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Regulation of c-Jun Phosphorylation by the IκB Kinase-ε Complex in Fibroblast-Like Synoviocytes

Susan E. Sweeney, Deepa Hammaker, David L. Boyle, and Gary S. Firestein

Rheumatoid arthritis (RA)³ is a chronic inflammatory disease marked by symmetric polyarthritis and joint destruction. Signaling pathways that regulate the production of inflammatory mediators such as cytokines and degradative enzymes have been implicated in the pathogenesis of RA and represent potential therapeutic targets (1). Among these, the transcription factors NF-κB (2) and AP-1 (3) are especially important and regulate an array of proinflammatory genes that participate in synovitis (4, 5). Understanding the critical signal transduction networks that modulate these host responses has stimulated the development of small molecule inhibitors as promising agents to treat RA (6–8). For instance, NF-κB blockade in RA has focused on the IκB kinase (IKK) complex, which contains IκK2, as a key convergence point for rapid NF-κB activation (9, 10). However, systemic inhibition of IκK2 activity poses significant potential safety concerns due to the role of NF-κB in host defense and apoptosis.

The possibility that alternative pathways might regulate NF-κB was in part stimulated by the discovery of IKKε (also known as IKKi). This kinase was originally identified as an inducible NF-κB-activating kinase that phosphorylates IκB (11, 12). IKKε along with TRAF-associated NF-κB activator-binding kinase 1 (TBK1) form a family of IκK-related kinases with ~30% structural homology to IκK1 and IκK2. There are clear functional differences between the classical IκKs and the IκK-related kinases. For instance, IκKε and TBK1 only phosphorylate serine 36 of IκB, but not serine 32, whereas IκK2 phosphorylates both residues (13). IκKε also has distinct substrate specificity and kinetics compared with IκK1 and IκK2 (14). IκKε can also phosphorylate other proteins involved in NF-κB signaling, including TRAF-interacting protein, and possibly IκK2 itself (15, 16). More recently, IκKε and the related kinase, TBK-1, were implicated as key initiators of anti-viral gene expression by virtue of their ability to activate IFN regulatory factor 3 (IRF3) (17, 18).

To determine a possible role for IκKε in RA, we previously studied its regulation in cultured fibroblast-like synoviocytes (FLS). We observed that this gene is constitutively expressed in FLS, in contrast to macrophages, but there is still little information on its function in these cells. Using in vitro kinase assays in human FLS, we now report that IκKε kinase activity is rapidly induced by cytokines and the TLR agonist LPS. Surprisingly, c-Jun, rather than IκB, was identified as an efficient downstream target of the IκKε immunoprecipitates. Gene expression studies in murine IκKε−/− FLS demonstrate a novel role for IκKε in regulation of matrix metalloproteinase (MMP) expression in human FLS. These data suggest diverse roles for IκKε in synovial inflammation and in the innate immune response.

Materials and Methods

Preparation of human FLS and murine IκKε knockout FLS

Synovial tissue and FLS were obtained from patients with osteoarthritis (OA) and RA at the time of total joint replacement or synovectomy as previously described (19). The diagnosis of RA conformed to ACR 1987 revised criteria (20). Synovium was minced and incubated with 1 mg/ml collagenase type VIII (Sigma-Aldrich) in serum-free RPMI 1640 (Invitrogen Life Technologies) for 1 h at 37°C, filtered, extensively washed, and cultured in DMEM (Invitrogen Life Technologies) supplemented with 10%
FCS (Gemini BioProducts), penicillin, streptomycin, gentamicin, and glucose in a humidified 5% CO₂ atmosphere. Cells were allowed to adhere overnight, and FLS were grown in DMEM containing 10% FCS and split at 1:3 when the cells were 70–80% confluent. FLS were used from passages 3–8, during which time they are a homogeneous population of cells (<1% CD11b positive, <1% phagocytic, and <1% FcRII and FcRII positive). In addition, multiple FLS lines were derived from IKKe knockout and wild-type mice (Phar macia) by microdissecting synovium and enzymatically dispersing the cells as previously described (21, 22). FLS were cultured in six-well plates or 100-mm dishes and used at 80% confluency. Cells were synchronized in 0.1% FCS for 24–48 h before the addition of cytokines or LPS.

