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TRAIL: a multifunctional cytokine

Uta Schaefer, Oksana Voloshanenko, Daniela Willen, Henning Walczak

Tumor Immunology Program, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany

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1. ABSTRACT

The Tumor necrosis factor (TNF)-Related Apoptosis Inducing Ligand, TRAIL, has gained much attention due to its specific anti-tumor potential without toxic side effects. TRAIL binds to a complex receptor system. In humans there are two death-inducing receptors for TRAIL while only one is present in mice. The signaling induced by these receptors leads to apoptosis but might also result in activation of survival signals. To assess the safety and possible side effects of TRAIL-based cancer therapy it is necessary to understand the physiological role of the TRAIL/TRAIL-R system. This has been addressed in mice deficient either for TRAIL or for its only murine apoptosis-inducing receptor, TRAIL-R (MK/mDR5). In this review we will discuss their phenotypes and the results of recent studies on the role of TRAIL in the homeostasis of the immune system, the influence of the TRAIL/TRAIL-R system on infection and autoimmune diseases and the still controversial role of TRAIL in tumorigenesis. Clinical trials with TRAIL and other TRAIL receptor agonists are now under way. It will be exciting to determine which TRAIL-R agonists, either alone or in combination with other anti-cancer therapeutics, will result in better outcome of cancer treatment in the future.

2. INTRODUCTION

TRAIL was independently identified by Wiley *et al.* and Pitti *et al.* in 1995 and 1996 as the third member of the TNF superfamily that induces apoptosis (1, 2). The attention to this novel cytokine amongst cancer researchers increased further following the discovery that TRAIL can kill tumor cells *in vivo* without being toxic (3). Following this study it was shown that TRAIL acted synergistically with standard chemotherapeutics, thereby achieving even more striking anti-tumor effects (3-8). Currently, different TRAIL-R agonists, including TRAIL itself, and various agonistic monoclonal antibodies against the two apoptosis-inducing human TRAIL receptors are now in clinical trials as novel cancer therapeutics, (9, 10). Several studies with knockout mice for TRAIL and its only apoptosis-inducing murine receptor have been conducted to unravel the physiological role of this potent cytokine. These studies revealed several influences of the TRAIL/TRAIL-receptor system in regulating the homeostasis of the immune system and in the immune surveillance of tumors which is going to be discussed in this review.

