Nuclear Factor κB Signaling in Atherogenesis
Menno P.J. de Winther, Edwin Kanters, Georg Kraal, Marten H. Hofker

Abstract—Atherosclerosis is an inflammatory disease, characterized by the accumulation of macrophage-derived foam cells in the vessel wall and accompanied by the production of a wide range of chemokines, cytokines, and growth factors. These factors regulate the turnover and differentiation of migrating and resident cells, eventually influencing plaque development. One of the key regulators of inflammation is the transcription factor nuclear factor κB (NF-κB), which, for a long time, has been regarded as a proatherogenic factor, mainly because of its regulation of many of the proinflammatory genes linked to atherosclerosis. NF-κB may play an important role in guarding the delicate balance of the atherosclerotic process as a direct regulator of proinflammatory and anti-inflammatory genes and as a regulator of cell survival and proliferation. Here we address recent literature on the function of NF-κB in inflammatory responses and its relation to atherosclerosis. (Arterioscler Thromb Vasc Biol. 2005;25:904-914.)

Key Words: atherosclerosis • signal transduction • immune system • macrophages

Atherosclerosis is a slowly progressing inflammatory disease of the medium- and large-sized arteries, resulting in the formation of fatty and fibrous lesions in the vessel wall. It is the major cause of cardiovascular diseases and is responsible for a large proportion of mortality in our Western society.

Inflammatory processes mark all stages of atherogenesis: from early endothelial activation by modified lipids to eventual rupture of the atherosclerotic plaque. In general, an inflammatory response is a normal physiological reaction to daily encountered micro-organisms, tissue damage, and modified self-proteins in an attempt of the body to restore and maintain homeostasis. Defense mechanisms will kill and clear most of the harmful substances within hours by activating the innate immune system. Additionally, the adaptive immune system may be activated, and an antigen-specific response will be mounted. The innate and the adaptive immune response, although often viewed as 2 separate systems, are intertwined and will greatly affect each other. It has become clear from many studies that both aspects of the immune system play a major role in atherosclerotic lesion development.

The initial inflammatory response in atherosclerosis may well be considered an attempt to resolve the potentially harmful situation caused by modified low-density lipoprotein (LDL). However, by still unknown mechanisms, this inflammatory response in the lesion will become a chronic inflammatory condition that remains unresolved, leading to progression of the atherosclerotic plaque.

As a major transcription factor in inflammatory responses, nuclear factor κB (NF-κB) is involved in the regulation of inflammatory and immune genes, apoptosis, and cell proliferation. The number of genes described to be regulated by NF-κB is >160, whereas the number of factors shown to induce the NF-κB activation pathway is even higher (for a recent update on these genes, visit http://people.bu.edu/gilmore/nf-κb/). Many of these NF-κB inducers and NF-κB-regulated genes have been implicated directly or indirectly in atherosclerosis. In this review, we discuss the transcription factor NF-κB and its potential role in atherosclerosis. It would be too elaborate to discuss all NF-κB–affected pathways and their role in atherosclerosis. We focus on the major atherosclerotic factors regulated by NF-κB and how they may affect different steps in the atherosclerotic process. Moreover, we discuss recent advances in understanding several signaling pathways to NF-κB using mouse models.

NF-κB Signaling Pathway
The transcriptional response in innate and adaptive immunity is dependent on the activation of various transcription factors. A crucial transcription factor is NF-κB, which controls the transcription of many genes with an established role in atherosclerosis, such as cytokines, chemokines, adhesion molecules, acute phase proteins, regulators of apoptosis, and cell proliferation. NF-κB was discovered in 1986 in the laboratory of David Baltimore and has from that time been the subject of extensive research. Many excellent reviews on NF-κB have been published for further reading.

