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REVIEW

Tumour necrosis factor superfamily members in ischaemic vascular diseases

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Abstract Current treatment of ischaemic vascular diseases such as coronary and peripheral artery disease includes angioplasty and bypass grafting, as well as lipid lowering therapies and control of other cardiovascular risk factors. Numerous members of the tumour necrosis factor superfamily (TNFSF) have recently shown emerging roles in both the protection and progression of such diseases. Understanding the role TNFSF members play in ischaemic vascular disease may provide insight into the development of novel therapeutics to prevent or treat diseases relating to atherosclerosis and ischaemia. This review summarizes the most recent findings relating to TNFSF members and the mechanisms that precede ischaemic vascular disease progression, particularly endothelial dysfunction, chronic inflammation, and atherosclerotic plaque development. This review also explores recent translational research on the role of TNFSF therapies in cardiovascular disease.

Keyword Ischaemia • Atherosclerosis • TNF superfamily

1. Introduction

Ischaemic vascular diseases are caused by stenosis, the abnormal narrowing of blood vessels, or vessel occlusion, with atherosclerosis the most common cause. Ischaemic vascular diseases come in multiple forms including blockage of the peripheral arteries (peripheral artery disease; PAD); blockage of the coronary arteries (coronary artery disease; CAD); and blockage of the carotid arteries. The various forms of ischaemic vascular disease are frequently found together. Diseases relating to atherosclerosis and ischaemia are now among the world's most dominant disorders for both mortality and morbidity.

Depending on disease severity and clinical context, current treatments include lifestyle modification, medical therapy, and more invasive treatments such as percutaneous angioplasty and bypass grafting. New therapies that target specific factors modulating ischaemic vascular disease may be useful in patients in which current treatments do not benefit. The tumour necrosis factor (TNF) superfamily (TNFSF) plays a key role in ischaemic pathophysiology, with both protective and destructive roles described. Targeting TNFSF signalling pathways by inhibiting or activating biological mechanisms involved in the progression of ischaemic vascular diseases may therefore provide therapeutic benefit. In this review, we outline recent advances in our understanding of TNFSF, specifically focusing on the most common ligand-receptor interactions described in ischaemic vascular disease, namely TNF-α, TNF-related apoptosis-inducing ligand (TRAIL), TNF-like weak-inducer of apoptosis (TWEAK), CD40L, and their cognate receptors.

2. The TNF superfamily

TNFSF comprise of ∼20 ligands and the ability to bind to at least one of the family's 30 related receptors (TNFRs). TNF ligands contain a shared amino acid sequence in their extracellular C-terminus, and are generally characterized as type II transmembrane proteins. This shared sequence is the family's hallmark, and the region to which each ligand binds its corresponding receptor(s). Although most ligands exist primarily as trimeric or multimeric membrane bound proteins, several members, for example TNF-α and TRAIL, are also biologically active following proteolytic cleavage to a soluble form. Irrespective of their form, TNF ligands operate to induce receptor aggregation and launch signal transduction pathways. Due to their possession of an extracellular N-terminus, most members of the TNFR superfamily fall into the type I transmembrane protein category. Some TNFRs, for example B cell activating factor receptor (BAFF-R), lack a signal peptide sequence, and thus fall into the type III transmembrane category. TNFRs share a hallmark sequence within their extracellular domain, characterized by the existence of ∼6 cysteine residues within a 40-amino acid sequence termed the cysteine-rich-domain. Most receptors primarily operate as homomeric trimers, but like the ligands, can be cleaved and exist in a secreted form.

