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Nature's TRAIL— On a Path to Cancer Immunotherapy

Minireview

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The TNF-related apoptosis-inducing ligand (TRAIL) offers great promise as a cancer therapeutic. Initially, soluble recombinant versions of the TRAIL molecule have exhibited specific tumoricidal activity against a variety of tumors alone, or in combination with other cancer treatments, and much anticipation awaits the outcomes from early clinical trials. More recently, the natural role of TRAIL has been explored in tumor and allogeneic bone marrow transplantation models in the mouse. Strikingly, the TRAIL effector pathway appears a vital component of immunosurveillance of spontaneous or resident tumor cells by both T cells and NK cells, stimulating more hope that manipulating TRAIL activity is a natural path to improved cancer immunotherapy.

TRAIL—A Promising Cancer Therapeutic

Members of the tumor necrosis factor (TNF) family of cytokines are expressed by effector lymphocytes and are important mediators of apoptosis that both shape and regulate the immune system. TNF-related apoptosis-inducing ligand (TRAIL), also known as Apo2 ligand, is a type II transmembrane protein belonging to the TNF superfamily (Wiley et al., 1995; Pitti et al., 1996). At least five receptors for TRAIL have been identified in humans (only one, DR5 [TRAIL-R2] in mice) and two of them, DR4 (TRAIL-R1) and DR5, are capable of transducing an apoptotic signal (Degli-Esposti, 1999; Ashkenazi, 2002). The other three receptors (TRAIL-R3, TRAIL-R4, and a soluble receptor called osteoprotegerin [OPG,TRAIL-R5]) lack death domains but may serve as decoy receptors to regulate TRAIL-mediated cell death. Investigation of the intracellular signaling pathways responsible for TRAIL receptor-induced apoptosis has produced controversial results, but most recent studies suggest DR5 signals through a FADD- and caspase-8-dependent pathway (Bodmer et al., 2000). Bax is required for TRAIL-induced apoptosis of certain cancer cell lines possibly by allowing release of second mitochondria-derived activator of caspases (Smac)/direct IAP binding protein with low pH (DIABLO) and antagonizing the inhibitor of apoptosis protein (IAP) family (Deng et al., 2002). Bax gene ablation led to resistance to TRAIL (Burns and El-Deiry, 2001; LeBlanc et al., 2002), and reintroduction of Bax into Bax-deficient cells restored TRAIL sensitivity (Deng et al., 2002).

The soluble recombinant TRAIL is of interest for cancer therapy and soon to enter clinical trials for a number of reasons. There are few agents that are truly cancer cell-specific in terms of efficacy or cell death induction. TRAIL is a rare example of such molecules that kill many transformed cells but spare most normal cells (Ashkenazi and Dixit, 1998; Wiley et al., 1995). Importantly, administration of soluble recombinant TRAIL in experimental animals, including mice and primates, induced significant tumor regression without systemic toxicity (Walczak et al., 1999; Ashkenazi et al., 1999). Not all cancer cells are sensitive to the cytotoxic effects of TRAIL. DR5 mutations have been found in some cancers of the head and neck, breast and lung, and Hodgkin's lymphoma (El-Deiry, 2001), and recent studies in the mouse suggest TRAIL immunoselects tumors for TRAIL resistance (Takeda et al., 2002) (see below). DR5 has been implicated in the cellular response to DNA-damaging radiation or chemotherapy as a target of p53 (Wu et al., 2000). Although one of the attractive features of TRAIL is its ability to kill cancers with mutations in the p53 gene, the combination of TRAIL with chemotherapeutic agents has been found to be particularly effective in killing cancers with wild-type p53, presumably through induction of DR5 expression (Nagane et al., 2001). In addition, Bax mutation in mismatch repair-deficient tumors can cause resistance to TRAIL therapy, but preexposure to chemotherapy rescues tumor sensitivity (LeBlanc et al., 2002). Synergy between TRAIL and anticancer drugs has been demonstrated against diverse tumor cell types in tumor xenograft experiments. While data showing the impressive selective anti-tumor activity in vitro of soluble TRAIL have generated considerable excitement and have resulted in the development of TRAIL as a novel anti-cancer agent, only now have a few key studies addressed the natural role of TRAIL in immunity against cancer.

