

■ R E V I E W

Role of TNF- α in vascular dysfunction

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A B S T R A C T

Healthy vascular function is primarily regulated by several factors including EDRF (endothelium-dependent relaxing factor), EDCF (endothelium-dependent contracting factor) and EDHF (endothelium-dependent hyperpolarizing factor). Vascular dysfunction or injury induced by aging, smoking, inflammation, trauma, hyperlipidaemia and hyperglycaemia are among a myriad of risk factors that may contribute to the pathogenesis of many cardiovascular diseases, such as hypertension, diabetes and atherosclerosis. However, the exact mechanisms underlying the impaired vascular activity remain unresolved and there is no current scientific consensus. Accumulating evidence suggests that the inflammatory cytokine TNF (tumour necrosis factor)- α plays a pivotal role in the disruption of macrovascular and microvascular circulation both *in vivo* and *in vitro*. AGEs (advanced glycation end-products)/RAGE (receptor for AGEs), LOX-1 [lectin-like oxidized low-density lipoprotein receptor-1] and NF- κ B (nuclear factor κ B) signalling play key roles in TNF- α expression through an increase in circulating and/or local vascular TNF- α production. The increase in TNF- α expression induces the production of ROS (reactive oxygen species), resulting in endothelial dysfunction in many pathophysiological conditions. Lipid metabolism, dietary supplements and physical activity affect TNF- α expression. The interaction between TNF- α and stem cells is also important in terms of vascular repair or regeneration. Careful scrutiny of these factors may help elucidate the mechanisms that induce vascular dysfunction. The focus of the present review is to summarize recent evidence showing the role of TNF- α in vascular dysfunction in cardiovascular disease. We believe these findings may prompt new directions for targeting inflammation in future therapies.

Key words: inflammation, macrovascular circulation, microvascular circulation, nitric oxide, reactive oxygen species (ROS), tumour necrosis factor- α (TNF- α).

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AGE, advanced glycation end-product; AMI, acute myocardial infarction; ASS, argininosuccinate synthase; CRP, C-reactive protein; DC, dendritic cell; EC, endothelial cell; EDHF, endothelium-dependent hyperpolarizing factor; EET, epoxyeicosatrienoic acid; EPC, endothelial progenitor cell; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HUVEC, human umbilical vein EC; ICAM-1, intercellular adhesion molecule-1; IHD, ischaemic heart disease; IL, interleukin; I/R, ischaemia/reperfusion; NF- κ B, nuclear factor κ B; I κ B, inhibitor of NF- κ B; IKK, I κ B kinase; NOS, NO synthase; cNOS, constitutive NOS; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS; O₂^{•-}, superoxide radical; ONOO⁻, peroxynitrite; PGI₂, prostacyclin; RA, rheumatoid arthritis; RAGE, receptor for AGEs; ROS, reactive oxygen species; SCF, stem cell factor; SK1, sphingosine kinase 1; Sph1P, sphingosine-1-phosphate; TNF, tumour necrosis factor; TNFR, TNF receptor; t-PA, tissue plasminogen activator; VCAM-1, vascular cell adhesion molecule-1; XO, xanthine oxidase; ZOF rat, Zucker Obese Fatty rat.

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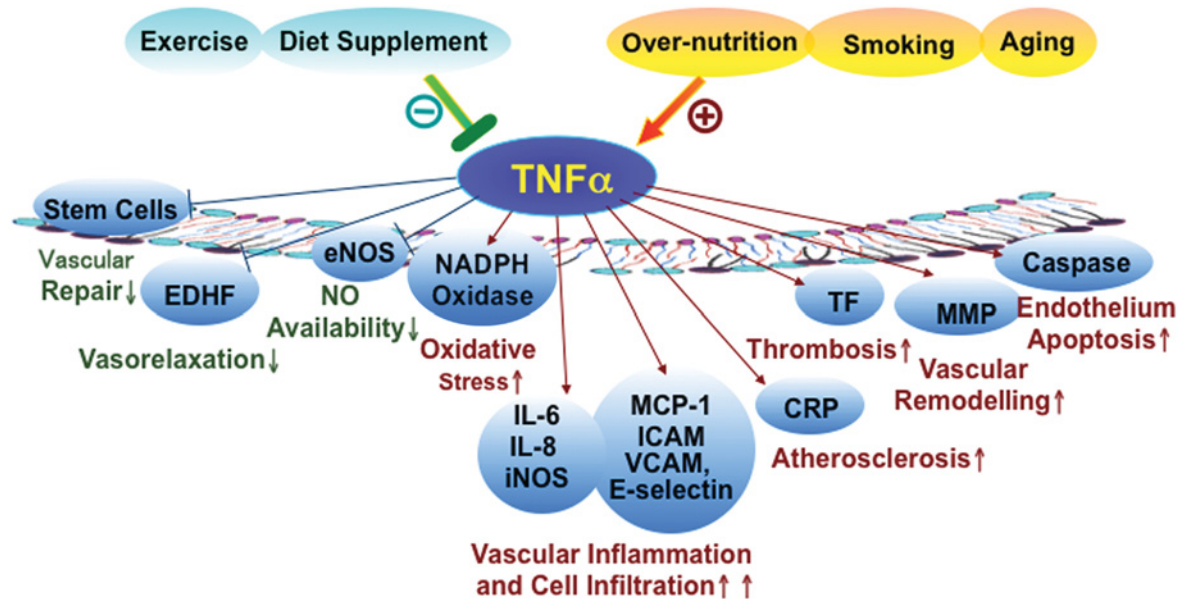


