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The CD95 Receptor: Apoptosis Revisited

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CD95 is the quintessential death receptor and, when it is bound by ligand, cells undergo apoptosis. Recent evidence suggests, however, that CD95 mediates not only apoptosis but also diverse nonapoptotic functions depending on the tissue and the conditions.

Introduction

Apoptosis is induced by a subgroup of the tumor necrosis factor (TNF) receptor superfamily. These so-called death receptors include CD95 (Fas/APO-1), DR3, TNF-R1, and two TRAIL receptors. A shared feature of death receptors is a conserved 80 amino acid sequence, the death domain, in the cytoplasmic tail of these molecules. CD95 is a quintessential death receptor that is constitutively expressed by most tissues. Upon ligation of CD95, sequential association of the adaptor molecule FADD (MORT1), pro-forms of caspases 8 and 10, and the caspase-8/10 regulator c-FLIP lead to the formation of a death-inducing signaling complex (DISC) (Peter and Krammer, 2003). The resulting oligomerization of procaspase-8 results in its activation, autoproteolytic processing, and release of an active heterotetrameric enzyme into the cytosol. The ensuing apoptotic program kills cells via two different pathways: either active caspase-8 directly cleaves and activates caspase-3 (type I) or caspase-3 cleavage is induced indirectly (type II). Alternatively, CD95 can trigger a non-apoptotic caspase-independent form of cell death (Holler et al., 2000).

Although CD95 has been viewed primarily as a death-inducing receptor, accumulating evidence suggests that ligation of CD95 can also mediate a variety of nonapoptotic activities and that its proapoptotic role in lymphocytes, pancreas, liver, and brain may have been overstated. Thus, although ligation of CD95 may induce apoptosis in some cell types under certain conditions, it may also protect cells and regulate tissue regeneration and proliferation. Here, we discuss the activities and signaling pathways of CD95 that support the notion that CD95 is not merely a dedicated death receptor but behaves like other members of the TNF receptor superfamily by mediating diverse functions in different tissues and under different conditions.

CD95: The Prototypic Death Receptor

The investigation of death receptors began with the isolation of agonistic CD95-specific monoclonal antibodies that ligate CD95 at the cell surface and induce apoptosis of cells from various tissues. An indication that CD95 is a proapoptotic molecule in vivo came from studies in which mice injected with the CD95 monoclonal antibody Jo2 exhibited massive apoptosis of liver cells. A primarily pro-death role for CD95 and its ligand, CD95L, in T cells was postulated based on the spontaneous development of autoimmune disease in three CD95/CD95L mutant mouse strains (Bidere et al., 2006). Autoimmunity in these models is characterized by massive accumulation of lymphocytes (primarily $CD4^-/CD8^-/B220^+$ T cells), production of high titer autoreactive antibodies, and various related pathologies. The gene mutation in the *lpr* (lymphoproliferation) mouse strain causes defective expression of CD95 due to insertion of a transposable element into intron 2. The second mouse strain (*gld*, generalized lymphoproliferative disorder) expresses a mutant form of CD95L. The third mouse strain with an *lpr*-like phenotype (lpr^{cg}) has a point mutation in the death domain of CD95 that abrogates recruitment of FADD, subsequent cleavage of caspase-8, and productive apoptotic signaling. In the related human condition, autoimmune lymphoproliferative syndrome (ALPS) (Bidere et al., 2006), ALPS type Ia patients carry dominant-neg-

ative mutations in CD95 and type Ib patients have mutations in CD95L, resembling mice with the *lpr*^{cg} and *gld* mutations, respectively. ALPS type II is caused by mutations in caspase-10, and ALPS type III patients (who show an in vitro defect in CD95-mediated apoptosis of T cells) have no known mutations in CD95, CD95L, caspase-8, or caspase-10.