TRAIL Expression—A Clue to Its Natural Role?

Three experimental tools have been key in defining the expression and physiological role of TRAIL in mice, including (1) a neutralizing anti-mouse (m)TRAIL monoclonal antibody (mAb) (N2B2) (Kayagaki et al., 1999c); (2) soluble recombinant human DR5 (Song et al., 2000); and (3) TRAIL gene-targeted mice (Cretney et al., 2002). Although TRAIL mRNA has been found in a variety of tissues and cells (Wiley et al., 1995), the anti-mTRAIL mAb has been extremely useful in determining which cells express TRAIL constitutively, and following activation. From studies in both mice and humans, we can now appreciate that freshly isolated T cells, NKT cells,

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B cells, dendritic cells (DC), monocytes, or NK cells do not express a detectable level of TRAIL on their surface (Takeda et al., 2001; Smyth et al., 2001; Kayagaki et al., 1999c; Griffith et al., 1999; Fanger et al., 1999). The exception appears to be a subset of mouse liver NK cells that express TRAIL constitutively in a type II IFN-dependent manner (Takeda et al., 2001; Smyth et al., 2001). It appears most likely that TRAIL expression on liver NK cells is regulated by IFN-γ secreted from NK cells in an autocrine manner, since a substantial proportion of liver NK cells constitutively produce both TRAIL and IFN-γ in wild-type and T cell-deficient mice (Takeda et al., 2001). The presence of a minor population of TRAIL$^+$ IFN-γ$^-$ cells suggests the contribution of some other mechanism. The exclusive constitutive expression of TRAIL on some liver NK cells raises the issue of what normal physiological role TRAIL may play in the liver. It is possible that liver NK cells may express TRAIL in response to gut-derived endogenous endotoxin or in early responses to infections, although analysis of germ-free mice revealed a subset of liver NK cells expressing TRAIL (K.T., unpublished data). Alternatively, a current hypothesis from the study of human NK cells is that immature NK cells express TRAIL and lose expression with maturity into granulated NK cells exerting perforin and FasL-mediated cytotoxicity (Zamai et al., 1998). Further studies will be required to determine whether mouse liver NK cells constitutively expressing TRAIL represent a distinct immature subset or stage of NK cell differentiation. TRAIL is highly expressed on most NK cells after stimulation with IL-2, IFNs, or IL-15 (Kayagaki et al., 1999a, 1999c; Zamai et al., 1998; Sato et al., 2001). Similarly, type I IFN-activated human peripheral blood T cells (Kayagaki et al., 1999b), CD11c$^+$ DC, and monocytes express TRAIL (Fanger et al., 1999; Griffith et al., 1999). These expression patterns reflect the broad role that TRAIL likely plays in innate immune responses involving IFNs, NK cells, and DC.

TRAIL—A Key Anti-Metastatic Effector Molecule of NK Cells

TRAIL was shown to be involved in the cytotoxic activity of activated NK cells against TRAIL-sensitive tumor cells in vitro (Kayagaki et al., 1999c) and, along with perforin and FasL, partly responsible for spontaneous NK cell activity against TRAIL-sensitive lines (Takeda et al., 2001). The anti-metastatic function of NK cells against TRAIL-sensitive tumor cells in mice was also shown to be partly dependent upon TRAIL, and natural TRAIL anti-metastatic activity was restricted to liver NK cells in several different models (Takeda et al., 2001) (Figure 1a). Both neutralizing anti-mTRAIL mAb and TRAIL gene-targeted mice supported a direct role for NK cell TRAIL in natural suppression of tumor metastasis, with no phenotype observed against all TRAIL-resistant cell lines examined in vivo (Takeda et al., 2001; Cretney et al., 2002). These findings provided evidence for the physiological function of TRAIL as a tumor suppressor. IFN-γ-mediated TRAIL induction on NK cells was also shown to play a significant role in IFN-γ-dependent anti-metastatic effects of IL-12 and the NKT cell activator, α-galactosylceramide (α-GalCer) in the liver, lung, and mammary gland (Smyth et al., 2001; Cretney et al., 2002) (Figure 1b). Therefore, TRAIL may be involved in the anti-tumor effects of many cytokines that act to stimulate NK cell activation and IFN-γ production. Thus, although the only NK cell subset to express TRAIL constitutively is found in the liver, many NK cells in the lung, liver, and spleen were induced to express TRAIL and thereby kill tumor cells in vivo (Smyth et al., 2001). In all cases, neutralization of TRAIL additively enhanced liver metastasis in perforin-deficient mice but not in IFN-γ-deficient mice (Smyth et al., 2001). These findings clearly place perforin and TRAIL as the two key cytotoxic effector pathways used by NK cells.