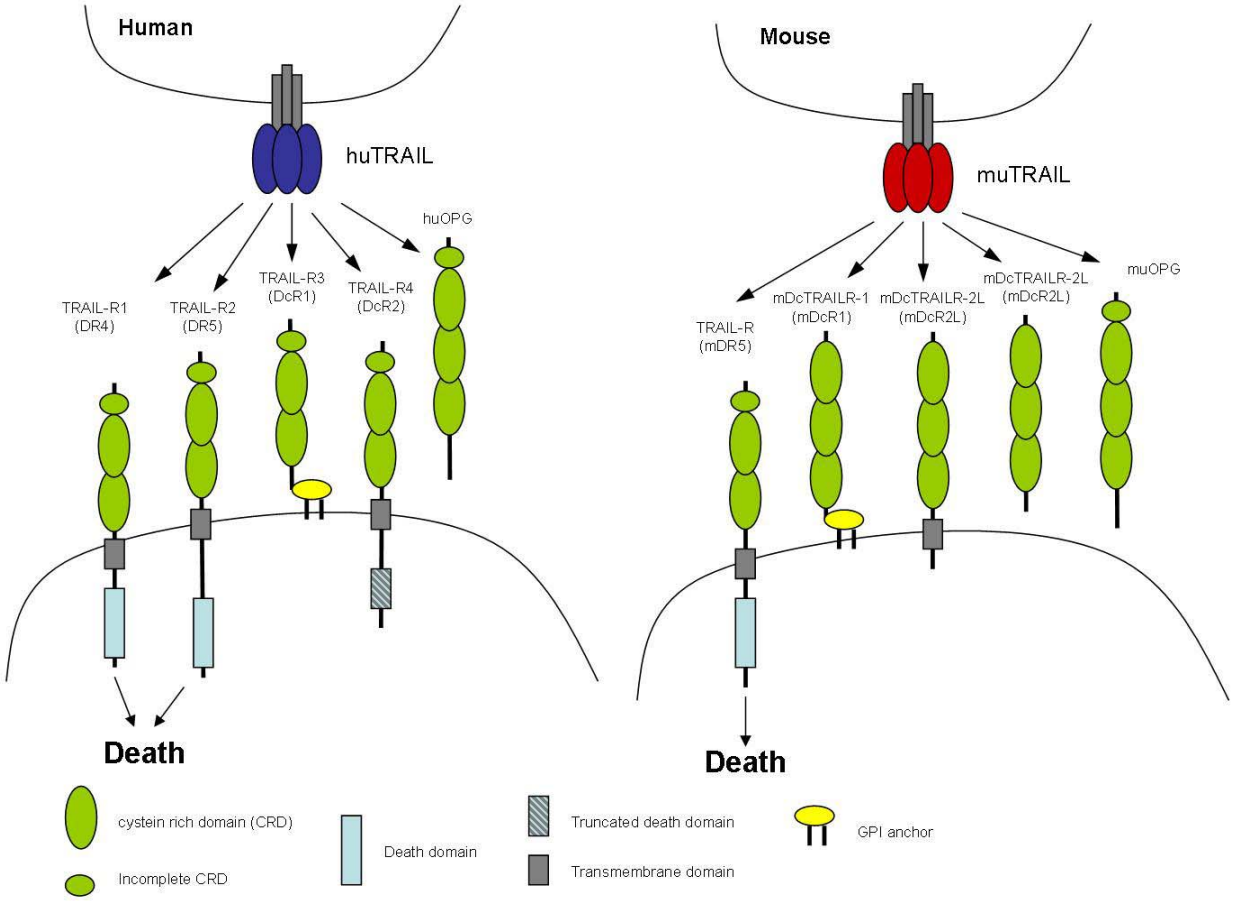

Figure 1 Overview of the TRAIL/TRAIL-R system in human and mouse. Schematic representation of binding of homotrimeric cell-bound TRAIL to its different receptors in both, man and mouse. In humans TRAIL binds to two apoptosis-inducing receptors, TRAIL-R1 and TRAIL-R2, which both contain a death domain (DD). Complete and incomplete cystein-rich sub-domains in the extracellular domains are indicated. TRAIL-R3 is GPI-anchored and lacks a cytoplasmic domain while TRAIL-R4 has a truncated DD. In mice there is only one apoptosis inducing receptor for TRAIL, murine TRAIL-R. The mouse system also contains two so-called "decoy" receptors, but the cystein domain structure is quite different from the one of human TRAIL-R3 and TRAIL-R4. Thus, they are only distantly related to each other.

2.1. TRAIL AND ITS RECEPTORS

TRAIL is expressed as a type II trans-membrane protein (C-terminus extra-cellular) but it also forms a soluble trimer. Compared to other members of the TNF superfamily, TRAIL binds to a complex system of receptors with differing affinities and possibly different signaling outcomes (Figure 1). Five TRAIL binding receptors are known in humans. These are the two apoptosis-inducing receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5/APO2/Killer/TRICK2), which contain an intracellular death domain (DD). The DD is as a prerequisite for apoptosis induction. TRAIL binding to TRAIL-R1 and/or TRAIL-R2 results in formation of the death-inducing signaling complex, the DISC. This apoptosis-initiating complex is discussed in more detail later. In addition, two non-apoptosis-inducing cell-bound receptors have been identified; TRAIL-R3 (TRID, LIT, DcR1) is GPI-anchored and lacks an intracellular domain, and TRAIL-R4 (TRUNDD, DcR2) contains only a truncated death domain and thus cannot transmit an apoptotic signal into the cell. These two receptors have been suggested to inhibit TRAIL death induction by overexpression. Therefore some authors refer to them also as decoy receptors. Others have proposed that these receptors are involved in the hindrance of proper preassembly of the apoptosis-inducing TRAIL receptors before ligand binding (11). A recent report by Merino *et al.* suggests two different modes of inhibiting TRAIL death signaling by TRAIL-R3 and TRAIL-R4; while TRAIL-R3 may titrate TRAIL within lipid rafts, TRAIL-R4 may interfere with initiator caspase activation and may prevent TRAIL-R1 recruitment to the TRAIL "death-inducing signaling complex" (DISC) (12). However, the role of these receptors, especially at physiological expression levels, is still not clear and might be more "regulatory" than "decoy" (13, 14).

TRAIL has also been shown to bind to osteoprotegerin (OPG), yet with rather low affinity (15, 16). OPG binds to and thereby inhibits another TNF family

member, RANKL (receptor activator of NF-κB Ligand) with high affinity. RANKL is involved in bone metabolism by stimulating osteoclast formation. OPG inhibits the RANKL-RANK interaction on osteoclasts and thereby interferes with osteoclastogenesis, (17). Considering that TRAIL and TRAIL-R deficient mice do not display a "bone phenotype", at least in vivo in the mouse, it is rather unlikely that the TRAIL-OPG interaction may also play a role in the TRAIL/TRAIL-R system.

The mouse TRAIL/TRAIL-R system appears to be similar to the human system at first glance, but in fact there are major differences: first of all, there is only one death-inducing TRAIL receptor in mice, namely TRAIL-R (MK, mDR5). This receptor is almost equally homologous to human TRAIL-R1 (76% identity) and TRAIL-R2 (79% identity) (18). Therefore it can not be regarded as the mouse orthologue of one or the other human TRAIL death receptor. For this reason we suggest to refrain from the misleading nomenclature of calling the murine TRAIL-R mDR5. The other murine TRAIL receptors, mouse decoy (mDc) TRAIL-R1 and mDcTRAIL-R2 seem to resemble the decoy functions of TRAIL-R3 and -R4. Yet the murine decoy TRAIL-Rs contain three complete cystein rich domains (CRDs) instead of two complete ones plus one incomplete CRD as it is seen in the human system and in the murine TRAIL-R (18, 19) (Figure 1). Thus, they are evolutionarily only distantly related to each other. However, since nature has apparently come up with this molecular concept at least twice it seems as if there is some advantage in also having non-apoptosis-inducing TRAIL receptors. Whether or not these different receptors serve different functions in mice as compared to humans remains to be determined.

2.2. TRAIL Signaling

TRAIL binding leads to the multimerization of TRAIL-R1 and/or TRAIL-R2 and subsequent recruitment of adaptor, effector and regulatory proteins. The TRAIL DISC is formed which initiates the "extrinsic" apoptosis pathway. The interactions between the DISC components are mediated by conserved homotypic protein-protein interaction motifs. Two important domains involved in these interactions are the DD and the death effector domain (DED).

