The central role of nuclear factor-κB in mesangial cell activation

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Background. Nuclear factor-κB (NF-κB) is a family of transcription factors that is recognized by the κB enhancer element. Numerous proinflammatory genes have binding sites for NF-κB, and the products of these genes are an integral part of cellular activation and inflammatory response systems. Because there is a close relationship between NF-κB and mediators of cell activation, it is possible that a disruption of NF-κB–activating pathways may effectively influence mesangial cell activation.

Methods. We reviewed available studies related to both NF-κB and mesangial cells in order to provide evidence for the role of NF-κB in mesangial cell activation.

Results. Studies reported by this laboratory and others showed that various experimental maneuvers that modulate NF-κB activation result in a parallel modulation of proinflammatory molecule production in cultured mesangial cells. Likewise, the ability of the inhibitors of NF-κB activation to down-regulate the inflammatory response in animal models of renal disease has been recently demonstrated.

Conclusions. These data suggest a pivotal role of NF-κB in mesangial cell activation and designate it as an obvious target for the modulation of this activation. Studies are necessary to characterize the role of NF-κB in human renal injury.

Mesangial cell activation has been demonstrated to occur in a large number of experimental models of renal disease, as well as in various human renal diseases [1, 2]. After mesangial injury caused by any of several factors, including immunological and metabolic factors, a plethora of adhesion molecules, chemokines, proinflammatory cytokines, and inflammatory enzymes appears to cause or at least contribute to the inflammatory and proliferative responses of the mesangial cell [1, 2]. These proinflammatory molecules are, in part, produced by mesangial cells [1, 2]. Therapeutic approaches aimed at antagonizing individual proinflammatory molecules, for example, by using neutralizing antibodies against platelet-derived growth factor (PDGF), have proven effective in reducing proliferation in experimental models [3]. However, because mesangial cell activation is likely to reflect a mosaic of numerous proinflammatory molecules, it may be difficult to target only one or two proinflammatory molecules for the therapy of renal disease. On the other hand, it may be possible to block intracellular signals, which are common to several proinflammatory molecules, and thereby inhibit induction and/or transcription of proinflammatory molecule genes that cause mesangial cell activation.

The regulatory repertoire of transcription factor families appears to be pivotal for the modulation of gene expression. One such regulatory network is composed of a family of ubiquitous gene transactivators known as nuclear factor-κB (NF-κB). Numerous proinflammatory genes have binding sites for NF-κB, and the products of these genes are an integral part of cellular activation and inflammatory response systems [4, 5]. Because there is a close relationship between NF-κB and mediators of cell activation, it is possible that disruption of NF-κB–activating pathways may effectively influence mesangial cell activation.

NUCLEAR FACTOR-κB

Nuclear factor-κB is a family of dimeric proteins that belong to the Rel family. They are distinguished by a homologous domain of approximately 300 amino acids, the Rel homology domain, that determines dimerization, nuclear localization, and binding to κB element (5′-GGGPyNNPyPyCC-3′) in the promoter and enhancer regions of target genes. By far the most common type of dimer comprises a p50 subunit bound to a p65 (Rel A) subunit; however, other Rel proteins (c-Rel, Rel B, v-Rel, and p52) are also described, and various combinations are possible [4–6]. It should be noted that p50 and

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p52 are synthesized as precursor proteins of 105 and 100 kDa, respectively, and are generated by proteolytic processing [4–6]. The other Rel proteins are not synthesized as precursors. NF-κB is normally held in the cytoplasm of unstimulated cells in an inactive form bound to an inhibitory protein, IκB, of which several types are recognized (IκB-α, IκB-β, and IκB-γ) [4–6]. IκB molecules contain ankryin-like repeats that interact with similar elements in Rel proteins and cover the nuclear localization signal [4–6].

Following stimulation of cells by several stimuli, including cytokines [for example, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)], lipopolysaccharide (LPS), immunoglobulin aggregates, or reactive oxygen species (ROS), IκB is phosphorylated. Phosphorylation of IκB occurs at two serine residues in the amino-terminal portion of IκB proteins, probably through the action of three newly described kinases (that is, NF-κB–inducing kinase and IκB kinase α and β) and the intracellular production of ROS [4–6]. Phosphorylated IκB is then ubiquitinated at nearby lysin residues and rapidly degraded by proteasomes [4–6]. The release of NF-κB from IκB results in the translocation of NF-κB into the nucleus, where it binds to specific sequences in the promoter regions of target genes. Remarkably, a large number of genes appear to be targets for the activation by NF-κB, among them those coding for adhesion molecules [for example, vascular-cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1)], chemokines [for example, monocyte chemotactant protein-1 (MCP-1) and IL-8], proinflammatory cytokines (for example, IL-1β and IL-6), and inflammatory enzymes [for example, inducible nitric oxide synthase (iNOS) and phospholipase A2 (PLA2)], which are involved in the inflammatory and proliferative responses of cells [4–6]. On the other hand, NF-κB is involved in antiapoptotic genomic response, although the putative antiapoptotic genes that are activated by NF-κB in response to apoptotic stimuli (for example, TNF-α) remain to be identified [4–6]. It should be noted that the IκB-α gene itself has a κB site and is activated by NF-κB, thus leading to increased synthesis of IκB-α and termination of NF-κB activation [4–6]. There are minor changes in the base composition of the NF-κB sites and surrounding sequences, and it is cooperation with other transcription factors, such as activator protein 1 (AP-1) and NF-IL6, may account for subtle regulation of specific genes in different cell types.

