TRAIL: not just for tumors anymore?

Chris A. Benedict and Carl F. Ware

Since the discovery of TNF-related apoptosis-inducing ligand (TRAIL) and its network of receptors, the majority of attention has focused on the clinical potential of manipulating this pathway in cancer therapy. However, the widespread expression of TRAIL under inflammatory conditions and the ability to induce both apoptotic and prosurvival signaling pathways has suggested that TRAIL plays broader roles in regulating immune processes. Two new studies now show that expression of TRAIL by neutrophils in the lung facilitates defenses against bacterial pathogens, whereas expression of TRAIL by cells within arterioles exacerbates vascular disease. These differentiating results highlight that the context of TRAIL signaling can determine whether the outcome is beneficial or pathogenic for the host.

TRAIL/Apo2L (*TNFSF10*) was originally identified in searches of EST databases for genes with homology to known TNF superfamily ligands (Wiley et al., 1995; Pitti et al., 1996). In humans, TRAIL binds two proapoptotic death receptors (DRs), TRAIL-R1 and –R2 (TNFRSF10A and 10B), as well as two other membrane receptors that do not induce death and instead may act as decoys for death signaling. Mice encode only a single TRAIL DR, with highest homology to TRAIL-R2, and also several decoy receptors (Schneider et al., 2003). Similar to the DR Fas, TRAIL binding to its cognate DRs induces formation of a death-inducing signaling complex, ultimately leading to caspase activation and initiation of apoptosis (Bodmer et al., 2000). However, a widely held consensus is that only tumor cells are susceptible to TRAIL-mediated apoptosis, although this varies widely depending on the type of cell, whereas nontransformed cells are largely resistant to death mediated by TRAIL DR. Decoy receptors can contribute to resistance to death signaling by competing for TRAIL and/or by activating cell survival pathways via NF-κB, ERK, and p38 (Shirley et al., 2011), and the TRAIL DR can also activate these pathways in certain situations, underscoring the potential of this pathway to regulate inflammation at multiple levels. This nonapoptotic signaling may parallel other DRs such as TNFR-1 and Fas, which can induce inflammation and necroptosis via RIP family kinases (Green et al., 2011; Mocarski et al., 2012). Nevertheless, the rationale for targeting the TRAIL system in cancer is based on its ability to induce apoptosis. This follows from much work showing that TRAIL preferentially induces apoptosis of transformed human cells and that tumorigenesis and metastasis is enhanced in TRAIL-deficient mice (*Trail*$^{-/-}$; Cretney et al., 2002). Consequently, recombinant TRAIL or agonistic antibodies targeting TRAIL receptors have entered phase I and II clinical trials (Fox et al., 2010; Gerspach et al., 2011). Although patients appear to tolerate these drugs quite well, in general it appears that they will have to be used in combination with additional targeted biologics or chemotherapy regiments to achieve clinical efficacy. This complexity in predicting the outcomes of signaling by DRs of the TNFR superfamily is illustrated in two papers published in this issue of the *JEM*.

Both studies investigate the role of TRAIL-mediated signaling in regulating immune responses in the lung. Steinwede et al. show that neutrophils rapidly recruited to the lung during *Streptococcus pneumoniae* infection release high levels of soluble TRAIL that induce apoptosis of alveolar macrophages, thereby limiting bacterial replication and restricting inflammation and the resulting lung damage (Steinwede et al., 2012). In contrast, work by Hameed et al. reveals that TRAIL can also play a harmful role in the lung. They find that TRAIL promotes disease development in several animal models of pulmonary arterial hypertension (PAH; Hameed et al., 2012). Consequently, the two papers illustrate the proverbial double-edged sword theme common among the TNF-family cytokines: a rapid, proapoptotic role for TRAIL that facilitates host defense versus a sustained, proinflammatory function that contributes to vascular disease. Notably, pharmacological modulation of TRAIL signaling showed significant benefit in both studies, highlighting potential new applications for TRAIL-targeted therapeutics.

The pros of TRAIL-mediated immunity...