NF-κB is the general name for a family of transcription factors consisting of 5 members: p65 (RelA), c-Rel, RelB, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100). All members share a Rel homology domain, mediating dimerization, association with inhibitory...
proteins, and DNA binding, whereas only the first 3 contain a transcriptional activation domain. The proteins can form different complexes of either homodimers or heterodimers. The most abundant complex, often referred to as being “NF-κB,” is p65/p50. In resting situation, the NF-κB dimer is kept inactive by an inhibitory protein: inhibitor of κB (IκB). Several isoforms of IκB exist, with IκBα being the most predominant and best studied. Association with IκB keeps NF-κB predominantly sequestered in the cytoplasm. This predominant presence in the cytoplasm in unstimulated cells is the resulting equilibrium from actions of nuclear localization signals on p50 and p65 and nuclear export signals on IκB. The classical activation of the canonical NF-κB pathway (Figure 1) can be initiated by a wide range of extracellular stimuli, including cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), but also viral products, bacterial components, and yeast products signaling through different Toll-like receptors (TLRs). These agents can activate the cells through their respective receptors, resulting in the activation of different signal transduction cascades, which will eventually activate the IκB kinase (IKK) complex. This complex will mediate the phosphorylation of IκB, resulting in its ubiquitination and degradation, leaving the NF-κB dimer free to translocate to the nucleus, where it can activate specific target genes through selective binding to the NF-κB consensus sequence GGGRNYYCC (R = purine; Y = pyrimidine). In such a way, NF-κB can regulate the transcription of many genes, of which cytokines, chemokines, adhesion molecules, and antiapoptotic genes are only a small portion.

The phosphorylation of IκB by the IKK complex is an essential step in the activation of the canonical NF-κB activation cascade. The IKK complex is composed of 3 subunits: IKK1 (IKKα), IKK2 (IKKβ), and NF-κB essential modulator (NEMO; IKKγ). IKK1 and IKK2 have kinase activity, although IKK2 is probably the most dominant kinase involved in phosphorylation of IκB, whereas IKK1 may show partial redundancy for the activation of the classical NF-κB pathway. Although NEMO does not have kinase activity, it is necessary for stimulus-mediated NF-κB activation.

More recently, IKK1 has been shown to be indispensable in an alternative NF-κB pathway (Figure 1). This pathway regulates the ubiquitin-mediated processing of p100 and is crucial for modulating the levels of relB-p52 heterodimers. In contrast to the classical p65-p50 dimers, relB-p52 dimers do not associate with IκB proteins but are kept in the cytoplasm as a relB-p100 dimer. The processing of p100 to p52 in this alternative NF-κB pathway is dependent on signaling through NF-κB–inducing kinase and IKK1, and the resulting relB-p52 dimers can transfer to the nucleus, where they mediate transcription of NF-κB–dependent genes. Several signals have been shown to activate the alternative pathway, including lymphotixin-β (LTβ), B-cell–activating factor, CD40 ligand, and lipopolysaccharide (LPS). Some of these factors can induce the classical and the nonclassical pathway, as was shown for LTβ, CD40 ligand, and LPS, and it is now thought that the 2 NF-κB pathways are activated in a sequential manner: the early response mediated by p65 containing dimers is later continued by a response with relB containing complexes to sustain the NF-κB activation.

As expected, the transcription factor NF-κB is also subjected to multilevel sophisticated control mechanisms. One of these regulatory mechanisms involves the auto feedback regulation of NF-κB by IκB. The rapid transcription of the inhibitory protein IκB on NF-κB activation is important in deactivating the activated transcription factor and thereby mediates the termination of NF-κB activation. Regulation of NF-κB also occurs in the nucleus, where phosphorylation, acetylation, and complexing with other factors regulate the transcriptional activity of the NF-κB dimer. The different heterodimers and homodimers may also affect transcription attributable to the different affinities to promoter regions affecting the transcriptional response.

Other transcription factors involved in atherogenesis, such as the peroxisome proliferator-activated receptors (PPARs), have also been shown to affect the NF-κB signaling pathway. PPARα has strong anti-inflammatory properties. It was shown to inhibit the expression of several NF-κB–dependent inflammatory genes through inhibition of NF-κB activation. The inhibitory effect of PPARα is mainly attributed to increased levels of IκBα. Moreover, cross-talk between NF-κB and PPARα was also shown because p65 overexpression was shown to inhibit PPAR-dependent expression.

Figure 1. NF-κB activation. Two NF-κB activation cascades can be discriminated. The classical NF-κB activation pathway (left) involves the activation of the IKK complex with the subsequent degradation of IκBα and nuclear translocation of the NF-κB dimer. The alternative NF-κB activation cascade (right) is mediated through IKK1 and results in the processing of p100 to p52, resulting in the nuclear transfer of the relB-p52 dimer. Ub indicates ubiquitination.

