Role of nuclear factor \( \kappa B \) in cardiovascular health and disease

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ABSTRACT

Cardiovascular pathologies are still the primary cause of death worldwide. The molecular mechanisms behind these pathologies have not been fully elucidated. Unravelling them will bring us closer to therapeutic strategies to prevent or treat cardiovascular disease. One of the major transcription factors that has been linked to both cardiovascular health and disease is NF-\( \kappa B \) (nuclear factor \( \kappa B \)). The NF-\( \kappa B \) family controls multiple processes, including immunity, inflammation, cell survival, differentiation and proliferation, and regulates cellular responses to stress, hypoxia, stretch and ischemia. It is therefore not surprising that NF-\( \kappa B \) has been shown to influence numerous cardiovascular diseases including atherosclerosis, myocardial ischemia/reperfusion injury, ischemic preconditioning, vein graft disease, cardiac hypertrophy and heart failure. The function of NF-\( \kappa B \) is largely dictated by the genes that it targets for transcription and varies according to stimulus and cell type. Thus NF-\( \kappa B \) has divergent functions and can protect cardiovascular tissues from injury or contribute to pathogenesis depending on the cellular and physiological context. The present review will focus on recent studies on the function of NF-\( \kappa B \) in the cardiovascular system.

INTRODUCTION

The NF-\( \kappa B \) (nuclear factor \( \kappa B \)) family

The NF-\( \kappa B \) family (Figure 1) consists of five members: p65 (RelA), RelB (RelB), c-Rel (Rel), p105/p50 (NFKB1) and p100/p52 (NFKB2). All members share an N-terminal RHD (Rel-homology domain), which is required for homo- and hetero-dimerization, nuclear translocation, association with inhibitory proteins, such as I\( \kappa B \) (inhibitory \( \kappa B \)), and DNA binding [1]. NF-\( \kappa B \) subunits can bind DNA sequences that contain the consensus motif 5'-GGGRNWYYCC-3' (where R is any...
purine, N is any nucleotide, W is adenine or thymidine and Y is any pyrimidine) [2–4]. In addition, p65, RelB and c-Rel contain a C-terminal TAD (transcription activation domain), which is necessary for transcriptional activation of target genes. p105 and p100 lack this domain and are synthesized as inactive precursors consisting of the RHD and C-terminal ankyrin repeats. The production of p50 and p52 requires removal of the ankyrin repeats, which relies on their modification at lysine residues with a small protein called ubiquitin. Modified versions of p100 or p105 are recognized by the proteasome, which leads to partial proteolytic processing and results in the release of the N-terminal portion of these molecules: p50 and p52 respectively. These mature forms can bind DNA. However, given they lack the TAD, they are incapable of transactivation. In fact, the p50–p50 homodimer can act as a repressor by competing for binding sites [5]. Nevertheless, p50 and p52 can positively regulate transcription by forming heterodimers with other molecules such as p65, RelB or c-Rel, or as homo- or hetero-dimers complexed with Bcl-3.

In unstimulated cells, NF-κB dimers are inactivated by binding to IκBα, which sequester NF-κB in the cytoplasm by masking the nuclear localization sequence. There are eight known IκBs: IκBα, IκBβ, IκBγ, IκBε, IκBζ, Bcl-3, p100 and p105, which bind to NF-κB via ankyrin repeats [6].

