Macrophages are an abundant source of cyclooxygenase-2 (COX-2) enzymatic products, but a specific mechanism for macrophage COX-2 gene expression has not been described. We examined whether PU.1, a myeloid-specific Ets family transcription factor, is involved. Sequence analysis revealed two potential c-Ets binding sites in the COX-2 promoter (COX-2p) which bind to immunoreactive PU.1. Chromatin immunoprecipitation analysis shows inducible PU.1 binding to these sites in response to lipopolysaccharide, and COX-2 protein production is augmented by ectopic expression of PU.1 but not by PU.1S148A, indicating that PU.1 phosphorylation is likely involved. Interestingly, expression of PU.1 results in acetylation of CCAAT/enhancer-binding protein-β (C/EBP-β) and increased production of COX-2 protein. Coimmunoprecipitation experiments suggest a role for p300 in C/EBP-β acetylation and COX-2 expression. In contrast, E1A inhibits acetylation of C/EBP-β and is correlated with decreased COX-2 expression. Together, these data suggest that PU.1 is activated by phosphorylation of Ser148 in response to lipopolysaccharide treatment and subsequently binds to sequences in the endogenous COX-2p in a time-dependent manner. Concomitantly, C/EBP-β becomes acetylated, and expression of the COX-2 gene increases. We speculate that a combinatorial role of PU.1 and C/EBP-β mediates the robust production of COX-2 products by macrophages which occurs in Gram-negative bacterial sepsis.

Proinflammatory stimuli induce COX-2 expression, which catalyzes the production of prostaglandins (1). Prostaglandins are involved in inflammation by influencing the recruitment of inflammatory cells and by increasing vascular permeability and inducing vasodilation (2–4). COX-2 expression is regulated largely at the level of transcription. The COX-2 promoter (COX-2p) contains many transcription factor binding motifs, suggesting that regulation of COX-2 gene expression could involve complex interactions among diverse transcription factors. Indeed, CREB, NF-κB, and C/EBP-β are known to be important in COX-2 gene expression (5–14). A recent study showed that C/EBP-β is required for the induction of COX-2 by stimuli in macrophages but not in fibroblasts (15), suggesting that there are possible functional interactions between C/EBP-β and macrophage-specific factors for COX-2 expression.

PU.1 is a member of the Ets transcription factor family whose expression is most abundant in B cells and macrophages (16–19). In the development of lymphoid and myeloid cells, PU.1 expression increases during myeloid differentiation of immature progenitor cells but decreases during erythroid differentiation (17, 19, 20). PU.1 also determines progenitor cell fate by functionally or physically interacting with other transcription factors such as GATA-1 and GATA-2 to suppress their transcriptional activities (21, 22). In addition, PU.1 inhibits acetylation of transcription factors by protein-protein interactions with CREB-binding protein (CBP)/p300 (23).

In some cases, PU.1 transcription activity is stimulated by phosphorylation. LPS treatment induces phosphorylation of PU.1 at Ser148, possibly by casein kinase II (24). Recruitment of PIP/IRF-4 to DNA and subsequent transcriptional synergy are dependent upon phosphorylation of PU.1 Ser148 (25). Accordingly, substitution of Ser148 with alanine abolishes transcriptional activity of PU.1 in association with PIP/IRF4 (25). Recent studies showed that PU.1 is also phosphorylated at Ser41 and Ser142 by Ras-phosphoinositide 3-kinase-dependent externally regulated kinase, Akt, and p38 mitogen-activated protein kinase-mediated pathways, respectively (26, 27).

Possible involvement of Ets family proteins in COX-2 gene expression was reported recently (28). PEA3, an Ets family protein, is a potent inducer of COX-2 in human breast cancer cell lines (29). The transcriptional activity of PEA3 requires an intact C/EBP-β binding motif in COX-2p (29). Although these results were from transient transfection of diverse reporter plasmids containing a partial COX-2p sequence, these results highlight a possible involvement of the ETS transcription factor family in COX-2 gene expression.

