International Journal of Surgery (2005) 3, 268–277

INTERNATIONAL JOURNAL OF SURGERY

www.int-journal-surgery.com

REVIEW

An introduction to death receptors in apoptosis

R. Kumar*, P.E. Herbert, A.N. Warrens

Department of Immunology, Hammersmith Hospital, Imperial College of Science, Technology and Medicine, Du Cane Road, London W12 0NN, UK

KEYWORDS
Apoptosis;
Death receptors;
TRAIL;
FasL

Abstract Apoptosis is a source of much research interest across many fields, including developmental biology, immunology and oncology. As the exact pathways of this process are identified, so too are potential avenues for therapeutic application. Death receptors are important in inducing apoptosis and together with their ligands have become a source of attention as potential therapeutic agents. This review provides an introduction to the role of death receptors in apoptosis, together with a look at possible areas where this information may be applied therapeutically.

Apoptosis, or programmed cell death, is the ultimate fate of many of our cells. Apoptosis is distinct from necrosis in that the former is a regulated active process, while the latter is not. From embryogenesis and beyond, apoptosis has a crucial role in maintaining normal cell turnover and in preventing the propagation of damaged or dangerous cells. The importance of apoptosis is evident by the array of conditions caused by disorders in apoptosis; certain forms of cancer, autoimmune disease, and neurodegenerative diseases where, rather than a failure in apoptosis, there is an excess of it.[1] Aside from programmed cell death, cells may also commit suicide in response to external noxious stimuli that result in damage.

* Corresponding author.
E-mail address: rajesh357@yahoo.com (R. Kumar).

Mechanisms of apoptosis

Caspases (cysteine-dependent aspartate-specific proteases) are the means by which apoptosis is effected (caspase-independent apoptosis is described, but will not be discussed here). Two major mechanisms exist by which caspases are activated: the 'intrinsic' and 'extrinsic' pathways.[2] The intrinsic pathway is activated by mitochondrial disruption with subsequent cytochrome c release. Initiators of this pathway include UV irradiation and cytotoxic drugs.[1] An 'apoptosome' is formed by the interaction of cytochrome c, Apaf-1, d-ATP/ATP and procaspase-9 with subsequent initiation of the caspase cascade.[3,4] This pathway is regulated by members of the Bcl-2 family.[5–7]

The extrinsic pathway involves the binding of ligands to cell surface 'death receptors' (DR) which

1743-9191/$ - see front matter
doi:10.1016/j.ijsu.2005.05.002

in turn initiates the caspase cascade.[8] Death receptors are part of the tumour necrosis factor (TNF) gene superfamily and provide a rapid and efficient route to apoptosis. The characteristics of death receptors are cysteine-rich extracellular domains and an intracellular cytoplasmic sequence known as the 'death domain'.[9,10] The death receptors best described to date are listed in Table 1 together with their various synonyms. In bold are the terms that will be used throughout this article.

The molecular pathways by which TNFR1 and Fas effect apoptosis have been best characterized, with the role of TRAIL receiving greater attention more recently and so these will be considered in further detail later. Fundamentally, the death receptors share a similar means by which the caspase enzymatic cascade is activated and this is discussed below.

Death receptors are type I transmembrane proteins and it is their intracytoplasmic death domain (DD) component that is essential for signal transduction and apoptosis to occur.[10,25] Upon binding its cognate ligand, Fas Ligand, the Fas receptor undergoes trimerisation, thus encouraging the binding of the adaptor protein FADD (Fas-associated DD protein, alternatively known as mediator of receptor-induced toxicity (MORT1)).[26,27] FADD not only has a DD, but also another domain known as the death effector domain (DED) which allows it to bind the DED of procaspase-8.[27] This aggregation of FADD and the initiator procaspase-8 is known as a death-inducing signaling complex (DISC).[28] Further recruitment of multiple procaspase-8 zymogen molecules results in cross-activation and autocatalysis with active caspase-8 generated and released into the cytoplasm.[29–31]

The other death receptors activate caspase-8 in a similar fashion with TRAIL-R1 and TRAIL-R2 directly binding FADD, although the TNF–TNFR1 complex utilises an extra intermediary. Following binding of TNF to TNFR1 and trimerisation, the adaptor molecule TRADD (TNFR-associated death domain) is recruited to facilitate binding of FADD to the receptor complex[32] with subsequent recruitment of procaspase-8 as before. A further difference between Fas- and TNFR1-mediated signaling is the ability of TRADD to recruit secondary adaptors such as RIP (a serine–threonine kinase receptor-interacting protein) and TRAF2 (TNF receptor-associated factor 2).[33] These, respectively, activate the NF-κB and JNK/AP-1 survival pathways,[33] i.e. can negate the apoptotic signal and ensure cell survival. A similarity between Fas and TRAIL systems is their ability to activate both the extrinsic and intrinsic apoptosis pathways. This occurs from the cleavage by procaspase-8 of the molecule Bid (a pro-death member of the Bcl-2 gene family).[34–38] The active part of the cleaved Bid translocates into mitochondria, binds Bax or Bak (pro-apoptotic members of the Bcl-2 family[39,40]) with resultant mitochondrial fragmentation, cytochrome c release and apoptosome formation as before.

Bid, therefore, acts as a bridge between the extrinsic and intrinsic pathways. In certain cells (known as type 1) activation of caspase-8 is sufficient to enable apoptosis to occur through its downstream activation of effector caspases-3 and -6. Other cells (termed type II) are less able to form the DISC and the extrinsic pathway requires amplification via the mitochondrial pathway, and it is here the role of Bid as a link between the pathways is relevant.[41]

Clearly, uncontrolled death receptor activation and apoptosis would be disastrous for an organism. To prevent this, signaling of these receptors is regulated in several ways, including anti-apoptotic proteins within cells, and the activation of transcription factors (Fig. 1).

