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Emerging Role for TNF- α in Erectile Dysfunction

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Abstract

Introduction—A role for cytokines in the pathophysiology of erectile dysfunction (ED) has emerged. Cytokines induce genes that synthesize other peptides in the cytokine family and several mediators, such as prostanoids, leukotrienes, nitric oxide, bradykinin, reactive oxygen species, and platelet-activating factor, all of which can affect vascular function. Consistent with the fact that the cavernosal tissue is a complex extension of the vasculature, risk factors that affect the vasculature have been shown to affect cavernosal function as well. Accordingly, the penile tissue has been recognized as an early sentinel for atherosclerosis that underlies coronary artery disease and cardiovascular diseases (CVD).

Aim—To review the literature pertaining to the role of tumor necrosis factor-alpha (TNF- α) in ED.

Methods—PubMed search for pertinent publications on the role of cytokines, particularly TNF- α , in CVD and ED.

Main Outcome Measures—Clinical and experimental evidence demonstrates that TNF- α may play a role in ED.

Results—TNF- α has been shown to play an important role in CVD, mainly due to its direct effects on the vasculature. In addition, high levels of TNF- α were demonstrated in patients with ED. In this review, we present a short description of the physiology of erection and the cytokine network. We focus on vascular actions of TNF- α that support a role for this cytokine as a potential candidate in the pathophysiology of ED, particularly in the context of CVD. A brief overview of

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its discovery, mechanisms of synthesis, receptors, and its main actions on the systemic and penile vasculature is also presented.

Conclusions—Considering that ED results from a systemic arterial defect not only confined to the penile vasculature, implication of TNF- α in the pathophysiology of ED offers a humoral linking between CVD and ED.

Keywords

Erectile Dysfunction; TNF- α ; Cytokines; Cardiovascular Disease; Coronary Artery Disease

The Physiology of Erection

Penile erection is determined by pressure changes in the cavernosal sinuses. The vasculature of the erectile tissue differs from most vascular beds as it is composed of arterioles and hollow blood-filled sinuses, both of which are lined with smooth muscle and endothelial cells [1-3]. In the absence of arousal stimuli, cavernosal vasoconstriction maintains the penis in the non-erect state. Contraction of the cavernosal smooth muscle, mainly in response to norepinephrine (NE) released from sympathetic nerve terminals, narrows the arteriolar lumen and sinusoidal cavities, restricting blood flow to maintain low intracavernosal pressure and a non-erect (flaccid) penis [1-3]. During sexual arousal or nocturnal tumescence, the release of nitric oxide (NO) (predominantly through the activation of neuronal nitric oxide synthase [nNOS] in nonadrenergic noncholinergic [NANC] nerves and local endothelial cells [endothelial nitric oxide synthase, eNOS]) stimulates smooth muscle relaxation [1-3]. The resulting dilation of the cavernosal arterioles and sinuses results in increased blood flow (driven by the force of the arterial blood pressure) and a subsequent rise in intracavernosal pressure. The erectile response ensues as the force of the elevated pressure expands the outer tunica albuginea of the penis, resulting in the increased penile length and diameter characteristic of erection.

Although various vasodilators have been implicated in the erectile response, NO is thought to be the principal stimulator of cavernosal vasodilation and penile erection [4-6]. NO is formed from the precursor amino acid, L-arginine, by enzymatic action of NOS, which exists as three main isoforms: nNOS, inducible nitric oxide synthase, and eNOS. All three isoforms have been detected in the penis; nevertheless, nNOS and eNOS are constitutive NOS enzymes expressed in penile tissues [5,7-9]. Upon its release, NO diffuses locally into adjacent smooth muscle cells of the corpus cavernosum and binds to soluble guanylyl cyclase, which catalyzes the conversion of guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP). This cyclic nucleotide then activates protein kinase G, also known as cGMP-dependent protein kinase I, which decreases cytosolic calcium (Ca^{2+}) by various mechanisms. The decay in cytosolic Ca^{2+} concentration induces relaxation of the vascular and cavernosal smooth muscle cells, leading to dilation of arterial vessels, increased blood flow into the sinuses of the corpora cavernosa, and penile erection [10,11].

NE, released from sympathetic nerve terminals, initiates contraction via a rise in the concentration of cytoplasmic Ca^{2+} and triggers smooth muscle contraction and flaccidity. Increased intracellular Ca^{2+} enhances the binding of Ca^{2+} to calmodulin, leading to activation of myosin light chain kinase and phosphorylation of the myosin light chain (MLC_{20}) of myosin II. The extent of MLC_{20} phosphorylation or force of contraction induced by agonist stimulation is normally higher than that predicted by the actual Ca^{2+} concentration in the cell, the so-called Ca^{2+} sensitization process. Activation of the Ca^{2+} -sensitizing pathway is regulated by Ras homolog gene family (Rho), member A (RhoA), a small G-protein from the Rho family that is activated upon the binding of GTP. Activation of RhoA, which is facilitated by Rho-specific guanine nucleotide exchange factors,

stimulates downstream effectors, such as Rho kinase, or ROCK. Activated ROCK phosphorylates the regulatory subunit of myosin light chain phosphatase and inhibits its activity, inhibiting MLC₂₀ dephosphorylation and facilitating smooth muscle cells contraction and flaccidity [10,11]. The importance of ROCK in the Ca²⁺ sensitization of corpus cavernosum smooth muscle cells and maintenance of penile flaccidity is well established [11-13]. All the components of RhoA/ROCK signaling pathway (RhoA, phosphatase inhibitor CPI-17, myosin phosphatase regulatory [MYPT-1] and catalytic [PP1delta] subunits, ROCK isoforms) are expressed in corpora cavernosa from several species and ROCK inhibition increases corpus cavernosum pressure in in vivo models [10,11]. Increased ROCK activity also plays critical roles in certain types of erectile dysfunction (ED) [14-19]. Rho-dependent pathways are activated by several stimuli and mediate cellular functions other than vascular smooth muscle cells contraction including actin cytoskeleton organization, cell adhesion, proliferation, migration, and inflammation, all of which are associated with vascular remodeling [20,21]. Recent evidence indicates that RhoA/ROCK is involved in the progression of target organ damage induced by vasoactive hormones, such as angiotensin II (Ang II) and aldosterone [22,23].

Based on the above information, it is reasonable to postulate that any substance that decreases relaxation mechanisms or increases contractile mechanisms in cavernosal smooth muscle cells may be a causative agent to the development of ED. In this sense, several cytokines, particularly tumor necrosis factor- α (TNF- α), appear as the most prominent candidate to the pathophysiology of ED due to its known effects on the vasculature.

Cytokines

Cytokines are soluble hormone-like proteins that provide communication between cells and the external environment. It is a term that comprises different protein families, such as lymphokines, monokines, interleukins (IL), colony-stimulating factors, interferons, chemokines, and TNF [24,25]. Although cytokine actions and interactions are extraordinarily complex and it seems to defy a simple understanding of their physiological function, some broad basic properties are commonly shared among cytokines: (i) pleiotropy, where a single cytokine can act on many different types of cells rather than a single cell type; (ii) redundancy, comprising a similar function that can be stimulated by different cytokines; and (iii) multifunctional referring to the fact that the same cytokine may be able to regulate several different immune functions [24].

Cytokines are secreted by white blood cells in the body in response to inflammatory stimuli [24-26]. However, it is now clear that other cells and tissues not only produce but are often major targets of cytokine effects. Among these tissues is the vascular endothelium, and a large body of evidence suggests that cytokines induce a variety of structural and functional alterations in endothelial cells (often called “endothelial activation”) and that cytokine-endothelial interactions may play important roles in immunity, inflammation, and vascular injury [27-29].