Here we examine whether PU.1 is a regulatory partner of C/EBP-β for COX-2 gene expression in macrophages. We show that PU.1 binds to the COX-2p in vitro as well as in vivo upon LPS treatment, which leads to COX-2 expression. We also provide evidence that C/EBP-β is acetylated in response to treatment with LPS, which is augmented further by PU.1. Our results suggest a novel mechanism by which PU.1 increases
COX-2 gene expression, at least in part, by augmenting acetylation of C/EBP-β.

EXPERIMENTAL PROCEDURES

Cell Culture—A murine macrophage cell line RAW264.7 (ATCC, Rockville, MD) was maintained in Dulbecco’s modified Eagle’s medium (Cellgro) containing 10% fetal bovine serum (HyClone), penicillin/streptomycin (Invitrogen), and 2 mM glutamine (Sigma).

Plasmids and Transfection—RAW264.7 cells (0.5 × 10^6) were transfected with plasmids by GenePORTER 2 (Gene Therapy Systems, Inc., San Diego), as specified by the manufacturer. Each transfection was normalized with appropriate empty vector plasmids. Transfected cells were incubated for 14–16 h in a 37 °C, CO2 incubator. For the LPS treatment, the cells were maintained further without serum overnight and subsequently treated with 1 µg/ml LPS in phosphate-buffered saline (Sigma) for different time points. To construct FLAG-tagged PU.1 and its mutant, pcDNA3.1 (+) was cut with NheI and HindIII (New England Biolabs) and ligated with a linker containing the FLAG sequence. Subsequently, murine PU.1 and PU.1S148A were amplified by (New England Biolabs) and ligated with a linker containing the FLAG sequence. Subsequently, murine PU.1 and PU.1S148A were amplified by PCR and were inserted downstream of the FLAG sequence in-frame after digestion with appropriate restriction enzymes. The expression of FLAG-tagged PU.1 and its mutant was determined by α-PU.1 antibody (Santa Cruz Biotechnology) as well as M2 antibody (Sigma). pcMV-murine PU.1 and PU.1S148A were described previously (25). pcMV-E1A12S/13S and -E1A13S were gifts from Dr. E. White (Rutgers University). All plasmids were prepared by an Endo-free Maxiprep kit (Qiagen).

Electrophoretic Mobility Shift Assay—The assay was performed as described previously (30). Two hairpin-structured oligonucleotides were designed. The complementary sequences underlined were separated by a guanidine and 4 nucleotides of thymidine that are constructed in a loop. The first oligonucleotide (5′-GAGGATCCAGTTTTC-CACCAGTACAGATG-3′) represents the sequence from -738 to -725 of mouse COX-2, which was named in this study as a proximal binding site. The second (5′-CCCACCTGGATCGGTTTCGCGCATCAC-TGGG-3′) represents the sequence from -333 to -318 of mouse COX-2, which was depicted as a proximal binding site.

RESULTS

PU.1 Binds to the Proximal COX-2 Promoter—Sequence analysis of the COX-2p by the TFSEARCH program (version 1.3, Tokyo University) revealed several potential c-Ets binding sites. Because most key transcription factor binding sites in the COX-2p are localized within proximal 1 kb of the promoter (5–15), we focused on two c-Ets binding sites located between -725 and -738 (5′-TAAGGATCCAGTTTTC-CACCAGTACAGATG-3′) and -529 and -320 (5′-ACTTGAGGATGGATGCGCAGTGAC-3′). The former is described in this study as the distal site and the latter as the proximal site. An NF-κB site is about 330 nucleotides downstream from the distal site and about 70 nucleotides upstream from the proximal site (Fig. 1).

To examine whether PU.1 recognizes these sites in vitro, we performed an electrophoretic mobility shift assay with probes containing the proximal or distal sites (data not shown).