Figure 1 Pivotal role of TNF- α in vascular dysfunction

Even though numerous risk factors, such as physical inactivity, smoking and over-nutrition, appear to contribute to the development of vascular dysfunction, normal aging is also an independent factor in the aetiology of cardiovascular diseases. There is evidence, however, that those seemingly diverse processes converge on modulating TNF- α signalling to lead to the generation of dysfunctional endothelium and the onset of vascular diseases. TNF- α induces the gene expression of various inflammatory cytokines and chemokines, either dependently or independently of the activation of transcriptional factors, such as NF- κ B and AP-1 (activator protein 1). This TNF- α -mediated signalling initiates and accelerates atherogenesis, thrombosis, vascular remodelling, vascular inflammation, endothelium apoptosis, vascular oxidative stress and impaired NO bioavailability, which contribute to the blunted vascular function. Dietary supplements and exercise favourably reduce the risk of vascular dysfunction by inhibiting TNF- α production and (or) TNF- α -mediated signalling. Risk factors in orange demonstrate those factors that converge on TNF- α to induce vascular dysfunction. Factors in green denote those that protect against vascular damage mediated by TNF- α expression and signalling. TNF- α -induced pathophysiological conditions related to vascular function are shown in blue. Both vascular risk factors and protective factors affect the regulation of vascular functions by modulating TNF- α production and downstream signalling. MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; TF, tissue factor.

INTRODUCTION

IHD (ischaemic heart disease) accounts for over 500 000 deaths annually in the United States. AMI (acute myocardial infarction), also known as a heart attack [1], is a common complication of IHD. AMI usually results from plaque rupture with thrombus formation in a coronary vessel, resulting in an acute reduction in blood supply to the downstream myocardium. Paradoxically, re-establishment of the blood supply can exacerbate vascular injury. Treatment of AMI, such as thrombolysis and other means of revascularization, often induce further vascular injury, which contributes to morbidity and mortality before normal cardiac function restores.

The endothelium is a functional barrier between the blood vessel and the blood stream, and was once considered to be relatively inert [2]. However, various functions of ECs (endothelial cells) have been elucidated, such as the control of fibrinolysis, coagulation, vascular tone, growth and immune response. The endothelium modulates vascular tone through several factors, including NO, PGI₂ (prostacyclin) and EDHF (endothelium-dependent hyperpolarizing factor). A hallmark of IHD is

the development of coronary vascular lesions, which are linked to well-known risk factors, such as diabetes and obesity conditions associated with increased levels of inflammatory markers (Figure 1). IHD accelerates the atherosclerotic process, the earliest event of which is endothelial dysfunction.

ROLE OF TNF- α (TUMOUR NECROSIS FACTOR- α) IN ENDOTHELIAL DYSFUNCTION

NO is a free radical generated by NOS (NO synthase) in a two-step five-electron oxidation of the terminal guanidino nitrogen of L-arginine. Three isoforms of NOS have been characterized: eNOS (endothelial NOS), nNOS (neuronal NOS) and iNOS (inducible NOS). eNOS and nNOS are also called cNOS (constitutive NOS) [3]. TNF- α regulates NOS expression and/or activity, which exerts direct effects on NO production; for example, human aortic ECs treated with TNF- α for 8 h had induced iNOS mRNA expression, but down-regulated eNOS expression [4]. Other studies have also shown that TNF- α significantly decreased eNOS expression in ECs

[5–8]. Unlike eNOS, iNOS is transcriptionally regulated and not normally produced in most cells. iNOS-derived RNS (reactive nitrogen species) initiate an ONOO⁻ (peroxynitrite)-mediated mechanism and therefore contribute to nitrate stress and impair endothelial function.