Table 1 Summary of the best characterized death receptors and their cognate ligand

Activating ligand	Death receptor
TNF	**TNFR1**/DR1/CD120a/p55[11]
FasL/CD95L	**Fas**/CD95/Apo1/DR2[12,13]
Apo3L/TWEAK	**DR3**/Apo3/WSL-1/TRAMP/LARD[13–17]
TRAIL/Apo2L	**TRAIL-R1**/DR4[18]
	TRAIL-R2/DR5/Apo-2/TRAILCK2/KILLER[19–23]
TRADD	DR6[24]

Death and decoy receptors

Control of activation of any receptor may be restricted by restricting the expression of the receptor or its respective ligand. TNFR1, Fas, TRAIL-R1 and TRAIL-R2 are expressed in a wide variety of tissues while the tissue distribution of TNF and FasL is more limited: TNF is expressed mainly by activated T cells and macrophages, and FasL by cytotoxic T cells, NK cells and antigen presenting cells. By contrast TRAIL is constitutively expressed in a wide range of human cells.[8,11,17,35,42–46]

TNF, FasL and TRAIL all exist as both soluble and membrane-bound forms. The relative functions of each are still being elucidated. Cleavage of the membrane-bound forms of TNF and FasL by metalloproteinases to the soluble form may

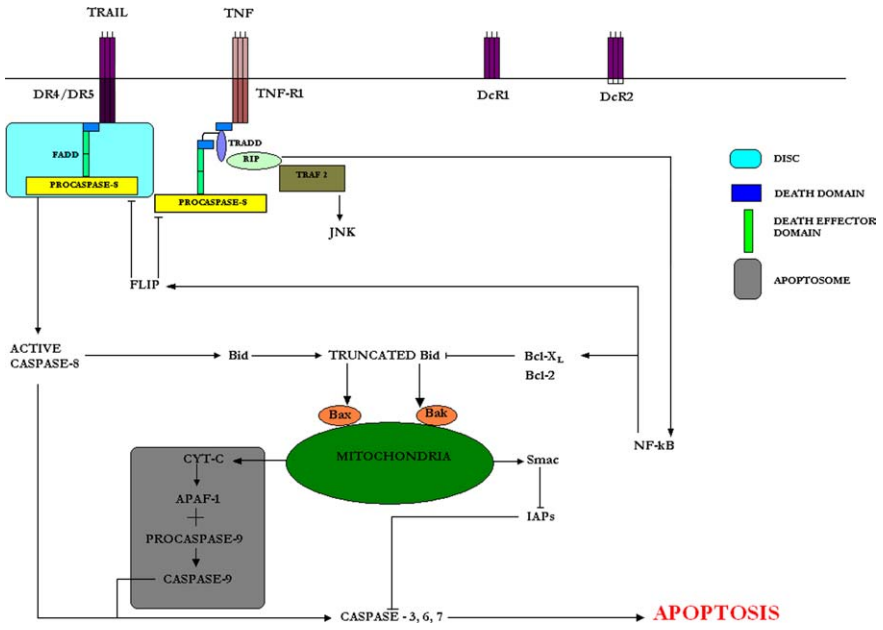

Figure 1 Simplified overview of events following death receptor activation.

represent a method of control since the soluble forms are less effective at initiating apoptosis.[47]

The wide range of cells on which both TRAIL and its death receptors are expressed (by contrast to TNF and FasL) implies that cells must employ mechanisms to protect themselves from unwanted self-destruction or death from neighbouring cells. One mechanism is the use of decoy receptors. DcR1 and DcR2 are cell surface receptors which bind TRAIL with high affinity, but are incapable of conveying an appropriate intracellular signal to induce apoptosis. DcR1 and DcR2 are structurally similar to TRAIL-R1 and TRAIL-R2, respectively, but the former has no cytoplasmic tail,[18,21,48–51] while the latter has only a truncated, ineffective DD.[48,52,53] The differential expression of decoy and death receptors which competitively bind TRAIL may offer an explanation of the observation that TRAIL ligation is fatal to some cells and not others; specifically, most normal cells compared to many cancer cells are resistant to TRAIL-induced death. However, this cannot be the sole regulatory mechanism as many tumour lines remain vulnerable to TRAIL-induced apoptosis while expressing high levels of decoy receptor.[54] Fas too has a decoy receptor, DcR3,[55] whose overexpression inhibits Fas-induced apoptosis.

The regulation of death

Moving to regulatory mechanisms within the cell, the silencer of death domains (SODD) is an inhibitory protein acting on DDs. It acts on the DD of TNFR1 and DR3 to prevent unwarranted aggregation of DDs leading to ligand-independent apoptosis.[56] Similar proteins may exist for the other death receptors.

c-FLIP is the cellular inhibitor of caspase-8 that contains two DEDs but lacks an active site and can bind Fas or TRAIL-DISCs to prevent activation of caspase-8 or -10.[57]

The Bcl-2 family of proteins control the release of cytochrome c,[58] which itself is released upon mitochondrial disruption and occurs with the cleavage of Bid. Bid, Bax, and Bak are members of the Bcl-2 family and are pro-apoptotic, while Bcl-2 and Bcl-X_L inhibit cytochrome c release and are anti-apoptotic. It seems that anti-apoptotic molecules such as Bcl-X_L compete for binding with the activated form of Bid, and upon doing so prevent it activating Bax or Bak, thereby avoiding the mitochondrial release of cytochrome c.[59] The outcome of this competition between pro-apoptotic and anti-apoptotic molecules determines whether the signal for cell death is successful.