Immunoprecipitation and Western Blotting—Total cell lysate was prepared using immunoprecipitation assay buffer (31). For immunoprecipitation analysis, 1–2 µg of appropriate antibodies was added to precleared cell lysate that had been normalized by protein contents and incubated overnight at 4 °C. Immune complexes were captured with 30 µl of protein A-Sepharose (Zymed Laboratories, Inc.) for 30 min at 4 °C and washed five times with radioligand precipitation buffer. Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane (Bio-Rad). Specific bands were revealed using enhanced chemiluminescence (ECL plus, Amer sham Biosciences). Nuclear extract was prepared after treating cells with hypotonic buffer (30). The amount of proteins was quantified by the Bradford assay (Bio-Rad) as specified by the manufacturer to ensure equal loading of samples. The antibodies used in this study were: normal rabbit IgG (Santa Cruz Biotechnology), α-COX-2 antibody (rabbit polyclonal from Santa Cruz Biotechnology), α-C/EBP-β and -δ (rabbit polyclonal from Santa Cruz Biotechnology), α-p300 antibody (rabbit polyclonal from Santa Cruz Biotechnology), and α-acetylated histone (mouse monoclonal from Upstate Biotechnology).

Cox2 Immunoprecipitation Assay—Reagents were obtained from Upstate Biotechnology, and the assay was performed as specified by the manufacturer. PU.1 binding to the COX-2p was analyzed by PCR with two sets of oligonucleotides. The first set of oligonucleotides (5′-TAGGAGATATCCAAAACACTT-3′ and 5′-TAGCTCAT-GAAAGCCTCAA-3′) encompassing the distal binding site generated a 408-bp PCR product. The second set of oligonucleotides (5′-CTAATTT- CACCGACGCGACGTGAC-3′ and 5′-ACTAGGAGGACTTCAGCCGAC-3′) encompassing the proximal binding site produced a 275-bp PCR product. PCR was performed at 94 °C for 30 s, 54 °C for 30 s, and 68 °C for 30 s with 40 cycles of reactions. PCR for the input was performed with 100 ng of genomic DNA.

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RAW264.7 cells were treated with 1 μg/ml LPS for various times, and nuclear extracts were prepared and then incubated with probes containing either the distal or proximal c-Ets binding sites. Both probes yielded protein-DNA complexes in the absence of LPS, and LPS treatment yielded little change in the binding pattern. To determine whether these complexes contained PU.1, the 2-h LPS sample with the proximal site probe was incubated with α-PU.1 antibody. Indeed, α-PU.1 incubation caused a complete supershift of the protein-DNA complex. Therefore, PU.1 binds, at least, to the proximal Ets site in the COX-2p.

A limitation of the electrophoretic mobility shift assay is that it examines DNA binding activity of proteins out of the cellular context and may not represent regulatory binding to the endogenous gene in response to diverse stimuli. To examine whether PU.1 recognized these two putative binding sites of the endogenous COX-2p in a regulatory fashion upon stimulus, we performed a ChIP assay (Fig. 2). After LPS treatment for indicated time points, RAW264.7 cells were fixed with formaldehyde. Protein-DNA complexes were immunoprecipitated with α-PU.1 antibody, and bound DNA fragments were recovered and subjected to semiquantitative PCR with oligonucleotides specific for the distal and proximal PU.1 binding sites (Fig. 2, A and B, respectively). As shown in Fig. 2, PU.1 binding to the proximal site was detected as early as 15 min, whereas binding of the distal site was observed within 1 h. Therefore, PU.1 is recruited to the COX-2p in response to treatment with endotoxin in a time-dependent manner, and its access to each site is regulated differentially.

