Review

Looking beneath the Surface: The Cell Death Pathway of Fas/APO-1 (CD95)

Ben Z. Stanger
Department of Genetics, Harvard Medical School and Howard Hughes Medical Institute, Boston, Massachusetts, U.S.A.

Summary: The biochemical basis of programmed cell death is poorly understood in mammals. The cell surface receptor Fas/APO-1 (CD95) is one molecule known to be central to a number of mammalian cell death processes. Several studies in the past year have led to insights about the role of Fas/APO-1 in vivo and have also given some clues about the biochemical components of the Fas/APO-1 death pathway. This article reviews those studies and discuss models of Fas/APO-1 signaling and function.

Background: Cell death occurs as a normal process in a wide variety of developmental and homeostatic contexts in metazoan organisms (1); it represents the timely and appropriate fate for many or even the majority of cells born in certain organ systems. Despite the importance and ubiquitous nature of such physiologic, or "programmed", cell death, little is known about the molecular events that mediate this process. That a conserved biochemical pathway exists is suggested by the observation that programmed cell death is almost always accompanied by a consistent set of morphologic changes, an appearance known as apoptosis (2).

The identification of the genes that control programmed cell death in higher eukaryotes has been hampered by several inherent difficulties. First, the genetic tools so useful in dissecting cell death pathways in *Caenorhabditis elegans* (3) and Drosophila (4) have not been available in higher eukaryotes. Second, the death-inducing properties of such genes makes genetic selection an impractical means of identification. Third, it appears that many cell death genes are constitutively expressed and present in an inactive form (5), making it unlikely that they could be discovered by techniques relying upon differential gene expression. Finally, genes identified by virtue of an ability to induce death when overexpressed must be subjected to rigorous criteria to determine whether the cell death is of physiologic importance, since it is likely that overexpression of certain proteins may lead to toxic effects that are distinct from the in vivo roles of those proteins.

Two approaches to date have yielded the most information about cell death processes: (i) identification of cell death genes by classical genetic means coupled with characterization of their mammalian homologs and (ii) screening for proteins capable of inducing cell death directly in mammalian cells. The Fas antigen/APO-1 is an example of a protein discovered using the latter approach, as it was first discovered as an inducer of cell death and later shown to be necessary and sufficient for certain programmed deaths in vivo. More recent studies have connected Fas to elements of cell death pathways in other species. It has been proposed that Fas is related to the Drosophila cell death protein Reaper, and that in signaling cell death Fas relies upon a relative of the *C. elegans* cell death protein CED-3. Fas may therefore represent an evolutionarily conserved component of a universal cell death pathway.

IN VIVO FUNCTIONS OF FAS AND ITS ROLE IN HUMAN DISEASE

Fas/APO-1 was identified independently by two groups performing monoclonal antibody screening. The antibodies were chosen for analysis based on the unusual property of being able to induce death in a wide variety of cell types. The cell surface molecule recognized by these antibodies was named APO-1 by one group (6), and Fas antigen by the other (7); subsequent cloning revealed Fas and APO-1 to be derived from the same gene (8,9). This gene encodes a transmembrane protein of about 45 kD that is widely expressed (although more abundant in certain tis-

Address correspondence and reprint requests to: Ben Z. Stanger, Harvard Medical School, Department of Genetics, 200 Longwood Avenue, Boston, MA 02115.

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sues such as thymus, lung, heart, and liver) and bears a strong similarity in its extracellular domain to members of the tumor necrosis factor family of receptors (TNF-Rs). This family includes the p75 nerve growth factor receptor, CD40, CD30, CD27, 4-1BB, and OX40 (among others) and is characterized by a cysteine-rich extracellular repeat motif; not surprisingly, the ligands for these receptors are also remarkably well conserved (reviewed in Ref. 10). At first glance, the cytoplasmic domain of Fas/APO-1 had no observed similarity to proteins outside of this family, although a relationship to several other proteins has now been appreciated (described below). Among members of the family, Fas/APO-1 bears the greatest resemblance (51% similarity) to the TNF-R type 1 (TNF-R1; p55 TNF-R) over a 45–amino acid stretch (8). Many of the models of Fas/APO-1 signaling have therefore been based on known components of the TNF-R1 pathway.