Caspases are initially synthesized in an inactive (procaspase) form and are activated by other caspases, or upon binding to the DISC, or apoptosome. Caspase regulation is also achieved by their interaction with inhibitors-of-apoptosis proteins (IAPs).[60] There are several human homologues — cIAP1, cIAP2, XIAP, NIAP, SURVIVIN and BRUCE. They act by binding to and inhibiting the activity of effector caspases-3, -6 and -7.[61] XIAP prevents activation of procaspase-9 by binding it while at

the apoptosome[62,63] and demonstrates inhibition of multiple effector caspases. The regulatory ability of IAPs is countered by the release of Smac/DIABLO from mitochondria, which sequesters IAPs to promote caspase activation.[64]

NF-κB is a transcription factor, the activation of which generates pro-survival signals.[65] It promotes the expression of c-FLIP, Bcl-X_L, XIAP, TRAF1 and TRAF2, whose anti-apoptotic roles have been mentioned above. There appears to be a differential ability in the capacity of the various death receptor ligands to induce NF-κB, with FasL being unable to do so, and TRAIL doing so to a lesser extent than TNF (perhaps explaining the stronger death-inducing capability of FasL compared to TNF).[66]

Pro-survival vs pro-death

Binding of ligands to cell surface death receptors and caspase activation is moderated by several anti-apoptotic mechanisms, some of which have already been described. As we have said, this is clearly necessary to circumvent widespread cell death within an organism, but apoptosis too is physiologically crucial, and failure here results in uncontrolled growth, neoplasia and immune disturbance. 'Constructive destruction' then is achieved by tipping the balance with the induced expression of death receptors and by caspase-mediated proteolysis of anti-apoptotic molecules. Caspases target the very molecules which mediate NF-κB activation as substrates,[67] together with inhibiting NF-κB itself and its products. In addition, both Bcl-2 and Bcl-X_L and IAPs are substrates for caspases.[60,68]

By understanding the mechanisms of apoptosis and the exact role of factors that either promote or antagonise this outcome, we may begin to influence this process in pathological states. Next, we will consider the physiologic role of death receptors and apoptosis in the immune system and in tumour surveillance before considering the therapeutic potential of these vital molecules.

Death receptors and ligands in the development of normal function of the immune system

Lymphocyte development, clonal expansion in response to a stimulus with subsequent control to maintain homeostasis, and appropriate receptor repertoire development all rely on death receptor-induced cell death. Immature T cells mature within the thymus and undergo T cell antigen receptor selection where death receptors are thought to be involved in inducing apoptosis in those cells which do not undergo appropriate T cell antigen receptor rearrangement.[69] Fas is thought to have a role in the peripheral deletion of T cells occurring during T cell development.[70,71] Following an immune response resulting in clonal expansion of antigen-specific T cells there needs to be a cull in the number of these cells to previous levels, and this occurs by 'activation-induced cell death' (AICD). AICD is mediated by Fas—FasL and TNF—TNFR interactions.[70,72—76]

As mentioned before, FasL is expressed on activated T cells and NK cells where its expression allows these cells to function within the immune system to eliminate infected cells, cancer cells and attack transplanted tissues which carry the Fas receptor.[35] FasL is also expressed within certain 'immune privileged' sites such as the eye, Sertoli cells of the testes, brain and placenta.[77,78]

Immune privileged sites are those that do not initiate an immune response when antigen is introduced. This is essential in a structure where inflammatory reactions could compromise the integrity and function of an organ. In the eye, several mechanisms to explain immune privilege have been proposed, including the ''blood-ocular barrier'' and direct lymphatic drainage. In addition, the cornea constitutively expresses functionally active FasL.[79] The Fas—FasL system can then cause apoptosis in invading Fas-positive inflammatory cells,[80] thus reducing the immune response. Indeed, it has been shown that a lack of FasL leads to damage during an immune response.[80,81]

FasL expression — role in graft protection

The association of FasL with immune privilege gave rise to the hope that the artificial expression of FasL on transplanted tissue might act as a local immunosuppressant, since invading activated lymphocytes express Fas, and would thus undergo apoptosis when they came into contact with the graft. However, some studies, but not all, demonstrated that islet cell grafts expressing FasL are rapidly destroyed, not by the classical lymphocyte-mediated rejection pathway, but rather by granulocytic destruction of the grafted cells.[82—84] Several groups have worked with the FasL molecule to establish if it could play a role in modifying the immune response to allografts. Particularly encouraging was work done on the eye.

In a corneal model of transplantation, FasL-positive grafts were accepted at a rate of 45—50%,

whereas FasL-negative grafts were rejected at around 100%.[85,86] However, experiments in organs other than the eye produced conflicting data, suggesting that FasL can be either immunoprotective or immunodestructive.[11,23,41]

There is some evidence that FasL is upregulated on non-haematopoietic cells in the context of inflammation[87,88] and work in our laboratory has investigated this further to show that it is upregulated on non-haematopoietic cells during the rejection of allogeneic skin. We have demonstrated that skin transplanted from FasL-defective mice is rejected at a more rapid rate than wild type skin. In part, this reflects the differential susceptibility of FasL-deficient and normal skin to the effector arm of the immune response. However, it is now clear that FasL's effects are not limited to the effector arm; it appears that the nature of the immune response generated if a graft has defective FasL is more vigorous than if FasL is present. Using bone marrow chimeras, in which skin is transplanted from FasL-defective mice which have been reconstituted with wild type marrow, or from wild type animals whose marrow has been reconstituted from a FasL-defective source, it appears that this effect on the afferent limb of the immune response is mediated by the presence of FasL on skin haematopoietic cells.