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Cooperation of NF-κB with other inflammatory transcription factors has also been demonstrated. The induction of C-reactive protein (CRP) by IL-1 or IL-6 was shown to be dependent on the interaction between p50 and CCAAT/enhancer binding proteins (C/EBP)-β.29,30 p50 enhanced and stabilized the binding of C/EBPβ to the CRP promoter and p50 and C/EBPβ binding sites in the promoter were necessary for full induction of the gene.29 For cyclooxygenase-2 (COX2), it was also shown that in macrophages, NF-κB and C/EBPβ collaborate for full and sustained induction of the gene.31 Moreover, it was shown recently that synergistic interaction between p65 and C/EBPβ is needed to activate the promoter of the human Mediterranean fever gene, a myeloid-specific TNF-induced inflammatory factor with so far unknown physiological function.32

It is clear that the eventual transcriptional response is not just simply the result of a single activated transcription factor. Many pathways are intertwined in orchestrating the transcriptional response, even when cells are activated with a single stimulus. Moreover, cells are often not subjected to a single stimulus but subjected to a range of factors acting on the cell. Understanding how these regulatory networks interact and what role NF-κB plays in these networks may provide us with more insight to fine tune the inflammatory response to the benefit of patients experiencing inflammatory-related diseases.

Atherogenic Properties of NF-κB

**Target Genes**

The support suggesting a role for the NF-κB system in atherosclerosis is based mainly on descriptive studies. Several years ago, activated NF-κB was demonstrated in human atherosclerotic lesions.33 Using an antibody specifically recognizing activated phosphorylated p65, NF-κB activation was shown in smooth muscle cells, macrophages, and endothelial cells. Moreover, hypercholesterolemia was shown to induce activated NF-κB in the vessel wall in a pig model for atherosclerosis.34 Using mice, Hajra et al showed that there was a colocalization of regions prone to develop atherosclerosis and increased levels of components of the NF-κB system,35 also indicative for a role of NF-κB in atherosclerosis.

NF-κB may not only contribute to the different stages in atherosclerosis development, it is also likely that its contribution will be cell type–dependent, leading to differential regulation of different genes (eg, in endothelial cells versus macrophages). We discuss how NF-κB may contribute to different steps in the atherosclerotic process (Figure 2).

**Initiation of Atherosclerosis**

Although direct evidence is lacking, NF-κB may be a crucial factor in the initiation of atherogenesis. The first steps in atherosclerosis are considered to be the modification of LDL in the vessel wall, leading to a local inflammation and resulting in the release of chemotactic factors and expression of adhesion molecules on the surface of endothelial cells. These processes lead to the attraction of monocytes to the site where the lesion will develop. NF-κB can be implicated in several of these steps. First, several enzymes demonstrated to be important in the early modification of LDL and the formation of inflammatory lipid mediators, including group IIa secretory phospholipase A2,36 5-lipoxygenase and 12-lipoxygenase,37 and COX238 are regulated by NF-κB. Second, monocyte chemoattractant protein-1 (MCP-1), which is a crucial chemokine for the attraction of monocytes in early lesion development,39,40 is regulated by NF-κB. Third, NF-κB has been shown to regulate expression of several adhesion molecules in response to inflammatory stimuli, including P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), all implicated in atherosclerosis development in mouse models.41–43 The potential importance of NF-κB in endothelial cells was further underscored by experiments showing that endothelial cells of regions in the aorta that are prone to developing atherosclerosis show higher levels of different components of the NF-κB system compared with regions that are less prone.35 Additionally, the high-prone regions showed more NF-κB activation after LPS treatment.
or feeding of a high-fat diet to atherosclerosis-susceptible mice.

The activation of NF-κB in endothelial cells during early atherogenesis or in other cells during later stages may be achieved through complex patterns of induction by many different stimuli.44 These agents include minimally modified LDL, advanced glycation endproducts resulting from hyperglycemia, inflammatory cytokines produced at the lesion site (also discussed below), or infectious agents such as cytomegalovirus or chlamydia.48,49 Also, the interaction of the endothelium with, for instance, platelets may contribute to atherogenesis. It was shown recently in mouse models that atherosclerosis is strongly increased by circulating activated platelets, and it had been shown previously that interaction of endothelial cells with activated platelets can induce ICAM-1, MCP-1, and E-selectin, all through NF-κB–dependent mechanisms.