The first component which is intracellularly recruited to the TRAIL DISC is the adaptor molecule FADD (Mort1). With its DD FADD binds to the DD of TRAIL-R1 or TRAIL-R2 thereby exposing a DED which then binds to the DED of pro-caspase-8 or -10 (20). These cystein-dependent aspartate-specific proteases are synthesized as inactive pro-enzymes but recruitment to the DISC induces their activation. Activation of caspase-8 then triggers a caspase cascade by inducing the cleavage and activation of the effector caspases 3 and 7. Active caspase-3 and -7 can further cleave a multitude of cellular substrates finally leading to the typical hallmarks of apoptosis like membrane blebbing and nuclear fragmentation (21).

Activated caspase-8 and -10 cleave the pro-apoptotic Bcl-2 family member Bid to its truncated form tBid which then moves to the outer membrane of the mitochondria. Thereby, Bid provides the link between the extrinsic death receptor apoptosis pathway and the "intrinsic" mitochondrial apoptosis pathway (22, 23). It is believed that tBid then most likely indirectly via neutralization of anti-apoptotic Bcl-2 family members activates the pro-apoptotic multidomain Bcl-2 proteins Bak and Bax which promote the release of cytochrome c from the mitochondrial intermembrane space into the cytoplasm. Bak and Bax are usually suppressed by the anti-apoptotic protein Bcl-2, the canonical member of this protein family which is often highly expressed in malignancies with bad prognosis (24).

Released cytochrome c together with Apaf-1 and caspase-9 are part of another destructive cellular machinery comparable to the DISC, the apoptosome. Once formed, the apoptosome mediates the activation of caspase-9 and subsequent cleavage of caspase-3 by this complex. In addition other proteins like Smac/Diablo (Second mitochondria derived activator of caspases/ direct IAP binding protein with low pI) are also released, which further promotes cell death. Smac/Diablo inhibits XIAP by direct interaction, thereby interfering with its binding to caspase-3 and -7. Thus, upon release of Smac/Diablo the inhibition of effector caspase maturation is relieved and caspase-3 and -7 can be fully activated.

However, the outcome of TRAIL receptor triggering is not necessarily cell death. It has been described that TRAIL can induce survival signals in some cell types especially by inducing the pro-survival and pro-inflammatory transcription factor NF-κB and also MAP-kinase pathways via JNK activation. This seems to be mediated via RIP, the receptor-interacting protein which was first discovered as an integral component of the TNF-R1 signaling complex (25). These non-apoptosis-inducing signaling pathways and also the death pathways are covered in detail in another review (26).

3. PHYSIOLOGICAL ROLE OF TRAIL

Several studies with mice deficient for TRAIL and its only apoptosis-inducing murine receptor in addition to experiments with TRAIL-blocking agents have led to the discovery of rather diverse functions of the TRAIL/TRAIL-R system *in vivo*. The so far observed phenotypes are summarized in Table 1.

In 2002 Cretney *et al.* and Sedger *et al.* published the first results obtained with a TRAIL-deficient mouse. Both studies showed no gross phenotype, as the mice were viable and fertile and did not display any developmental defects, showing that there is no crucial role for TRAIL in embryonic development (27, 28). Interestingly, bone density was not influenced in these mice as mentioned in the introduction. Thus, the OPG-TRAIL interaction does not seem to be important for these OPG-mediated effects (27). The same is true for TRAIL-R-deficient mice (29, 30).

Table 1. Overview of TRAIL and TRAIL-R knockout experiments

TRAIL or TRAIL-R knockout	*Phenotype*	*References*
TRAIL $^{-/-}$	increased tumor growth and experimental liver metastasis with 4T1 and Renca tumors, higher susceptibility to MCA-induced tumors	27
TRAIL $^{-/-}$	increased tumor growth and experimental liver metastases of A20 cell line, normal bone density, no spontaneous tumor development	28
TRAIL$^{-/-}$	increased experimental liver metastases after intrasplenic injection of the TRAIL sensitive renal cancer cell line, no difference in lung metastases	68
TRAIL $^{-/-}$	defect in negative selection, increased susceptibility to autoimmune diseases: collagen induced arthritis and streptozotocin induced diabetis.	63
TRAIL$^{-/-}$	normal thymocyte negative selection, no sign of autoimmunity in aged TRAIL$^{-/-}$ mice	60
TRAIL $^{-/-}$	partial resistance to Listeria monocytogenes infection, markedly lower bacterial numbers, larger spleens due to less apoptosis during infection	48
TRAIL-R $^{-/-}$	increased clearance of MCMV, increased levels of IL-12, IFN-α, IFN-γ, enhanced macrophage cytokine production	29
TRAIL-R $^{-/-}$	no spontaneous tumor development, no difference in p53$^{-/-}$ induced lymphomas and APC$^{+/min}$ induced intestinal adenoma development	71
TRAIL-R $^{-/-}$	reduced tissue apoptosis after radiation in thymus, spleen, Peyers patches and white matter of the brain	30
TRAIL $^{-/-}$	more lymphoid malignancies in aged mice, increased mixed sarcomas and lymphoid malignancies in the p53$^{+/-}$ background, no differences in Her2/neu crossbred mice	70
TRAIL $^{-/-}$	increased severity of EAE in remitting and non-remitting disease	64
TRAIL $^{-/-}$	increased sensitivity to CD95 induced liver damage	87
TRAIL $^{-/-}$	no influence in antigen specific CD8^{+} T-cell response after LCMV infection, "helpless" CD8^{+} T cells prolong their memory function (i.e. undergo secondary expansion)	55

The main roles of the TRAIL/TRAIL-R system were found in the immune system. This was not surprising as it was already suggested by the inducible expression of TRAIL in immune cells. TRAIL has been shown to influence infections and to contribute to the development of autoimmune diseases. TRAIL is also implicated in the immune surveillance against tumors and metastasis. These findings are now discussed below in detail.

