

TNF- α in the cardiovascular system: from physiology to therapy

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Abstract: Signaling pathways induced by the proinflammatory cytokine tumor necrosis factor alpha (TNF- α) play a key role in the cellular responses to inflammation and injury. In the cardiovascular system, TNF- α -activated signal transduction pathways may contribute to vascular dysfunction, development and progression of atherosclerosis, and adverse cardiac remodeling following myocardial infarction and heart failure. This review addresses the role of TNF- α in vascular physiology and disease. Furthermore, the therapeutic benefits of systemic TNF- α antagonism in cardiovascular and autoimmune inflammatory diseases are summarized and critically discussed.

Keywords: TNF- α , vascular inflammation, atherosclerosis, shear stress, heart failure, TNF- α antagonists

TNF- α signaling

Tumor necrosis factor alpha (TNF- α) was described in 1975 as a circulating antitumor-origenic cytokine.¹ Originally, TNF- α was thought to be produced mainly by immune cells like activated macrophages and lymphocytes,^{2,3} but further studies reported its expression also in endothelial and epithelial cells,^{4,5} smooth muscle cells,⁶ and cardiac myocytes.⁷ More recent investigations demonstrated that TNF- α is a key proinflammatory cytokine and an important part of the innate immune system which, upon stimulation of pattern recognition receptors, increases the expression of genes required to control tissue inflammation and injury.

The human TNF- α gene, first cloned in 1985,⁸ is located on chromosome 6p21.3, spans about three kb and contains four exons, whereby more than 80% of the secreted protein is coded for by the last exon.⁹ TNF- α is expressed as a 26 kDa transmembrane protein arranged in stable homotrimers that are biologically active.¹⁰ Upon proteolytic cleavage by the metalloprotease TNF- α -converting enzyme (TACE),¹¹ the soluble cytokine is released into the extracellular space. This secreted 17 kDa protein forms triangular pyramid-shaped trimers and activates two distinct TNF- α receptors,¹² ie, TNFR1 (p55) and TNFR2 (p75).^{13,14} TNFR1 is expressed in nearly all cells, and can be activated by both the membrane-bound and soluble trimeric forms of TNF- α , whereas TNFR2 is found mainly in cells of the immune system and the heart, and responds to the membrane-bound form of TNF- α . Both TNF receptors lack intrinsic enzyme activity and require the recruitment of adaptor molecules to initiate signaling.¹³ Upon binding TNF- α , TNFR1 forms trimers and the resulting conformational change leads to dissociation of the inhibitory protein SODD (silencer of death domains) from the intracellular death domain. This dissociation enables the adaptor protein TRADD (TNFR1-associated death

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domain) to bind to the death domain and to initiate signaling by nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs) or caspases (Figure 1).^{15,16}

During activation of NF-κB, the adaptor protein TRADD recruits TRAF2 (TNFR-associated factor 2) and the serine-threonine kinase RIP (receptor-interacting protein kinase). Subsequently, IκB kinase is recruited by TRAF2 and activated by RIP, and phosphorylates IκBα (inhibitor of κB), an inhibitory protein that binds to NF-κB and inhibits its translocation. Upon phosphorylation IκBα is degraded, releasing the transcription factor NF-κB, which translocates to the nucleus to mediate the transcription of proteins involved in cell activation, proliferation, and survival.

Among the three major MAPK cascades, TNF-α induces the strongest activation of the c-Jun N-terminal kinases (JNKs) responsive to stress stimuli, whereas the responses evoked in p38-MAPK and extracellular signal-regulated kinase (ERK) activation are moderate to low. The TRAF2/Rac axis phosphorylates the JNK-inducing upstream kinases, which then activate JNK. Upon translocation to the nucleus, JNK activates transcription factors such as c-Jun

and activating transcription factor 2. The JNK pathway is involved in apoptosis, neurodegeneration, cell differentiation, and proliferation, but also in more specific regulation of cell functions, eg, chemokine production mediated by AP-1 (activator protein-1), including RANTES (regulated on activation, normal T-cell expressed and secreted), interleukin-8, and granulocyte macrophage colony-stimulating factor.

Although the death-inducing capability of TNF-α is weak compared with other TNF family members (such as Fas), its binding to TNFR1, which contains an intracellular death domain, can induce cell death signaling in certain conditions.¹⁷ In this process, TRADD binds FADD (Fas-associated protein with death domain), which then recruits caspase-8. A high concentration of caspase-8 induces its autoproteolytic activation and subsequent cleaving of effector caspases, thus mediating cell apoptosis. It must be noted, however, that compared with its overwhelming functions in the inflammatory response, TNF-α-induced cell death signaling is usually of minor importance in cell physiology.

TNF-α-mediated effects induced by the pathways downstream of TNFR1 are often conflicting, indicating an extensive