Mutations in the *fas* gene are responsible for the recessive *lymphoproliferation* (*lpr*) phenotype in mice (11). lpr mice develop a progressive lymphadenopathy and immune complex syndrome that resembles the human autoimmune disease systemic lupus erythematosus. Two mutations can lead to the phenotype: an insertion, which prevents normal processing of precursor RNA, and a point mutation in the cytoplasmic domain that renders Fas/APO-1 incapable of transmitting the death signal. When the ligand for Fas/APO-1 was cloned (FasL [12]), a mutation in this gene was found to cause the phenotype seen in the mouse mutant *gld*, a non-allelic phenocopy of *lpr* (13,14). These studies demonstrated that both the receptor and its ligand are needed for the elimination of immune cells capable of recognizing self-antigen—a process known as "tolerance" to self-antigen.

How do mutations in these cell death genes lead to autoimmune disease? The answer to this question may rest with alterations in the process of activation-induced cell death (AICD), which occurs when certain T cells are triggered through the T-cell receptor (15). In vivo, AICD may serve to clear the circulation of mature autoimmune T cells that have escaped earlier selection and normal activated T cells that have already done their job, both of which pose a potential hazard to the organism. The abnormal T cells of *lpr* mice arise from a defect in elimination at a mature developmental stage (16), and indeed, activated T cells from *lpr* and *gld* mice are unable to undergo AICD (17). Soluble Fas/APO-1 can block AICD in a number of T-cell lines (18–20), demonstrating that Fas/APO-1 is directly responsible for the death of these mature T cells. Fas/APO-1 is also required for the elimination of B cells that have become nonspecifically activated by a process known as "bystander" activation (21), as well as the specific elimination (clonal deletion) of self-reactive B cells from the periphery (22). These results indicate that autoantibody production in *lpr* animals (23) may be directly attributable to abnormal B-cell activity rather than to a secondary effect orchestrated by autoimmune T cells.

In addition to its role in maintaining tolerance, Fas/APO-1 is required for cytotoxic T cells (CTLs) to kill their targets, forming an auxilliary pathway to the well characterized granule exocytosis pathway (reviewed in Ref. 24). It is not currently known which settings in vivo utilize the Fas/APO-1 pathway versus the granule exocytosis pathway for CTL activity. Whether the participation of Fas/APO-1 in CTL killing is also responsible in part for the *lpr* autoimmune phenotype likewise remains to be determined. In addition, Fas is capable of triggering hepatocyte cell death (25), and mice bearing a targeted mutation (which renders them true nulls for *fas*) develop liver hyperplasia (26), implying that Fas may play a role in liver homeostasis. (See also the article by Leist et al. in this issue [27] suggesting that the Fas-mediated death of hepatocytes does not depend upon the expression of TNF-R1).

Thus, Fas/APO-1 is required for at least two physiologic processes in vivo: the maintenance of tolerance to self-antigen and cytotoxic T-cell activity. The range of human disease attributable to mutations in *fas* is just beginning to be discovered. For example, a subset of lupus patients synthesize elevated levels of a variant Fas antigen lacking a transmembrane domain (28). This variant protein may interfere with Fas/FasL interactions, and could play a role in the pathogenesis of autoimmune disease by blocking autoreactive cell death. Recent studies suggest that mutations in human Fas are associated with lymphoproliferative syndromes that appear to be the human equivalents of the murine *lpr* phenotype (29,30). The Fas system also has potential applications in certain clinical situations; in particular, overexpression of FasL on donor tissues may be an effective inhibitor of post-transplantation graft rejection (31).

Most intriguing, however, is the prospect that Fas/APO-1 may participate in the pathogenesis of disease caused by human immunodefi-

ciency virus (HIV). $CD4^+$ lymphocytes from asymptomatic HIV-infected patients are abnormally susceptible to AICD (32), a process now known to be regulated by Fas and its ligand (see above). More recent studies have demonstrated that T cells from HIV-infected individuals are more sensitive to Fas-induced killing than those of normal individuals (33), and that the HIV proteins Tat and gp120 can directly sensitize cells to the effects of Fas/APO-1 (34). A cell death pathway involving Fas/APO-1 may therefore underlie the T-cell depletion seen in AIDS, making the elucidation of this pathway all the more important.

EVIDENCE THAT FAS/APO-1 SELF-ASSOCIATES

The sequence of the Fas/APO-1 cytoplasmic domain contains no obvious clues about the mechanism of cell killing; in particular, the intracellular portion of Fas/APO-1 has no recognizable kinase or phosphatase domains, no SH2 or SH3 docking motifs, nor homology to other membrane proteins involved in regulating cell death (e.g., *bcl*-2). However, as mentioned above, the Fas/APO-1 cytoplasmic domain does have a region with significant similarity to the TNF-R1 cytoplasmic domain. On the basis of this homology, deletion and mutation analyses were performed which showed that this homology region is important for the ability of both molecules to induce cell death (35), and gave rise to the name "death domain" to refer to this conserved region (36). It was hypothesized that the death domain may serve as a protein-protein interaction motif, with the attractive possibility that both Fas/APO-1 and TNF-R1 may signal cell death through their interaction with a common intracellular ligand.