These data show that the different steps in early atherosclerosis, lipid modification, chemotaxis, and adhesion may be highly affected by NF-κB. Future experiments using cell-specific knockout models for NF-κB may shed light on the regulation of these processes in the different cells in atherogenesis.

**Foam Cell Formation**

On entering the lesion site, monocytes differentiate into macrophages. A major factor involved in monocyte-to-macrophage differentiation is macrophage colony-stimulating factor (CSF; M-CSF or CSF-1), which was shown to be regulated by NF-κB. Its importance was shown in different atherosclerosis models in which virtually no atherosclerosis developed in the absence of M-CSF.55,56 For optimal migration of monocyte/macrophages into tissues, degradation of extracellular matrix is a requirement. A crucial enzyme in this process is, matrix metalloproteinase-9 (MMP-9), the transcription of which is regulated by NF-κB. Absence of MMP-9 in apolipoprotein E–deficient (apoE−/−) mice led to reduced atherosclerosis, impaired macrophage infiltration, and reduced media damage.58 In addition to mediating macrophage infiltration, MMP-9 is also facilitating smooth muscle cell migration, which may contribute to the fibrotic cap formation discussed later.

The differentiation of monocytes to macrophages is characterized by increased expression of 2 major scavenger receptors on the surface of cells: scavenger receptor class A (SR-A) and CD36. Both bind different forms of modified LDL, and they mediate the scavenging of modified lipids from the vessel wall, thereby mediating the process of foam cell formation. Interestingly, the role of NF-κB in the scavenging of modified lipids or foam cell formation has not been studied extensively. It was shown only recently that in the absence of p50, activated macrophages take up less modified LDL because of downregulation of SR-A. However, detailed analyses of the exact function of NF-κB in macrophage cholesterol influx are still lacking. Moreover, not much is known about whether the cholesterol efflux mechanisms, for instance, through ATP-binding cassette transporter 1 (ABCA1), are also affected by NF-κB activation, although LPS-induced repression of ABCA1 was shown to be reversed by NF-κB inhibitors.61 Because the 2 processes of cholesterol influx and efflux are considered to be crucial in atherogenesis, insights in this will be interesting for future experiments.

Although the role of NF-κB in the turnover of modified LDL in macrophages has not been studied thoroughly, the effects of modified LDL on NF-κB–mediated inflammatory responses has been the subject of many studies and turned out to be dual-faceted. It has been shown that minimally modified LDL can enhance activation of endothelial cells. Moreover, short-term incubation or usage of low concentrations of oxidized LDL can also enhance NF-κB activation in monocytes.62,63 However, longer incubations and usage of more severely oxidized LDL or components of severely oxidized LDL have been shown by many groups to inhibit NF-κB–mediated inflammatory responses in monocytes and macrophages. These contrasting effects and the potential effects of severity of modification, length of incubation, concentration of lipoproteins, and maybe also differences between primary cells and human or mouse cell lines make it currently difficult to draw unequivocal conclusions on the in vivo effects of modified LDL on NF-κB activation in the atherosclerotic plaque.

**Inflammation**

Atherosclerosis is an inflammatory disease, but the mechanism by which inflammatory cytokines affect atherogenesis is still poorly understood. Although in many studies, the effects of absence of increased levels of inflammatory cytokines on atherosclerosis were investigated, the exact inflammatory pathways are still unclear. The sequence in time, cellular source, cellular target, and inducing agents are for many cytokines in atherosclerosis still a puzzle. We discuss the major NF-κB–regulated cytokines studied in atherogenesis: TNF, IL-1β, IL-6, IL-10, IL-12, and interferon-γ (IFNγ). TNF is multifunctional, and one of the most important proinflammatory and immune modulatory cytokines.68 Therefore, it should have a major role in atherogenesis. Surprisingly, no effect was found in the first article studying atherogenesis in a mouse model deficient for TNF.69 Although the experiments were not performed on an atherosclerosis–susceptible background and used a very severe diet containing cholate, they revealed that such an important cytokine as TNF is not absolutely crucial for the development of atherosclerosis. Interestingly, later studies showed that absence of TNF in apoE−/− mice can reduce atherosclerosis.70 These studies were complemented by investigations studying the role of the major TNF receptor p55 in atherogenesis.69,71 Surprisingly, absence of p55 was found to increase atherosclerotic lesion formation 2- to 3-fold. So although absence of TNF did not affect atherosclerosis, the absence of another p55 ligand, LTα, actually reduced atherosclerosis. Hence, these studies indicate that signaling through the TNF–p55 axis may protect against or enhance atherogenesis, maybe depending on the genetic background of the mouse strain used. It is clear that TNF...
signaling is not an obvious proatherosclerotic pathway, but the exact mechanisms remain to be elucidated.

