Mini Review

On the TRAIL to apoptosis

Tudor M. Baetu, John Hiscott*

Terry Fox Molecular Oncology Group, Lady Davis Institute for Medical Research, Jewish General Hospital and Department of Microbiology and Immunology, McGill University, 3755 Cote St. Catherine, Montreal, Que., Canada H3T 1E2

Abstract

Apoptosis in mammalian cells can be initiated through two major interrelated pathways, one involving engagement of the TNF family of death receptors, the other involving the release of cytochrome c from mitochondria. Unlike other members of the TNF ligand family, TNF-related apoptosis-inducing ligand (TRAIL) preferentially induces apoptosis in tumor cell lines, but not in normal cells, suggesting that TRAIL could potentially represent a powerful cancer therapeutic. Recent experiments have revealed that one of the key regulators of TRAIL expression in lymphocytes is the NF-kB transcription factors. Several TRAIL receptors have been identified: two of these receptors, TRAIL-R1/DR4 and TRAIL-R2/DR5, contain cytoplasmic death domains and signal apoptosis, while two other decoy receptors, TRAIL-R3/DcR1 and TRAIL-R4/DcR2 lack a functional death domain and do not mediate apoptosis. Many cancer cell lines preferentially express TRAIL-R1 and TRAIL-R2, suggesting differential regulation of the death and decoy receptors. Further knowledge of the regulation and physiological role of TRAIL and TRAIL receptors may aid in the rational design of regimens that utilize the TRAIL signaling pathway to eliminate tumor cells. © 2002 Published by Elsevier Science Ltd.

Keywords: TRAIL; Apoptosis; NF-κB; TNF

1. Overview of the major apoptotic pathways

Apoptosis, or programmed cell death, is a key mechanism allowing multicellular animals to tightly regulate cell growth, preventing pathological processes such as cancer, immunodeficiency and auto-reactivity. In mammalian cells, apoptosis can be initiated through two major pathways, one involving engagement of the death receptors, the other the release of cytochrome c from the mitochondria. Both pathways are triggered by external and internal cues, such as DNA damage. Pro- and anti-apoptotic members of the Bcl-2 family of proteins (Bcl-2 family) and pro- and anti-apoptotic proteins, are characterized by one or more conserved Bcl-2 homology (BH) domains and have been classified into three functional groups: members of group I, such as Bax and Bak, are pro-apoptotic; group II consists of diverse proteins whose only common feature is the presence of the BH3 domain [10–13]. In the event of apoptosis, cytochrome c is released from mitochondria, associates with Apaf-1 and caspase-9, leading eventually to the activation of caspase-9 [14–16].

The death receptor pathway is initiated by the TNF-α family of cytokines, such as TNF-α, Fas-ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), all of which can act as extracellular activators of apoptosis upon binding to their respective receptors [17,18]. These “death-inducing” receptors, which include TNF receptor 1 (TNFR1), Fas, TRAIL receptors 1 and 2 (TRAIL-R1 and TRAIL-R2), TNF receptor-related apoptosis-mediating protein (TRAMP) and death receptor 6 (DR6) harbor extracellular cysteine-rich domains as well as cytoplasmic death domains responsible for the recruitment of the death-inducing signaling complex through interaction with adaptor proteins such as Fas-associated death domain protein (FADD) [19–21] and TNFR1 associated death domain protein (TRADD) [22]. Upon binding of its ligand, TRNF1 recruits FADD through TRADD, while Fas recruits FADD directly. In turn, FADD recruits multiple caspase-8 molecules, resulting in caspase-8 activation through induced proximity [23,24]. Caspase-8 is thought to propagate the apoptosis signaling pathway either by direct processing and activation of caspase-3, or by cleaving Bid, a group III...
Fig. 1. Control of apoptosis by NF-xB. Apoptosis can be triggered upon engagement of such death receptors as TNFR, Fas or TRAIL-R1 and TRAIL-R2. Binding of their respective ligands induces receptor clustering and the recruitment of multiple procaspase-8 molecules via the adaptor protein FADD, resulting in caspase-8 activation. Caspase-8 activation can be blocked by the degenerate caspase homologue c-FLIP. Alternatively, in response to extracellular cues or internal insults, pro- and anti-apoptotic Bcl-2 family members gather at the surface of mitochondria to regulate cytochrome c release. Once released, cytochrome c associates with Apaf-1 and procaspase-9, leading to caspase-9 activation. The death receptor and mitochondrial pathways converge at the level of caspase-3 activation. Caspase-3 activity is antagonized by the IAP proteins. Several anti-apoptotic genes are regulated by NF-xB, including c-IAP1 and 2, TRAF1 and TRAF2, as well as Bcl-2 homologue A1/Bfl-1. NF-xB can also act to promote apoptosis, as it plays an active role in the regulation of Fas receptor and FasL, TNF-a and TRAIL death receptor ligands.