A model soon developed that held Fas/APO-1 aggregation as the principle means of receptor activation (Fig. 1A). Studies of receptor tyrosine kinases had already given precedent to the notion that receptor signaling may occur through aggregation and subsequent association with cytoplasmic proteins through interaction domains (reviewed in Ref. 37). Several observations suggested that aggregation is likewise important in Fas/APO-1 signaling. Dhein et al. (38) demonstrated that crosslinking is required for transmission of the Fas/APO-1 death signal, such that $F(ab')_2$ preparations of anti–Fas/APO-1 antibodies are unable to induce apoptosis unless an anti–Ig $F(ab')_2$ crosslinking antibody is also present. The crystal structure of ligand-bound TNF-R1 extracellular domain showed that this complex exists as a trimer (39). Moreover, immunofluorescence studies of transiently transfected cells expressing either wild-type Fas/APO-1 or an interleukin 4 receptor (IL-4R) Fas chimera have shown a punctate membrane staining pattern as though receptor clustering occurs even in the absence of ligand (Stanger and Leder, unpublished results).

More recently, studies using chimeric receptors have demonstrated that the extracellular domains of CD40 (40), the erythropoietin receptor (41), and IL-4R (Stanger and Leder, unpublished results), can all be substituted for the extracellular domain of Fas/APO-1 without any loss in death-inducing activity. These studies suggest that the extracellular domain serves merely to aggregate Fas/APO-1 intracellular domains when ligand is present. Although the possibility remains that ligand binding may lead to other, more complex, alterations in receptor conformation, the aggregation model remains the simplest explanation for preserved function with multiple distinct extracellular domains.

THE DEATH DOMAIN AS A MEDIATOR OF PROTEIN-PROTEIN INTERACTIONS

Modular binding domains such as the Src and Pleckstrin homology domains are utilized in many signal transduction proteins, and the death domain may represent an analogous domain in cell death signal transduction. Binding modules provide not only a convenient framework for interactions between various proteins, but can also determine the specificity of their interactions. Unfortunately, there is little direct evidence to support the Fas aggregation hypothesis. Attempts to document Fas/APO-1 self-association by co-immunoprecipitation from cells have been disappointing. The best evidence to date for an interaction comes from the yeast two hybrid method (42). Experiments utilizing this system have shown a capacity for self-association of the cytoplasmic domains of both Fas/APO-1 (43,44) and TNF-R1 (43,45), as well as a weak association between the cytoplasmic domains of the two receptors (43). The ability of a death domain to self-associate has also been demonstrated in vitro, as fusion proteins consisting of the cyto-