Furthermore, several reports have demonstrated that cytokines modulate the tonus of the vasculature in different vascular beds [30,31], and the vascular response appears to be related to the balance between all the vasoactive factors released under the influence of cytokines. Thus, regional differences in release and responsiveness to these factors appear to contribute to the dilator or constrictor response observed within a specific vascular bed [32]. In addition, the presence of a low-grade inflammatory process is associated with many cardiovascular diseases (CVD) and, accordingly, cytokines levels, such as TNF- α , are increased in response to inflammation and contribute to the changes in vascular reactivity observed in CVD [29,33-35].

TNF- α : Its Receptors, and Signaling Pathway

TNF- α , named for its ability to cause rapid necrotic tumor regression [36], is the founding member of the TNF ligand superfamily, which is known to have pleiotropic functions including cell proliferation, differentiation, activation, and apoptosis [37]. TNF- α is primarily produced by macrophages, lymphocytes, neutrophils, keratinocytes, and fibroblasts during acute inflammatory reactions [38]. However, it has been demonstrated that TNF- α is produced in cardiac myocytes, smooth muscle cells, and endothelial cells in response to endotoxin independent of the presence of inflammatory cells in ex vivo and in vitro cardiac studies [39,40]. Its synthesis is initiated by the transcriptional activation of the TNF- α gene, which is largely driven by several nuclear factor-kappa B (NF- κ B) responsive elements in the 5' promoter region [41]. Currently, two isoforms of TNF (TNF- α and TNF- β) have been identified and share similar inflammatory activities [42]. TNF- α is the smaller and more abundant of the two peptides, and TNF- β (first described as lymphotoxin- α) [43] is less abundant, and is thought to be produced mainly by T-cells. TNF- β will not be discussed further in this review. TNF- α receptors (TNFRs) signal as homotrimers, and can exist either as membrane-bound or as truncated soluble forms [44]. Two distinct surface receptors mediate the effects of TNF- α , TNFR-1 (p55), and TNFR-2 (p75), both receptor subtypes are found in endothelial cells [45], smooth muscle [46], human and rat cardiac myocytes [47,48]. Furthermore, gene expression of TNFR-1 has been demonstrated in cavernosal tissue [49]. The wide repertoire of signaling responses elicited by TNF results from the ability to recruit a range of adapter proteins to the receptor. Both TNFRs and associated adapter proteins contain various defined regions or motifs that have been shown to elicit specific signaling events (Figure 1). Usually, the first protein recruited to TNFR1 activation is TNFR1-associated death domain protein (TRADD), which serves as a platform to recruit at least three additional mediators, receptor-interacting protein 1, Fas-associated death domain protein and TNF-receptor-associated factor 2 (TRAF2) [50-53]. TRAF2 plays a central role in early events, common to TNFR1 and TNFR2, that lead to I κ B kinase (IKK) and mitogen-activated protein kinase (MAPK) activation. Structural and biochemical analysis of the TRADD-TRAF2 complex reveals that TRAF2 has a higher affinity for TRADD than it has for TNFR1 or TNFR2 [52,54]. This might explain why TNF- α is a better activator of TNFR1, which uses TRADD as an adaptor, than TNFR2, which does not interact with TRADD. NF- κ B activation represents one of the most central proinflammatory responses elicited by TNF- α , particularly in endothelial cells [55]. NF- κ B is regulated primarily by phosphorylation of inhibitory proteins, the I κ Bs, which retain the transcription factor in the cytoplasm of nonstimulated cells [56]. In response to TNF- α and other agonists, the I κ Bs are phosphorylated by the IKK complex, resulting in their ubiquitination, degradation, and nuclear translocation of the freed NF- κ B [56]. Once in the nucleus, NF- κ B transcriptional activity can be modulated further through phosphorylation by various protein kinases that are TNF- α responsive, such as the p38 subgroup of MAPK, providing a point for cross talk with other signaling pathways. Although other signaling pathways are activated by TNF- α , such as MAPK, PI-3 Kinase/Akt, and sphingomyelinase, detailed discussion on this topic is beyond the scope of this review (more information about this topic can be obtained in comprehensive reviews [38,52,55,57-60]).

TNF- α Actions on the Vasculature

TNF- α is an important contributor to many CVD, such as myocardial ischemia-reperfusion injury, chronic heart failure, atherosclerosis, and sepsis-associated cardiovascular disorders [61-63].

Endothelial dysfunction is a key event in the pathophysiology of ED and, importantly, endothelial function is impaired in the presence of increased oxidative stress and inflammatory conditions [64]. The vascular endothelium is a major target for the actions of

TNF- α in these diseases, in which TNF- α plasma levels are significantly elevated. Administration of TNF- α in vivo induces impairment of endothelium-dependent vasorelaxation in a variety of vascular beds and decreases the release of NO [65]. TNF- α has the ability to increase arterial reactive oxygen species generation, which likely accounts for some of the reduction in NO levels. Moreover, TNF- α suppresses eNOS expression by inhibiting the gene promoter activity [66,67], predominantly through destabilization of eNOS mRNA in endothelial cells [68]. In addition to its effects on endothelial function, a substantial proatherogenic role for TNF- α has recently been demonstrated [69]. The presence of TNFR1 in the vascular wall causes atherosclerosis [69].

In endothelial cells, TNF- α not only induces inflammatory gene transcription but also activation of RhoA and ROCK [70]. ROCK activation plays a role in TNF- α -induced increases in junctional permeability [71,72], as well as in TNF- α -induced actomyosin rearrangement and apoptosis [73]. Additionally, pharmacological inhibition of ROCK as well as dominant negative RhoA overexpression dramatically reduced TNF- α -induced endothelial cell permeability and apoptosis [71,73]. Degradation of inhibitor kappa-B followed by translocation of NF- κ B into the nucleus and activation of gene expression is essential in TNF- α signaling. In human umbilical cord vein endothelial cells, inhibition of RhoA reduces TNF- α -induced NF- κ B binding to DNA, via blockade of NF- κ B-translocation [74]. Finally, TNF- α leads to increased Ca²⁺ sensitivity, via activation of the RhoA/ROCK pathway, a mechanism that may contribute not only to TNF- α -induced airway hyperresponsiveness and hyperreactivity [75,76]. Conversely, inhibitors of ROCK also block TNF- α production by lipopolysaccharide-stimulated monocytes [77] and burn-injured cardiomyocytes [78]. ROCK inhibitors attenuate TNF- α -induced leukocyte infiltration by inhibiting both cytoskeletal rearrangement of endothelial cells [79] and by a suppressive effect on the functions of polymorphonuclear leukocytes and expression of inflammatory cytokines [77,80-82]. All together, these observations suggest that ROCK inhibitors may display anti-inflammatory properties, by blocking TNF- α actions.

ED and Markers of CVD (Inflammatory Mediators)

ED, defined as the inability to attain and/or maintain penile erection [83], is a multifactorial condition that is estimated to affect more than 150 million men worldwide, with this number expected to double by 2025 [84,85]. Considered a major public health problem, which seriously affects the quality of life of patients and their partners, ED becomes increasingly prevalent with age. The presence of chronic illness (e.g., CVD), as well as smoking, alcohol or drug abuse, and sedentary lifestyle are major risk factors for ED [84,86].