Reagents

Affinity-purified goat polyclonal anti-IKKα (IKKe) Ab (Sc-5694), rabbit polyclonal anti-IKKβ/2 Ab (SC-7607), mouse anti-JNK2 mAb (SC-7345), rabbit polyclonal anti-MAPK kinase 4 (anti-MKK-4) Ab (SC-964), goat polyclonal anti-c-Jun Ab (SC-44-G), and secondary Abs were purchased from Santa Cruz Biotechnology. GST-c-Jun, GST-IkBα, and the JNK inhibitor, SP600125 were obtained from Celgene. TNF-α and IL-1 were purchased from R&D Systems. LPS was obtained from Sigma-Aldrich. Dominant negative (dn) IKKe adenovirus containing a K38A mutation (provided by Dr. C. Tripp, Pharmacia, St. Louis, MO) and dnIKKβ adenovirus (9) were also used.

Immunohistochemistry

Immunostaining was performed as previously described (23, 24). Briefly, cryosections (5 μm) of synovial tissue from RA and OA patients were fixed with 4% paraformaldehyde for 10 min, then incubated with anti-CD68 for the isotype control Ab overnight at 4°C. Sections were washed, and endogenous peroxidase was depleted with 0.1% H₂O₂ and 0.1% NaN₃. The sections were then incubated with secondary HRP-conjugated anti-goat Ab, washed, and developed with diaminobenzidine (Vector Laboratories).

Western blot analysis

Western blot was performed as described previously (25). FLS were cultured in DMEM with 10% FCS in 100-mm dishes at 80% confluence and split cultured in six-well plates or 100-mm dishes and used at 80% confluency. Earlier studies in FLS indicated that IKKe function in FLS readily detected, with similar levels present in RA and OA samples (see Fig. 1A). As shown in Fig. 1B, immunohistochemical analysis confirmed tissue expression of IKKe, with prominent staining in the synovial intimal lining of RA and OA tissue as well as scattered sublining mononuclear cells. Because FLS are located in the synovial intimal lining, we also evaluated cultured FLS for IKKe expression. As previously described, IKKe was constitutively expressed by both RA and OA FLS (Fig. 2) (27).

Immunoprecipitation assay

FLS lysates were prepared as described above for kinase assay. The lysates were precleared with 150 μl of protein G-agarose slurry and 10 μg of isotype control Ab overnight at 4°C. The precleared lysates were then incubated with Ab at 5 μg/ml with 50 μl of a 1:1 slurry of protein G-agarose beads at 4°C overnight. The immunoprecipitates were washed five times with lysis buffer, incubated in nonreducing Laemmli sample buffer, and heated for 5 min at 95°C. The samples were subjected to SDS-PAGE, and Western blot analysis was performed as described above.

EMSA

After serum starvation for 48 h, IKKe knockout and wild-type murine FLS were treated with medium, TNF-α (100 ng/ml), or LPS (1 μg/ml) for 1 h. Cells were then washed with cold PBS and nuclear extracts were prepared as previously described (9, 21). A nuclear extraction kit (Chemicon International) was used to isolate extracts, and EMSA was performed using the gel-shift assay system kit (Promega) with control HeLa nuclear extract and cold competition oligonucleotides to demonstrate specificity. The protein concentration was determined, and lane loading was normalized appropriately. The nuclear extract was mixed with the appropriate purified α3′-P-labeled oligonucleotides, resolved by 4% PAGE, dried, and visualized by autoradiography.

Quantitative PCR

IKKe wild-type and knockout murine FLS were cultured in six-well plates and incubated with TNF-α (100 ng/ml) or LPS (1 μg/ml) for 6 h. RNA isolation and RT-PCR were performed as previously described using TaqMan PCR analysis and the GeneAmp 5700 Sequence Detection System (Applied Biosystems) (25, 26). Forward and reverse primers for MMP3, MMP13, and IFN-inducing protein 10 (IP-10) as well as fluorogenic TaqMan FAM/TAMRA-labeled hybridization probes were used (Assays on Demand, Applied Biosystems). To control for sample cellularity, hypoxanthine guanine phosphoribosyltransferase (HGPRT) forward and reverse primers and a TaqMan-labeled probe were included in separate PCRs. Each 20 μl of PCR mix also included 1X TaqMan universal PCR Master Mix with AmpliTaq Gold DNA polymerase, uracil-N-glycosylase (Ung), and dUTP, and a passive reference to minimize background fluorescence fluctuations. Thermal cycle conditions were 2 min at 50°C to allow activation of uracil-N-glycosylase, 10 min at 95°C to activate AmpliTaq polymerase, and 40 cycles of 95°C for 15 s at 60°C for 1 min. The threshold cycle was determined for each sample using GeneAmp software. Standard curves are generated by linear regression using log (threshold cycle) vs log (cell number). The cell equivalent (CE) number for samples was calculated using the standard curve. Data are expressed as the ratio between the gene of interest CE and the HGPRT CE, yielding the relative expression.

