Cardiovascular disease is the leading cause of morbidity and mortality in the United States. In most instances, heart failure is the final consequence of many underlying disease etiologies such as long-standing hypertension, coronary heart disease, valvular insufficiency, arrhythmia, viral myocarditis, and mutations in sarcomere-encoding genes. A compensatory enlargement of the myocardium, or hypertrophy, typically accompanies many of the predisposing insults discussed above and is a leading predictor for the development of more serious and life-threatening disease.2,3 Given the strong association between the presentation of pathological cardiac hypertrophy and a progressive deterioration in myocardial function, investigators have focused on understanding the molecular mechanisms that initiate the compensatory growth response or that precipitate the transition to heart failure. A number of signal transduction pathways are thought to play pivotal roles in mediating the hypertrophic growth of the myocardium in response to pathological stimuli.4 In this issue of Circulation, Cook et al5 focus their attention on the nuclear factor (NF)-κB signaling pathway as a potentially important intracellular mediator of cardiac hypertrophy.

NF-κB is a transcription factor that can directly regulate the expression of immediate-early genes and genes involved in the stress response following a variety of physiological or pathological stimuli. Traditionally, the NF-κB pathway has been implicated as a pivotal intracellular mediator of the inflammatory response associated with septic shock, ischemia-reperfusion injury, acute respiratory distress syndrome, viral-induced cytotoxic effects, and cytokine-mediated chronic disease states.5 These disease associations have made NF-κB an attractive target for pharmacological inhibition with the goal of preserving end organ function following acute injury or resulting from chronic inflammatory disorders. Recent studies by several investigators have also implicated NF-κB activation as a causal event in the cardiac hypertrophic response, as modeled in cultured cardiac myocytes.6–9 This linkage between NF-κB and myocyte hypertrophy is especially interesting given the long-standing hypothesis that inflammatory cytokines (eg, tumor necrosis factor [TNF]–α, interleukin [IL]–1) are relevant mediators of cardiomyopathic disease states and that NF-κB itself is dominantly regulated by the cytokine TNF-α.10 For example, heart failure patients typically show elevated levels of circulating TNF-α in the blood10 and have significant myocardial NF-κB activation.10,11 Another important consideration is that NF-κB inhibition or activation can sensitize cardiac myocytes to apoptosis depending on the nature of the stimulus. This is especially relevant given the hypothesis that cardiac myocyte apoptosis can exacerbate or even initiate the phenotypic manifestations of heart failure.12

NF-κB transcription factors are homo- or heterodimers that translocate to the nucleus and bind DNA through a Rel-homology domain. There are 5 mammalian members of the NF-κB/Rel family that have been identified: NFκB1 (p50 and its precursor p105), NFκB2 (p52 and its precursor p100), c-Rel, RelA (p65), and RelB. In most resting cells, NF-κB is a p50/RelA dimer that is retained in the cytoplasm bound to IκBα, which function as cytoplasmic inhibitors of NF-κB.5,13 On stimulation, IκB becomes phosphorylated on specific serine residues through the action of the IKK complex, which comprises IKKα and IKKβ, and a 48-kDa regulatory protein termed IKKγ.13 Phosphorylation of IκB triggers its ubiquitination and degradation by the proteosome, which permits NF-κB translocation to the nucleus where it activates transcription of inflammatory and immune response target genes (eg, TNF-α, IκBα, angiotensinogen, IL-2, and IL-8).13 NF-κB signaling is also critically involved in the apoptotic response of a cell to stress or inflammatory stimuli. In fact, a large number of both pro- and antiapoptotic gene products are directly regulated by NF-κB. These include cellular inhibitors of apoptosis (c-IAPs), antiapoptotic Bcl2 family members (A1, Bcl-xl), and the FLICE inhibitory protein (FLIP).14 Many of these proteins can themselves directly influence the NF-κB pathway or block the apoptosis signaling cascade. Alternatively, NF-κB upregulates the expression/activity of proapoptotic genes such as Fas, FasL, caspase 8, caspase 11, and TNF-α.11 NF-κB can also regulate the expression of signaling accessory proteins such as A20. Interestingly, A20 is directly upregulated by NF-κB itself,15 where it functions to then suppress NF-κB by interfering with the upstream signaling events that normally promote activation.16

In this issue, Cook et al5 set out to investigate the importance of NF-κB signaling in the heart as a potential mediator of hypertrophy and/or apoptosis through a manipulation of the NF-κB inhibitory protein A20. Interestingly, they observed an increase in A20 mRNA after 3 hours of...
pressure overload to the heart, which returned to near-basal levels by 6 hours. They also observed a decrease in IκB-α protein levels after pressure overload that was normalized by 6 hours, potentially corresponding to an attenuation of NF-κB signaling through A20 upregulation at this later time point. To examine the mechanistic implications of A20 expression, Cook et al.17 infected cultured cardiomyocytes with a recombinant adenovirus expressing this inhibitory protein. The authors found that A20 inhibited TNF-α–induced transcription of NF-κB target genes, ICAM1 and VCAM1, similar to the use of a dominant-negative IKKβ-encoding adenovirus.