The autoimmune pathologies seen in the mutant mice and in human patients correlate with an in vitro assay in which pre-activated T cells undergo cell death upon restimulation (a phenomenon called activation-induced cell death or AICD). This process, which affects mostly a CD95-expressing Th1 subset of CD4$^+$ T cells (Devadas et al., 2006), is defective in T cells from CD95/CD95L mutant mice or from human ALPS patients. These observations led to the proposal that CD95 plays an essential role in lymphocyte homeostasis. However, after 18 years of CD95 research and numerous studies of other members of the TNF receptor superfamily, we propose that CD95 may have elaborate tissue-specific functions including, but not limited to, the induction of apoptosis. Increasing evidence suggests that not only CD95 but also CD95L contributes to nonapoptotic functions of the CD95/CD95L system through "retrograde" or "reverse" signaling. In this situation, the receptor CD95 acts as a ligand for the membrane-bound form of CD95L. For example, in activated T cells expressing CD95L, CD95L can transduce signals and together with antigen-mediated activation of the T cell receptor helps to drive T cell proliferation (Sun et al., 2006).

Nonapoptotic Consequences of CD95 Signaling

Nearly all CD95-specific reagents were selected (or designed) because of their ability to induce apoptosis. None of the available CD95L preparations have the exact primary structure of the physiological soluble (sCD95L) or membrane-bound (mCD95L) ligand. They are either tagged (e.g., with FLAG) or are fusion proteins (e.g., leucine zipper CD95L). Recent work shows that neither physiological sCD95L nor mCD95L efficiently kills certain cells that are readily killed by commercial anti-CD95 reagents, suggesting that in vivo CD95L may have nonapoptotic as well as apoptotic activities (Algeciras-Schimnich et al., 2003). Consistent with this, there are data suggesting that CD95 expressed on T and B cells does not just mediate apoptosis, at least not in a situation of an acute immune response. Loss of CD95 in either T or B cells or transgenic expression of a caspase inhibitor in these cells does not seem to cause an *lpr*-like syndrome (Chen et al., 2006; Hao et al., 2004). Thus, while CD95 is critical for AICD in vitro, the evidence to support its role in the acute death of activated T cells in vivo is less convincing. Although there is indirect evidence that the *lpr* syndrome could be caused by resistance of CD95-deficient dendritic cells to apoptosis (Chen et al., 2006), intrinsic apoptosis pathway components (such as the BH3 protein BIM) are more important than CD95 for apoptosis of T cells. So, what then is the function of the dramatic upregulation of CD95 by T cells shortly after activation? Several studies demonstrate that T cell proliferation induced by suboptimal anti-CD3 stimulation is enhanced when CD95 is triggered (Alderson et al., 1993). Furthermore, deletion of CD95 in T cells causes lymphopenia in mice (Hao et al., 2004), suggesting that CD95 expression by T cells is required for their survival, proliferation, and/or activation. Alternatively, CD95L, which is upregulated in T cells from these mice, could either generate an environment that does not allow naive T cells to survive or it could mediate nonapoptotic activities such as inducing proinflammatory cytokine production in tissues expressing CD95 that then indirectly cause lymphopenia (Matsumoto et al., 2007).

The highest constitutive expression of CD95 is in liver cells (hepatocytes). Given that the anti-CD95 antibody Jo2 causes massive apoptosis of liver cells, CD95 was postulated to induce apoptosis of hepatocytes. Liver cells also die through CD95-mediated apoptosis during viral hepatitis, liver cirrhosis, and Wilson's disease. However, CD95 is involved in liver regeneration subsequent to partial hepatectomy (Desbarats and Newell, 2000). Indeed, injection of the normally hepatotoxic Jo2 antibody into partially hepatectomized mice actually accelerates liver regeneration. Liver damage is associated with the activation of antiapoptotic signaling pathways (Akt, STAT3, and NF-κB) that protect against CD95-mediated cell death and thus may help to switch CD95-mediated signals from primarily apoptotic to nonapoptotic. While it is possible that the accelerated regenerative response caused by injection of Jo2 into partially hepatectomized animals may be an indirect effect by an as yet unidentified mechanism, these data strongly suggest a nonapoptotic activity for CD95 in the liver. Such findings on the function of CD95L/CD95 parallel those for the TNF/TNFR1 system (Diehl, 2000).