Several mechanisms have been suggested for the induction/activation of NOS by TNF- α . Yoshizumi et al. [9] demonstrated that TNF- α markedly reduced mRNA levels of cNOS in HUVECs (human umbilical vein ECs) in a dose- and time-dependent manner without changing the rate of cNOS gene transcription. TNF- α appears to decrease cNOS mRNA levels by increasing the rate of mRNA degradation. Another study, however, suggested that TNF- α increases eNOS activity in HUVECs [10]. Activation of eNOS by TNF- α requires activation of Akt (protein kinase B), a known eNOS activator, via Sph1P (sphingosine-1-phosphate) receptor activation. Sph1P receptor is activated by Sph1P, a sphingolipid involved in proliferation, survival, migration and differentiation of these cells, generated through N-SMase2 (neutral sphingomyelinase 2) and SK1 (sphingosine kinase 1) activation [10]. TNF- α -mediated activation of eNOS is accompanied by increased NO generation, which exerts protective effects on DC (dendritic cell) adhesion to endothelium induced by TNF- α itself. It has also been suggested that TNF- α may increase iNOS expression by activating NF- κ B (nuclear factor κ B) [11]. TNF- α -induced iNOS mRNA expression in microvascular ECs could be decreased by rooperol (a dicatchol from the South African plant *Hypoxis rooperi*) administration, which is an anti-inflammatory agent in the treatment of several inflammatory disorders [12]. In HUVECs, the effect of TNF- α on iNOS expression was not affected by statin treatment, whereas reduced eNOS expression was reversed by rosuvastatin and ceruvastatin by inhibiting HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase and subsequent blocking of isoprenoid synthesis [13].

Evidence suggests that TNF- α impairs endothelium-dependent and NO-mediated vasodilation in various vascular beds, e.g. mouse coronary arterioles [14], rat coronary arterioles [15], cat carotid arteries [16] and bovine small coronary arteries [17]. Picchi et al. [15] demonstrated that endothelial dysfunction in pre-diabetic metabolic syndrome is a result of the effects of TNF- α and the subsequent production of O₂^{•-} (superoxide radical). We have assessed the role of TNF- α in I/R (ischaemia/reperfusion) injury in TNF 1.6 mice, which overexpress TNF- α in cardiac tissue. Myocardial I/R initiated the increase in the expression of TNF- α , which induced activation of XO (xanthine oxidase) and the production of O₂^{•-}, leading to coronary endothelial dysfunction [18]. Gao et al. [14] showed that AGE (advanced glycation end-product)/RAGE (receptor for AGEs) and NF- κ B signalling play a pivotal role in elevating circulating and/or local vascular TNF- α production. The increased TNF- α expression induces the

production of ROS (reactive oxygen species), leading to endothelial dysfunction in Type 2 diabetes. Endothelial dysfunction associated with TNF- α in pathophysiological conditions is linked to excess production of ROS and a decrease in NO bioavailability.

TNF- α appears to decrease the bioavailability of NO by (i) diminishing the production of NO [6,15,17,19], and (ii) enhancing the removal of NO [14]. Picchi et al. [15] reported that the real-time production of NO in isolated coronary arteries from ZOF rats (Zucker Obese Fatty rats; a model of pre-diabetic metabolic syndrome) and ACh (acetylcholine)-induced NO production were significantly lower in ZOF rats compared with the lean control rats. This result suggested that higher concentrations of circulating and protein expression of TNF- α diminished NO bioavailability in ZOF rat coronary arteries via the decreased expression of eNOS (Figure 2). Many studies have shown that the direct effects of TNF- α on eNOS are via down-regulating eNOS expression and diminishing NO production in diverse vasculatures [6,10,14,15]. In addition to eNOS, other factors are also involved in regulating NO production, and one of those factors is a functional citrulline/NO cycle [20–23]. The citrulline/NO cycle is regulated by ASS (argininosuccinate synthase). NO is synthesized from the conversion of L-arginine into L-citrulline mediated by eNOS, and ASS catalyses the rate-limiting step in the arginine regeneration through the citrulline/NO cycle and appears to be co-ordinately regulated with eNOS activity [24] (Figure 2). Goodwin et al. [6] have shown that TNF- α diminished the protein and mRNA expression of ASS in aortic ECs and directly resulted in the reduced production of NO. Gao and co-workers [14,15] reported that TNF- α impaired NO-mediated vasodilation in Type 2 diabetic coronary arterioles. A neutralizing antibody to TNF- α decreased the formation of ROS (O₂^{•-}, ONOO⁻ and H₂O₂) and improved NO-mediated vasodilation. TNF- α stimulates the endothelial generation of ROS by activation of NADPH oxidase, perhaps via the subunits gp91^{phox}, NOX-1, p47^{phox} and p22^{phox} (Figure 2).