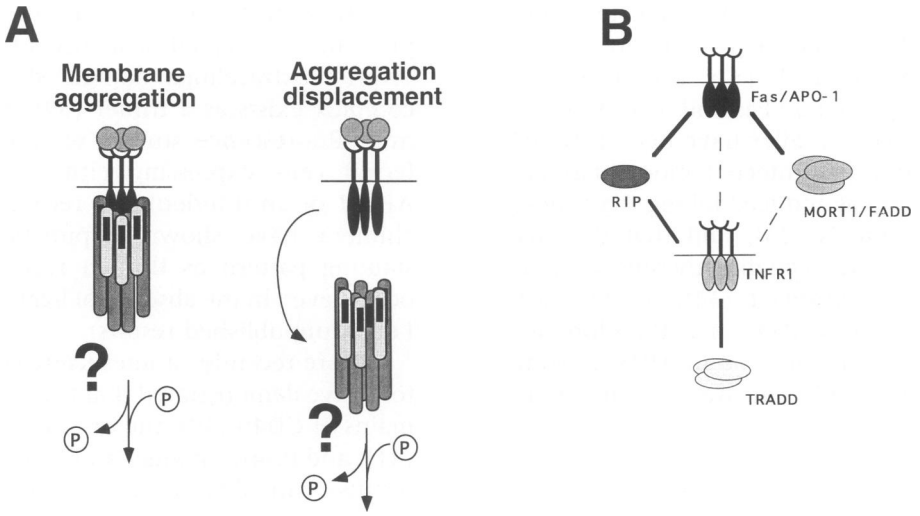

FIG. 1. Fas/APO-1–interacting proteins

(A) Models of membrane proximal events in Fas/APO-1 signaling. On the left, Fas ligand binding has caused the aggregation of Fas/APO-1 cytoplasmic domains and/or the heterotypic association of other death domain-containing proteins (e.g., RIP, MORT1/FADD). Other Fas-associated proteins or modifiers of the Fas/APO-1 signal (e.g., FAP-1, HCP) may also be components of the complex, and may serve in trans to activate the signaling cascade. On the right, this complex had existed preformed and upon ligand binding was displaced from the membrane by the homotypic association of Fas/APO-1 death domains. The complex can now freely act in the cytoplasm. (B) Interactions between Fas- and TNF-R1-associated proteins. Known interactions are shown with solid lines, while interactions for which there is some evidence are shown with dotted lines. Proteins for which self-association has been demonstrated are indicated as trimers. Degree of shading represents the extent of amino acid identity with the Fas/APO-1 death domain (the order is: RIP > TNF-R1 = MORT1/FADD > TRADD).

plasmic domain of TNF-R1 fused to the *E. coli* maltose binding protein or glutathione-S-transferase are capable of binding to each other (43).

Additional support for the notion that death domains can mediate protein oligomerization comes from the finding that two proteins capable of interacting with the Fas/APO-1 cytoplasmic domain (MORT1/FADD and RIP) and one capable of interacting with the TNF-R1 cytoplasmic domain (TRADD) all contain death domains (44,46–48). Although extensive pairwise tests-of-association have not been performed with death domain-containing proteins, there is some specificity to their binding: the death domain-containing proteins identified so far are capable of interacting with some but not all other members of the family (Fig. 1B).

Both MORT1/FADD and RIP are incapable of binding a mutant Fas/APO-1 bearing a mutation analogous to the inactivating point mutation in the *lpr* death domain. Moreover, MORT1/FADD and RIP can bind to a mutant Fas/APO-1 missing the 15 C-terminal amino acids, which are not required for Fas/APO-1 to induce cell death (35). However, these novel proteins do not bind to Fas mutants bearing more extensive C-terminal deletions which block Fas/APO-1 signaling (44,48), indicating that their interactions bear relevance to signaling in vivo. Finally, TRADD, MORT1/FADD, and RIP are all capable of inducing cell death upon transient overexpression, making them strong candidates for proximal signaling components of the Fas and/or TNF-R1 death pathway.

However, the most convincing evidence of a role for these proteins in Fas/APO-1 signaling has yet to be obtained: (i) do MORT1/FADD, RIP, and TRADD interact with Fas or TNF-R1 physiologically and if so under what conditions (e.g., ligand binding); and (ii) does disruption of any of these candidate genes lead to a block in the signal transmitted by either receptor? It has recently been proposed that the cytoplasmic domains of Fas/APO-1 and TNF-R1 (and by extension these three newly identified death domain-containing proteins) represent mammalian homologs of the Drosophila Reaper protein (49). The *reaper* gene was identified through a genetic screen for mutants deficient in cell death during embryogenesis, and seems to be both necessary and sufficient

for much of the cell death during Drosophila development (50). Close inspection of the 65–amino acid Reaper polypeptide has revealed a limited degree of similarity with the death domain–containing proteins of mammals (49,51), raising the possibility that Reaper-like sequences have been conserved and incorporated into various proteins as a death cassette. Insertion of this death domain cassette into transmembrane proteins may have permitted ligand-regulated cell death induction; by inducing aggregation, the death domain of Fas/APO-1 or TNF-R1 may serve as a nucleus for the association of other Reaper-like molecules that transmit the actual death signal.

MODELS OF KINASE AND PHOSPHATASE INVOLVEMENT

The events following Fas receptor crosslinking are still not known, but it appears at least that signals generated by Fas/APO-1 ligation do not proceed directly to the nucleus, since cells enucleated with cytochalasin B still undergo cytoplasmic changes characteristic of apoptosis (52,53). Moreover, a number of post-receptor processes modulate Fas/APO-1 signaling, as cells expressing high cell surface levels of Fas may vary in their susceptibility to anti-Fas depending upon the degree of T-cell activation (54,55) or with the nature of B-cell co-stimulation (21).

Nevertheless, several scenarios can be envisioned for the steps following Fas/APO-1 ligation. One model is that Fas/APO-1 signaling is achieved through the action of kinases and/or phosphatases. Kinases participate in signaling by several membrane receptors that are themselves enzymatically inert, including JAK kinases in cytokine receptor signaling and Src-family kinases in antigen receptor signaling (56). TNF-R1 acts through a kinase cascade that includes a sphingomyelinase-induced ceremide intermediate (reviewed in Ref. 57) and a family of kinases capable of phosphorylating c-Jun—the stress activated kinases (58,59). Although the participation of these protein kinases in TNF-R signaling is well established, it is unclear whether they are relevant to Fas signaling. In addition, the death domain of TNF-R1 itself has been shown to interact with a Ser/Thr protein kinase (60,61).