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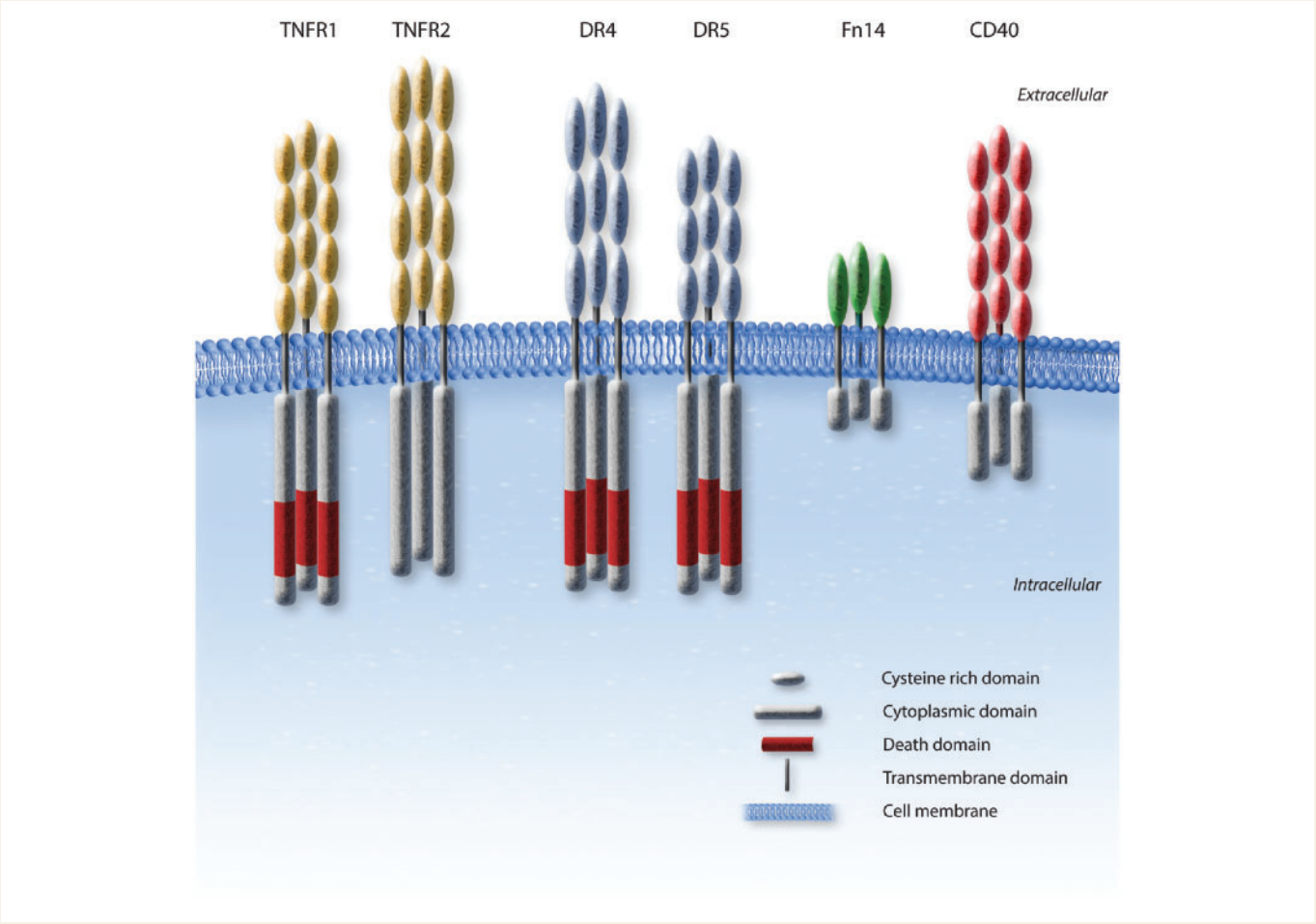

Figure 1 Receptor structures for TNF-α, TRAIL, TWEAK, and CD40L. TNFR1 and TNFR2 bind to TNF-α; DR4 and DR5 bind to TRAIL; Fn14 binds to TWEAK; and CD40 bind to CD40L.

Following receptor ligation, TNFR signals exhibit pro-apoptotic and non-apoptotic behaviour. Three groups have been identified, with the first set of receptors referred to as death receptors, as they act to stimulate apoptosis via the interaction of their cytoplasmic death domains (DD) with accessory proteins. Multiple death receptors, for example death receptor 4 (DR4), death receptor 5 (DR5), and Fas, can induce pro-survival behaviour alongside their stereotypical apoptotic behaviours. The second group recruit TNFR-associated-factors (TRAFs) that act to stimulate pro-survival signalling through their TRAF-interacting-motif (TIM) domains. The third group are decoy receptors, lacking functional cytoplasmic domains, and act as competitive inhibitors. See *Table 1* for examples of TNF ligand-receptor interactions. Structures of signalling receptors for TNF-α, TRAIL, TWEAK, and CD40L are described in *Figure 1*.

