The E2F family of transcription factors (E2Fs) plays an important role in the regulation of cell proliferation and apoptosis and includes 6 structurally related E2F proteins (E2F1 through 6). E2Fs function as heterodimers with members of the DP family (DP-1 and DP-2) to transactivate or repress gene expression and play important roles in regulating both cell proliferation and antiproliferative processes such as apoptosis and senescence.1 Atherosclerosis represents a defective reparative process in response to repeated injuries to the vessel wall.2 Central to the resulting inflammatory reaction are proinflammatory cytokines, bacterial and viral products, and reactive oxygen intermediates, all of which activate nuclear factor kappa-B (NFkB), which controls the transcription of over 100 genes that encode mediators of innate immune and inflammatory responses.3 Among these induced genes are leukocyte adhesion molecules, metalloproteinases (MMPs), and proinflammatory cytokines, including tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6), which, in turn, can activate NFkB.4 Hence, NFkB not only promotes the recruitment and activation of inflammatory cells, but also serves as a central switch within a positive feedback loop that regulates the expression of proinflammatory factors in the development of atherosclerosis. In addition, NFkB works in concert with other transcription factors, activator protein-1 (AP-1) in particular, to activate the expression of pro-atherosclerotic genes5. In this context, atherosclerosis can be viewed as an inflammatory process that is critically dependent on the transcriptional activation of cytokine genes under the control of NFkB and other transcription factors.

See p 2707

The relevance of NFkB to atherosclerosis is supported by numerous in vitro and in vivo studies. For example, activated NFkB and its regulated inflammatory mediators, such as cytokines, inducible NO synthase, and leukocyte adhesion molecules, have been detected in macrophages, smooth muscle cells, and endothelial cells in human atherosclerotic plaques but not in healthy vessels.6–8 Pathogenically important factors, such as reactive oxygen species involved in low-density lipoprotein oxidation, and components of microorganisms such as Chlamydia pneumoniae, can directly activate NFkB in cells isolated from atherosclerotic plaques.9,10 Furthermore, NFkB serves as a signaling molecule in apoptosis transduction pathways, resulting in increased turnover of vascular cells located in atherosclerotic plaque caps, which then renders such plaques vulnerable to rupture, contributing to the development of unstable coronary syndromes4. Moreover, NFkB has been implicated in the pathophysiology of myocardial ischemia-reperfusion injury, ischemic preconditioning, and heart failure, as well as non-cardiovascular diseases, such as autoimmune arthritis, glomerulonephritis, asthma, lung fibrosis, septic shock, and carcinogenesis.11 Inhibition of NFkB nuclear translocation (and therefore activity), and probably of other transcription factors, should therefore provide a mechanism that inhibits the expression of a battery of proinflammatory genes induced in response to many injurious stimuli. Such inhibition may delay or even prevent the initiation and progression of atherosclerotic lesions and other diseases involving innate and maladaptive immunity.

The NFkB family consists of p50, p52, p65 (RelA), c-Rel, and RelB, which form various homo- and heterodimers. In resting cells, the NFkB dimers reside in the cytoplasm in an inactive form bound to inhibitory proteins known as IκB3,12. To date, 6 IκB proteins have been reported. The well characterized IκBα and IκBβ have 2 N-terminal serine residues that are phosphorylated in response to diverse stimuli. The phosphorylated IκBs then undergo multiquitination and proteolytical degradation, which liberates NFkB. Consequently, NFkB translocates to the nucleus and binds to promoter or enhancer regions of specific genes, initiating or repressing transcription. Thus, phosphorylation of IκBα by a 700- to 900-kDa multimeric complex, referred to as the IκB kinase (IKK) complex, represents a crucial step in NFkB activation12.

The article by Chen et al13 demonstrates that adenovirus-mediated gene transfer of E2F-1 markedly inhibits the phosphorylation of IκBα and reduces NFkB p65 nuclear translocation in response to TNFα in human aortic endothelial cells (HAECs). This study reveals a novel function of E2F-1, the best characterized member of the E2F family of transcription factors, and positions E2F-1 as an inhibitor for NFkB activation. Importantly, these authors showed that E2F-1 overexpression in HAECs counteracted the TNFα-induced endothelial intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin expression and inhibited the adhesion of monocytic U937 cells to HAECs.13 These data provide evidence for an important link between cytokines, E2F1, NFkB, and the proinflammatory transformation of endothelial cells, and suggest that E2F1 functions as an inhibitory regulator for NFkB activation and the ensuing inflammatory response.