2. TNF-related apoptosis-inducing ligand (TRAIL)

The recently discovered ligand TRAIL, also known as Apo2 ligand, is a 40 kDa type II transmembrane protein that is structurally related to the TNF family of cytokines. The extracellular domain of TRAIL shares the highest amino acid homology with the FasL (28%), TNF-a (23%), lymphotoxin-a (23%) and lymphotoxin-b (22%), all known to initiate apoptosis of transformed cells and activated lymphocytes through the death receptor pathway. Although TRAIL mRNA is detected in various cells and tissues, member of the Bcl-2 family; truncated Bid (tBid) in turn targets mitochondria for cytochrome c release. Both the death receptor and the mitochondrial pathways converge at the level of effector caspase-3 activation [16,25-28].

Activated caspases can be considered the central executors of the apoptotic pathway: inhibition of caspase activity results in an inhibition of apoptosis. All caspases possess an active-site cysteine and selectively cleave substrates after aspartic acid residues [1-2]. Following the activation of caspases, cleavage of the inhibitory subunit of the caspase-activated DNase (CAD) occurs, leading to the activation of CAD, which enters the nucleus and cleaves DNA to produce the DNA laddering characteristic of apoptotic cells [3-5]. Cleavage of nuclear lamins is responsible for nuclear shrinking and budding [6,7], while cleavage of cytoskeletal proteins fodrin and gelsolin may be responsible for the overall loss of cell shape [8]. Finally, cleavage of PAK2 is sought to be responsible for the blebbing observed in apoptotic cells [9].
inflammation and joint tissue destruction [54]. Recent results seem to indicate that, like FasL, TRAIL may play an important role in preventing auto-immunity. Indeed, blocking endogenous TRAIL function in mice enhances proliferation of autoreactive B cells [52,53].

2.1. Biomedical importance of TRAIL

Like other members of the TNF ligand family, TRAIL induces apoptosis in a variety of cell lines in vitro, including several tumor cell lines resistant to chemotherapeutic agents or ionizing radiation due to mutations in the p53 tumor suppressor gene [30–32]. However, while the utility of Fasl and TNF-α is limited by their acute toxic effects on normal tissue, TRAIL preferentially induces apoptosis in tumor cell lines, but not in normal cells [33], suggesting that it may prove to be a powerful cancer therapeutic.

Systemically administered anti-Fas antibodies cause lethal liver damage [34], while TNF-α is known to cause a lethal inflammatory response, mediated through the ability of TNF to activate the proinflammatory NF-κB transcription factor [35]. These complications severely limit the use of these apoptosis-inducing ligands in anticancer therapy. Although it is not established whether TRAIL causes liver toxicity in humans [36,37], pre-clinical studies are very promising. Recombinant human TRAIL, protein systemically injected in mice and non-human primates promotes potent apoptosis-inducing activity against tumor cells without significant cytotoxicity to normal organs or tissues [33,38]. Moreover, newly developed anti-TRAIL-R2 antibodies exhibit strong anti-tumoral activity both in vitro and in vivo, without hepatotoxic cytotoxicity [39].