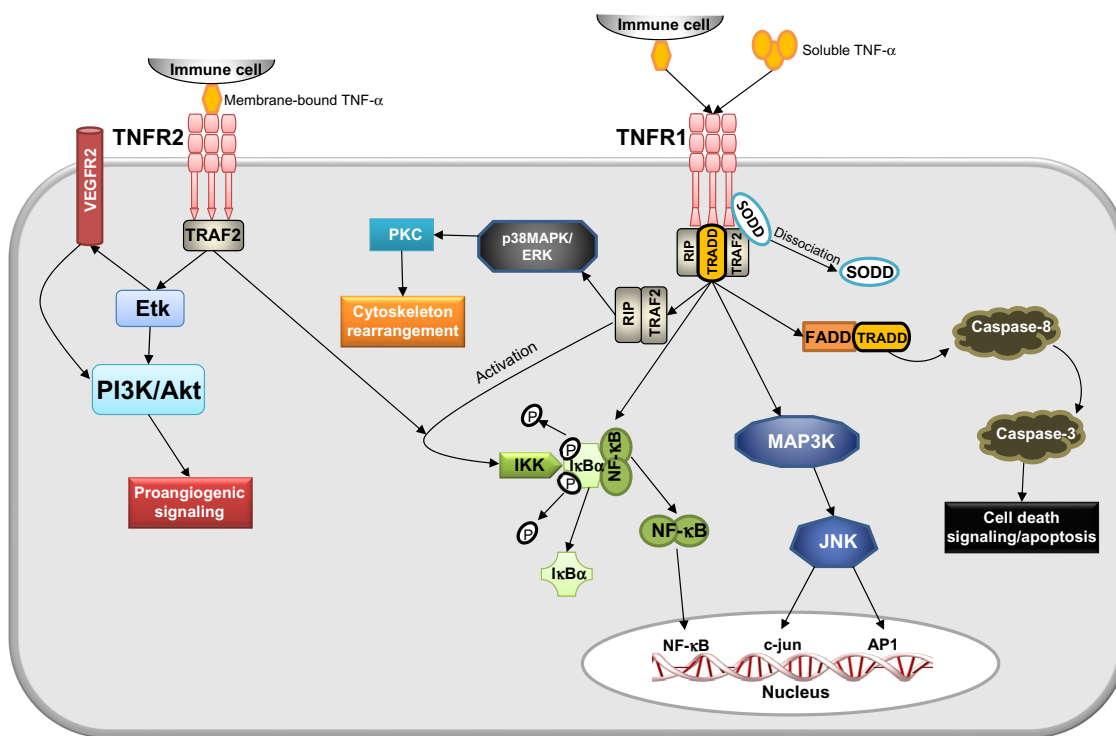


Figure 1 TNF-α-initiated signal transduction. TNF-α (soluble or membrane-bound) activates two different TNF-receptors, ie, TNFR1 and TNFR2. After binding of TNF-α, TNFR1 recruits distinct adaptor molecules (TRAF2, TRADD) at the intracellular death domain, thereby activating three major signaling pathways: NF-κB-signaling, MAPK/C-Jun-signaling, and caspase/apoptotic signaling. TNFR2 activation by membrane-bound TNF-α leads to activation of PI3K/Akt and proangiogenic pathways (eg, VEGF/VEGFR2), and is furthermore able to interfere with NF-κB signaling.

Abbreviations: Akt, protein kinase B; AP-1, activator protein-1; Etk, endothelial/epithelial tyrosine kinase; ERK, extracellular signal-regulated kinase; FADD, Fas-associated protein with death domain; IKK, IκB kinase; IκBα, inhibitor of κB; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; RIP, receptor-interacting protein kinase; SODD, silencer of death domains; TNF-α, tumor necrosis factor alpha; TNFR, TNF-α receptor; TRADD, TNFR1-associated death domain protein; TRAF2, TNFR-associated factor 2; VEGFR2, vascular endothelial growth factor receptor 2.

cross-talk and context dependency. As an example, NF- κ B enhances the transcription of proteins that interfere with cell death signaling. In contrast, activated caspases modulate NF- κ B signaling by cleaving several components of the NF- κ B pathway. Furthermore, the balance between proapoptotic and antiapoptotic signaling can be shifted by activation of TNFR2, which uses Etk (endothelial/epithelial tyrosine kinase, a non-receptor tyrosine kinase family member), to promote cell adhesion, migration, proliferation, survival, and angiogenesis. Activation of TNFR2 by TNF- α may lead to transactivation of vascular endothelial growth factor receptor 2 and Etk. Subsequently, downstream signaling by PI3K/Akt (phosphatidylinositol 3-kinase/Akt) is initiated, which stimulates endothelial migration and tube formation.¹⁸ Other factors, such as increased production of reactive oxygen species (ROS), concurrent cytokine/growth factor expression, or cell type can additionally modulate the cellular effects of TNF- α . Thanks to such complicated regulation of TNF- α signaling, various cells with vastly diverse functions can respond appropriately to inflammation and injury.¹³

Role of TNF- α in the cardiovascular system

The cardiovascular effects of TNF- α include its effect on endothelial function and interactions with inflammatory cells, as well as effects on vasodilation, smooth muscle cell (SMC) physiology, cardiac myocyte function, and glucose homeostasis. Direct evidence of TNF- α -stimulated vascular dysfunction was provided by a study of intra-arterial TNF- α administration in humans. In healthy volunteers, an acute local vascular inflammation was observed upon intra-arterial infusion of high-dose TNF- α (80 or 240 ng/min) for 30 minutes. In parallel, impaired endothelium-dependent vasomotion and a sustained increase in endothelial tissue plasminogen activator release were detected.¹⁹ Administration of a lower TNF- α dose (17 ng/min) for 60 minutes induced an increase in basal vascular resistance in healthy subjects, which was blocked by pretreatment with a nonselective cyclooxygenase inhibitor or a nitric oxide (NO) synthase (NOS) inhibitor.²⁰ The authors concluded that the observed effects of TNF- α were likely mediated not only by the reduced bioavailability of NO, but also by increased cyclooxygenase-dependent production of vasoconstrictors, as also reported in more recent studies.^{21,22}

Role of TNF- α in atherogenesis

Vascular injury associated with atherosclerotic disease is characterized by formation and release of inflammatory cytokines, activation of ROS production, and reduced availability of NO,

leading to endothelial dysfunction. Among the proinflammatory cytokines, TNF- α is a key player maintaining low-level systemic inflammation. Several mechanisms were described that contribute to the proatherogenic effects of TNF- α on the endothelium, including its role in ROS production, reducing the bioavailability of NO, and increasing the endothelial permeability to circulating blood components and cells. These proinflammatory atherogenic mechanisms are briefly highlighted below.

TNF- α -induced endothelial barrier dysfunction

TNF- α regulates vascular permeability in order to control inflammation, since the increased permeability of microvessels allows blood macromolecules and inflammatory cells to enter the injured tissues. However, increased permeability also contributes to formation of atherosclerotic plaques, which are initiated by the subendothelial accumulation of blood lipids and inflammatory cells. TNF- α contributes to disruption of the endothelial barrier by several mechanisms. Activation of TNFR1 has been reported to induce protein kinase C-dependent rearrangement of the actin cytoskeleton,²³ to increase the monomeric to filament actin pool ratio,²⁴ and to destabilize microtubules *in vitro*.²⁵ Destabilization of the endothelial cytoskeleton by TNF- α induces formation of intercellular gaps²⁶ and leads to a dose-dependent and time-dependent increase in paracellular movement of macromolecules across endothelial monolayers.²⁶

In parallel with rearrangement of the actin cytoskeleton, TNF- α increases tyrosine phosphorylation of vascular endothelial cadherin, the key molecule in endothelial cell-cell contacts, which allows paracellular passage of blood macromolecules.²⁷ Another mechanism contributing to increased endothelial activation and permeability in response to TNF- α is the degradation of glycocalyx.²⁸ Moreover, TNF- α was recently shown to increase the transcytosis of low-density lipoprotein across human endothelial cells and to contribute to early atherosclerosis by enhancing subendothelial retention of low-density lipoprotein in the vascular walls of apolipoprotein E-deficient mice.²⁹ These effects of TNF- α were substantially blocked not only by transcytosis inhibitors, but also by NF- κ B inhibitors and peroxisome proliferator-activated receptor- γ inhibitors, indicating a cross-talk between these pathways.