Considering its central role in inflammation, several strategies have been developed to antagonize NFkB nuclear import. These include chemical pyrrolidine dithiocarbamate, adenovirus specifying IκBα, inhibitors for upstream NFkB activators, such as tyrosine kinase inhibitors, and chemical inhibitors such as small molecule inhibitors of IκB kinase.

Editorial

E2F1
A Magic Bullet for Atherosclerosis?

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kinase (TK) and protein kinase C (PKC) inhibitors, NFκB decoy oligonucleotides, and a synthetic peptide containing a cell membrane-permeable motif and nuclear sequence, SN 50, to name a few.\textsuperscript{4,12,14} Although these approaches have been successful in inhibiting NFκB nuclear import in response to various stimuli, the consequence of blocking NFκB activation on the development of atherosclerosis turned out to be paradoxical, considering the general expectation of reduced atherosclerotic lesion formation on blocking atherosclerosis turned out to be paradoxical, considering the general expectation of reduced atherosclerotic lesion formation on blocking NFκB activation. For example, Schreyer et al.\textsuperscript{15} demonstrated that C57BL/6 mice lacking TNF receptor p55—a factor thought to play a primary role in activating NFκB and hence in inflammatory processes—had aortic sinus lesions 2.3-fold larger than C57BL/6 wild-type mice when fed an atherogenic diet. Furthermore, they found that the uptake and degradation of acetylated low-density lipoprotein was increased by 3-fold in cultured peritoneal macrophages isolated from p55-null mice versus wild-type mice. These paradoxical findings are likely due to the multifaceted effects of NFκB in vascular cells, with regards to cell survival and apoptosis in particular.

Indeed, NFκB has been implicated in both promoting and inhibiting apoptosis. In endotoxinemia, acute and overwhelming NFκB activation leads to widespread endothelial cell death with permeability disturbances and disseminated coagulation.\textsuperscript{16} TNFα-induced apoptosis is paralleled by increased NFκB activation.\textsuperscript{4} On the other hand, inhibition of NFκB increased, rather than reduced, TNFα-induced cell death in multiple cell types.\textsuperscript{17} Such a protective role of NFκB was also observed in p65/RelA knockout mice, which died embryonally from extensive liver apoptosis.\textsuperscript{18} Hence, it is apparent that NFκB exerts dual effects on the regulation of cell viability, and such dual effects stress the need for ways to manipulate NFκB activity that would selectively induce cell death in one cell type, while protect cells from undergoing apoptosis in another cell type in the same organ to achieve maximal therapeutic efficacy with minimal side effects. In the case of atherosclerosis and related conditions, preservation of the endothelium with simultaneous induction of smooth muscle cell apoptosis may be beneficial by promoting re-endothelialization and reducing platelet adhesion and thrombus formation while suppressing smooth muscle cell proliferation in the intima. Remarkably, E2F-1 itself has been shown to function as both an oncogene and a tumor suppressor,\textsuperscript{20} by promoting re-endothelialization and reducing platelet adhesion: potential anti-inflammatory activity of the transcription factor, E2F-1. Mouse embryos null for both Rb-\textsuperscript{1/2} and E2F-1 display much reduced apoptosis. Interestingly, in these mouse embryos, there is tissue specificity, with cell proliferation/tumor incidence increasing within certain tissues and cell apoptosis/tumor incidence decreasing in others in the absence of E2F-1, suggesting that E2F-1 serves as either an oncogene/apoptosis inhibitor or tumor suppressor/apoptosis promoter depending on the tissue/cell context.\textsuperscript{20} Instructively, overexpression of E2F-1 in coronary vascular smooth muscle cells induces apoptosis, whereas E2F-1 overexpression exerts a survival effect in proliferating endothelial cells and restores cell-cycle progression of such cells. These observations, together with the findings by Chen et al.\textsuperscript{13} support the theory that E2F-1 could serve as a “magic bullet” that exerts multiple beneficial effects in the vasculature, including inhibition of endothelial cell apoptosis, inhibition of smooth muscle cell proliferation, and inhibition of inflammatory cytokine and adhesion molecule expression, all of which prevent the development of atherosclerosis. Although further work is needed to dissect the pathways and mechanisms underlying the differential effects of E2F-1 in these different cellular activities with regard to the involvement of NFκB or other transcription factors, as well as to examine the in vivo anti-atherogenic effects of E2F-1 overexpression, the intriguing discovery presented in this article\textsuperscript{13} regarding the anti-inflammatory role of E2F-1 in relation to NFκB activation certainly gives pause for thought.

References


Key Words: Editorials – atherosclerosis – inflammation – signal transduction
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Circulation. 2002;106:2640-2641
doi: 10.1161/01.CIR.0000043247.87843.AA
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
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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