Traditionally, ED is thought of as an outcome of occlusive systemic vascular disease that occurs as a late consequence of atherosclerosis. Prospective results from the Massachusetts Male Aging Study [87] demonstrated that ED and coronary artery diseases (CAD) share common risk factors in humans. In addition, the prevalence of ED ranges from 42% to 75% in patients with established CAD [88-91] and severity of CAD is associated with the severity of ED [91-93]. Furthermore, Montorsi and coworkers [94] reported that CAD and atherosclerotic burden determine the rate of ED: it is low in acute coronary symptoms and one-vessel disease and high in chronic coronary syndrome. However, vasculogenic ED may result not only from occlusion of the cavernosal arteries by atherosclerosis (structural vascular ED), but also from impairment of endothelial function/smooth muscle relaxation (functional vascular ED) [95-97]. Current and emerging clinical studies show that the penile vascular bed may be indeed a sensitive indicator of early systemic endothelial cell and/or smooth muscle dysfunction. In other words, ED is considered an early clinical manifestation of generalized vascular disease and carries an independent risk for cardiovascular events [98-100]. While it is clear that ED is multifactorial, longitudinal population-based studies clearly demonstrate that cardiovascular risk factors such as hypertension, dyslipidemia,

central obesity, and insulin resistance are major risk factors for vasculogenic ED [101]. Furthermore, clustering of these factors, as occurs in patients with metabolic syndrome, increases the risk for the development of ED even further [102,103].

Recent evidence points to a low-grade inflammatory process as an important pathophysiologic component of CVD. Accordingly, inflammatory markers are increased in patients with hypertension and metabolic disorders, and predict the development of CVD [104]. Patients with CVD present with increased expression and plasma concentration of several inflammatory mediators, which include C-reactive protein (CRP) and adhesion molecules, such as selectins (P-selectin, E-selectin, and L-selectin), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). Moreover, increased plasma levels of the primary inflammatory cytokine TNF- α and of the secondary inflammatory cytokine IL-6, as well as ICAM-1, VCAM-1, E-selectin, von Willebrand factor (vWF), and CRP have been demonstrated in patients with hypertension [104]. High levels of inflammatory mediators are considered independent risk factors for the development of hypertension and may also be associated with increased risk of diabetes, CVD, and ED. Available evidence identifies inflammation as a common pathophysiologic mechanism linking ED, metabolic syndrome, and CAD [34,105-112]. Patients with ED also present with increased expression and plasma concentration of inflammatory markers and mediators, including CRP [34,106-108,110,111], adhesion molecules (P-selectin, ICAM-1, VCAM-1) [107,111], and cytokines (TNF- α , IL-6, IL-8, IL-18) [34,108,111]. Importantly, plasma levels of these inflammatory markers are significantly higher in patients with ED (vs. subjects without ED) matched for age and coronary risk score [109]. In addition, levels of inflammatory markers are also increased in men with ED but without cardiovascular risk factors or overt vascular damage [107]. Finally, sexual performance has also been negatively correlated with circulating levels of endothelial prothrombotic and inflammatory parameters such as fibrinogen, vWF, IL-1 β , and IL-6 [34]. Therefore, therapeutic approaches to control vascular inflammation, particularly in patients with ED and CVD, may provide significant clinical benefits.

TNF- α and ED

Figure 2 illustrates the complex interplay between ED and CAD, where the incidence of ED and the levels of TNF- α are simultaneously correlated to age [84,113,114]. It is noteworthy that CAD appears right after ED and after the levels of TNF- α start to increase [87,89,94]. Thus, TNF- α may represent not only a common link between ED and CAD, but its increasing levels associated with ED may be a predictor of cardiovascular events.

The association between low-grade inflammation and altered endothelial–prothrombotic state on one side and ED on the other in patients with and without CAD has been recently reviewed [34]. Several lines of evidence suggest that TNF- α plays a key role in inducing endothelial dysfunction. First, clinically stable heart transplant patients exhibit a strong positive relation between plasma TNF- α and impaired vascular response to acetylcholine [115]. Second, infusion of TNF- α alone impaired endothelial function in healthy subjects, whereas infusion of IL-6 did not [116]. Of major importance, TNF- α levels are increased in serum of patients with moderate to severe ED [34,112,117], and levels of TNF- α and endothelial–prothrombotic parameters are inversely associated with sexual performance [34].

Experimental studies have demonstrated that TNF- α knockout (KO) mice exhibit changes in cavernosal reactivity that would facilitate erectile responses: decreased responses to adrenergic nerve stimulation and increased NANC and endothelium-dependent relaxation that are associated with increased corporal eNOS and nNOS protein levels [118].

While eNOS is mainly involved in endothelium-dependent relaxation, nNOS is considered the major mediator of NANC relaxation in the penis [4-6]. Recently, it has been shown that TNF- α decreases nNOS expression in corpora cavernosa [49]. On the other hand, TNF- α knockout animals have increased nNOS expression in cavernosal tissue [118], which suggests that TNF- α down-regulates nNOS expression in cavernosal tissue (Figure 3). Accordingly, it has been demonstrated that TNF- α infusion significantly induces an increase in mean arterial pressure and a reduction in renal nNOS protein expression, within both the cortex and medulla from pregnant rats [119].

Hayward and coworkers [120] have shown that a mouse model that overexpresses human tumor necrosis factor alpha (hTNF- α) not only exhibits decreased induced erections but also decreased mounting behavior and number of intromissions. Conversely, TNF- α KO mice demonstrate increased number of spontaneous erections [118]. Additionally, isolated corpora cavernosa from TNF- α -infused mice display decreased NANC-dependent relaxation and increased sympathetic-mediated contractions in vitro (Figure 4), which would favor penile detumescence to occur [49]. Increased direct adrenergic responses were also observed in cavernosal tissue from TNF- α -infused mice [49]. Although TNF- α increases Ca²⁺ sensitivity in airway smooth muscle cell, the exact role on cavernosal smooth muscle remains speculative (Figure 4). Downregulation of eNOS and nNOS seems to be the mechanism underlying the functional changes in cavernosal strips from TNF- α -infused mice [49].

It is interesting to note that the penile vasculature may not be only a target of widespread inflammation generated elsewhere, but it may actively participate in the whole process. Accordingly, the human corpus cavernosum has a local renin-angiotensin system (RAS) [121] and Ang II, one of the final products and the main known mediator of the RAS, which induces vascular injury through several mechanisms, including vasoconstriction, cell growth, oxidative stress production, and inflammation [122]. Similarly, the penile smooth muscle cells not only respond to but also synthesize endothelin-1 (ET-1), its converting enzyme (ECE-1), and both ET_A and ET_B receptor subtypes [123]. ET-1 not only induces vasoconstriction, but it also stimulates the expression of adhesion molecules and activates transcriptional factors responsible for the coordinated increase in the expression of many cytokines and enzymes, which can in turn lead to the production of inflammatory mediators [124,125]. Both peptides have been shown to increase TNF- α levels and this pro-inflammatory cytokine also positively regulates release of these vasoactive peptides [126-129]. More importantly, TNF- α inhibition slows the progression of hypertension and renal damage Ang II salt-sensitive hypertension [130] as well as in mineralocorticoid hypertension [131], a model where ET-1 plays a major role in end-organ damage [124,125,132,133]. In deoxycorticosterone-acetate (DOCA)-salt hypertensive animals, blockade of TNF- α actions with etanercept treatment reduces most indexes of renal inflammation independent of any blood pressure-lowering effect. In DOCA-salt hypertensive rats, TNF- α inhibition reduces monocyte chemoattractant protein-1 excretion and lowers renal cortical NF- κ B activity. Urinary ET-1 excretion also decreases in DOCA-salt hypertensive rats on etanercept treatment along with reductions in renal cortical ICAM-1 expression [131].

