

## The Transcription Factor NF- $\kappa$ B and the Regulation of Vascular Cell Function

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**Abstract**—A variety of pathophysiological situations that affect cells of the vasculature, including endothelial and smooth muscle cells, leads to the expression of genes such as adhesion molecules and chemokines that are dependent on members of the nuclear factor (NF)- $\kappa$ B family of transcription factors. The corresponding gene products mediate important biological functions such as immune and inflammatory reactions, smooth muscle cell proliferation, and angiogenesis. The beneficial and usually transient NF- $\kappa$ B-dependent gene expression may be exaggerated in pathological situations and results in damage to the vessel wall and impaired vascular cell function. In this review, we will capitalize on the favorable and adverse roles of NF- $\kappa$ B in the context of vascular disease, eg, chronic and localized inflammation, arteriosclerosis, and neoangiogenesis. (*Arterioscler Thromb Vasc Biol.* 2000;20:e83-e88.)

**Key Words:** endothelial cells ■ smooth muscle cells ■ inflammation ■ arteriosclerosis ■ angiogenesis

### NF- $\kappa$ B Family Members and Mode of Activation

Nuclear factor (NF)- $\kappa$ B is a family of transcription factors that was originally identified in B cells but was then rather rapidly discovered to be ubiquitously expressed and also phylogenetically conserved down to *Drosophila*. Family members include RelA (p65), RelB, c-Rel, NF- $\kappa$ B1 (p50), and NF- $\kappa$ B2 (p52), the latter two being synthesized from the inactive precursor molecules p105 and p100, respectively, as well as their inhibitory subunits I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , and I $\kappa$ B $\epsilon$ . NF- $\kappa$ B subunits form homo- and heterodimers, the most prominent one being the p65/p50 heterodimer, and bind to the decameric consensus sequence GGGRNNTYCC (R=G or A, Y=C or T, N is any nucleotide) that displays a certain degree of specificity toward subunit compositions. The dimer is retained in the cytoplasm in an inactive state through interaction with I $\kappa$ B. NF- $\kappa$ B is rapidly activated in response to a variety of inflammatory and other stimuli that lead to degradation of I $\kappa$ B (the Figure). Important stimuli in regard to vascular biology include tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, bacterial lipopolysaccharide (LPS), advanced glycation end products (AGEs), hyperglycemia, platelet-activating factor, shear stress, oxidized lipids, oxidant stress, and hypoxia/reperfusion.<sup>1</sup> A peculiarity of NF- $\kappa$ B is the very rapid and (with few exceptions) transient nature of its activity, which makes it well suited for the expression of many immune and “stress”-response genes, because they need to be upregulated only on demand for a limited period of time and then shut down (a summary of those stimuli relevant for vascular biology, along with the target genes regulated by

NF- $\kappa$ B, is given in the Table). In turn, prolonged activation of NF- $\kappa$ B, which may occur either through persistence of the stimulating agent(s) or through impairment of the mechanisms of downregulation (see below), is a hallmark of many chronic inflammatory and vascular diseases, calling for a more detailed investigation of the NF- $\kappa$ B regulatory circuits.

Our current understanding of the regulatory steps that lead to activation of NF- $\kappa$ B include the following (the Figure): On stimulation, NF- $\kappa$ B (eg, the p65/p50 heterodimer) is released from an inactive cytoplasmic complex that contains the inhibitory subunit I $\kappa$ B $\alpha$ , - $\beta$ , or - $\epsilon$ . I $\kappa$ B's, when bound to NF- $\kappa$ B, mask the NF- $\kappa$ B nuclear localization signal and thus prevent its nuclear translocation. Liberation of NF- $\kappa$ B is initiated by I $\kappa$ B phosphorylation on two serine residues, Ser 32 and 36, in I $\kappa$ B $\alpha$  and corresponding sites in I $\kappa$ B $\beta$ ,<sup>2</sup> followed by recognition by the  $\beta$ -TrCP-like component of an E3 ubiquitin ligase complex (Skp1/Cul1/ROC1/F-box protein FWD1), ubiquitination, and degradation via the 26S proteasome.<sup>3</sup> A major breakthrough in the field has been the identification of two kinases (IKK1/IKK $\alpha$  and IKK2/IKK $\beta$ ) that specifically phosphorylate I $\kappa$ B $\alpha$ .<sup>4</sup> These kinases form homo- and heterodimers through their leucine zipper domains and are bound to a third protein (NEMO/IKK $\gamma$ ) that couples the I $\kappa$ B kinases to upstream activators.<sup>5</sup> This I $\kappa$ B kinase complex (IKC), consisting of IKK1, IKK2, and NEMO, is included within a high-molecular-weight (500 to 700 kDa) complex termed signalosome, which contains other proteins such as the catalytic subunit of protein kinase A (csPKA) and phosphotyrosine-containing proteins.<sup>4</sup> Genetic evidence from knockout mice has revealed a prominent role for IKK2 in