NO has been implicated as the major mediator of endothelium-dependent relaxation, but EDHF also plays an important role in regulating vascular tone and vaso-reactivity, particularly in resistance blood vessels, where a small change in membrane potential causes a significant change in diameter [25]. A number of different factors have been considered as candidates for EDHFs, such as K⁺ ions, EET (epoxyeicosatrienoic acid) and H₂O₂ [26]. Current evidence suggests that EDHF-induced responses may be mediated by one or a combination of several factors in different vasculatures [25]. Type 2 diabetes impairs EDHF-mediated vasodilation [27]; however, the mechanisms have not been clearly elucidated. For example, the role of TNF- α in EDHF-mediated vascular dysfunction is controversial. Wimalasundera et al. [28] reported that TNF- α did not inhibit EDHF-dependent

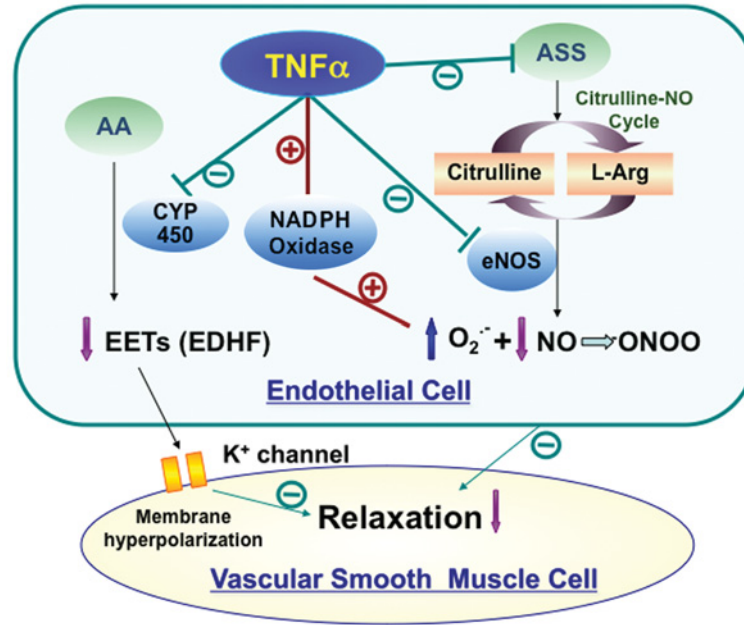


Figure 2 Role of TNF- α in endothelial dysfunction

TNF- α reduces the production of NO through the inhibition of the enzyme activities of ASS and eNOS, and enhances the removal of NO through the increase in NADPH-dependent $O_2^{\bullet-}$ production to react with NO to form $ONOO^-$. As a consequence, TNF- α decreases the bioavailability of NO to induce relaxation of smooth muscle in the vasculature. TNF- α also diminishes EETs, one of the candidate EDHFs, via the inhibition of cytochrome P450 (CYP 450) enzyme activity. AA, arachidonic acid.

vasodilation, whereas Gillham et al. [29] measured a direct effect of TNF- α on EDHF-mediated vasodilation by incubation of 1 nmol/l TNF- α for 1 or 2 h with blood vessels from human omental arteries and showed that TNF- α impaired EDHF-mediated dilation. In addition, Kessler et al. [30] found that TNF- α reduced EDHF synthesis with direct measurement of hyperpolarization from porcine coronary arteries, and Park et al. [30a] have shown that EDHF-mediated dilation in coronary arterioles from Type 2 diabetic mice null for TNF- α (db^{TNF-}/db^{TNF-}) was enhanced compared with Type 2 diabetic (db/db) mice. The possible mechanism of impaired EDHF-mediated vasodilation by TNF- α may be via EETs, one of the candidates for EDHF. EETs are synthesized in the ECs from AA (arachidonic acid) through cytochrome P450 oxygenase. TNF- α down-regulated the protein expression of cytochrome P450 2C, which is the major family of cytochrome P450 mono-oxygenases in porcine aortic ECs [30] (Figure 2).

EFFECT OF TNF- α ON ROS PRODUCTION

The production of ROS can stimulate a cytokine cascade through NF- κ B-induced transcriptional events, which then induce the expression of TNF- α [31,32]. TNF- α stimulates $O_2^{\bullet-}$ production in neutrophils and ECs, reportedly via CAPK (ceramide-activated protein kinase),

NADPH oxidase [33], XO [34], NOS [35,36] etc. Many experimental studies suggest that increased $O_2^{\bullet-}$ production accounts for a significant proportion of the NO deficit in diabetic vessels. Potential sources of vascular $O_2^{\bullet-}$ production include NADPH-dependent oxidases [37,38], XO [39], lipoxygenase, mitochondrial oxidase and uncoupled NOS [40]. NADPH oxidase appears to be the principal source of $O_2^{\bullet-}$ production in several animal models of vascular disease, including diabetes [41]. Furthermore, NADPH oxidase proteins and activity are present in human blood vessels, including atherosclerotic coronary arteries [42], and in saphenous veins and mammary arteries from patients with coronary artery disease [43], which suggests that this oxidase system plays an important role in cardiovascular diseases [44]. Guzik et al. [45] have described the mechanisms of increased $O_2^{\bullet-}$ production in human diabetes mellitus. They found that basal $O_2^{\bullet-}$ release was significantly elevated in vessels from patients with diabetes. Western immunoblot analysis showed increased levels of the p22^{phox} membrane-bound subunit and the p67^{phox} and p47^{phox} cytosolic subunits in both veins and arteries from patients with diabetes. Moreover, engagement of RAGE triggers signalling cascades in which activation of NADPH oxidase recruits multiple downstream pathways, including p21ras, the MAPKs (mitogen-activated protein kinases), the JAK (Janus kinase)/STAT (signal transducer and activator of transcription) pathway, PI3K

(phosphoinositide 3-kinase), cdc42/rac and nuclear translocation of NF- κ B [46]. As mentioned previously, NF- κ B can be considered as a link between TNF- α and AGE/RAGE signalling because TNF- α enhanced RAGE expression by NF- κ B activation [32,47–50].

