Death Receptors and Their Ligands in Atherosclerosis

Mary M. Kavurma, Nicole Y. Tan, Martin R. Bennett

Abstract—Atherosclerosis is characterized by the accumulation of a fibro-fatty plaque consisting of immune cells, vascular smooth muscle cells (VSMCs), vascular endothelial cells (ECs), and extracellular matrix, surrounding a lipid-rich core. The complexity of atherosclerosis is highlighted by the multifaceted effects that apoptosis and proliferation of specific cell types can have on vessels at different stages of the disease. Death receptors are membrane-bound protein complexes that on binding their cognate ligand, activate an intracellular signaling cascade that results in apoptosis. More recently, signaling from these receptors has been shown to activate multiple other processes, including cell proliferation. This review summarizes our current understanding of signaling events after death receptor activation and the role of death receptors and their ligands in atherosclerosis. (Arterioscler Thromb Vasc Biol. 2008;28:1694-1702)

Key Words: apoptosis ■ TNF/TNFR signalling ■ proliferation ■ atherosclerosis

There are currently more than 40 tumor necrosis factor (TNF)-ligand/TNF-receptor (TNFR) members, with biological roles ranging from inflammation, apoptosis, autoimmunity, and organogenesis. TNFR superfamily members are type I transmembrane proteins and can be classified into 3 groups (reviewed in1,2). Members of the first group include receptors such as TNFR1, TNF-related apoptosis-inducing ligand (TRAIL) death receptors (DR) DR4 and DR5, and CD95/Fas. These proteins contain a death domain (DD) within their cytoplasmic tail which provides the capacity for protein–protein interactions with other DD-containing proteins. The second group of proteins include TNFR2 and receptor activator of NFκB (RANK), where the cytoplasmic tail of these proteins do not contain a DD. Instead, these receptors contain TIM domains (TNF receptor-associating protein–protein interaction motifs), which leads to recruitment of TRAF proteins and activation of signal transduction pathways such as NFκB, Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), and p38. The third group of proteins does not contain functional intracellular signaling domains and is termed decoy receptors (DCR). These receptors can compete with their corresponding TNFR for ligand binding and include DcR1, DcR2, and osteoprotegerin (OPG) for TRAIL, and DcR and soluble DcR3 for Fas. TNFRs are characterized by highly conserved cysteine-rich domains (CRD), containing 1 to 4 CRDs within their extracellular domain among the various family members, which are required for ligand binding and self association. TNFRs can form trimers or oligomers on the cell surface, and this oligomerisation defines the potency of subsequent signaling events.1

Most TNF ligands are type II transmembrane proteins (reviewed in1,2) and include TNF (for TNFR1), TRAIL (for TRAIL receptors [TRAIL-Rs]), and Fas ligand (FasL, for Fas). Some of these ligands are released from the membrane after proteolytic cleavage to produce biologically active soluble trimers that can trigger receptor activation (Figure 1). The main signals transmitted from TNF death receptors such as TNFR1, TRAIL-R, and Fas result in an apoptotic response characterized by direct activation of intracellular cysteine proteases (caspases), without directly involving the mitochondrial death pathway. However, these death receptors have also been shown to initiate survival signals via the activation of transcription factors NFκB and Ap1,1 and consequently their function is far more complicated than originally described (below).

TNFR Family Signaling

Induction of Apoptosis

Emerging evidence now indicates that TNFR members exist as preassembled oligomers before ligand stimulation.3 Studies of Fas and TNFR1 have identified a ligand-independent oligomerisation domain termed the preligand assembly domain (PLAD), which is believed to assist in the specificity and speed of the apoptotic response, because the receptors can preassemble and induce supertrehering at the cell membrane after ligand engagement.3 Subsequently apoptosis is initiated via the formation of the death-inducing signaling complex (DISC), which comprises the ligand, death receptor, and the Fas-associated death domain (FADD) protein (Figure 2). FADD contains a death effector domain (DED) motif that binds procaspase-8. Recruitment of caspase-8 to the DISC leads to the activation of caspase-8 and subsequent activation
of effector caspases (-3, -6, and -7). Similarly, caspase-10 recruitment also requires FADD and can initiate an apoptotic signal in the absence of caspase-8.4 This receptor-mediated pathway has been termed the extrinsic death pathway.

