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PERSPECTIVES IN BASIC SCIENCE

Transcription factor-κB (NF-κB) and renal disease

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Transcription factor-κB (NF-κB) and renal disease. Nuclear factor-κB (NF-κB) comprises a family of dimeric transcription factors that regulate the expression of numerous genes involved in inflammation and cell proliferation. Although NF-κB was initially identified in lymphocytes, it has been found to be a transcription factor present in virtually all cell types. In resting cells, NF-κB dimers remain in the cytoplasm in an inactive form bound to the inhibitory subunit IκB. Upon stimulation, IκB is phosphorylated, ubiquitinylated, and ultimately degraded by proteolytic cleavage by the proteasome system. As a result, NF-κB dimers are translocated into the nucleus and activate the transcription of target genes. Increasing data suggest a pivotal role for NF-κB in a variety of pathophysiological conditions in which either inflammation or cell number control are critical events. NF-κB has been found to be activated in experimental renal disease. Importantly, both in vivo and in vitro, NF-κB activation can be modulated by pharmacological maneuvers. Indeed, it is now widely acknowledged that the anti-inflammatory action of steroids is basically obtained through the inhibition of the transactivation of NF-κB–dependent genes. In addition, some of the beneficial effects of angiotensin-converting enzyme inhibitors and statins may, at least in part, be mediated by an inhibition of NF-κB activation. A better understanding of the mechanisms involved in NF-κB regulation and its modulation may provide new tools to improve the treatment of renal diseases with a better sound pathophysiological approach.

Nuclear factor-κB (NF-κB) was initially identified as a nuclear factor bound to the enhancer of the immunoglobulin (Ig) κ light chain gene of B lymphocytes [1]. It soon became clear that NF-κB is present in virtually every cell type, although sequestered in an inactive form in the cytoplasm [2–5]. Upon stimulation, NF-κB is released from an inhibitory subunit (IκB) and translocates into the nucleus, where it promotes the transcriptional activation of target genes. The signal is eventually terminated by the new synthesis of IκB. A variety of extracellular stimuli is able to induce the activation of IκB.

Key words: nuclear factor-κB, inflammation, cell proliferation, dimeric transcription factors, transactivation of NF-κB, kidney disease.

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Nuclear factor-κB promotes the expression of a number of genes involved in inflammation, such as cytokines and adhesion molecules. Not surprisingly, clinical and experimental data are confirming the presence of activated NF-κB in a variety of chronic, inflammatory disorders [6].

Nuclear factor-κB has also been found in an inactive form in renal cells and activated upon stimulation, both in vivo and in vitro. Although the evidence linking NF-κB activation to human renal disease is limited, increasing data suggest that NF-κB plays a pivotal role in many nephropathies [7, 8].

In recent years, a large number of publications have increased our understanding on the structure, ways of activation, regulation, and transcriptional activation of NF-κB [2–6, 9]. Although a variety of different stimuli may elicit NF-κB activation, all major activation pathways present several common features. A detailed description of the molecular mechanisms involved in NF-κB activation and its regulatory properties is beyond the scope of the present review. However, a succinct description of the structure of NF-κB and an overview of the consensus NF-κB activation pathway are presented. In addition, we summarize the experimental and clinical evidence linking NF-κB and renal disease.

STRUCTURE, FUNCTION, AND REGULATION

Structure and function

NF-κB proteins. In mammals, active NF-κB is present as a homodimer or heterodimer of the five identified members of the NF-κB/Rel family (Fig. 1) [2–5, 9]. In spite of an intensive search, no new members of the family have been found in recent years, suggesting that no more genes of the NF-κB/Rel family exist in the mammalian genoma. By far the most abundant dimer in most cell types, and the most widely studied, it is composed of subunits p50 and p65. Hence, although "NF-κB" applies to all of the members of the family, it is often used to refer to the p50-p65 dimer. All of these proteins share a highly conserved Rel homology region (RHR) of about 300 amino acids, composed of two Ig-like domains. This region is responsible for interaction with other mem-

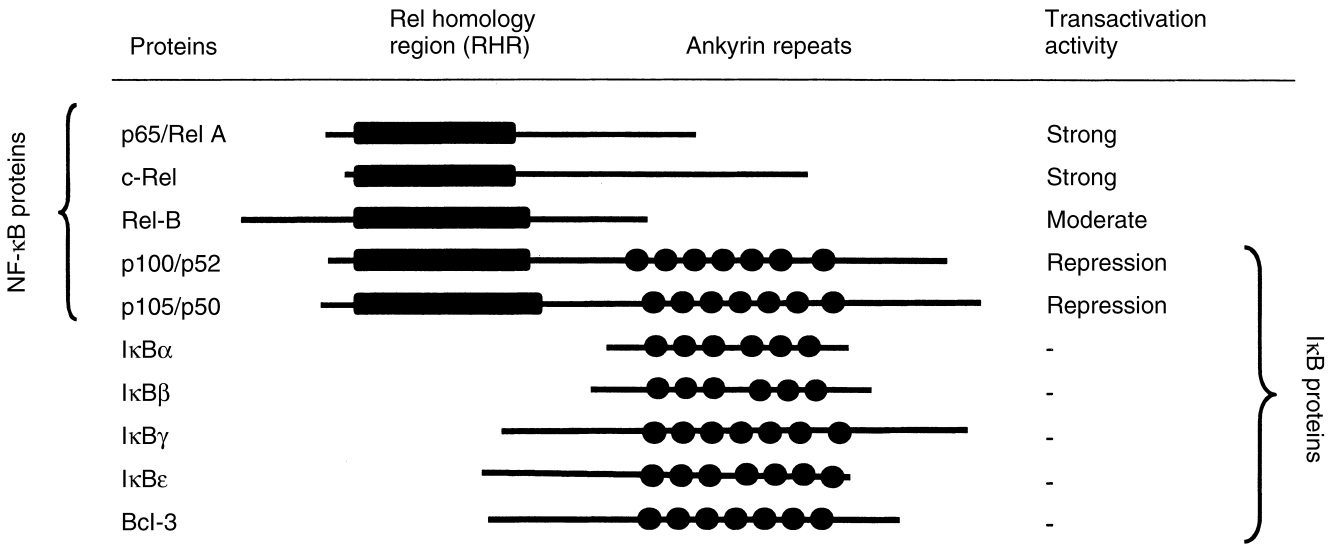

Fig. 1. Schematic representation of the nuclear factor-κB (NF-κB) and inhibitory subunit (IκB) family of proteins (modified from Rothwarf and Karin [9], with permission).

bers of the family (dimerization), DNA (DNA binding and transactivation), and the inhibitory proteins (IκB). In addition, the RHR also contains a nuclear localization sequence (NLS). NF-κB dimerize by extensive hydrophobic interactions in the carboxy-terminal domain RHR, whereas the amino-terminal domain is responsible for DNA sequence recognition. NF-κB dimers bind to the DNA consensus sequence (κB site) GGGRNNYYCC, where R is purine, Y is pyrimidine, and N is any base. Various NF-κB dimers present different affinities to κB sites and also vary in their ability to activate the transcription of target genes (Table 1). For instance, p50 and p52 lack the transactivation domain, are unable to promote transcriptional activity, and are considered to mediate transcriptional repression. Conversely, p65/RelA and c-Rel are potent transcriptional activators. Finally, RelB produces transcriptional activation in certain cell types. Knockout mice for all of the *NF-κB* genes have been obtained, indicating specific roles for each NF-κB protein. Interestingly, only the p65 knockout is lethal, suggesting some functional redundancy among other members of the family.