3. TNF signalling

TNFSF ligands produce both pro-apoptotic and pro-survival signals.[1] *Figure 2* depicts pathways shared by TNF-α, TRAIL, TWEAK, and CD40. TNFSF signalling is often modelled by the family's founding member, TNF-α, and its receptors TNF-R1 and TNF-R2. TNF-R1 belongs to the DD-containing subset of TNFRs, stimulating apoptosis. Following ligand binding, the receptor, which is ubiquitously expressed on most cell types, recruits TNFR-associated-DD (TRADD) to its trimerized DD. When acting to initiate apoptosis, TRADD associates with a second adaptor protein called Fas-associated protein with DD (FADD) to activate caspase-8/10, commencing a series of caspase cleavages that culminate in cellular apoptosis. The complex of FADD in association with procaspase-8/10 is referred to as the death-inducing signalling complex (DISC). In cells where caspase-8/10 is abundant, apoptosis can occur via the extrinsic pathway. If caspase-8/10 is sparse, apoptosis may result through the mitochondria, initiated with BH3 interacting domain (BID) cleavage.

Activation of DR4 and DR5 by TRAIL occurs in a similar fashion to TNF-R1. However, DR4 and DR5 do not require binding of TRADD to stimulate apoptosis, but instead, associate immediately with the DISC. In addition to DR4 and DR5, TRAIL binds three decoy receptors: decoy receptor 1 (DcR1), decoy receptor 2 (DcR2), and soluble osteoprotegerin (OPG). Decoy signalling is thought to limit apoptotic functions of TRAIL. Like TNF-α and TRAIL, TWEAK can weakly stimulate apoptosis; however, the action through which this ligand may stimulate apoptosis is often disputed. Initial reports indicated TWEAK binds death receptor 3 (DR3) to stimulate apoptosis, yet TWEAK's only reported receptor is Fn14. Apoptosis via Fn14 is poorly understood, but believed to involve JAK-STAT signalling and the presence of interferon (IFN)-γ.

Table 1 Examples of TNFSF ligand-receptor interactions

Ligands	Death receptor	Receptor containing TIM	Decoy receptor
TNF-α	TNFR1	TNFR2	–
LT-α	TNFR1	TNFR2 HMVEM	
FasL (CD95L)	Fas (CD95)	–	DcR3
TRAIL (Apo2L/CD53)	DR4 (TRAILR1/CD261) DR5 (TRAILR2/CD262)	–	DcR1 (TRAILR3) DcR2 (TRAILR4) OPG
TWEAK	DR3	Fn14	–
CD40L	–	CD40	–
Ox40L (CD252)	–	Ox40 (CD134)	–
Light	–	HMVEM	DcR3
RankL (TRANCE/CD254)	–	RANK (CD265)	OPG
BAFF (CD257)	–	BAFF-R (BR3/CD268)	–

TIM, TRAF-interacting motif; LT-α also known as TNF-β; HVEM, herpesvirus entry mediator; LIGHT, also referred to as tnfsf14.