A role for TRAIL in promoting host defense to pathogens is not unprecedented. Several studies in mice have shown that TRAIL can promote or hamper immunity to influenza infection (Ishikawa et al., 2005; Herold et al., 2008; Brincks et al., 2011). Interestingly, early replication of influenza in the lung is not increased in the absence of TRAIL, and can even be reduced (Ishikawa et al., 2005), but ultimately virus clearance is significantly delayed. However, whether TRAIL ultimately promotes or

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attenuates inflammation, apoptosis, and lung damage during flu infection is not entirely clear, as differing results have been reported (Herold et al., 2008; Brincks et al., 2011). Paralleling aspects of influenza infection, control of West Nile virus is also normal at early times in *Trail*$^{-/-}$ mice, but clearance from the central nervous system is delayed due to a reduced ability of CD8 T cells to kill infected cells (Shrestha et al., 2012). Better control of the *Listeria* bacterium in the spleen and liver of *Trail*$^{-/-}$ mice is also observed, commensurate with decreased macrophage apoptosis and increased inflammatory cytokine levels (Zheng et al., 2004). The TRAIL-dependent suppression and killing of macrophages that is seen in these infections is likely mediated through its cognate DR. Consistently, *Trail-r*$^{-/-}$ mice show enhanced inflammatory cytokine production and control of the β-herpesvirus cytomegalovirus at early times, likely caused by increased NK cell activation resulting from hyperactivated myeloid cells (Diehl et al., 2004). Consequently, pathogen defenses in the absence of TRAIL or its DR are unhindered in the early stages of infection, or even enhanced, as a result of an increased innate response. However, if infection is not effectively controlled in the initial period, which likely depends on characteristics of the specific pathogen, then adaptive immunity suffers, clearance is delayed, and tissue damage is increased.

A new piece of this rather complicated mechanistic puzzle has now been added by the work of Steinwede et al. (2012). These authors show that during *S. pneumoniae* infection of mice, neutrophils that are rapidly recruited to the lung express high levels of TRAIL, which is released in soluble form into the airways. Secreted TRAIL then promotes the apoptosis of alveolar macrophages within the first 24 h of infection (Fig. 1 A). Alveolar macrophages are one of the first cell types infected by *S. pneumoniae*, and their rapid death is required to limit bacterial replication and pathogenesis (Marriott et al., 2006). Macrophages in the lungs of *Trail*$^{-/-}$ mice showed much less apoptosis, and instead appeared to die more frequently by necrosis. This "switch" in the amount and apparent mechanism of macrophage death at this very early time in mice lacking TRAIL resulted in higher levels of *S. pneumoniae* replication in the lung, more lung damage due to inflammatory cytokines, and increased mortality. Interestingly, neutrophils themselves are subject to TRAIL-dependent apoptosis in models of LPS-induced lung inflammation/damage (McGrath et al., 2011), but this was not observed in *Trail*$^{-/-}$ mice infected with *S. pneumoniae*. Depletion of neutrophils during *S. pneumoniae* lung infection resulted in substantially decreased levels of soluble TRAIL, supporting the claim that neutrophils are the major source of TRAIL in the lung in this model. Moreover, macrophage apoptosis in the airways decreased after neutrophil depletion, although the impact of this on *S. pneumoniae* infection was not evaluated. The clinically relevant observation of this study is the demonstration that administration of recombinant TRAIL to wild-type mice carrying a heavy burden of invasive *S. pneumoniae* improved lung function and reduced pathology, resulting from enhanced macrophage apoptosis, and decreased bacterial loads. Similar enhanced resistance was also seen when an agonistic antibody to the mouse TRAIL DR was administered. This approach was effective even in mice depleted of neutrophils, suggesting therapeutic potential of targeting the TRAIL system in immune-compromised patients suffering from severe *S. pneumoniae* infection.

