Interactions between CBP, NF-κB, and CREB in the lungs after hemorrhage and endotoxemia

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The transcriptional regulatory factor nuclear factor (NF)-κB has a central role in modulating expression of proinflammatory mediators that are important in acute lung injury. In vitro studies have shown that competition between NF-κB and cAMP response element binding protein (CREB) for binding to the coactivator CREB-binding protein (CBP) is important in regulating transcriptional activity of these factors. In the present study, we examined in vivo interactions between CBP, CREB, and NF-κB in hemorrhage- or endotoxemia-induced acute lung injury. Association of CBP with CREB or the p65 subunit of NF-κB increased in the lungs after hemorrhage or endotoxemia. Inhibition of xanthine oxidase before hemorrhage, but not before endotoxemia, decreased p65-CBP interactions while increasing those between CREB and CBP. These alterations in CREB-CBP and p65-CBP interactions were functionally significant because xanthine oxidase inhibition before hemorrhage resulted in increased expression of the CREB-dependent gene c-Fos and decreased expression of macrophage inflammatory protein-2, a NF-κB-dependent gene. The present results show that the coactivator CBP has an important role in modulating transcription in vivo under clinically relevant pathophysiological conditions.

acute lung injury; inflammation; gene expression regulation; transcription factors; reactive oxygen species; adenosine 3’-5’-cyclic monophosphate response element binding protein binding protein; nuclear factor-κB

ACUTE LUNG INJURY OCCURS FREQUENTLY after severe infection or blood loss (27). Pulmonary findings characteristic of acute lung injury include massive accumulation of neutrophils (8, 18), increased proinflammatory cytokine levels (14, 15, 32a), and release of reactive oxygen intermediates (ROIs) (34). Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and macrophage inflammatory protein (MIP)-2 are increased in the lung after hemorrhage or endotoxemia and contribute to lung inflammation and injury (31). Binding elements for the transcriptional regulatory factor nuclear factor (NF)-κB are present in the promoter regions of these cytokines and have important functions in modulating their expression (5, 29). Increased nuclear translocation of NF-κB occurs in the lungs after endotoxemia or hemorrhage (31) and in lung cell populations of patients with acute lung injury (28). NF-κB appears to play an important role in the induction of acute lung injury because inhibition of its activation is associated with decreased expression of proinflammatory cytokines and amelioration of neutrophilic alveolitis in experimental models (7, 31).

ROIs contribute to increased pulmonary production of proinflammatory cytokines when ischemia-reperfusion injury is a major component of the pathophysiological process, as occurs with blood loss (31). The role of ROIs in the pathogenesis of acute lung injury appears to be less important when the ischemic insult is less intense, such as after endotoxemia. For example, we found that inhibition of the ROI-generating enzyme xanthine oxidase diminished hemorrhage- but not endotoxemia-induced increases in the expression of IL-1β, MIP-2, and TNF-α among lung neutrophils (31). In part, the role of ROIs in contributing to the development of acute lung injury may be related to their role in activating NF-κB, thereby increasing proinflammatory cytokine expression (30).

The activity of many inducible transcription factors, including NF-κB, is regulated through their association with cellular coactivators (10, 16, 17, 20, 32, 38). Interaction with the coactivator cAMP response element binding protein (CREB)-binding protein (CBP) appears to be necessary to optimize the transcriptional activity of NF-κB (32, 38). In addition to interacting with NF-κB, CBP also associates with TATA box-binding protein (16) and transcription factor (TF) IIB (23), becoming part of the general transcriptional apparatus. The interaction of the p65 (Rel A) subunit of NF-κB with CBP involves the KIX region of CBP, which is the same region responsible for binding the transcriptionally active serine-133-phosphorylated form of CREB (32, 38). Disruption of the KIX-based interactions between CREB or NF-κB and CBP by...
mutagenesis significantly decreases the efficiency of NF-κB- or CREB-dependent transcription (38).

Because CBP is present in limiting amounts in the nucleus, competition between NF-κB and CREB for binding to CBP has been postulated to be important in regulating the transcriptional activity of these factors (38). In vitro studies have directly shown such competition between p65 and CREB for limiting amounts of CBP (38). Evidence for competitive interactions between NF-κB and CREB has also been provided by Ollivier et al. (22) and Parry and Mackman (24). In their experiments, activation of protein kinase A resulted in increased amounts of phosphorylated CREB and decreased NF-κB-mediated transcription of TNF-α, endothelial leukocyte adhesion molecule-1, and vascular cell adhesion molecule-1 but did not prevent nuclear translocation of NF-κB heterodimers. However, there is little information available on in vivo interactions between CBP, CREB, and NF-κB.