Effects of TNF- α on NO formation and bioavailability

NO is the major mediator of endothelium-dependent vasorelaxation. *In vivo* studies demonstrated that

endothelium-dependent vasodilation is impaired by TNF- α in a NO-dependent manner, in various vessels of the arterial tree.^{30–33} TNF- α decreases the bioavailability of NO both by reducing its production^{31,34,35} and by enhancing its removal.³⁰ Decreased NO generation results from TNF- α -mediated inhibition of endothelial NO synthase (eNOS) expression³⁶ and activity. TNF- α signaling suppresses gene promoter activity and destabilizes eNOS mRNA, thus reducing eNOS protein expression.^{37,38} These processes are mediated by TNFR1 signaling, whereas TNFR2 has little effect on eNOS expression.³⁹ Moreover, TNF- α decreases NO bioavailability by accumulation of the endogenous eNOS inhibitor ADMA (asymmetric dimethylarginine)⁴⁰ and by enhanced removal of NO, for example via its reaction with superoxide, in which peroxynitrite is generated.⁴¹

Role of TNF- α in ROS generation

TNF- α stimulates vascular superoxide production by increasing the activity of NADPH-dependent oxidases (NOX) in endothelial cells,^{30,31,42} smooth muscle cells,⁴³ and neutrophils.⁴⁴ NOX catalyze the transfer of electrons from NADPH to molecular oxygen, resulting in generation of superoxide. Other potential sources of TNF- α -induced vascular superoxide production include ceramide-activated protein kinase, xanthine oxidase,⁴⁵ lipoxygenase, mitochondrial oxidase, and uncoupled eNOS.⁴⁶

Expression of different NOX subunits (NOX4 [Nox 4A and 4B],⁴⁷ NOX2, NOX5⁴⁸), as well as the regulatory proteins associated with NOX, namely p22phox, p47phox, and p67phox, has been described in human arterial endothelial cells. Upon exposure to TNF- α , expression of several of these proteins (NOX4A, p22phox, p47phox, and p67phox) is upregulated, and the activity of NOX is increased by nearly threefold.⁴⁷ NOX activation by TNF- α is furthermore Rac1-dependent, as shown in endothelial cells *in vitro*⁴⁹ and in cardiac-specific Rac1-deficient mice.⁵⁰

Furthermore, studies in human microvascular endothelial cells demonstrated that TNF- α induces rapid phosphorylation of p47phox, which then binds to TRAF4 and translocates to the membrane, resulting in increased p47phox-p22phox complex formation and NOX activation. Binding of phosphorylated p47phox by TRAF4 occurs in a protein kinase C-dependent manner,^{51,52} which may link superoxide generation to the pathways involved in endothelial barrier disruption by TNF- α .⁵³ This is further underscored by the fact that TNF- α -induced vascular permeability is prevented by an antiperoxynitrite agent.⁵⁴

Apart from increasing monolayer permeability and decreasing NO bioavailability, the generation of superoxide upon TNF- α exposure has further implications for induction of proatherogenic pathways in endothelium, as activation of NOX has been shown to contribute to TNF- α -induced NF- κ B activation.⁵⁵

TNF- α -induced endothelial-leukocyte interactions

Vascular endothelial cells are the primary cellular target for the actions of circulating proinflammatory cytokines. TNF- α binding to TNFR1 initiates the expression of many proinflammatory, proatherogenic proteins^{55,56} via activation of the transcription factors NF- κ B and c-Jun/ATF-2,^{57–60} and thus contributes to increased recruitment of leukocytes by the endothelium.⁶¹

Proinflammatory TNF- α signaling induces rapid endothelial expression of cell adhesion molecules, which mediate the recruitment and transmigration of circulating leukocytes into the vascular wall.⁶² Adhesion molecules, including E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular cell adhesion molecule 1 (ICAM-1) are synthesized *de novo* within 30–120 minutes of TNF- α stimulation⁶³ and transported to the endothelial surface.

Chandrasekharan et al⁶² investigated in detail the involvement of the two TNF- α receptors in endothelial adhesion molecule expression. Studies utilizing cultured endothelial cells derived from wild-type, TNFR2-deficient, or TNFR1-deficient mice showed that TNFR2 was required for TNF- α -induced E-selectin, VCAM-1, and ICAM-1 expression. Furthermore, TNF- α -stimulated leukocyte rolling, firm adhesion to the endothelium, and transmigration were dramatically reduced in TNFR2-deficient mice, indicating that endothelial TNFR2 is also essential for TNF- α -induced leukocyte-endothelial cell interaction.⁶²

Role of shear stress in endothelial responses to TNF- α

TNF- α plays an important role in regulating the endothelial response to inflammation and injury by promoting migration of immune cells into the diseased or injured tissues in order to protect the tissues from harmful pathogens, damaged cells or irritants. However, the chronic presence of increased systemic levels of TNF- α in the context of cardiovascular or inflammatory diseases may be deleterious to the endothelium in medium and large arteries, where constant low-level stimulation of endothelial activation and vascular permeability contributes to atherogenesis. There are several endothelial

protection mechanisms that prevent the inflammatory response in arteries exposed to TNF- α , the most important of them being the signaling pathways activated by laminar shear stress (Figure 2).

Laminar shear stress exerts a profound antiapoptotic effect on endothelial cells treated with TNF- α via upregulation of NO synthesis^{64,65} and post-transcriptional activation of eNOS.⁶⁶ Immediately after the onset of shear stress, eNOS is activated by phosphorylation at serine 1177 through PI3K/Akt, which increases NO formation within seconds. Moreover, laminar flow mediates prosurvival signaling by inhibiting TNF- α -induced caspase-3 and JNK activity in vitro and ex vivo.⁶⁷ Downstream of TNFR1, laminar shear stress furthermore reduces c-Jun and NF- κ B transcriptional activation in vitro.⁶⁸ Ex vivo studies on perfused rabbit aortas⁶⁹ showed that pre-exposure to laminar shear stress (12 dyne/cm²) inhibited TNF- α -induced activation of JNK, p38-MAPK, and ERK, and prevented VCAM-1 expression in endothelial cells. Furthermore, laminar shear stress was shown to prevent the association of TNFR1 with TRAF-2⁶⁹ and to

suppress the capacity of TNF- α to induce proinflammatory gene expression in endothelial cells by enhancing induction of NF- κ B-dependent cytoprotective transcripts.⁷⁰

In vivo studies showed that endothelial VCAM-1 expression depends on NF- κ B activation in response to disturbed shear stress.⁷¹ Hajra et al⁷² detected high levels of NF- κ B proteins in endothelial cells at sites of low shear in the aortic arch and suggested that these regions may be primed for enhanced NF- κ B activation in response to proinflammatory agonists. In accordance with this, significantly stronger VCAM-1 expression was detected at the atherosclerosis-prone region of the murine aorta (lesser curvature of the aortic arch), as compared with the greater curvature region with a low probability of atherosclerosis.⁷³ This corresponds to the observation that the pattern of shear stress determines the localization of atherosclerotic plaques at the arterial wall regions typically characterized by non-uniform blood flow and low shear stress, ie, at bifurcations and curvatures. Our in vitro studies demonstrated that prolonged preconditioning of endothelial cells with either laminar or non-uniform shear stress strongly affects