Fas ligation results in the rapid tyrosine phosphorylation of many cellular proteins, and the tyrosine kinase inhibitor herbimycin A has been shown to interfere with DNA fragmentation and cell death in Jurkat cells (62), although protection was not seen in L929 cells treated with herbimycin A (63). At least two proteins known to interact with Fas/APO-1 in a yeast two-hybrid assay are apparently involved in the transfer of phosphate groups: RIP, which encodes a putative protein kinase (44) and FAP-1, which encodes a protein phosphatase (64). The kinase domain of RIP contains regions of similarity to both Ser/Thr and Tyr kinases, an unusual feature among kinases which it shares with the soybean kinase GmPK6 (65). In the subdomains that appear to mediate hydroxyamino acid recognition (66), RIP resembles a Ser/Thr kinase, while in the framework residues outside the active site, RIP is more similar to Tyr kinases. However, the kinase domain of RIP is clearly not needed for its ability to induce cell death in transfected cells, since deletion mutants lacking the catalytic domain can still kill cells (44).

FAP-1 is capable of binding to the C-terminal 15 amino acids of Fas/APO-1, a region previously shown to inhibit Fas signaling (35). FAP-1 is identical to a portion of PTP-BAS, a tyrosine phosphatase cloned from human basophils that is expressed in many tissues (67). The interaction is mediated by one of the GLGF repeats of PTP-BAS, in a domain that appears to be important for regulation of phosphatase activity. High levels of FAP-1 expression are correlated with resistance to Fas-induced killing in several cell lines, and FAP-1 overexpression can render Jurkat cells Fas-resistant in an expression-dependent manner, consistent with an inhibitory role for FAP-1 in Fas signaling (64). The possibility that RIP and FAP-1 may act antagonistically on the same substrate remains to be explored.

Nevertheless, a simple model of pro-apoptotic kinases and anti-apoptotic phosphatases does not accurately represent Fas/APO-1 signaling. Instead, signaling is likely to be regulated by a dynamic site-specific crosstalk between kinases and phosphatases. This complexity is already apparent in the role that phosphatases seem to have in Fas/APO-1 signaling. In contrast to the FAP-1 phosphatase, which has an inhibitory effect on Fas/APO-1 killing, other phosphatases appear to promote or mediate the Fas death signal, since phosphatase inhibitors can block Fas-mediated death (63,68). The hematopoietic cell protein tyrosine phosphatase (HCP) is a candidate for such an apoptotic phosphatase. Its expression is positively correlated with Fas sensitivity in a number of cell lines and overexpres-

sion of HCP can render a Fas-resistant cell line sensitive to anti-Fas antibodies (68). Moreover, an autoimmune phenotype is seen in mice of the strain *motheaten*, which have mutations in the HCP gene (69).

Other membrane-proximal pathways may be used to transduce the Fas/APO-1 death signal. Fas/APO-1 ligation causes the rapid activation of sphingomyelinase, which makes ceramide by cleaving the phosphocholine-linked sphingoid fatty acid sphingomyelin (70,71). Sphingomyelinase and ceramide are themselves pro-apoptotic, leading to cell death and DNA fragmentation within 3 to 6 hr (72,73). However, the role of this fatty acid intermediate in Fas/APO-1 signal transduction is still undetermined, and at least one study failed to find ceramide release during Fas- or TNF-induced killing of the breast carcinoma line MCF7 (74). In addition, overexpression of a *ras* dominant negative mutant or microinjection of anti-Ras antibodies was able to partially block cell death, suggesting that part of the Fas/APO-1 pathway utilizes Ras (75). Most Tyr phosphorylation events following Fas ligation on Jurkat cells are not altered by blocking Ras, although overexpression of the dominant negative Ras did inhibit the tyrosine phosphorylation of one ~97-kD protein; notably, this protein has the same molecular weight as ceramide-activated protein kinase (76).

Thus, many possibilities still exist for the initial steps in Fas/APO-1 signaling, including death-inducing proteins capable of interacting directly with Fas, and complex interactions among kinases, phosphatases, fatty acids, and possibly Ras. Furthermore, the observation that under certain circumstances Fas can transmit activation as well as death signals (77–80) highlights the regulatory complexity that is likely to underly Fas/APO-1 signaling.