The extrinsic pathway should be contrasted with that activated when cells undergo DNA damage for example, whereby translocation of the proapoptotic protein Bax to the mitochondria results in the loss of transmembrane potential, and induces the release of cytochrome c and Smac/DIABLO into the cytosol. Cytochrome c and Apaf-1 then activate caspase-9 with subsequent proteolytic activation of caspases -3, -6, and –7 (Figure 2). This pathway has been termed the intrinsic death pathway because it involves the mitochondria. Interestingly, cross-talk between the extrinsic and intrinsic pathways has been observed. In cells with insufficient activation of caspase-8 or -10 after death receptor activation, cleavage of Bid results in translocation to the mitochondria where it can activate Bax and Bak to stimulate apoptosis.

These apoptotic signaling pathways are illustrated in Figure 2.

Cell Survival and Proliferation
Other than programmed cell death, TNFRs also present survival/activation signals (Figure 2), typically through activation of JNK and NFκB. Interestingly, TNFR1 also transduces survival signals by recruiting adapter molecules TRAFs. At least 6 mammalian TRAFs have been identified to date1 and they interact directly with the TNFR-associated-DD protein (TRADD), a 34-kDa adapter protein that binds to TNFR1 via its DD. TRADD then recruits downstream signaling molecules including FADD and receptor-interacting protein (RIP). Subsequently, TRAFs are capable of mediating activation of JNK and NFκB. In apoptosis signaling, the cellular FADD-like interleukin (IL)-1beta-converting enzyme (FLICE)-like inhibitory protein (c-FLIP) binds to the DISC and blocks caspase-8 processing and activation, and subse-
sequently inhibits Fas-induced apoptosis. However, c-FLIPs also interact with TRAF-1, TRAF-2, and RIP, resulting in activation of NFκB and extracellular signal regulated kinase (ERK). This inhibition of caspase 8 and activation of NFκB and ERK switches the signal from apoptosis to nonapoptotic pathways. Like TNFR1, activation of NFκB and JNK via TRAIL death receptors DR4 or DR5 is mediated by recruitment of TRADD, RIP, and TRAF2 and can occur independently of caspase-8 or -10. Once in the nucleus, NFκB can mediate the activation of genes shown to suppress apoptosis including cellular inhibitor of apoptosis proteins 1 and 2 (c-IAP1 and c-IAP2), X-linked mammalian inhibitor of apoptosis protein (XIAP), TRAF1 and TRAF2, c-FLIP, and B-cell leukemia-extra long (Bcl-xL).

In addition to the cell-execution death pathway, FasL and Fas are also capable of activating other cellular processes including proliferation. For example, activation of Fas enhances proliferation of TCR-stimulated T cells and thymocytes by caspase activation without the induction of apoptosis. Interestingly, the strongly conserved proline-rich cytoplasmic tail of FasL interacts with SH3 (Src homology 3)-domain containing proteins to stimulate activation of Akt, ERK1/2, and JNK. Although TRAIL, DR4, and DR5 can also activate ERK1/2, MAPK, JNK, and Akt, the mechanisms for the antiapoptotic role of FasL/Fas and TRAIL/TRAIL-R at present are largely undefined.