Received April 3, 2000; revision accepted August 2, 2000.

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*Arterioscler Thromb Vasc Biol.* is available at <http://www.atvbaha.org>

**Activators and Target Genes of NF- $\kappa$ B**Inducers of NF- $\kappa$ B

Interleukins, growth factors, mitogens	IL-1, TNF- $\alpha$ , lymphotoxin, IFN- $\gamma$ , phorbol esters, lectins, PDGF, VEGF
Viruses, microorganisms, and their products	LPS, <i>Chlamydia pneumoniae</i> , <i>Vaccinia</i> virus, <i>Borrelia burgdorferi</i> , <i>Shigella dysenteriae</i> , <i>Plasmodium falciparum</i>
Physical factors	UV, $\gamma$ -irradiation, shear stress (?), heat shock, cold shock
Others	Ox-LDL, AGEs, thrombin, double-strand RNA, homocysteine, hypoxia/reperfusion, leptin, heavy metals (Ni, Co), complement, xenoreactive natural antibodies

Genes regulated by NF- $\kappa$ B

Interleukins and growth factors	IL-1, IL-6, IL-8, TNF- $\alpha$ , G-CSF, M-CSF, GM-CSF, MCP-1, RANTES, MGSA/ <i>gro</i> - $\alpha$
Cytokine and cell adhesion receptors	E-selectin, ICAM-1, VCAM-1, MAdCAM-1, Lox-1, RAGE
Apoptosis related	A20, A1, XIAP, c-IAP1, c-IAP2
Immunomodulatory	MHC-I, MHC-II, IRF-1
Others	iNOS, COX-2, tissue factor, PAI-1, I $\kappa$ B $\alpha$ , MnSOD, MMP-2, MMP-9, PTX3

CSF indicates colony-stimulating factor; GM, granulocyte/macrophage; RANTES, regulated upon activation normal T lymphocyte expressed and secreted; MGSA, melanoma growth-stimulating activity; MAd, mucosal addressin; XIAP, X-linked inhibitor of apoptosis; IRF, interferon regulatory factor; SOD, superoxide dismutase; and PTX, pertussis toxin.

inflammatory signaling, whereas IKK1 appears to be involved in developmental processes.<sup>6,7</sup> In addition to the IKKs, pp90rsk and DNA-PK have been demonstrated to be capable of directly phosphorylating and activating I $\kappa$ B.<sup>8,9</sup> Recently, two novel IKKs have been identified, one that is inducible by LPS on the mRNA level in macrophages (IKK-i)<sup>10</sup> and one (TBK1) that forms a complex with TNF receptor-associated factor (TRAF)2 and TRAF family member-associated NF- $\kappa$ B activator (TANK) and thus, represents an alternative signaling pathway for TNF- $\alpha$ , leading to NF- $\kappa$ B activation.<sup>11</sup>