To examine whether recruited PU.1 on the COX-2p results from an increased amount of PU.1 after LPS treatment, we measured total PU.1 levels in cytoplasmic and nuclear protein fractions of RAW cells treated with LPS by Western blot analyses against α-PU.1 antibodies. As shown in Fig. 3, the expression level of PU.1 was not affected significantly by LPS treatment, and immunoreactive PU.1 was confined to the nuclear protein fraction, indicating that PU.1 is expressed constitutively in RAW264.7 cells. Taken together, these data show that PU.1 binds to c-Ets binding sites in the COX-2p and that the LPS induction of PU.1 binding to the endogenous COX-2p is not the result of a simple increase in PU.1 expression.

PU.1 Enhances COX-2 Gene Expression—The LPS induction of PU.1 binding to the endogenous COX-2p suggested that PU.1 might regulate COX-2 expression. To test this, RAW264.7 cells were transiently transfected with a PU.1-expression plasmid with or without LPS treatment for 4 h (Fig. 4). Although LPS induced COX-2 expression, ectopic expression of PU.1 alone was not sufficient to induce COX-2 (Fig. 4A). However, PU.1 augmented the LPS induction of COX-2 50–100% as the amount of the PU.1 expression plasmid increased (Fig. 4, B and C). These data suggest that a modification of PU.1 induced by LPS treatment is functionally more important than an increased amount of PU.1.

To address this possibility, because phosphorylation at PU.1 Ser148 is known to be crucial for PU.1 transcriptional activity including its association with PIP/IRF-4 (24), we examined whether mutations of Ser148 would influence COX-2 gene expression (Fig. 5). RAW264.7 cells were transfected with plasmids expressing either FLAG-tagged wild type PU.1 or the PU.1<sup>S148A</sup> mutant and treated with LPS for 4 h. Equivalent expression of these proteins after transfection was assessed by Western blot with M2 antibody (Fig. 5A). As shown in Fig. 5A, PU.1<sup>S148A</sup> did not increase COX-2 expression in the presence of LPS compared with the induction observed with wild type PU.1. In three additional similar experiments that employed
PU.1- and PU.1S148A-expressing plasmids, we obtained comparable results (Fig. 5B). Together, the above results show that PU.1 is involved in COX-2 expression and that phosphorylation of PU.1 upon LPS treatment is likely to be important for its transcriptional activity so to induce COX-2 expression.

C/EBP-β Is Acetylated in Response to Treatment with Endotoxin—C/EBP-β is required for the induction of COX-2 by stimuli in macrophages (15), suggesting that there are possible functional interactions between C/EBP-β and PU.1, resulting in increased COX-2 gene expression in LPS-treated macrophages. C/EBP-β physically interacts with CBP/p300 that have histone acetyltransferase activity (31–33). Preliminary data showed that inhibition of histone deacetyltransferase activity by sodium butyrate markedly augmented COX-2 expression elicited by LPS (data not shown). These data prompted us to examine whether C/EBP-β becomes acetylated in response to treatment with LPS.

RAW264.7 cells were treated with LPS, and total cell lysate was prepared for immunoprecipitation of C/EBP-β. Immune complexes of C/EBP-β captured by protein A were washed extensively and analyzed by SDS-PAGE and Western blotting with α-acetyllysine antibody to reveal acetylated products. LPS treatment rapidly increased acetylation of C/EBP-β within 15 min (Fig. 6A). To confirm this observation further, a reciprocal experiment was performed (Fig. 6B). Acetylated proteins were immunoprecipitated by the β-acetyllysine antibody from total cell lysate and then analyzed by Western blot with α-C/EBP-β antibody to reveal acetylated C/EBP-β. Consistent with the data shown in Fig. 6A, acetylation of C/EBP-β was detected as early as 15 min of LPS stimulation and remained detectable 1 h after stimulation. In these experiments, the total amount of immunoreactive C/EBP-β was not significantly affected by LPS treatment, but acetylation of C/EBP-β was increased in a time-dependent fashion by LPS treatment (Fig. 7), indicating that acetylation of C/EBP-β is not the result of increased expression C/EBP-β. Taken together, these data show that acetylation of C/EBP-β takes place in response to LPS treatment.