In previous work with murine models of acute lung injury, our laboratory (31) found that hemorrhage resulted in increased expression of NF-κB-dependent proinflammatory cytokines, enhanced nuclear translocation of NF-κB, and increased levels of serine-133-phosphorylated CREB in the lungs. Although xanthine oxidase blockade prevented hemorrhage-induced increases in the expression of NF-κB-dependent cytokines, such antioxidant therapy did not affect nuclear translocation of NF-κB. However, inhibition of xanthine oxidase before hemorrhage was found to result in further increases in the amounts of phosphorylated CREB in the lungs. A possible explanation for such findings is that the increased amounts of phosphorylated CREB produced by xanthine oxidase inhibition before hemorrhage might compete with NF-κB for limiting amounts of CBP, thereby reducing NF-κB-dependent transcription. In the present studies, we directly show that such interactions between NF-κB, CREB, and CBP occur in vivo and are capable of modulating the transcriptional activity of NF-κB and CREB.

**METHODS**

**Mice.** Male BALB/c mice, 8–12 wk of age, were purchased from Harlan Sprague Dawley (Indianapolis, IN). The mice were kept on a 12:12-h light-dark cycle with free access to food and water. All experiments were conducted in accordance with institutional review board-approved protocols.

**Materials.** Isoflurane was obtained from Abbott Laboratories (North Chicago, IL). *Escherichia coli* 0111:B4 endotoxin and octylphenoxy poly(ethylenoxy)ethanol were obtained from Sigma (St. Louis, MO). The allopurinol-supplemented diet was purchased from ICN Biomedicals (Costa Mesa, CA). RPMI 1640 medium containing 25 mM HEPES and 300 mg/ml of l-glutamine was obtained from BioWhittaker (Walkersville, MD). Protein A magnetic beads were obtained from Dynal (Lake Success, NY). The Comassie Plus protein assay reagent, SuperSignal West Femto maximum sensitivity substrate, and anti-mouse secondary antibody conjugated to horseradish peroxidase were purchased from Pierce (Rockford, IL). The Hybond nitrocellulose and anti-rabbit secondary antibody conjugated to horseradish peroxidase were obtained from Amersham (Piscataway, NJ). Anti-CBP (A22), anti-CBP (C-1), and anti-CREB (24H4B) antisera were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-p65 antiserum was obtained from BIOMOL (Plymouth Meeting, PA). The RNeasy kit was from QIAGEN (Valencia, CA). MIP-2, c-Fos, and rRNA control primers and probes, murine leukemia virus reverse transcriptase, and AmpliTaq Gold polymerase were purchased from PerkinElmer (Foster City, CA).

**Models of hemorrhage and endotoxemia.** The murine hemorrhage model used in these experiments was developed in our laboratory and has been reported previously (2, 31). With this model, 30% of the calculated blood volume (−0.55 ml for a 20-g mouse) is withdrawn by cardiac puncture from an isoflurane-anesthetized mouse over a 60-s period. The period of isoflurane anesthesia is <2 min in all cases. The mortality rate with this hemorrhage protocol is ~12%.

The model of endotoxemia was used as reported previously (31). Mice received an intraperitoneal injection of lipopolysaccharide at a dose of 1 mg/kg in 200 μl of PBS. This dose has previously been demonstrated to produce acute neutrophilic alveolitis, histologically consistent with acute lung injury in mice (11, 12).

**Allopurinol supplementation.** To assess the effects of xanthine oxidase on cytokine expression and transcriptional factor activation, mice were pair-fed an allopurinol-supplemented diet (2.5 g/kg chow) or a normal control diet for 1 wk before hemorrhage or endotoxemia (4). Measured xanthine oxidase activity (33) in the lung was reduced from 7.4 ± 0.7 mU/g in control fed mice to essentially undetectable levels (0.2 ± 0.2 mU/g) in allopurinol-fed mice.

**Excision of lungs.** Briefly, the chest of the mouse was opened, and the lung vascular bed was flushed with 3−5 ml of chilled (4°C) PBS injected into the right ventricle. Lungs were then excised, avoiding the paratracheal lymph nodes, and washed twice in RPMI 1640 medium containing 25 mM HEPES-300 mg/ml of l-glutamine with penicillin-streptomycin.