TRAIL-Mediated Immunosurveillance of Cancer Initiation

Immune surveillance against tumors is mediated by both innate and adaptive components of cellular immunity. The adaptive component mainly consists of CD8$^+$ cytotoxic T lymphocytes (CTL) that recognize tumor antigens presented by major histocompatibility complex (MHC) class I molecules on tumor cells. NK cells have long been implicated in innate immunity against tumors, especially MHC class I-deficient variants. Recent studies have substantiated a pivotal role of NK cells, perforin, and IFN-γ in natural protection from primary tumor development induced by a chemical carcinogen methylchoranthrene (MCA) (Street et al., 2001) (Figure 1c). Neutralization of TRAIL promoted tumor development in mice inoculated with MCA and this protective effect of TRAIL was at least partly mediated by NK cells and totally dependent on IFN-γ (Takeda et al., 2002; Cretney et al., 2002). Heterogeneous tumor cells with different TRAIL sensitivity are expected to arise during the MCA-induced tumor development in individual mice; however, the preferential emergence of TRAIL-sensitive fibrosarcoma cells in TRAIL-deficient and IFN-γ-deficient mice strongly suggested an immunoselection pressure against TRAIL-sensitive cells during tumor development (Takeda et al., 2002) (Figure 1c). In summary, IFN-γ may regulate TRAIL-mediated tumor surveillance, not only by regulating TRAIL expression on effector cells but also by sensitizing tumor cells to TRAIL-mediated cytotoxicity. The mechanism by which IFN-γ sensitizes tumor cells to TRAIL remains to be determined. A substantial contribution of TRAIL to immune surveillance against spontaneous tumor development caused by p53 mutation was also recently demonstrated (Takeda et al., 2002). In contrast to perforin that selectively protected from disseminated lymphoma (Smyth et al., 2000), TRAIL, like IFN-γ, additionally suppressed sarcoma formation. Collectively, these studies demonstrated a role for TRAIL in NK cell-mediated immunosurveillance against tumors. Since monocytes and DC also express TRAIL after stimulation with IFNs, a possible contribution of TRAIL on these cells to anti-tumor activity in vivo must now be evaluated. In particular, some tumor models already examined (Takeda et al., 2002) have suggested that TRAIL expressed on non-T/NK cells might contribute to the natural suppression of liver metastasis, although the identity of these effector cells remains unclear. Tumors where IFN-γ and monocytes control tumor growth are worthy of further attention. Further studies on the molecular mechanisms by which IFNs and TRAIL contribute to immune surveillance against tumors may provide a novel strategy to prevent tumor development.