**NUCLEAR FACTOR-κB AND MESANGIAL CELL ACTIVATION**

**In vitro evidence**

Several studies have shown that the well-known inducers of NF-κB activation also stimulate NF-κB activation in cultured mesangial cells [7–17]. We have demonstrated that LPS stimulated human mesangial cells to exhibit an NF-κB–like activity, as assessed by electrophoretic mobility shift assays (EMSA) and supershift assays with antibodies against p50 and p65 subunits of NF-κB [7]. Two recent works have established that the exposure of mouse and human mesangial cells to aggregates of immunoglobulin G and A rapidly activated a NF-κB complex constituted of p50 and p65 subunits [8, 9]. IL-1β and TNF-α have also been shown to induce NF-κB activation in rodent and human mesangial cells [8, 10–17]. The generation of superoxide anion by xanthine oxidase has been shown to induce NF-κB activation in mouse mesangial cells [8]. However, neither hydrogen peroxide nor diamide were capable to induce NF-κB activation in mouse or human mesangial cells [8, 18]. Hence, proinflammatory molecules that stimulate mesangial cell activation also induce NF-κB activation in such cells.

The importance of NF-κB activation in mesangial cell inflammation and proliferation has also been demonstrated indirectly by the modulation of NF-κB activation in mesangial cell cultures. Rovin et al have demonstrated that the inhibition of NF-κB activation by the protease inhibitor tosyl-phe-chloromethylketone, which prevents the proteolytic degradation of IκB, or by an antisense oligonucleotide to p65 was correlated with a dose-dependent reduction in IL-1β–induced MCP-1 mRNA expression in human mesangial cells [10]. Duque et al have shown that human mesangial cell pretreatment with the antioxidant pyrrolidine dithiocarbamate (PDTC) inhibited NF-κB activation and MCP-1, IL-8, and interferon-inducible protein 10 mRNA expression [9]. Kunz et al found an association between the suppression of IL-1β–induced iNOS expression and the reduction of NF-κB activation by cyclosporine A in rat mesangial cells [11]. We recently reported that lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitor, prevents LPS-induced NF-κB activation in mesangial cells and that lovastatin inhibits mesangial cell expression and production of MCP-1 and IL-6 (abstract: Massy et al, *J Am Soc Nephrol* 7:1859, 1996) [7]. On the other hand, a recent study has shown that FK506 activated NF-κB and induced the production of IL-6 in mouse mesangial cells (in contrast to the situation in T cells) [19]. Thus, various experimental maneuvers that modulate NF-κB activation result in a parallel modulation of proinflammatory molecule production in mesangial cells (Fig. 1).

**In vivo evidence**

In animal models of renal disease, the effects of NF-κB modulation on mesangial cell activation have not been extensively evaluated [20–23]. Nevertheless, NF-κB activation has been shown in glomeruli of rats injected with antiglomerular basement membrane sera [20, 21] and in
Fig. 1. Schematic representation of the role of NF-κB in mesangial cell activation. Following stimulation of mesangial cells by cytokines, lipopolysaccharide, immunoglobulin aggregates, angiotensin II, or reactive oxygen species, IκB is phosphorylated and degraded, releasing NF-κB for translocation to the nucleus where it is transcriptionally active. In the nucleus, NF-κB interacts with a number of promoters, among them those for adhesion molecules, chemokines, proinflammatory cytokines, and inflammatory enzymes, which are involved in the inflammatory and proliferative responses of mesangial cells. The use of various inhibitors of NF-κB activation results in a parallel reduction of the inflammatory response of mesangial cells. Abbreviations are: ACEI, angiotensin-converting enzyme inhibitors; HMG-CoAri, hydroxy-3-methylglutaryl coenzyme A reductase inhibitors.

Conclusions

Numerous experimental studies in vitro and in vivo have established the role of NF-κB in mesangial cell activation. Moreover, various experimental maneuvers that inhibit NF-κB activation result in a parallel reduction of inflammatory response of mesangial cells. Collectively, these data suggest a pivotal role of NF-κB in mesangial cell activation and designate this transcription factor as an obvious target for the modulation of the inflammatory response of mesangial cells. The use of various inhibitors of NF-κB activation results in a parallel reduction of the inflammatory response of mesangial cells. Conclusions

massy et al: NF-κB and mesangial cells