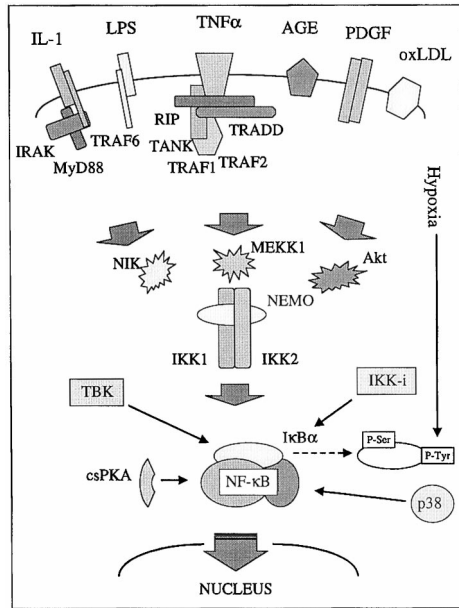
In different cell types, multiple interactions between the IKKs, predominantly IKK2, and their upstream activators have been described. These include the mitogen-activated protein kinase kinase kinase (MAP3K)-type kinases NIK (NF- $\kappa$ B-inducing kinase) and MEKK1 (MAPK/extracellular signal-regulated kinase kinase-1), the transforming growth factor (TGF)- $\beta$ -inducible kinase TAK1, as well as Akt and the protein kinase C (PKC) isoforms  $\zeta$  and  $\Theta$ ,<sup>12-17</sup> reflecting both heterogeneity but also a certain degree of specificity of the NF- $\kappa$ B signaling pathway in regard to different cell types and stimuli. These upstream activators couple to different components of the receptor-specific cytoplasmic signaling complexes that are recruited to the receptors on stimulation, eg, TRAF1, TRAF2, and TNF receptor-1-associated death domain protein (TRADD) for TNF receptors and TRAF6 for IL-1 and Toll family receptors.

However, it should be noted that besides this main pathway of NF- $\kappa$ B activation, alternative routes can lead to or be required for full NF- $\kappa$ B activity. These include the phosphorylation-dependent proteolytic cleavage of the p105 and p100 precursor molecules that involves Tpl2/COT as well as the phosphorylation of serine residues in RelA (eg, Ser 276 and 529), leading to an increase of the transactivation properties of NF- $\kappa$ B. The latter involves csPKA as well as kinases of the p38 MAPK pathway and may regulate the interaction of RelA with the transcriptional coactivators p300/CBP.<sup>18,19</sup> Moreover, nuclear translocation of NF- $\kappa$ B was shown to be a result of tyrosine phosphorylation of I $\kappa$ B $\alpha$  in response to hypoxia,

which may be relevant for intercellular adhesion molecule (ICAM)-1 expression in the context of reperfusion injury.<sup>20</sup> Recently, continuous nucleocytoplasmic shuttling of the NF- $\kappa$ B/I $\kappa$ B $\alpha$  complex has been demonstrated, along with the possibility of activation of nuclear NF- $\kappa$ B by TNF- $\alpha$ .<sup>21</sup> It remains to be established whether these additional pathways are of physiological relevance for the regulated activation of NF- $\kappa$ B.

**NF- $\kappa$ B and the Inflammatory Response**

It was noted from very early on that the majority of the transcriptionally regulated genes expressed in endothelial cells (ECs) in response to inflammatory mediators such as LPS, IL-1, or TNF- $\alpha$  contained NF- $\kappa$ B binding sites in their promoter regions. Subsequently, reporter gene studies using deletion and substitution mutants have revealed that NF- $\kappa$ B is of functional importance for the expression of these proinflammatory genes. Moreover, pharmacological (antioxidants or proteasome inhibitors; see below) or genetic inhibition of NF- $\kappa$ B by overexpression of stabilized I $\kappa$ B $\alpha$  mutants<sup>22,23</sup> resulted in highly efficient inhibition of EC activation. Examples of NF- $\kappa$ B-regulated genes include vascular cell adhesion molecule (VCAM)-1, E-selectin, IL-1, IL-6, IL-8, tissue factor, plasminogen activator inhibitor (PAI)-1, cyclooxygenase (COX)-2, and inducible nitric oxide synthase (iNOS); the Table). Although NF- $\kappa$ B is necessary but usually not alone sufficient for the expression of these genes, it is of central importance for regulated inflammatory gene expression. The transcriptional regulation of NF- $\kappa$ B involves, in part, physical interaction with other transcription factors, eg, SP-1, activator protein (AP)-1, or CREB and with transcriptional coactivators such as p300/CBP.<sup>18</sup> The latter have been demonstrated to act through their histone acetyltransferase, because inhibitors of histone deacetylase, such as trichostatin A, enhance NF- $\kappa$ B-dependent IL-6, IL-8, and E-selectin expression.<sup>19</sup> In some cases, the physical interaction with other proteins can also have a "silencing" function, as in the case of the peroxisome proliferator-activated receptor