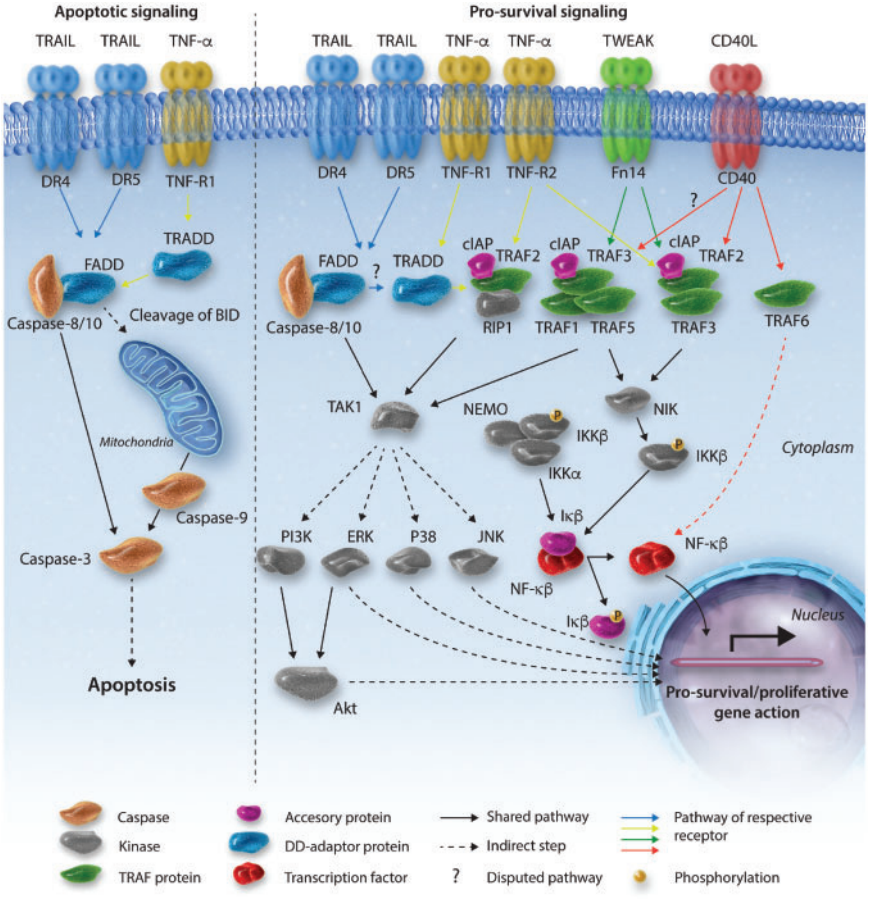

Figure 2 Apoptotic and non-apoptotic signalling by TNF-α, TRAIL, TWEAK and CD40L. Apoptosis for the TWEAK-R is not shown as its mechanism is not currently understood. TNFSF members also produce pro-survival signals via activation of NF-κB, PI3K and MAPK, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38.

The action of TNF-R1 in pro-survival gene transcription is often used as the paradigm for TNFSF signalling. As with apoptosis, binding of TNF-α to the receptor results in trimerization and binding of TRADD to the DD. Association with TRADD then promotes recruitment of accessory proteins, TRAF2 and receptor-interacting protein-1 (RIP1). Activation of cellular inhibitor of apoptosis proteins (cIAPs) with TRAF2 results in ubiquitination of RIP1, allowing association of the complex with transforming growth factor β-associated kinase-1 (TAK1). Through TAK1, the complex stimulates nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) translocation and activation, with NF-κB stimulation following either the canonical or non-canonical pathway. Canonical NF-κB activation induces degradation of NF-κB inhibitor (IκB) via IκB kinase (IKK) complex, whereas the non-canonical pathway is dependent on the stimulation of IKKα by NF-κB kinase (NIK). Crosstalk between the two pathways has been reviewed elsewhere.[2] Canonical stimulation appears to be the more common, and is the path which TNF-R1 is known to follow.

DR4 and DR5 also stimulate pro-survival signals in a similar manner to TNF-R1 that is by forming complexes involving TRAF2, RIP1, and TAK1. However, there is debate as to whether TRAIL-receptors recruit accessory molecules via the action of FADD, TRADD, or a combination of the two. TRAIL-receptors can also induce pro-survival actions independent of NF-κB, including, activation of mitogen activated protein kinase (MAPK) pathways or activation of Akt via phosphoinositide 3-kinase (PI3K).

Receptors lacking a DD, for example TNF-R2, interact directly with TRAF proteins and stimulate NF-κB through both canonical and non-canonical means. The activated TNF-R2 trimer interacts directly with both TRAF1 and TRAF2 to stimulate cIAP ubiquitination and promote canonical activation via PI3K or non-canonical activation through recruitment of TRAF3 and NIK. How TNF-R2 results in NF-κB stimulation at present is unclear. Fn14 (TWEAK-R) can also activate NF-κB, whereby association with TRAF1/3/5 activates the canonical pathway, whilst association with TRAF2 stimulates the non-canonical pathway. As previously mentioned, TWEAK ligation can also induce MAPK signals, with Kumar *et al.*[3] describing a significant reduction in TWEAK-induced activation of both JNK and p38, but not ERK, in TAK1 deficient MEFs. This indicates a signalling mechanism for TWEAK that is comparable to both TNF-α and TRAIL.

CD40 belongs to the TRAF binding subset of TNFR, also capable of triggering both canonical and non-canonical NF-κB activation. Stimulation of the receptor by CD40L often results in the recruitment of TRAF2 in complex with TRAF3 and cIAPs. Degradation of TRAF3 through cIAP action, and self-degradation of TRAF2, can result in activation of NIK, promoting NF-κB activity in a non-canonical manner. Recruitment of TRAF2 can also stimulate JNK and p38 pathways, in a manner not dissimilar to that of TWEAK ligation. Canonical activation occurs when the only TRAF associating with CD40 to the cytoplasmic domain is TRAF2. TRAF6 has also been implicated in the stimulation of NF-κB by CD40; however, the mechanism(s) of this interaction are not well understood. Relationships between various TRAF molecules and CD40 have been reviewed in greater detail by Elgueta *et al.*[4]

4. TNF interactions and ischaemic vascular disease

4.1 TNF-α

Although essential to the immune response, TNF-α is considered pro-atherosclerotic due to its pro-inflammatory action. Clinically, elevation of the cytokine above baseline levels is associated with a higher risk of CAD, acute myocardial infarction (MI) and heart failure.[5,6] This is supported by pre-clinical studies, with apolipoprotein E knockout mice (*Apoe*$^{-/-}$) administered TNF-α showing increased atherosclerotic plaque size approximately five-fold compared with saline injected controls,[7] whereas TNF-α deletion in *Apoe*$^{-/-}$ mice reduced lesion size.[8]