### Lipid Rafts: The Switch From Apoptosis to Survival?

One of the major unanswered questions from these studies has been how signaling events from the same ligand receptor can activate diametrically opposed pathways, either apoptosis or survival, and at what level such regulation is exerted. Recent studies have suggested that at least some of this regulation occurs at the level of incorporation of specific protein components into lipid rafts. During apoptosis, 4 sequential molecular ordering events occur in Fas-mediated signaling: (1) formation of Fas microaggregates at the cell surface induced by FasL, (2) actin-dependent assembly of FADD and DISC components, (3) formation of higher-order receptor clusters that are caspase-8 dependent, and (4) actin-independent FasL-Fas internalization via an endosomal pathway. Recent evidence has implicated Fas translocation and clustering into lipid rafts in Fas signaling. Lipid rafts are membrane microdomains capable of forming platforms, consisting of a dynamic pool of cholesterol and sphingolipids. The tight interaction of cholesterol and sphingolipids determines the transition of the microdomains to a liquid-ordered phase; lipid rafts may thus act as platforms for recruiting and concentrating signaling molecules including cell surface receptors. Consistent with these findings, expression of sphingomyelin causes Fas-mediated apoptosis with translocation of Fas into lipid rafts and subsequent Fas clustering. Redistribution of TNFR1 and TRAIL receptors into lipid rafts have also been observed.

However, the role of signal transduction events after receptor translocation into lipid rafts is controversial. For example, multiple drugs induce the translocation of Fas and TRAIL receptors into lipid rafts to initiate an apoptotic signal. In contrast, TNFRI initiates the activation of NFκB, ERK2, and MAPK, and FasL-SH3-domain containing protein recruitment to lipid rafts involves activation of Akt, ERK1/2, and JNK, signaling pathways involved in survival. Interestingly, a recent study reports that TRAIL-receptor-DISC assembly in lipid rafts initiates an apoptotic signal in nonsmall cell lung carcinoma, whereas nonrafts mediate TRAIL induced activation of NFκB and ERK1/2. Furthermore, the assembly of RIP and c-FLIP to TRAIL-receptor interactions mediates the nonapoptotic signals of TRAIL. These findings suggest that redistribution of TNFR and DISC components from nonrafts to lipid rafts can control the switch between survival signals and apoptosis.

### Role of Death Receptors in the Vasculature

Apoptosis and proliferation are intimately coupled processes involved in normal tissue homeostasis within the vasculature. The balance between aberrant proliferation and apoptosis is accountable for mediating profound changes in the development of atherosclerosis. The consequences of abnormal apoptosis or proliferation of cells within atherosclerotic plaques is either beneficial or detrimental to lesion development, depending on the stage of lesions, localization, and the cell type involved. For example, elimination of VSMCs from a vessel via FasL/Fas-induced apoptosis can inhibit intimal hyperplasia after vascular injury. On the other hand, aberrant apoptosis of VSMCs cells induced during the advanced stage of atherosclerosis can promote plaque rupture. Some of these effects are outlined in Table 1.

