Transpl Int (2000) 13 [Suppl 1]: S3–S6

I. Condo
R. Testi

Intracellular mediators of programmed cell death initiated at the cell surface receptor Fas

I. Condo, R. Testi (✉)
Department of Experimental Medicine and Biochemical Sciences,
University of Rome "Tor Vergata",
I-00133 Rome, Italy
e-mail: tesrob@flashnet.it,
Fax: + 39–06–72596505

Abstract Apoptosis is a programmed cell death process, which plays a pivotal role in development, in tissue homeostasis and in several human diseases. Fas (CD95/Apo-1) is a member of the "death receptors" family, a group of cell surface proteins that trigger apoptosis upon binding with their natural ligands. In the immune system, intracellular signal transduction triggered from Fas splits into two different pathways. The proteolytic pathway is mediated by a family of cysteine proteases, the caspases, responsible for the morphological changes occurring in the apoptotic process. To complete this death program, another series of events, involving a lipid pathway, is necessary. Upon Fas stimulation, a sequential activation of specific enzymes results in the accumulation of ceramides and GD3 ganglioside. GD3 directly induces mitochondrial damage and triggers the release of apoptogenic factors, allowing efficient execution of Fas-mediated apoptosis.

Key words Apoptosis · Fas · Mitochondria · GD3 ganglioside

Introduction

Programmed cell death, or apoptosis, is a tightly regulated process which occurs at various physiological stages in the life cycle of multicellular organisms. Morphologically, apoptosis is characterized by several changes including cell shrinkage, membrane blebbing, condensation of nuclear chromatin, fragmentation of chromosomal DNA and formation of membrane-enclosed vesicles, called apoptotic bodies. Eventually apoptotic bodies are recognized and phagocytized by neighboring cells to prevent inflammatory or immune reactions.

This type of cell death differs from necrosis, which results in cell lysis followed by release of the cytoplasmic content, which triggers an inflammatory response. Programmed cell death plays a key role both in development and in homeostasis [12], being the most common way to eliminate unwanted or harmful cells. Aberration or disregulation of this process is the cause of many human disorders such as cancer, autoimmunity, and neurodegenerative diseases [28, 37].

Death receptors as mediators of apoptosis

The apoptotic process initiated from surface receptors may be divided into three stages:

1. Interaction of the specific signal with the cell
2. Signal transduction within the cell, and
3. Execution of the death program

Mammals have evolved a family of surface receptor proteins, the so-called "death receptors", that trigger programmed cell death upon binding with their natural ligands [1]. Members of this family, such as Fas (CD95/APO-1), TNF-R1, DR3 (APO-3), DR4 (TRAIL-R1) and DR5 (TRAIL-R2), are transmembrane proteins sharing structural and functional homology. Each death receptor contains cystein-rich domains in the extracellular portion and a motif, termed "death domain", in the cytoplasmic region. Death domains are involved in homophilic and eterophilic protein-protein interactions. Associations between death domains occur upon recep-

tor-ligand binding, and this interaction is necessary and sufficient for apoptosis triggering [11, 35].

The best characterized death receptor is Fas, a cell surface protein widely expressed on both lymphoid and nonlymphoid cells [24]. Its natural ligand is FasL [32], a homotrimeric molecule that belongs to the TNF family of cytokines. Triggering of Fas plays an important role in several physiological and pathological processes [23], such as killing by cytotoxic T cells and by natural killer cells, controlling T cell numbers during the immune response, and maintenance of immune privilege in organs such as the eye, the placenta and the testis. Fas is also implicated in the failure of tumor cell clearance [15] and in autoimmune diseases, such as Hashimoto's thyroiditis [9] or autoimmune diabetes [31].

Signal transduction of Fas-mediated apoptosis

Stimulation of Fas occurs after binding with atrimeric FasL molecule and leads to crosslinking of three receptor molecules. This results in the clustering of intracellular death domains, inducing the recruitment of two signaling proteins that, together with Fas, form the so-called "death-inducing signaling complex" (DISC) [21]. One member of the DISC is the adapter protein FADD (Fas-associated death domain). This protein is directly recruited through binding of its own death domain to the death domain of Fas. Evidence for a key role of FADD in Fas-induced apoptosis comes from both knockout [39, 40] and transgenic mice [25]: deletion of the wild type genes or expression of a dominant negative mutant of FADD leads to complete resistance upon Fas stimulation. FADD also contains a "death effector domain", another motif involved in homophilic and eterophilic interactions between proteins.