Basic signaling pathways leading to activation of NF- $\kappa$ B. Binding of soluble mediators, eg, inflammatory cytokines, to their receptors triggers the assembly of cytoplasmic receptor-specific adapter molecules (eg, TRAFs) that activate members of the MAP3 (NIK, MEKK1) and other kinases. The latter further activate IKKs that phosphorylate I $\kappa$ B $\alpha$  on amino-terminal serine residues, leading to its proteasome-mediated degradation. Thereby NF- $\kappa$ B is liberated from its cytoplasmic complex and translocates to the nucleus. For details and additional signaling components, see text.

(PPAR)- $\alpha$ . PPAR- $\alpha$  activators that include certain fatty acids and fibric acid derivatives have been shown to reduce VCAM-1 expression, for example, and may have implications for pharmacological inhibition of NF- $\kappa$ B.<sup>24</sup>

Equally important as the mechanisms leading to NF- $\kappa$ B activation are the ways in which its activity can be downregulated. Evidently, this is of relevance for an organism to avoid overshooting reactions of the immune response. Indeed, self-limiting feedback mechanisms have been described that can act on several levels: First, receptors that trigger NF- $\kappa$ B can be internalized and degraded or shed from the cell surface. Second, NF- $\kappa$ B-dependent transcriptional activation and resynthesis of I $\kappa$ B $\alpha$  leads to termination of NF- $\kappa$ B activity in the nucleus and reshuttling to the cytoplasm.<sup>25,26</sup> The general importance of I $\kappa$ B $\alpha$  in NF- $\kappa$ B downregulation is supported by the findings that constitutive NF- $\kappa$ B activation in Reed-Sternberg cells of Hodgkin's disease patients can be due to mutations or aberrant expression of I $\kappa$ B $\alpha$  and that fibroblasts from I $\kappa$ B $\alpha$ -deficient mice show a sustained activation of NF- $\kappa$ B in response to TNF- $\alpha$ .<sup>27,28</sup> Third, hyperphosphorylation of IKKs in the HLH domain inhibits their kinase activity.<sup>29</sup> Fourth, NF- $\kappa$ B-dependent expression of COX-2 at later stages of the inflammatory episode directs the generation of cyclopentenone prostaglandins that exert anti-inflammatory activity.<sup>30</sup> However, the quantitative contribution and balance between activating and repressing signals in the course of downregulation of NF- $\kappa$ B activity are still poorly understood. It will be of central importance for the understanding of chronic inflammatory diseases to further investigate these positive and negative regulatory mechanisms.

## NF- $\kappa$ B and Arteriosclerosis

Arteriosclerosis can be viewed as a multistep, chronic inflammatory disease that involves the interplay between various soluble mediators, monocytes, ECs, and smooth muscle cells (SMCs). Endothelial dysfunction is regarded as an initial step that can be caused by various stimuli like dyslipidemia, AGEs, high glucose levels, hyperhomocysteinemia, and generation of free radicals that are associated with many of the extensively studied risk factors or infectious agents. Subsequently, deregulated expression of cell adhesion molecules and chemotactic cytokines can attract T cells and monocytes that transmigrate into the intima of the vascular wall. Uptake of oxidized LDL (ox-LDL) by activated monocytes via the scavenger receptor or CD36 will lead to their activation, release of TNF- $\alpha$ , and transition into foam cells, a hallmark of the early arteriosclerotic lesion. Monocyte-derived cytokines and growth factors, eg, TNF- $\alpha$ , will further affect the integrity of the vascular wall by directly or indirectly stimulating SMC proliferation and migration via release of IL-6, for example.<sup>31</sup> Monocytes may also secrete matrix metalloproteinases that, at later stages of atherosclerosis, lead to plaque instability and rupture.<sup>32</sup>