Current data suggest a potential future use for membrane-bound FasL in graft protection,[89] though its effects have still to be fully elucidated. One model developed by Swenson et al.,[90] used a viral gene transfer method to induce expression in allotransplanted rat kidneys. The successfully transfected kidneys showed a significantly prolonged survival time.

However, it is also worth mentioning the other side of the story, the role of FasL-expressing allospecific cytotoxic T-lymphocytes, which may bind to Fas expressed on transplanted organs, leading to apoptosis of cells within these tissues.[91] This leads to the suggestion that the down-regulation of FasL and TNF-alpha in allografts rendered tolerant by donor-specific transfusion plays a role in acute allograft rejection.[92] Other work has concluded that Fas-mediated cytotoxicity is not required for the rejection of murine nonvascularised heterotopic cardiac allografts.[93,94]

Death receptors and ligands in tumour surveillance and anticancer therapy

Murine experiments have demonstrated TRAIL expression on liver NK cells (NK cells playing an important role in the control of tumour spread), but not other NK cells, T cells or conventional T cells.[95] TRAIL, perforin and FasL together are responsible for NK cell-mediated inhibition of liver metastasis.[95] Blocking expression of either TRAIL or FasL with monoclonal antibodies prevents liver NK cell cytotoxicity in vitro, and increases hepatic metastases of several tumour cell lines.[95] Crucial to expression of TRAIL and FasL on NK cells is interferon-γ (IFN-γ).[96] Experiments in TRAIL-deficient mice have further substantiated the important role of TRAIL against tumour development and metastasis.[97]

Interferons are cytokines with important antitumour roles and it seems one of the ways they carry out their role is by using TRAIL as a mediator. IFN-γ induces TRAIL expression on cytotoxic T cells[98] and TRAIL expression is reduced on NK cells in mice deficient in IFN-γ.[95] $CD4^+$ T cell-dependent intraocular rejection of a particular tumour cell line does not require $CD8^+$ T cells, B cells, TNF, perforin, FasL, or NK cells, but is mediated by TRAIL-induced apoptosis and susceptibility to this killing is enhanced by IFN-γ.[99] Melanoma cells resistant to FasL have been shown to be susceptible to $CD4^+$ T cell killing mediated by TRAIL-induced apoptosis.[100]

Human cancers develop ways of evading host defence mechanisms and conventional therapies, e.g. by the loss of the p53 tumour suppressor gene.[101] p53 mainly initiates apoptosis via the intrinsic pathway and its intact function is required for many DNA-damaging drugs to be effective. Inactivation of p53 can lead to resistance. Death receptors offer a way around this by initiating apoptosis independent of p53, especially useful in cells already resistant to chemo- and radiotherapy.

The successful therapeutic administration of death receptor ligands or antibodies to these receptors in vivo, however, will depend on a differential effect on cancer cells compared to normal cells. Administering TNF causes a serious inflammatory response and systemic FasL causes hepatic apoptosis. TRAIL, however, has become an anticancer agent with promising potential. TRAIL appears to demonstrate the required ability of killing tumour cells while leaving normal cells alone,[102,103] and in addition induces apoptosis in a number of cancer cell lines regardless of their p53 status.[104] In addition, animal experiments have failed to show any serious adverse systemic effects of TRAIL administration.[102,103]

The exact mechanisms for the resistance of normal cells to TRAIL have yet to be fully elucidated, but the previously mentioned decoy receptors may play a part. The expression of DcR1 and DcR2 may confer protection against TRAIL and their overexpression is a possible mechanism for tumour evasion, with DcR1 increased in

gastrointestinal cancers.[105] High levels of FLIP have also been implicated in protecting normal cells[106] and overexpression in certain tumours and in melanoma results in TRAIL resistance.[107]

Given the host of anti-apoptotic mechanisms in place described earlier, there are a number of potential avenues for cancer cells to evade death and proliferate. Aside from FLIP, Bax is another target for inactivation often found in cancers with deficient DNA mismatch repair.[108,109] Upregulation of Bcl-X_L is another method.[110] Remembering that NF-κB orchestrates induction of proteins such as FLIP and Bcl-X_L, it is not unreasonable to expect TRAIL resistance to be found in tumours expressing high levels of NF-κB.[111]

Understanding such mechanisms of resistance also provides the possibility for sensitizing TRAIL-resistant cells, perhaps with NF-κB inhibitors,[112] by reducing FLIP expression, or with the use of chemotherapeutic agents to upregulate p53, TRAIL-R2 and Bak.[113] Such combinations may provide a synergistic effect on killing cancer cells. Other strategies under investigation include targeting Bcl-2,[114,115] XIAP,[116] Smac[117] and increasing APAF-1 expression.[118]

Promising results have been seen with human TRAIL-R1 monoclonal antibody (HGS-ETR1) experiments.[119] In vitro treatment of tumour cell lines enhanced the cytotoxic activity of chemotherapeutic agents, even where HGS-TR1 alone was not effective. Corresponding results were found in vivo with tumour regression or repression with HGS-TR1 treatment. Combination treatment with chemotherapy again produced enhanced anti-tumour activity.[119] Such potential against a broad range of malignancies is under further investigation and Phase II clinical trials of HGS-TR1 are underway in patients with non-small cell lung cancer, colorectal cancer and non-Hodgkin's lymphoma.