IL-1β is also a proinflammatory cytokine and has been studied in atherosclerosis using either IL-1β–deficient mice or mice lacking the IL-1 receptor. Absence of IL-1β in apoE−/− mice was shown to reduce atherosclerosis by ≈30%. This effect was accompanied by decreased expression of one of the major adhesion molecules in atherosclerosis, VCAM-1, and chemokine MCP-1. In line with these data, absence of IL-1 receptor was shown recently to have strong reducing effects on atherosclerosis development in apoE−/− mice. It should be noted that TNF and IL-1 are regulated by NF-κB, but they are also activators of the NF-κB pathway. Hereby, these cytokines may affect atherosclerosis by regulating many other NF-κB dependent genes and amplify the inflammatory response.

In line with previous cytokines, IL-6 is often also classified as proinflammatory. To study IL-6 in atherogenesis, Elhage et al crossed IL-6–deficient mice onto an apoE−/− background. Interestingly, early lesion formation was not affected by the absence of IL-6. In contrast, when atherosclerosis was examined at 1 year of age, the atherosclerotic lesions were larger in the apoE−/− IL-6–deficient mice compared with controls, making IL-6 an antiatherogenic factor. Interestingly, Xing et al showed that IL-6–deficient mice showed increased induction of proinflammatory cytokines in 2 models of local and systemic endotoxic shock. This observation indicates that IL-6 can function as an anti-inflammatory cytokine required for controlling inflammation. Huber et al showed that administration of recombinant IL-6 aggravates atherosclerosis, but it is not clear whether this is a direct result of IL-6 or merely a reflection of the proatherogenic effects of systemic inflammation that might be induced by IL-6. This is a problem also encountered in similar studies in which proinflammatory substances are administered systemically, making the direct effects on atherosclerosis difficult to interpret.

Importantly, NF-κB activation may also mediate the expression of genes that are clearly anti-inflammatory. Some of these genes have been linked to atherogenesis. The expression of the anti-inflammatory cytokine IL-10 was initially described to be NF-κB independent. However, more recently, it was shown that TNF- and apoptosis ligand–related leukocyte-expressed ligand 1, a member of the TNF family, may induce IL-10 in an NF-κB–dependent manner. Moreover, deletion of IKK2 from macrophages was shown to strongly reduce IL-10 secretion after LPS stimulation. Whether this effect is direct or indirect through, for instance, a reduced autocrine induction by TNF should be elucidated. However, these data show that inhibition of NF-κB activation may also reduce IL-10. The effect of IL-10 on atherogenesis has been studied quite extensively using different mouse models, and all these studies confirmed that IL-10 is an antiatherogenic factor. This shows that modification of NF-κB may also affect antiatherosclerotic inflammatory cytokines.

Atherosclerosis is generally considered to be a Th1-driven disease because Th1 cytokines dominate the atherosclerotic lesions. Two major NF-κB–driven Th1 cytokines, IL-12 and IFNγ, have been studied in atherosclerosis. When crossed to an apoE−/− background, IL-12−deficient animals had smaller lesions at different time points and at different atherosclerotic locations. Interestingly, the same article shows that absence of the Th2 cytokine IL-4 also reduced atherosclerosis, indicating that Th2 cytokines cannot be considered anti-inflammatory. When studying IFNγ, Whitman et al found that absence of this cytokine in apoE−/− animals profoundly reduced atherosclerosis in the aortic root. Surprisingly, this effect was restricted to male mice only. Administration of IFNγ to male apoE−/− mice increased atherosclerosis.