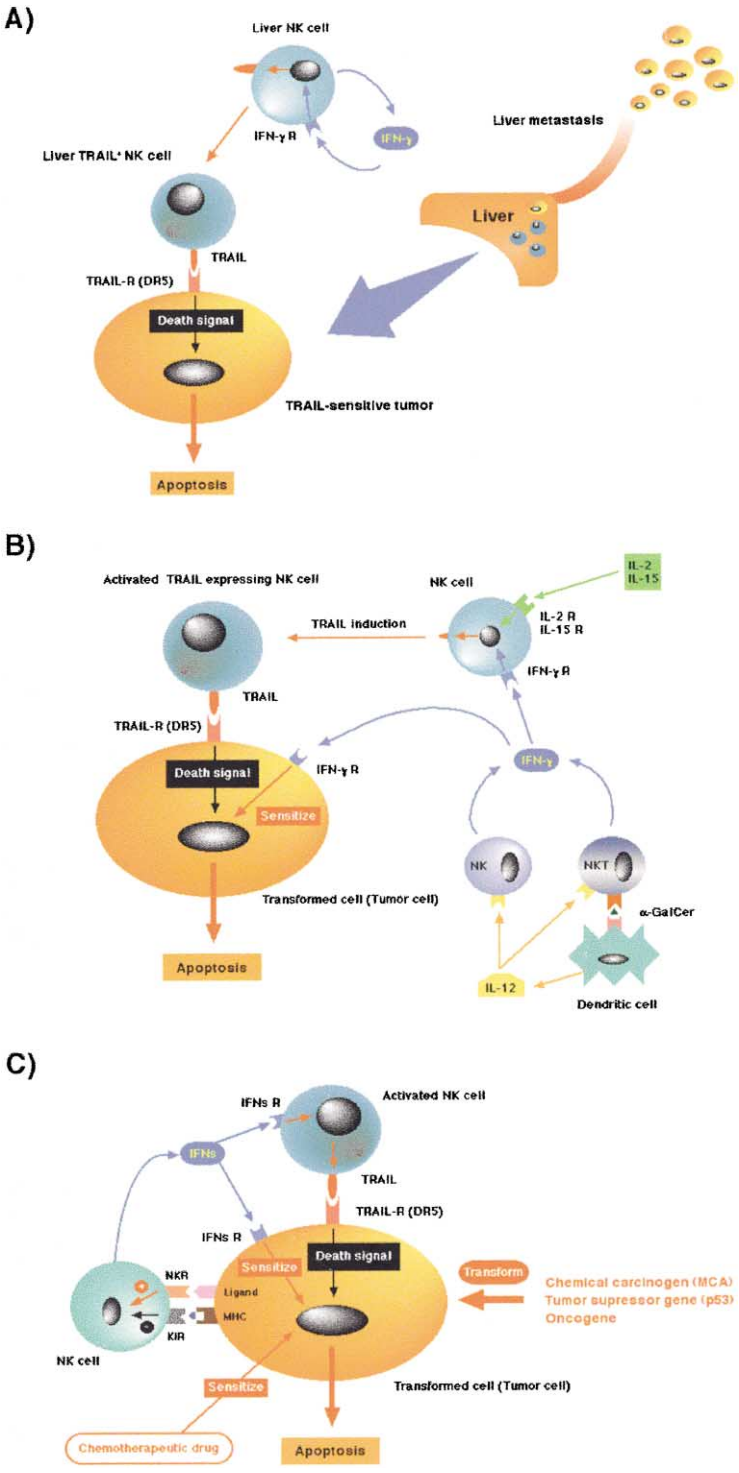

Figure 1. The Role of TRAIL-Mediated Cytotoxicity in Tumor Suppression by NK Cells

(A) TRAIL-mediated anti-metastatic effect by liver NK cells. Mouse liver NK cells express TRAIL constitutively and they are responsible for the anti-metastatic function against TRAIL-sensitive tumor cells. The TRAIL expression on liver NK cells is regulated by endogenously produced IFN-γ.

(B) Relative contribution of TRAIL-mediated cytotoxicity by NK cells in IFN-γ-inducing immunotherapies. During effective immunotherapy with IL-12 or α-GalCer, the induction of IFN-γ controls tumor metastasis by virtue of TRAIL induction on NK cells. IL-2 and IL-15 may also induce TRAIL on NK cells and stimulate NK cell proliferation.

(C) Model of role for TRAIL in immune surveillance against tumor development. Both NK cells and IFN-γ have been implicated in natural protection from primary tumor development. TRAIL is partly responsible for the NK-cell-mediated and IFN-γ-dependent mechanism of tumor elimination. Type I IFNs also induce TRAIL expression on NK cells. NK cell activation is strictly regulated by positive and negative signals through NK cell activating receptors (NKR) and killer inhibitory receptors (KIR), respectively. NK cells recognize some ligands on transformed cells and then are activated to eliminate transformed ("dangerous") cells in a TRAIL-dependent manner.