**Signalling to NF-κB**

NF-κB is activated by several signalling pathways that cause it to be released from IκB (usually by ubiquitination and degradation of IκB), allowing NF-κB to translocate to the nucleus. These pathways are classified as canonical (classical) and non-canonical (alternative) (Figure 2). In the non-canonical pathway, the IKK (IκB kinase) complex (a homodimer of IKKa [7]) is phosphorylated by NIK (NF-κB-inducing kinase) which is activated upon the degradation of its negative regulators TRAF [TNF (tumour necrosis factor)-receptor-associated factor] 2, TRAF3, c-IAP (cellular inhibitor of apoptosis protein) 1 and c-IAP2 [8] in response to LT-β (lymphotoxin-β), BAFF (B-cell-activation factor), CD40 or RANKL (receptor activator of activation of NF-κB ligand). Activation of the IKK complex leads to proteosomal processing of the RelB–p100 heterodimer to RelB–p52, which then translocates to the nucleus and regulates a specific subset of target genes [6]. In the canonical pathway the IKK dimer (a IKKa–IKKβ heterodimer) is activated by signalling through receptors, such as TNFR (TNFα receptor) 1, IL-1R [IL (interleukin)-1 receptor], TLRs (Toll-like receptors) and other pro-inflammatory receptors, in response to their cognate ligands, including cytokines, viral, yeast and microbial products [1]. Engagement of IL-1R and TLRs leads to the recruitment of signal adaptors, including MyD88 (myeloid differentiation primary response gene 88), TOLLIP (Toll-interacting protein) and IRAKs (IL-1R-associated kinases), resulting in the activation of TRAF6. TRAF6 contains a RING finger that functions as an E3 ligase that can catalyse auto-ubiquitination. Engagement of TNFR1 leads to the recruitment of other signalling intermediaries, including TRADD (TNF-associated death domain), TRAF2 and RIP (receptor-interacting protein). TRAF2, like TRAF6, contains a RING finger E3 ligase which drives polyubiquitination of RIP [9]. The ubiquitin chains on RIP and TRAF6 may act as a scaffold for binding of the pro-inflammatory kinases TAK1 [TGF (transforming growth factor)-β-activated kinase], complexed with the adaptor protein TAB2 (TAK1-binding protein 2), and IKK [10]. This allows TAK1 to interact with the polyubiquitin chain and IKK to complex with NEMO.
NF-κB in cardiovascular health and disease 595

Figure 2 NF-κB signalling

The left-hand side shows the canonical signalling pathway. IL-1R and TLR recruit signal adaptors (including MyD88, TOLLIP and IRAKs) leading to the modification TRAF6 with Lys63-linked polyubiquitin chains (Ub). Signal transduction through TNFRs relies on the recruitment of signalling intermediaries (including TRAF2) which leads to Lys63-linked polyubiquitination of RIP. The ubiquitin chains act as a scaffold for oligomerization of TAB2–TAK1 and IKK–NEMO complexes. This may create an optimal configuration for TAK1-mediated phosphorylation of IKK or neighbouring TAK1 molecules. Finally, the activated IKK complex phosphorylates IκBα which is subsequently modified with Lys48-linked polyubiquitin chains and then transported to the proteasome where it is degraded, thus liberating NF-κB for nuclear entry.

NF-κB activation is regulated by multiple negative feedback mechanisms, including NF-κB-dependent IκBα expression. Newly synthesized IκBα removes NF-κB from the nucleus, thereby terminating NF-κB activation. The deubiquitinating enzymes A20, CYLD and cezanne block IKK activation by removing polyubiquitin chains from upstream intermediaries and are therefore negative regulators of NF-κB. The right-hand side shows the non-canonical pathway. A homodimer of IKKα is phosphorylated by NIK which is activated upon the degradation of its negative regulators c-IAP, TRAF2 and TRAF3, which occurs in response to LT-β, BAFF, CD40 or RANKL receptor activation. Activation of the IKK complex leads to proteasomal processing of NF-κB, which then translocates to the nucleus to regulate target genes.

The circled P indicates phosphorylation events.

(NF-κB essential modulator). TAK1 is a MAPKKK [MAPK (mitogen-activated protein kinase) kinase kinase] that can activate IKK through phosphorylation. This process may position TAK1 in an optimal configuration for phosphorylation of IKK.