It is noteworthy that diseases associated with high levels of TNF- α such as psoriasis, psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, and chronic obstructive pulmonary disease also have been associated with ED in males and, in many cases, with sexual dysfunction in females [117,134-138]. Although these evidence indicate that TNF- α may be the causal agent of ED, TNF- α is not alone in the list of cytokines, and a myriad of effects must coexist in all of these conditions.

Further studies are essential to determine whether TNF- α plays a detrimental role in ED associated with CVD such as hypertension, diabetes, CAD, and heart failure. Finally, a key role for TNF- α in mediating smooth muscle and endothelial dysfunction is of interest not only because markedly elevated serum levels of TNF- α have been documented in patients with ED, but also because we now have access to targeted anti-TNF- α therapies.

References

1. Burnett AL. Role of nitric oxide in the physiology of erection. *Biol Reprod* 1995;52:485–9. [PubMed: 7756443]
2. Andersson KE. Pharmacology of penile erection. *Pharmacol Rev* 2001;53:417–50. [PubMed: 11546836]
3. Andersson KE, Stief CG. Neurotransmission and the contraction and relaxation of penile erectile tissues. *World J Urol* 1997;15:14–20. [PubMed: 9066089]
4. Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochem Biophys Res Commun* 1990;170:843–50. [PubMed: 2166511]
5. Rajfer J, Aronson WJ, Bush PA, Dorey FJ, Ignarro LJ. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *N Engl J Med* 1992;326:90–4. [PubMed: 1309211]
6. Burnett AL, Nelson RJ, Calvin DC, Liu JX, Demas GE, Klein SL, Kriegsfeld LJ, Dawson VL, Dawson TM, Snyder SH. Nitric oxide-dependent penile erection in mice lacking neuronal nitric oxide synthase. *Mol Med* 1996;2:288–96. [PubMed: 8784782]
7. Alm P, Larsson B, Ekblad E, Sundler F, Andersson KE. Immunohistochemical localization of peripheral nitric oxide synthase-containing nerves using antibodies raised against synthesized C- and N-terminal fragments of a cloned enzyme from rat brain. *Acta Physiol Scand* 1993;148:421–9. [PubMed: 7692702]
8. Burnett AL, Tillman SL, Chang TS, Epstein JI, Lowenstein CJ, Bredt DS, Snyder SH, Walsh PC. Immunohistochemical localization of nitric oxide synthase in the autonomic innervation of the human penis. *J Urol* 1993;150:73–6. [PubMed: 7685426]
9. Dail WG, Barba V, Leyba L, Galindo R. Neural and endothelial nitric oxide synthase activity in rat penile erectile tissue. *Cell Tissue Res* 1995;282:109–16. [PubMed: 8581913]
10. Leite R, Giachini FR, Carneiro FS, Nunes KP, Tostes RC, Webb RC. Targets for the treatment of erectile dysfunction: Is NO/cGMP still the answer? *Recent Patents Cardiovasc Drug Discov* 2007;2:119–32.
11. Priviero FB, Leite R, Webb RC, Teixeira CE. Neurophysiological basis of penile erection. *Acta Pharmacol Sin* 2007;28:751–5. [PubMed: 17506932]
12. Chitaley K, Wingard CJ, Clinton Webb R, Branam H, Stopper VS, Lewis RW, Mills TM. Antagonism of Rho-kinase stimulates rat penile erection via a nitric oxide-independent pathway. *Nat Med* 2001;7:119–22. [PubMed: 11135626]
13. Wang H, Eto M, Steers WD, Somlyo AP, Somlyo AV. RhoA-mediated Ca^{2+} sensitization in erectile function. *J Biol Chem* 2002;277:30614–21. [PubMed: 12060659]
14. Chitaley K, Webb RC, Mills TM. Rho-kinase as a potential target for the treatment of erectile dysfunction. *Drug News Perspect* 2001;14:601–6. [PubMed: 12806426]
15. Bivalacqua TJ, Champion HC, Usta MF, Celtek S, Chitaley K, Webb RC, Lewis RL, Mills TM, Hellstrom WJ, Kadowitz PJ. RhoA/Rho-kinase suppresses endothelial nitric oxide synthase in the penis: A mechanism for diabetes-associated erectile dysfunction. *Proc Natl Acad Sci U S A* 2004;101:9121–6. [PubMed: 15184671]
16. Rajasekaran M, White S, Baquir A, Wilkes N. Rho-kinase inhibition improves erectile function in aging male Brown-Norway rats. *J Androl* 2005;26:182–8. [PubMed: 15713824]
17. Seftel A. RhoA/Rho-kinase suppresses endothelial nitric oxide synthase in the penis: A mechanism for diabetes-associated erectile dysfunction. *J Urol* 2005;173:928–9. [PubMed: 15711333]

18. Jin L, Liu T, Lagoda GA, Champion HC, Bivalacqua TJ, Burnett AL. Elevated RhoA/Rho-kinase activity in the aged rat penis: Mechanism for age-associated erectile dysfunction. *FASEB J* 2006;20:536–8. [PubMed: 16396994]
19. Park K, Kim SW, Rhu KS, Paick JS. Chronic administration of an oral Rho kinase inhibitor prevents the development of vasculogenic erectile dysfunction in a rat model. *J Sex Med* 2006;3:996–1003. [PubMed: 17100932]
20. Kobayashi N, Nakano S, Mita S, Kobayashi T, Honda T, Tsubokou Y, Matsuoka H. Involvement of Rho-kinase pathway for angiotensin II-induced plasminogen activator inhibitor-1 gene expression and cardiovascular remodeling in hypertensive rats. *J Pharmacol Exp Ther* 2002;301:459–66. [PubMed: 11961044]
21. Loirand G, Rolli-Derkinderen M, Pacaud P. RhoA and resistance artery remodeling. *Am J Physiol Heart Circ Physiol* 2005;288:H1051–6. [PubMed: 15706042]
22. Nakano S, Kobayashi N, Yoshida K, Ohno T, Matsuoka H. Cardioprotective mechanisms of spironolactone associated with the angiotensin-converting enzyme/epidermal growth factor receptor/extracellular signal-regulated kinases, NAD(P)H oxidase/lectin-like oxidized low-density lipoprotein receptor-1, and Rho-kinase pathways in aldosterone/salt-induced hypertensive rats. *Hypertens Res* 2005;28:925–36. [PubMed: 16555582]
23. Ohtsu H, Mifune M, Frank GD, Saito S, Inagami T, Kim-Mitsuyama S, Takuwa Y, Sasaki T, Rothstein JD, Suzuki H, Nakashima H, Woolfolk EA, Motley ED, Eguchi S. Signal-crosstalk between Rho/ROCK and c-Jun NH2-terminal kinase mediates migration of vascular smooth muscle cells stimulated by angiotensin II. *Arterioscler Thromb Vasc Biol* 2005;25:1831–6. [PubMed: 15994438]
24. Tayal V, Kalra BS. Cytokines and anti-cytokines as therapeutics—An update. *Eur J Pharmacol* 2008;579:1–12. [PubMed: 18021769]
25. Kelso A. Cytokines: Principles and prospects. *Immunol Cell Biol* 1998;76:300–17. [PubMed: 9723771]
26. O'Shea JJ, Murray PJ. Cytokine signaling modules in inflammatory responses. *Immunity* 2008;28:477–87. [PubMed: 18400190]
27. Cotran RS, Pober JS. Cytokine-endothelial interactions in inflammation, immunity, and vascular injury. *J Am Soc Nephrol* 1990;1:225–35. [PubMed: 2104268]
28. Cotran RS, Pober JS. Effects of cytokines on vascular endothelium: Their role in vascular and immune injury. *Kidney Int* 1989;35:969–75. [PubMed: 2651773]
29. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. *Basic Res Cardiol* 2008;103:398–406. [PubMed: 18600364]
30. Glembo T, Britt LD, Hill MA. Endotoxin interacts with tumor necrosis factor- α to induce vasodilation of isolated rat skeletal muscle arterioles. *Shock* 1996;5:251–7. [PubMed: 8721384]
31. Minghini A, Britt LD, Hill MA. Interleukin-1 and interleukin-6 mediated skeletal muscle arteriolar vasodilation: In vitro versus in vivo studies. *Shock* 1998;9:210–5. [PubMed: 9525329]
32. Vila E, Salaices M. Cytokines and vascular reactivity in resistance arteries. *Am J Physiol Heart Circ Physiol* 2005;288:H1016–21. [PubMed: 15706038]
33. Zhang C, Xu X, Potter BJ, Wang W, Kuo L, Michael L, Bagby GJ, Chilian WM. TNF- α contributes to endothelial dysfunction in ischemia/reperfusion injury. *Arterioscler Thromb Vasc Biol* 2006;26:475–80. [PubMed: 16385082]
34. Vlachopoulos C, Aznaouridis K, Ioakeimidis N, Rokkas K, Vasiliadou C, Alexopoulos N, Stefanadi E, Askitis A, Stefanadis C. Unfavourable endothelial and inflammatory state in erectile dysfunction patients with or without coronary artery disease. *Eur Heart J* 2006;27:2640–8. [PubMed: 17056702]
35. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, Dellsperger KC, Zhang C. Role of TNF- α in vascular dysfunction. *Clin Sci (Lond)* 2009;116:219–30. [PubMed: 19118493]
36. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 1975;72:3666–70. [PubMed: 1103152]
37. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: Integrating mammalian biology. *Cell* 2001;104:487–501. [PubMed: 11239407]

38. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol* 2001;11:372–7. [PubMed: 11514191]
39. Giroir BP, Johnson JH, Brown T, Allen GL, Beutler B. The tissue distribution of tumor necrosis factor biosynthesis during endotoxemia. *J Clin Invest* 1992;90:693–8. [PubMed: 1522226]
40. Kapadia S, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor- α gene and protein expression in adult feline myocardium after endotoxin administration. *J Clin Invest* 1995;96:1042–52. [PubMed: 7635940]
41. Shakhov AN, Collart MA, Vassalli P, Nedospasov SA, Jongeneel CV. Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor α gene in primary macrophages. *J Exp Med* 1990;171:35–47. [PubMed: 2104921]
42. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996;334:1717–25. [PubMed: 8637518]
43. Ruddle NH, Waksman BH. Cytotoxic effect of lymphocyteantigen interaction in delayed hypersensitivity. *Science* 1967;157:1060–2. [PubMed: 6036235]
44. Baker SJ, Reddy EP. Transducers of life and death: TNF receptor superfamily and associated proteins. *Oncogene* 1996;12:1–9. [PubMed: 8552378]
45. Gaeta ML, Johnson DR, Kluger MS, Pober JS. The death domain of tumor necrosis factor receptor 1 is necessary but not sufficient for Golgi retention of the receptor and mediates receptor desensitization. *Lab Invest* 2000;80:1185–94. [PubMed: 10950109]
46. Amrani Y, Ammit AJ, Panettieri RA Jr. Tumor necrosis factor receptor (TNFR) 1, but not TNFR2, mediates tumor necrosis factor- α -induced interleukin-6 and RANTES in human airway smooth muscle cells: Role of p38 and p42/44 mitogen-activated protein kinases. *Mol Pharmacol* 2001;60:646–55. [PubMed: 11562425]
47. Krown KA, Yasui K, Brooker MJ, Dubin AE, Nguyen C, Harris GL, McDonough PM, Glembotski CC, Palade PT, Sabbadini RA. TNF α receptor expression in rat cardiac myocytes: TNF α inhibition of L-type Ca^{2+} current and Ca^{2+} transients. *FEBS Lett* 1995;376:24–30. [PubMed: 8521959]
48. Torre-Amione G, Kapadia S, Lee J, Bies RD, Lebovitz R, Mann DL. Expression and functional significance of tumor necrosis factor receptors in human myocardium. *Circulation* 1995;92:1487–93. [PubMed: 7664431]
49. Carneiro FS, Zemse S, Giachini FR, Carneiro ZN, Lima VV, Webb RC, Tostes RC. TNF- α infusion impairs corpora cavernosa reactivity. *J Sex Med* 2009;6(3 suppl):311–9. [PubMed: 19267854]
50. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 1996;84:299–308. [PubMed: 8565075]
51. Rothe M, Sarma V, Dixit VM, Goeddel DV. TRAF2-mediated activation of NF- κ B by TNF receptor 2 and CD40. *Science* 1995;269:1424–7. [PubMed: 7544915]
52. Bradley JR, Pober JS. Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene* 2001;20:6482–91. [PubMed: 11607847]
53. Stanger BZ, Leder P, Lee TH, Kim E, Seed B. RIP: A novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. *Cell* 1995;81:513–23. [PubMed: 7538908]
54. Park YC, Ye H, Hsia C, Segal D, Rich RL, Liou HC, Myszka DG, Wu H. A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. *Cell* 2000;101:777–87. [PubMed: 10892748]
55. Madge LA, Pober JS. TNF signaling in vascular endothelial cells. *Exp Mol Pathol* 2001;70:317–25. [PubMed: 11418010]
56. Baldwin AS Jr. The NF- κ B and I κ B proteins: New discoveries and insights. *Annu Rev Immunol* 1996;14:649–83. [PubMed: 8717528]
57. Pfizenmaier K, Wajant H, Grell M. Tumor necrosis factors in 1996. *Cytokine Growth Factor Rev* 1996;7:271–7. [PubMed: 8971482]
58. Chen G, Goeddel DV. TNF-R1 signaling: A beautiful pathway. *Science* 2002;296:1634–5. [PubMed: 12040173]