Multiple cell types of the vessel wall express TNF-ligands and TNFRs including FasL/Fas, TNF-α/TNFRI, and TRAIL/TRAIL-R, in both normal vessels and lesions of atherosclerosis. Although not fully established, factors evident in atherosclerotic lesions can influence the expression of these death receptors and their ligands (Table 2), and some of these factors include TNF-α itself, insulin, oxLDL, IFN family members, homocystein, IL-1, and NFκB. Increased or decreased expression of TNFR or TNF ligands may contribute to their physiological role to induce death or survival of cells; however, vascular cells are frequently resistant to TNFR-mediated death. For example, TNF- or Fas-receptor signaling alone did not trigger apoptosis of vascular smooth muscle cell (VSMC) but required the contribution of other cytokines to induce vascular cell death. Additionally, death receptor signaling in some cell types may be proatherogenic but antiatherogenic in other cells. This means that studies in which a particular gene or protein is overexpressed or inhibited in multiple cell types are difficult to interpret. For example, global deficiency of FasL and TNF-α alone does not alter atherosclerotic lesion development even after a high-fat diet. Interestingly, plasma from patients with acute myocardial function, unstable angina pectoris, and hypertension have elevated levels of soluble FasL linking the presence of the less active soluble form of FasL with unstable coronary syndromes, although the significance of this finding is unclear. However, we do know that FasL−/−ApoE−/− mice and Fas−/−ApoE−/− mice display enhanced atherosclerotic lesions compared to ApoE−/− mice.
TNFR1 knockout mice display increased atherosclerosis, suggesting that the presence of this receptor is ultimately antiatherogenic. Death receptor signaling is further complicated in TNF-α−/− ApoE−/− double-knockout mice which display reduced lesion size and diminished development of atherosclerosis. The effects of TRAIL in atherosclerosis in vivo also remains uncertain, although the finding that serum soluble TRAIL levels are reduced in patients predisposed to coronary artery disease implicates a protective role for TRAIL in cardiovascular disorders. In addition to the apparently contradictory effects of manipulations of specific TNFs, TNFRs attributable to changes in multiple cell types within the vessel wall, global deficiency of TNFRs or TNF ligands may predispose to atherosclerosis via systemic effects. For example, Fas-deficient (lpr) and FasL-deficient (gld) mice have a lupus-like syndrome, resulting in the production of neoepitopes that may cross-react with oxidized low density lipoprotein (OxLDL), and thus may promote inflammation.

In contrast to the effects of global absence of death receptors or ligands in all vessel walls in vivo, effects of death ligand receptor interactions have been studied in individual cell types in culture. ECs, VSMCs, and inflammatory cells including macrophages and T cells express death ligands and death receptors to a variable extent. Our understanding of death receptor signaling in ECs, VSMCs, and inflammatory cells is summarized below.

### Table 1. Abnormal Apoptosis and Proliferation of Vascular Cells in Atherosclerosis

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Apoptosis</th>
<th>Proliferation</th>
<th>Physiological Benefit</th>
<th>Pathological Damage</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>√</td>
<td>√</td>
<td>Increased infiltration of inflammatory cells</td>
<td>Accelerated atherogenesis</td>
<td>51–53</td>
</tr>
<tr>
<td>EC</td>
<td>√</td>
<td>√</td>
<td>Reduced inflammatory cell infiltration</td>
<td>Intimal thickening</td>
<td>68–70</td>
</tr>
<tr>
<td>VSMC</td>
<td>√</td>
<td>√</td>
<td>Reduction in vessel wall renarrowing</td>
<td>Plaque rupture</td>
<td>29–31,62,87,89,90,100</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>√</td>
<td>√</td>
<td>Inhibition of atherogenesis</td>
<td>Thrombosis</td>
<td>74–76,101</td>
</tr>
</tbody>
</table>

### Table 2. Expression of TNFR and TNF-Ligands Induced by Atherogenic and Antiatherogenic Factors in Vascular Cells

<table>
<thead>
<tr>
<th>Factors</th>
<th>Ligands/Receptors</th>
<th>Expression</th>
<th>Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>Fas</td>
<td>Increase</td>
<td>EC apoptosis</td>
<td>62</td>
</tr>
<tr>
<td>OxLDL</td>
<td>Fas/FasL</td>
<td>Increase</td>
<td>VSMC apoptosis</td>
<td>102,103</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Fas</td>
<td>Increase</td>
<td>EC apoptosis</td>
<td>104–106</td>
</tr>
<tr>
<td></td>
<td>DRA/DR5</td>
<td>Decrease</td>
<td>Inhibition of EC apoptosis</td>
<td></td>
</tr>
<tr>
<td>TNFR1</td>
<td>Increase</td>
<td>Macrophage apoptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>TRAIL</td>
<td>Decrease</td>
<td>?</td>
<td>107</td>
</tr>
<tr>
<td>IFNα</td>
<td>TRAIL</td>
<td>Increase</td>
<td>VSMC death</td>
<td>108</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>TRAIL</td>
<td>Increase</td>
<td>VSMC death</td>
<td>109</td>
</tr>
<tr>
<td>IL-1</td>
<td>Fas</td>
<td>Increase</td>
<td>VSMC apoptosis</td>
<td>110</td>
</tr>
<tr>
<td>NF-κB</td>
<td>TRAIL</td>
<td>Increase</td>
<td>Inhibition of VSMC apoptosis</td>
<td>96</td>
</tr>
</tbody>
</table>