....and the cons

The study by Hameed et al. (2012) shows that TRAIL signaling does not always benefit the host. In several rodent models of PAH, TRAIL expression promoted the progression of PAH, and blocking TRAIL ameliorated disease. PAH is a debilitating vascular disease that affects ∼1–5 in 100,000 persons in the United States, preferentially impacting females (∼4:1), in which vasoconstriction of distal lung arterioles leads to increased arterial pressure and eventual death resulting from right ventricular failure of the heart (Frumkin, 2012). It is largely accepted that chronic inflammation plays a key role in PAH pathogenesis, likely promoting the proliferation of vascular smooth muscle cells that contributes to occlusion (Price et al., 2012). Familial PAH results from mutations in the bone morphogenic type II receptor (Newman et al., 2001), a member of the TGF-β family, further supporting a contributing role for inflammation. However, the nature of the effector cells, cytokines, and signaling pathways that initiate PAH remain unclear.

Hameed et al. (2012) show that in a toxin-induced model of PAH in rats, TRAIL expression is enhanced in lung endothelial and epithelial cells at times corresponding to the peak of vascular remodeling and increased right ventricular blood pressure, and administration of a TRAIL-blocking antibody dramatically reduced pulmonary hypertension. Mice exposed to several weeks of hypoxic conditions also develop a PAH-like disease, and the pathology in this model was markedly reduced in *Trail*$^{-/-}$ mice. Work from this group showed that TRAIL also regulates vascular disease in atherosclerosis-susceptible *Apoe*$^{-/-}$ mice. These mice develop an IL-1–dependent PAH-like disease when fed an intermediate fat diet (Lawrie et al., 2011), which is abolished when they are crossed to *Trail*$^{-/-}$ mice. Supporting a key role for TRAIL expression by lung epithelial or endothelial cells in this model, bone marrow chimeras generated with *Apoe*$^{-/-}$/*Trail*$^{-/-}$ mice indicated that TRAIL expression by nonhematopoietic cells was required. Interestingly, in two of these animal models, intact TRAIL signaling correlated with increased smooth muscle cell proliferation and lung remodeling and with reduced numbers of apoptotic cells in the small arteries. Administering a blocking TRAIL antibody in both the rat and mouse models reduced established lung pathology, suggesting a potential therapy in human lung disease. Collectively, the authors put forward a model whereby TRAIL expression in endothelial or epithelial cells of the lung vasculature promotes the proliferation of smooth muscle cells, remodeling of the small arteries and arterioles, and the development of PAH (Fig. 1 B).