59. Ihnatko R, Kubes M. TNF signaling: Early events and phosphorylation. *Gen Physiol Biophys* 2007;26:159–67. [PubMed: 18063842]
60. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ* 2003;10:45–65. [PubMed: 12655295]
61. Ferrari R. Tumor necrosis factor in CHF: A double facet cytokine. *Cardiovasc Res* 1998;37:554–9. [PubMed: 9659437]
62. Meldrum DR, Meng X, Dinarello CA, Ayala A, Cain BS, Shames BD, Ao L, Banerjee A, Harken AH. Human myocardial tissue TNF alpha expression following acute global ischemia in vivo. *J Mol Cell Cardiol* 1998;30:1683–9. [PubMed: 9769224]
63. Berk BC, Abe JI, Min W, Surapisitchat J, Yan C. Endothelial atheroprotective and anti-inflammatory mechanisms. *Ann N Y Acad Sci* 2001;947:93–109. discussion 09–11. [PubMed: 11795313]
64. Trepels T, Zeiher AM, Fichtlscherer S. The endothelium and inflammation. *Endothelium* 2006;13:423–9. [PubMed: 17169774]
65. Chia S, Qadan M, Newton R, Ludlam CA, Fox KA, Newby DE. Intra-arterial tumor necrosis factor-alpha impairs endothelium-dependent vasodilatation and stimulates local tissue plasminogen activator release in humans. *Arterioscler Thromb Vasc Biol* 2003;23:695–701. [PubMed: 12692009]
66. Anderson HD, Rahmutula D, Gardner DG. Tumor necrosis factor-alpha inhibits endothelial nitric-oxide synthase gene promoter activity in bovine aortic endothelial cells. *J Biol Chem* 2004;279:963–9. [PubMed: 14581470]
67. Neumann P, Gertzberg N, Johnson A. TNF-alpha induces a decrease in eNOS promoter activity. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L452–9. [PubMed: 14555463]
68. Yoshizumi M, Perrella MA, Burnett JC Jr, Lee ME. Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. *Circ Res* 1993;73:205–9. [PubMed: 7685252]
69. Zhang L, Peppel K, Sivashanmugam P, Orman ES, Brian L, Exum ST, Freedman NJ. Expression of tumor necrosis factor receptor-1 in arterial wall cells promotes atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;27:1087–94. [PubMed: 17442899]
70. Mong PY, Petruccio C, Kaufman HL, Wang Q. Activation of Rho kinase by TNF-alpha is required for JNK m activation in human pulmonary microvascular endothelial cells. *J Immunol* 2008;180:550–8. [PubMed: 18097057]
71. Petrache I, Birukova A, Ramirez SI, Garcia JG, Verin AD. The role of the microtubules in tumor necrosis factor-alpha-induced endothelial cell permeability. *Am J Respir Cell Mol Biol* 2003;28:574–81. [PubMed: 12707013]
72. McKenzie JA, Ridley AJ. Roles of Rho/ROCK and MLCK in TNF-alpha-induced changes in endothelial morphology and permeability. *J Cell Physiol* 2007;213:221–8. [PubMed: 17476691]
73. Petrache I, Crow MT, Neuss M, Garcia JG. Central involvement of Rho family GTPases in TNF-alpha-mediated bovine pulmonary endothelial cell apoptosis. *Biochem Biophys Res Commun* 2003;306:244–9. [PubMed: 12788095]
74. Hippenstiel S, Schmeck B, Seybold J, Krull M, Eichel-Streiber C, Suttrop N. Reduction of tumor necrosis factor-alpha (TNF-alpha) related nuclear factor-kappaB (NF-kappaB) translocation but not inhibitor kappa-B (Ikappa-B)-degradation by Rho protein inhibition in human endothelial cells. *Biochem Pharmacol* 2002;64:971–7. [PubMed: 12213594]
75. Hunter I, Cobban HJ, Vandenabeele P, MacEwan DJ, Nixon GF. Tumor necrosis factor-alpha-induced activation of RhoA in airway smooth muscle cells: Role in the Ca²⁺ sensitization of myosin light chain20 phosphorylation. *Mol Pharmacol* 2003;63:714–21. [PubMed: 12606782]
76. Morin C, Sirois M, Echave V, Gomes MM, Rousseau E. EET displays anti-inflammatory effects in TNF-alpha stimulated human bronchi: Putative role of CPI-17. *Am J Respir Cell Mol Biol* 2008;38:192–201. [PubMed: 17872494]
77. Doe C, Bentley R, Behm DJ, Lafferty R, Stavenger R, Jung D, Bamford M, Panchal T, Grygielko E, Wright LL, Smith GK, Chen Z, Webb C, Khandekar S, Yi T, Kirkpatrick R, Dul E, Jolivet L, Marino JP Jr, Willette R, Lee D, Hu E. Novel Rho kinase inhibitors with anti-inflammatory and vasodilatory activities. *J Pharmacol Exp Ther* 2007;320:89–98. [PubMed: 17018693]

78. Horton JW, Maass DL, Ballard-Croft C. Rho-associated kinase modulates myocardial inflammatory cytokine responses. *Shock* 2005;24:53–8. [PubMed: 15988321]
79. Thorlacius K, Slotta JE, Laschke MW, Wang Y, Menger MD, Jeppsson B, Thorlacius H. Protective effect of fasudil, a Rho-kinase inhibitor, on chemokine expression, leukocyte recruitment, and hepatocellular apoptosis in septic liver injury. *J Leukoc Biol* 2006;79:923–31. [PubMed: 16641138]
80. Kawaguchi A, Ohmori M, Fujimura A. Partial protective effect of Y-27632, a Rho kinase inhibitor, against hepatic ischemia-reperfusion injury in rats. *Eur J Pharmacol* 2004;493:167–71. [PubMed: 15189778]
81. Shiotani S, Shimada M, Suehiro T, Soejima Y, Yosizumi T, Shimokawa H, Maehara Y. Involvement of Rho-kinase in cold ischemia-reperfusion injury after liver transplantation in rats. *Transplantation* 2004;78:375–82. [PubMed: 15316365]
82. Tasaka S, Koh H, Yamada W, Shimizu M, Ogawa Y, Hasegawa N, Yamaguchi K, Ishii Y, Richer SE, Doerschuk CM, Ishizaka A. Attenuation of endotoxin-induced acute lung injury by the Rho-associated kinase inhibitor, Y-27632. *Am J Respir Cell Mol Biol* 2005;32:504–10. [PubMed: 15778497]
83. NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence. *JAMA* 1993;270:83–90. [PubMed: 8510302]
84. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: Results of the Massachusetts Male Aging Study. *J Urol* 1994;151:54–61. [PubMed: 8254833]
85. Ayta IA, McKinlay JB, Krane RJ. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. *BJU Int* 1999;84:50–6. [PubMed: 10444124]
86. Shabsigh R, Fishman IJ, Schum C, Dunn JK. Cigarette smoking and other vascular risk factors in vasculogenic impotence. *Urology* 1991;38:227–31. [PubMed: 1887536]
87. Feldman HA, Johannes CB, Derby CA, Kleinman KP, Mohr BA, Araujo AB, McKinlay JB. Erectile dysfunction and coronary risk factors: Prospective results from the Massachusetts male aging study. *Prev Med* 2000;30:328–38. [PubMed: 10731462]
88. Hodges LD, Kirby M, Solanki J, O'Donnell J, Brodie DA. The temporal relationship between erectile dysfunction and cardiovascular disease. *Int J Clin Pract* 2007;61:2019–25. [PubMed: 17997808]
89. Montorsi F, Briganti A, Salonia A, Rigatti P, Margonato A, Macchi A, Galli S, Ravagnani PM, Montorsi P. Erectile dysfunction prevalence, time of onset and association with risk factors in 300 consecutive patients with acute chest pain and angiographically documented coronary artery disease. *Eur Urol* 2003;44:360–4. discussion 64–5. [PubMed: 12932937]
90. Kloner RA, Mullin SH, Shook T, Matthews R, Mayeda G, Burstein S, Peled H, Pollick C, Choudhary R, Rosen R, Padma-Nathan H. Erectile dysfunction in the cardiac patient: How common and should we treat? *J Urol* 2003;170:S46–50. discussion S50. [PubMed: 12853773]
91. Solomon H, Man JW, Wierzbicki AS, Jackson G. Relation of erectile dysfunction to angiographic coronary artery disease. *Am J Cardiol* 2003;91:230–1. [PubMed: 12521639]
92. Akilli H, Gok H, Soylu A, Kayrak M. Severity of coronary artery disease and symptoms of erectile dysfunction in males with a positive exercise treadmill test. *Int J Urol* 2007;14:733–7. [PubMed: 17681065]
93. Greenstein A, Chen J, Miller H, Matzkin H, Villa Y, Braf Z. Does severity of ischemic coronary disease correlate with erectile function? *Int J Impot Res* 1997;9:123–6. [PubMed: 9315488]
94. Montorsi P, Ravagnani PM, Galli S, Rotatori F, Veglia F, Briganti A, Salonia A, Deho F, Rigatti P, Montorsi F, Fiorentini C. Association between erectile dysfunction and coronary artery disease. Role of coronary clinical presentation and extent of coronary vessels involvement: The COBRA trial. *Eur Heart J* 2006;27:2632–9. [PubMed: 16854949]
95. Sullivan ME, Thompson CS, Dashwood MR, Khan MA, Jeremy JY, Morgan RJ, Mikhailidis DP. Nitric oxide and penile erection: Is erectile dysfunction another manifestation of vascular disease? *Cardiovasc Res* 1999;43:658–65. [PubMed: 10690337]