CYSTEINE PROTEASES

One guiding hypothesis in cell death research has been the prospect (and hope) that an evolutionarily conserved final common pathway may serve as the convergence point for multiple cell death stimuli (81,82). Two genes identified through mutant analysis and known to be required for most programmed cell deaths in *C. elegans*, *ced*-3 and *ced*-4 (3), have been obvious candidates for such a process. The *ced*-3 gene has been cloned (83) and encodes a protein which resembles the mammalian interleukin 1β–converting enzyme (ICE) (84), a cysteine protease that cleaves after Asp residues. ICE belongs to a family of cysteine proteases that includes Nedd2/ICH-1, CPP32, and ICH-2 (reviewed recently in Ref. 85). Transient overexpression of most members of the family induces cell death (Ref. 86 and those above). Of note, the cytotoxic granule protein Fragmentin 2/granzyme B is also a protease with Asp specificity (87). Fragmentin 2 is capable of inducing cell death (88), and appears to do so by cleaving CPP32 (89).

Strong support for the notion that a cysteine protease is required for Fas/APO-1 killing came from the finding that both the cowpox-encoded ICE-inhibitor CrmA and/or a noncleavable IL-1β transition state analogue (Ac-Tyr-Val-Ala-Asp-aldehyde) could prevent Fas-induced apoptosis (90–92). Several approaches have been taken to determine which protease(s) are responsible, including an in vitro system in which many of the morphologic and biochemical hallmarks of apoptosis are preserved (93). One such hallmark is the cleavage by an Asp-specific protease of poly-(ADP-ribose) polymerase (PARP) to an 85-kD fragment both in vivo and in vitro. The enzyme which carries out this cleavage can be blocked by tetrapeptide inhibitors of ICE and is thus presumed to be a cysteine protease. This protease is distinct from ICE (since purified ICE could not cleave PARP) and was called prICE, for protease resembling ICE (93).

Recent studies have suggested that prICE is identical to the CED-3 relative CPP32 (also called Yama or apopain), and that CPP32 is the key protease required for Fas/APO-1–mediated killing. Tewari et al. (94) showed that PARP is cleaved following Fas crosslinking and that purified, activated CPP32 can cleave PARP in a CrmA-inhibitable manner. More direct evidence that prICE is CPP32 came from the biochemical purification of prICE using peptide binding and PARP cleavage as assays (95). CPP32 is produced as a 32 kD proenzyme that is cleaved at two Asp residues to yield the active p17 and p12 subunits. Among known cysteine proteases, CPP32 and the *C. elegans* CED-3 protein are most closely related, as might be expected for the mammalian homolog of the nematode cell death protease.

Mice bearing a targeted mutation in the ICE gene have a relative resistance to anti-Fas induced killing, suggesting that ICE itself plays some role in the Fas/APO-1 pathway (96), although several forms of thymocyte death appear to be normally intact in ICE–"knock-out" mice (97). ICE is capable of cleaving CPP32 to its ac-

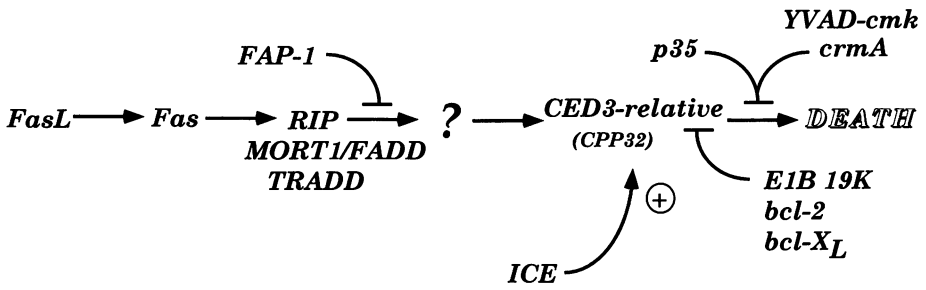

FIG. 2. Model of Fas/APO-1 signaling
See text for details.

tive form (94), and thus it may activate CPP32 in vivo. However, osteosarcoma cells containing high levels of active CPP32 have no detectable ICE (95), suggesting that other enzymes are capable of catalyzing cleavage of the CPP32 proenzyme.

OTHER MODULATORS OF FAS/APO-1 SIGNALING

Another evolutionarily conserved component of cell death regulation involves the Bcl-2 family of membrane proteins, which are capable of forming homo- and hetero-dimers that serve as a "rheostat" to positively or negatively influence cell death decisions (reviewed in Ref. 98). Bcl-2 is homologous to the protein encoded by the *ced*-9 gene of *C. elegans* (81), which acts as a negative regulator of *ced*-3–induced cell death (99). Since Fas killing requires a *ced*-3–like cysteine protease, it has been postulated that Bcl-2 family members also regulate death signals from Fas.