Graft versus Tumor—Contribution of T Cell TRAIL

Although several years ago activated T cells were shown to exert cytolysis of target cells through the TRAIL pathway (Kayagaki et al., 1999b), only now has a clear role for TRAIL in the T cell-mediated immune defense against tumor been formally shown (Schmaltz et al., 2002) (Figure 2). Allogeneic hematopoietic cell transplantation (AHCT) is an important therapy for a variety of malignant diseases. The anti-tumor activity of allogeneic donor T cells (graft versus tumor, GVT) provides arguably (a) the best evidence for T cell-mediated anti-tumor activity with clinical relevance and (b) is at present the most potent immunotherapy of cancer available. GVT activity is triggered by the recognition of tumor-specific antigens or alloantigens expressed on malignant cells, and thus graft versus host (expressing alloantigens) disease (GVHD), involving systemic illness and target organ damage to skin, liver, and intestines, is the single most

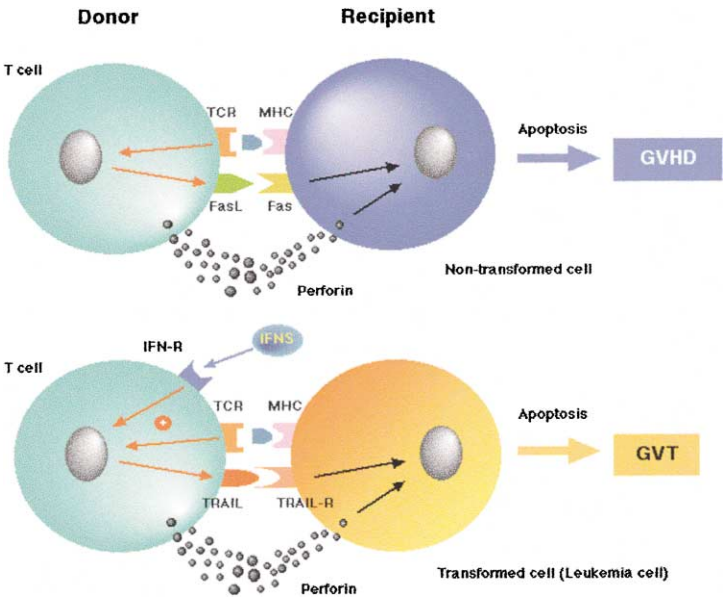

Figure 2. Role of TRAIL on Allo-Reactive T Cells in Graft versus Host Disease and Graft versus Tumor Activity after Allogeneic Hematopoietic Cell Transplantation

The reactivity of the donor T cell against recipient normal cells and transformed (leukemia) cells is essential in the development of GVHD and GVT. Cytolytic activity of donor T cells is mediated through different effector mechanisms in those responses. The Fas/FasL and perforin pathways are mainly responsible for donor T cell-mediated GVHD activity. The perforin pathway plays an important role in GVT activity. TRAIL on donor T cells is selectively required for optimal GVT activity, but not for GVHD.

important complication of AHCT. In three different mouse bone marrow transplantation (BMT) models, TRAIL has been shown to be required for optimal GVT activity by donor T cells (Schmaltz et al., 2002). By contrast, TRAIL played little or no role in the GVHD activity of donor T cells and the TRAIL pathway was not required for GVHD target organ pathology. Given that TRAIL-deficient T cells have an intact proliferative and cytokine response to mitogen and alloantigens in vitro and in vivo, these data may represent a very important biological illustration that the TRAIL pathway can selectively kill transformed cells preferentially over normal cells. Donor $CD8^+$ T cells expressing perforin mediate GVT, and $CD4^+$ or $CD8^+$ T cells expressing FasL and/or perforin cause GVHD (Schmaltz et al., 2001). TRAIL expression has been detected in both $CD4^+$ and $CD8^+$ T cells after allogeneic stimulation in vitro or in vivo. This illustration of natural TRAIL activity is supported by the ability of recombinant TRAIL to reduce the numbers of myeloid colonies from patients with acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and myelodysplastic syndromes, but not from normal BM (Plasilova et al., 2002). In the context of other molecules of the TNF/TNFR superfamilies, implicated in GVHD and GVT, a rational therapeutic strategy will now be to minimize GVHD by neutralizing the FasL/Fas pathway and enhance GVT by augmenting the TRAIL/TRAIL-R pathway. Although type I IFN stimulate TRAIL in vitro, it is unclear whether cotreatment with type I IFN in vivo may further enhance the GVT benefit of TRAIL. With the recent demonstration in allo-BMT models that NK cell allo-transplants mismatched at KIR can enhance GVT and minimize GVHD, it is quite likely that part of the success of this approach similarly relies on the selectivity of TRAIL for transformed cells. Alternatively, it is also possible that mismatched NK cells might eliminate bone marrow-derived antigen-presenting cells by a TRAIL-dependent mechanism, thereby preventing these cells from presenting recipient antigens to grafted T cells and thus limiting GVHD. This enthusiasm for TRAIL as a therapy in allo-BMT therapy must be balanced by the knowledge that many leukemias (ALL, AML) from patients are not sensitive to TRAIL in vitro (Wuchter et al., 2001) and thus combination therapies may need to be assessed.