Upon Fas crosslinking, the death effector domain of FADD allows the recruitment of procaspase-8, the second signaling protein of DISC. Procaspase-8 binds to FADD through its amino-terminal death effector domains, and undergoes oligomerization. Procaspase-8 is the zymogen form of caspase-8 (FLICE/MACH) [3], which is an enzyme belonging to the family of caspases [38], cysteine proteases that specifically cleave substrates after aspartic acid residues. Recruitment to the DISC and oligomerization then trigger maturation of the proenzyme into the catalytic active form. Active caspase-8 is in fact generated proteolytically by a self-cleavage reaction, which leads to release of the active subunits into cytosol [22].

Following DISC formation and caspase-8 activation, Fas signaling splits into two different pathways, both dependent on caspase-8 activation. One branch is the proteolytic pathway mediated by the caspases cascade, while a second pathway involves mediators of a lipidic nature (Fig. 1).

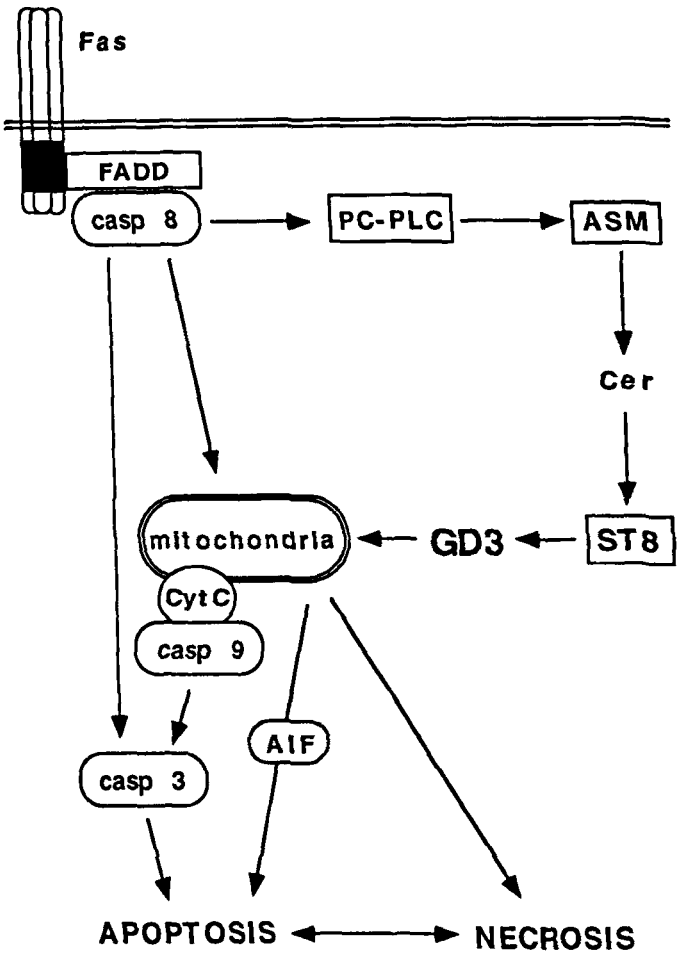

Fig. 1 Schematic representation of CD95-induced signaling (*ASM* acidic sphingomyelinaser, *PC-PLC* phosphatidylcholine-specific phospholipase C, *CytC* cytochrome C, *casp* caspase, *Cer* ceramide; *AIF* apoptosis inducing factor, *ST8* α-2,8-sialyltransferase)

The caspases pathway

Presently, 14 members of the caspase family have been described. Caspases can be classified as initiators or effectors, depending on their relative role in the apoptotic process. Initiator caspases, for example caspase-8, are involved in signaling and regulation of pro-apoptotic stimuli, while effector members function in the execution of cell death, through ordinated disassembly of cell structures [38]. Genetic and biochemical evidence supports a cascade model for caspase activation in Fas-mediated apoptosis. Downstream caspase-8 activation, the proteolytic pathway, has been shown to involve activation of effector caspases such as caspase-3, caspase-6 and caspase-7. Notably, caspase-3 plays a fundamental role in the execution of programmed cell death, as demonstrated by knockout mice having lethal defects in the extensive apoptosis that occurs during brain development [16]. Caspase cascade then leads to cleavage of

key cellular substrates, mainly resulting in the activation of pro-apoptotic proteins and in the inactivation of anti-apoptotic and structural proteins.

Besides activation of executioner procaspases, caspase-8 directly cleaves a cytosolic protein, called Bid, a pro-apoptotic member of the Bcl-2 family [27]. Caspase-8 cleavage generates a truncated form of Bid, which translocates from the cytosol to mitochondrial membranes [17, 20], triggering mitochondrial inner transmembrane potential ($\Delta\Psi m$) collapse, the opening of a large conductance channel, known as mitochondrial permeability transition pore (PTPC), and cytochrome *c* release. Mitochondrial changes are often associated with the release of apoptosis-inducing factor (AIF) [34] and the release and activation of mitochondrial caspases [33], which ultimately trigger the fragmentation of chromosomal DNA.