Statistical analysis

Statistics were generally performed using paired Student’s t test. A comparison was considered significant at p < 0.05.

Results

IKKe expression in synovial tissue and synoviocytes

To assess the potential role of IKKe in synovial inflammation and arthritis, initial studies were performed to determine whether the IKKe protein is expressed in RA and OA synovial tissue. Western blot analysis was performed on RA and OA synovial lysates using polyclonal anti-IKKα Ab. Immunoreactive IKKe protein was readily detected, with similar levels present in RA and OA samples (see Fig. 1A). As shown in Fig. 1B, immunohistochemical analysis confirmed tissue expression of IKKe, with prominent staining in the synovial intimal lining of RA and OA tissue as well as scattered sublining mononuclear cells. Because FLS are located in the synovial intimal lining, we also evaluated cultured FLS for IKKe expression. As previously described, IKKe was constitutively expressed by both RA and OA FLS (Fig. 2) (27).

IKKe function in FLS

Earlier studies in FLS indicated that IKKe mRNA expression is increased by cytokines, but that the effects on protein levels are less pronounced (27). These data suggested that IKKe activity, like
As shown in Fig. 3, both IKKε expression in RA and OA synovial tissue. Expression of actin is included as a control for protein loading. Analysis demonstrates similar levels of IKKε expression in RA and OA synovial tissue. To evaluate this possibility, FLS were stimulated with TNF-α for 15 min, and lysates were immunoprecipitated with anti-IKKε Ab, and kinase assays were performed. The dnIKKε blocked cytokine-stimulated c-Jun phosphorylation, whereas LacZ and dnIKK2 had no effect (n = 2).

**c-Jun phosphorylation by IKKε immunoprecipitates**

Because IκBα appeared to be a relatively poor substrate for IKKε and did not alter the canonical pathway of NF-κB activation, other substrates were evaluated. Among the potential candidates, we were surprised to find that IKKε immunoprecipitates rapidly and efficiently phosphorylated c-Jun, which was evaluated because it plays a key role in the regulation of MMP gene expression as well as activation of the antiviral program (29, 30). Fig. 5A shows a representative experiment demonstrating that IL-1 and TNF-α increase c-Jun activation (10.4 ± 3.5-fold; p < 0.001; n = 4) with a return to baseline in 24 h. Phosphorylation of c-Jun was also observed when FLS were stimulated with the TLR4 ligand LPS for 15 min (see Fig. 5B for a representative experiment). Additional evidence that IKKε directly contributes to c-Jun phosphorylation was provided in experiments using an adenoviral construct expressing dnIKKε. As shown in Fig. 5C, human FLS were infected with adenoviruses encoding dnIKKε, LacZ, or dnIKK2. After stimulating infected cells with TNF-α for 15 min, cell lysates were immunoprecipitated with anti-IKKε Ab, and kinase assays were performed. The dnIKKε blocked cytokine-stimulated c-Jun phosphorylation, whereas LacZ and dnIKK2 had no effect (n = 2).

**Contribution of JNK to c-Jun phosphorylation by IKKε immunoprecipitates**

One potential explanation for the phosphorylation of c-Jun by IKKε immunoprecipitates is that JNK might coprecipitate with the complex. To evaluate this possibility, in vitro kinase reactions were performed in the presence or the absence of the JNK inhibitor SP600125 (6). Because MKK4 and JNK are known to coprecipitate (25, 31), anti-MKK4 Ab was used as a positive control. As shown in Fig. 6A, phosphorylation of c-Jun by the IKKε complex is not blocked by the JNK inhibitor, whereas activity of the MKK4-JNK complex is markedly decreased. As a second approach, we performed Western blot analysis to determine whether JNK is present in the complexes. Anti-IKKε or control IgG immunoprecipitates were probed with anti-JNK2 mAb, but JNK was not detected in the complexes (Fig. 6B).