The canonical NF-κB pathway can be activated by other stimuli, including hypoxia [11–13], ROS (reactive oxygen species) [14], for instance induced by G-protein-coupled receptor agonists [15], and mechanical forces [16]; however, the signalling pathways that underlie these responses are relatively poorly characterized.

Negative regulation of NF-κB

NF-κB activation is tightly regulated by negative feedback loops which control the duration of NF-κB nuclear localization in response to a stimulus. NF-κB dynamics are important because they influence the magnitude and specificity of target gene expression [17,18]. Negative feedback mechanisms include NF-κB-dependent induction of IκBα, A20 [19–21] and cezanne [22,23]. IκBα, which is induced at a transcriptional level by NF-κB, has a nuclear export sequence enabling it to remove NF-κB from the nucleus, thereby terminating NF-κB activation [24]. This generates a feedback loop which results in oscillations of NF-κB between the nucleus and cytoplasm. Studies have revealed that the deubiquitinating enzymes CYLD (cylindromatosis gene), A20 and cezanne [25,26] are critical negative regulators of NF-κB. They are induced by pro-inflammatory signalling and can block IKK activation by removing polyubiquitin chains from upstream intermediaries [22,27–29]. This dampens
NF-κB oscillations by stabilizing re-synthesized IκBα for NF-κB inactivation.

**Transcriptional targets of NF-κB in cardiovascular cells**

NF-κB regulates many processes in the cardiovascular system, including inflammation, cell survival, differentiation and proliferation. Pro-inflammatory cytokines and microbial products induce NF-κB signalling, resulting in transcriptional regulation of pro-inflammatory genes, including cytokines, chemokines and adhesion molecules (Table 1), which promote the inflammatory process. NF-κB also controls cell viability through transcriptional activation of inhibitors of apoptosis (Table 1). This explains the observation that IKK−/− and NF-κB p65−/− mice die in utero due to excessive hepatocyte apoptosis [30,31], and the finding that TNFR1 signalling leads to apoptosis in the absence of NF-κB activation [32].

**NF-κB AND CARDIOVASCULAR DISEASE**

The effect of the NF-κB on cell survival/apoptosis may have consequences for the development of the cardiovascular system. Studies of transgenic mice with cardiac-specific expression of a stabilized form of IκBα (to block NF-κB activation) did not result in congenital heart defects [33]. In contrast, chemical inhibition of NF-κB activation in chicken embryos did affect cardiac development. Inhibition of NF-κB activation by the BAY 11–7085 inhibitor during a crucial stage of heart development resulted in defects in the cardiac outflow tract at 6 days after administration of the inhibitor [34]. However, which particular NF-κB subunit is involved in cardiac outflow tract development is unclear. Moreover, genetic studies using organ- or cell-specific knockouts are now required to substantiate observations made using the pharmacological NF-κB inhibitor and to further study the effect of NF-κB on heart development.

In the adult, NF-κB can have both beneficial and detrimental roles in the cardiovascular system. In certain disease settings the NF-κB pathway is over- or under-stimulated, leading to alterations in signalling and consequently gene expression, contributing to disease pathology. The non-canonical pathway has mainly been studied in lymphocytes and information on the role in other cell types is lacking; therefore we have focussed on the involvement of the canonical pathway in cardiovascular health and disease.

**Atherosclerosis**

Atherosclerosis is a chronic lipid-driven inflammatory disease characterized by accumulation of lipids in arterial walls which can lead to a heart attack or stroke [35,36]. The importance of the role of NF-κB-regulated inflammation in atherosclerosis is evident because pharmacological inhibition or genetic knockdown of the pro-inflammatory proteins ICAM-1 (intracellular adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1), selectins, MCP-1 (monocyte chemoattractant protein-1), TNFα, IL-1β and/or their receptors leads to a reduction in lesion size, macrophage infiltration and disease progression in experimental models of atherosclerosis [37–48]. The NF-κB pathway is activated in most cell types involved in the different stages of lesion development, indicating that NF-κB might be involved in vascular inflammation and the initiation and progression of atherosclerotic lesion formation [1,49,50].