The data on these NF-κB–driven inflammatory cytokines show that the role of these cytokines and of NF-κB in these processes is not as clear as initially expected. NF-κB drives factors that may protect against atherosclerosis, such as IL-6 and IL-10, but also factors that enhance atherosclerosis, such as IL-1, IL-12, and IFNγ. The exact role of TNF signaling remains to be elucidated. It should be noted that a major problem when interpreting these data on the role of cytokines in atherosclerosis is the fact that the local effect of the cytokine in the lesion cannot be easily discriminated from systemic effects of the cytokines.

Interestingly, it was shown recently that NF-κB may indeed be regulating anti-inflammatory processes. Using an air pouch model for inflammation, 2 waves of NF-κB activation were shown. The first wave during the onset of inflammation was associated with the attraction of inflammatory cells and the induction of several proinflammatory genes. The second wave of NF-κB activation came later and was associated with the induction of anti-inflammatory cytokines, such as transforming growth factor-β, a cytokine that also has antiatherogenic effects. Blockade of NF-κB during this second wave of NF-κB activation actually enhanced inflammation, as measured by the number of inflammatory cells. These findings may lead to a new and more complete portrayal of NF-κB in inflammation showing a role in the onset as well as the resolution of inflammation. The implications for atherosclerosis remain to be elucidated.

Cell Death

In later stages of atherosclerosis, cell turnover and especially cell death become an important issue. Death of large lipid laden cells is considered to be a clinically important process because it determines the stability of atherosclerotic plaques by causing the formation of the necrotic core. Foam cell death can be either through necrosis or apoptosis (ie, programmed cell death). In contrast to necrosis, apoptosis is generally considered to be a silent event and not to evoke cellular activation, and is thereby important for maintaining homeostasis. However, in atherosclerosis, apoptosis may be detrimental. Apoptotic cells need to be scavenged and cleared from the damaged site, and in the atherosclerotic lesion,
scavenging of large lipid-filled cells may be inefficient. Moreover, large lipid-laden macrophages might not undergo the complete apoptotic process, resulting in secondary necrosis. The result of necrosis is the release of all intracellular content and proinflammatory debris. As a consequence, the inflammatory response accompanying atherosclerosis might be augmented. In addition, cell death in the plaque results in the formation of necrotic cores, which are a critical determinant of plaque stability. Finally, apoptosis in the plaque appears to release factors that increase thrombus formation by activation of platelets, and it has been associated with sites of plaque ruptures in human atherosclerotic plaques.

Embryonic lethality of the initial mouse models in which the different components of the NF-κB system were ablated emphasized the general importance of this transcription pathway. Mice deficient for p65 die in utero because of severe TNF-dependent liver apoptosis, and IKK2- and NEMO-deficient mice also showed very similar phenotypes. These data show that NF-κB is an important cellular survival factor, and this is probably because of the fact that NF-κB regulates many different antiapoptotic factors, such as cellular inhibitors of apoptosis (c-IAP), caspase inhibitors (c-FLIP), and Bcl-2 family members (Bcl-2, Bfl-1, and Bcl-xL). To overcome the embryonic lethality of IKK2-deficient animals, mice were generated in which the IKK2 allele was flanked by loxP sites, and these animals were crossed with Cre-recombinase transgensics to get cell-specific ablation of IKK2. Using this conditional approach, it was shown that IKK2 signaling is essential for survival of mature T cells and B cells. Also, enterocyte-specific deletion of IKK2 was shown to prevent the systemic inflammation in response to gut ischemia–reperfusion but gave severe tissue damage because of increased apoptosis in response to reperfusion. In addition, it was shown recently that deletion of IKK2 from macrophages resulted in increased cell death after treatment with different stimuli. These data show that in different cell types, NF-κB signaling is important for survival under certain circumstances.

Inhibition of NF-κB activation in atherosclerosis might result in cells that are sensitive to cell death. Because of the potentially detrimental effect of cell death on atherogenesis, the antiapoptotic function of the NF-κB system may therefore be seen as antiatherosclerotic, and care should be taken when considering NF-κB inhibition as treatment.