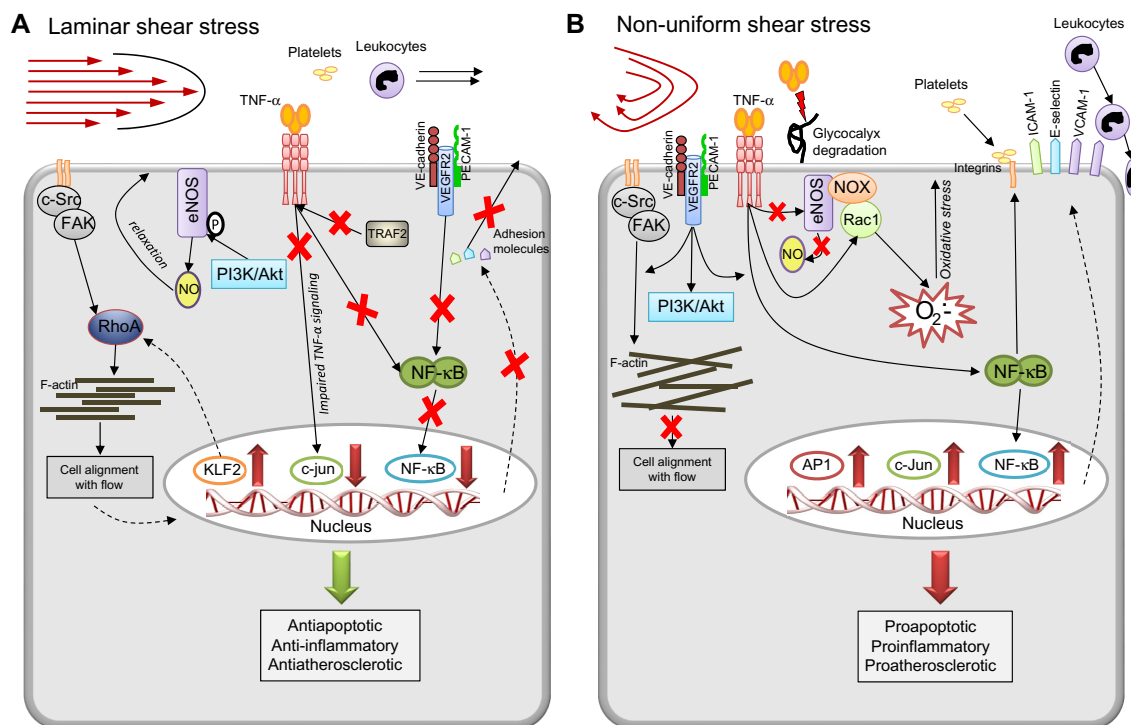


Figure 2 Shear stress dependency of TNF- α signaling in endothelial cells. **(A)** Laminar shear stress prevents proinflammatory activation of endothelial cells by TNF- α by inhibiting NF- κ B signaling (eg, preventing expression of adhesion molecules and inducing production of NO) and therefore results in a quiescent endothelial phenotype. **(B)** Proatherogenic non-uniform shear stress synergistically enhances proinflammatory TNF- α signaling by activating NF- κ B, AP-1, or c-Jun, leading to endothelial dysfunction characterized by increased permeability, enhanced oxidative stress, lack of cell alignment with flow and adhesion molecule expression, followed by adhesion of leukocytes to the activated endothelium.

Abbreviations: Akt, protein kinase B; AP-1, activator protein-1; c-Src, proto-oncogene tyrosine-protein kinase Src; eNOS, endothelial nitric oxide synthase; FAK, focal adhesion kinase; ICAM-1, intercellular adhesion molecule-1; KLF-2, Krüppel-like factor 2; NF- κ B, nuclear factor- κ B; NO, nitric oxide; NOX, NADPH-dependent oxidase; PECAM-1, platelet-endothelial adhesion molecule-1; PI3K, phosphatidylinositol 3-kinase; Rac1, Ras-related C3 botulinum toxin substrate 1; RhoA, Ras homolog family member A; TNF- α , tumor necrosis factor alpha; TRAF, TNFR-associated factor 2; VCAM-1, vascular cell adhesion molecule-1; VE-cadherin, vascular endothelial cadherin; VEGFR2, vascular endothelial growth factor receptor 2.

their responsiveness to circulating TNF- α .⁷⁴ Endothelial cells exposed to laminar shear stress were essentially protected against TNF- α -induced upregulation of adhesion molecules and recruitment of monocytic cells. In contrast, in cells exposed to non-uniform shear stress, TNF- α induced a dramatic increase in the numbers of adhering monocytic cells, and in the expression of VCAM-1 and E-selectin, which was sensitive to NF- κ B inhibition. These findings indicated that the TNF- α -activated signaling pathways are largely convergent with non-uniform shear stress-triggered signaling involving NF- κ B, c-Jun, or AP-1. Additionally, upon exposure to non-uniform shear stress, endothelial glycocalyx becomes stiffer and loses its buffering function,^{75,76} thus further potentiating the glycocalyx-degrading effect of TNF- α .²⁸ Taken together, chronic exposure to laminar shear stress strengthens endothelial cell resistance to TNF- α , whereas non-uniform shear stress increases endothelial susceptibility to TNF- α stimulus (Figure 2).⁷⁴ In endothelial cells constantly exposed to blood flow, shear stress-activated mechanisms are thus major modulators of the biologic responses to cytokines.

Role of TNF- α in apoptosis of endothelial cells

TNF- α normally acts as a potent activator of endothelial cells (Figure 3) and does not induce endothelial apoptosis.⁵⁷

However, when combined with agents that release ceramide (eg, ultraviolet irradiation or cytotoxic drugs) or with ceramide mimetics such as lipopolysaccharide, TNF- α can mediate cell death in endothelial cells.⁵⁷ According to studies by Csiszar et al^{32,77} in the carotid arteries of young rats, recombinant TNF- α elicits endothelial dysfunction, oxidative stress, and proinflammatory gene expression. In aged coronary arteries, TNF- α expression is increased and contributes to apoptosis of endothelial cells by caspase-3 activation. Furthermore, elevated levels of plasma TNF- α during septic shock can induce both microvascular dysfunction and NF- κ B-dependent endothelial apoptosis,⁷⁸ which is associated with the severity and outcome of sepsis. The balance between activation and injury of endothelial cells induced by TNF- α and other cytokines may thus govern local vascular responses to these mediators in disease conditions in vivo.