TNF- α activates the transcription of NF- κ B, which regulates the expression of genes involved in inflammation, oxidative stress and endothelial dysfunction [51–53]. TNF- α initiates the signalling cascades via the IKK [I κ B (inhibitor of NF- κ B) kinase] complex, which contains IKK α and IKK β . TNF- α predominantly initiates signalling cascades acting through IKK β [54,55]. The inhibitory protein I κ B α is phosphorylated, ubiquitinated and degraded by the proteasome, releasing NF- κ B to translocate into the nucleus. Under normal physiological conditions, the inflammatory response is terminated by binding NF- κ B with the inhibitory protein I κ B [56,57]. In ECs, NF- κ B regulates the inducible expression of genes encoding TNF- α , IL (interleukin)-6, MCP-1 (monocyte chemoattractant protein-1) and adhesion molecules in diabetic mice [58]. Shoelson et al. [59] have detailed the role of IKK β in inflammation-induced insulin resistance in obesity and Type 2 diabetes, with the genetic disruption of the IKK β signalling pathways shown to improve insulin resistance. We have shown that blockade of IKK β activity by sodium salicylate not only prevented insulin resistance, but also preserved coronary arteriolar vasodilation in Type 2 diabetic mice (J. Yang, Y. Park, H. Zhang, X. Xu, G.A. Laine, K.C. Dellsperger and C. Zhang, unpublished work). Furthermore, obese insulin-resistant subjects have endothelial dysfunction and resistance to endothelium-dependent insulin-mediated vasodilation [60,61]. Augmentation of insulin signalling may contribute to endothelium-dependent NO-mediated vasodilation in diabetic mice treated with sodium salicylate (J. Yang, Y. Park, H. Zhang, X. Xu, G.A. Laine, K.C. Dellsperger and C. Zhang, unpublished work). NF- κ B induces TNF- α signalling to accentuate oxidative stress and endothelial dysfunction induced via an IKK β -dependent mechanism, which may be associated with inflammatory and insulin signalling pathways seen in Type 2 diabetes. AGE/RAGE signalling, as noted above, stimulates the production of O₂^{•-}, which could exacerbate both oxidative stress and impaired bioavailability of NO. Thus it appears that impaired NO function may be the direct result of the overproduction of O₂^{•-} induced by TNF- α and AGE/RAGE. O₂^{•-} is the chemical precursor to many ROS, such as H₂O₂ and ONOO⁻.

ROLE OF TNF- α IN LIPID METABOLISM

Both clinical observations and basic research have indicated a potential link between inflammation and lipid metabolism. TNF- α acts as a key cytokine that affects and mediates intermediary metabolism, and a close

relationship between TNF- α and lipid metabolism is supported by several studies. In patients with hyperlipidaemia, TNF- α levels correlated significantly with the concentrations of VLDL (very-low-density lipoprotein) -triacylglycerol (triglyceride) and -cholesterol, and negatively with HDL (high-density lipoprotein)-cholesterol [62]. Simvastatin and atorvastatin decrease TNF- α levels in subjects with hyperlipidaemia and hypercholesterolaemia [63–65]. Furthermore, patients with type IIa and IIb dyslipidaemia have an abnormal pattern of TNF- α . HMG-CoA reductase inhibitors (statins) and PPAR- α (peroxisome-proliferator-activated receptor- α) activators (fibrates) normalize TNF- α levels [66]. A high-cholesterol diet induces high levels of serum TNF- α concentration, whereas the mRNA expression of TNF- α is significantly reduced by atorvastatin treatment in hypercholesterolaemic rabbits [67]. TNF- α blockade could significantly affect lipid metabolism. Short-term administration of adalimumab, a fully human anti-TNF- α monoclonal antibody, to patients with active RA (rheumatoid arthritis), significantly increased HDL-cholesterol concentrations; in addition, the atherogenic index decreased [68]. Infliximab, a chimaeric anti-TNF- α monoclonal antibody, had similar results [69–71]. Administration of TNF- α has been demonstrated to directly interfere with the plasma lipid level and metabolic pathways. In mice, administration of TNF- α results in an acute increase in plasma triacylglycerol concentrations of 85%, and inhibition of TNF- α activity blocked the increase in serum triacylglycerols that is characteristically observed after LPS (lipopolysaccharide) treatment [72,73]. The effect of TNF- α on lipid metabolism is complicated, and the mechanisms are complex and take place at different levels and through different steps, from affecting protein expression to inhibiting enzyme activity. Collectively, these studies prompt the question: why does TNF- α produce different responses in different situations? With this controversial background and in conjunction with previous studies, there is ample rationale to study the role of TNF- α in lipid metabolism.