**Initiation**

An important event in the initiation of atherosclerosis is the recruitment of leucocytes from the circulation to the vessel wall for subsequent migration into the subendothelial layer. This process is termed the leucocyte adhesion cascade. Quiescent inactive ECs (endothelial cells) resist leucocyte adhesion; however, ECs can become activated in response to various stimuli and express adhesion proteins, chemokines and other pro-inflammatory proteins to facilitate leucocyte adhesion [51,52]. This process involves activation of NF-κB in
ECs in response to pro-atherogenic molecules, including TNFα, IL-1, CD40, cytokines, bacterial and viral infections [53], oxLDLs (oxidized low-density lipoproteins) [54], ROS [23,55] and AGEs (advanced glycation end-products) [56]. These molecules activate NF-κB via distinct signalling pathways that converge to activate IKK. In addition, EC dysfunction in response to oxLDL, ROS and pro-atherogenic agents can activate NF-κB indirectly through uncoupling of eNOS (endothelial NO synthase) with NO production, thus reducing production of NO which is a negative regulator of NF-κB [57–61]. Mechanical forces can also influence vascular inflammation by regulating NF-κB expression and activity in ECs. For example, cultured HAECs (human aortic ECs) exposed to pro-atherogenic mechanical conditions (low shear stress) show a significant increase in NF-κB (p50 and p65) activation compared with HAECs exposed to anti-atherogenic conditions (high shear stress) [62]. Similarly, NF-κB activity in ECs is elevated at regions of the arterial tree that are exposed to disturbed blood flow and are susceptible to atherosclerosis compared with athero-protected regions exposed to steady flow [16]. In addition, NF-κB activation in ECs has been associated with hypertension [63], which is one of the major risk factors for atherosclerosis. Indeed, AngII (angiotensin II), an important mediator of the RAAS (renin–angiotensin–aldosterone system), which is associated with hypertension [63], which is one of the major risk factors for atherosclerosis. Indeed, AngII (angiotensin II), an important mediator of the RAAS (renin–angiotensin–aldosterone system), which is elevated in hypertension, can activate NF-κB by inducing intracellular ROS [64–66].

NF-κB signalling in ECs leads to expression of genes that induce recruitment of inflammatory cells to the vessel wall, including ICAM-1, VCAM-1, P- and E-selectins, cytokines (e.g. TNFα, IL-1, IL-6 and IL-8), chemokines (e.g. MCP-1), growth factors and MMPs (matrix metalloproteinases) [1,37,49]. Endothelium-specific inhibition of NF-κB p65 activation by ablation of NEMO or overexpression of dominant-negative IkBα leads to the suppression of adhesion molecule expression on ECs, impaired macrophage recruitment to atherosclerotic plaques, and decreased expression of cytokines and chemokines in the aorta of ApoE (apolipoprotein E)-null mice [50]. Lesion progression in mice is strongly increased by circulating activated platelets [67]. Interaction between these platelets and the endothelium results in an NF-κB-dependent increase in ICAM-1 [68], MCP-1 [69] and E-selectin [70]. These results indicate that NF-κB activation in ECs plays an essential role in vascular inflammation and atherosclerosis by regulating the expression of pro-inflammatory molecules. In addition to the regulation of inflammatory activation, NF-κB activation may also enhance EC viability by inducing anti-apoptotic genes [71]. These results raise the possibility that therapeutic targeting of NF-κB in ECs to prevent vascular inflammation may also have adverse effects by sensitizing ECs to apoptosis.