Role in vascular insulin resistance

Chronic inflammation contributes to vascular insulin resistance. Upon binding to its receptor on endothelial cells, insulin enhances microvascular perfusion of skeletal muscle by activating PI3K/Akt, which mediates an antiapoptotic effect and also increases gene expression and activation of eNOS.⁷⁹ TNF- α interferes with endothelial insulin signaling

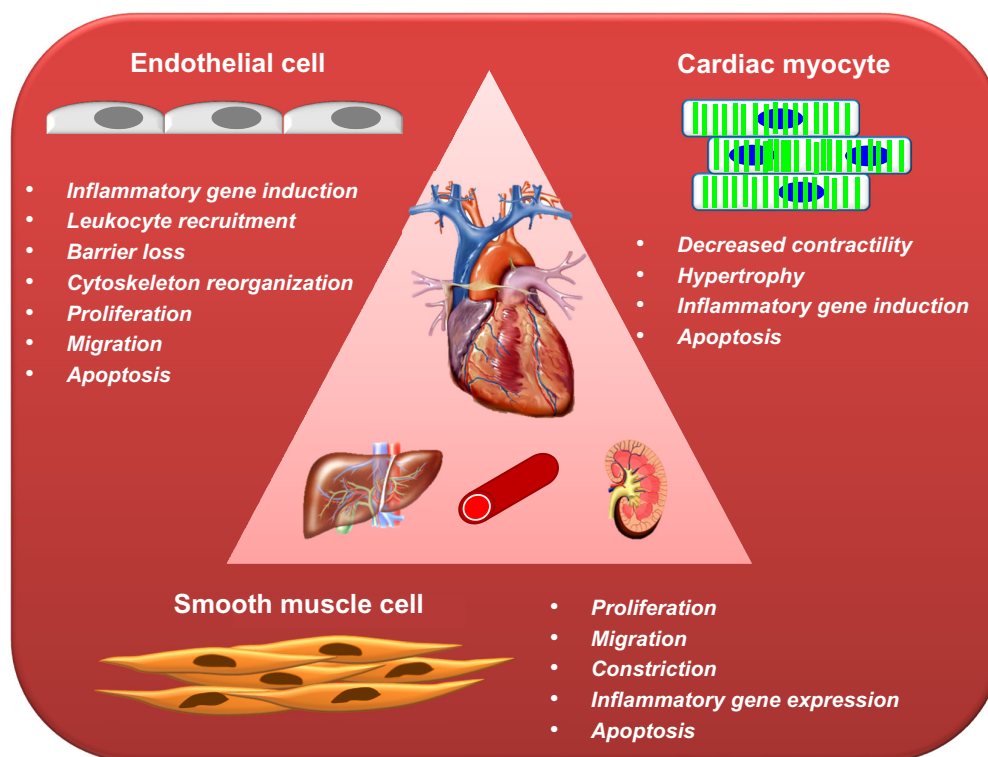


Figure 3 Schematic presentation of the most important effects elicited by TNF- α in the cells of cardiovascular system.

and induces insulin resistance both *in vivo*⁸⁰ and *in vitro*.⁸¹ In bovine aortic endothelial cells, TNF- α increases p38-MAPK phosphorylation and potently stimulates serine phosphorylation of the insulin receptor, thus inactivating signaling. In parallel, insulin-stimulated receptor tyrosine phosphorylation, Akt phosphorylation, and eNOS activity,⁸¹ as well as insulin uptake,⁸² are strongly inhibited by TNF- α in a p38-MAPK-dependent manner. TNF- α -mediated vascular insulin resistance may thus contribute to generalized endothelial dysfunction, eg, in diabetes and the cardiometabolic syndrome.

Effects on vascular smooth muscle cells

Phenotypic transition of vascular SMCs from a differentiated to a proliferative state during vascular injury and inflammation plays an important role in early atherosclerosis. TNF- α signaling induces SMC migration and proliferation mediated by TRAIL, ERK, and p38MAPK activation downstream of TNFR1.^{23,83,84} Moreover, TNF- α stimulates NOX and superoxide dismutase in SMCs, thus enhancing the formation of ROS and H₂O₂, which further induce activation of ERK and p38MAPK.⁸⁵ During the development of atherosclerotic lesions, matrix metalloproteinase (MMP)-9 is upregulated and is required for migration of SMCs. TNF- α signaling upregulates nuclear FoxO4, which in turn activates transcription of the MMP-9 gene, inducing the ability of SMCs to migrate *in vitro* and *in vivo*.⁸⁶

SMCs are normally resistant to Fas or cytokine-induced apoptosis, and even prolonged stimulation with a cytokine cocktail (interferon- γ , TNF- α , and interleukin-1 β) or sFas ligand did not induce any significant change in SMC morphology or viability *in vitro*.⁸⁷ However, in the context of atherosclerosis, SMCs isolated from the neointima of injured rat aorta were characterized by inhibited DNA synthesis and a decrease in cell numbers in response to TNF- α , as compared with medial SMCs.⁸⁸ TNF- α also induced terminal dUTP nick end-labeling (TUNEL) positivity and caspase-3 expression in intimal SMCs, indicating increased cell death by apoptosis, whereas medial SMCs were markedly less sensitive to the TNF- α stimulus. Apoptosis in intimal SMCs was effectively inhibited by treatment with blocking antibodies against TNF- α receptors, suggesting that endogenous activation of TNF- α receptors may represent a way to limit hyperplasia in injured arteries.⁸⁸ However, this mechanism may also be involved in intimal SMC apoptosis in advanced atherosclerotic plaques. Taken together, TNF- α affects SMC functions in multiple ways (Figure 3), consequently inducing the proatherogenic SMC phenotype.

Role in cardiac myocytes

In healthy individuals, cardiac TNF- α concentrations are low and do not affect contractile function.^{89–91} The presence of TNF- α protein and transcripts is usually restricted to microvessels in the normal heart.⁹² However, human cardiac myocytes express functional TNFR1 and respond to TNF- α .⁹³ *In vivo* administration of exogenous TNF- α exerts cardiodepressant effects in rat^{94,95} and dog^{96,97} hearts. TNF- α dose-dependently reduces sarcoplasmic reticulum Ca²⁺ uptake and myofilament Ca²⁺ sensitivity,^{98–100} and this effect is mediated by p38 MAPK, a negative regulator of cardiac contractility. Intracellular calcium handling is suppressed by TNF- α via inhibition of sarcoplasmic reticulum calcium adenosine triphosphatase expression and activity.¹⁰¹ Furthermore, leakage of Ca²⁺ from the sarcoplasmic reticulum, which contributes to the depressed Ca²⁺ transient and reduced contractility is also increased by TNF- α .¹⁰² This effect is mediated by increased activation of cardiac caspase-8, leading to myocardial NO and mitochondrial ROS production, which results in S-nitrosylation of the ryanodine receptor and Ca²⁺ leak from the sarcoplasmic reticulum.¹⁰³ TNF- α exposure is also associated with another NO-independent, but calcium-dependent, early depressant mechanism that is manifested by reduced contractile function and cyclic adenosine monophosphate response to beta-adrenergic stimulation.¹⁰⁴

Apart from reducing contractility in cardiac myocytes (Figure 3), TNF- α induces cell hypertrophy manifested by cell enlargement, increased global protein synthesis, induction of sarcomere organization, and expression of embryonic genes, such as ANF (atrial natriuretic factor, a marker of hypertrophy).¹⁰⁵ These phenomena are mediated by activation of p38MAPK and NF- κ B¹⁰⁶ in a ROS-dependent manner.^{105,107–109} In line with these findings, *in vivo* studies demonstrated a hypertrophic response in cardiac myocytes, leading to left ventricular hypertrophy and dilatation upon cardiac-specific overexpression or chronic infusion of high doses of TNF- α .^{94,105,110}

Cardiac myocytes have further been shown to undergo apoptosis after stimulation with TNF- α *in vitro*.¹¹¹ Al-Lamki et al⁹² investigated in detail the independent regulation and differential functions of TNFRs in myocardium. In biopsies from normal human hearts, TNFR1 but not TNFR2, was predominantly and strongly expressed by cardiac myocytes and rarely seen in fibroblasts. In organ cultures of the wild-type mouse heart, TNF- α stimulation upregulated TNFR2, activated signaling downstream of both receptors, and caused apoptosis and entry into the cell cycle. In TNFR1-deficient mouse hearts treated with TNF- α , activation of TNFR2,

negligible apoptosis, and increased entry into the cell cycle were observed. In contrast, TNFR2-deficient organ cultures showed increased TUNEL positivity and lower levels of cell cycle entry.⁹² These findings are thus consistent with TNFR1-mediated cardiac cell death and TNFR2-mediated repair.