Unprocessed, full-length p105 and p100 form stable dimers with other members of the NF-κB family hindering their NLS and sequestering the dimers within the cytoplasm. By poorly understood mechanisms, NF-κB activating stimuli promote the processing of p105 and p100 by the proteasome. This process resembles the degradation of IκB because it requires ubiquitinylation, although it is relatively slow and partial, yielding the p50 and p52 subunits. NF-κB dimers containing p52 or p50 are then transferred to the nucleus, where they bind to the specific recognition sequences. Proinflammatory cytokines modestly promote the processing of p105 and p100, but their contribution to the global regulation of NF-κB activity is unclear.

Table 1. Nuclear factor-κB (NF-κB) target genes

NF-κB target genes
IgG κ light chain
Cytokines
Interleukins IL-1, IL-2, IL-6, and receptors (IL-2R)
Chemokines IL-8, GRO, IP-10, MCP-1, RANTES, MIP-1, eotaxin
Colony-stimulating factors M-CSFm GM-CSF, G-CSF
TNF-α, TNF-β, interferon-β
Surface molecules involved in immune function
T-cell receptor β chain
β_2-microglobulin
Adhesion molecules (selectin, ICAM, VCAM, ELAM)
Major histocompatibility complex antigens (class I, class II)
Inflammatory enzymes
Inducible nitric oxide synthase
Inducible cyclooxygenase 2
5-Lipooxygenase
Cytosolic phospholipase A
Acute phase response genes
Serum amyloid A
Angiotensinogen
Complement (B,C4)
Ferritin
Tissue factor
Metalloproteinases
Oncogene, transcription factors and related genes
c-rel
c-myc
IFR
IκBα

IκB proteins. Seven members of the IκB family have been identified in mammals: IκBα, IκBβ, IκBγ, IκBε, Bcl-3, and the precursors of NF-κB1 (p105) and NF-κB2 (p100; Fig. 1). All of them contain six or seven ankyrin repeats by which they bind to the RHR of NF-κB masking the NLS, thereby sequestering NF-κB in the cyto-

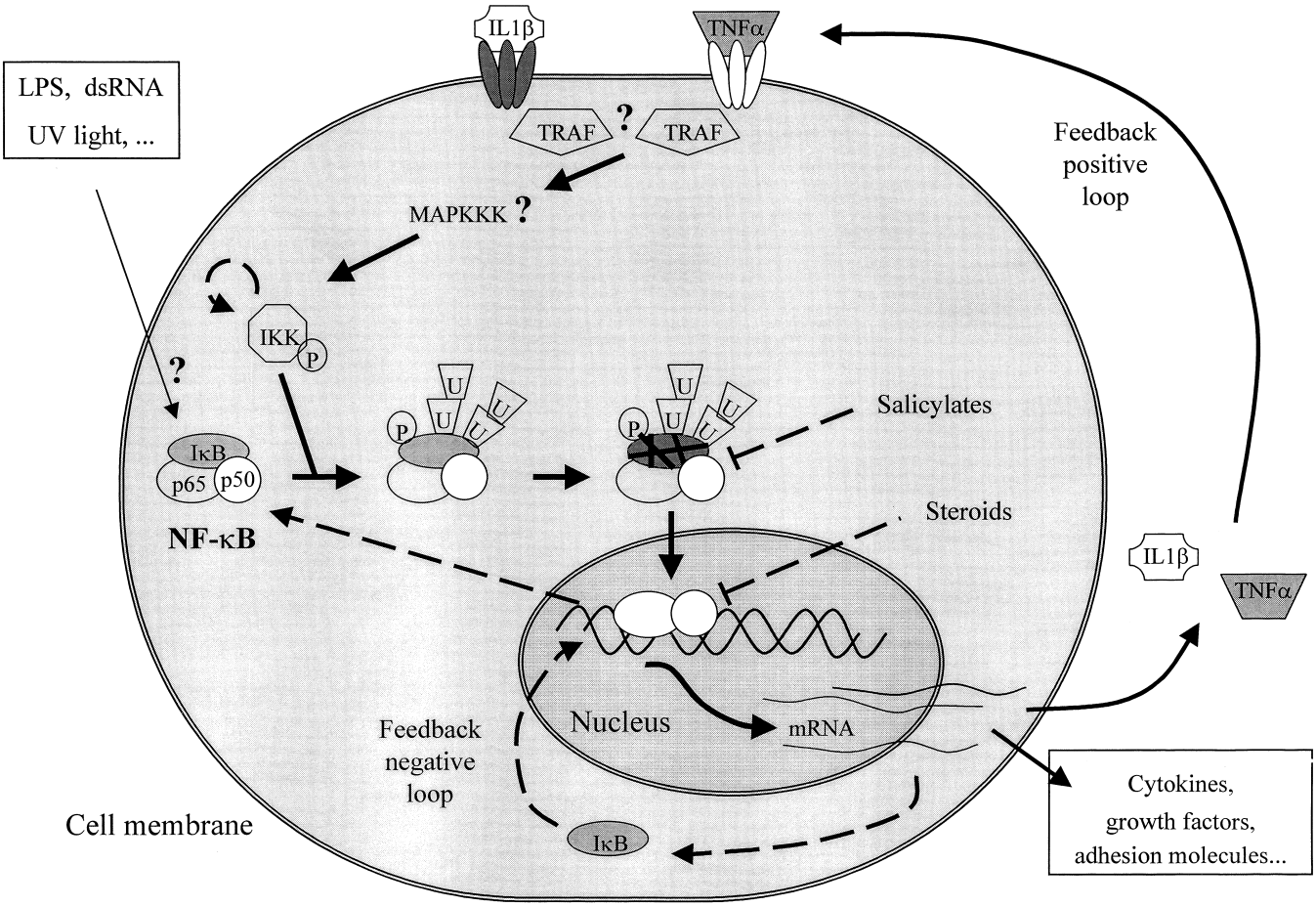

Fig. 2. Schematic representation of the main pathway of NF-κB activation. Proinflammatory cytokines such as TNF-α or IL-1β activate IκB kinase kinase (IKK) by phosphorylation, possibly through the action of TRAF (TNF-α receptor associated factor) and one mitogen-activated kinase kinase kinase (MAPKKK). Active IKK phosphorylates (P) IK, leading to its polyubiquitinylation (U) and degradation by the proteasome. As a result, free NF-κB is translocated into the nucleus where it binds and regulates the expression of target genes. Some of these genes code for inflammatory cytokines such as IL-1β and TNF-α, closing the circle of a self-sustained pro-inflammatory loop. On the other hand, NF-κB promotes the expression of IκB. Newly synthesized IκB binds to NF-κB in the nucleus and the NF-κB:IκB complex is exported to the cytoplasm, ending NF-κB action. Other stimuli [bacterial lipopolysaccharide (LPS), double-stranded RNA (dsRNA), ultraviolet (UV) light] result in NF-κB activation by poorly understood mechanisms. Salicylates inhibit the degradation of IκB by the proteasome. The physical association of the glucocorticoid receptor and NF-κB prevents the binding of NF-κB to DNA and the expression of inflammatory genes.

plasm and rendering it inactive. IκBα, IκBβ, and IκBε present amino-terminal regulatory regions required for stimulus-induced degradation. By far, IκBα is the best characterized member of the group, and most of the literature refers to IκBα when IκB is mentioned. The regulation and potential roles for the rest of IκB members of the family are poorly understood. IκBα knockout mice are born normally, but usually die a few days after birth because of extensive skin inflammation consistent with persistent NF-κB activation [10]. Interestingly, the IκBα gene expression is under the control of NF-κB. By inducing IκBα expression, active NF-κB promotes a negative regulatory loop able to terminate its own activity (Fig. 2). In fact, newly synthesized IκBα binds to active DNA-bound NF-κB, and the trimer is exported to the cytoplasm, ending the NF-κB–dependent transactivation [11].