**Regulation of MMP expression by IKKε**

Because IKKε immunoprecipitates phosphorylated c-Jun, which plays a key role in MMP gene expression and subsequent joint...
MMP induction was significantly greater in the IKK blocked by small molecule inhibitors and thereby target an array of IKK, known as IKK could alter pathogenic gene transcription while leaving basal ex-
survival. This issue led us to evaluate alternative pathways that could have a major impact on normal immune responses and cell 

destruction in arthritis, we evaluated the role of IKKε in MMP production (21, 32). Murine IKKε−/− and IKKε+/+ FLS lines were generated by synovial microdissection. Western blot analysis of murine FLS isolated from joints of IKKε−/− and IKKε+/+ mice confirmed the absence of the IKKε protein in the knockout cells (Fig. 7). Because c-Jun is a key regulator of MMP gene expression, we focused our attention on MMP3 and MMP13. IKKε−/− and IKKε+/+ FLS were stimulated with LPS or TNF-α for 6 h, and MMP3 and MMP13 mRNA levels were quantified by real-time quantitative PCR. Fig. 8, A (MMP3) and B (MMP13), shows that MMP induction was significantly greater in the IKKε+/+ FLS compared with IKKε−/− FLS after either TNF-α or LPS stimulation (n = 4). Fig. 8C demonstrates that IP-10 (CXCL-10) expression is not attenuated in the murine IKKε−/− FLS, indicating that the results of the MMP experiments are not due to nonspecific effects on cell viability or gene transcription. The lack of effect on IP-10 is surprising, but might reflect a more important role of TBK-1 for this gene in FLS.

Discussion

Therapy for RA has improved dramatically over the last decade with the advent of anti-cytokine therapy (33). However, many pa-
tients have persistent disease despite optimal treatment. The search for novel therapeutic targets to address this unmet medical need has focused attention on the intracellular machinery that regulates the production of pathogenic mediators. Transcription factors such as NF-κB and AP-1 are especially important because of their pivotal role in cytokine and MMP gene expression (34, 35). Kinase cascades that modulate these pathways could potentially be blocked by small molecule inhibitors and thereby target an array of genes involved in the disease pathogenesis (36).

One concern with inhibition of major pathways such as NF-κB is that many homeostatic and host defense mechanisms might also be affected (37). For instance, targeting NF-κB through IKK2 could have a major impact on normal immune responses and cell survival. This issue led us to evaluate alternative pathways that could alter pathogenic gene transcription while leaving basal expression intact. Hence, we considered whether a novel inducible IKK, known as IKKε, might represent a safer way to modulate, rather than ablate, transcription factor activation. IKKε/IKKi is an IKK-related kinase and was initially identified as an inducible protein that could phosphorylate IκB in LPS-stimulated macrophages (11, 13). However, NF-κB-independent functions were suggested by additional studies demonstrating that IKKε can phosphorylate other proteins, including TRAF-interacting protein (15). This along with recent data demonstrating that MEFs lacking IKKε retain normal activation of the canonical NF-κB pathway after TNF-α stimulation (28) suggested that IKKε has many other effects.

Although IKKε is usually considered an inducible gene, our previous studies of IKKε in arthritis demonstrated constitutive mRNA and protein expression in FLS (27). IL-1 and TNF-α markedly induced IKKε mRNA, but protein levels only modestly increased. These data raised the possibility that post-translational activation of IKKε might have a more important role in FLS. Even though the cytokines known to induce IKKε in vitro are present in RA tissues, IKKε RNA transcript levels are similar in RA and OA. In the present study we extended these observations by demonstrating that IKKε protein expression in inflammatory and nonin-
flammatory synovial tissue is similar. Immunohistochemistry also showed abundant immunoreactive IKKε in the synovial intimal lining, suggesting that FLS produce the protein in vivo. These protein studies are the first demonstration of IKKε protein expres-
sion and distribution in human disease.

The similar levels of IKKε protein in OA and RA led us to evaluate whether its functional status is modulated in FLS after cytokine or TLR ligand stimulation. In vitro kinase assays showed that IKKε function is rapidly induced by IL-1 and TNF-α as well as LPS stimulation. Although IκBα could serve as a substrate, we were struck by the observation that IKK2 was significantly more efficient than IKKε at generating phospho-IκBα. In addition, NF-κB activation was not altered by the absence of IKKε in FLS. This raised the possibility that alternative substrates and signaling cascades might be important for IKKε-mediated functions in FLS. Surprisingly, we found that c-Jun served as an excellent substrate for the IKKε immunoprecipitate, with a 10-fold increase in activity after cytokine stimulation. As a component of the transcription factor AP-1, c-Jun is a key regulator of MMP gene expression and has been implicated as a major pathway responsible for extracellular matrix destruction in animal models of arthritis (32). These data suggest that rather than NF-κB, IKKε regulates c-Jun-driven gene expression in FLS.
be phosphorylated in cytokine-stimulated IKK. For instance, preliminary studies suggest that Ser63 and Ser73 can be phosphorylated by IKK, which is important. Studies to identify the specific residues on c-Jun that are phosphorylated by IKK are in progress.