A Role for TRAIL-Mediated Anti-Tumor Immunity in Humans?

A recent paper has reported that TRAIL expression is lower on $CD4^+$ lymphocytes from patients with advanced melanoma and immune cells that can express TRAIL are observed in regressing primary melanoma and in metastases of patients responding to IFN-α2 therapy (Hersey and Zhang, 2001). In addition, TRAIL expression was upregulated on $CD56^+$ NK cells in the peripheral blood of hepatocellular carcinoma patients responding to a combination of 5-fluorouracil and IFN-α (H.Y., unpublished data). While such findings are interesting, they are too preliminary to make a general conclusion. Comparing the TRAIL expression on immune cells from patients with different prognoses may be of limited value and similar attempts to correlate the levels of other effector molecules such as perforin/granzyme and FasL with cancer progression have not yet been particularly informative. Some human cancer cell lines and primary human epithelial tumors are sensitive to TRAIL-induced apoptosis ex vivo; however, significant heterogeneity in TRAIL sensitivity has been reported for a variety of human cancers. It remains to be proven whether the immune system does have a significant impact on epithelial malignancy in humans and therefore one might not expect such human tumors to be TRAIL resistant. In human tumor types that are recognized as immunogenic, such as melanoma, it is clear that many fresh isolates from patients are indeed TRAIL resistant and this resistance appeared to be associated with low TRAIL receptor expression (Nguyen et al., 2001). Tumors have many ways to escape immune surveillance without needing to become TRAIL resistant and in the human, non-death-inducing TRAIL-R expressed on normal cells may confer protection from TRAIL during malignant transformation. The isolation of patients genetically de-

fective in TRAIL or TRAIL-R expression may or may not reveal a cancer-susceptible phenotype (like FasL-Fas); however, no such condition has yet been reported.

Manipulation of TRAIL function in humans has not yet been addressed in any clinical setting. We now await the administration of recombinant human TRAIL to cancer patients, but additional trials comparing the efficacy of TRAIL-expressing NK cells and T cells in humans following adoptive transfer in either solid tumor or allo-BMT transplant settings may also be of interest. Recombinant forms of TRAIL have been shown to kill tumor cells in either a type I (not protected by bcl-2 family proteins)- and II (protected by bcl-2 family proteins)-dependent manner. These different forms of target cell sensitivity are not well understood molecularly and which of these pathways is used by natural TRAIL expressed on lymphocytes remains unclear. Defining the contexts in which TRAIL may eliminate or spare normal tissues is also of great importance when considering manipulating the TRAIL pathway in novel cancer therapies. The ability of some soluble forms of TRAIL to kill normal cells such as hepatocytes (Lawrence et al., 2001), and the possible role of TRAIL in other immune responses (Hilliard et al., 2001; Song et al., 2000), raises some concerns about simplistic approaches. The demonstrated killing activity of anti-human DR5 mAbs against liver cancer cells, but not normal hepatocytes (Ichikawa et al., 2001), at least offers hope that rational engineering of recombinant molecules will provide new strategies to exploit the TRAIL/TRAIL-R pathway in cancer cells.

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