**Progression**

After transmigration to the intima, monocytes derive the morphological characteristics of macrophages [35]. These macrophages express scavenger receptors [72], which internalize modified lipoprotein particles leading to the enrichment of cholesteryl esters in the cytoplasm; this triggers differentiation of macrophages into foam cells (lipid-laden macrophages) [73]. The resulting fatty streaks (early lesions) are characterized by the subendothelial accumulation of lipids, foam cells and T-lymphocytes. More advanced atherosclerotic lesions are defined by the deposition of fibrous tissue. This stage of lesion development is driven by SMCs (smooth muscle cells) which migrate from the media into the intima where they proliferate and synthesize extracellular matrix [74], controlling the progression of a lipid-rich atherosclerotic lesion into a fibrotic plaque [73].

In human aortic atherosclerotic lesions, activated NF-κB (p65) was first detected by Brand et al. [75]. They demonstrated nuclear NF-κB in ECs overlying early lesions, and in SMCs, macrophages and T-cells in more advanced lesions. Bourcier et al. [54] detected active nuclear p65 and p50 in SMCs in human lesions, whereas NF-κB was inactive in SMCs in healthy tissues. In cells isolated from human atherosclerotic tissue active NF-κB p65, p50 and c-Rel were detected, but not p52 and RelB [49]. The consequences of NF-κB activation in plaques are likely to be complex. Activity of the canonical pathway results in up-regulation of pro-inflammatory (TNFα, IL-6 and IL-8) and pro-thrombotic (MMPs and TF (tissue factor)) mediators, which are pro-atherogenic [49]. NF-κB signalling in ECs may promote recruitment and activation of inflammatory cells, whereas NF-κB activity in SMCs led to their proliferation [76]. Interestingly, inhibition of NF-κB activity in macrophages, by IKK2 deletion, in mice with an LDLR (low-density lipoprotein receptor)-null background increased atherosclerosis, suggesting NF-κB activation in macrophages is beneficial [77]. The genetic deletion of IKK2 led to reduced expression of the anti-inflammatory cytokine IL-10, suggesting that NF-κB may exert protective effects in macrophages via IL-10-dependent suppression of inflammation [77]. In contrast, genetic deletion of NF-κB1 in macrophages in the LDLR-null background resulted in a decrease in atherosclerosis. The NF-κB1-deficient macrophages show a reduced uptake of oxLDL but a prolonged production of TNFα in response to a pro-inflammatory lipopolysaccharide stimulus [78].

**Plaque rupture**

Rupture of an atherosclerotic lesion can lead to infarction/ischaemia of the area downstream of the lesion site. Patients with unstable angina, who are at risk of plaque rupture, have high levels of activated NF-κB in their white blood cells [79]. Plaque rupture has been associated with high MMP activity in macrophages.
Excessive MMP production leads to the destruction of the extracellular matrix, which plays an important role in plaque rupture. Several MMPs (MMP1, 3 and 9) proposed to be instrumental in this process are transcriptionally regulated by NF-κB [80], suggesting that NF-κB activation may influence rupture via MMP induction.

**MI (myocardial infarction) and reperfusion injury**

MI occurs following interruption of blood supply to the heart, typically as a result of atherosclerotic plaque rupture in a coronary artery. Reduced perfusion of the myocardium can subsequently lead to regional cardiomyocyte death and loss of function (infarction). Current treatments employed for restoring blood flow in ischaemic myocardium include thrombolytic therapy, PCI (percutaneous coronary intervention) and CAGB (coronary artery bypass graft) surgery [81–83]. Paradoxically, reperfusion can also cause tissue injury by triggering myocardial inflammation and cardiomyocyte apoptosis.