Because c-Jun is known to regulate MMP gene expression, we evaluated the functional role of IKKε by assessing MMP3 and MMP13 gene expression in cultured FLS. Small molecule inhibitors of IKKε are not currently available; therefore, we focused our attention on stimulated FLS isolated from wild-type and IKKε knockout mice. We noted that MMP induction was strikingly lower in the IKKε knockout FLS compared with wild-type cells after either cytokine or LPS stimulation even though basal expression was similar. Hence, our original search for alternative pathways to pathogenic NF-κB activation ultimately led us to a novel mechanism that regulates c-Jun and MMP production. Because IKKε is expressed in the rheumatoid intimal lining, which is the primary source of MMPs and proinflammatory cytokines, this could represent an interesting therapeutic target. Based on studies with IKKε−/− embryonic fibroblasts and our own studies in FLS, however, IKKε blockade will probably not have a major impact on the canonical NF-κB pathway (28).

As noted above, our studies of the function of IKKε in RA focused initially on its participation in NF-κB signaling and subsequently the alternative activation pathways. However, we were also intrigued by the data indicating that the IKK-related kinases play an unanticipated role in innate immunity (17, 18). For instance, TLR3 ligation and formation of a virus-activated kinase complex leads to IKKε and TBK-1 activation, followed by IRF phosphorylation. In fact, TLRs have also been implicated in the pathogenesis of RA, and many TLR ligands, such as peptidoglycans, are present in the rheumatoid joint and activate synovial fibroblasts (38). TLR and cytokine signaling pathways are convergent, and the clinical efficacy of cytokine defects can be bypassed by the innate immune system to induce arthritis (39). Our results demonstrating that LPS induces IKKε kinase activity in FLS and

FIGURE 5. TNFα, IL-1, and LPS-mediated activation of c-Jun phosphorylation by IKKε. FLS were stimulated with cytokines or LPS, and IKKε immunoprecipitates were evaluated for the ability to phosphorylate GST-c-Jun in vitro. A, GST-c-Jun phosphorylating activity increased within 15 min of IL-1 or TNFα stimulation, with a return to baseline in 24 h (n = 4). IgG immunoprecipitates did not have IκB-phosphorylating activity (data not shown). B, LPS stimulation also results in IKKε-mediated c-Jun phosphorylation (n = 2). GST-c-Jun phosphorylation by JNK is included as a positive control (right lane). C, IKKε-mediated phosphorylation of c-Jun is inhibited by dnIKKε. FLS were infected overnight with adenoviral vectors encoding LacZ or dnIKKε, then stimulated with TNFα for 15 min. The dnIKK2 adenoviral vector was included as an additional control. In vitro kinase assays were performed with IKKε immunoprecipitates using GST-c-Jun substrate. DnIKKε, but not dnIKK2, inhibited c-Jun phosphorylation by IKKε immunoprecipitates. The medium controls for adenovirus-infected and uninfected FLS were similar in multiple experiments (data not shown).

Although IKKε might phosphorylate c-Jun directly, it is also possible that other kinases coprecipitate with IKKε. One major concern was that JNK could associate with the IKKε complex because it phosphorylates c-Jun while binding to many other kinases, including MEKK1, MEKK2, MKK4, and MKK7 (25, 31). Three lines of evidence suggest that this is not the case. First, phosphorylation of c-Jun by the IKKε immunoprecipitate was not inhibited by a selective JNK inhibitor. Second, immunoreactive JNK was not detected in the IKKε complex by Western blot analysis. Finally, a dominant negative IKKε construct blocked c-Jun phosphorylation in vitro. Additional studies are needed to determine whether IKKε or an undefined associated kinase is responsible for the kinase activity resulting in phosphorylation of c-Jun. For instance, preliminary studies suggest that Ser63 and Ser73 can be phosphorylated in cytokine-stimulated IKKε−/− cells (data not shown), suggesting that the extent or kinetics of phosphorylation or the specific residues activated by the IKKε complex might be important. Studies to identify the specific residues on c-Jun that are phosphorylated by IKKε immunoprecipitates are in progress.