Aside from use of the recombinant ligand and agonist antibodies to the TRAIL death receptors, gene therapy may offer another treatment modality. High levels of transgene expression using an adenoviral vector expressing TRAIL and causing apoptosis in a variety of breast cancer cell lines have been demonstrated.[120] Gene therapy remains a novel technique and its application in this form may be restricted to local therapy.[121,122]

Bone metabolism, fracture and wound healing

Bone remodeling requires the resorptive actions of osteoclasts and the laying down of bone matrix by osteoblasts. Here too members of the TNF ligand family have a significant role to play. These include receptor activator of NF-κB ligand (RANKL), the receptor RANK and the soluble decoy receptor osteoprotogerin (OPG).

Animals injected with OPG, or transgenic mice overexpressing OPG are found to have increased bone mass[123,124] while OPG knockout mice show severe osteoporosis.[125] OPG, thus, appears to be important in inhibiting osteoclast bone resorption. Within bone, RANKL serves to stimulate mature osteoclast activity[126] and inhibit osteoclast apoptosis.[127]

Osteoclasts and osteoblasts arise from separate cell lineages, the former from haematopoietic precursors and the latter from mesenchymal stem cells. It is the interaction between RANKL found on osteoblasts and its receptor RANK, expressed on osteoclast precursors, that is essential for osteoclastogenesis. Together with OPG, RANKL and RANK play a crucial role in bone metabolism and are also involved in fracture healing.

In a mouse tibia fracture model, RANKL expression was strongly induced throughout the period of fracture healing, but was almost undetectable in non-fractured bone.[128] While OPG shows constitutive expression even in unfractured bone, it too is upregulated during fracture repair. Aside from fracture healing, an area where such knowledge may be applied is in structural musculoskeletal grafts. Bone grafts can often be harvested from allogeneic cadaveric donors, however, vascularised fresh autologous grafts are significantly better to graft, with improved healing and remodeling.[129,130] Allografts are lacking several factors important in angiogenesis and bone remodeling and RANKL has recently been demonstrated to be necessary for complete autograft healing.[131] Furthermore, recombinant adeno-associated virus, as a vector for RANKL, when applied to the surface of allografts showed significant remodeling and vascularisation, leading to a new bone collar around the graft.[131]

Unsurprisingly, given the inflammatory response that occurs with injury and the necessity for resolution of this response, apoptosis is of importance in wound healing too.[132,133] One of the factors mediating apoptosis and controlling the inflammatory response is transforming growth factor β (TGFβ).[134] The pro-apoptotic effects of TGFβ appear to involve caspases and its effects may be inhibited by Bcl-X_L.[135,136] TGFβ is under further investigation because of its differential expression in embryonic tissue compared to adult tissue. Skin wounds early in gestation on a mammalian fetus heal without scarring and the growth factor profile here is different to the adult

situation. With embryonic wounds there are low levels of TGFβ1 and TGFβ2, low levels of platelet-derived growth factor (PDGF) and high levels of TGFβ3.[137] Therapeutic manipulation of wounds in animals by neutralizing PDGF, TGFβ1 and TGFβ2, and adding exogenous TGFβ3 results in scar-free healing.[137] Pharmaceutical molecules based on these experiments have entered trials on wound healing in humans.[137]

Conclusion

The dynamic balance between death receptors and anti-apoptotic mechanisms is vital to regulated cell death, disturbance of which can be severely detrimental to an organism. As the exact molecular events are delineated and the players in this process identified, so too potential therapeutic avenues open up. The manipulation of death receptors and their ligands has possible future implications in a variety of areas, including the treatment of cancers and inflammatory disorders, bone and soft tissue healing and in transplant rejection.