NF-κB is a central regulator of cardiac responses to ischaemia and reperfusion. It is activated by pro-inflammatory cytokines (e.g. TNFα and IL-1) and endogenous ligands for TLRs that are generated in response to ischaemia/reperfusion. The signalling pathways that regulate TNFR and TLR signalling to NF-κB are described above. In addition, ischaemia/reperfusion leads to alterations in oxygen availability that can activate NF-κB. In normoxic conditions, IKKα/β is degraded in response to hydroxylation by an enzyme called PHD1 (prolyl hydroxylase 1) [13]. Ischaemia leads to hypoxia which can suppress PHD1 function, thereby enhancing IKKβ expression for NF-κB activation [13]. Hypoxia also activates NF-κB by phosphorylation of IκBα on tyrosine residues [84]. In addition to these mechanisms, hypoxia also elevates the expression of NF-κB subunits through HIF1 (hypoxia-inducible factor 1)-dependent transcriptional activation of NF-κB subunit genes [85]. Re-oxygenation of hypoxic tissues during reperfusion can also activate NF-κB via induction of ROS. The molecular mechanisms underlying NF-κB activation by ROS operate at multiple levels and include ROS-dependent activation of IKK [86,87] and ASK1 (apoptosis signal-regulating kinase 1, a MAPKKK) proteins [15]. In addition, our laboratory and others have demonstrated that ROS promote NF-κB activation by suppressing the activities of redox-sensitive enzymes that negatively regulate the NF-κB pathway [23,88].

Thus myocardial ischaemia or ischaemia/reperfusion leads to activation of NF-κB in several cell types in the myocardium, which in turn induces pro-inflammatory proteins, including the adhesion proteins ICAM-1 and P-selectin, in coronary ECs, resulting in leucocyte recruitment [89]. This process has been documented in numerous clinical studies. For example, Valen et al. [90] demonstrated NF-κB activation (p50/p65), accompanied by an increase in the expression of ICAM-1, TNFα and IL-1β, upon myocardial ischaemia/reperfusion in human atrial tissue sampled during open heart surgery. As inflammation can lead to tissue injury, it has also been suggested that NF-κB activation during myocardial ischaemia/reperfusion is damaging. This idea is consistent with the observation that blocking NF-κB using pharmacological inhibitors [91,92] or decoy oligonucleotides [93] can reduce MI in animal models. In addition, mice lacking NF-κB1 have less heart failure and lower mortality compared with wild-type mice after MI [94]. However, therapeutic interventions to block NF-κB should be used with caution because NF-κB also exerts protective effects in myocardial ischaemia/reperfusion. Indeed, studies in a murine MI model revealed that activation of NF-κB was essential for the protection of cardiomyocytes from apoptosis via induction of cytoprotective genes including c-IAP1 and Bcl-2 [95]. NF-κB also positively regulates the release of SCF (stem cell factor), which is responsible for homing of (potentially beneficial) cardiac stem cells to the site of injury [92].

**IP (ischaemic preconditioning)**

IP was first described by Murry et al. in 1986 [96] as short periods of coronary occlusion followed by reperfusion that can protect the myocardium from ischaemia in response to prolonged ischaemia. IP has been shown to reduce infarction sizes in all species tested so far, including humans [97,98]. The mechanism underlying IP is uncertain but is believed to involve NF-κB. However, this relationship is complex because NF-κB plays a role in IP and is also a target molecule that is suppressed by IP. For example, several studies revealed that NF-κB activation and inflammation in response to prolonged ischaemia was reduced by IP [99–103]. On the other hand, NF-κB inhibitors can attenuate the cardioprotective effects of IP [99,104]. This apparent paradox may be resolved by considering the negative feedback loops that are induced by NF-κB signalling. Several negative regulators of NF-κB including IκBα and A20 are induced by NF-κB [19–21]. Thus it is plausible that NF-κB activation during IP leads to the induction of negative regulators that subsequently reduce NF-κB activity in response to prolonged ischaemia. In support of this idea, our group recently demonstrated that ischaemia/reperfusion in the kidney leads to the induction of A20, which subsequently suppresses NF-κB dependent inflammation and injury [105]. In addition, A20 has been shown to protect the heart from ischaemia/reperfusion injury [106].