Because c-Jun is known to regulate MMP gene expression, we evaluated the functional role of IKKε by assessing MMP3 and MMP13 gene expression in cultured FLS. Small molecule inhibitors of IKKε are not currently available; therefore, we focused our attention on stimulated FLS isolated from wild-type and IKKε knockout mice. We noted that MMP induction was strikingly lower in the IKKε knockout FLS compared with wild-type cells after either cytokine or LPS stimulation even though basal expression was similar. Hence, our original search for alternative pathways to pathogenic NF-κB activation ultimately led us to a novel mechanism that regulates c-Jun and MMP production. Because IKKε is expressed in the rheumatoid intimal lining, which is the primary source of MMPs and proinflammatory cytokines, this could represent an interesting therapeutic target. Based on studies with IKKε−/− embryonic fibroblasts and our own studies in FLS, however, IKKε blockade will probably not have a major impact on the canonical NF-κB pathway (28).

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FIGURE 6. IKKε-mediated c-Jun phosphorylation is independent of JNK. A, TNFα-mediated activation of c-Jun phosphorylation by IKKε immunoprecipitates. In vitro kinase assays were performed on IKKε immunoprecipitates of TNFα-activated FLS using GST-c-Jun substrate. IKKε immunoprecipitates were prepared from control and cytokine-stimulated FLS, and kinase assays were performed in the presence and the absence of the JNK inhibitor SP600125 (left). The JNK inhibitor did not block c-Jun phosphorylation mediated by IKKε immunoprecipitates. As a positive control, SP600125 did block c-Jun phosphorylation by the MKK4 immunoprecipitate (which contains JNK) (right) (31). This figure is representative of three experiments. B, Western blot analysis of the complex using anti-JNK2 mAb indicated that JNK is not present in the IKKε complex (the control is FLS lysates on the same blot). These data suggest that IKKε complex phosphorylates c-Jun independent of JNK (n = 3).
that MMP expression in IKKε knockout synoviocytes is suppressed suggest that these pathways might be operative in human cells as well. TLR signaling pathways are emerging as small molecule targets for the development of new therapeutics that modify the innate immune response (40).

After phosphorylation by the IKKε/IKKβ-1 complex, the IRFs bind to IFN-stimulated response elements in promoters of various antiviral genes, including IFN-stimulated genes, IFN-β, and RANTES. Of interest, c-Jun as a heterodimer with ATF-2 has also been implicated in activation of these genes as a component of the IFN enhanceosome (29, 30). The presence of c-Jun and IRF3 in the enhanceosome provides a link among IKKε, c-Jun, the antiviral program, and MMP expression. By participating in the IRF signaling cascade as a result of TLR ligation, IKKε can potentially regulate IFN-β and RANTES expression (17, 18). The impact of these observations on RA is still not certain. However, IFN-β might have a protective role in synovitis and represent a counter-regulatory pathway that suppresses synovial inflammation in animal models of arthritis (41, 42). IFN-β is expressed in RA synovium; however, it is not clear whether the beneficial effects of IKKε blockade and proinflammatory cytokine and MMP suppression will be offset by the decreased IFN-β production.

Yet another potential proinflammatory link between IKKε and RA was suggested in IKKε knockout MEFs. Interaction between NF-κB and C/EBP signaling pathways activated by LPS was altered in IKKε knockout MEFs (28). Cells deficient in IKKε retained normal activation of the canonical NF-κB pathway, but failed to induce C/EBPβ-specific DNA binding and transcription of C/EBP and NF-κB target genes after LPS stimulation. In MEFs, IKKε deficiency resulted in a reduction of LPS-induced mRNA expression of cyclooxygenase-2, IL-1, IL-6, TNF-α, IP-10, and RANTES (28). C/EBPβ has also been implicated in MMP expression, and inhibition of MMP production in IKKε-deficient MEFs might also involve C/EBP-mediated effects on transcription (43). These pathways are obviously of interest and will be the subject of future studies.

Based on these intriguing data, we propose that IKKε could participate in multiple novel signaling pathways that are nearly unexplored in chronic inflammation. IKKε also represents a potential therapeutic target in RA with interesting pharmacology that could link innate immunity, extracellular matrix destruction, and cell recruitment. It is activated by factors known to be present in the rheumatoid joint and initiates a cascade that modulates innate and adaptive immune responses. Multiple transcription factor families, including NF-κB, AP-1, C/EBP, and IRF, interact with the IKK-related kinase IKKε. These data indicate that IKKε and other related kinases could serve as therapeutic targets in RA.

**Disclosures**

The authors have no financial conflict of interest.