AGING AND TNF- α

Epidemiological studies have shown that even normal aging is an independent risk factor for cardiovascular diseases [74]. An aging-induced pro-inflammatory shift plays an important role in vascular regulatory mechanisms. Previous studies have suggested that circulating levels of TNF- α are elevated in the elderly [75]. Increased TNF- α production has been demonstrated in carotid arteries, aortic wall [76] and coronary arteries [77] of aged rodents. Age-related up-regulation of TNF- α in rat coronary arteries induced endothelial apoptotic cell death, which may lead to impaired endothelial function in the elderly [77]. At the cellular level, TNF- α reduced the growth rate and *in vitro* life span of ECs in both

dose- and treatment-length-dependent manners, suggesting that the aging of ECs is modified by TNF- α exposure [78]. In contrast, inhibition of TNF- α exerts beneficial effects in aging-related pathophysiological changes. *In vivo*, chronic TNF- α inhibition by etanercept improves flow-mediated arterial dilation in resistance arteries of aged female rats [79], as well as down-regulates the expression of inflammatory markers, including iNOS and ICAM-1 (intercellular adhesion molecule-1), which are abundantly expressed in aged vessels. In carotid arteries of young animals, recombinant TNF- α induced endothelial dysfunction, oxidative stress and increased apoptosis and pro-inflammatory gene expression, mimicking many of the symptoms of vascular aging [74]. Thus dysregulation of TNF- α expression is associated with vascular aging, and anti-TNF- α treatment exerts anti-aging vasculoprotective effects.

To sum up, aging is an independent factor in vascular dysfunction. In the presence of other risk factors, such as smoking and over-nutrition, the development of endothelial dysfunction might be accelerated. Those risk factors converge on TNF- α to cause vascular oxidative stress, vascular remodelling, thrombosis, cell infiltration, apoptosis, vascular inflammation etc., and therefore lead to vascular damage (Figure 1).

TNF- α AND HIGH-FAT AND HIGH-CARBOHYDRATE DIETS

The effects of over-nutrition on endothelial dysfunction in healthy subjects and subjects with dyslipidaemia, the metabolic syndrome and diabetes have been examined in many studies. Endothelial function was markedly impaired by a high-fat meal that caused an acute hypertriacylglycerolaemia. This impairment was evident in patients with dyslipidaemia with baseline hypertriacylglycerolaemia, but not in controls with normotriacylglycerolaemia [80]. Compared with the control group, subjects with metabolic syndrome had reduced endothelial function, as assessed using the L-arginine test, and higher circulating levels of TNF- α . Following the high-fat meal, both triacylglycerol and TNF- α levels increased more in subjects with the metabolic syndrome than in normal subjects, whereas endothelial function decreased more in subjects with the metabolic syndrome [81]. Moreover, in healthy subjects, the high-fat meal increased plasma levels of TNF- α , IL-6, ICAM-1 and VCAM-1 (vascular cell adhesion molecule-1), while the high-carbohydrate meal had no effects in these subjects. In patients with diabetes, both meals significantly increased cytokine and adhesion molecule levels, but the increase lasted longer following the high-fat meal [82]. On the basis of the significant relationship between increases in TNF- α levels and decreases in endothelial function in subjects with the metabolic syndrome and diabetes [81], the mechanisms of

TNF- α -induced endothelial dysfunction following high-energy diets has been extensively studied at the molecular and cellular levels. Intraluminal butter administration significantly increased TNF- α expression in lamina propria macrophage and lymphocyte adherence to intestinal microvessels, accompanied by increases in the expression levels of ICAM-1, MAdCAM-1 (mucosal adhesion cell adhesion molecule-1) and VCAM-1. Furthermore, anti-TNF- α treatment attenuated the enhanced expression of adhesion molecules induced by butter administration [83]. Therefore high-energy diets may cause endothelial dysfunction, as well as potentiate TNF- α -mediated EC injury [84]. Reducing saturated fat and dietary cholesterol intake and avoiding excess calories remains the cornerstone of the dietary approaches to decrease the risk of vascular diseases.