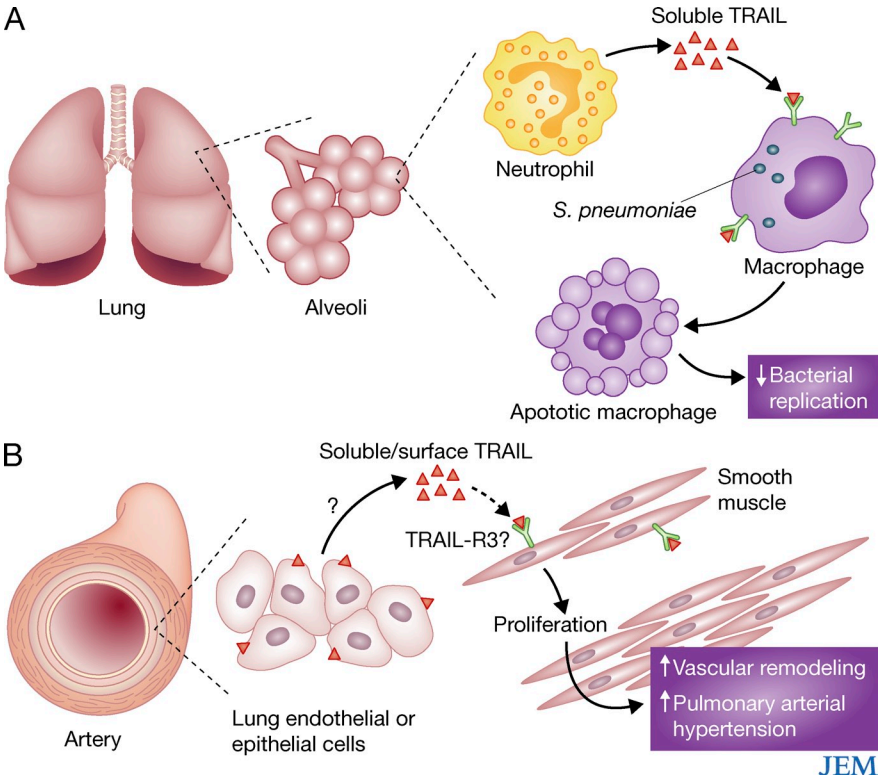

Figure 1. Protective and pathogenic roles for TRAIL signaling. (A) During infection with *S. pneumoniae*, neutrophils that migrate to the lungs produce soluble TRAIL, which then promotes apoptosis of infected alveolar macrophages. Macrophage apoptosis limits bacterial spread and restricts the magnitude and duration of pathology-inducing inflammation. (B) TRAIL (soluble or membrane-bound) produced by structural cells in small arteries induces the proliferation of smooth muscle cells, possibly via TRAIL-R3, and may also promote the survival of other cells in the arterioles, resulting in increased vascular remodeling and PAH.

What factors differentiate the nature of TRAIL signaling in these two studies that ultimately result in either benefit or detriment for the host? First, TRAIL expression in the context of PAH is likely sustained for a longer time period than during *S. pneumoniae* infection. Consequently, chronic activation of proinflammatory TRAIL signaling pathways, as opposed to proapoptotic signaling, may be a key underlying difference. TRAIL induces proliferation, but not apoptosis, of human smooth muscle cells via activation of NF-κB (Secchiero et al., 2004; Kavurma et al., 2008). In contrast, tumor or pathogen-infected cells oftentimes die in response to TRAIL. This is exemplified by the sensitivity of lung-resident macrophages to TRAIL-induced apoptosis during *S. pneumoniae* infection. What, precisely, induces macrophage sensitivity remains an open question. Interestingly, TRAIL-dependent killing of macrophages has also been observed during HIV infection (Laforge et al., 2011; Zhu et al., 2011). Whether TRAIL is soluble or cell-associated, which cell type is exposed to TRAIL, and whether TRAIL decoy receptors contribute in these two scenarios are also likely to be key determinants. Increased expression of TRAIL-R3 (a gpi-linked decoy receptor for TRAIL) was observed in smooth muscle cells isolated from PAH patients in the Hameed et al. (2012) study, and its blockade modestly reduced TRAIL-induced proliferation. However, the operable mechanisms for this decoy are not known.

Together these studies reveal TRAIL and its receptors as a complex, multicomponent signaling network, a conserved theme in studies of the TNF superfamily. Here, related cytokine networks, including the BAFF-APRIL and TNF-LT-LIGHT networks (Ware, 2005), also function as double-edged swords to coordinate host defense and homeostasis.

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REFERENCES

Bodmer, J.L., N. Holler, S. Reynard, P. Vinciguerra, P. Schneider, P. Juo, J. Blenis, and J. Tschopp. 2000. TRAIL receptor-2 signals apoptosis through FADD and caspase-8. *Nat. Cell Biol.* 2:241–243. http://dx.doi.org/10.1038/35008667

Brincks, E.L., P. Gurung, R.A. Langlois, E.A. Hemann, K.L. Legge, and T.S. Griffith. 2011. The magnitude of the T cell response to a clinically significant dose of influenza virus is regulated by TRAIL. *J. Immunol.* 187:4581–4588. http://dx.doi.org/10.4049/jimmunol.1002241

Cretney, E., K. Takeda, H. Yagita, M. Glaccum, J.J. Peschon, and M.J. Smyth. 2002. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. *J. Immunol.* 168:1356–1361.