CD95 is also widely expressed in the central nervous system (CNS). Neurons express CD95, and many CNS-derived tumor cells (such as glioblastoma) are sensitive to CD95-mediated apoptosis in vitro. Surprisingly, injection of CD95-specific monoclonal antibodies into mice with experimental sciatic nerve crush injury may actually accelerate functional recovery (Desbarats et al., 2003). In vitro, CD95 ligation induces neurite outgrowth in sensory neurons through activation of MAP kinases. The MAP kinase signaling pathway is activated in neural progenitor cells after CD95 ligation (Tamm et al., 2004), and neuronal branching in CNS neurons is stimulated by CD95 during development both in vitro and in vivo (Zuliani et al., 2006). Thus, accumulating data suggest a physiologic role for CD95 in regulating neuronal development, growth, differentiation, and regeneration in the CNS. CD95 is also highly expressed and has been shown to mediate nonapoptotic activities in other tissues such as heart, pancreas, and colon (Badorff et al., 2002; Apostolou et al., 2003).

Nonapoptotic, CD95-mediated signaling promotes chronic inflammatory arthritis. The severity of disease in a DBA/1$^{lpr/lpr}$ mouse model of arthritis induced by injection of collagen is markedly reduced compared to heterozygous controls despite the known resistance of *lpr* cells to apoptosis (Ma et al., 2004). Macrophages express both CD95 and CD95L; interactions between CD95 receptors and the CD95L on adjacent macrophages leads to sequestration of FADD to the DISC complex but no apoptosis. Blocking CD95-CD95L interactions in cultured wild-type macrophages suppresses their activation by interleukin-1 receptor 1 (IL-1R1) or toll-like receptor (TLR) 4 and the subsequent interaction of FADD with the TLR adaptor protein MyD88 (Ma et al., 2004). This suggests that CD95-CD95L interactions may promote chronic inflammation through a unique mechanism in which CD95 ligation promotes activation via the IL-1R1–TLR4 pathway.

CD95 and CD95L in Cancer

Almost all human tumors express CD95. Increased expression of CD95L in solid tumors with concomitant downregulation of CD95 was interpreted as a way for tumor cells to mount a "counterattack" against tumor-infiltrating lymphocytes (Green and Ferguson, 2001). The model is attractive because T cells are more sensitive to CD95-mediated apoptosis (in vitro) when activated and presumably would be killed by the tumor cells upon interaction with them. This "tumor strikes back" model was tested experimentally by engineering tumors to express high levels of surface CD95L. Unexpectedly, these CD95L bearing tumors were often rejected more efficiently after injection into mice compared to their untransfected counterparts. Rejection of the tumors is largely independent of the adaptive immune system and is associated with rapid infiltration of neutrophils and other granulocytes. In addition, a variety of studies show that ligation of CD95 expressed by tumor cells induces them to produce chemotactic factors such as IL-8 and MCP1 rather than undergo apoptosis, resulting in the recruitment of more proinflammatory cells thus linking CD95 to inflammation (Matsumoto et al., 2007). Many human cancers are associated with inflammation, which may in turn contribute to tumor growth. Thus, it is reasonable to hypothesize that in some cases CD95L via its interaction with CD95 could contribute to tumor growth in cancers associated with inflammation in part through the induction of chemotactic factors. Whether stimulation of cancer cells through CD95 leads to rejection of tumors or inflammation and tumor growth may depend on the tumor type and its stage of progression.

Tumor progression may be promoted by nonapoptotic signals emanating from CD95 that include (but are not limited to) activation of NF-κB and all three major MAPK pathways: ERK1/2, p38, and JNK1/2 (Barnhart et al., 2004). Activation of CD95 in apoptosis-resistant tumor cells results in the upregulation of a distinct set of genes whose products have been implicated in invasion, metastasis, and apoptosis resistance. Many tumor cells increase their motility and invasiveness in vitro when nonapoptotic pathways are activated through CD95, demonstrating that activation of these pathways is physiologically important.

An increase in the serum concentration of sCD95L in cancer patients suggests a possible immunosuppressive role for this molecule (although it is not clear whether the sCD95L exists in the biologically active trimerized form) (Barnhart et al., 2004). However, the generalized immune suppression that would be expected from this situation (rather than specific suppression of the immune response to the tumor) is not seen in these patients, and thus it may be that the increase in CD95L expression in tumor tissues plays a more direct role in tumor progression. CD95 has been reported to act as a tumor promotor in lung cancer (Lee et al., 2003), thyroid cancer (Mitsiades et al., 2006), and ovarian cancer (M.E.P., unpublished data).