The lipid pathway

Another series of events, involving sphingomyelin hydrolysis and ceramide accumulation, is required for the progression of apoptosis induced by Fas-FasL interaction. Sphingomyelin is a major phospholipid of cellular membranes [18]; hydrolysis of this molecule is catalyzed by specific phospholipases, the sphingomyelinases, which generate phosphocoline and ceramide [30, 36]. After Fas crosslinking, two different sphingomyelinases are activated within the first 15 min [4, 5]: a neutral sphingomyelinase (NSM) located at the cytoplasmatic membrane [13] and an acidic sphingomyelinase [ASM] mainly located in acidic membrane subdomains [19]. ASM also requires the upstream activation of PC-PLC, a phosphatidylcholine-specific phospholipase C. However, DISC formation is required only for ASM activation and not for NSM, as demonstrated in Fas-resistant cellular mutants expressing death domain-defective receptors [5]. The sequential PC-PLC/ASM activation leads to the accumulation of ceramide, a diffusible messenger sufficient to induce programmed cell death in hematopoietic cells [10, 14, 26]. The key role of ASM-derived ceramide is also demonstrated in cells from individuals affected by Niemann-Pick disease (NPD), genetically deficient in ASM activity, which do not undergo normal Fas-mediated apoptosis. In these cells, an efficient Fas-dependent apoptotic program can be reconstituted by direct replacement of ASM [7].

ASM-derived ceramide is then targeted to the Golgi apparatus and induces neosynthesis of the disialoganglioside GD3 [6], a glycosphigolipid containing two sialic acid residues. GD3 ganglioside is able to induce $\Delta\Psi m$ loss and apoptosis in intact cells [6], whereas other gangliosides do not. Moreover, overexpression of GD3 synthase (α-2, 8-sialyltransferase or ST8), which catalyzes the synthesis of GD3 from GM3 ganglioside, can directly trigger cell death, while suppression of endogenous ST8 substantially prevents ceramide- and Fas-mediated apoptosis [6]. GD3 accumulation is impaired in ASM-deficient cells from NPD patients, but reconstitution of ASM activity in these cells restores both GD3 neosynthesis and optimal Fas-dependent apoptosis [7], indicating that ASM-derived ceramide is utilized for biosynthesis of GD3. Neosynthesized GD3 ganglioside then localizes within mitochondrial membranes, where it is able to induce the opening of PTPC, leading to release of both cytochrome *c* and AIF, and consequent DNA fragmentation [29].

Conclusions

Accumulation of sphingomyelin-derived ceramides during acute stress responses occurs in simple organisms that lack caspases or bcl-2 family members [2, 8]. Since lipid and glycolipid mediators travel almost exclusively within cellular membranes, membrane-directed delivery of death messages through the lipid pathway may represent an evolutionary ancient mechanism for the recruitment of mitochondria to the cell death program.

In higher organisms, death receptor signaling involves apoptotic effectors of both proteic and lipidic nature, which converge at the mitochondrial level. Similarly to proteic inducers of mitochondrial permeability transition, GD3 ganglioside likely acts at the level of PTPC to activate post-mitochondrial caspases and cell dismantling.

Thus, different pathways, using mediators of different nature, simultaneously converge on the mitochondria to irreversibly commit the cell to apoptosis.

References

1. Ashkenazi A, Dixit VM (1998) Death receptors: signaling and modulation. Science 281: 1305–1308
2. Basu S, Kolesnick R (1998) Stress signals for apoptosis: ceramide and c-Jun kinase. Oncogene 17: 3277–3285
3. Boldin MP, Goncharov TM, Goltsev YV, 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
4. Cifone MG, De Maria R, Roncaioli P, Rippo MR, Azuma M, Lanier LL, Santoni A, Testi R (1994) Apoptotic signaling through CD95 (Fas/APO-1) activates an acidic sphingomyelinase. J Exp Med 180: 1547–1552