**Proliferation of Smooth Muscle Cells and Fibrous Cap Formation**

In more advanced atherosclerotic lesions, the migration of smooth muscle cells and the formation of a fibrous cap contribute to the stability of atherosclerotic lesions. On the other hand, proliferation in restenosis is unwanted. Many important experiments on the role of NF-κB in proliferation have been conducted. It was shown that antisense oligos against p65 could inhibit smooth muscle cell proliferation and neointima formation in rat carotid arteries. NF-κB is induced during smooth muscle cell proliferation, and several groups have used different ways of inhibiting NF-κB to confirm the essential role of NF-κB in this process. Recently, Zuckerbraun et al. used an adenovirus expressing a dominant-negative form of IκBα in their rat model for intimal hyperplasia. They show that the so-called IκBα super repressor could inhibit intimal hyperplasia in vivo and smooth muscle cell proliferation in vitro. They also show an upregulation of the cyclin-dependent kinase inhibitors p21 and p27, which may explain the reduced proliferation. No clear effect of the IκBα super repressor on cell death was observed. They speculate that the inhibition of NF-κB may be of future therapeutic interest in the treatment of vascular diseases. This may certainly be true for diseases when hyperproliferation is a problem, but it should be mentioned that SMC proliferation in atherosclerosis may mediate cap formation, which is considered to be a “good” plaque-stabilizing process. Hence, NF-κB may be a positive factor in this aspect of the atherosclerotic process.

**Recent Advances Using Mouse Models for Activation of NF-κB**

As indicated previously, many different signaling pathways can activate NF-κB. Recently, some advances have been made in examining the role of these pathways in atherosclerotic mouse models. Simplifying the activation routes to NF-κB, 3 major pathways can be distinguished (Figure 3).
Mouse Experiments Investigating Components of the NF-κB Activation Cascade

<table>
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<tr>
<th>Gene</th>
<th>Model</th>
<th>Lesion Area</th>
<th>Background</th>
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<td>idlr⁻/⁻</td>
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Summary of the experimental data on the effects of different NF-κB-inducing pathways and NF-κB components.

Indicated are the gene studied, the model used (Wt indicates wild-type; KO, knockout; BA, blocking antibody; mKO, macrophage knockout; hKO, hematopoietic knockout; idlr, LDL receptor; TNFRI, TNF receptor type I; TNFRII, TNF receptor type II; IL-1R, IL-1 receptor), the effect on atherosclerotic lesion area, the atherosclerosis-susceptible background used, some remarks on the characteristics of the lesions, and the relevant references.

All these receptor pathways activate different activation pathways, including NF-κB, resulting in activation of the IKK complex. An overview of the results studying different branches of NF-κB activation is given in the Table.

TNF uses 2 receptors (p55 and p75) to transduce its signal onto cells. Most cells express p55, whereas p75 is highly regulated. Both have been studied in atherosclerosis and were discussed above in the inflammation section. Interestingly, whereas absence of p75 had no effect, p55-deficient animals developed more atherosclerosis compared with controls. In the same article, 2 ligands for p55 were also studied, and it was shown that absence of TNF did not affect atherosclerosis, whereas absence of LTα actually reduced atherosclerosis and could not explain the increase in the absence of p55. It was speculated that p55 may bind alternative ligands that mediate atheroprotective mechanisms.

The second branch involves different receptors, including IL-1 and IL-18 receptors and TLRs, that all share the use of the adaptor molecule myeloid differentiation factor 88 (Myd88). Using Myd88-deficient animals on an apoE⁻/⁻ background, it was shown recently that this NF-κB activation branch is proatherogenic. Björkbacka et al. showed that atherosclerosis in Myd88–apoE double knockout mice was strongly reduced. This coincided with reduced expression in the aortic arch of several atherosclerosis-related chemokines, including MCP-1. Circulating levels of MCP-1 were also reduced. The same article also studied CD14 deficiency, but this did not affect atherosclerosis. Michelsen et al. also used Myd88-deficient mice to study atherosclerosis and also found a strong reduction in the absence of Myd88. They could correlate reduced atherosclerosis with reduced monocyte adherence, reduced levels of COX2, and again lower levels of MCP-1. They also found similar effects, although less pronounced in the absence of TLR4. These experiments show that Myd88 is an important molecule in atherosclerosis and highly affects atherosclerotic chemokine expression. Particularly, TLR signaling is becoming more of interest to the atherosclerosis field. For instance, it was shown recently that saturated fatty acids can signal through TLR-mediated pathways to induce NF-κB, whereas unsaturated fatty acids did not show this effect. In line with the important role for Myd88 signaling in atherosclerosis, inhibition of IL-1 or IL-18 signaling, both potent inducers of NF-κB through Myd88, has been shown to result in decreased atherosclerosis.