ROLE OF EXERCISE IN CARDIOVASCULAR DISEASE

Pro-inflammation events, such as the increases in TNF- α , CRP (C-reactive protein), IL-6 and resistin, appear to produce their harmful effects, at least in part, by inducing endothelial dysfunction and also by decreasing endothelial NO generation. NO inhibits platelet adherence and aggregation, suppresses vasoconstriction, reduces the adherence of leucocytes to the endothelium and suppresses the proliferation of VSMCs (vascular smooth muscle cells) [85]. Of these pro-inflammatory cytokines, TNF- α is a key player in systemic low-level inflammation through stimulating the expression of adhesion molecules on ECs and thereby inducing endothelial dysfunction [85,86]. TNF- α is a strong biological driver of the metabolic syndrome, which is characterized by abdominal obesity, hypertension, a reduced level of HDL, elevated triacylglycerols and high-fasting glucose, and constitutes an important risk factor in atherosclerosis and Type 2 diabetes [86]. Keller et al. [87] have reported that TNF- α overexpression returned to normal levels after 1 h of acute swimming exercise in TNFR (TNF receptor)-knockout mice. In addition, chronic exercise appears to suppress pro-inflammatory factors, such as TNF- α , CRP and IL-6, and augment anti-inflammatory factors, including IL-4, IL-10, TGF- β (transforming growth factor- β) and adiponectin, even though these results showed discrepancies according to the modes, intensity and time duration of exercise [86,88–90]. Therefore the anti-inflammatory effects of exercise may offer protection against TNF- α -induced insulin resistance and the secondary development of cardiovascular dysfunction. In summary, regular exercise contributes to the prevention of cardiovascular dysfunction by controlling traditional cardiovascular risk factors, including HDL- and LDL (low-density lipoprotein)-cholesterol, improving antioxidant factors, such as SOD (superoxide

dismutase) and glutathione peroxidase, elevating the anti-inflammatory effect and suppressing TNF- α , which is the main pro-inflammatory cytokine (Figure 1).

TNF- α AND STEM CELLS/PROGENITOR CELLS

Stem and progenitor cells possess the ability to self-regenerate and differentiate into many cell types, including cardiovascular-specific forms. The role of stem cells and progenitor cells in cardiovascular diseases has been widely investigated. As inflammation is involved in most cardiovascular diseases, understanding the communication and interaction between TNF- α and stem cells is important. Clinical evidence has shown that the serum TNF- α level was negatively correlated with peripheral blood CD34⁺ stem cells and circulating EPCs (endothelial progenitor cells) in the early and late stages of congestive heart failure, which might be related to the myelosuppressive effect of TNF- α [91]. A similar observation was also reported in a mouse model of congestive heart failure, with increased serum TNF- α levels and decreased bone marrow progenitor cells [92]. *In vitro* studies have indicated a causal relationship between TNF- α and suppression of haemopoietic stem cell growth. Rusten et al. [93] reported that TNF- α directly inhibited SCF (stem cell factor)-stimulated proliferation of CD34⁺ haemopoietic progenitor cells. Similar results were also demonstrated in human CD34⁺ myeloid leukaemia cells and primitive human bone marrow progenitor cells (CD34⁺CD38⁻) [94,95]. Interestingly, the inhibitory effects of TNF- α in these studies were consistently mediated by TNFR-I, but not TNFR-II. On the contrary, the TNFR-II signalling pathway has a protective profile on stem cell function. Chen et al. [96] have demonstrated that TNF- α -overexpressing cardiomyocytes attracted increased numbers of embryonic stem cells, mediated by TNFR-II in the embryonic stem cells. Treatment with TNFR-II-overexpressing mesenchymal stem cells attenuated cardiac dysfunction after myocardial infarction [97]. The expression of TNFR-II on bone-marrow-derived progenitor cells was required for ischaemia-induced neovascularization [98]. Thus distinct effects of TNF- α are mediated by different subtypes of TNFRs in stem cells, whereas the overall effect might be dependent on the expression level and ratio of these two receptors.

Apart from the direct effects, TNF- α is able to indirectly influence the fate of stem cells. TNF- α markedly stimulates production of GM-CSF (granulocyte/macrophage colony-stimulating factor), a strong mobilizer of stem cells from bone marrow [99]. Activation of the TNF- α /Fas pathway in lymphocytes in the bone marrow may play a pathogenic role in suppressing haemopoiesis [92]. In the peripheral circulation, EPC adhesion to

HUVECs was significantly increased by TNF- α pretreatment with HUVECs; the adhesion was mediated by the up-regulation of E-selectin on the cells. Interestingly, when EPCs rather than HUVECs were stimulated with TNF- α , EPC adhesion to HUVECs was not induced [100]. TNF- α also has effects on stem cell differentiation: administration of TNF- α switched the differentiation of these cells from granulocytes to almost complete production of macrophages when mouse Lin⁻Sac⁺ haemopoietic progenitor cells were cultured with SCF and IL-7 [101].

In summary, TNF- α plays an important role in regulating stem-cell-mediated vascular repair and remodelling; however, the overall effect of TNF- α on stem cell mobilization, proliferation and function is complicated, depending on the TNFR subtypes and the presence of other cytokines as well as other cells (Figure 1).