3.1. Role of TRAIL in the immune system

3.1.1. TRAIL in innate immunity

TRAIL is found to be expressed on a variety of innate immune cells in a stimulation-dependent manner. Monocytes for example express mainly soluble but also surface-bound TRAIL upon stimulation with LPS, and type I and type II interferons and show killing activity against tumor cell lines (31, 32).

Plasmacytoid dendritic cells (DCs), the main players which link innate and adaptive immunity, can also express TRAIL after stimulation with different agents like e.g. oligodeoxynucleotides (ODN) containing the CpG motif. Activation of DCs leads to IFN-α production which stimulates TRAIL expression (33). In addition, IFN-β stimulation of DCs can lead to TRAIL surface expression, thereby enhancing the direct cytotoxic activity of these DCs against tumor cells (34).

Recently, a new subtype of DCs was identified which can produce IFN-γ and has cytolytic activity. These cells were hence called IFN-γ-producing killer dendritic cells (IKDCs) (35, 36). Their killer potential has been shown to be in part due to the IFN-γ-mediated expression of TRAIL (36).

Importantly, immature NK cells express TRAIL on their cell surface after stimulation with IFN-γ. TRAIL together with CD95 and perforin is responsible for most of the cytotoxic effect of NK cells (37). Liver NK cells express TRAIL constitutively as they produce IFN-γ in an autocrine fashion (38). Also cytokines like IL-2 and IL-15 induce human or mouse NK cells to upregulate TRAIL on their surface (39-41). Furthermore, activated NK cells are resistant to TRAIL-induced apoptosis even though they express TRAIL-R1 and -R2 on their surface (42).

Recently, it was shown that fetal and neonatal mice have mostly immature NK cells which are TRAIL-positive in the liver and spleen, while adult mice only have a sub-population of TRAIL-expressing immature NK cells in the liver. Takeda *et al.* therefore suggest that TRAIL^{-} NK cells develop from TRAIL^{+} NK precursor cells. Thus, TRAIL expression may be a marker for immature NK cells**.** In contrast, TRAIL-positive NK cells in the adult liver do not change their phenotype (43). One intriguing possible role of an early stage TRAIL expressing NK cell could be the elimination of immature DCs, as immature DCs are sensitive to TRAIL-induced apoptosis (44, 45).

3.1.2. TRAIL in infectious diseases

Many cell types, which are involved in the fight against infections, e.g. NK cells and cytotoxic T-cells (CTLs) express TRAIL when they are activated and, at least in part, exert their killer function via TRAIL. The first *in vivo* demonstration for a direct role of TRAIL in viral infections was performed by Sato *et al*. in 2001. Mice infected with encephalomyocarditis virus (EMCV) had higher viral titers and died significantly earlier after treatment with TRAIL-blocking antibodies. The main source for TRAIL seemed to be NK cells, because NK cell depletion resulted in a similar reduction of EMCV load as TRAIL inhibition. Since no additive effect was found by blocking TRAIL and depleting NK cells, TRAIL was in fact identified as the main functional killing molecule employed by NK cells to fight EMCV infection (46).

Type I and Type II interferons are the main mediators of this TRAIL effect by inducing TRAIL expression on immune cells and, on the other hand, by sensitizing usually resistant cells for TRAIL. This was shown by Sedger *et al.* who suggested that TRAIL may play a direct role in the defense against viral infections by showing that fibroblasts, which are primarily TRAIL-

resistant, were rendered TRAIL sensitive by infection with human cytomegalovirus (HCMV) (47).

The role of TRAIL-R in immune responses to different infections was intensively studied by Diehl *et al*. (29). TRAIL-$R^{-/-}$ mice and wild type littermates were challenged with a variety of pathogens: *Listeria monocytogenes*, *Salmonella typhimurium*, encephalomyocarditis virus (EMCV), *Mycobacterium bovis,* Bacillus Calmette-Guérin (BCG) and murine cytomegalovirus (MCMV). The only difference was found in the enhanced resistance of TRAIL-$R^{-/-}$ mice against MCMV. This finding appears to be counterintuitive since previous results, as discussed above, suggested that when virus-infected cells could not be cleared via TRAIL-induced apoptosis the negative effects of infection should rather be increased. Interestingly, serum IL-12 and IFN-γ levels were elevated in TRAIL-$R^{-/-}$ mice 24h after infection. IL-12 was presumably produced by DCs which then in turn could induce IFN-γ production by NK cells. In addition, macrophages and DCs derived from TRAIL-$R^{-/-}$ mice showed increased production of TNFα and IL-12 after stimulation with different Toll-like receptor stimuli (29). Thus, the presence of TRAIL-R on these antigen-presenting cells seems to exert a regulatory function.

These results go in line with another study in which ameliorated *Listeria monocytogenes* infection and reduced apoptosis could be found in $TRAIL^{-/-}$ mice (48). An enhanced innate immune response could also account for the role of TRAIL in this model even if the overall results differ in both studies, possibly due to differences with respect to the immunological requirements to successfully cope with a specific pathogenic challenge (29, 48). Yet, the mechanism behind this increased cytokine production are still elusive. As Diehl *et al.* did not find any differences in the numbers of various immune cell populations, a gross effect on the composition of the immunologic cells can be excluded and the individual ornament of the lymphocytes will have to be studied in detail.

