Malorni W and Fais S (2000) CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. EMBO J. 19: 5123–5134
120. Okura T, Gong L, Kamitani T, Wada T, Okura I, Wei CF, Chang HM and Yeh ET (1996) Protection against Fas/APO-1- and tumor necrosis factor-mediated cell death by a novel protein, sentrin. J. Immunol. 157: 4277–4281
121. Wright DA, Futcher B, Ghosh P and Geha RS (1996) Association of human fas (CD95) with a ubiquitin-conjugating enzyme (UBC-FAP). J. Biol. Chem. 271: 31037–31043
122. Becker K, Schneider P, Hofmann K, Mattmann C and Tschopp J (1997) Interaction of Fas(Apo-1/CD95) with proteins implicated in the ubiquitination pathway. FEBS Lett. 412: 102–106