**Coimmunoprecipitation.** Interactions of CBP and p65 or CREB were demonstrated with a modification of the method of Gerritsen and coworkers (13). Lungs were homogenized in 1 ml of lysis buffer [25 mM HEPES-KOH, pH 7.2, 150 mM potassium acetate, 2 mM EDTA, 0.1% octylphenoxy poly(ethylenoxy)ethanol, 10 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml of leupeptin, 10 μg/ml of aprotinin, 1.5 μg/ml of pepstatin, and 5 mM dithiothreitol], incubated on ice for 10 min, and centrifuged at 12,000 g at 4°C. Lung extracts were isolated, and protein concentration was determined with Coomassie Plus protein assay reagent standardized to bovine serum albumin according to the manufacturer’s protocol. Lung extracts (8 μg) were rotated for 1 h at 4°C with 20 μg of anti-CBP (A22) cross-linked to protein A magnetic beads according to the manufacturer’s protocol. The beads were washed four times with 1 ml of lysis buffer and boiled for 5 min in SDS-denaturing sample buffer. Proteins were run through SDS-7.5% polyacrylamide gels and transferred to Hybond nitrocellulose for 16 h at 4°C. The membranes were blocked with 1% bovine serum albumin in a buffer containing 20 mM Tris-HCl, pH 7.6, 137 mM sodium chloride, and 0.5% Tween 20 (TBS-T) and incubated at a 1:1,000 dilution with anti-p65, anti-CREB, or anti-CBP (C-1) for 1 h at 20°C. Blots were washed three times with TBS-T buffer, incubated for 1 h with an anti-mouse or anti-rabbit secondary antibody conjugated to horseradish peroxidase, and washed 10 times in TBS-T. Proteins were visualized by incubation with SuperSignal West Femto maximum sensitivity substrate and with a ChemiDoc chemiluminescence detection system (Bio-Rad, Richmond, CA).
Quantitative PCR. RNA was isolated from whole lungs with an RNaseasy kit according to the manufacturer’s protocol. Briefly, lungs were homogenized for 30 s on ice in 300 μl of a buffer containing 25% guanidinium thiocyanate and 1% 2-mercaptoethanol. Samples were incubated with proteinase K at 55°C for 10 min, centrifuged at 12,000 g for 3 min, washed, incubated with DNase for 15 min at room temperature, and washed again. RNA was eluted from the membrane in 40 μl of RNase-free water, and the quantity of RNA was determined at an absorbance of 260 nm.

Primers and probes for MIP-2 and c-Fos were designed with the Primer Express software supplied by PerkinElmer. The MIP-2 primers and probes consisted of forward primer, 5'-TGTTACGGCCCCAGGA-3'; reverse primer, 5'-AACCTTTTTGACCCGGTCTGAG-3'; and probe, 5'-TGCTGCCCAGAGAAGGTCTAGGGCCA-3'. The c-Fos primers and probes consisted of forward primer, 5'-GGAGAAGCTGAGGCCGCTTGC-3'; reverse primer, 5'-CCTCTTCAGGAGATAGCTGCTCAGCGCT-3'; and probe, 5'-CTCTCCGGGATGCTCTGCGCT-3'.

Based on primer optimization, conducted as described in the manufacturer’s protocol, the primer concentration in the PCR was 10 nM forward primer and 450 nM reverse primer of the MIP-2 amplicon and 450 nM for both the forward and reverse primers of c-Fos. In each experiment, ribosomal control probe and forward and reverse primers at 50 nM were used to normalize the amount of RNA in each sample. One-step RT-PCRs of 50 μl consisted of reverse transcription for 30 min at 48°C with murine leukemia virus RT at 0.25 U/μl, followed by incubation for 10 min at 95°C and 40 cycles of amplification (95°C for 15 s and 60°C for 1 min) with AmpliTaq Gold polymerase at 0.025 U/μl. The quantity of MIP-2 and c-Fos mRNA was determined from a standard curve with 10-fold dilutions of known amounts of target RNA with each primer and probe set. RNA amounts were determined with software provided with the GeneAmp 5700 sequence detection system (ABI PRISM, Applied Biosystems, Foster City, CA). Quantification was determined by normalizing the amount of MIP-2 or c-Fos RNA by the amount of 18S rRNA.