2.2. Physiological roles of TRAIL

Closely linked to its involvement in apoptosis, recent studies have revealed that the cytotoxic effect of a variety of immune cells, including CD4+ T cells, NK cells, macrophages and dendritic cells, is at least partly dependent on TRAIL expression, strongly suggesting that TRAIL acts as a tumor suppressor in vivo [40–45]. More specifically, either alone or in conjunction with FasL, TRAIL could play a substantial role in suppressing tumor metastasis [46–49]. Members of the TNF family are involved in the modulation of host defense mechanisms, including inflammation, T-cell co-stimulation, induction of B cell proliferation, macrophage activation, as well as elimination of unwanted immune cells by apoptosis. For example, while upregulation of TNF-α expression initiates and exacerbates autoimmune responses [50,51], modulation of expression of FasL on T cells is involved in activation-induced cell death (AICD) of mature T cells as well as T-cell mediated apoptosis of autoreactive B cells [52,53]. Recent results seem to indicate that, like Fasl, TRAIL may play an important role in preventing auto-immunity. Indeed, blocking endogenous TRAIL function in mice enhances proliferation of autoreactive lymphocytes or synovial cells, leading to arthritic inflammation and joint tissue destruction [54].

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Inducers of TRAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inducer</strong></td>
<td><strong>References</strong></td>
</tr>
<tr>
<td><strong>Viral inducers</strong></td>
<td><strong>HIV-1</strong> [55–58]</td>
</tr>
<tr>
<td></td>
<td><strong>HTLV-1 Tax</strong> [59]</td>
</tr>
<tr>
<td></td>
<td><strong>Reovirus</strong> [60]</td>
</tr>
<tr>
<td></td>
<td><strong>Measles virus</strong> [61]</td>
</tr>
<tr>
<td></td>
<td><strong>Cytomegalovirus</strong> [62]</td>
</tr>
<tr>
<td></td>
<td><strong>Adenoviruses</strong> [63,64]</td>
</tr>
<tr>
<td></td>
<td><strong>Human papillomavirus-16 E7</strong> [65]</td>
</tr>
<tr>
<td><strong>Bacterial inducers</strong></td>
<td><strong>Superoxide denitrogen B</strong> [82]</td>
</tr>
<tr>
<td><strong>Chemical agents</strong></td>
<td><strong>Phorbol ester</strong> [82,85]</td>
</tr>
<tr>
<td></td>
<td><strong>PHA</strong> [82,85]</td>
</tr>
<tr>
<td></td>
<td><strong>Concanavalin A</strong> [85]</td>
</tr>
<tr>
<td></td>
<td><strong>Immunocyn</strong> [82,85]</td>
</tr>
<tr>
<td></td>
<td><strong>α-Galactosylceramide</strong> [44]</td>
</tr>
<tr>
<td><strong>Physiological stimulation</strong></td>
<td><strong>T-cell receptor stimulation</strong> [46,87,122]</td>
</tr>
<tr>
<td></td>
<td><strong>Type I interferon</strong> [66,87]</td>
</tr>
<tr>
<td></td>
<td><strong>IL-2</strong> [42]</td>
</tr>
<tr>
<td></td>
<td><strong>IL-15</strong> [42]</td>
</tr>
<tr>
<td></td>
<td><strong>TNF-α</strong> [82,85]</td>
</tr>
</tbody>
</table>

TRAIL may also play an important role in virus induced disease and TRAIL expression is upregulated as a consequence of infection by multiple viruses (Table 1). In this regard, TRAIL may be responsible for the activation-induced cell death (AICD) of bystander T cells during AIDS [55–57]. Exogenously supplied HIV-1 Tat upregulates TRAIL production in primary human macrophages, suggesting that secretion of Tat by infected cells may in turn upregulate TRAIL in uninfected cells thus, inducing apoptosis of bystander T cells [58]. Similarly, the Tax oncoprotein encoded by human T-cell leukemia virus type I (HTLV-1) was shown to induce apoptosis of T cells through an increased expression of TRAIL [59]. Related studies also demonstrate that reovirus, measles virus and human cytomegalovirus infected cells are rendered cytotoxic via the TRAIL pathway [60–62] and indicate that virus infected cells express enhanced levels of TRAIL, which are responsible, at least in part, for virus induced apoptosis, possibly contributing to viral pathogenesis [55,56,60–62]. Moreover, several viral gene products are able to either sensitize or protect cells to the effects of TRAIL. For example, the adenovirus E1A oncogene and the human papillomavirus-16 E7 gene product enhance killing of tumor cells by TRAIL, while the adenoviral encoded RID/E3 proteins induce internalization of TRAIL-R1. Such a modulation of TRAIL inhibits apoptosis and favors acute and persistent infections [63–65]. These results suggest a role for TRAIL in viral disease, although in many cases little is known about the physiological significance of the virus-mediated modulation of TRAIL expression.
2.3. Regulation of TRAIL