NF- $\kappa$ B has been demonstrated to be constitutively active in SMCs *in vitro*, and its inhibition by overexpression of I $\kappa$ B $\alpha$  leads to apoptosis in low-density but not high-density cultures.<sup>33</sup> It thus appears to be essential for SMC proliferation initiated by serum, thrombin, or TNF- $\alpha$ .<sup>34,35</sup> NF- $\kappa$ B is expressed in arterial SMCs after balloon injury and is responsible for the expression of several genes, including ICAM-1, VCAM-1, and macrophage chemoattractant protein (MCP)-1, the latter of which can mediate the infiltration of monocytes.<sup>36</sup> High levels of NF- $\kappa$ B are also present in SMCs of the arteriosclerotic lesion,<sup>37</sup> especially in those derived from the intima, which also show enhanced iNOS expression and I $\kappa$ B $\alpha$  turnover (Z. Yan, unpublished data, 2000). Both platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) generate signals that lead, via the serine/threonine kinase Akt, to NF- $\kappa$ B activation and NF- $\kappa$ B-dependent expression of antiapoptotic genes, thus protecting cells from apoptosis during (aberrant) proliferation.<sup>38</sup>

Recently, the group of Collins and Cybulsky has investigated NF- $\kappa$ B expression in regions of high probability (HP regions) for the development of arteriosclerotic plaques in comparison with adjacent regions where plaques are unlikely to develop. These HP regions, which coincide with areas exposed to high shear stress, were found to express highly elevated levels of RelA. Surprisingly, RelA localization was cytoplasmic, translocating to the nucleus only after additional stimulation with LPS or feeding of an atherogenic diet.<sup>39a</sup> This observation suggests that shear stress may prime certain regions of the vessel wall by inducing steady-state levels of NF- $\kappa$ B that later, on an additional threat, becomes activated to mediate expression of inflammatory cytokines and adhesion molecules.

Thus, there are multiple ways in which NF- $\kappa$ B can contribute to the initiation and progression of arteriosclerosis. First, the majority of proinflammatory genes expressed in ECs during the initial phase of the lesion and in response to inflammatory mediators is dependent on NF- $\kappa$ B. Second,

activation of infiltrating monocytes leads to expression of (again, mainly NF- $\kappa$ B dependent) genes that, in the case of TNF- $\alpha$ , for example, promote SMC proliferation. Furthermore, SMC proliferation itself has been functionally associated with NF- $\kappa$ B. Last but not least, prevention of apoptosis in SMCs may involve NF- $\kappa$ B through the regulation of antiapoptotic genes such as cellular inhibitor of apoptosis protein (cIAP)-1.<sup>33</sup>

### NF- $\kappa$ B and Angiogenesis

In the adult organism, angiogenesis occurs only in few physiological situations, eg, wound healing, pregnancy, or the female reproductive cycle, and in pathological cases like neovascularization of solid tumors. The process of capillary sprouting is likely to involve a multitude of regulatory molecules to mediate the distinct steps of extracellular matrix remodeling, EC migration, proliferation, lumen formation, and blood vessel maturation. It is mediated by key angiogenic factors of the VEGF family, basic fibroblast growth factor (bFGF), and their receptors. Moreover, because cells loosen their contact to the extracellular matrix during migration and proliferation, they are prone to apoptosis and thus, require mechanisms to prevent them from undergoing programmed cell death.<sup>38</sup>

NF- $\kappa$ B has been implicated in cell proliferation in several ways. For example, a number of tumors show increased or aberrant NF- $\kappa$ B activity due to the presence of either oncogenic v-Rel in chicken reticular disease, p52 translocations in T- and B-cell lymphomas, or nonfunctional I $\kappa$ B $\alpha$  in Reed-Sternberg cells in Hodgkin's disease.<sup>39b,40,27</sup> Although it may be argued that the main function of NF- $\kappa$ B in tumors is to prevent apoptosis, recent findings of NF- $\kappa$ B-dependent regulation of the cyclin D1 promoter have provided evidence for a more direct link between this transcription factor and the cell cycle.<sup>41</sup> In SMCs, inhibition of NF- $\kappa$ B through overexpression of I $\kappa$ B $\alpha$  inhibited proliferation.<sup>35</sup> However, in other cell types, I $\kappa$ B $\alpha$  could be stably expressed without significant effects on proliferation.