References

1. MacFarlane M. TRAIL-induced signalling and apoptosis. *Toxicol Lett* 2003;**139**(2—3):89—97.
2. Bratton SB, et al. Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. *Exp Cell Res* 2000;**256**(1):27—33.
3. Cain K, et al. Caspase activation involves the formation of the aposome, a large (approximately 700 kDa) caspase-activating complex. *J Biol Chem* 1999;**274**(32):22686—92.
4. Zou H, et al. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J Biol Chem* 1999;**274**(17):11549—56.
5. Green DR, Reed JC. Mitochondria and apoptosis. *Science* 1998;**281**(5381):1309—12.
6. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 1999;**13**(15):1899—911.
7. Green DR. Apoptotic pathways: paper wraps stone blunts scissors. *Cell* 2000;**102**(1):1—4.
8. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science* 1998;**281**(5381):1305—8.
9. Brakebusch C, et al. Cytoplasmic truncation of the p55 tumour necrosis factor (TNF) receptor abolishes signalling, but not induced shedding of the receptor. *EMBO J* 1992; **11**(3):943—50.
10. Itoh N, Nagata S. A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. *J Biol Chem* 1993;**268**(15):10932—7.
11. Smith CA, Farrah T, Goodwin RG. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell* 1994;**76**(6):959—62.
12. Nagata S. Apoptosis by death factor. *Cell* 1997;**88**(3): 355—65.
13. Chinnaiyan AM, et al. FADD/MORT1 is a common mediator of CD95 (Fas/APO-1) and tumor necrosis factor receptor-induced apoptosis. *J Biol Chem* 1996;**271**(9): 4961—5.
14. Kitson J, et al. A death-domain-containing receptor that mediates apoptosis. *Nature* 1996;**384**(6607):372—5.
15. Marsters SA, et al. Apo-3, a new member of the tumor necrosis factor receptor family, contains a death domain and activates apoptosis and NF-kappa B. *Curr Biol* 1996; **6**(12):1669—76.
16. Bodmer JL, et al. TRAMP, a novel apoptosis-mediating receptor with sequence homology to tumor necrosis factor receptor 1 and Fas(Apo-1/CD95). *Immunity* 1997;**6**(1): 79—88.
17. Screaton GR, et al. LARD: a new lymphoid-specific death domain containing receptor regulated by alternative pre-mRNA splicing. *Proc Natl Acad Sci U S A* 1997;**94**(9): 4615—9.
18. Pan G, et al. The receptor for the cytotoxic ligand TRAIL. *Science* 1997;**276**(5309):111—3.
19. Pan G, et al. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* 1997; **277**(5327):815—8.
20. Screaton GR, et al. TRICK2, a new alternatively spliced receptor that transduces the cytotoxic signal from TRAIL. *Curr Biol* 1997;**7**(9):693—6.
21. Sheridan JP, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* 1997; **277**(5327):818—21.
22. Walczak H, et al. TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. *EMBO J* 1997;**16**(17):5386—97.
23. Wu GS, et al. KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. *Nat Genet* 1997;**17**(2): 141—3.
24. Pan G, et al. Identification and functional characterization of DR6, a novel death domain-containing TNF receptor. *FEBS Lett* 1998;**431**(3):351—6.
25. Tartaglia LA, et al. A novel domain within the 55 kd TNF receptor signals cell death. *Cell* 1993;**74**(5):845—53.
26. Boldin MP, et al. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J Biol Chem* 1995;**270**(14): 7795—8.
27. Chinnaiyan AM, et al. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 1995;**81**(4):505—12.
28. Medema JP, et al. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO J* 1997;**16**(10):2794—804.
29. Martin DA, et al. Membrane oligomerization and cleavage activates the caspase-8 (FLICE/MACHalpha1) death signal. *J Biol Chem* 1998;**273**(8):4345—9.
30. Muzio M, et al. An induced proximity model for caspase-8 activation. *J Biol Chem* 1998;**273**(5):2926—30.
31. Yang X, Chang HY, Baltimore D. Autoproteolytic activation of pro-caspases by oligomerization. *Mol Cell* 1998;**1**(2): 319—25.
32. Hsu H, et al. TRADD—TRAF2 and TRADD—FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 1996;**84**(2):299—308.
33. Yeh WC, et al. Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* 1997;**7**(5): 715—25.
34. Wang CY, Mayo MW, Baldwin Jr AS. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. *Science* 1996;**274**(5288):784—7.

35. Li JH, et al. The regulation of CD95 ligand expression and function in CTL. *J Immunol* 1998;**161**(8):3943–9.
36. Luo X, et al. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 1998; **94**(4):481–90.
37. Gross A, et al. Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. *J Biol Chem* 1999;**274**(2):1156–63.
38. Roy S, Nicholson DW. Cross-talk in cell death signaling. *J Exp Med* 2000;**192**(8):F21–5.
39. Eskes R, et al. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. *Mol Cell Biol* 2000;**20**(3):929–35.
40. Wei MC, et al. tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. *Genes Dev* 2000;**14**(16):2060–71.
41. Scaffidi C, et al. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J* 1998;**17**(6):1675–87.
42. Leithauser F, et al. Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. *Lab Invest* 1993;**69**(4):415–29.
43. Krammer PH. CD95's deadly mission in the immune system. *Nature* 2000;**407**(6805):789–95.
44. Golstein P. Cell death: TRAIL and its receptors. *Curr Biol* 1997;**7**(12):R750–3.
45. Jeremias I, et al. TRAIL/Apo-2-ligand-induced apoptosis in human T cells. *Eur J Immunol* 1998;**28**(1):143–52.
46. Martinez-Lorenzo MJ, et al. Involvement of APO2 ligand/ TRAIL in activation-induced death of Jurkat and human peripheral blood T cells. *Eur J Immunol* 1998; **28**(9):2714–25.
47. Nagata S. Fas ligand-induced apoptosis. *Annu Rev Genet* 1999;**33**:29–55.
48. Degli-Esposti MA, et al. Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J Exp Med* 1997;**186**(7):1165–70.
49. MacFarlane M, et al. Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL. *J Biol Chem* 1997;**272**(41):25417–20.
50. Mongkolsapaya J, et al. Lymphocyte inhibitor of TRAIL (TNF-related apoptosis-inducing ligand): a new receptor protecting lymphocytes from the death ligand TRAIL. *J Immunol* 1998;**160**(1):3–6.
51. Schneider P, et al. Characterization of two receptors for TRAIL. *FEBS Lett* 1997;**416**(3):329–34.
52. Marsters SA, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr Biol* 1997;**7**(12): 1003–6.
53. Pan G, et al. TRUNDD, a new member of the TRAIL receptor family that antagonizes TRAIL signalling. *FEBS Lett* 1998;**424**(1–2):41–5.
54. Griffith TS, Lynch DH. TRAIL: a molecule with multiple receptors and control mechanisms. *Curr Opin Immunol* 1998;**10**(5):559–63.
55. Pitti RM, et al. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. *Nature* 1998; **396**(6712):699–703.
56. Jiang Y, et al. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. *Science* 1999; **283**(5401):543–6.
57. Irmler M, et al. Inhibition of death receptor signals by cellular FLIP. *Nature* 1997;**388**(6638):190–5.
58. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science* 1998;**281**(5381):1322–6.
59. Cheng EH, et al. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. *Mol Cell* 2001;**8**(3):705–11.
60. Deveraux QL, Reed JC. IAP family proteins – suppressors of apoptosis. *Genes Dev* 1999;**13**(3):239–52.
61. Roy N, et al. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J* 1997; **16**(23):6914–25.
62. Bratton SB, et al. Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. *EMBO J* 2001;**20**(5):998–1009.
63. Srinivasula SM, et al. A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature* 2001;**410**(6824):112–6.
64. Du C, et al. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* 2000;**102**(1):33–42.
65. Karin M, et al. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2002;**2**(4): 301–10.
66. Almasan A, Ashkenazi A. Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy. *Cytokine Growth Factor Rev* 2003;**14**(3–4):337–48.
67. Lin Y, et al. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. *Genes Dev* 1999;**13**(19):2514–26.
68. Chen Y, et al. Mutations within a furin consensus sequence block proteolytic release of ectodysplasin-A and cause X-linked hypohidrotic ectodermal dysplasia. *Proc Natl Acad Sci U S A* 2001;**98**(13):7218–23.
69. Newton K, Strasser A. Ionizing radiation and chemotherapeutic drugs induce apoptosis in lymphocytes in the absence of Fas or FADD/MORT1 signaling. Implications for cancer therapy. *J Exp Med* 2000;**191**(1): 195–200.
70. Singer GG, Abbas AK. The fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity* 1994;**1**(5): 365–71.
71. Van Parijs L, Ibraghimov A, Abbas AK. The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. *Immunity* 1996;**4**(3):321–8.
72. Alderson MR, et al. Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* 1995; **181**(1):71–7.
73. Brunner T, et al. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* 1995;**373**(6513):441–4.
74. Dhein J, et al. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* 1995;**373**(6513):438–41.
75. Ju ST, et al. Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 1995;**373**(6513):444–8.
76. Zheng L, et al. Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* 1995;**377**(6547): 348–51.
77. Chen JJ, Sun Y, Nabel GJ. Regulation of the proinflammatory effects of Fas ligand (CD95L). *Science* 1998; **282**(5394):1714–7.
78. Nagata S, Golstein P. The Fas death factor. *Science* 1995; **267**(5203):1449–56.
79. Niederkorn JY. Immune privilege in the anterior chamber of the eye. *Crit Rev Immunol* 2002;**22**(1):13–46.
80. Griffith TS, et al. Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 1995;**270**(5239): 1189–92.