### Death Receptor Signaling in ECs of the Vessel Wall

The endothelium controls inflammation, vascular remodeling, and vascular tone, and ECs can retard atherogenesis by acting as barriers to inflammatory T-cells and macrophages. Because vascular ECs are primary targets for proinflammatory actions within the vessel wall, EC dysfunction may lead to the infiltration of inflammatory cells and lipids. After damage to the endothelium, VSMC migration and proliferation is also induced within the denuded lesion, suggesting that EC dysfunction can regulate VSMC proliferation leading to increased intimal thickening. Indeed, Fas activation in ECs sensitizes to Fas-mediated death under hypercholesterolemic conditions, promoting EC loss, lead-
ing to accelerated atherogenesis. EC loss can also induce local thrombosis, and may thus partly underlie vessel occlusion after plaque erosion.

Although the role of EC apoptosis in vivo needs further elucidation, multiple strands of evidence suggest that TNFR-induced apoptosis of ECs is important in vivo. For example, in vitro TNFR/ligand-initiated apoptosis of vascular ECs has been observed. Human plaque microparticles carry active TNF-α-converting enzyme that enhances cell surface processing of TNF and TNFRI on ECs, and ligation of TNF-α to TNFRI on ECs can induce apoptosis. Finally, activation of TNFRI can induce apoptosis and inhibit migration of ECs.

In contrast to TNF-α, ECs express FasL and low levels of Fas, but appear to be resistant to FasL/Fas-mediated apoptosis. However, on stimulation with cytokines such as TNF-α or interferon gamma (IFNγ), Fas expression and apoptosis is induced in ECs. Furthermore, OXLDL that is evident in atherosclerotic lesions induces EC apoptosis by sensitizing these cells to Fas-mediated cell death. Interestingly this Fas-mediated EC apoptosis occurs through decreased expression of c-FLIP.

Human aortic ECs (HAECs) and human umbilical vein ECs (HUVECs) also express TRAIL and TRAIL-R (DR4, DR5, DcR1, and DcR2). Although there are reports of TRAIL-induced EC death in culture, the majority of studies implicate TRAIL in nonapoptotic pathways in ECs. For example, recent evidence suggests that TRAIL may play a role in leukocyte/EC adhesion. In part through NFκB, 70% of EC that did not die after TRAIL treatment in culture demonstrated increased NFκB activity and increased expression of adhesion molecules on ECs. Not only is the proadhesive role of TRAIL in ECs supported by other studies, this effect is also seen with the death receptor DcR3. DcR3 activation increases monocyte adhesion to ECs via NFκB activation and transcriptional upregulation of ICAM1, VCAM1, and IL-8.

In contrast, FasL on vascular endothelium inhibits TNF-α-induced leukocyte extravasation and in carotid artery allografts delayed infiltration of inflammatory cells and diminished intimal hyperplasia, suggesting anti-inflammatory functions for TNFR and their ligands in atherosclerosis.

TNF ligands and TNFR have other multiple actions in ECs. Endothelial nitric oxide synthase (eNOS) maintains vascular tone, promotes EC survival and migration, and has antithrombotic and anti-inflammatory activity in atherosclerosis. TRAIL in particular is implicated in NO production resulting in migration of HUVECs and subsequent activation of eNOS in an Akt-dependent manner. TRAIL-inducible NO occurs by redistribution of eNOS from the plasma membrane to the cytoplasm without inducing apoptosis. TRAIL has also been implicated in EC proliferation in part by activating Akt and ERK but not p38, JNK, or NFκB. These recent findings have significant implications in TNF ligand/TRAIL-mediated EC biology and subsequent cardiovascular function.