Statistical analysis. Because of inherent variability between groups of mice, for each experimental condition, the entire group of animals was prepared and studied at the same time. For each experimental condition, mice in all groups had the same birth date and had been housed together. Separate groups of mice were used for coimmunoprecipitations and quantitative PCR. For quantitative PCR, lung RNA was isolated from each individual animal and analyzed before group data were calculated. Data are presented as means ± SE for each experimental group. One-way analysis of variance, Tukey-Kramer test, Student-Newman-Keuls test, Dunnett’s multiple comparisons, or Student’s t-test were used for comparisons between data groups. P < 0.05 was considered significant.

RESULTS

Interactions between p65 or CREB with CBP after hemorrhage or endotoxemia. Coimmunoprecipitation was used to determine the relative amounts of CREB or p65 associated with CBP in the lungs after hemorrhage or endotoxemia. As shown in Figs. 1 and 2, there were no changes in pulmonary levels of CBP compared with those found in control unmanipulated mice during the 60 min after endotoxin administration or blood loss.

There was increased association of both p65 and CREB with CBP after hemorrhage but with differing kinetics for each of these transcriptional regulatory factors (Fig. 1). The amounts of p65 associated with CBP began to increase 15 min after blood loss, with p65-CBP interactions becoming maximal 30 min posthemorrhage (P < 0.05 vs. control) and then decreasing slightly at 60 min. In contrast, CREB-CBP interactions were at similarly high levels 5, 15, 30, and 60 min after hemorrhage (P < 0.01 vs. control).

Interactions of p65 or CREB with CBP increased in the lungs after endotoxemia but with time courses different from those found after blood loss (Fig. 2). Association of significant amounts of p65 with CBP was found only at 30 min after endotoxin administration (P < 0.05 vs. control). CREB-CBP interactions increased 15 and 30 min (P < 0.05 vs. control) after endotoxemia before decreasing back to baseline at 60 min.
Xanthine oxidase inhibition modifies p65-CBP and CREB-CBP interactions after hemorrhage. In a previous study (31), we found that xanthine oxidase-derived ROIs were responsible for hemorrhage- but not endotoxin-induced increases in the pulmonary expression of proinflammatory cytokines. Despite the fact that transcription of such cytokines is dependent on NF-κB, xanthine oxidase inhibition did not affect translocation of NF-κB to the nucleus after hemorrhage. Because hemorrhage-induced CREB phosphorylation was further increased after xanthine oxidase blockade, we hypothesized that increased amounts of serine-133-phosphorylated CREB displaced NF-κB from the limiting amounts of CBP present in the nucleus, thereby decreasing NF-κB-dependent transcription.

Using coimmunoprecipitation studies, we found that xanthine oxidase inhibition altered hemorrhage-induced interactions between NF-κB or CREB with CBP in the lungs (Figs. 3B and 4). In these experiments, mice were fed either an allopurinol-containing xanthine oxidase-inhibiting or control diet and were then subjected to endotoxin administration or hemorrhage. CREB-CBP and p65-CBP interactions were determined 30 min after hemorrhage or endotoxia, the time points when maximal interactions between CBP and these transcriptional factors were seen in our initial experiments (see Figs. 1 and 2). Allopurinol feeding did not produce any changes in CREB-CBP and p65-CBP relationships compared with those found in mice fed a control diet (Fig. 3A).

When xanthine oxidase was inhibited before hemorrhage, interactions between p65 and CBP were significantly reduced and those between CREB and CBP were significantly increased compared with such associations in mice fed a control diet (Fig. 3). In contrast, xanthine oxidase inhibition had no effect on p65-CBP or CREB-CBP interactions after endotoxia (Fig. 4). Baseline pulmonary CBP levels were not affected by xanthine oxidase inhibition.

Modulation of p65-CBP and CREB-CBP interactions in vivo affects CREB- and NF-κB-dependent gene transcription. Because association with CBP is required for CREB- or NF-κB-dependent transcription (10, 16, 17, 20, 32, 38), the effects of xanthine oxidase inhibition in increasing CREB-CBP and decreasing p65-CBP interactions should result in enhanced expression of CREB-dependent genes and diminished expression of those dependent on NF-κB. To determine if alterations in the association of CREB or p65 with CBP were functionally important and produced alterations in gene expression in vivo, we examined levels of c-Fos and MIP-2 mRNA in the lungs of hemorrhaged mice fed either a control or allopurinol-containing xanthine oxidase-inhibiting diet. c-Fos transcription is dependent on CREB (25), whereas that of MIP-2 is NF-κB dependent (36).