ANTI-TNF- α TREATMENT IN CLINICAL STUDIES

As a potent pro-inflammatory trigger, the central role of TNF- α in vascular dysfunction has been demonstrated by the ability of agents that block the action of TNF- α to treat a range of cardiovascular disorders and inflammatory conditions, including AMI, heart failure, RA, diabetes, hyperlipidaemia and COPD (chronic obstructive pulmonary disease).

Intra-arterial TNF- α infusion in healthy volunteers and patients provides direct evidence about TNF- α -stimulated vascular dysfunction. In healthy volunteers, intra-arterial TNF- α at a dose of 80 or 240 ng/min for 30 min resulted in an acute local vascular inflammation that was associated with impaired endothelium-dependent vasomotion, as well as a sustained and substantial increase in endothelial t-PA (tissue plasminogen activator) release [102]. A dose of 17 ng/min for 60 min increased the basal bioavailability of the vasoconstrictor prostaglandin and reduced the basal bioavailability of NO, although it had no effects on endothelium-dependent vasomotion in healthy subjects [103]. In patients with coronary heart disease, intra-arterial TNF- α at a dose of 80 ng/min for 60 min caused an increase in t-PA concentrations without affecting blood flow [104]. In patients with Type 2 diabetes, intra-arterial infusion with TNF- α (10 ng·100 ml⁻¹ of forearm volume·min⁻¹ for 2 h) induced the impairment of endothelial function in resistance vessels [105].

On the basis of the important role of TNF- α in inducing endothelial dysfunction, clinical trials are under way to investigate the use of the three currently available TNF- α inhibitors (infliximab, etanercept or adalimumab), as well as others, in vascular disorders accompanied by several diseases. Chronic anti-inflammatory treatment with the anti-TNF- α antibody infliximab improved

endothelial function of the brachial artery in patients with RA [106,107], systemic vasculitis [108] and Crohn's disease [109]. Anti-TNF- α therapy with etanercept, a recombinant TNFR that binds to and functionally inactivates TNF- α , and adalimumab, a fully human monoclonal antibody directed against TNF- α , were also able to improve endothelial function in patients with RA [110,111]. In patients with advanced heart failure, etanercept improves systemic endothelial vasodilator capacity [112], although studies using anti-TNF- α antibodies in chronic heart failure have been terminated prematurely owing to a lack of benefit on the rate of death or hospitalization [113]. However, although short-term etanercept treatment had a significant beneficial effect on systemic inflammatory markers, no improvement in vascular or metabolic insulin sensitivity was observed in obese patients with Type 2 diabetes [114]. In addition to TNF- α inhibitors, various statins, such as atorvastatin and simvastatin, and the ACEI (angiotensin-converting enzyme inhibitor) quinapril were reported to decrease circulating TNF- α levels, as well as improve endothelial function in patients with Type 2 diabetes, congestive heart failure, RA or hyperlipidaemia [65,115–121]. Therefore, although the efficacy and safety of anti-TNF- α biologicals have been extensively studied in treating vascular complications of inflammatory diseases, such as RA and Crohn's disease, the use of TNF- α inhibitors and antibodies in cardiovascular disease and Type 2 diabetes may need to be evaluated further by randomized controlled clinical trials or long-term observational studies. Furthermore, direct evidence may be needed to address the causal relationship between the effects of statins and ACEIs in attenuating TNF- α production and improving endothelial function.

To sum up, the evidence above suggests an important role of TNF- α in vascular dysfunction. These studies may support the long-term use of drugs that block TNF- α function to reduce the high incidence of cardiovascular disorders and vascular complications in various diseases.

CONCLUDING REMARKS

Recent research in animal models and humans provides compelling evidence identifying TNF- α as one of several regulators of vascular homeostasis. Major progress has been made in unveiling the molecular mechanisms that underlie the multiple vasculoprotective actions of anti-TNF- α . However, many of the mechanisms proposed in the present review are based on *in vitro* studies and, thus, the physiological relevance of these findings remains to be confirmed *in vivo*. Our understanding of TNF- α and TNFR, especially with respect to structure–function relationships and their pathophysiological role in vascular dysfunction, is still in its infancy. There have been trials

using TNF- α antagonists in heart failure, but there are very few trials using TNF- α antagonists (soluble receptors) in vascular disease. Although TNF- α antagonists (soluble receptors) have been shown to lack benefits on the rate of death or hospitalization in chronic heart failure, there have been trials using TNF- α antagonists in vascular complications of inflammatory diseases, such as RA and Crohn's diseases. These studies suggested beneficial effects of anti-TNF- α treatment in improving vascular function; however, there are very few trials applying anti-TNF- α treatment in vascular diseases and cardiovascular disorders related to Type 2 diabetes and the metabolic syndrome. Is it time to revisit antagonism of TNF- α in cardiovascular diseases? We believe that further investigations in this exciting field could facilitate the development of selective TNF- α antagonists with therapeutic potential in the management of diabetes and other vascular diseases.

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