**PCI**

PCIs such as balloon angioplasty or stent placement are commonly performed to restore blood flow in
stenoed arteries. A major complication of PCI is the occurrence of restenosis; a process that involves SMC proliferation and migration from the media into the neointima. Several animal models suggest that NF-κB is involved in the restenosis process. Upon PCI a marked increase in oxidative stress is observed, leading to NF-κB activation in SMCs [106a], which is correlated to a subsequent increase in ICAM-1 expression and SMC proliferation [106b]. These results are consistent with clinical trials which demonstrated a reduced severity of restenosis in coronary arteries that received stents and were transfected with NF-κB decoy oligonucleotides [106c–106e], presumably by reducing SMC proliferation and improving re-endothelialization. Thus animal and clinical studies suggest that NF-κB is a positive regulator of restenosis after angioplasty or stenting.

CPB (cardiopulmonary bypass) and vein grafting
CABG surgery is another procedure to manage ischemic heart disease. In most cases it is carried out with the aid of CPB which provides a motionless bloodless field for the surgeon to operate on and circulates oxygenated blood to the rest of the body while the heart is arrested. CPB is associated with a systemic inflammatory response and the production of inflammatory mediators, such as IL-1, IL-6, IL-8, TNFα and activation of complement. The mechanism of the inflammatory response in CPB is multifactorial, it combines operative trauma, contact activation of circulating blood components by the artificial surface of the bypass circuit, ischaemia/reperfusion injury to major organs and endotoxin release from the gut [107–110]. The consequences of this inflammatory response can include acute lung injury, renal impairment, neurocognitive effects, multiple organ failure and death [108,110–112]. Given the critical role of NF-κB in inflammation, it is not surprising that elevated NF-κB activation has been observed during CPB [113–118]. In addition, corticosteroids can attenuate the pro-inflammatory response in patients undergoing cardiac surgery, possibly by inhibiting NF-κB activity [116–118].

Although vein bypass grafts are commonly used as conduits in cardiac and vascular surgery, [119] their use is complicated by intimal hyperplasia and accelerated atherosclerosis, which cause late failure rates of up to 40% of grafts within 10 years of surgery [120]. The process of vein grafting leads to activation of NF-κB [121], endothelial expression of adhesion molecules [122] and chemokines [123], and to the recruitment of inflammatory cells within 6–24 h following surgery [124–126]. It has been suggested that the inflammatory process contributes to pathogenesis because vein graft disease can be reduced by depletion of macrophages [127] or by genetic deletion of pro-inflammatory genes such as ICAM-1 [128] or TNFR [129]. Several studies have suggested that NF-κB influences vein graft disease. Indeed, treatment using pharmacological inhibitors of NF-κB such as salicylate [130] or administration of NF-κB decoy oligonucleotides [131,132] reduced the recruitment of macrophages and the accumulation of intimal smooth muscle cells in experimental vein grafts. Hypertrophy and heart failure
Prolonged exposure of hearts to physiological stresses such as heightened pressure or ischaemia can initiate cardiac hypertrophy, a process of remodelling that leads to thickening of the heart muscle to increase stroke volumes. Thus it has been suggested that cardiac hypertrophy is an adaptive response to ensure adequate perfusion in stressed hearts, thus maintaining physiological homoeostasis. Physiological cardiac hypertrophy is observed in healthy individuals (e.g. during pregnancy or in response to exercise), but can also precede further remodelling that is associated with cardiomyocyte apoptosis and can lead to cardiac dilation and failure [133].