Compared with control hemorrhaged animals, inhibition of xanthine oxidase before hemorrhage produced increased expression of the CREB-dependent gene c-Fos (Fig. 5A), whereas expression of MIP-2, a NF-κB-dependent gene, was decreased (Fig. 5B). In contrast, xanthine oxidase blockade before endotoxia resulted in no changes in c-Fos (Fig. 6A) or MIP-2 (Fig. 6B) mRNA levels compared with those found in control mice after endotoxin administration.

DISCUSSION

Transcriptional activation of both NF-κB and CREB requires association with the coactivator CBP (10, 16, 17, 20, 32, 38). Transcriptional coactivators such as CBP function by facilitating or bridging sequence-specific activators such as NF-κB or CREB to the basal transcriptional machinery, including the RNA polymerase II holoenzyme, and altering chromatin structure. CBP contains histone acetyltransferase (HAT) domains and has strong HAT activity (6). CBP can
recruit the CBP-associated factor (p/CAF), which also has potent HAT activity, indicating that complexes with multiple HAT activities can be formed (32). The relative importance of the HAT activity of each coactivator appears to vary depending on the transcriptional factor involved. For example, CREB requires the HAT activity of CBP but not that of p/CAF (19), whereas NF-κB utilizes the HAT activity of p/CAF but not that of CBP (32).

Phosphorylation of NF-κB or CREB stimulates transcriptional activity by promoting interactions with CBP. For NF-κB, the required phosphorylation event involves serine-276 on the p65 subunit (38). CBP has two sites that can interact with p65, an NH₂-terminal domain that associates with unphosphorylated p65, and an S domain involving the KIX region, which can only interact with serine-276-phosphorylated p65 (38). The p50 subunit of the NF-κB heterodimer fails to recruit CBP (32). Phosphorylation of serine-133 of CREB, leading to transcriptional activation (9, 21, 26, 38).

Previous studies (13, 32, 38) showing association of CBP with CREB or NF-κB have all used in vitro or cell culture conditions, and little information was available concerning interactions between CBP and transcriptional factors under in vivo conditions relevant to disease processes. In the present experiments, we examined p65-CBP and CREB-CBP interactions in the lungs and found that hemorrhage or endotoxemia resulted in increased association of NF-κB or CREB with CBP. The enhanced interactions of NF-κB or CREB with CBP after hemorrhage or endotoxemia appeared to have functional importance because transcription of NF-κB- and CREB-dependent genes was contemporaneously increased.

In the present experiments, interactions between NF-κB, CREB, and CBP were examined in whole lung homogenates. In our laboratory's previous study (31), we demonstrated increased activation of NF-κB and CREB in neutrophils that accumulate in the lungs after hemorrhage or endotoxemia. Inhibition of xanthine oxidase resulted in increased activation of CREB in lung neutrophils after hemorrhage but not after endotoxemia (31). Similar to the results in the present...
In the present experiments, we found that inhibition of xanthine oxidase before hemorrhage could modulate association of CBP with CREB or NF-κB, providing a means to examine the importance of CREB-CBP and NF-κB-CBP interactions on transcriptional activity in vivo. As predicted by previous in vitro studies (22, 24, 38), decreased association of NF-κB with CBP diminished NF-κB-dependent transcription, whereas increased interaction of CREB with CBP led to enhanced expression of the CREB-dependent gene c-Fos.

Transfection experiments showing that increased expression of CBP results in enhanced NF-κB-dependent transcription indicate that the amounts of CBP present in the nucleus are limiting (20). The present experiments, coupled with our laboratory’s previous study (31), support this hypothesis and suggest that limiting levels of CBP are important in regulating transcription in vivo. If the amounts of CBP present in the lungs were not limiting, conditions that increase amounts of phosphorylated CREB should result in enhanced transcription of CREB-dependent genes without affecting NF-κB-dependent transcription, a finding not observed in these experiments.