3.1.3. TRAIL in the adaptive immune system

Also T cells show TRAIL expression after appropriate stimulation, while it is absent in naive T cells. For instance, human and mouse T cells express TRAIL on their surface when stimulated with anti-CD3 and type I interferons (37, 49, 50). The same was true for freshly isolated human T-cells when stimulated with phytohemagglutinin (PHA) and IL-2 or when activated with LPS which also shows a type-I interferon dependency (31).

Recent studies suggest a physiological role for the TRAIL/TRAIL-R system in T cells, particularly in "helpless" $CD8^+$ cytotoxic lymphocytes (CTLs). These "helpless" CTLs are primed in the absence of $CD4^+$ T cells. Therefore they do not undergo a second round of clonal expansion when restimulated by their cognate antigen (51, 52). Janssen *et al.* showed that without help by $CD4^+$ T cells during the primary response $CD8^+$ T cells can undergo the secondary expansion when TRAIL is absent (53). The usual phenotype of helpless $CD8^+$ T cells might therefore be due to an activation-dependent death mediated by TRAIL.

Interestingly, Hamilton and others could strengthen a role of TRAIL in helpless $CD8^+$ T cells in a homeostatic proliferation setting. This occurs for example in neonates or lymphopenics. *Leishmenia monooxogynes* specific for the OVA peptide could be cleared in the lymphopenic setting only in the presence of help provided by $CD4^+$ T cells. Also here, TRAIL deficiency could restore secondary proliferation in the "helpless" case (54).

However, another study claimed that TRAIL only delayed but not completely abolished the deletion of "helpless" $CD8^+$ T-cells (55), suggesting that the regulation of "helplessness" might be more complicated than initially thought (53, 54).

The TRAIL/TRAIL-R system also seems to be involved in the regulation of T_H1 and T_H2 responses (56, 57). Upon activation with anti-CD3 in-vitro-differentiated T_H2 but not T_H1 cells upregulated TRAIL. In addition, T_H2 cells were more resistant to TRAIL-induced apoptosis than T_H1 cells, possibly due to increased expression of $FLIP_L$ after anti-CD3 stimulation.

A function of the TRAIL/TRAIL-R system in thymic negative selection was first proposed by Lamhamedi-Cherradi *et al.* in 2003. The main fact which raised this theory was that TRAIL-deficient mice are more susceptible to autoimmune diseases (58, 59). However, using four different models Cretney *et al.* convincingly showed that there is no role of the TRAIL/TRAIL-R system in thymic negative selection (29, 60). In addition, it now seems highly unlikely that negative selection involves apoptosis induction by any death receptor since transgenic mice which express a dominant-negative version of FADD under a T-cell specific promoter did not show any defect in negative selection (61).

3.1.4. TRAIL in autoimmune diseases

Autoimmune diseases result from the inappropriate immune recognition of self-antigens. Some of the most dangerous players in these diseases are auto-reactive T-lymphocytes which attack the body's own cells and elicit an immune response without an actual infection. Despite the fact that no spontaneous autoimmune diseases could be observed in TRAIL- and TRAIL-R-deficient mice, many studies have identified profound effects when autoimmunity was induced in $TRAIL^{-/-}$ mice or in the presence of TRAIL or TRAIL-R-blocking agents.

Multiple sclerosis (MS) is characterized by the infiltration of immune cells into the CNS (central nervous system) and subsequent destruction of the myelin around axons which are formed by oligodendrocytes. An often used mouse model which mimics this disease by an artificial immunization with myelin derived antigens or peptides, is experimental autoimmune encephalomyelitis (EAE).

Table 2. The role of the TRAIL/TRAIL receptor system in tumorigenesis

Tumor type	*TRAIL or TRAIL-R knockout or knockdown*	*Effect*	*References*
The TRAIL/TRAIL-R system plays a role:			
Xenograft of TRAIL-sensitive human cell lines	Injection of TRAIL-R agonists (e.g. recombinant TRAIL)	various tumor cell lines from different tissues, e.g. colonic HCT116 and mammary MDA-231, showed increased tumor growth when injected into SCID mice	7,3
Injection of syngeneic TRAIL-sensitive cell lines	Neutralizing TRAIL-antibodies and TRAIL $^{-/-}$ mice	e.g. the mammary carcinoma cell line 4T1 and renal Renca cells showed increased tumor growth and experimental metastasis formation	69, 27
Fibrosarcomas	Injection of MCA into TRAIL $^{-/-}$ mice	increased frequency of fibrosarcomas	27
Spontaneous lymphomas	TRAIL $^{-/-}$ mice	aged TRAIL$^{-/-}$ mice show more lymphomas at the age of 300-500 days, p53$^{+/-}$ mice show more lymphoid malignancies in the TRAIL$^{-/-}$ background	70
Sarcomas	Injection of TRAIL neutralizing antibody And TRAIL $^{-/-}$ mice	more mixed sarcomas in TRAIL $^{-/-}$ p53 $^{+/-}$ mice	70
The TRAIL/TRAIL-R system does not play a role:			
Spontaneous tumors	Young TRAIL and TRAIL-R $^{-/-}$ mice	no spontaneous incidence of any tumors in young mice	27-29
Intestinal adenomas	TRAIL-R $^{-/-}$ mice	no difference in tumor development in APC$^{+/min}$ mice	71
Mammary carcinomas	TRAIL $^{-/-}$ mice	no difference in tumor development in Her2/neu overexpressing mice	70
Lymphomas	TRAIL-R $^{-/-}$ mice	no difference in p53$^{-/-}$ induced lymphomas	71

Studies with TRAIL-blocking antibodies and TRAIL-deficient mice showed that TRAIL is mostly involved in preventing disease progression by inhibiting the autoreactive immune response (62-64). Systemic blockage of TRAIL led to a severe increase of diverse disease phenotypes which pointed to a role for TRAIL in regulating immune cell homeostasis and autoimmune reactions. In contrast, Aktas *et al.* found the opposite when they blocked TRAIL directly in the CNS by injecting TRAIL-R2-Fc intracisternally into mice in which EAE had been induced. Here, inhibition of TRAIL could nearly prevent the disease (65). Therefore, the TRAIL/TRAIL-R system might have a dual role in MS, on the one hand controlling self-reactive immune cells in the periphery and on the other hand by contributing to the irreversible CNS damage during neuroinflammation and the killing of oligodendrocytes and neurons in the CNS.