Activation of NF-κB

NF-κB activation pathways. NF-κB can be activated by a number of physiological and nonphysiological stimuli, including cytokines, mitogens, viruses, mechanical, and oxidative stress, and a variety of chemical agents (Table 2). At present, it is unclear how various intracellular and extracellular stimuli converge to trigger NF-κB activation. However, several common features have been identified for the major activation pathways [9]. First, after a potent stimulus, such as the one provided by interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), or lipopolysaccharide (LPS), IκBα is rapidly degraded within minutes [2–5]. The degradation of IκB is achieved by a series of consecutive steps: (*1*) IκB phosphorylation at serine residues 32 and 36 by a specific kinase, (*2*) recognition of phosphorylated IκB by the type 3 ubiquitin-protein ligase complex, (*3*) polyubiquitinylation of IκB

Table 2. NF-κB stimuli

NF-κB stimuli
Bacterial lipopolysaccharide (LPS)
Virus: herpes, Cytomegalovirus, adenovirus, hepatitis B, human immunodeficiency virus
Chemical stress: oxidative, osmotic (glucose, albumin)
Mitogens: phorbol esters (PKC stimuli), serum, PHA
dsRNA
UV light
Cytokines: IL-1, IL-2, TNF-α, IFN-γ
Membrane protein activation: adhesion molecules, fibronectin, CD2, CD3, CD28, αβTCR
Vasoactive agents: angiotensin II, thrombin, endothelin
Lipoproteins (LDL, VLDL)

at lysines 21 and 22, and (*4*) degradation by the 26S proteasome. All four steps are required to unmask the nuclear recognition sequences of NF-κB, resulting in the translocation of active NF-κB into the nucleus, through the interaction with karyopherins (Fig. 2). In addition to this pathway, two other less well defined (atypical) pathways have been identified [9]. In both cases, NF-κB activation is much slower and weaker as compared with the typical one mentioned previously in this article. In one of the atypical pathways, hypoxia-induced NF-κB activation is achieved through IκBα tyrosine phosphorylation and removal by interaction with phosphoinositide 3-kinase (in the absence of degradation by the proteasome) [12]. In the second, ultraviolet light exposure promotes IκBα degradation by the proteasome in the absence of phosphorylation [13]. Additional mechanisms involved in the regulation of NF-κB have been described such as calpain-dependent IκBα proteolysis [14] and the direct phosphorylation of p65 [15], but their description is beyond the scope of the present article. Furthermore, many other stimuli (Table 2) promote the activation of NF-κB. However, the molecular mechanisms involved in the activation are poorly understood and will not be addressed in this review.

IκB phosphorylation. In the typical pathway, IκB kinase (IKK) seems to be the most likely responsible for IκB phosphorylation [16], although several additional candidates have been proposed. IKK is composed of one regulatory and two functional subunits. A detailed description of the characteristics of this kinase is beyond the scope of this article, and the readers are referred to a recent extensive review on the topic [9]. IKK is again activated through phosphorylation probably by a kinase belonging to the mitogen activated protein kinase kinase kinase family (MAPKKK). IKK appears to be the most likely point of convergence of many NF-κB activators.

At present, we have a reasonable, albeit incomplete, understanding of the pathway of activation of IKK by inflammatory cytokines. TNF-α and IL-1β activate NF-κB primarily through interaction with their respective type 1 receptors (TNFR1, IL-1R1) [17, 18]. Both receptors, through interaction with some adapter proteins, can bind different molecules of the TNF receptor-associated factors (TRAF) family (TRAF 2 for TNF-α and TRAF6 for IL-1β) [17, 19]. TRAF proteins can interact with MAPKK kinases likely involved in IKK phosphorylation [20, 21]. However, a full understanding of this pathway has not been achieved. For instance, it seems clear that the NF-κB activation by TNF-α or IL-1β involves the formation of reactive oxygen species and that this step can be inhibited by substances such as pyrrolidine dithiocarbamate (PDTC) [21–25]. The precise step in the activation cascade that requires the formation of reactive oxygen species has not been defined [26].

IκB kinase appears to be rate limiting for NF-κB activation. Most stimuli cause a peak of IKK activation limited in time [16]. More intense stimuli produce more transient responses. This regulation may have important physiological reasons: Intense and persistent NF-κB activation may result in deleterious effects. The termination of IKK activation is mediated by (auto) phosphorylation in the carboxy-terminal region, which takes place once the natural competitors for the phosphorylation (IκBs) are exhausted.

Nuclear localization and transcriptional activity. Once NF-κB is released from the inhibitory unit IκB, the dimer is translocated into the nucleus, probably by the interaction of the newly exposed NLSs with the karyopherins (responsible for nucleocytoplasmic transport). Different NF-κB dimers bind with diverse affinity to the variety of κB sites in DNA, resulting in subtle cell and gene-specific modulations of gene expression. NF-κB dimers do not promote gene transcription by themselves, but as a part of a complex of several coactivators such as the cAMP response element binding protein (CREB)-binding protein (CBP) [27]. Moreover, NF-κB interacts with a variety of other transcription factors in a positive or negative manner. One of the factors most commonly involved in the activation of NF-κB target genes is activator protein-1 (AP-1). Both NF-κB and AP-1 are activated in response to some proinflammatory stimuli, but they differ in their response to oxidative stress [28]. In addition, NF-κB can physically interact with other transcription factors. In some cases, such as with nuclear factor IL-6 (NF-IL6), the interaction results in a synergistic stimulation of the transcription of inflammatory genes (cytokines, inducible nitric oxide synthase) [29–35]. Interestingly, the physical association of NF-κB and the glucocorticoid receptor (another transcription factor) inhibits the binding of NF-κB to DNA and prevents NF-κB–dependent transactivation [36].

Thus far, all of the previous discussion has referred to NF-κB as if it were a single molecule. It is clear that several dozens of NF-κB:IκB complexes may exist within the cells, providing an enormous diversity of options and allowing for subtle regulations in the NF-κB system.