More importantly, inhibition of NF-κB through A20 overexpression inhibited the hypertrophic response of cultured neonatal cardiac myocytes after agonist stimulation. This result is also consistent with as many as 4 other studies in which NF-κB inhibition was shown to attenuate or block the hypertrophic response of cultured cardiac myocytes. For example, TNF-α, parameters of hypertrophy induced by angiotensin II, endothelin-1, phenylephrine, and myo- trophin were all inhibited through overexpression of a degradation-resistant mutant of IκBα.6–9 Collectively, these data make a convincing case for NF-κB as a necessary mediator of the cardiac growth response in culture. In contrast, De Keulenaer et al.17 recently reported that overexpression of iex-1, an NF-κB–dependent target gene that is normally induced by NF-κB signaling, reduces strain-phenylephrine-, and endothelin-1–mediated myocyte hypertrophy in culture. This result indirectly suggests that NF-κB signaling also has an anti hypertrophic regulatory component, although the dominance of the proposed iex-1 inductive mechanism is uncertain, especially given the convincing data set discussed above in which primary inhibition of NF-κB is itself anti hypertrophic.

Although most in vitro studies now support the contention that NF-κB inhibition represents a desirable therapeutic approach for antagonizing cardiac hypertrophy, especially of an inflammatory etiology, a significant complication requires discussion. NF-κB activation is a double-edged sword in that it can mediate both anti- and proapoptotic effects. In this manner, NF-κB inhibition might either predispose or protect the myocardium to apoptosis depending on the nature of the stimulus. For example, studies conducted in neonatal rat cardiac myocytes have shown that NF-κB inhibition promoted apoptosis after TNF-α treatment or hypoxia/reoxygenation stimulation.18,19 Cook et al.14 also documented that overexpression of dominant-negative IKKβ promoted apoptosis of cultured cardiac myocytes after serum deprivation or ischemia/reoxygenation. These results suggest that NF-κB is normally a pro survival factor in cultured cardiac myocytes. In contrast, Condorelli et al.20 failed to identify a proapoptotic effect associated with NF-κB inhibition after TNF-α stimulation. Furthermore, in vivo transfection of an NF-κB “decoy” oligonucleotide into the rat or pig heart reduced NF-κB activity, which was associated with a reduction in ischemia/reperfusion injury, suggesting that NF-κB is normally proapoptotic and not protective.21,22

The results discussed above not only highlight the complexity associated with NF-κB signaling in the apoptotic response of cardiac myocytes, but they also underscore the potential pitfalls that might underlie a therapeutic strategy aimed at NF-κB. However, in the case of A20, inhibition of NF-κB did not sensitize cardiac myocytes to apoptosis after either serum deprivation or ischemia/reoxygenation. This result suggests that A20 might function in a more selective manner in blocking NF-κB activity, such that prohypo- tropic pathways are significantly antagonized, whereas apoptotic pathways are not induced (or survival pathways are not inhibited). In this manner, a better understanding of how NF-κB couples to various effector pathways in the heart, either through direct transcriptional mediators or otherwise, will undoubtedly implicate novel therapeutic approaches. However, enthusiasm for a therapeutic approach directed against the A20: NF-κB interface should be somewhat guarded until the full scope of A20 biological functions is elucidated. In other words, it is uncertain whether the sole function of A20 is to regulate NF-κB potency.

Cook et al.14 have added critical mechanistic data to our understanding of the role of NF-κB in mediating cardiac hypertrophy. In the future, it will also be important to extend such an approach to the whole animal, as cultured cardiac myocytes have certain limitations. It will be interesting to determine both the hypertrophic and apoptotic responses in NF-κB–inhibited mice, such as in transgenic mice expressing a degradation-resistant form of IκB-α in the heart.23 By comparison, it will also be critical to generate and characterize cardiac-specific A20 expressing transgenic mice, potentially representing a more selective means of blocking NF-κB hypertrophic effects, while sparing apoptotic pathways. It will also be desirable to generate mice that are selectively missing A20 from the heart as a means of understanding its biological importance, especially given the observation that traditionally targeted A20 mice have severe inflammation and die prematurely.24 In any event, there is little doubt that NF-κB represents an attractive target for treating certain forms of heart disease provided that the inhibitory approach is properly directed with the desired specifity.

References


Is Nuclear Factor κB an Attractive Therapeutic Target for Treating Cardiac Hypertrophy?
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Circulation. 2003;108:638-640
doi: 10.1161/01.CIR.0000085362.40608.DD
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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