TNF- α in myocardial infarction

Occlusion of coronary arteries rapidly induces expression of proinflammatory cytokines, including TNF- α .¹¹² However, conflicting findings were reported regarding the role of TNF- α in animal models of myocardial infarction (MI). TNF- α deficiency was shown to either decrease¹¹³ or have no effect on infarct size as compared with wild-type mice.^{114–117} Similarly, TNFR1-deficiency either reduced¹¹⁵ or did not affect infarct size.^{117–119} In a mouse model of coronary ligation, TNFR2-deficiency did not influence infarct size.¹¹⁸ Interestingly, knockout of both TNFRs in mice was shown to increase infarct size after 1 day, and this increase was associated with enhanced apoptosis of cardiac myocytes.¹²⁰ These data are consistent with the finding that whereas an early increase in TNF- α post-MI contributes to stabilization of left ventricular function, prolonged stimulation with TNF- α induces left ventricular dysfunction in the later phase post-MI.¹²¹ In a recent study by Zhang et al, knockouts of TNFR1 or TNFR2 were shown to have opposite effects on cardiac function post-MI, whereby deletion of TNFR1 improved the cardiac dysfunction and the absence of TNFR2 increased the cardiac injury.¹²² Thus, both TNFR appear to play an important role post-MI, explaining at least in part the conflicting findings from *in vivo* knockout studies. Ideally, upregulation of TNF- α post-MI should be rapid and transient, in order to activate expression of TNFR2 and cardioprotective signaling. However, chronic exposure to increased TNF- α levels leads to TNFR1-mediated left ventricular dysfunction and increased MMP-2 activity, resulting in degradation of the matrix, and finally increased apoptosis of cardiomyocytes.^{117,123}

TNF- α in heart failure

Heart failure is characterized by a debilitating decline in cardiac function. TNFR1 signaling is detrimental in murine models of heart failure, whereas TNFR2 mediates cardioprotection.¹²⁴ Contractile dysfunction downstream of TNFR1 involves β -adrenergic receptor uncoupling,¹²⁵ as well as increased oxidative stress due to ROS formation and enhanced inducible NOS synthesis leading to production of NO and peroxynitrite.¹²⁶ Furthermore, TNF- α downregulates the expression of contractile proteins, such as α -myosin heavy chain and cardiac α -actin.¹²⁷ Apart from reducing

contractility, TNF- α enhances the transcription of hypertrophic genes in the failing heart.¹⁰⁵ Together with increased cardiac myocyte apoptosis¹²⁸ and cardiac fibrosis,¹²⁹ this hypertrophic effect contributes to structural alterations and heart failure. Consequently, long-lasting neutralization of TNF- α was shown to attenuate cardiac myocyte hypertrophy and adverse myocardial remodeling in a rat model of heart failure.¹³⁰

Contribution of TNF- α signaling to hyper/hypotension

In sepsis, massive overproduction of TNF- α provokes life-threatening refractory hypotension, and according to clinical trials, this hypotensive effect constitutes a limiting factor for the use of TNF- α as a systemic antitumor agent. Upon administration in dogs, TNF- α (at 60 mg/kg body weight) induces cardiovascular collapse leading to the lethal shock syndrome.¹³¹ This hypotensive effect of TNF- α is mediated by excessive production of NO and ROS and activation of caspase.¹³²

In contrast, a constant low-level increase in TNF- α production in the vascular system activates multiple pathways that contribute to vasoconstriction and can induce hypertension. Serum TNF- α is significantly and independently associated with blood pressure in apparently healthy subjects.^{133,134} In a study by Bautista et al,¹³⁴ mean plasma levels of TNF- α were four times higher in hypertensive subjects than in non-hypertensive controls. The prevalence of hypertension increased progressively from 30% in the lowest TNF- α quartile to nearly 53% in the highest quartile, corresponding to an almost twofold significant increase in the prevalence.

In physiological conditions, a complex cross-regulation between the renin-angiotensin system and TNF- α signaling takes place. TNF- α was shown to inhibit renin gene expression in adrenal cells¹³⁵ and renal juxtaglomerular cells,¹³⁶ although TNF- α deficiency did not affect physiological regulation of renin expression by salt.¹³⁶ Furthermore, a reduction of angiotensinogen production in renal proximal tubular cells by TNF- α was reported.¹³⁷ However, this reduction may be counterbalanced by increased angiotensinogen expression in hepatocytes,¹³⁸ which is an important contributor to circulating angiotensinogen levels. In the heart, no effects of cardiac-specific overexpression of TNF- α on renin or angiotensin production were observed, but the activation of myocardial renin-angiotensin system was increased, leading to enhanced left ventricular remodeling, increased collagen content, and apoptosis of cardiac myocytes.¹³⁹

In multiple animal studies, TNF- α was found to promote hypertension, both in the presence and absence

of renal injury.^{140,141} According to a recent report, kidney-derived TNF- α plays a decisive role in promoting the angiotensin II-induced increase in blood pressure.¹⁴² In a mouse model of chronic hypertensive kidney disease, TNF- α deficiency resulted in a blunted hypertensive response and reduced organ damage, mediated by enhanced eNOS expression and NO bioavailability. The same effect, along with reduced cardiac hypertrophy, was observed in wild-type animals transplanted with a TNF- α -knockout kidney, confirming that TNF- α produced by renal parenchymal cells potentiates the hypertensive response by suppressing bioavailability of NO within the kidney.

In the vasculature, TNF- α is known to participate in vascular contraction, acting either at the level of the endothelium or the SMCs. The major pathways involved in this effect include those for induction of vasoconstrictive preproendothelin-1 mRNA expression and endothelin-1 protein secretion in endothelial cells.^{143,144} Activation of the NF κ B pathway is furthermore responsible for TNF- α -induced upregulation of expression of the endothelin B2 receptor in SMCs.¹⁴⁵ The endothelin B2 receptor mediates vasoconstriction^{146,147} and is not expressed in SMCs under normal conditions, but is upregulated in patients with cardiovascular disease (eg, peripheral artery disease,¹⁴⁸ pulmonary hypertension,¹⁴⁹ ischemic heart disease,¹⁵⁰ and cerebral ischemic stroke¹⁴⁶), suggesting possible involvement of TNF- α signaling. Moreover, TNF- α was reported to induce production of thromboxane A2 in endothelial cells²¹ and SMCs²² and to abolish insulin-mediated vasodilation in a JNK-dependent manner.¹⁵¹ Taken together, the existing studies indicate that TNF- α is an important regulator of blood pressure, both at the level of the kidney and at the level of the vasculature and myocardium.