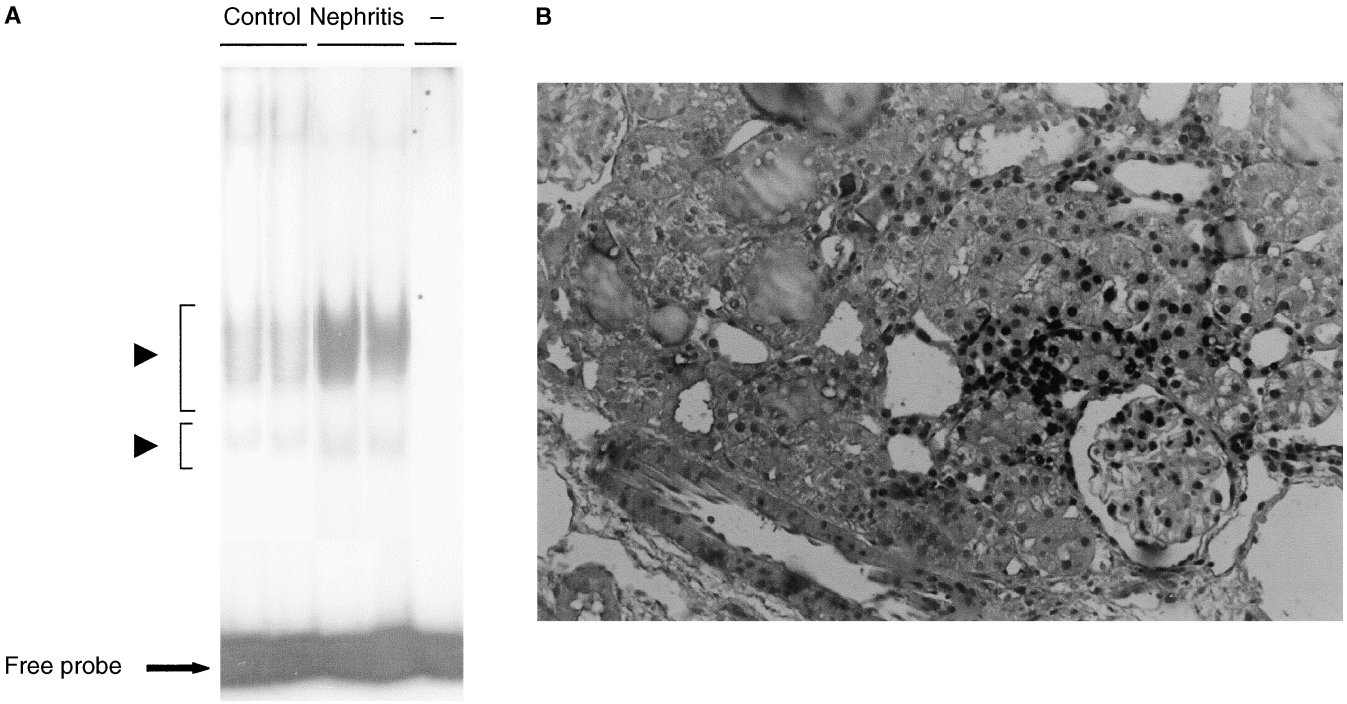

Fig. 3. In vivo detection of NF-κB activation. (*A*) EMSA. Incubation of renal cortex extracts from rats with tubulointerstitial nephritis demonstrates the presence of NF-κB activation by its ability to bind to radiolabeled consensus sequences and "retard" their electrophoretic mobility. Arrowheads indicate different activated NF-κB complexes. Lanes 1 and 2, control rats; lanes 3 and 4, rats with protein-overload proteinuria; lane 5, negative control of the assay, where no protein extract is added. Adapted from Morrissey and Klahr [7], with permission. (*B*) Southwestern histochemistry. Tissular active NF-κB is demonstrated by its ability to bind to a digoxigenin-labeled probe in rats with tubulointerstitial nephritis. Digoxigenin is developed by incubation with antidigoxigenin alkaline phosphatase conjugated antibodies. This technique allows the study of NF-κB activation, its structural distribution, and the identification of the specific cell types involved. Adapted from Hernández-Presa, Gómez-Guerrero, and Egido [38], with permission from the International Society of Nephrology.

NF-κB AND DISEASE

Detection of NF-κB activity

As mentioned previously in this article, NF-κB regulates the transcription of a large array of genes, in particular many of those that are involved in immune and inflammatory responses (Table 1). Until very recently, the evidence of the role of NF-κB in human disease was indirect and based on the altered expression of many of these genes in a variety of disorders and the crucial role of NF-κB in their regulation obtained basically from in vitro studies. The most common way of studying NF-κB activation relates to the ability of active NF-κB to bind to specific DNA sequences [37]. This can be assessed by changes in the electrophoretic mobility of DNA probes containing κB sites when incubated with cell extracts containing active NF-κB (Fig. 3A). This technique [electrophoretic mobility shift assay (EMSA)], although relatively straightforward for in vitro studies, has some difficulties that have precluded its wide use with tissue samples. Recently, two new techniques have begun to be used to assess the presence of active NF-κB in vivo. The first one is based on immunohistochemistry with specific antibodies that can recognize only the active form of NF-κB. More recently, the presence of active NF-κB in tissue sections has been demonstrated by incubation with labeled probes containing κB sites (Southwestern analysis; Fig. 3B) [38]. In addition to the detection of NF-κB activity, the last two methods are compatible with conventional histologic exams providing simultaneous structural information. In addition, the specific cell types showing increased NF-κB activation can be detected by simultaneous immunostaining.

NF-κB and inflammatory diseases

The role of NF-κB in a variety of nonrenal chronic inflammatory diseases, such as rheumatoid arthritis, asthma, or inflammatory bowel disease, has been recently reviewed [6] and will not be addressed in the present article. It must be stressed that in addition to these obvious inflammatory diseases, a lower degree of inflammation is increasingly considered important in other disorders such as atherosclerosis [39]. Indeed, markers of ongoing inflammation are extremely powerful predictors of the long-term presence of cardiovascular events [40–44]. In agreement with this, active NF-κB has been found in human and experimental atherosclerotic lesions [38, 45–48]. Intriguingly, most of the benefits of aspirin and pravastatin in the prevention of cardiovascular events

were restricted to patients with markers of inflammation [40, 42]. Indeed, long-term pravastatin treatment was associated with decreased C-reactive protein levels [49]. In addition, experimental evidence lends further support for an anti-inflammatory action of angiotensin-converting enzyme (ACE) inhibitors and statins through NF-κB modulation in models of atherosclerosis [48, 50, 51].

NF-κB and apoptosis

In recent years, NF-κB has been found to play an important role in the control of cell proliferation and death, more specifically in protecting from programmed cell death [17, 52–54]. It is very likely that the anti-apoptotic effects of NF-κB have been minimized by the fact that many studies have used TNF-α as the main stimulus for NF-κB activation. When the investigators have been able to dissect the different pathways activated by TNF-α, it has become evident that TNF-α may promote both cell death and survival. The anti-apoptotic effect of TNF-α is mediated by NF-κB, although the genes involved in this action are not well characterized [55–57]. In fact, p65 knockout mice die because of massive hepatic apoptosis [58]. Since apoptosis is increasingly recognized as a mechanism of disease and healing [59], disregulation of NF-κB–mediated cell survival signals may contribute to renal disease [60, 61].

NF-κB AND THE KIDNEY

NF-κB and kidney cells

Several studies have demonstrated that, in addition to infiltrating leukocytes, a variety of stimuli may induce NF-κB also in renal resident cells. Most of the studies have been conducted in mesangial cells [8, 21, 62–72]. Although a variety of NF-κB–like activities have been described by EMSA, most of the studies have identified p50 and p65 subunits as the predominant NF-κB components in mesangial cells. Bacterial LPS, IL-1β, and TNF-α are among the traditional stimuli that are consistently associated with increased mesangial NF-κB activation. Importantly, the increased NF-κB activation correlates with increased expression of a variety of inflammatory genes such as IL-1β, IL-6, IL-8, TNF-α, monocyte chemoattractant protein-1 (MCP-1), interferon invasive protein-10 (IP-10), and inducible nitric oxide synthase. Recently, angiotensin II and angiotensin II degradation products such as angiotensin III have been added to the list of physiologically important molecules that activate NF-κB [70, 73–75]. Angiotensin II seems to stimulate NF-κB activation by both angiotensin II type 1 and 2 (AT1 and AT2) receptors, as suggested by the inhibitory effect of specific antagonists. These new findings provide the basis for a new "anti-inflammatory" action of angiotensin converting enzyme (ACE) inhibitors and angiotensin II that may contribute to renal protection by a mechanism unrelated to vascular tone regulation. In addition to ACE inhibitors, another family of widely used drugs, the HMG-CoA reductase inhibitors or statins, have been found to inhibit mesangial cell NF-κB activation and the expression of pro-inflammatory genes [63, 76, 77].

Besides the pro-inflammatory actions of NF-κB activation, recent data also support a role for NF-κB in the control of mesangial cell proliferation and apoptosis directly or by analogy with the closely related vascular smooth muscle cells [34, 63, 77–83]. First, as mentioned, many mesangial cell mitogens such as angiotensin II activate NF-κB. Second, several agents that inhibit mesangial cell proliferation such as ACE inhibitors or statins also inhibit NF-κB activation. Finally, interference with NF-κB activation by different mechanisms results in inhibition of cell proliferation and apoptosis.