Another experimental autoimmune disease is EAT (experimental autoimmune thyroiditis). CBA/J mice are immunized with murine thyroglobulin, and then spleen cells from these mice are subsequently injected into irradiated recipient CBA/J mice which develop EAT after ten days. Systemic treatment of mice with recombinant TRAIL decreased apoptosis of thyroid cells. Thus again, the effect of systemic TRAIL treatment was preventive rather than exacerbating the disease. The authors attributed this effect to reduced IFN-γ levels which in turn reduced the T_H1 response (66). In fact, here a deletion of autoreactive T cells was achieved by TRAIL treatment. The same was true in autoimmune diabetes induced by a low dose of streptozotocin in TRAIL-deficient mice. An increase in diabetes incidence could be observed (63). Also soluble TRAIL-R2-Fc which blocked TRAIL in non-obese-diabetic (NOD) mice exacerbated the development of type-I diabetes (58, 59).

The same groups also induced arthritis by different stimuli. TRAIL-deficient mice developed the typical symptoms when treated with collagen whereas wildtype C57BL/6 mice were not susceptible (63). As outlined above, the first explanations for this suppressive function of TRAIL on autoimmunity involved the possible role of TRAIL in negative selection. However, since this does not seem to be the case (60), it remains to be shown how TRAIL and its receptor regulate innate versus adaptive immunity with such profound consequences for development and suppression of arthritis.

3.2. TRAIL in tumorigenesis and cancer surveillance

The first hint that TRAIL may play an important role in tumorigenesis were based on the finding that soluble recombinant TRAIL showed a reduction of tumor growth against human TRAIL-sensitive tumor xenografts in SCID mice (3, 7). Next, Sedger *et al*. could show that a syngeneic tumor transplant of a B cell lymphoma line grows much faster in the absence of TRAIL and leads to more liver metastasis (28). Also Cretney *et al.* found increased tumor growth and experimental metastasis incidence by using TRAIL-sensitive Renca, mouse renal cancer cells, and 4T1 mouse mammary carcinoma cell lines in TRAIL-deficient mice or mice treated with a TRAIL-neutralizing antibody (27, 38, 67, 68).

This tumor prevention was IFN-γ-dependent and was independent of perforin. IL-12 also influences the anti-metastatic activity of NK cells (68). This activity of IL-12 depends on activation of NK cells which results in IFN-γ production and in turn induces the production of TRAIL. NKT cells which are selectively activated by α-galactosylceramide (α-GalCer) significantly reduced the numbers of experimental Renca and 4T1 liver metastases. Also this effect was dependent on TRAIL (27, 69).

Even so, the role of the TRAIL/TRAIL-R system seems to be more complicated, especially in the light of several studies using autochthonous tumor models, which are induced or are developing spontaneously. A summary of tumor models which addressed the role of the TRAIL/TRAIL-R system in tumor surveillance is presented in Table 2.

TRAIL$^{-/-}$ and TRAIL-R$^{-/-}$ mice do not spontaneously develop tumors at early age (27-29). Yet,

TRAIL-deficient mice develop more lymphomas when they are aged after 500 days (70). When tumors were induced with the carcinogen MCA (methyocholanthrene) an increased frequency of fibrosarcomas was found in TRAIL$^{-/-}$ mice (27).

In contrast, in a number of experimental settings no tumor-suppressive function for TRAIL could be found. Deletion of TRAIL-R does not effect the formation of intestinal adenomas in *Apc*$^{Min/+}$ transgenic mice (71). Furthermore, the presence of TRAIL does not delay the onset of mammary carcinogenesis in Her2/neu-transgenic mice despite the fact that these mammary tumors expressed MHC class I and were TRAIL-sensitive (70).

The results of an inhibited TRAIL/TRAIL-R system on spontaneous tumor induction by loss of p53 are controversial. p53$^{+/-}$ animals primarily develop sarcomas, whereas p53$^{-/-}$ mice develop lymphomas. The first experiments with TRAIL-neutralizing antibodies in p53$^{+/-}$ mice showed a promoting effect in the induction of sarcomas (67). This result could be confirmed by using TRAIL-deficient p53$^{+/-}$ mice, which develop more sarcomas and lymphomas (70). But this was not the same when TRAIL-R deficient mice were crossed to p53$^{-/-}$ mice, because no increase in the frequencies of lymphomas or thymomas was found (71). The discrepancy between these studies could either be due to unknown effects of the TRAIL-blocking antibodies or due to different tumor types. TRAIL may therefore be more important in the control of p53-dependent non-lymphoid tumors, but this suggestion is inconsistent with the role of TRAIL in hematological malignancies (70). One may speculate that the tissue in which the tumor occurs and the presence of TRAIL expressing cells within this tissue may be important. At any rate, further investigation, especially in models of autochthonous tumor development, will be needed to shed some light on the role of TRAIL in tumorigenesis.