With the use of in vitro (eg, tube formation in 2- or 3-dimensional gels) and in vivo models of angiogenesis, several lines of evidence suggest a functional role for NF- $\kappa$ B: it was found to be necessary for capillary tube formation in vitro<sup>42</sup> and in a retinal neovascularization model in mice.<sup>43</sup> Furthermore, inhibition of NF- $\kappa$ B by antisense oligonucleotides blocked capillary tube formation in collagen gels.<sup>42</sup> Although these experiments do not allow us to determine which steps are NF- $\kappa$ B dependent, Scatena et al<sup>44</sup> have shown that NF- $\kappa$ B is essential for inhibition of apoptosis. By culturing ECs on matrices of different composition, using blocking antibodies, and inhibiting NF- $\kappa$ B, these authors could demonstrate that osteopontin and fibronectin promote the  $\alpha$ v $\beta$ 3 integrin-mediated activation of NF- $\kappa$ B as well as cell survival. In accordance with those observations, we could show that adenovirus-mediated overexpression of either I $\kappa$ B $\alpha$  or dominant-negative IKK2 inhibits tube formation in vitro (W. Oitzinger, unpublished data, 2000). Additional mechanisms by which NF- $\kappa$ B may regulate angiogenesis include the induction of matrix metalloproteinases and of the VEGF receptor flk-1.<sup>45</sup>

### Development of Pharmacological and Genetic Inhibitors of NF- $\kappa$ B

Therefore, it would be desirable in regard to several aspects of vascular pathology to specifically inhibit NF- $\kappa$ B. Indeed, a number of novel, low-molecular-weight inhibitors have been identified that, according to their mode of action, can be grouped into distinct classes. The first NF- $\kappa$ B inhibitors described were antioxidants and radical scavengers, eg, *N*-acetylcysteine and the more potent pyrrolidine dithiocarbamate.<sup>22</sup> More recently, resveratrol, a polyphenolic compound, has been identified as a constituent of red wine that may be responsible for the suggested beneficial effect of moderate red wine consumption on cardiovascular function.<sup>46</sup> However, notwithstanding numerous reports, the precise mode of action of antioxidants and radical scavengers remains ambiguous, as the role of oxygen radicals in activating NF- $\kappa$ B is not fully understood. It has been discussed whether phosphatases, which are more susceptible to oxygen radicals compared with protein kinases, might play a role in this context.

The second major group are proteasome inhibitors that function to inhibit I $\kappa$ B $\alpha$  degradation and p105 processing. These include broad-specificity inhibitors like lactacystin, a series of peptide aldehyde inhibitors of the catalytic subunit of the proteasome (eg, MG132), PS-341, a dipeptide boronic acid inhibitor, as well as epoxomicin, an epoxy- $\beta$ -aminoketone isolated from *Actinomycetes* that is used in antitumor therapy.<sup>47-49</sup> The latter preferentially block the chymotrypsin-like activity of the proteasome, which is the relevant activity for I $\kappa$ B $\alpha$  degradation. However, most proteasome inhibitors appear to be toxic when applied for prolonged periods of time, probably owing to their still-broad spectrum of inhibition. Based on our growing understanding of the heterogeneity of the proteasome, it should be possible to develop proteasome inhibitors that are more selective for the NF- $\kappa$ B/I $\kappa$ B system.

The third group comprises inhibitors of the IKC that act through binding and/or inhibition of I $\kappa$ B kinase activity. Examples include curcumin and epoxyeicosatrienoic acids.<sup>50,51</sup> One group of compounds studied best in this regard are cyclopentenones, which were shown to specifically inhibit IKK2. Interestingly, endogenous cyclopentenones are generated by COX-2 at later stages of the inflammatory response and thus, may also represent one of several endogenous shutdown mechanisms to limit the inflammatory reaction.<sup>30</sup>