The NF-κB system has been less extensively studied in renal tubular epithelial cells, although it is clear that these cells may express a number of NF-κB–dependent genes. Recent studies have also demonstrated active NF-κB in renal tubular epithelial cells, as well as urothelial cells, after a variety of stimuli and, when assessed, in association with increased expression of inflammatory genes [84–87]. Recent reports indicate that high albumin concentrations may induce NF-κB activation, suggesting a mechanism for tubular injury in proteinuric states [88, 89]. In addition to classic stimuli, we have found that the vasoactive substances angiotensin II and endothelin also induce NF-κB activation in tubular epithelial cells (Gómez Garre, unpublished data).

The kidney presents other cell types common to other organs such as epithelial cells, vascular smooth muscle cells, fibroblasts, and leukocytes in which NF-κB activation may also play an important role. However, the regulation of NF-κB in these cells does not differ essentially from other organs and will not be specifically addressed in this review.

NF-κB and renal disease

In recent years, increased interest on NF-κB and improved detection techniques are providing direct evidence of the in vivo involvement of NF-κB in experimental and human renal diseases. In the following sections, we have arbitrarily classified the evidence according to the initial and/or major mechanism leading to renal damage.

Models of systemic inflammation. In rabbits, chronic inflammation by means of repeated injections of $AgNO_3$ is associated with renal NF-κB activation and increased serum levels of amyloid A [90]. In a murine model of endotoxemia, renal NF-κB activation correlated with increased expression of tissue factor and fibrin deposition [91]. In the same model, after somatic gene transfer with the NF-κB inhibitor IκBα, there was a reduction in tissue factor expression, fibrin deposition, and mortality, strongly

supporting a critical role for NF-κB in experimental endotoxemia. Interestingly, preliminary reports in a few patients with sepsis suggest that progressive NF-κB activation in circulating mononuclear cells is associated with increased mortality [91]. In a similar model, Khachigian, Collins, and Fries have described that LPS injection is associated with NF-κB activation and increased expression of vascular cell adhesion molecule-1 (VCAM-1) by mesangial cells [92]. In addition, treatment of mice with N-acetyl cysteine prevented NF-κB activation as well as VCAM-1 expression. These results demonstrate that some inhibitors of NF-κB activation in vitro may also work in vivo.

Nephritis. Coming down from models of systemic inflammation, NF-κB activation has also been described in a variety of models of nephritis. Sakurai et al have reported increased NF-κB activation, as well as AP-1 (by EMSA), in rat glomeruli shortly after nephrotoxic serum administration. The effect peaked at days 3 to 5, lasted for about two weeks, and was associated with increased expression of IL-1β, MCP-1, intercellular adhesion molecule-1, and inducible NOS, and the development of proteinuria. Treatment with PDTC or steroids prevented the previously described alterations [25, 93]. In antithymocyte glomerulonephritis, increased activation of NF-κB and AP-1 has been reported [94]. Finally, increased AP-1 but not NF-κB activity, as assessed by Southwestern immunohistochemistry, has been reported in immune complex nephritis [38, 72]. Whether this reflects a different pattern of transcription factor activation in this model or merely reflects a somewhat lower sensitivity of the Southwestern analysis is at present unclear. In any event, in this model treatment with ACE inhibitors resulted in the attenuation of NF-κB activation as assessed by EMSA [70]. Preliminary data from our laboratory further support a role of angiotensin II in vivo by the demonstration of increased glomerular activation of NF-κB and AP-1, as well as TNF-α, IL-1β, and IL-6 expression, after systemic administration of angiotensin II. Both angiotensin II receptors (AT1 and AT2) seem to be involved in these effects since they could be partially prevented by both AT1 and AT2 specific antagonists. Finally, preliminary data from patients with IgA nephropathy indicate that, as expected, active NF-κB is also found in human glomerulonephritis as assessed by Southwestern histochemistry (abstract; Ashizawa et al, *J Am Soc Nephrol* 10:95A, 1999). NF-κB staining was particularly prominent in areas with increased mesangial cellularity, suggesting again a role for NF-κB in controlling mesangial cell proliferation. Moreover, preliminary data from our laboratory (Mezzano and Egido, personal communication) have also confirmed the presence of activated NF-κB (Southwestern histochemistry) and increased expression of proinflammatory genes in renal biopsies from proteinuric patients with minimal change disease or membranous nephropathy.

Tubulointerstitial disorders. Hydronephrosis is followed by interstitial inflammation and eventually fibrosis. In models of unilateral ureteral obstruction, increased NF-κB and AP-1 activation has been detected by EMSA in cortical samples shortly after the intervention, but not in the contralateral kidney or in sham-operated animals [7, 73, 95, 96]. Again, the activation of transcription factors correlated with increased expression of target genes such as tissue factor, TNF-α and MCP-1 [97, 98]. Interestingly, pharmacological blockade of angiotensin actions (also as therapeutic agents once renal damage is already present) attenuated the activation of NF-κB and markedly improved tubulointerstitial damage.

Proteinuria. Proteinuric states are a clear demonstration of the interaction between primarily glomerular and interstitial disorders. In this regard, glomerular damage may result in proteinuria. Proteinuria by itself may result in interstitial damage (involving NF-κB activation). Finally, interstitial damage results in the loss of glomerular function. In adriamycin-induced nephrosis, activation of NF-κB in renal cortex has been demonstrated by EMSA [99]. In this model, when rats with established proteinuria were treated with PDTC for two weeks, NF-κB activation was reduced in parallel with an improvement in the score of interstitial damage.

Interestingly, in adriamycin-induced nephrosis, lovastatin treatment is associated with an attenuation of glomerular MCP-1 mRNA expression and monocyte infiltration [100]. In overload proteinuria, we have demonstrated both NF-κB and AP-1 activation by EMSA as well as by Southwestern histochemistry (Gómez Garre, unpublished data). Preliminary data from our laboratory show that the degree of proteinuria rather than the histologic picture (membranous nephropathy vs. minimal change disease) is a major determinant of NF-κB activation in patients with proteinuria (Mezzano and Egido, personal communication).

NF-κB AS A THERAPEUTIC TARGET

Since NF-κB plays such a pivotal role in the pathophysiology of a variety of disorders, it is conceivable that exogenous modulation of NF-κB activation may help to devise new therapeutic approaches. In this regard, the first target of actions is the prevention of the initial insult or stimuli leading to NF-κB activation. Most often the therapeutic action must take place once the initial injury has already taken place. However, we may still modulate some additional stimuli contributing to NF-κB activation. Rather surprisingly, some apparently NF-κB–unrelated agents are now emerging as potential modulators of the NF-κB pathway. For instance, ACE inhibitors, angiotensin II antagonists, and HMG-CoA reductase inhibitors

also may exhibit salutary effects in conditions in which neither hypertension nor hyperlipidemia are pathogenetically important, perhaps by their ability to attenuate NF-κB activation.

When the original or ongoing stimuli for NF-κB activation cannot be fully controlled, it is still possible to lessen their effects by interfering with the pathways leading to NF-κB activation. This is the basis for the treatment with antioxidants to prevent NF-κB activation. The biochemical characterization of the IKK may prompt the search for specific inhibitors. Recent experimental studies have also been successful in preventing NF-κB activation by adenovirus-mediated transfer of IκBα [91]. Additionally, new drugs are targeted to prevent the degradation of IκB and hence the nuclear translocation of active NF-κB. For instance, preliminary data suggest that the interference with NF-κB activation by specific inhibitors of the proteasome degradation of IκB may have favorable effects in asthma [101]. Some widely used immunosuppressive agents such as cyclosporine A and tacrolimus exert their anti-inflammatory action at least partially by preventing IκB degradation and the subsequent translocation of active NF-κB into the nucleus [102–104]. This effect is not restricted to lymphocytes, since similar findings have been reported in renal cells [67, 105, 106]. Finally, once active NF-κB reaches the nucleus, it is still possible to modulate the transcriptional activation of genes by the interaction with other transcription factors. In fact, we have been manipulating the NF-κB system for decades without knowing it by the use of glucocorticoids.