TNF-α's ability to enhance inflammation, stimulate foam cell development and induce vascular cell apoptosis may be responsible for complications associated with ischaemic vascular disease. For example, TNF-α augments the expression of chemokines and adhesion molecules necessary for recruitment and migration of pro-inflammatory monocytes to the vessel intima; in particular vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), E-Selectin, and monocyte chemoattractant protein-1 (MCP-1),[9,10] as evidenced by reduced expression of these molecules in *Tnf-α*$^{-/-}$*Apoe*$^{-/-}$ mice. Increased vascular permeability is also a characteristic of atherosclerosis. Angelini *et al.*[11] showed that TNF-α enhanced permeability of human endothelial cell (EC) monolayers, reporting tyrosine phosphorylation of E-cadherin, β-catenin, γ-catenin, and p120^{ctn}, as the mechanism. Interestingly, TNF-α-induced inflammatory responses occur via NF-κB.[12] TNF-α also indirectly stimulates the differentiation of monocytes into foam cell macrophages,[13] whereas anti-TNF-α antibody treatment attenuated monocyte differentiation.[14] Furthermore, excess TNF-α levels in coronary heart disease was associated with increased oxidized-LDL (oxLDL) uptake by macrophages, with increased scavenger receptor expression associated with TNF-induced monocytic lipid uptake.[14]

TNF-α is also associated with cellular apoptosis, a process that results in vascular damage associated with vascular calcification,[15] and an unstable atherosclerotic plaque phenotype.[16] Amorphous calcification, osteogenic differentiation and bone formation in the vessel wall is commonly associated with ischaemic vascular diseases such as PAD.[17] Because TNF-α is also important in bone formation, it may also regulate vascular calcification. Indeed, TNF-α stimulated calcium deposition in vascular cells, in part, via cyclic adenosine monophosphate activation,[18] and by increasing expression of osteogenic markers in the vessel wall.[19] This suggests that in addition to apoptosis, TNF-α may regulate vascular cell differentiation and calcification in the vessel wall.

Although TNF-α-induced calcification involves TNF-R1,[20] the role of TNF-α receptors in atherosclerosis is conflicting. For example, TNF-R1 knockout mice displayed an approximately two-fold greater lesion size with a 30% reduction in cell nuclei number-to-lesion area ratio, suggesting a greater lipid content with TNF-R1 deficiency compared with wild type in response to a high fat diet.[21] In contrast, deletion of TNF-R1 in *Ldlr*$^{-/-}$ mice resulted in significant reduction in atherosclerosis, ∼40% compared with littermate controls.[22] These mice also displayed reduced VCAM-1, ICAM-1, and inflammatory marker expression.[22] Since TNF-α is involved in many processes, the cytokine and its cognate receptors could perhaps offer a potential target area for therapies against progression of atherosclerosis and ischaemia.

4.2 TRAIL (or Tnfsf10)

TRAIL can regulate many processes including vasodilation, angiogenesis, and inflammation, with TRAIL regulated effects being both protective or detrimental in ischaemic vascular disease.[23] For example, TRAIL is capable of inducing apoptosis of human EC and influences vascular inflammation *in vitro*.[24] However, *in vivo*, *Trail*$^{-/-}$ vessels displayed more severe tissue inflammation with increased expression of TNF-α, IL-1β, and IL-6, in response to a high fat diet.[25] Basally, increases in MCP-1, IL-1β, and VCAM-1 were observed with TRAIL deficiency.[25] Alongside its pro-

inflammatory action, the association of TRAIL with NF-κB signalling has been linked to promotion of vascular smooth muscle cell (VSMC) proliferation and survival.[26] The nature of VSMC proliferation in injured or diseased vessels is controversial; immediate to injury proliferation is unfavourable, whereas in established lesions, VSMC proliferation promotes fibrous cap development, decreasing the likelihood of plaque rupture. Indeed, TRAIL expression is associated with increased VSMC content in stable plaque; α-smooth muscle actin staining, collagen, and cap thickness was reduced with TRAIL deletion in *Apoe*$^{-/-}$ mice,[27] whereas administration of TRAIL to diabetic mice significantly increased VSMC content and stabilized atherosclerotic lesions.[28]

In addition to stabilizing atherosclerotic plaque, TRAIL plays a role in EC function. Recent studies showed that *Trail*$^{-/-}$*Apoe*$^{-/-}$ vessels displayed increased oxidative stress, impaired vasorelaxation and vascular permeability, with reduced VE-cadherin expression.[29] Pre-treatment of EC with recombinant human TRAIL also inhibited angiotensin II (AngII)-induced monocyte adhesion, in part by reducing VCAM-1.[29] Importantly, these findings identified that TRAIL inhibited AngII-induced reactive oxygen species production; however, the mechanisms are currently unclear.