Strikingly, TRAIL can even exert a metastasis promoting function in TRAIL-resistant cells. This resistance to TRAIL-induced apoptosis can be achieved by high expression of FLIP, XIAP, anti-apoptotic members of the Bcl-2 family or down-regulation of pro-apoptotic members of this family (72-75). Resistance to apoptosis can unmask other TRAIL-induced signals which can lead to alternative functions of this protein like the induction of survival and invasion pathways (76).
In the case of human pancreatic ductal adenocarcinoma which express Bcl-xL at extremely high levels, TRAIL induces a strong increase in the number and volume of metastases in a xenograft model using SCID mice. The authors attribute this effect to the induction of survival pathways induced by TRAIL via upregulation of IL-8 and monocyte chemoattractant protein (MCP). In another study, TRAIL could enhance the invasive phenotype of cholangiocarcinomas (77). These studies are supported by the finding that in non-transformed cells as vascular smooth muscle cells or endothelial cells TRAIL can promote a significant increase in proliferation and migration (78, 79). Given these data, concerns that TRAIL therapy might also induce a metastatic potential have to be taken into account when planning the clinical application of agonists of the two TRAIL death receptors.

Even if it is still unclear under which circumstances the TRAIL/TRAIL-R system plays a role in tumorigenesis, especially when tumor cells are TRAIL-resistant, a recent study pointed out how TRAIL-R agonists may be used in an combinatory immunotherapy. Uno *et al.* used a triple antibody therapy comprising agonistic TRAIL-R-, CD40- and CD137 (4-1BB)-specific antibodies. The use of anti–mouse-TRAIL-R antibody MD5-1 had already previously been shown to be active against TRAIL-sensitive syngeneic tumors and experimental metastasis in immunocompetent mice without systemic toxicity (69).
Agonistic CD40- and CD137-specific monoclonal antibodies, which stimulate cytotoxic T lymphocyte (CTL)-mediated anti-tumor effects by stimulating antigen-presenting cells (APCs) and costimulating T-cells together with MD5-1 antibodies, induced the rejection of established subcutaneous syngeneic 4T1 mammary tumors in mice. Most strikingly, this triple antibody therapy caused rejection of co-established apoptosis-resistant tumors, suggesting a crucial requirement of TRAIL-R-mediated apoptosis and activated CTLs cells for tumor immunity and complete tumor cell eradication, i.e. also of TRAIL-resistant tumor cells, in the context of this therapy (80).

In summary, the TRAIL/TRAIL-R system does not seem to play a general role in tumor suppression as most studies failed to show such a role especially in the autochthonous background. Thus, involvement of the TRAIL/TRAIL-R system in early tumorgenicity might be tissue-specific, but could also depend on the presence of TRAIL-expressing immune cells. Further studies which examine all steps in autochthonous tumorigenesis and also examine metastasis formation are now needed to reveal a potential role of the TRAIL/TRAIL-R system in the prevention of tumors and/or metastasis and, consequently, to lead the way to the best possible therapeutical use of TRAIL-R agonists.

4. TRAIL AS A THERAPEUTIC AGENT

A number of biotech and pharmaceutical companies develop therapeutic agents designed to activate the death program in cancer cells. TRAIL-receptor-targeted therapies are currently being pursued in clinical studies by at least four different companies: Human Genome Sciences (HGS), Daiichi Sankyo, Genentech and Amgen. Some of these studies have already shown very promising results (Table 3).

Recombinant TRAIL/Apo2L as well as humanized agonistic mAb targeting TRAIL-R1 or TRAIL-R2 are currently being evaluated in Phase I and Phase II clinical trials.

HGS has completed three Phase II clinical trials of HGS-ETR1, a fully humanized agonistic antibody against TRAIL-R1, as monotherapy in heavily pretreated patients with non-Hodgkin's-Lymphoma (NHL), colorectal cancer and non-small cell lung cancer (NSCLC). The

Table 3. Therapeutic approaches targeting the two apoptosis inducing human TRAIL-Rs in cancer therapy

Molecule	*Companies*	*Description*	*Clinical trial status*
Single agent			
HGS-ETR1 (Mapatumumab)	Human Genome Sciences	humanized anti-TRAIL-R1 agonistic mAb	Phase II completed: NHL, colorectal cancer, NSCLC
HGS-ETR2	Human Genome Sciences	humanized anti-TRAIL-R2 agonistic mAb	Phase I: advanced solid tumors
HGS-TR2J	Human Genome Sciences	humanized anti-TRAIL-R2 agonistic mAb	Phase I: advanced solid tumors
TRA-8 (CS-1008)	Daiichi Sankyo Inc.	humanized anti-TRAIL-R2 mAb	Phase I: advanced solid tumors and lymphomas (not yet recruiting)
Apo2L/TRAIL (AMG 951)	Genentech/ Amgen	soluble TRAIL, activates TRAIL-R1 and TRAIL-R2	Phase Ib
AMG 655	Amgen	humanized anti-TRAIL-R2 agonistic mAb	Phase I: (initiated in 2005)
Combination with Chemotherapy			
HGS-ETR1 + Paclitaxel + Carboplatin	Human Genome Sciences	humanized anti-TRAIL-R1 agonistic mAb + chemotherapy	Phase Ib: advanced solid tumors
HGS-ETR1 + Gemcitabine + Cisplatin	Human Genome Sciences	humanized anti-TRAIL-R1 agonistic mAb + chemotherapy	Phase Ib: advanced solid tumors
HGS-ETR1 + Bortezombib (Velcade®)	Human Genome Sciences	humanized anti-TRAIL-R1 mAb + proteasome inhibitor	Phase II: advanced MM (recruiting since Oct. 2006)
Apo2L/TRAIL + Rituximab	Genentech/ Amgen	soluble TRAIL that activates TRAIL-R1 and TRAIL-R2 + anti-C20	Phase Ib/II: NHL (recruiting since June 2006)

results of these trials show that HGS-ETR1 is well tolerated and HGS-ETR1 could be administered safely and repetitively. No dose-limiting toxicities were observed up to the highest dose tested (10mg/kg). Stable disease could be found in 29% of the patients who participated in the NSCLC study and in 32% of the patients that participated in the colorectal cancer study. Clinical response or stable disease was detected in 14/17 patients with NHL diagnosed with follicular lymphomas.