As mentioned previously in this article, the physical interaction of NF-κB and the glucocorticoid receptor prevent the binding of NF-κB to DNA and the subsequent gene transactivation [36]. In addition, glucocorticoids also stimulate the transcription of the inhibitory IκBα [107, 108]. The interaction between steroids and the NF-κB system provides a molecular basis for the pleiotropic effects of steroids in pro-inflammatory genes lacking steroid response elements. In fact, this appears to be a major mechanism responsible for the anti-inflammatory action of steroids [6].

However, all of these are unselective attempts to block NF-κB activation. Given the importance of NF-κB in the immune response, such an approach may be dangerous. A better understanding of the mechanisms of specific activation of the various forms of NF-κB in different cell lines, as well as the interaction with other transcription factors, may provide the means to achieve a more selective blockade.

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REFERENCES

1. Sen R, Baltimore D: Inducibility of the immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. *Cell* 47:921–928, 1986
2. Baeuerle PA, Henkel T: Function and activation of NF-κB in the immune system. *Annu Rev Immunol* 12:141–179, 1994
3. Baldwin AS: The NF-κB and IκB proteins: New discoveries and insights. *Annu Rev Immunol* 14:649–681, 1996
4. Ghosh S, May MJ, Kopp EB: NF-κB and Rel proteins: Evolutionary conserved mediators of immune responses. *Annu Rev Immunol* 16:225–260, 1998
5. Grilli M, Chiu JJS, Lenardo M: NF-κB and Rel: Participants in a multiform transcriptional regulatory system. *Int Rev Cytol* 143:1–62, 1993
6. Barnes PJ, Karin M: Nuclear factor-κB: A pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336:1066–1071, 1997
7. Morrissey J, Klahr S: Transcription factor NF-κB regulation of renal fibrosis during ureteral obstruction. *Semin Nephrol* 18:603–611, 1998
8. Massy ZA, Guijarro C, O'Donnell MP, *et al*: The central role of nuclear factor-κB in mesangial cell activation. *Kidney Int* 56:S76–S79, 1999
9. Rothwarf DM, Karin M: The NF-κB activation pathway: A paradigm in information transfer from membrane to nucleus. *Sci Signal Transduction Knowledge Environ* 5:1–16, 1999, *http://www.stke.org/cgi/content/full/OC_sigtrans;1999/5/re1*
10. Klement JF, Rice NR, Car BD, *et al*: IκBα deficiency results in a sustained NF-κB response and severe widespread dermatitis in mice. *Mol Cell Biol* 16:2341–2349, 1996
11. Chiao PJ, Miyamoto S, Verma IM: Autoregulation of IκBα activity. *Proc Natl Acad Sci USA* 91:28–32, 1994
12. Imbert V, Rupec RA, Livolsi A, *et al*: Tyrosine phosphorylation of IκBα activates NF-κB without proteolytic degradation of IκBα. *Cell* 86:787–798, 1996
13. Li N, Karin M: Ionizing radiation and short wavelength UV activate NF-κB through two distinct mechanisms. *Proc Natl Acad Sci USA* 95:13012–13017, 1998
14. Shumway SD, Maki M, Miyamoto S: The PEST domain of IκBα is necessary and sufficient for in vitro degradation by mu-calpain. *J Biol Chem* 274:30874–30881, 1999
15. Naumann M, Scheidereit C: Activation of NF-κB *in vivo* is regulated by multiple phosphorylations. *EMBO J* 13:4597–4607, 1994
16. Didonato JA, Hayakawa M, Rothwarf DM, *et al*: A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. *Nature* 388:548–554, 1997
17. Hsu H, Shu HB, Pan MG, *et al*: TRADD-TRAF2 and TRADD–FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 84:299–308, 1996
18. Stylianou EO, Neill LA, Rawlinson L, *et al*: Interleukin 1 induces NF-κB through its type I but not its type II receptor in lymphocytes. *J Biol Chem* 267:15836–15841, 1992
19. Cao Z, Xiong J, Takeuchi M, *et al*: TRAF6 is a signal transducer for interleukin-1. *Nature* 383:443–446, 1996
20. Rovin BH, Wilmer WA, Danne M, *et al*: The mitogen-activated protein kinase p38 is necessary for interleukin 1β-induced monocyte chemoattractant protein 1 expression by human mesangial cells. *Cytokine* 11:118–126, 1999
21. Wilmer WA, Tan LC, Dickerson JA, *et al*: Interleukin-1β induction of mitogen-activated protein kinases in human mesangial cells: Role of oxidation. *J Biol Chem* 272:10877–10881, 1997
22. Satriano JA, Shuldiner M, Hora K, *et al*: Oxygen radicals as second messengers for expression of the monocyte chemoattractant protein, JE/MCP-1, and the monocyte colony-stimulating