TRAIL's role in angiogenesis has also been described. *Trail*$^{-/-}$ mice had impaired ability to recover following femoral artery ligation, a model of PAD, compared with wild-type controls, with adenoviral delivery of TRAIL significantly improving blood flow and capillary density post-ligation.[30] Moreover, TRAIL increased EC processes relating to angiogenesis *in vitro*, including proliferation, migration, and tubule formation,[30–32] in some cases better than VEGF or FGF-2.[31] The mechanism of action is likely to involve NOX4 and nitric oxide-dependent pathways.[30,33] This is significant, as activation of NOX4 and endothelial nitric oxide synthase (eNOS) are cardio-protective at low levels, strengthening the importance of TRAIL in protecting against ischaemic vascular disease progression, and highlighting TRAIL as a potential therapeutic target in ischaemic vascular disease, in particular PAD.

Like TNF-α, TRAIL is implicated in differentiation of vascular cells into bone forming cells. In this instance, the absence of TRAIL stimulates vascular calcification. For example, *Trail*$^{-/-}$*Apoe*$^{-/-}$ mice fed a high fat diet developed more vascular calcification in atherosclerotic plaque than fat-fed *Apoe*$^{-/-}$ mice.[34] It has been identified that TRAIL deletion in VSMC promoted mineralization *in vitro*, in part by up-regulating the TNF family member receptor activator of nuclear factor kappa-β (RANK) ligand (RANKL),[34] which activates osteoclastogenesis by binding its receptor RANK. Of note, OPG, a decoy receptor of TRAIL, inhibits osteoclastogenesis. Clinical evidence also supports a protective role for TRAIL. Indeed, patients with CAD have decreased TRAIL levels.[29] The InCHIANTI study of elderly individuals, showed those in the lowest quartile of TRAIL concentrations had a two-fold increased risk of all-cause cardiovascular mortality compared with those in the highest quartile,[35] and it was postulated that soluble TRAIL levels may be a good predictor for risk of death in sufferers of stable angina, stroke, and PAD. Thus, targeting these pathways may identify new strategies for the treatment of complications associated with ischaemic vascular disease.

4.3 TWEAK (or Tnfsf12)

Participation of TWEAK in ischaemic vascular disease is generally deemed to be detrimental. TWEAK's primary receptor, Fn14, is physiologically expressed at low levels. Following vascular damage such as in atherosclerosis,[36] expression of both the receptor and ligand is known to be unregulated; however, TWEAK-mediated signalling is relatively unexplored. Generally, TWEAK-receptor interactions result in pro-inflammatory signals. In a manner similar to both TNF-α and TRAIL, TWEAK can increase IL-8, MCP-1, ICAM-1, VCAM-1, and E-selectin in ECs,[3,37] in part via activation of NF-κB.

The TWEAK-Fn14 axis is also associated with elevated vascular calcification, arterial stiffening,[38] and atherosclerosis.[39] In human atherosclerotic plaques, TWEAK and its receptor are co-localized to both VSMC and macrophages,[36] and ligand-receptor interactions linked to up-regulation of the DNA binding protein high-mobility group box 1 (HMGB1).[40] Blocking TWEAK in atherosclerotic mice has resulted in contrasting findings. Schapira *et al.*[41] found that using Fn14-Fc fusion protein to inhibit TWEAK resulted in both reduced fibrotic and VSMC content in atherosclerotic lesions, suggesting that it promotes plaque stability, while Fernández-Laso *et al.* demonstrated that blocking TWEAK promoted an unstable plaque phenotype.[42] Up-regulation of HMGB1 via TWEAK can shift the phenotype of infiltrating monocytes towards an M1 state.[40] TWEAK signalling has also been linked to foam cell development, with positive immunostaining of Fn14 on pro-inflammatory macrophages, as well as lipid-rich foam cells.[41] Importantly, these macrophages contained less oxLDL, suggesting reduced lipid uptake.[41] A better understanding of this cytokine's mechanisms and signalling is required.