A number of important genes involved in immunoregulation, cytokine and chemokine gene expression are under the control of NF-κB. Moreover, NF-κB has been shown to play an important role in regulating anti-apoptotic and pro-apoptotic events, depending on the physiological circumstances [67]. Initial studies established an anti-apoptotic role for NF-κB in immature B-lymphoma cells: inhibitors of NF-κB sensitized these cells to anti-IgM induced apoptosis, while overexpression of c-Rel had a protective effect by upregulating c-myca expression [68,69]. Further investigation revealed that a large number of anti-apoptotic genes are upregulated by NF-κB (Fig. 1), including cellular inhibitor of apoptosis proteins 1 and 2 (c-IAP1 and c-IAP2), TNFR associated factors 1 and 2 (TRAF1 and TRAF2), as well as the Bcl-2 homologue A1/Bfl-1 [70–73]. As expected, inhibition of NF-κB activity resulted in an increased sensitivity of cells to apoptosis induced by TNF-α and DNA damaging agents such as etoposide and doxorubicin [74–77]. Surprisingly enough, NF-κB can also act as a pro-apoptotic factor. It has been shown that NF-κB plays an active role in the regulation of Fas, FasL, and TNF-α, factors involved in death receptor induced apoptosis [78–80]. These results indicate that NF-κB may have a pro-apoptotic role in AICD [81]. For example, NF-κB is actively involved in the removal of mature T cells by upregulating the expression of Fas-ligand (Fasl). Furthermore, the promoter of human Fasl, contains κB sites and can be upregulated by AICD in T cells [79,81] and the modulation of expression of FasL on T cells can influence T cell mediated apoptosis of autoreactive B cells [53]. As stated earlier, the cytotoxic effect of various immune cells, including CD4+ T cells, NK cells, macrophages and dendritic cells is dependent on the expression of such apoptosis inducing ligands as TRAIL and FasL, [41–43,47,66]. Several experiments have shown that T cells activated via the TCR, PMA/ionomycin or PHA stimulation display increased levels of TRAIL mRNA [82–84] (Table 1). Further investigation revealed that activation of NF-κB following T-cell receptor engagement is responsible for this upregulation [59,85,86]. TRAIL expression induced by a variety of stimuli known to activate NF-κB transcription activity was inhibited in Jurkat T cells stably expressing a transdominant form of IκBα, as well as in primary T lymphocytes treated with a variety of pharmacological inhibitors of NF-κB. Moreover, NF-κB dependent expression of TRAIL was linked to the presence of two κB binding sites within the TRAIL promoter and mutation of either of these sites resulted in the loss of inducibility of the TRAIL promoter [85]. Also, T cells lacking IκB kinase gamma (IKKγ), an essential component of the NF-κB signaling pathway, failed to express TRAIL following stimulation, thus, demonstrating that TRAIL expression in activated T cells is NF-κB dependent [59,86]. Therefore, one of the key regulators of TRAIL expression in lymphocytes is NF-κB (Fig. 1).