Bcl-2 can partially protect cells from Fas/APO-1–mediated apoptosis (100), and coexpression of the Bcl-2 binding protein BAG-1 fully protects cells (101). The Bcl-2 relative Bcl-X_L is also capable of inhibiting Fas-mediated cell death (74,102). However, the role that Bcl-2 itself plays in modulating Fas signals in vivo is unresolved. Mapara et al. (78) studied the expression of Fas/APO-1 and *bcl*-2 in human leukemic B cells activated with *Staphylococcus aureus* Cowan I (SAC) and interleukin 2 (IL-2). Following activation, cell surface expression of Fas/APO-1 increased, *bcl*-2 mRNA decreased, and cells became susceptible to Fas-mediated killing. However, one cell line did not down-regulate *bcl*-2 in response to SAC plus IL-2 and was paradoxically stimulated to proliferate in response to anti-Fas antibodies. Thus, down-regulation of *bcl*-2 may be permissive for induction of apoptosis through Fas/APO-1. However, there are clearly other means of achieving Fas-resistance, since variability in Fas-responsiveness has been seen despite a uniform absence of Bcl-2 expression in a number of non-hematopoietic tumors (79).

Viruses, in addition to a well-characterized exploitation of mitosis-promoting genes in the viral life cycle, also use anti-apoptotic genes to protect against host cell death (98). The adenovirus E1B 19K protein appears to be encoded by one such gene, and can protect cells from death induced by several stimuli, including TNF (103). E1B 19K can also protect cells from anti-Fas mediated apoptosis (104), presumably as a result of its similarity to Bcl-2 (105). The insect baculovirus p35 protein can inhibit Fas/APO-1–induced apoptosis and PARP cleavage, suggesting that it too may act as a global inhibitor of cell death initiated by many stimuli (106), and is likely to do so by forming a stable complex with members of the ICE/CED-3 family (107). In addition, open reading frames within the Epstein-Barr virus and African Swine Fever virus encode members of the Bcl-2 family (108), and it remains to be determined whether these homologs can protect cells from Fas/APO-1–mediated apoptosis.

Other insights into the regulation of Fas signaling have come from studies of cytotoxic granules proteins. One such protein is TIA-1, an RNA-binding protein that is capable of inducing cell death in permeabilized thymocytes (109); it is phosphorylated by FAST, a serine/threonine kinase that is itself activated by rapid dephosphorylation following Fas ligation (110). FAST may therefore be a substrate of the pro-apoptotic phosphatases described above. Moreover, the TIA-1–related protein TIAR is translocated from the nucleus to the cytoplasm within 30 min of Fas signaling (111). It is possible that TIA-1, TIAR, and FAST are direct mediators of the Fas

LIST OF WHO'S WHO:

FAS	Ligand of Fas/Apo-1 receptor	Activates cell death
FAS/Apo-1/ CD95	Transmembrane receptor Activated by binding of its ligand (FasL)	Transmits death signal
TNF-R1	Tumor necrosis factor receptor type 1 Has homology to Fas/Apo-1	Transmits death signal
MORT1/FADD	"Death domain" containing protein Interacts with Fas/Apo-1	Candidate signaling component in the Fas-mediated death pathway
RIP	Protein kinase Contains "death domain" Interacts with Fas/Apo-1	Candidate signaling component in the Fas-mediated death pathway
TRADD	"Death domain" containing protein Interacts with TNF-R1	Candidate signaling component in the TNF-R1–mediated death pathway
Reaper	Drosophila protein Has homology to "death domain"	Necessary and sufficient for cell death in Drosophila development
FAP-1	Protein phosphatase Interacts with Fas/Apo-1	Inhibits Fas-mediated signaling pathway
HCP	Hematopoietic cell protein tyrosine phosphatase	Promotes Fas-mediated signaling pathway
Ras	Oncogene product	Appears to promote Fas-mediated signaling pathway
Ceramide	Fatty acid intermediate produced by shingomyelinase	Appears to promote Fas-mediated signaling pathway
CED-3	*C. elegans* gene product Resembles mammalian ICE protein	Promotes cell death in *C. elegans* development
ICE	Mammalian interleukin 1β–converting enzyme, related to C. Elegans Ced-3 gene product Cyteine protease that cleaves after Asp residues May cleave and activate related cysteine protease CPP32	May play role in Fas-mediated death pathway
Nedd2/ICH-1	Cysteine protease of the ICE family	
ICH-2	Cysteine protease of the ICE family	
CPP32/prICE/ Yama/Apopain	Cysteine protease of the ICE family Cleaves PARP	Key protease in Fas-mediated death pathway
PARP	Poly(ADP-ribose)polymerase Substrate of CPP32 protease Cleaved during apoptosis	
Fragmentin 2/ GranzymeB	Cytotoxic granule protein Protease with Asp specificity Causes cleavage of CPP32	Promotes cell death
CrmA	Cowpox encoded ICE-inhibitor Inhibits PARP cleavage	Inhibits Fas-mediated death pathway
CED-9	*C. elegans* gene product Negative regulator of Ced-3	Inhibits cell death
Bcl-2	Mammalian homolog of Ced-9 "Rheostat" control of cell death	Partially protects cells from Fas-mediated apoptosis
BAG-1	Binds to Bcl-2	With Bcl-2, protects cells from Fas-mediated apoptosis
Bcl-X_L	Protein related to Bcl-2	Protects cells from Fas-mediated apoptosis
E1B 19K	Adenovirus protein similar to Bcl-2	Protects cells from Fas-mediated apoptosis
p35	Insect baculovirus protein Forms stable complex with CED-3 family members	Protects cells from Fas-mediated apoptosis