factor, CSF-1, in response to tumor necrosis factor-α and immunoglobulin G: Evidence for involvement of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase. *J Clin Invest* 92:1564–1571, 1993
23. Satriano J, Schlöndorff D: Activation and attenuation of transcription factor NF-κB in mouse glomerular mesangial cells in response to tumor necrosis factor-α, immunoglobulin G, and adenosine 3′:5′-cyclic monophosphate: Evidence for involvement of reactive oxygen species. *J Clin Invest* 94:1629–1636, 1994
24. Rovin BH, Dickerson JA, Tan LC, *et al*: Modulation of IL-1-induced chemokine expression in human mesangial cells through alterations in redox status. *Cytokine* 9:178–186, 1997
25. Sakurai H, Hisada Y, Ueno M, *et al*: Activation of transcription factor NF-κB in experimental glomerulonephritis in rats. *Biochim Biophys Acta* 1316:132–138, 1996
26. Suzuki YJ, Mizuno M, Packer L: Signal transduction for nuclear factor-κB activation: Proposed location of antioxidant-inhibitable step. *J Immunol* 153:5008–5015, 1994
27. Sheppard KA, Rose DE, Haque ZK, *et al*: Transcriptional activation by NF-κB requires multiple coactivators. *Moll Cell Biol* 1999:6367–6378, 1999
28. Schenk H, Klein M, Erdbrugger W, *et al*: Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-κB and AP-1. *Proc Natl Acad Sci USA* 91:1672–1676, 1994
29. Akira S, Isshiki H, Sugita T, *et al*: A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. *EMBO J* 9:1897–1906, 1990
30. Leclair KP, Blanar MA, Sharp PA: The p50 subunit of NF-κB associates with the NF-IL6 transcription factor. *Proc Natl Acad Sci USA* 89:8145–8149, 1992
31. Matsusaka T, Fujikawa K, Nishio Y, *et al*: Transcription factors NF-IL6 and NF-κB synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. *Proc Natl Acad Sci USA* 90:10193–10197, 1993
32. Dunn SM, Coles LS, Lang RK, *et al*: Requirement for nuclear factor (NF)-κB p65 and NF-interleukin-6 binding elements in the tumor necrosis factor response region of the granulocyte colony-stimulating factor promoter. *Blood* 83:2469–2479, 1994
33. Godambe SA, Chaplin DD, Takova T, *et al*: Upstream NFIL-6-like site located within a DNase I hypersensitivity region mediates LPS-induced transcription of the murine interleukin-1β gene. *J Immunol* 153:143–152, 1994
34. Grassl C, Luckow B, Schlondorff D, *et al*: Transcriptional regulation of the interleukin-6 gene in mesangial cells. *J Am Soc Nephrol* 10:1466–1477, 1999
35. Dlaska M, Weiss G: Central role of transcription factor NF-IL6 for cytokine and iron-mediated regulation of murine inducible nitric oxide synthase expression. *J Immunol* 162:6171–6177, 1999
36. Ray A, Prefontaine KE: Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. *Proc Natl Acad Sci USA* 91:752–756, 1994
37. Papavassiliou AG: Molecular medicine: Transcription factors. *N Engl J Med* 332:45–47, 1995
38. Hernández-Presa MA, Gómez-Guerrero C, Egido J: In situ non-radioactive detection of nuclear factors in paraffin sections by Southwestern histochemistry. *Kidney Int* 55:209–214, 1999
39. Ross R: Atherosclerosis: An inflammatory disease. *N Engl J Med* 340:115–126, 1999
40. Ridker PM, Cushman M, Stampfer MJ, *et al*: Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 336:973–979, 1997
41. Ritchie ME: Nuclear factor-κB is selectively and markedly activated in humans with unstable angina pectoris. *Circulation* 98:1707–1713, 1998
42. Ridker PM, Rifai N, Pfeffer MA, *et al*: Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels: Cholesterol Recurrent Events (CARE) Investigators. *Circulation* 98:839–844, 1998
43. Koenig W, Sund M, Fröhlich M, *et al*: C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: Results from the MONICA (monitoring trends and determinants in cardiovascular disease) augsburg cohort study, 1984 to 1992. *Circulation* 99:237–242, 1999
44. Ridker PM, Hennekens CH, Buring JE, *et al*: C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 342:836–843, 2000
45. Bourcier T, Sukhova G, Libby P: The nuclear factor-κB signaling pathway participates in dysregulation of vascular smooth muscle cells in vitro and in human atherosclerosis. *J Biol Chem* 272:15817–15824, 1997
46. Brand K, Page S, Rogler G, *et al*: Activated transcription factor nuclear factor-κB is present in the atherosclerotic lesion. *J Clin Invest* 97:1715–1722, 1996
47. Brand K, Page S, Walli AK, *et al*: Role of nuclear factor-κB in atherogenesis. *Exp Physiol* 82:297–304, 1997
48. Bustos C, Hernández-Presa MA, Ortego M, *et al*: HMG-CoA reductase inhibition by atorvastatin reduces neointimal inflammation in a rabbit model of atherosclerosis. *J Am Coll Cardiol* 32:2057–2064, 1998
49. Ridker PM, Rifai N, Pfeffer MA, *et al*: Long-term effects of pravastatin on plasma concentration of C-reactive protein: The Cholesterol Recurrent Events (CARE) Investigators. *Circulation* 100:230–235, 1999
50. Hernández-Presa MA, Bustos C, Ortego M, *et al*: Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-κB activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. *Circulation* 95:1532–1541, 1997
51. Hernández-Presa MA, Bustos C, Ortego M, *et al*: ACE inhibitor quinapril reduces the arterial expression of NF-κB-dependent proinflammatory factors but not of collagen I in a rabbit model of atherosclerosis. *Am J Pathol* 153:1825–1837, 1998
52. Beg AA, Baltimore D: An essential role for NF-κB in preventing TNF-α-induced cell death. *Science* 274:782–748, 1996
53. Liu Z, Hsu H, Goeddel DV, Karin M: Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. *Cell* 87:565–576, 1996
54. Van Antwerp DJ, Martin SJ, Kafri T, *et al*: Suppression of TNF-α-induced apoptosis by NF-κB. *Science* 274:787–789, 1996
55. Wang CY, Mayo MW, Korneluk RG, *et al*: NF-κB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 281:1680–1683, 1998
56. Wu MX, Ao Z, Prasad KV, *et al*: IEX-1L, an apoptosis inhibitor involved in NF-κB-mediated cell survival. *Science* 281:998–1001, 1998
57. Bertrand F, Desbois-Mouthon C, Cadoret A, *et al*: Insulin antiapoptotic signaling involves insulin activation of the nuclear factor κB-dependent survival genes encoding tumor necrosis factor receptor-associated factor 2 and manganese-superoxide dismutase. *J Biol Chem* 274:30596–30602, 1999
58. Beg AA, Sha WC, Bronson RT, *et al*: Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. *Nature* 376:167–170, 1995
59. Thompson CB: Apoptosis in the pathogenesis and treatment of disease. *Science* 267:1456–1462, 1995
60. Ortiz A, Lorz C, Catalan MP, *et al*: Role and regulation of apoptotic cell death in the kidney: Y2K update. *Front Biosci* 5:D735–D749, 2000
61. Lieberthal W, Levine JS: Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. *Am J Physiol* 271:F477–F488, 1996
62. Guijarro C, Kim Y, Kasiske BL, *et al*: Central role of the transcription factor nuclear factor-κB in mesangial cell production of chemokines. *Contrib Nephrol* 120:210–218, 1997
63. Guijarro C, Kim Y, Schoonover CM, *et al*: Lovastatin inhibits lipopolysaccharide-induced NF-κB activation in human mesangial cells. *Nephrol Dial Transplant* 11:990–996, 1996
64. Khachigian LM, Collins T, Fries JWU: Nuclear factor-κB mediates induction of vascular cell adhesion molecule-1 in glomerular mesangial cells. *Biochem Biophys Res Commun* 206:462–467, 1995
65. Rovin BH, Tan LC, Dickerson JA, *et al*: Activation of nuclear factor-κB correlates with MCP-1 expression by human mesangial cells. *Kidney Int* 48:1263–1271, 1995