The involvement of other transcriptional activators such as the interferon regulated factors (IRFs) and/or NF-κB proteins provide an additional level of complexity to the regulation of TRAIL surface expression. Sequence analysis of the TRAIL promoter revealed several potential NF-κB/AP-1 binding sites, as well as several interferon stimulated response elements (ISRE) and an IFN-γ activated sequence (GAS). In addition, type I interferons, as well as IL-2 and IL-15 stimulation, induced expression of TRAIL by NK cells [42,87] and constitutive expression of TRAIL on liver NK cells may be dependent on the endogenous production of IFN-γ [48]. It has also been reported that peripheral blood T cells and dendritic cells stimulated with type I interferons display an enhanced cytotoxicity activity through an upregulation of surface TRAIL expression [47,87] (Table 1). Taken together, these results suggest the existence of additional levels of TRAIL regulation that involve several distinct signaling pathways which have yet to be characterized.

3. TRAIL receptors

To date, several TRAIL receptors have been identified (Fig. 2). Two of these receptors TRAIL-R1/DR4 and TRAIL-R2/DR5 contain cytoplastic death domains and signal apoptosis through a caspase-dependent pathway [88–91], while two other receptors, TRAIL-R3/DcR1 and TRAIL-R4/DcR2 lack a functional death domain and do not mediate apoptosis upon binding of TRAIL [89]. The extracellular domains of these four receptors are highly homologous and map to the human chromosome 8p22-21, indicating that they have arisen by gene duplication [92–94]. A fifth TRAIL receptor, osteoprotegerin (OPG, TRAIL-R5), a secreted TNFR homologue involved in bone homeostasis, may also act as a decoy receptor protecting from TRAIL-induced apoptosis [95,96].

3.1. TRAIL death receptors

Engagement of TRAIL-R1 or TRAIL-R2 by TRAIL results in the recruitment and activation of caspase-8, as well as cleavage of Bid and cytochrome c release from mitochondria, events that subsequently lead to the activation of the caspase cascade [97,98]. It has been proposed that TRAIL-R1 and TRAIL-R2 signal apoptosis through the Fas-associated death domain protein (FADD) [99–104]. However, fibroblasts derived from FADD deficient mice undergo apoptosis upon overexpression of TRAIL-R1, indicating the existence of a FADD independent signaling mechanism [105]. This experimental discrepancy may stem from differences in the experimental system used by different investigators. Recent evidence suggests that DAP3, a GTP-binding protein, may play a role in linking death inducing TRAIL receptors to FADD and downstream apoptotic machinery, thereby acting as an adaptor molecule required for TRAIL-induced apoptosis [106]. Binding of
Fig. 2. TRAIL signaling pathways. Engagement of TRAIL-R1/DR4 and TRAIL-R2/DR5 by TRAIL results in the recruitment and activation of caspase-8. To date, it has not been established whether FADD or some other adaptor protein is required for the recruitment of caspase-8 to the death receptor complex. In addition, binding of TRAIL to these receptors results in the activation of NF-κB, a process mediated by a TRAF2-NIK-IKK dependent signaling cascade. TRAIL-R3/DcR1 exists as a glycosylphosphatidylinositol anchored surface protein and is unable to signal cell death, thus, acting as a decoy receptor. TRAIL-R4/DcR2 contains a truncated death domain and does not mediate apoptosis upon binding of TRAIL, however, TRAIL-R4/DcR2 retains the ability to signal NF-κB activation, suggesting that it may inhibit TRAIL-induced apoptosis by upregulating anti-apoptotic genes.

TRAIL to TRAIL-R1 or TRAIL-R2 also results in the activation of NF-κB and c-Jun N-terminal kinase (JNK) [107], indicating that TRAIL receptors can signal both apoptosis and gene transcription. TRAIL induces activation of NF-κB through a TRAF2-NIK-IKK dependent signaling cascade [108]. It should be noted though that the activation of NF-κB alone is not sufficient to block apoptosis induced by TRAIL receptors [99,101].