The immunomodulatory actions of xanthine oxidase activation observed in the present studies were not unexpected. Previous work from our laboratory (31) and others (3) has shown that xanthine oxidase is activated by hemorrhage and that such activation is important in increasing transcription of NF-κB-dependent proinflammatory cytokines such as TNF-α and MIP-2. Xanthine oxidase-derived ROS also appear to modulate CREB phosphorylation through their inhibitory effects on mitogen-activated protein kinase kinase (MEK) 1/2 and ERK2 activity (1). ERK1/ERK2 can phosphorylate serine-133 of CREB (9, 21, 26, 37). Our laboratory (1) previously found that inhibition of xanthine oxidase before blood loss, but not endotoxemia, enhanced MEK 1/2 and ERK2 activity, providing a mechanism by which hemorrhage could affect CREB activation. In those experiments, interventions that increased or decreased ERK2 activation after hemorrhage produced parallel changes in the levels of phosphorylated CREB in the lungs.

An ancillary finding of the present experiments is that hemorrhage appears to modulate interactions between CBP and CREB or NF-κB by mechanisms that are different from those initiated by endotoxemia. Inhibition of xanthine oxidase before hemorrhage resulted in increased association of CREB with CBP and decreased amounts of NF-κB bound to CBP. In contrast, no such effects of xanthine oxidase blockade were found after endotoxemia. These results indicate that the mechanisms leading to acute inflammatory lung injury after blood loss or infection are distinct, even though the clinical presentation in each setting is similar. Such findings also suggest that early interventions aimed at preventing acute lung injury may need to be different depending on the clinical setting, with antioxidants having a greater role when ischemic injury predominates, such as after severe blood loss.

Fig. 4. Effect of xanthine oxidase inhibition on interactions of p65 or CREB with CBP after endotoxemia. LPS, lipopolysaccharide. Amounts of p65 or CREB associated with CBP were determined using lung extracts from control or allopurinol-fed mice 30 min after endotoxemia. A: representative experiment of 6. B: relative amounts of p65 or CREB normalized to CBP. Values are means ± SE.
The present results indicate that coactivators such as CBP have important roles in modulating transcription in vivo under clinically relevant pathophysiological conditions. As shown in these experiments, reciprocal interactions between CREB and NF-κB for binding to CBP occur in vivo, providing an explanation for the ability of manipulations that increase CREB phosphorylation to diminish NF-κB-dependent transcription. Such findings indicate that even though translocation of NF-κB to the nucleus and phosphorylation are not necessary for NF-κB-dependent transcription, coactivator binding to CBP is essential for optimal transcriptional activity.

Fig. 5. c-Fos (A) and macrophage inflammatory protein (MIP)-2 (B) expression in the lungs after hemorrhage in mice fed an allopurinol-containing xanthine oxidase-inhibiting or control diet. A and B, a: c-Fos and MIP-2 mRNA levels, determined by quantitative PCR, were normalized to those for rRNA. Representative PCRs are shown. R, reaction threshold. A and B, b: results from a representative experiment with 5 mice/group. Values are means ± SE. Two additional experiments with separate sets of mice gave similar results. **P < 0.01 and ***P < 0.001 vs. unmanipulated mice fed a control diet.

Fig. 6. Lack of effect of xanthine oxidase inhibition on c-Fos (A) and MIP-2 (B) expression in the lungs after endotoxemia. Mice were fed either an allopurinol-containing xanthine oxidase-inhibiting or control diet. A and B, a: c-Fos and MIP-2 mRNA levels, determined by quantitative PCR, were normalized to those for rRNA. Representative PCRs are shown. A and B, b: results from a representative experiment with 5 mice/group. Values are means ± SE. Two additional experiments with separate sets of mice gave similar results. **P < 0.01 vs. unmanipulated mice fed a control diet.
lation of p65 are necessary to initiate transcription of NF-κB-dependent genes, such events are not sufficient because additional regulatory events independent of NF-κB activation may affect recruitment of CBP and assembly of the transcriptional apparatus. In the present experiments, interventions that increased levels of phosphorylated CREB resulted in decreased association of NF-κB with CBP, suggesting that therapies that increase CREB phosphorylation could be useful in diminishing NF-κB-driven inflammatory responses in vivo. Other transcriptional factors such as p53 can also compete with NF-κB, and presumably with CREB, for interaction with CBP (35). Competitive interactions between transcriptional factors for association with a coactivator such as CBP may have important therapeutic implications because interventions that modulate activation of one transcriptional factor are likely to affect expression of additional genes not directly regulated by that factor.

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REFERENCES


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