CD95-Linked Signaling Pathways

Surprisingly little is known about the molecular links that connect CD95 to activation of the ERK, JNK, p38, and NF-κB signaling pathways. FADD, caspase-8, and c-FLIP (components of DISC) are known to link CD95 to nonapoptotic pathways (Matsumoto et al., 2007). A number of mouse models and mutant mice reveal nonapoptotic activities for these DISC components (for review see Park et al., 2005). FADD, caspase-8, and c-FLIP are essential for mice to develop beyond embryonic day 11.5, and studies now show that all three proteins are important for the survival of activated T and B cells. In addition, both caspase-8 (Ben Moshe et al., 2007) and FADD (Schuchmann et al., 2005) are required for effective liver regeneration. There is evidence that CD95 can be connected to the nonapoptotic activities of these DISC components. Both FADD and caspase-8 are required for proliferation of early bone marrow-derived hematopoietic progenitor cells (Pellegrini et al., 2005), and CD95L can protect this cell population from dying (Josefsen et al., 1999). Whether the DISC components act through CD95, through other death receptor family members, or independently of surface receptors is unknown, but the findings emphasize that the downstream components of apoptosis can also play a role in lymphocyte or hepatocyte survival.

The ability of CD95 signaling to mediate either death or growth signals and to play a critical role in development pathways may hinge on its ability to regulate downstream caspase activation. A reasonable candidate for mediating the regulation of signaling consequences may be c-FLIP, which at high concentrations can inhibit CD95-mediated apoptosis (for review see Park et al., 2005). Another possible switch between proapoptotic and nonapoptotic CD95 signaling may be the posttranslational modifications that regulate the ability of CD95 to become internalized after its activation by CD95L. Two acceptor sites for posttranslational modifications in the intracellular domain of CD95 regulate nonapoptotic activities of CD95. Tyr291 is important for internalization of CD95 (Lee et al., 2006), and Cys199 regulates receptor aggregation and

localization to lipid rafts, both requisite steps for CD95 internalization (Chakrabandhu et al., 2006). Internalization of CD95 into an endosomal compartment may determine which signaling pathways are engaged. When internalization of CD95 is blocked, the receptor cannot induce apoptosis and instead remains fully engaged in activating nonapoptotic pathways (Lee et al., 2006). CD95 therefore seems to be similar to other internalizing receptors such as the epidermal growth factor receptor (EGFR). Internalization of EGFR targets activated receptors to the endocytic compartment and contributes to both the intensity of signaling and the assembly of signaling complexes (Miaczynska et al., 2004).

What mechanism decides which signaling pathways will be activated by CD95? It is likely that multiple signaling pathways, including those activated by caspases and NF-κB, are simultaneously triggered by ligation of CD95, and that only under certain conditions is the signaling threshold to induce apoptosis reached. Alternatively, nonapoptotic outcomes may result in response to, for example, inhibition at the receptor level (e.g., by c-FLIP), inappropriate concentrations of caspase-8/caspase-10 or of downstream proapoptotic proteins such as Bax, upregulation of protective molecules (e.g., Bcl-2 or IAP family members), or activation of protective pathways (e.g., ERK, NF-κB).

Another mechanism for nonapoptotic pathways to prevail may be through mutation or downregulation of CD95 itself, a situation frequently found in many human cancers (Muschen et al., 2000) enabling them to evade destruction by the immune system. However, tumor cells rarely completely lose CD95 expression and so may be able to regulate this receptor to establish a level of CD95 signaling that is too weak to induce receptor internalization and apoptosis when engaged by CD95L but still sufficient for full activation of nonapoptotic pathways, which could lead to tumor promotion.

Conclusion

We suggest it is time to reappraise the physiological functions of the death receptor CD95 in various tissues. We argue that important functions of CD95 have been widely ignored and that the available data do not support the conclusion that the induction of apoptosis is the sole function, or perhaps, in some tissues, even the primary function, of CD95 in vivo. Indeed, in some cases CD95 can act as a protector of tissues rather than as their destroyer. Next, we need to identify the signaling components that connect CD95 to nonapoptotic signaling pathways because these may provide new targets to treat diseases, such as inflammation and cancer, for which nonapoptotic activities of CD95 are proving important.

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