PU.1 Increases Acetylation of C/EBP-β by Facilitating a Protein-Protein Interaction between C/EBP-β and CBP/p300—Because PU.1 interacts with CBP/p300 and modulates acetylation of other transcription factors that interact with CBP/p300 (23, 34), we tested whether PU.1 affects acetylation of C/EBP-β (Fig. 8). RAW264.7 cells were transfected with different amounts of a PU.1 expression vector and stimulated with LPS for 1 h, a time point when acetylation of C/EBP-β declined close to basal levels (Fig. 6). Total cell lysate was immunoprecipitated with α-C/EBP-β antibody, and immune complexes were analyzed by Western blot with the α-acetyllysine antibody to reveal the acetylated form of C/EBP-β. As shown in Fig. 8A, acetylation of C/EBP-β increased in a dose-dependent fashion.
with transfected PU.1 expression plasmid, which supports a conclusion that PU.1 increases acetylation of C/EBP-β.

To examine whether PU.1-dependent acetylation of C/EBP-β resulted from increased protein-protein interaction between C/EBP-β and CBP/p300, RAW264.7 cells were transfected with 8 μg of either an empty vector plasmid or 1–8 μg of PU.1, and each transfection was normalized with pCMV vector to 8 μg. At 24 h post-transfection, transfected cells were incubated with serum overnight and then treated with 1 μg/ml LPS for 1 h. Total cell extract was prepared, and an equal amount of proteins was used for immunoprecipitation (IP) of C/EBP-β. Immune complexes were analyzed by SDS-PAGE and Western blotting (WB) with α-acetylated antibody to reveal an acetylated form of C/EBP-β. A, PU.1 increases acetylation of C/EBP-β. RAW264.7 cells were transfected with either 8 μg of empty vector plasmid or 1–8 μg of PU.1, and each transfection was normalized with pCMV vector to 8 μg. At 24 h post-transfection, transfected cells were incubated without serum overnight and then treated with 1 μg/ml LPS for 1 h. Total cell extract was prepared, and an equal amount of proteins was used for immunoprecipitation (IP) of C/EBP-β. Immune complexes were analyzed by SDS-PAGE and Western blotting (WB) with α-acetylated antibody to reveal an acetylated form of C/EBP-β. B, PU.1 enhances interaction between p300 and C/EBP-β. Total cell extract was prepared from RAW264.7 cells either transfected with 8 μg of pCMV (lanes 1 and 2) or the PU.1 expression vector (lane 3 and 4) in the presence or absence of 1 μg/ml LPS for 1 h. Equal amounts of proteins were used for immunoprecipitation of C/EBP-β. As a positive control, p300 immunoprecipitation was also performed (lane 5). Immune complexes were analyzed by SDS-PAGE and Western blotting with α-p300 antibody to reveal that p300 interacts with C/EBP-β.

Fig. 7. Expression of C/EBP-β is not affected by LPS treatment. RAW264.7 cells were treated with 1 μg/ml LPS, and cytoplasmic and nuclear fractions were prepared. 20 μg of proteins were employed for Western blot (WB) analyses against C/EBP-β and -δ antibodies. Three isoforms of C/EBP-β, full-length (FL), liver-enriched activating protein (LAP), and liver-enriched inhibitory protein (LIP) are shown in the top panel, and expression of C/EBP-β upon LPS treatment is shown in the bottom panel.

E1A Inhibits Acetylation of C/EBP-β and COX-2 Expression—The adenovirus E1A protein binds to CBP/p300 and suppresses transcription by either blocking histone acetyltransferase activity or sequestering CBP/p300 (35–37). It is also known that E1A inhibits transcriptional activity of C/EBP-β by competing for the CH3 domain on CBP/p300 where both E1A and C/EBP-β bind (32). Therefore, it was possible that E1A might interfere with acetylation of C/EBP-β leading to a decrease in COX-2 gene expression.