5. Cifone MG, Roncaioli P, De Maria R, Camarda G, Santoni A, Ruberti G, Testi R (1995) Multiple signaling originate at the Fas/Apo-1 (CD95) receptor: sequential involvement of phosphatidylcholine-specific phospholipase C and acidic sphingomyelinase in the propagation of the apoptotic signal. EMBO J 14: 5859–5868
6. De Maria R, Lenti L, Malisan F, d'Agostino F, Tomassini B, Zeuner A, Rippo MR, Testi R (1997) Requirement for GD3 ganglioside in CD95- and ceramide-induced apoptosis. Science 277: 1652–1655
7. De Maria R, Rippo M, Schuchman E, Testi R (1998) Acidic sphingomyelinase (ASM) is necessary for Fas-induced GD3 ganglioside accumulation and efficient apoptosis of lymphoid cells. J Exp Med 187: 897–902
8. Fraser A, James C (1998) Fermenting debate: do yeast undergo apoptosis. Trends Cell Biol 8: 219–221
9. Giordano C, Stassi G, De Maria R, Todaro M, Richiusa P, Papoff G, Ruberti G, Bagnasco M, Testi R, Galluzzo A (1997) Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. Science 275: 960–963
10. Hannun YA (1996) Functions of ceramide in coordinating cellular responses to stress. Science 274: 1855–1859
11. Itoh N, Nagata S (1993) A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. J Biol Chem 268: 10932–10937
12. Jacobson MD, Weil M, Raff MC (1997) Programmed cell death in animal development. Cell 88: 347–354
13. Jayadev S, Linardic CM, Hannun YA (1994) Identification of arachidonic acid as a mediator of sphingomyelin hydrolysis in response to tumor necrosis factor. J Biol Chem 269: 5757–5763
14. Kolesnick R, Fuks Z (1995) Ceramide: a signal for apoptosis or mitogenesis? J Exp Med 181: 1949–1952
15. Krammer PH, Dhein J, Walczak H, Berhmann I, Mariani S, Matiba B, Fath M, Daniel P, Knipping E, Westendorp MO, Stricker K, Bäumler C, Hellbardt S, Germer M, Peter ME, Debatin K-M (1994) The role of APO-1-mediated apoptosis in the immune system. Immunol Rev 142: 175–191
16. Kuida K, Zheng TS, Na S, Kuan C-Y, Yang D, Karasuyama H, Rakic P, Flavell RA (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. Nature 384: 368–372
17. Li H, Zhu H, Xu CJ, Yuan J (1998) Cleavage of Bid by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94: 491–501
18. Linardic CM, Hannun YA (1994) Identification of a distinct pool of sphingomyelin in the sphingomyelin cycle. J Biol Chem 269: 23530–23537
19. Liu P, Anderson RGW (1995) Compartmentalized production of ceramide at the cell surface. J Biol Chem 270: 27179–27185
20. Luo X, Budihardjo I, Zou H, Slaughter C, 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
21. Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME (1997) FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 16: 2794–2804
22. Muzio M, Stockwell BR, Stennicke HR, Salvesen GS, Dixit VM (1998) An induced proximity model for caspase-8 activation. J Biol Chem 273: 2926–2930
23. Nagata S (1997) Apoptosis by death factor. Cell 88: 355–365
24. Nagata S, Golstein P (1995) The Fas death factor. Science 267: 1449–1456
25. Newton K, Harris AW, Bath ML, Smith KGC, 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
26. Obeid LM, Linardic CM, Karolak LA, Hannun YA (1993) Programmed cell death induced by ceramide. Science 259: 1769–1771
27. Reed JC (1997) Double identity for proteins of the Bcl-2 family. Nature 387: 773–776
28. Reed JC (1999) Mechanisms of apoptosis avoidance in cancer. Curr Opin Oncol 11: 68–75
29. Rippo MR, Malisan F, Ravagnan L, Condo' I, Tomassini B, Costantini P, Todaro M, Susin SA, Rufini A, Kroemer G, Testi R GD3 ganglioside directly targets mitochondria and induces the release of apoptogenic factors. Submitted
30. Spence MW (1993) Sphingomyelinases. Adv Lipid Res 26: 3–23
31. Stassi G, De Maria R, Trucco G, Rudert W, Testi R, Galluzzo A, Giordano C, Trucco M (1997) Nitric oxide primes pancreatic β cells for Fas-mediated destruction in insulin-dependent diabetes mellitus. J Exp Med 186: 1193–1200
32. Suda T, Takahashi T, Golstein P, Nagata S (1993) Molecular cloning and expression of the Fas ligand, a novel member of the Tumor Necrosis Factor family. Cell 75: 1169–1178
33. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Brenner C, Larochette N, Prevost MC, Alzari PM, Kroemer G (1999) Mitochondrial release of caspase-2 and -9 during the apoptotic process. J Exp Med 189: 381–394
34. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397: 441–446
35. Tartaglia LA, Ayres TM, Wong GH, Goeddel DV (1993) A novel domain within the 55 kD TNF receptor signals cell death. Cell 74: 845–853
36. Testi R (1996) Sphingomyelin breakdown and cell fate. Trends Biochem Sci 21: 468–471
37. Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267: 1456–1462
38. Thornberry N, Lazebnik Y (1998) Caspases: enemies within. Science 281: 1312–1316
39. Yeh WC, Pompa JL, McCurrach ME, Shu HB, Elia AJ, Shahinian A, Ng M, Wakeham A, Khoo W, Mitchell K, El-Deiry WS, Lowe SW, Goeddel DV, Mak TW (1998) FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science 279: 1954–1958
40. Zhang J, Cado D, Chen A, Kabra NH, Winoto A (1998) Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. Nature 92: 296–300