Inactivation of TNF- α in atherosclerosis, heart failure, and inflammatory diseases

TNF- α promotes the inflammatory response, thus contributing to the clinical problems related to cardiovascular diseases and autoimmune disorders, which are strongly associated with cardiovascular comorbidity. The currently available TNF- α inhibitors include two types of biologic therapeutics, whereby blockade of TNF- α is achieved using either monoclonal antibodies (eg, infliximab, adalimumab, certolizumab, golimumab) or a soluble recombinant fusion protein consisting of the extracellular ligand-binding domain of TNFR2 and the Fc fragment of human IgG1 (etanercept).

TNF- α blockade in animal models

Experimental data show that genetic inactivation of TNF- α diminishes the size of atherosclerotic lesions, both in wild-type mice deficient in TNF- α and fed an atherogenic diet and in apolipoprotein E/TNF- α double-deficient mice, irrespective of the type of diet.^{152–155}

The effect of anti-TNF- α antibodies on neointima formation following balloon injury in rabbits was investigated in detail by Zhou et al.¹⁵⁶ Balloon angioplasty increased tissue expression of TNF- α by 100,000-fold over baseline, and this increase persisted over 6 days following arterial injury. Anti-TNF- α treatment neutralized tissue TNF- α activity by 60%–75% and suppressed macrophage infiltration, but did not inhibit neointima formation.¹⁵⁶

Regarding the vascular dysfunction associated with increased insulin resistance, inhibition of TNF- α was shown to result in a marked increase in insulin sensitivity in obese rats. Moreover, neutralization of TNF- α by etanercept significantly reduced TNF- α levels in β 2-adrenergic receptor-deficient mice, leading to increased insulin receptor tyrosine phosphorylation¹⁵⁷ and alleviation of insulin resistance.

TNF- α antagonism was also shown to confer cardioprotection. A single administration of soluble TNFR-Fc fusion protein at the time of reperfusion after MI was able to limit infarct size and to reduce early left ventricular diastolic dysfunction in rats subjected to 60 minutes of coronary occlusion.¹⁵⁸ Moreover, systemic TNF- α inhibition was cardioprotective in rats subjected to a continuous TNF- α infusion,⁹⁴ in mice with cardiac-restricted overexpression of TNF- α ,¹⁵⁹ and in animal models of heart failure.^{126,160}

Collectively, these preclinical observations suggest that blockade of TNF- α might inhibit progression of atherosclerotic lesions, prevent insulin resistance and accelerated atherosclerosis in the metabolic syndrome, and improve clinical outcomes in patients with heart failure.

Clinical studies in patients with heart failure

Circulating levels of TNF- α and soluble TNFRs are independent predictors of mortality in patients with heart failure.¹⁶¹ Although preclinical data suggested that TNF- α blockade in heart failure would be beneficial, randomized trials of anti-TNF- α therapy (etanercept in the RENEWAL trial¹⁶² and infliximab in the ATTACH trial)¹⁶³ in human heart failure unexpectedly demonstrated a time-related and dose-related increase in mortality and heart failure-related hospitalization. In further studies, the mechanisms

of new and/or worsening heart failure in etanercept-treated patients were investigated,¹⁶⁴ demonstrating a significant 70-fold increase in plasma levels of immunoreactive TNF- α . Moreover, etanercept enhanced TNF- α bioactivity and increased the immunoreactive mass of homotrimeric TNF- α in heart failure patients, and this was suggested as a potential mechanism of the detrimental effects of this therapy in some patients.¹⁶⁴ In light of the unique and divergent inflammatory responses evoked specifically by each TNF- α receptor in the failing heart, global TNF- α inhibition can therefore dysregulate both adverse and protective signaling, leading to dramatic worsening of cardiac function.

TNF- α antagonism in autoimmune diseases

Anti-TNF- α therapy is approved for use in autoimmune inflammatory conditions, such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, and psoriasis.¹⁶⁵ These disorders are often associated with an increased risk of cardiovascular comorbidities, and the benefits of TNF- α inhibition, apart from anti-inflammatory efficacy and improved survival,¹⁶⁶ are thought to include cardiovascular protection. Consequently, many studies have been conducted to investigate the vascular effects of TNF- α inhibition in these patients, and they have produced an abundance of controversial results.

In patients with rheumatoid arthritis, use of anti-TNF- α therapy (etanercept or infliximab) was shown to reduce the incidence of first cardiovascular events.¹⁶⁷ Moreover, in rheumatoid arthritis patients with pre-existing cardiovascular disease, the risk of heart failure was significantly lower in anti-TNF- α -treated patients than in the remaining patients.¹⁶⁸ Other studies reported an increased risk of developing heart failure, after adjustment for traditional cardiovascular risk factors, in patients who had a higher disease activity score at follow-up,¹⁶⁹ and a slightly increased relative risk of heart failure in a large population of relatively young rheumatoid arthritis patients.¹⁷⁰

Increased aortic stiffness, an independent predictor of cardiovascular mortality, is a common feature of inflammatory diseases. Several studies have demonstrated improved aortic stiffness in patients with inflammatory arthropathies treated by TNF- α antagonists,^{171–174} whereas carotid intima-media thickness was either reduced¹⁷⁴ or remained stable.¹⁷⁵ In contrast, arterial stiffness, lipid profiles, and other traditional cardiovascular risk factors were not improved after 6 and 12 months of anti-TNF- α therapy in patients with ankylosing spondylitis, although the treatment decreased their disease activity.¹⁷⁶

Regarding endothelial function, flow-mediated dilatation in patients with inflammatory arthropathies is strongly impaired when compared with healthy controls.^{177,178} Anti-TNF- α treatment was shown to significantly improve endothelium-dependent vasodilation in patients with rheumatoid arthritis.^{175,179,180} Other studies in rheumatoid arthritis patients showed a significant but transient effect of infliximab infusion on endothelium-dependent vasodilatation, which returned to baseline within 2–4 weeks post-infusion.^{177,181} Beneficial effects on capillary recruitment and endothelium-dependent vasodilatation were also reported in ankylosing spondylitis patients treated either with etanercept,¹⁷⁸ or with infliximab, which was furthermore shown to reduce nitrite concentrations.¹⁸² In contrast, Irace et al reported transient vasoconstriction and increased wall shear stress in the common carotid and brachial arteries following treatment with infliximab in patients with rheumatoid arthritis, with no effect on flow-mediated dilatation.¹⁸³ Progression of atherosclerosis in psoriatic arthritis patients despite TNF- α blockade was also observed; these patients showed no recovery of flow-mediated dilatation after 2 years of anti-TNF- α treatment, and their serum TNF- α levels were increased at 2 years when compared with baseline.¹⁸⁴ There was a significant progression in both mean intima-media thickness and mean maximum intima-media thickness, despite improvement in the disease score and the lipid profile.