81. Griffith TS, et al. CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. *Immunity* 1996;**5**(1):7—16.
82. Kang SM, et al. Fas ligand expression in islets of Langerhans does not confer immune privilege and instead targets them for rapid destruction. *Nat Med* 1997;**3**(7): 738—43.
83. Kang SM, et al. Fas ligand expression on islets as well as multiple cell lines results in accelerated neutrophilic rejection. *Transplant Proc* 1998;**30**(2):538.
84. Allison J, et al. Transgenic expression of CD95 ligand on islet beta cells induces a granulocytic infiltration but does not confer immune privilege upon islet allografts. *Proc Natl Acad Sci U S A* 1997;**94**(8):3943—7.
85. Stuart PM, et al. CD95 ligand (FasL)-induced apoptosis is necessary for corneal allograft survival. *J Clin Invest* 1997; **99**(3):396—402.
86. Yamagami S, et al. Role of Fas—Fas ligand interactions in the immunorejection of allogeneic mouse corneal transplants. *Transplantation* 1997;**64**(8):1107—11.
87. Gochuico BR, et al. Airway epithelial Fas ligand expression: potential role in modulating bronchial inflammation. *Am J Physiol* 1998;**274**(3 Pt 1):L444—9.
88. Bonfoco E, et al. Inducible nonlymphoid expression of Fas ligand is responsible for superantigen-induced peripheral deletion of T cells. *Immunity* 1998;**9**(5):711—20.
89. Gregory MS, et al. Membrane Fas ligand activates innate immunity and terminates ocular immune privilege. *J Immunol* 2002;**169**(5):2727—35.
90. Swenson KM, et al. Fas ligand gene transfer to renal allografts in rats: effects on allograft survival. *Transplantation* 1998;**65**(2):155—60.
91. Tannapfel A, et al. Apoptosis and the expression of Fas and Fas ligand (FasL) antigen in rejection and reinfection in liver allograft specimens. *Transplantation* 1999;**67**(7):1079—83.
92. Josien R, et al. Fas ligand, tumor necrosis factor-alpha expression, and apoptosis during allograft rejection and tolerance. *Transplantation* 1998;**66**(7):887—93.
93. Krupnick AS, et al. Mechanism of T cell-mediated endothelial apoptosis. *Transplantation* 2002;**74**(6):871—6.
94. Djamali A, Odorico JS. Fas-mediated cytotoxicity is not required for rejection of murine nonvascularized heterotopic cardiac allografts. *Transplantation* 1998; **66**(12):1793—801.
95. Takeda K, et al. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nat Med* 2001;**7**(1): 94—100.
96. Kayagaki N, et al. Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: a novel mechanism for the antitumor effects of type I IFNs. *J Exp Med* 1999;**189**(9): 1451—60.
97. Cretney E, et al. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. *J Immunol* 2002;**168**(3):1356—61.
98. Ruiz-Ruiz C, Lopez-Rivas A. Mitochondria-dependent and -independent mechanisms in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis are both regulated by interferon-gamma in human breast tumour cells. *Biochem J* 2002;**365**(Pt 3): 825—32.
99. Wang S, et al. Role of TRAIL and IFN-gamma in CD4+ T cell-dependent tumor rejection in the anterior chamber of the eye. *J Immunol* 2003;**171**(6):2789—96.
100. Thomas WD, Hersey P. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in Fas ligand-resistant melanoma cells and mediates CD4 T cell killing of target cells. *J Immunol* 1998;**161**(5):2195—200.
101. Kaufmann SH, Earnshaw WC. Induction of apoptosis by cancer chemotherapy. *Exp Cell Res* 2000;**256**(1):42—9.
102. Ashkenazi A, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. *J Clin Invest* 1999; **104**(2):155—62.
103. Walczak H, et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat Med* 1999;**5**(2):157—63.
104. Wang S, El-Deiry WS. Requirement of p53 targets in chemosensitization of colonic carcinoma to death ligand therapy. *Proc Natl Acad Sci U S A* 2003;**100**(25): 15095—100.
105. Sheikh MS, et al. The antiapoptotic decoy receptor TRID/TRAIL-R3 is a p53-regulated DNA damage-inducible gene that is overexpressed in primary tumors of the gastrointestinal tract. *Oncogene* 1999;**18**(28):4153—9.
106. Kim K, et al. Molecular determinants of response to TRAIL in killing of normal and cancer cells. *Clin Cancer Res* 2000; **6**(2):335—46.
107. Bullani RR, et al. Selective expression of FLIP in malignant melanocytic skin lesions. *J Invest Dermatol* 2001;**117**(2): 360—4.
108. Meijerink JP, et al. Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. *Blood* 1998; **91**(8):2991—7.
109. Packham G, Stevenson FK. Bodyguards and assassins: Bcl-2 family proteins and apoptosis control in chronic lymphocytic leukaemia. *Immunology* 2005;**114**(4):441—9.
110. Burns TF, El-Deiry WS. Identification of inhibitors of TRAIL-induced death (ITIDs) in the TRAIL-sensitive colon carcinoma cell line SW480 using a genetic approach. *J Biol Chem* 2001;**276**(41):37879—86.
111. Ravi R, et al. Regulation of death receptor expression and TRAIL/Apo2L-induced apoptosis by NF-kappaB. *Nat Cell Biol* 2001;**3**(4):409—16.
112. Ravi R, Bedi A. Sensitization of tumor cells to Apo2 ligand/TRAIL-induced apoptosis by inhibition of casein kinase II. *Cancer Res* 2002;**62**(15):4180—5.
113. LeBlanc H, et al. Tumor-cell resistance to death receptor-induced apoptosis through mutational inactivation of the proapoptotic Bcl-2 homolog Bax. *Nat Med* 2002;**8**(3):274—81.
114. Jansen B, et al. Bcl-2 antisense therapy chemosensitizes human melanoma in SCID mice. *Nat Med* 1998;**4**(2): 232—4.
115. Banerjee D. Genasense (Genta Inc). *Curr Opin Investig Drugs* 2001;**2**(4):574—80.
116. Zhang XD, et al. Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of human melanoma is regulated by smac/DIABLO release from mitochondria. *Cancer Res* 2001;**61**(19):7339—48.
117. Fulda S, Meyer E, Debatin KM. Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression. *Oncogene* 2002;**21**(15): 2283—94.
118. Soengas MS, et al. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* 2001;**409**(6817): 207—11.
119. Pukac L, et al. HGS-ETR1, a fully human TRAIL-receptor 1 monoclonal antibody, induces cell death in multiple tumour types in vitro and in vivo. *Br J Cancer* 2005; **92**(8):1430—41.
120. Lin T, et al. Long-term tumor-free survival from treatment with the GFP-TRAIL fusion gene expressed from the hTERT promoter in breast cancer cells. *Oncogene* 2002;**21**(52): 8020—8.