Preclinical studies and a large number of in vitro studies demonstrate that many chemotherapeutics and radiation can sensitize cancer cells for TRAIL-induced apoptosis. TRAIL/Apo2L acts synergistically when combined with chemotherapeutics (e.g. etoposide, cisplatin, irinotecan or oxaliplatin), proteasome inhibitors (MG321 and bortezomib) and γ-irradiation (81-85). The mechanisms conferring this synergy include enhanced DISC formation capacity, death receptor upregulation, modulation of Bcl-2 family members, caspase upregulation and inhibition of IAP family members or downregulation of c-FLIP (84). Therefore the combinatorial treatment with TRAIL-receptor agonists plus chemotherapeutics and/or radiotherapy can be regarded as one of the most promising new strategies to treat cancer in the future. Recently HGS has initiated two Phase Ib trials evaluating the safety and tolerability of HGS-ETR1 in combination with chemotherapeutic agents (Paclitaxel + Carboplatin and Gemcitabine + Cisplatin) in the treatment of patients with advanced solid tumors. Interim results of the Phase Ib studies demonstrate that the combination of HGS-ETR1 + chemotherapeutics are well tolerated and HGS-ETR1 can be administered safely and repetitively at doses up to 20 mg/kg intravenously. Furthermore HGS is currently recruiting patients with advanced multiple myeloma (MM) to investigate the efficacy and safety of HGS-ETR1 in combination with the proteasome inhibitor bortezomib compared to bortezomib alone. Preliminary results have recently been announced by HGS showing no toxicity when HGS-ETR1 is applied together with chemotherapeutics.

The theoretical advantage of using TRAIL/Apo2L as a bio-therapeutic agent is that it targets both death receptors, TRAIL-R1 and TRAIL-R2. Yet the group of MacFarlane *et al.* has recently synthesized mutant forms of TRAIL which selectively bind to either TRAIL-R1 or TRAIL-R2 (86). They could show that different tumors signal apoptosis either through TRAIL-R1 or TRAIL-R2. Therefore it is important to determine whether primary tumor cells signal via TRAIL-R1 or TRAIL-R2 to target the right receptor when applying TRAIL-receptor targeted therapy.

The safety and efficacy of TRAIL/Apo2L is currently evaluated in a Phase Ib trial conducted by Genentech and Amgen. Furthermore, a PhaseIb/II trial by Genentech/Amgen is currently recruiting rituximab-refractory NHL patients to investigate the efficacy and safety of a combined treatment of rituximab + TRAIL/Apo2L. An important point one has to consider is the difference in the *in vivo* half life of the different TRAIL-R agonists. In non-human primates the ligand TRAIL/Apo2L has a plasma half-life which is drastically shorter ($t_{1/2}$ ~30min) than the half-life of the agonistic antibodies ($t_{1/2}$ of serum IgG is ~21 days) which has to be taken into account when evaluating the efficacy of TRAIL-Receptor targeted therapy. An increased plasma half-life might improve localization to the target and reduces the frequency of administration to the patient. But despite the rapid renal clearance and therefore reduced plasma half-life of TRAIL/Apo2L, high anti-tumor activity is observed *in vivo,* pointing to a significant tumor penetration of TRAIL/Apo2L (84), a possible advantage of the cytokine over monoclonal antibodies.

In summary, a large body of preclinical evidence has now been gathered on the potential of TRAIL and anti-TRAIL-R agonistic mAbs as novel therapeutic agents for the treatment of cancer. Given the resulting clinical development programs initiated in several biotech and pharmaceutical companies, there is abundant optimism that TRAIL-R-targeted therapies will prove to be effective for patients suffering from at least some types of cancer. Success of TRAIL-R agonists as stand-alone therapy will

most likely be limited, since most primary tumors seem to be TRAIL-resistant. Thus, combinatorial treatment protocols which comprise TRAIL-R agonists together with other therapeutic approaches will lead the way to future to cancer therapy.

5. PERSPECTIVE

The last decade of research on TRAIL has revealed that this cytokine is truly an interesting molecule with a multitude of functions in both cancer and immunity. Despite the fact that much progress has been made with regard to understanding TRAIL signaling and its crosstalk with other signaling pathways as well as to its role in the immune homeostasis, autoimmunity, cancer immune surveillance and tumor suppression, it seems as if we have only discovered the tip of the iceberg so far. It will be fascinating to dive deeper into the biology of this intriguing cytokine to further unravel its function hopefully culminating the identification of better treatments for both autoimmune diseases and cancer.

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Send correspondence to: Henning Walczak, Ph.D., Division of Apoptosis Regulation (D040), Tumor Immunology Program, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany, Tel: 49-69221-423700, Fax : 49-69221-423699, E-mail : h.walczak@dkfz-heidelberg.de

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