Diehl, G.E., H.H. Yue, K. Hsieh, A.A. Kuang, M. Ho, L.A. Morici, L.L. Lenz, D. Cado, L.W. Riley, and A. Winoto. 2004. TRAIL-R as a negative regulator of innate immune cell responses. *Immunity*. 21:877–889. http://dx.doi.org/10.1016/j.immuni.2004.11.008

Fox, N.L., R. Humphreys, T.A. Luster, J. Klein, and G. Gallant. 2010. Tumor Necrosis Factor-related apoptosis-inducing ligand (TRAIL) Receptor-1 and Receptor-2 agonists for cancer therapy. *Expert Opin. Biol. Ther.* 10:1–18. http://dx.doi.org/10.1517/14712590903319656

Frumkin, L.R. 2012. The pharmacological treatment of pulmonary arterial hypertension. *Pharmacol. Rev.* 64:583–620. http://dx.doi.org/10.1124/pr.111.005587

Gerspach, J., K. Pfizenmaier, and H. Wajant. 2011. Therapeutic targeting of CD95 and the TRAIL death receptors. *Recent Patents Anticancer. Drug Discov.* 6:294–310. http://dx.doi.org/10.2174/157489211796957739

Green, D.R., A. Oberst, C.P. Dillon, R. Weinlich, and G.S. Salvesen. 2011. RIPK-dependent necrosis and its regulation by caspases: a mystery in five acts. *Mol. Cell.* 44:9–16. http://dx.doi.org/10.1016/j.molcel.2011.09.003

Hameed, A.G., N.D. Arnold, J. Chamberlain, J.A. Pickworth, C. Paiva, S. Dawson, S. Cross, L. Long, L. Zhao, N.W. Morrell, et al. 2012. Inhibition of tumor necrosis factor related apoptosis-inducing ligand (TRAIL) reverses experimental pulomonary hypertension. *J. Exp. Med.* 209:1919–1935.

Herold, S., M. Steinmueller, W. von Wulffen, L. Cakarova, R. Pinto, S. Pleschka, M. Mack, W.A. Kuziel, N. Corazza, T. Brunner, et al. 2008. Lung epithelial apoptosis in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing

ligand. *J. Exp. Med.* 205:3065–3077. http://dx.doi.org/10.1084/jem.20080201

Ishikawa, E., M. Nakazawa, M. Yoshinari, and M. Minami. 2005. Role of tumor necrosis factor-related apoptosis-inducing ligand in immune response to influenza virus infection in mice. *J. Virol.* 79:7658–7663. http://dx.doi.org/10.1128/JVI.79.12.7658-7663.2005

Kavurma, M.M., M. Schoppet, Y.V. Bobryshev, L.M. Khachigian, and M.R. Bennett. 2008. TRAIL stimulates proliferation of vascular smooth muscle cells via activation of NF-kappaB and induction of insulin-like growth factor-1 receptor. *J. Biol. Chem.* 283:7754–7762. http://dx.doi.org/10.1074/jbc.M706927200

Laforge, M., L. Campillo-Gimenez, V. Monceaux, M.C. Cumont, B. Hurtrel, J. Corbeil, J. Zaunders, C. Elbim, and J. Estaquier. 2011. HIV/SIV infection primes monocytes and dendritic cells for apoptosis. *PLoS Pathog.* 7:e1002087. http://dx.doi.org/10.1371/journal.ppat.1002087

Lawrie, A., A.G. Hameed, J. Chamberlain, N. Arnold, A. Kennerley, K. Hopkinson, J. Pickworth, D.G. Kiely, D.C. Crossman, and S.E. Francis. 2011. Paigen diet-fed apolipoprotein E knockout mice develop severe pulmonary hypertension in an interleukin-1-dependent manner. *Am. J. Pathol.* 179:1693–1705. http://dx.doi.org/10.1016/j.ajpath.2011.06.037

Marriott, H.M., P.G. Hellewell, S.S. Cross, P.G. Ince, M.K. Whyte, and D.H. Dockrell. 2006. Decreased alveolar macrophage apoptosis is associated with increased pulmonary inflammation in a murine model of pneumococcal pneumonia. *J. Immunol.* 177:6480–6488.

McGrath, E.E., H.M. Marriott, A. Lawrie, S.E. Francis, I. Sabroe, S.A. Renshaw, D.H. Dockrell, and M.K. Whyte. 2011. TNF-related apoptosis-inducing ligand (TRAIL) regulates inflammatory neutrophil apoptosis and enhances resolution of inflammation. *J. Leukoc. Biol.* 90:855–865. http://dx.doi.org/10.1189/jlb.0211062

Mocarski, E.S., J.W. Upton, and W.J. Kaiser. 2012. Viral infection and the evolution of caspase 8-regulated apoptotic and necrotic death pathways. *Nat. Rev. Immunol.* 12:79–88.