121. McCormick F. Cancer gene therapy: fringe or cutting edge? *Nat Rev Cancer* 2001;**1**(2):130—41.
122. Armeanu S, et al. Adenoviral gene transfer of tumor necrosis factor-related apoptosis-inducing ligand overcomes an impaired response of hepatoma cells but causes severe apoptosis in primary human hepatocytes. *Cancer Res* 2003;**63**(10):2369—72.
123. Simonet WS, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997;**89**(2):309—19.
124. Yasuda H, et al. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. *Endocrinology* 1998;**139**(3):1329—37.
125. Bucay N, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998;**12**(9):1260—8.
126. Lacey DL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998;**93**(2):165—76.
127. Fuller K, et al. TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. *J Exp Med* 1998;**188**(5):997—1001.
128. Kon T, et al. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. *J Bone Miner Res* 2001;**16**(6):1004—14.
129. Garbuz DS, Masri BA, Czitrom AA. Biology of allografting. *Orthop Clin North Am* 1998;**29**(2):199—204.
130. Goldberg VM, Stevenson S. The biology of bone grafts. *Semin Arthroplasty* 1993;**4**(2):58—63.
131. Ito H, et al. Remodeling of cortical bone allografts mediated by adherent rAAV-RANKL and VEGF gene therapy. *Nat Med* 2005;**11**(3):291—7.
132. Greenhalgh DG. The role of apoptosis in wound healing. *Int J Biochem Cell Biol* 1998;**30**(9):1019—30.
133. Edwards M, Jones D. Programmed cell death in human acute cutaneous wounds. *J Cutan Pathol* 2001;**28**(3):151—5.
134. Shah M, Foreman DM, Ferguson MW. Control of scarring in adult wounds by neutralising antibody to transforming growth factor beta. *Lancet* 1992;**339**(8787):213—4.
135. Chipuk JE, et al. Bcl-xL blocks transforming growth factor-beta 1-induced apoptosis by inhibiting cytochrome c release and not by directly antagonizing Apaf-1-dependent caspase activation in prostate epithelial cells. *J Biol Chem* 2001;**276**(28):26614—21.
136. Kim SG, et al. Caspase-mediated Cdk2 activation is a critical step to execute transforming growth factor-beta1-induced apoptosis in human gastric cancer cells. *Oncogene* 2001;**20**(10):1254—65.
137. Ferguson MW, O'Kane S. Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. *Philos Trans R Soc Lond B Biol Sci* 2004;**359**(1445):839—50.