66. Tetsuka T, Srivastava SK, Morrison AR: Tyrosine kinase inhibitors, genistein and herbimycin A, do not block interleukin-1β-induced activation of NF-κB in rat mesangial cells. *Biochem Biophys Res Commun* 218:808–812, 1996
67. Walker G, Kunz D, Pignat W, *et al*: Suppression by cyclosporin A of interleukin 1β-induced expression of group II phospholipase A2 in rat renal mesangial cells. *Br J Pharmacol* 121:787–793, 1997
68. Auwardt RB, Mudge SJ, Chen CG, *et al*: Regulation of nuclear factor κB by corticosteroids in rat mesangial cells. *J Am Soc Nephrol* 9:1620–1628, 1998
69. Duque N, Gómez-Guerrero C, Egido J: Interaction of IgA with Fc α receptors of human mesangial cells activates transcription factor nuclear factor-κB and induces expression and synthesis of monocyte chemoattractant protein-1, IL-8, and IFN-inducible protein 10. *J Immunol* 159:3474–3482, 1997
70. Ruiz-Ortega M, Bustos C, Hernández-Presa MA, *et al*: Angiotensin II participates in mononuclear cell recruitment in experimental immune complex nephritis through nuclear factor-κB activation and monocyte chemoattractant protein-1 synthesis. *J Immunol* 161:430–439, 1998
71. Dorsam G, Taher MM, Valerie KC, *et al*: Diphenyleneiodium chloride blocks inflammatory cytokine-induced up-regulation of group IIA phospholipase A(2) in rat mesangial cells. *J Pharmacol Exp Ther* 292:271–279, 2000
72. Gómez-Guerrero C, Duque N, Casado MT, *et al*: Administration of IgG Fc fragments prevents glomerular injury in experimental immune complex nephritis. *J Immunol* 164:2092–2101, 2000
73. Klahr S, Morrissey JJ: Comparative study of ACE inhibitors and angiotensin II receptor antagonists in interstitial scarring. *Kidney Int* 52(Suppl 63):S111–S114, 1997
74. Ruiz-Ortega M, Lorenzo O, Egido J: Angiotensin III increases MCP-1 and activates NF-κB and AP-1 in cultured mesangial and mononuclear cells. *Kidney Int* 57:2285–2298, 2000
75. Ruiz-Ortega M, Lorenzo O, Ruperez M, *et al*: Angiotensin II activates nuclear transcription factor κB through AT(1) and AT(2) in vascular smooth muscle cells: Molecular mechanisms. *Circ Res* 86:1266–1272, 2000
76. Massy ZA, Kim Y, Guijarro C, *et al*: Low-density lipoprotein-induced expression of interleukin-6, a marker of human mesangial cell inflammation: Effects of oxidation and modulation by lovastatin. *Biochem Biophys Res Commun* 267:536–540, 2000
77. Ortego M, Bustos C, Hernández-Presa MA, *et al*: Atorvastatin reduces NF-κB activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. *Atherosclerosis* 147: 253–261, 1999
78. Bellas RE, Lee JS, Sonenshein GE: Expression of a constitutive NF-κB-like activity is essential for proliferation of cultured bovine vascular smooth muscle cells. *J Clin Invest* 96:2521–2527, 1995
79. Tsai JC, Jain M, Hsieh CM, *et al*: Induction of apoptosis by pyrrolidinedithiocarbamate and N-acetylcysteine in vascular smooth muscle cells. *J Biol Chem* 271:3667–3670, 1996
80. Massy ZA, Guijarro C, O'Donnell MP, *et al*: Regulation of mesangial cell proliferation by the mevalonate pathway. *Contrib Nephrol* 120:191–196, 1997
81. O'Donnell MP, Kasiske BL, Kim Y, *et al*: Lovastatin inhibits proliferation of rat mesangial cells. *J Clin Invest* 91:83–87, 1993
82. Sugiyama H, Savill JS, Kitamura M, *et al*: Selective sensitization to tumor necrosis factor-α-induced apoptosis by blockade of NF-κB in primary glomerular mesangial cells. *J Biol Chem* 274:19532–19537, 1999
83. Guijarro C, Blanco-Colio LM, Ortego M, *et al*: 3-Hydroxy-3-methylglutaryl coenzyme A reductase and isoprenylation inhibitors induce apoptosis of vascular smooth muscle cells in culture. *Circ Res* 83:490–500, 1998
84. Oertli B, Beck-Schimmer B, Fan X, *et al*: Mechanisms of hyaluronan-induced up-regulation of ICAM-1 and VCAM-1 expression by murine kidney tubular epithelial cells: Hyaluronan triggers cell adhesion molecule expression through a mechanism involving activation of nuclear factor-κB and activating protein-1. *J Immunol* 161:3431–3437, 1998
85. Otieno MA, Anders MW: Cysteine S-conjugates activate transcription factor NF-κB in cultured renal epithelial cells. *Am J Physiol* 273:F136–F143, 1997
86. Woods JS, Ellis ME, Dieguez-Acuna FJ, *et al*: Activation of NF-κB in normal rat kidney epithelial (NRK52E) cells is mediated via a redox-insensitive, calcium-dependent pathway. *Toxicol Appl Pharmacol* 154:219–227, 1999
87. Rackley RR, Bandyopadhyay SK, Fazeli-Matin S, *et al*: Immunoregulatory potential of urothelium: Characterization of NF-κB signal transduction. *J Urol* 162:1812–1816, 1999
88. Zoja C, Donadelli R, Colleoni S, *et al*: Protein overload stimulates RANTES production by proximal tubular cells depending on NF-κB activation. *Kidney Int* 53:1608–1615, 1998
89. Wang Y, Rangan GK, Tay YC, *et al*: Induction of monocyte chemoattractant protein-1 by albumin is mediated by nuclear factor κB in proximal tubule cells. *J Am Soc Nephrol* 10:1204–1213, 1999
90. Ray A, Ray BK: Persistent expression of serum amyloid A during experimentally induced chronic inflammatory condition in rabbit involves differential activation of SAF, NF-κB, and C/EBP transcription factors. *J Immunol* 163:2143–2150, 1999
91. Böhrer H, Qiu F, Zimmermann T, *et al*: Role of NFκB in the mortality of sepsis. *J Clin Invest* 100:972–985, 1997
92. Khachigian LM, Collins T, Fries JW: N-acetyl cysteine blocks mesangial VCAM-1 and NF-κB expression in vivo. *Am J Pathol* 151:1225–1229, 1997
93. Sakurai H, Shigemori N, Hisada Y, *et al*: Suppression of NF-κB and AP-1 activation by glucocorticoids in experimental glomerulonephritis in rats: Molecular mechanisms of anti-nephritic action. *Biochim Biophys Acta* 1362:252–262, 1997
94. Seto M, Kim S, Yoshifusa H, *et al*: Effects of prednisolone on glomerular signal transduction cascades in experimental glomerulonephritis. *J Am Soc Nephrol* 9:1367–1376, 1998
95. Wendt T, Zhang YM, Bierhaus A, *et al*: Tissue factor expression in an animal model of hydronephrosis. *Nephrol Dial Transplant* 10:1820–1828, 1995
96. Morrissey JJ, Klahr S: Rapid communication. Enalapril decreases nuclear factor κB activation in the kidney with ureteral obstruction. *Kidney Int* 52:926–933, 1997
97. Guo G, Morrissey J, McCracken R, *et al*: Role of TNFR1 and TNFR2 receptors in tubulointerstitial fibrosis of obstructive nephropathy. *Am J Physiol* 277:F766–F772, 1999
98. Morrissey JJ, Klahr S: Differential effects of ACE and AT1 receptor inhibition on chemoattractant and adhesion molecule synthesis. *Am J Physiol* 274:F580–F586, 1998
99. Rangan GK, Wang Y, Tay YC, *et al*: Inhibition of nuclear factor-κB activation reduces cortical tubulointerstitial injury in proteinuric rats. *Kidney Int* 56:118–134, 1999
100. Park YS, Guijarro C, Kim Y, *et al*: Lovastatin reduces glomerular macrophage influx and expression of monocyte chemoattractant protein-1 mRNA in nephrotic rats. *Am J Kidney Dis* 31:190–194, 1998
101. Elliott PJ, Pien CS, McCormack TA, *et al*: Proteasome inhibition: A novel mechanism to combat asthma. *J Allergy Clin Immunol* 104:294–300, 1999
102. Hughes K, Antonsson A, Grundstrom T: Calmodulin dependence of NFκB activation. *FEBS Lett* 441:132–136, 1998
103. Meyer S, Kohler NG, Joly A: Cyclosporine A is an uncompetitive inhibitor of proteasome activity and prevents NF-κB activation. *FEBS Lett* 413:354–358, 1997
104. Marienfeld R, Neumann M, Chuvpilo S, *et al*: Cyclosporin A interferes with the inducible degradation of NF-κB inhibitors, but not with the processing of p105/NF-κB1 in T cells. *Eur J Immunol* 27:1601–1609, 1997
105. Holschermann H, Durfeld F, Maus U, *et al*: Cyclosporine a inhibits tissue factor expression in monocytes/macrophages. *Blood* 88:3837–3845, 1996
106. Kunz D, Walker G, Eberhardt W, *et al*: Interleukin 1β-induced expression of nitric oxide synthase in rat renal mesangial cells is suppressed by cyclosporin A. *Biochem Biophys Res Commun* 216:438–446, 1995
107. Auphan N, Didonato JA, Rosette C, *et al*: Immunosuppression by glucocorticoids: Inhibition of NF-κB activity through induction of I κB synthesis. *Science* 270:286–290, 1995
108. Scheinman RI, Cogswell PC, Lofquist AK, *et al*: Role of transcriptional activation of I κB α in mediation of immunosuppression by glucocorticoids. *Science* 270:283–286, 1995