To test this possibility, RAW264.7 cells were transfected with plasmids expressing human adenovirus 5 E1A 12S and E1A 13S (38) followed by treatment with LPS for 15 min before preparation of total cell extracts (Fig. 9A). As shown in Fig. 9A,
E1A suppressed acetylation of C/EBP-β induced by LPS treatment. To examine whether E1A down-regulated COX-2 expression, RAW264.7 cells transfected with the E1A 13S-expressing vector were incubated with LPS for 4 h to induce COX-2 protein expression. As shown in Fig. 9, B and C, E1A suppressed COX-2 gene expression elicited by LPS treatment. Taken together, these results suggest that E1A is able to interfere with acetylation of C/EBP-β elicited by LPS treatment, which leads to down-regulation of COX-2 gene expression.

**DISCUSSION**

COX-2 is induced by diverse stimuli that are relevant to the pathogenesis of severe sepsis, such as tumor necrosis factor-α, interleukin-1β, and LPS, in various cell types including macrophages (1). COX-2 is induced in lung in response to LPS challenge, indicating that COX-2 may be involved in pathogenesis of the acute respiratory distress syndrome (39, 40). COX-2 expression, in part, results from complex regulatory mechanisms of transcription, which differ depending on stimuli and cell type. In macrophages, COX-2 gene expression is regulated by C/EBP-β and its cognate binding site at position −138/130, whereas CREB and NF-κB binding sites seem to have nonessential roles (5, 15). Macrophages from C/EBP-β homozygous null mice are unresponsive to LPS stimulation, but fibroblastic cells respond normally (15). This result indicates that there are macrophage-specific regulators for COX-2 expression. In transcriptional regulation, macrophages differ from many cell types by the presence of myeloid cell type-specific Ets family protein PU.1 (16). Sequence analysis revealed the existence of c-Ets binding sites in the COX-2p. Therefore, the present study examined whether PU.1 is involved in COX-2 expression.

Our data show that PU.1 binds to the COX-2p in vivo upon LPS stimulation and that expression of PU.1 in the presence of LPS increases acetylation of C/EBP-β, which is closely correlated to COX-2 induction. The present results, combined with published data, suggest a possible mechanism by which PU.1 increases COX-2 gene expression (Fig. 10). LPS treatment induces phosphorylation of PU.1 that subsequently binds to its cognate binding sites in the COX-2p. Recruitment of PU.1 to
the promoter may increase the local concentration of CBP/p300, providing greater access to C/EBP-β. Acetylation of this key transcription factor seems to be involved in COX-2 gene expression.

Results of ChIP analyses showed that the proximal binding site is more accessible than the distal site, suggesting that recruitment of PU.1 in COX-2 expression. Although this may be caused by differential modification of PU.1 upon stimuli, it is more likely that PU.1 binding is controlled by the context of a COX-2 promoter. It is noteworthy that the proximal site is located between the NF-κB and C/EBP-β binding sites, which are involved in the transcriptional regulation of COX-2 gene expression. It is possible that these two factors and transcription coactivators such as CBP/p300 cooperate to open up the proximal PU.1 binding site.

Similar to previously published results (24), our data show that the level of PU.1 expression is not changed by LPS treatment. Interestingly, ectopic expression of PU.1 failed to induce COX-2 expression. Similarly, the level of C/EBP-β is not changed significantly upon LPS treatment, and overexpression of C/EBP-β also failed to induce COX-2 gene expression. In a sharp contrast, overexpression of inhibitor of C/EBP-β activation NF-κB fully induced COX-2 gene expression (data not shown). These results might highlight importance of modifications of involved transcription factors upon stimuli. Consistent with this notion, we found that phosphorylation of PU.1 at Ser148 mediates COX-2 gene expression. In addition, differential phosphorylation sites of PU.1 were reported in diverse cell types and stimuli (26, 27). Therefore, it is likely that PU.1 transcriptional activity is controlled by signal-dependent modifications rather than a simple increase of the amount of PU.1.