Death Receptor Signaling in Inflammatory Cells of the Vessel Wall

Inflammatory cells are found in all stages of atherogenesis, and the recruitment of monocytes and lymphocytes into the vessel wall is a critical factor in the progression of atherosclerosis. Once in the lesion, T-cells and macrophages can generate reactive oxygen species and cytokines that can subsequently amplify the inflammatory response. It has been generally perceived that loss of macrophages through systemic apoptosis may promote plaque stability. For example, direct induction of monocyte/macrophage apoptosis in ApoE⁻/⁻ mice either reduced plaque development in atherogenesis or had no effect on plaque size or inflammation when induced in established lesions. Indeed, administration of TRAIL to ApoE⁻/⁻ diabetic mice induced macrophage apoptosis and markedly reduced plaque formation, suggesting that macrophage apoptosis may be beneficial in reducing plaque development. This concept is consistent with previous studies showing that atheroma/macrophage recruitment and/or reduction of macrophages in mice lacking chemoattractant proteins such as monocyte chemoattractant protein-1 (MCP-1) inhibits atherosclerosis, and CD4 T-cell depletion results in reduced lesion formation.

However, there is some controversy regarding this because apoptotic inflammatory cells colocalize to regions both of inflammation and to sites of plaque rupture, and apoptosis increases the release of tissue factor from macrophages. Furthermore, secondary necrosis of unphagocytosed apoptotic macrophages, for example when phagocytosis is delayed, is suggested to be a potent proinflammatory event. The increase in apoptosis of macrophages at these sites is believed to contribute to atheroplasia via cellular debris accumulation, increased inflammation, and growth of the necrotic core. Thus, the contribution of inflammatory cell apoptosis both local and systemic in the development of atherosclerosis is a multi-faceted complex process dependent on different stages of the disease.

The role of death receptors in inflammatory cell apoptosis in vivo and in vitro is also not well established. T lymphocytes and macrophages express some TNFR/ligands in plaques, and TNFR pathways have been implicated in T cell and macrophage death. In particular, FasL overexpression on the endothelium significantly attenuated T cell and macrophage accumulation in atherosclerosis, suggesting that Fas-L on ECs engages Fas on migrating inflammatory cells, inducing apoptosis as they adhere. Furthermore, NO-induced macrophage death was inhibited by Fas-Fc chimeras, and macrophages from Fas-deficient mice are more resistant to NO-induced death. As mentioned above, administration of TRAIL in diabetic ApoE-null mice also demonstrated apoptosis of infiltrating macrophages. In contrast to these findings suggesting that the major effect of Fas/FasL on inflammatory of immune cells is induction of apoptosis, activation of Fas enhances proliferation of TCR-stimulated T-cells and thymocytes. In particular, the proline-rich intracellular domain of FasL can stimulate antigen-specific T-cell proliferation by inducing the phosphorylation of Akt, ERK1/2, JNK, and FasL itself. Fasl can also transduce a costimulatory signal by activating the tran-
scription factors NFAT and AP-1, and by enhancing IFN-γ production. These studies suggest that Fas-Fasl signaling may induce apoptosis of macrophages but also promote proliferation and change phenotype of T cells, ultimately promoting atherosclerosis. Indeed, adenovirus administration of FasL to hypercholesterolaemic rabbits enhances atherosclerosis.