In addition, some drugs that are already in clinical use have turned out to function, at least in part, by inhibition of NF- $\kappa$ B. Prominent examples include the potent anti-inflammatory glucocorticoids, which were reported to directly bind to the RelA and NF- $\kappa$ B1 subunits of NF- $\kappa$ B, thereby preventing DNA binding and transactivation. An additional mechanism involves the induction of I $\kappa$ B $\alpha$  at the transcriptional level.<sup>52</sup> However, these latter results, which had been obtained in epithelial cells and were also reported for monocytes, could not be confirmed in several others including ECs, suggesting that in different cell types, different mechanisms of inhibition may be operative.<sup>53</sup> Salicylates were reported to inhibit NF- $\kappa$ B through binding to IKK2 and also through inhibition of RSK2.<sup>54,55</sup> Atorvastatin, a synthetic 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor that lowers plasma cholesterol levels by inhibiting endogenous cholesterol synthesis, prevented activation of NF- $\kappa$ B in SMCs through stabilization of I $\kappa$ B $\alpha$ .<sup>56</sup> An entirely distinct

mode of action is the basis for the activity of mesalamine, an anti-inflammatory aminosalicylate: it inhibits phosphorylation of residues in the RelA transactivation domain, which occurs by an as-yet-unidentified protein kinase and which is required for full transcriptional activity.<sup>57</sup> Mesalamine may therefore be valuable not only as a component of a combination therapy<sup>44</sup> but also as a tool to study the emerging role of additional signaling pathways leading to NF- $\kappa$ B activation.

Thus, known NF- $\kappa$ B inhibitors act on several different levels of the signaling pathway, including scavenging of oxygen radicals, inhibition of the I $\kappa$ B kinases, inhibition of (subsets of) the proteasome, binding to the transcription factor, and interference with transactivation, as well as induction of I $\kappa$ B $\alpha$ . Because many of today's inhibitors, eg, antioxidants, lack specificity, it will be of great interest to identify compounds that specifically block NF- $\kappa$ B without interfering with other cellular functions.

This highly desirable objective may be accomplished more easily by the use of genetic inhibitors. Based on our current understanding of the activation process, several candidate genes are available and have in part already been tested. The drawback of the genetic approach for clinical applications is that it implies the use of vectors and delivery systems that have not yet been developed to the extent of being applicable in broad clinical use. Genetic approaches include the overexpression of mutated, stabilized I $\kappa$ B $\alpha$  genes; of dominant-negative mutant I $\kappa$ B $\alpha$  kinases, a RelA construct lacking the transactivation domain; and of superoxide dismutase. With the use of conventional lipid-based transfection as well as recombinant adenoviral and retroviral vectors, these genes have been successfully introduced into cells of the vasculature and demonstrated to inhibit NF- $\kappa$ B both in vitro and in vivo<sup>23,58</sup> (W. Oitzinger, unpublished data, 2000). The successful use of antisense oligonucleotides and double-stranded transcription factor decoys (eg, dumbbell oligonucleotides) has also been reported.<sup>42,59</sup> Genetic inhibition of NF- $\kappa$ B might be considered in settings where local and transient expression of the inhibitor is desirable, eg, for the prevention of restenosis after balloon angioplasty or of transplant arteriosclerosis after coronary bypass grafting.

### Concluding Remarks

During the past few years, the NF- $\kappa$ B family of transcription factors has become increasingly recognized as a regulator of major importance in many different cell types. Cells of the vessel wall are no exception; even more, NF- $\kappa$ B plays a central role in a variety of diseases, including inflammation, arteriosclerosis, restenosis, and reperfusion injury, as well as formation of new blood vessels. Through mediating the inflammatory response, NF- $\kappa$ B is part of the innate arm of the immune system. The importance of NF- $\kappa$ B is recognized by the pharmaceutical industry and reflected by the flourishing discovery of novel, low-molecular-weight inhibitors that promise novel therapeutic possibilities. However, the involvement of NF- $\kappa$ B in such a variety of cellular functions also raises concerns about possible side effects of inhibitory drugs. It may be argued that NF- $\kappa$ B is a "stress response" factor that is needed only during certain pathological "defense" situations and is dispensable during the unchallenged physiological state; clinical studies will need to address these concerns. Nevertheless, as the past few years have also seen a diversification of NF- $\kappa$ B not only in terms of different subunit composition but also in regard to the pathways that

lead to its activation, cell type and stimulus-specific pathways—along with their respective inhibitors—can be envisaged. To further delineate these pathways will be one of the challenging future objectives in the field.

### Acknowledgments

This work was supported by grants from the Austrian Science Foundation (SFB5-12) and the Jubiläumsfonds der Oesterreichischen Nationalbank (#6912). The authors wish to apologize to those contributors in the field who have been cited only indirectly owing to space limitations.

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