3.2. TRAIL decoy receptors

In contrast with TRAIL-R1 and TRAIL-R2, TRAIL-R3/DcR1 exists as a glycosylphosphatidylinositol anchored surface protein that is unable to signal cell death, thus, acting as a decoy receptor [89,97,109–111]. A fourth TRAIL receptor, TRAIL-R4/DcR2 contains only a partial death domain and does not mediate apoptosis upon binding of TRAIL: this member retains the ability to activate NF-κB, suggesting that it may inhibit TRAIL-induced apoptosis by inducing anti-apoptotic genes (Fig. 2) [93]. In fact, transfection of non-signaling TRAIL-R3 or TRAIL-R4 results in a decrease in the amount of cell death. Furthermore, TRAIL-R3 mRNA is preferentially found in normal cells, but not in transformed cells, suggesting that these decoy receptors might be responsible for the resistance of normal cells to TRAIL-induced apoptosis [89,90,109]. These results suggest a complex regulation of TRAIL-induced apoptosis at the level of expression of the various TRAIL receptors.

3.3. Regulation of the various TRAIL receptors

While both the TRAIL death and decoy receptors are co-expressed in normal human tissues, many cancer cell lines preferentially express TRAIL-R1 and TRAIL-R2, known to signal apoptosis [89,90,112], suggesting a differential regulation of the death and decoy receptors. As yet,
the details this differential regulation of various TRAIL receptors are not well established.

TRAIL-R2 expression has been linked to p53 [113], a transcription factor known to modulate the expression of a variety of genes involved in growth arrest and apoptosis in response to DNA damage [114,115]. Further investigation revealed functional p53 binding sites within the TRAIL-R2 promoter, thus, establishing TRAIL-R2 as a direct p53 target gene that signals apoptotic death [116]. These observations suggest that TRAIL-R2 may play a role in p53-mediated tumor suppression and that loss of p53 function and TRAIL-R2 expression may lead to tumor progression and resistance to chemotherapy. However, p53 has also been shown to induce the expression of TRAIL-R3 and TRAIL-R4 [117]. Since these receptors abrogate the anti-apoptotic function of TRAIL, the significance of their upregulation by p53 remains controversial [118].

In addition to confirming the role of NF-κB as a regulator of apoptosis, recent experiments have shown that the c-Rel subunit of the NF-κB transcription factor is able to induce expression of both TRAIL-R1 and TRAIL-R2, while overexpression of transdominant mutant of the inhibitory protein IκBα or a transactivation deficient mutant of c-Rel abrogates this effect and protects cells from TRAIL-induced apoptosis [119]. Thus, NF-κB may activate the TRAIL apoptotic cascade in different cell lines by upregulating both TRAIL-R1 and its two death receptors, TRAIL-R1 and TRAIL-R2. In contrast to these results, others have shown that activation of NF-κB induces an upregulation of TRAIL-R3, but not of TRAIL-R1 and TRAIL-R2, and thus, protects against apoptosis [120].

The key to resolving these contradictory results may lie in the fact that not only are the various TRAIL receptors differentially expressed, but their function may also be regulated depending on cellular localization. For example, following treatment of human melanoma cells with TRAIL, TRAIL-R1 and TRAIL-R2 are internalized into endosomes, whereas TRAIL-R3 and TRAIL-R4 move from the nucleus to the cell surface, suggesting the existence of more complex levels of regulation [121]. A complete understanding of these regulatory circuits will require extensive analysis of TRAIL-mediated signaling events and the characterization of TRAIL receptor knockouts.

4. Conclusion

While the current studies clearly illustrate a role for TRAIL and its receptors in cell growth homeostasis, their precise function in tumor surveillance, immunoregulation and viral infection are yet to be established, particularly in the context of NF-κB and p53 mediated modulation of apoptosis. Further knowledge of the regulation and physiological role of TRAIL and TRAIL receptors may aid in the rational design of regimens that utilize the TRAIL signaling pathway to eliminate tumor cells, while at the same time sparing normal tissues.

Acknowledgements

The authors thank members of the Molecular Oncology Group at the Lady Davis Institute, McGill University for helpful discussions and comments during the preparation of this review. This research program is supported by research grants and training fellowships from the Canadian Institutes of Health Research, the National Cancer Institute of Canada, Fonds de la Recherche en Sante du Quebec, and CANVAC, the Canadian Network for Vaccines and Immunotherapeutics.

References