Newman, J.H., L. Wheeler, K.B. Lane, E. Loyd, R. Gaddipati, J.A. Phillips III, and J.E. Loyd. 2001. Mutation in the gene for bone morphogenetic protein receptor II as a cause of primary pulmonary hypertension in a large kindred. *N. Engl. J. Med.* 345:319–324. http://dx.doi.org/10.1056/NEJM200108023450502

Pitti, R.M., S.A. Marsters, S. Ruppert, C.J. Donahue, A. Moore, and A. Ashkenazi. 1996. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J. Biol. Chem.* 271:12687–12690. http://dx.doi.org/10.1074/jbc.271.22.12687

Price, L.C., S.J. Wort, F. Perros, P. Dorfmüller, A. Huertas, D. Montani, S. Cohen-Kaminsky, and M. Humbert. 2012. Inflammation in pulmonary arterial hypertension. *Chest.* 141:210–221. http://dx.doi.org/10.1378/chest.11-0793

Schneider, P., D. Olson, A. Tardivel, B. Browning, A. Lugovskoy, D. Gong, M. Dobles, S. Hertig, K. Hofmann, H. Van Vlijmen, et al. 2003. Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J. Biol. Chem.* 278:5444–5454. http://dx.doi.org/10.1074/jbc.M210783200

Secchiero, P., C. Zerbinati, E. Rimondi, F. Corallini, D. Milani, V. Grill, G. Forti, S. Capitani, and G. Zauli. 2004. TRAIL promotes the survival, migration and proliferation of vascular smooth muscle cells. *Cell. Mol. Life Sci.* 61:1965–1974. http://dx.doi.org/10.1007/s00018-004-4197-6

Shirley, S., A. Morizot, and O. Micheau. 2011. Regulating TRAIL receptor-induced cell death at the membrane : a deadly discussion. *Recent Patents Anticancer. Drug Discov.* 6:311–323. http://dx.doi.org/10.2174/157489211796957757

Shrestha, B., A.K. Pinto, S. Green, I. Bosch, and M.S. Diamond. 2012. CD8+ T cells Use TRAIL to restrict West Nile virus pathogenesis by controlling infection in neurons. *J. Virol.* 86:8937–8948. http://dx.doi.org/10.1128/JVI.00673-12

Steinwede, K., S. Henken, J. Bohling, R. Maus, B. Ueberberg, C. Crumshagen, E.L. Brincks, T.S. Griffith, T. Welte, and U.A. Maus. 2012. TNF-related apoptosis-inducing ligand (TRAIL) exerts therapeutic efficacy for the treatment of pneumococcal pneumoniae in mice. *J. Exp. Med.* 209:1937–1952.

Ware, C.F. 2005. Network communications: lymphotoxins, LIGHT, and TNF. *Annu. Rev. Immunol.* 23:787–819. http://dx.doi.org/10.1146/annurev.immunol.23.021704.115719

Wiley, S.R., K. Schooley, P.J. Smolak, W.S. Din, C.P. Huang, J.K. Nicholl, G.R. Sutherland, T.D. Smith, C. Rauch, C.A. Smith, et al. 1995. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity.* 3:673–682. http://dx.doi.org/10.1016/1074-7613(95)90057-8

Zheng, S.J., J. Jiang, H. Shen, and Y.H. Chen. 2004. Reduced apoptosis and ameliorated listeriosis in TRAIL-null mice. *J. Immunol.* 173:5652–5658.

Zhu, D.M., J. Shi, S. Liu, Y. Liu, and D. Zheng. 2011. HIV infection enhances TRAIL-induced cell death in macrophage by down-regulating decoy receptor expression and generation of reactive oxygen species. *PLoS ONE.* 6:e18291. http://dx.doi.org/10.1371/journal.pone.0018291