4.4 CD40L

Although primarily recognized for its role in immune cell regulation, biologically functional CD40L and its receptor, CD40, are abundant in multiple cell types within human atherosclerotic lesions, including ECs, VSMCs, and macrophages. In early stages of atherosclerosis, CD40L-CD40 signals associate with dysfunction of human coronary artery EC, while treatment with soluble CD40L down-regulated eNOS expression.[43] This is significant as eNOS and subsequent nitric oxide production are protective within the vasculature, reducing oxidative stress in the vessel wall. CD40L also increased IL-1, IL-6, IL-8, TNF-α, MCP-1, VCAM-1, ICAM-1, and E-selectin expression and release,[44,45] in part via NF-κB.[43] Blocking CD40 signalling in mice reduced atherosclerosis and produced a stable plaque phenotype.[46–48]

In addition to altering cytokine levels, CD40L can influence monocyte and macrophage function.[49] CD40L can stimulate scavenger receptor type A (SRA), and in turn increase lipid uptake and foam cell development.[50] This is supported by studies using CD40 small interfering RNA or anti-CD40L antibody which reduced monocyte-to-foam cell conversion.[50] Further studies are required to identify the impact of this ligand-receptor complex on the onset and progression of ischaemic vascular diseases.

5. Translational aspects of TNFSF therapy in coronary disease

5.1 TNF-α

The primary source of data on TNFSF therapy in coronary disease comes from secondary analysis of coronary events in rheumatoid arthritis (RA) patients undergoing therapy with TNF-α inhibition. RA is a systemic inflammatory disease characterized by chronic articular inflammation, associated with worsened ischaemic vascular disease and a significantly increased risk of cardiovascular events.[51,52] TNF is a key mediator of inflammation in RA, driving pro-inflammatory cytokines, cell adhesion molecules, osteoclastogenesis, and matrix metalloproteases activation.[53] A growing number of studies have demonstrated that a reduced risk of cardiovascular events in RA patients treated with TNF

inhibitors, although controversy remains regarding whether this is an effect of the drugs themselves, or rather reflects better disease control.[54–56]

Two meta-analyses have evaluated cardiovascular events in RA patients treated with TNF inhibitors. Barnabe *et al.*[57] demonstrated a reduced risk of all cardiovascular events for TNF inhibitor use across cohort studies (RR 0.46, 95% CI 0.28, 0.77); however, analysis of three randomized control trials did not reach significance, suggested by the authors to be due to low power to assess for cardiovascular events in these trials. Roubille *et al.*[58] also demonstrated a reduction in all cardiovascular events for RA patients treated with TNF inhibitors across 17 studies (RR 0.70, 95% CI 0.54, 0.90), as well as a reduction in MI, stroke, and major adverse cardiac events.

Since these meta-analyses, several large cohort studies have been published analysing TNF inhibitor use in national registries. Ljung *et al.*[59] analysed coronary events in 6864 patients treated with TNF inhibitors in the Swedish Biologics Register, compared with a group of 34 229 general population referents. It was demonstrated that RA patients who exhibited a good response to TNF inhibition had a similar rate of acute coronary syndrome (ACS) events as the population controls; however, RA patients with only moderate or no response to TNF inhibition had a 2.7×-increased risk of ACS. Earlier, limited, analysis of this registry did not demonstrate a significant difference in cardiovascular events between a combined cohort of RA patients on TNF inhibitors and population referents.[60] Lee *et al.* assessed data in 4140 consecutive Australian patients with RA, psoriatic arthritis, or ankylosing spondylitis, 57% of who were on anti-TNF therapy at baseline, 3% on alternate biologics, and the remainder biologic-naïve. Multi-variate analysis demonstrated a reduction in coronary events with both TNF inhibition (HR 0.85, 95% CI 0.76, 0.95) and other biologic therapy (HR 0.81, 95% CI 0.7, 0.95) across all three inflammatory conditions, however, this benefit was not sustained after therapy was ceased.[61] British registry data were analysed by Low *et al.*, including 11 200 RA patients on TNF inhibitor therapy, demonstrating a reduced rate of MI when compared with a propensity-decile matched group of 3058 patients on other DMARD therapy (adjusted HR 0.61, 95% CI 0.41, 0.89).[56] This study was unable to delineate whether a TNF inhibitor-specific effect, or better disease control, was attributable for the risk reduction. An earlier paper from the same group did not demonstrate a significant reduction in the overall rate of MI with TNF inhibitor use in 8659 patients, compared to 2170 patients on other DMARDS (incidence rate ratio 1.44, 95% CI 0.56, 3.57).[55] However, it was demonstrated that responders to TNF inhibition had a significantly lower rate of MI than non-responders (incidence rate ratio 0.36, 95% CI 0.19, 0.69).[55] Further research is required to determine whether reduced cardiovascular events in these patients are in fact due to TNF inhibitors themselves, or reflect better disease control.