signal transduction cascade; at the very least, the behavior of these proteins make them interesting markers of Fas signaling events.

Finally, as mentioned above, the nature and degree of T- and B-cell activation can modulate cellular responses to Fas/APO-1. While the Bcl-2 "rheostat" may control part of the response, most of the signals controlling the decision are unknown. Identification of other molecules whose activity or subcellular localization changes with anti-Fas treatment will provide a panel of candidates that can be tested for their ability to modulate the Fas/APO-1 signal.

IS THERE A COMMON SIGNALING PATHWAY FOR FAS/APO-1 AND TNF-R1?

The extent to which the Fas/APO-1 and TNF-R1 pathways overlap remains an open question. There are several similarities between signals generated by anti-Fas and TNF. Cell deaths caused by these treatments occur with indistinguishable kinetics and morphology, a similarity which accounts for the initial proposal that TNF's cytotoxicity is mediated by Fas (7). Other studies have shown that cell death signaled by both Fas/APO-1 and TNF-R1 can be blocked by a number of proteins, including Bcl-2 (100), Bcl-X_L (74), p35 (106), E1B 19K protein (104), and the ICE inhibitor CrmA (90).

However, several pieces of evidence argue against a shared pathway. Chimeric proteins consisting of the extracellular domain of CD40 fused to the cytoplasmic domain of either Fas/APO-1 or TNF-R1 are both capable of inducing cell death in the presence of the ligand gp39 (as can the cytoplasmically unrelated TNF-R2), but these chimeras kill with different kinetics (40). Moreover, conditions have been described under which cell death via Fas/APO-1 can be inhibited while cell death via TNF-R1 is unaffected (63,112).

At the very least, TNF-R1 signaling is likely to have several cellular effects, such as NFκB activation, that are not shared by Fas. It is possible that FAS/APO-1 and TNF-R share elements of the same pathway, but engage them with different efficiency. For example, the affinity of Fas/APO-1 or TNF-R1 for a potent death-inducing intermediate may differ. Alternatively, regions outside the death domain may modify the strength of the apoptotic signal; the inhibitory C terminus of Fas is an example of such a modifier domain.

CONCLUSIONS

The Fas/APO-1 receptor plays a central role in mammalian tolerance to self-antigen and cell-mediated cytotoxicity. Over the past year, several studies have elucidated aspects of Fas/APO-1 signaling which make it possible to draw a speculative model for the Fas/APO-1 pathway (Fig. 2). Following ligand binding, receptor signals are first generated by the aggregation of the Fas death domain. A number of cytoplasmic death domain-containing proteins have been identified which may associate with Fas to form a signaling-competent complex; phosphorylation and dephosphorylation events modulate signals transmitted by the active complex. Further downstream, signals engage a member of the CED-3 family of cysteine proteases, possibly the CPP32 protease; a number of cellular and viral proteins are able to block cell death at this point. It is still unknown whether the protease itself catalyzes all additional reactions necessary for cell death or whether it activates additional signaling intermediates.

By analogy with the signaling processes governing proliferation and differentiation, the signal for programmed cell death seem to converge on an evolutionarily conserved set of reactions. Thus, the details of programmed cell death regulation in other organisms are relevant to Fas/APO-1 signaling. Many unresolved issues remain: (i) which, if any, of the known Fas-interacting proteins are used to transduce Fas/APO-1 signals, and how does signal modification occur, (ii) how do these signals lead to the activation of the protease, and (iii) how does the protease induce cell death? Answers to these questions will permit not only a better understanding of this basic biological process, but also the prospect of developing therapeutic agents for those conditions in which programmed cell death plays a role.

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