96. Montorsi P, Montorsi F, Schulman CC. Is erectile dysfunction the “tip of the iceberg” of a systemic vascular disorder? *Eur Urol* 2003;44:352–4. [PubMed: 12932935]
97. Kirby M, Jackson G, Simonsen U. Endothelial dysfunction links erectile dysfunction to heart disease. *Int J Clin Pract* 2005;59:225–9. [PubMed: 15854201]
98. Billups KL, Bank AJ, Padma-Nathan H, Katz S, Williams R. Erectile dysfunction is a marker for cardiovascular disease: Results of the minority health institute expert advisory panel. *J Sex Med* 2005;2:40–50. discussion 50–2. [PubMed: 16422903]
99. Pohnholzer A, Temml C, Obermayr R, Wehrberger C, Madersbacher S. Is erectile dysfunction an indicator for increased risk of coronary heart disease and stroke? *Eur Urol* 2005;48:512–8. discussion 17–8. [PubMed: 15998563]
100. Thompson IM, Tangen CM, Goodman PJ, Probstfield JL, Moynour CM, Coltman CA. Erectile dysfunction and subsequent cardiovascular disease. *JAMA* 2005;294:2996–3002. [PubMed: 16414947]
101. Grover SA, Lowenstein I, Kaouache M, Marchand S, Coupal L, DeCarolis E, Zoccoli J, Defoy I. The prevalence of erectile dysfunction in the primary care setting: Importance of risk factors for diabetes and vascular disease. *Arch Intern Med* 2006;166:213–9. [PubMed: 16432091]
102. Jackson G. The metabolic syndrome and erectile dysfunction: Multiple vascular risk factors and hypogonadism. *Eur Urol* 2006;50:426–7. [PubMed: 16644094]
103. Heidler S, Temml C, Broessner C, Mock K, Rauchenwald M, Madersbacher S, Pohnholzer A. Is the metabolic syndrome an independent risk factor for erectile dysfunction? *J Urol* 2007;177:651–4. [PubMed: 17222651]
104. Savoia C, Schiffrin EL. Vascular inflammation in hypertension and diabetes: Molecular mechanisms and therapeutic interventions. *Clin Sci (Lond)* 2007;112:375–84. [PubMed: 17324119]
105. Sullivan ME, Miller MA, Bell CR, Jagroop IA, Thompson CS, Khan MA, Morgan RJ, Mikhailidis DP. Fibrinogen, lipoprotein (a) and lipids in patients with erectile dysfunction. A preliminary study. *Int Angiol* 2001;20:195–9. [PubMed: 11573052]
106. Billups KL, Kaiser DR, Kelly AS, Wetterling RA, Tsai MY, Hanson N, Bank AJ. Relation of C-reactive protein and other cardiovascular risk factors to penile vascular disease in men with erectile dysfunction. *Int J Impot Res* 2003;15:231–6. [PubMed: 12934049]
107. Bocchio M, Desideri G, Scarpelli P, Necozione S, Properzi G, Spartera C, Francavilla F, Ferri C, Francavilla S. Endothelial cell activation in men with erectile dysfunction without cardiovascular risk factors and overt vascular damage. *J Urol* 2004;171:1601–4. [PubMed: 15017230]
108. Giugliano F, Esposito K, Di Palo C, Ciotola M, Giugliano G, Marfella R, D’Armiento M, Giugliano D. Erectile dysfunction associates with endothelial dysfunction and raised proinflammatory cytokine levels in obese men. *J Endocrinol Invest* 2004;27:665–9. [PubMed: 15505991]
109. Chiurlia E, D’Amico R, Ratti C, Granata AR, Romagnoli R, Modena MG. Subclinical coronary artery atherosclerosis in patients with erectile dysfunction. *J Am Coll Cardiol* 2005;46:1503–6. [PubMed: 16226175]
110. Esposito K, Giugliano F, Martedì E, Feola G, Marfella R, D’Armiento M, Giugliano D. High proportions of erectile dysfunction in men with the metabolic syndrome. *Diabetes Care* 2005;28:1201–3. [PubMed: 15855589]
111. Eaton CB, Liu YL, Mittleman MA, Miner M, Glasser DB, Rimm EB. A retrospective study of the relationship between biomarkers of atherosclerosis and erectile dysfunction in 988 men. *Int J Impot Res* 2007;19:218–25. [PubMed: 16915303]
112. Vlachopoulos C, Rokkas K, Ioakeimidis N, Stefanadis C. Inflammation, metabolic syndrome, erectile dysfunction, and coronary artery disease: Common links. *Eur Urol* 2007;52:1590–600. [PubMed: 17707576]
113. Johannes CB, Araujo AB, Feldman HA, Derby CA, Kleinman KP, McKinlay JB. Incidence of erectile dysfunction in men 40–69 years old: Longitudinal results from the Massachusetts male aging study. *J Urol* 2000;163:460–3. [PubMed: 10647654]

114. Paolisso G, Rizzo MR, Mazziotti G, Tagliamonte MR, Gambardella A, Rotondi M, Carella C, Giugliano D, Varricchio M, D'Onofrio F. Advancing age and insulin resistance: Role of plasma tumor necrosis factor- α . *Am J Physiol* 1998;275:E294–9. [PubMed: 9688632]
115. Holm T, Aukrust P, Andreassen AK, Ueland T, Brosstad F, Froland SS, Simonsen S, Gullestad L. Peripheral endothelial dysfunction in heart transplant recipients: Possible role of proinflammatory cytokines. *Clin Transplant* 2000;14:218–25. [PubMed: 10831080]
116. Bhagat K, Vallance P. Inflammatory cytokines impair endothelium-dependent dilatation in human veins in vivo. *Circulation* 1997;96:3042–7. [PubMed: 9386173]
117. Karadag F, Ozcan H, Karul AB, Ceylan E, Cildag O. Correlates of erectile dysfunction in moderate-to-severe chronic obstructive pulmonary disease patients. *Respirology* 2007;12:248–53. [PubMed: 17298458]
118. Carneiro FS, Sturgis LC, Giachini FR, Carneiro ZN, Lima VV, Wynne BM, San Martin S, Brands MW, Tostes RC, Webb RC. TNF- α knockout mice have increased corpora cavernosa relaxation. *J Sex Med* 2009;6:115–25. [PubMed: 19170842]
119. Alexander BT, Cockrell KL, Massey MB, Bennett WA, Granger JP. Tumor necrosis factor- α -induced hypertension in pregnant rats results in decreased renal neuronal nitric oxide synthase expression. *Am J Hypertens* 2002;15:170–5. [PubMed: 11863253]
120. Hayward MD, Jones BK, Saparov A, Hain HS, Trillat AC, Bunzel MM, Corona A, Li-Wang B, Strenkowski B, Giordano C, Shen H, Arcamone E, Weidlick J, Vilensky M, Tugusheva M, Felkner RH, Campbell W, Rao Y, Grass DS, Buiakova O. An extensive phenotypic characterization of the hTNF α transgenic mice. *BMC Physiol* 2007;7:13. [PubMed: 18070349]
121. Kifor I, Williams GH, Vickers MA, Sullivan MP, Jodbert P, Dluhy RG. Tissue angiotensin II as a modulator of erectile function. I. Angiotensin peptide content, secretion and effects in the corpus cavernosum. *J Urol* 1997;157:1920–5. [PubMed: 9112563]
122. Schiffrin EL, Touyz RM. From bedside to bench to bedside: Role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension. *Am J Physiol Heart Circ Physiol* 2004;287:H435–46. [PubMed: 15277186]
123. Granchi S, Vannelli GB, Vignozzi L, Crescioli C, Ferruzzi P, Mancina R, Vinci MC, Forti G, Filippi S, Luconi M, Ledda F, Maggi M. Expression and regulation of endothelin-1 and its receptors in human penile smooth muscle cells. *Mol Hum Reprod* 2002;8:1053–64. [PubMed: 12468637]
124. Schiffrin EL. Vascular endothelin in hypertension. *Vascu Pharmacol* 2005;43:19–29. [PubMed: 15955745]
125. Tostes RC, Muscara MN. Endothelin receptor antagonists: Another potential alternative for cardiovascular diseases. *Curr Drug Targets Cardiovasc Haematol Disord* 2005;5:287–301. [PubMed: 16101562]
126. Marsden PA, Brenner BM. Transcriptional regulation of the endothelin-1 gene by TNF- α . *Am J Physiol* 1992;262:C854–61. [PubMed: 1566813]
127. Ferreri NR, Zhao Y, Takizawa H, McGiff JC. Tumor necrosis factor- α -angiotensin interactions and regulation of blood pressure. *J Hypertens* 1997;15:1481–4. [PubMed: 9431855]
128. Luo B, Liu L, Tang L, Zhang J, Ling Y, Fallon MB. ET-1 and TNF- α in HPS: Analysis in prehepatic portal hypertension and biliary and nonbiliary cirrhosis in rats. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G294–303. [PubMed: 14715521]
129. Kagawa T, Takao T, Horino T, Matsumoto R, Inoue K, Morita T, Hashimoto K. Angiotensin II receptor blocker inhibits tumour necrosis factor- α -induced cell damage in human renal proximal tubular epithelial cells. *Nephrology (Carlton)* 2008;13:309–15. [PubMed: 18331441]
130. Elmarakby AA, Quigley JE, Pollock DM, Imig JD. Tumor necrosis factor α blockade increases renal Cyp2c23 expression and slows the progression of renal damage in salt-sensitive hypertension. *Hypertension* 2006;47:557–62. [PubMed: 16415373]
131. Elmarakby AA, Quigley JE, Imig JD, Pollock JS, Pollock DM. TNF- α inhibition reduces renal injury in DOCA-salt hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R76–83. [PubMed: 17989143]