CD40 is a member of the TNF receptor family and is expressed on different cell types in atherosclerosis. It is activated by CD40 ligand (CD40L) and signals to NF-κB, both through the classical and the alternative pathway. Different experiments have investigated the CD40–CD40L axis in atherogenesis. Surprisingly, direct data using CD40-deficient animals are still lacking, and most studies have focused on modifying CD40L, either by using CD40L-deficient animals or administration of CD40L-inhibiting antibodies. All these studies show that inhibition of CD40L results in smaller atherosclerotic lesions with a more stable plaque phenotype, characterized by a smaller lipid core and a thicker fibrous cap. These data show that the CD40–CD40L system is a proatherogenic signaling cascade that also highly affects plaque stability.
Only recently, the role of NF-κB in atherosclerosis was studied directly using mouse models. Macrophage-specific deletion of the main NF-κB–activating kinase IKK2 was shown to increase atherosclerotic lesions in LDL receptor-deficient mice.78 IKK2 deletion led to an increase in lesion size in the absence of macrophage IKK2. This confirms the role of NF-κB as a survival factor. Analysis of lesion progression showed a more advanced plaque phenotype, with increased macrophage influx in early lesions in the IKK2-deleted group. Although deletion of IKK2 was not complete in macrophages, indicating selection against homozygous IKK2 knockout cells, isolated macrophages showed reduced NF-κB activation and reduced TNF and IL-10 secretion. These data show that inhibition of NF-κB activation in macrophages may result in increased cell death, and not only affects proinflammatory pathways but also anti-inflammatory cytokines. The combination of these 2 effects may be the main cause of the observed increased atherosclerosis. Interestingly, A20 was identified recently as an atherosclerosis-susceptibility locus in a quantitative trait loci analysis.114 A20 is an inhibitor of NF-κB activation and is involved in the termination of NF-κB responses. A20-deficient mice develop severe inflammation and cachexia and die prematurely.115 and A20 was shown recently to have ubiquitinating and deubiquitinating activity for RIP1 and TRAF6.116,117 The group of Jan Breslow found that less efficient termination of NF-κB activation by a polymorphism in A20 was associated with reduced atherosclerosis. Hence, these data suggest that prolonged NF-κB activation is antiatherogenic rather than proatherogenic.

Finally, p50 was also identified recently as an important factor in the progression of atherosclerosis and the prevention of inflammation in the lesion.61 The p50 subunit has DNA binding activity but lacks transcriptional activity. Therefore, p50 is regarded as an important regulator of NF-κB activation, as shown by the repressive properties of p50 homodimers and the downregulation of, for instance, TNF expression by p50 homodimers.118,119 Especially in macrophages, p50 is regarded as a silencer of the inflammatory response. Studies performed in mice deficient for p50 in the cells of the hematopoietic system resulted in reduced atherosclerosis, although the atherosclerotic lesions were more inflamed and contained more B and T lymphocytes.61 These results emphasize the complex role of NF-κB in many processes in the lesion.

Conclusions and Future Directions

The data on NF-κB signaling discussed above show how different routes can affect atherosclerosis. Moreover, they underscore that NF-κB signaling, per se, is not proatherosclerotic, and that some aspects of the atherosclerotic process may be affected by NF-κB in a proatherogetic way, whereas others are more antiatherogenic. The role of NF-κB as cell survival factor may be especially important in understanding how NF-κB affects the atherosclerotic process. Moreover, it should be noted that future experiments focusing more on cell-specific conditional knockouts for parts of the NF-κB activation pathway will shed light on how NF-κB in the different cells can modulate atherosclerosis. General inhibition of NF-κB as a future antiatherosclerotic therapy may be too risky, also because of the broad function of NF-κB in inflammation and immunity. However, understanding the contribution of pathways signaling to NF-κB and pathways activated by NF-κB may help understand how the inflammatory balance during atherosclerosis is orchestrated. Subsequent zooming in on these specific pathways may yield novel ways to modify atherosclerosis development.

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Menno P.J. de Winther, Edwin Kanters, Georg Kraal and Marten H. Hofker

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