Several clinical studies have found a strong link between NF-κB activation and heart failure. Failed human hearts contain activated forms of NF-κB, whereas normal hearts do not [134,135], and polymorphisms in the promoter of the NFKB1 gene are a risk factor for dilated cardiomyopathy [136]. Perhaps the most significant findings were obtained from a study of patients that received left ventricular assist devices which demonstrated that reverse remodelling (enhanced function) was associated with a decline in cardiac NF-κB activity [137]. Studies using transgenic or knockout mice have attempted to define a causal relationship between NF-κB and cardiac hypertrophy; however, the results generated are complex and their interpretation uncertain. Several studies demonstrated that genetic deletion of NF-κB1 led to reduced cardiac hypertrophy in response to prolonged exposure to TNFα, AngII [138] or MI [94], suggesting that NF-κB1 is a positive regulator of hypertrophy. However, these studies are difficult to interpret because NF-κB1 was deleted from all cells and the function of NF-κB1 in cardiomyocytes, ECs, leucocytes and other cell types was not studied in isolation. Secondly, NF-κB1 has complex effects on NF-κB activity because it encodes an inhibitor of NF-κB (p105) as well as an active NF-κB subunit (p50; see Figure 1). Finally, a recent study using NF-κB1-null mice suggested that NF-κB1 is a negative regulator of cardiac hypertrophy in response to MI [139]; however, after MI, increased levels of TNFα were observed in these mice, suggesting NF-κB signalling may still be active. The reason for the discrepancy between this and previous reports is uncertain but may be due to differing genetic backgrounds.

A clearer picture has emerged from studies of conditional transgenic and knockout mice in which
NF-κB was targeted specifically in cardiomyocytes. Overexpression of a non-degradable form of IkBα in cardiomyocytes simultaneously reduced cardiac hypertrophy and promoted heart failure in response to MI [140,141], suggesting that NF-κB protects the heart by participating in compensatory remodelling. A similar conclusion was drawn from studies of cardiomyocyte-specific NEMO- [142] and IKKβ- [143] knockouts which were characterized by cardiomyocyte apoptosis and spontaneous development of dilated cardiomyopathy in the absence of cardiac hypertrophy. The phenotype was rescued by the administration of antioxidants, suggesting that NF-κB may protect against cardiomyopathy through the activation of antioxidant genes to reduce apoptosis. This idea is consistent with the observation that NF-κB positively regulates hypertrophy in vivo [144,145] and in vitro [15,146], and induces several anti-apoptotic genes including A20 and Bcl-2 in isolated cardiomyocytes in vitro [147].

It is clear that NF-κB is involved in cardiac hypertrophy and failure, but further studies are required to establish its precise role and to inform therapeutic targeting of NF-κB in these processes. In particular, it should also be recognized that NF-κB is likely to have multiple divergent effects and may promote or suppress pathogenic processes. Further studies of isolated cells in vitro and lineage-specific knockouts should now be carried out to define the transcriptional targets and function of NF-κB in interstitial cells, leucocytes and ECs as well as cardiomyocytes.

CONCLUDING REMARKS

The widespread involvement of NF-κB in cardiovascular disease establishes the pathway as a therapeutic target to more effectively manage cardiovascular disease. Indeed, several clinical therapies used today (e.g. against atherosclerosis) have been shown to affect NF-κB signalling. However, emerging reports reveal that the function of NF-κB and its influence on disease processes varies according to the cell types in which it is activated. For example, in atherosclerosis, NF-κB activity in ECs is pro-atherogenic, whereas NF-κB activity in macrophages can be anti-atherogenic. Therefore the cell-type-specific effects on NF-κB are poorly characterized and future work should focus on conditional knockouts to assess NF-κB function in particular cell types and on genomic approaches to determine the transcriptional targets of NF-κB in cardiovascular cells. Although there are abundant compounds that inhibit NF-κB signalling they lack specificity. Although blocking signalling could be beneficial in certain cardiovascular diseases, maintenance of NF-κB activity is critical for immune and inflammatory responses and homeostasis. Therefore strategies to target NF-κB therapeutically in humans should be handled with great care, focusing on cell-specific-targeted therapies. New information on the molecular details of the NF-κB pathway will hopefully enable the development of more specific inhibitors of NF-κB signalling at the level of upstream receptors or adaptors, which will inhibit the detrimental elements of signalling while preserving the beneficial processes.

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