132. Carneiro FS, Giachini FR, Lima VV, Carneiro ZN, Nunes KP, Ergul A, Leite R, Tostes RC, Webb RC. DOCA-salt treatment enhances responses to endothelin-1 in murine corpus cavernosum. *Can J Physiol Pharmacol* 2008;86:320–8. [PubMed: 18516094]
133. Carneiro FS, Nunes KP, Giachini FR, Lima VV, Carneiro ZN, Nogueira EF, Leite R, Ergul A, Rainey WE, Clinton Webb R, Tostes RC. Activation of the ET-1/ETA pathway contributes to erectile dysfunction associated with mineralocorticoid hypertension. *J Sex Med* 2008;5:2793–807. [PubMed: 18823320]
134. Ermertcan AT. Sexual dysfunction in dermatological diseases. *J Eur Acad Dermatol Venereol* 2009;9:985–1117.
135. Blake DJ, Maisiak R, Koplan A, Alarcon GS, Brown S. Sexual dysfunction among patients with arthritis. *Clin Rheumatol* 1988;7:50–60. [PubMed: 3409645]
136. Dincer U, Cakar E, Kiralp MZ, Dursun H. Assessment of sexual dysfunction in male patients with Ankylosing Spondylitis. *Rheumatol Int* 2007;27:561–6. [PubMed: 17094004]
137. Cakar E, Dincer U, Kiralp MZ, Taskaynatan MA, Yasar E, Bayman EO, Ozgul A, Dursun H. Sexual problems in male ankylosing spondylitis patients: Relationship with functionality, disease activity, quality of life, and emotional status. *Clin Rheumatol* 2007;26:1607–13. [PubMed: 17285222]
138. Pirildar T, Muezzinoglu T, Pirildar S. Sexual function in ankylosing spondylitis: A study of 65 men. *J Urol* 2004;171:1598–600. [PubMed: 15017229]

Figure 1.

Illustration demonstrates the sequential events in the activation of TNFR1 and TNFR2 receptors by tumor necrosis factor- α (TNF- α). Usually, TNFR1-associated death domain protein (TRADD) is the first recruited protein after TNFR1 receptor activation, which acts like an anchor of at least three additional mediators: receptor interaction protein 1 (RIP1), TNF-receptor-associated factor 2 (TRAF2), and Fas-associated death domain protein (FADD). TRAF2 stimulates antiapoptotic and inflammatory pathways, whereas FADD stimulates caspases inducing apoptotic and anti-inflammatory effects. TRAF2 is also recruited by TNFR2 receptor with TNF-receptor-associated factor 1 (TRAF1) causing inflammatory and antiapoptotic effects.

Figure 2.

Graph demonstrates simultaneous increase in the levels of tumor necrosis factor-alpha (TNF- α), erectile dysfunction (ED) and coronary artery disease (CAD). Correlation between age and plasma TNF- α levels (straight line). The dashed line illustrates the correlation between the incidence of ED and age. The age range for the first clinical symptoms of CAD is indicated by the shaded area. Adapted results from references [70,75,80-82].

Molecular mechanisms targeted by tumor necrosis factor- α (TNF- α) causing impairment of relaxation in the corpora cavernosa. During sexual arousal or sleep-related tumescence acetylcholine and nitric oxide (NO) are released from parasympathetic and nonadrenergic noncholinergic (NANC) nerves, respectively. Whereas acetylcholine causes NO generation by endothelial nitric oxide synthase (eNOS) activation on endothelial cells, neuronal nitric oxide synthase (nNOS) is responsible for NO production in NANC nerves. Upon its release, NO diffuses locally into adjacent smooth muscle cells of the corpus cavernosum and binds to soluble guanylyl cyclase, which catalyzes the conversion of guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP). TNF- α decreases eNOS and nNOS expression on endothelial cells and NANC nerves, respectively, causing impairment of cavernosal relaxation. Pro-inflammatory status is evoked by TNF- α , which induces adhesion molecules, nuclear factor-kappa B (NF- κ B) and increases leukocyte migration. TNF- α induces changes in extracellular matrix deposition by smooth muscle cells and initiates a fibrotic state with reduced elasticity and compliance, which finally can lead to the impairment of erectile responses.

Figure 4.

Molecular mechanisms activated by tumor necrosis factor- α (TNF- α) which causes hypercontractility of cavernosal smooth muscle cells. Norepinephrine release from sympathetic nerve terminals activates α -adrenergic receptors that lead to the subsequent activation of specific G proteins and phospholipase C (PLC), which causes a rise in the concentration of cytoplasmic Ca^{2+} . Increased intracellular Ca^{2+} then activates myosin light chain (MLC) and triggers smooth muscle contraction and subsequent penile flaccidity. Activation of Ras homolog gene family member A (RhoA) which is facilitated by guanine nucleotide exchange factors (GEFs), stimulates downstream effectors, such as Rho-kinase. Activated Rho-kinase phosphorylates the regulatory subunit of myosin light chain phosphatase and inhibits its activity, thus facilitating smooth muscle cells contraction and flaccidity. TNF- α increases sympathetic- and α -adrenergic agonist-mediated contractions in corpora cavernosa and activates the RhoA/Rho-kinase signaling pathway.