**VSMCs**

Although apoptosis of VSMCs is seen less frequently than in macrophages in atherosclerosis, recent studies have clarified the role of VSMC apoptosis in all phases of atherosclerosis. Apoptosis of VSMCs in advanced lesions can reduce VSMC production of extracellular matrix and collagen, thinning the fibrous cap, expanding the necrotic core, and subsequently promoting plaque instability and potential rupture. Intense VSMC apoptosis can also promote both local and systemic inflammation. Low level VSMC apoptosis can induce vulnerable plaque features as plaques develop or in established lesions, and profound changes in vessel media, resulting in acceleration of vascular calcification and medial degeneration. FasL and Fas are expressed in VSMCs from normal vessels and atherosclerotic plaques, and FasL-induced apoptosis can reduce VSMC accumulation and inhibit intimal hyperplasia after vascular injury. In contrast, VSMC apoptosis induced by the FasL/Fas in established plaques can promote plaque rupture.

FasL–Fas–mediated apoptosis in VSMCs has also been observed in vitro. For example, macrophages expressing FasL can induce apoptosis of plaque-derived VSMCs, and IFN-γ, a cytokine abundant in atherosclerotic lesions and secreted by T lymphocytes, can also induce apoptosis of VSMC in a Fas-dependent manner both in vitro and in human arteries transplanted into immunodeficient mice in vivo. Unlike in inflammatory cells, Fas and some other death receptors are predominantly intracellular in VSMCs, requiring priming of the cell with either expression of specific gene products (e.g., the tumor suppressor gene p53 or inflammatory cytokines such as IFN-γ, NO, and IL-1β) to relocate it to the cell surface where it is active. Induced Fas trafficking requires PI3K, Akt, and Jak-2. Furthermore, apoptosis of VSMCs is induced after increased expression of FasL by the zinc-finger transcription factor Sp1, which in turn is phosphorylated by the atypical protein kinase C-ζ (PKC-ζ). Indeed, human carotid atherosclerotic plaques demonstrate increased expression of PKC-ζ and FasL in terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)–positive VSMCs.

Like FasL–Fas, TRAIL and TRAIL-R have been implicated in VSMC death. In vitro studies demonstrate that VSMCs express all TRAIL-Rs, and VSMCs are susceptible to cell death induced by TRAIL. TRAIL is expressed on the surface of immune cells and adaptive transfer of CD4 T cells to immunodeficient mice implanted with human carotid plaque tissue induces plaque VSMC apoptosis, suggesting that immune cells invading the plaque may kill VSMCs in lesions. Interestingly, VSMC apoptosis was blocked using antibodies targeting TRAIL and DR5, and other studies have shown that TRAIL-R death pathways are active in VSMCs.

Recently, nonapoptotic functions of TRAIL have been identified. TRAIL administration increases the VSMC content in plaques of diabetic ApoE−/− mice, implicating a protective role for TRAIL in VSMC biology. Although this phenomenon may be attributable in part to the reciprocal induction of macrophage apoptosis, TRAIL has been shown to increase VSMC proliferation and migration in vitro, via activation of ERK and Akt. In support of these findings, we recently showed that TRAIL-induced VSMC proliferation involved the activation of NFκB and increased expression of the antiapoptotic growth factor receptor, insulin-like growth factor-1 receptor (IGF1R). Interestingly, the proliferative effects of TRAIL on VSMCs were biphasic. Increased proliferation of VSMCs was dependent on DR4 and DcR1 and only observed at low concentrations (0.1 to 1 ng/mL), an effect no longer evident at higher subapoptotic concentrations (100 to 400 ng/mL).

**Conclusions**

TNFR signaling is implicated in both the development and consequences of atherosclerosis, and it is now clear that death receptor signaling initiates multiple pathways resulting in apoptosis or proliferation of cells. However, the multiple TNF-ligand/TNFRs, and different cell types that express them, make interpretation of studies with global administration or knockdown of particular TNF-ligand/TNFR in a complex disease such as atherosclerosis difficult to interpret. In contrast, future studies involving cell-selective administration or inhibition of specific TNFR and their ligands in animal models are required to analyze the contribution of TNF-ligands and TNFR in atherosclerosis and related disorders. However, the widespread expression and the profound effects of targeting TNFR in atherosclerosis may potentially lead to useful therapies for its prevention or treatment.

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**Disclosures**

None.

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