TNF inhibition has also been prospectively studied in patients with heart failure with no demonstrable benefit, and even a trend towards harm. Two randomized control trials (RECOVER and RENNAISANCE) analysed the use of etanercept in a combined 2048 patients with NYHA class III/IV heart failure; however, both studies were stopped prematurely after no benefit was shown at two years.[62] One smaller trial (ATTACH) investigated the use of infliximab in 150 patients with NYHA III/IV heart failure, receiving either placebo, low- or high-dose infliximab. At the 28-week mark, patients in the high-dose infliximab group actually demonstrated worsened heart failure and increased mortality, with the subsequent conclusion drawn that infliximab should be avoided in heart failure.[63] Interestingly, recent meta-analysis of TNF inhibitor use in RA has not demonstrated an increased risk of heart failure.[58] The current general recommendation is to avoid TNF inhibitors in patients with moderate to severe heart failure.

While the above data certainly indicate a positive effect of TNF inhibitors on ischaemic vascular disease in RA patients, it does remain to be seen whether this benefit is a direct action of the drugs themselves, or simply reflective of better disease control. It is unknown whether a future trial will address the use of TNF inhibition in coronary disease, with this controversy unlikely to be definitively answered until such a time. It is also important to recognize that TNF inhibitors are well-known to cause a wide range of side effects, including infusion reactions, serious infections, and cytopenias, which may limit their wide-spread use in future practice.[53]

5.2 TRAIL

TRAIL therapy in oncology has been studied in phase I/II trials using soluble forms of the protein; however, efficacy has been disappointing, while usage has also been limited by physiochemical instability and tumour resistance.[64,65] To the author's knowledge, there are as of yet no data on the effect of TRAIL therapies in ischaemic vascular disease. Nevertheless, TRAIL therapy maintains a high translatable potential, particularly given its protective effect and theoretically lower risk of side effects such as infection compared with other TNFSF targets. As TRAIL therapy matures further and undergoes further trials, we hope to see analysis of cardiovascular outcomes to elicit whether this may be a useful future avenue for development.

5.3 TWEAK

BIIB 023 is a humanized monoclonal antibody to TWEAK which has been studied in several phase I and II trials in rheumatological disease.[66,67] BIIB 023 did not demonstrate adverse cardiovascular events, however, due to lack of efficacy has ceased development. Enavatuzumab, a humanized monoclonal antibody to TweakR, the TWEAK receptor, is a partial agonist that modulates cytokine expression, with no pro-angiogenic effects. Enavatzumab has recently been studied in a phase I clinical trial analysing safety in a small number of patients with solid organ cancers.[68] This trial did not demonstrate any negative cardiovascular events, however, over 40% of patients had dose-limiting hepatic and pancreatic side effects, leading to discontinuation of development of the drug in its current form. TWEAK remains a potential future target in ischaemic vascular disease; however, it may be some time before adequate data are available on future agents.

5.4 CD40L and CD40

IDEC-131 and BG9588 are humanized monoclonal antibodies to CD-40L, developed for use in inflammatory conditions such as systemic lupus erythematosus and Crohns' disease. Although some promising markers of efficacy were shown, phase II trials of both drugs were stopped when several participants experienced thromboembolic events, including several MIs in patients treated with BG9588.[69,70] It was speculated at the time that prothrombotic tendencies of these drugs were due to cross-linking of CD40L expressed by platelets,[70] rendering them unlikely to be of benefit in ischaemic vascular disease.

These findings have led to the development of newer agents specifically targeting CD40, and not its ligand. Phase I trials of CD40 inhibitors have recently been conducted in rheumatological disease, showing some promise, with phase II trials currently underway.[71] It remains to be seen whether these agents may be of benefit in cardiovascular disease, with

secondary analysis of larger trials likely to provide further information in the future.

6. Translatable potential and future directions

While TNFSF members have been considered potential drug targets in ischaemic vascular disease for many years, data on their effects on the cardiovascular system are generally lacking. A 'translational gap' currently exists for this family of ligands, with no trials yet conducted on their use in patients with ischaemic vascular disease. While the TNFSF members remain promising targets for the prevention and treatment of ischaemic vascular disease, more research is required to understand their role in the vasculature under both normal and abnormal conditions.

Conflict of interest: none declared.

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