The effects of systemic blockade of TNF- α on insulin resistance remain controversial. While some studies reported improved insulin sensitivity in infliximab-treated patients with rheumatoid arthritis,^{185,186} other authors found that insulin resistance was not alleviated by antagonism of TNF- α .¹⁸⁷ Gonzalez-Gay et al¹⁸⁸ suggested that the effects may depend on the degree and severity of insulin resistance before the start of therapy, whereby the beneficial effect is observed in patients with the most severe disease.

In obese insulin-resistant patients, no improvement in insulin sensitivity was observed following a single intravenous administration of soluble TNFR-Fc fusion protein.¹⁸⁹ Similarly, no effects of systemic TNF- α blockade on vascular or metabolic insulin sensitivity were detected in either healthy obese patients¹⁹⁰ or in obese patients with type 2 diabetes mellitus.¹⁹¹

The effects of direct TNF- α inhibition on lipid metabolism in patients with rheumatoid arthritis are similarly controversial: In two independent studies,^{183,192} adverse effects were observed, including elevated triglycerides and reduced high-density lipoprotein cholesterol levels. One further study in infliximab-treated patients with rheumatoid arthritis and

ankylosing spondylitis showed no influence on the overall lipid profile.¹⁹³ In contrast, several studies demonstrated increases in serum concentrations of high-density lipoprotein cholesterol upon treatment with infliximab.¹⁹⁴⁻¹⁹⁶ Similar beneficial effects of etanercept treatment on the lipid profile were observed in patients with ankylosing spondylitis.¹⁹⁷ The detailed interplay of the proinflammatory activity of TNF- α with insulin resistance and lipid metabolism in the context of atherosclerosis and cardiometabolic disorders thus warrants further investigation.

Toward the next generation of anti-TNF- α therapies

The currently available TNF- α blockers neutralize the activity of soluble TNF- α and prevent its binding to both TNFR1 and TNFR2. Although these compounds are effective in the treatment of inflammatory arthritis, ankylosing spondylitis, and psoriasis, long-term use of TNF- α blockers can have adverse effects due to inhibition of the pleiotropic functions of TNF- α . Safety considerations here include an increased incidence of pulmonary and skin infections, new-onset autoimmune diseases, malignancies, and congestive heart failure, resulting from inhibition of the immunomodulatory effects of TNF- α .¹⁹⁸ Since many of the pathological and protective signals diverge either at the ligand level (soluble versus membrane-bound TNF- α) or at the receptor level (TNFR1 or TNFR2), selective blockade of soluble TNF- α or of TNFR1 signaling has been postulated to be preferable to complete TNF- α blockade in the treatment of chronic inflammatory diseases.^{199,200}

Based on the results obtained in animal models of myocardial infarction¹²² and rheumatoid arthritis,²⁰¹ therapeutic strategies that target TNFR1 while sparing TNFR2 are expected to both inhibit inflammation and promote the activity of regulatory T-cells, and might be superior to systemic TNF- α blockade. Consequently, there is a growing interest in use of specific receptor-targeting compounds as anti-TNF- α therapeutic agents. These compounds are still in the research and development phase, but many recent studies in animal models of inflammatory disease indicate that increased therapeutic benefit and a reduced risk of side effects can be achieved by specific inhibition of soluble TNF or TNFR1, while leaving TNFR2 signaling fully functional. A variety of approaches to the pharmacological blockade of TNFR1 have been proposed thus far, among them monoclonal antibodies (atrosab)²⁰² and antagonistic TNF- α mutants specific for TNFR1.^{203,204} Therapeutic administration of soluble TNFR1 PLAD (preligand-binding assembly domain) protein potently

inhibited inflammatory arthritis in animal models.²⁰⁵ In addition to these inhibitory strategies, a short hairpin RNA directed against TNFR1 was successfully used to downregulate TNFR1 expression and to reduce experimental arthritis in vivo.²⁰⁶

Furthermore, several reports from the field of targeted drug delivery and nanomedicine appear to open promising possibilities for local or tissue-targeted TNF- α gene silencing. Two different topical delivery systems (cationic amphiphilic lipid particles²⁰⁷ and capsaicin-loaded cationic lipid-polymer hybrid nanoparticles)²⁰⁸ have recently demonstrated efficacy in local transport of anti-TNF- α small interfering (si)RNA and in the treatment of experimental psoriasis. Moreover, the problem of insufficient endothelial siRNA transfection efficiency in vivo has recently been overcome by Dahlman et al,²⁰⁹ who developed a polymeric nanoconstruct (7C1) capable of delivering siRNAs specifically to endothelial cells, which allowed concurrent silencing of multiple endothelial genes. This approach was successfully utilized for the purpose of RNA-based therapies, as demonstrated in animal models of various diseases involving endothelial dysfunction.²⁰⁹ It seems plausible that the combination of specific siRNAs with local delivery systems that allow blocking of TNF- α /TNFR1 signaling in vascular cells without affecting gene expression in other tissues or immune cells may prove a safe and efficient strategy to control cardiovascular inflammation in the future.

Conclusion

TNF- α -induced signaling plays an important role in the cellular response to inflammation and injury, being central to the development and homeostasis of the immune system. The protective mechanisms induced by TNF- α include the host defense against infections and its inhibitory role in tumorigenesis. However, persistent overproduction of TNF- α has deleterious effects on tissues by inducing a chronic inflammatory response. In the cardiovascular system, TNF- α -activated signal transduction pathways contribute to vascular dysfunction, atherogenesis, hypertension, and adverse cardiac remodeling after myocardial infarction. The existing clinical studies seem to support the notion that treatment with anti-TNF- α may have a favorable effect on endothelial dysfunction and atherosclerotic processes in patients with inflammatory arthropathies. However, an increased risk of heart failure, as a consequence of systemic TNF- α blockade, remains a major concern in patients with cardiovascular disease. Selective inhibition of TNFR1 with specific antibodies, antagonists, or siRNAs provides a promising opportunity to neutralize the proinflammatory, proatherogenic effects of

TNF- α , while maintaining the cardioprotective immunomodulatory responses mediated by TNFR2. Further studies on TNF- α inhibition in atherosclerosis and cardiometabolic disease are therefore urgently needed in order to assess the safety and efficacy of novel therapeutic strategies focused on blocking the responses specific to TNFR1, as compared with global TNF- α inhibition.

Acknowledgments

This work was supported by the German Research Foundation (DFG CI 162/2-1), ELAN-Fonds of University Hospital Erlangen (research grant to KU), and by the EU “NanoAthero” project (FP7-NMP-2012-LARGE-6-309820). The authors would like to thank Margarete Goppelt-Struebe for critical reading and editing of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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