Our results cannot exclude the possibility that phosphorylation at other sites is also involved because Akt and p38 mitogen-activated protein kinase-mediated pathways phosphorylate PU.1 at Ser41 and Ser142 in B cells, respectively. It is not well understood how multiple phosphorylation events affect transcriptional activity of PU.1, but phosphorylation of Ser148 enables PU.1 to interact with PIP/IRF-4, which synergistically increases transcriptional activity of composite PU.1/IRF-4 sites (25, 41, 42). However, this mechanism may not be relevant to COX-2 expression in macrophages because PU.1/IRF-4 composite binding site is not present in the proximal COX-2 promoter, and the expression of IRF-4 in macrophages is controversial (41, 43–45). Alternatively, phosphorylation of PU.1 may increase its interaction with CBP/p300, the complex of which may easily bind to the PU.1 site in the COX-2 promoter. CBP/p300 recruited by PU.1 may increase the probability for other transcription factors to serve as a substrate for histone acetyltransferase activity of CBP/p300. Our data support this possibility because PU.1 activated by LPS enhances the interaction between CBP/p300 and C/EBP-β and accordingly increases acetylation of C/EBP-β.

Recent studies also show a close relationship between histone acetylation and gene activation (46–48). On the other hand, acetylation of transcription factors results in either activation or repression of transcription depending on the nature of transcription factors and acetylation sites (49, 50). Our results show that PU.1 with LPS treatment augments acetylation of a key transcription factor C/EBP-β and results in increased production of immunoreactive COX-2, suggesting that acetylation of C/EBP-β by PU.1 contributes to COX-2 expression. Expression of C/EBP-β generates three isoforms: full-length, liver-enriched activating protein, and liver-enriched inhibitory protein by promiscuous initiation of translation (5). Because a single band of acetylated C/EBP-β was detected in this study, it is possible that acetylation occurs upstream of the second Met residue in the N-terminal region where liver-enriched activating protein starts. The exact location of acetylated lysine residues is under investigation.

Our data show that adenovirus E1A inhibits acetylation of C/EBP-β elicited by LPS treatment, which concurrently leads to a decrease in COX-2 expression. E1A can modulate transcription via inhibition of CBP/p300 function either by sequestering CBP/p300 and/or inhibiting histone acetyltransferase activity (35–38). Because C/EBP-β binds to the same CH3 domain of CBP/p300 where E1A binds (32), it is likely that E1A suppresses acetylation of C/EBP-β by sequestering CBP/p300.

Functional similarities between E1A and PU.1 have been reported in blocking erythroid cell differentiation. The similarity stems from, in part, the fact that E1A and PU.1 share binding motifs on CBP/p300 which play a crucial role in erythroid cell differentiation (21, 51–53). A recent study also demonstrated that, like E1A, PU.1 suppresses acetylation of GATA-1 in vivo and other related transcription factors in vitro. In the present study we show that PU.1 increases acetylation of C/EBP-β, whereas E1A suppresses acetylation (23). This difference may be for several reasons. Because a PU.1 binding motif on CBP/p300 is not mapped precisely, it is possible that the PU.1 binding motif does not completely overlap with that of C/EBP-β on CBP/p300. Even if the two binding motifs overlap, it is possible that C/EBP-β has higher affinity to CBP/p300 than PU.1. Thus, PU.1 may not effectively sequester CBP/p300 from C/EBP-β. It is also possible that modification of PU.1 in response to stimuli regulates interaction between PU.1 and CBP/p300, which makes their interactions temporary. Finally, it is possible that DNA affects kinetics of interaction between PU.1 and CBP/p300 so that DNA bound PU.1 could have different affinity to CBP/p300. Unlike E1A, PU.1 binding sites are present in other macrophage-specific gene enhancer regions. In the proximal COX-2 promoter, at least two PU.1 binding sites lie near the C/EBP-β binding site.

In summary, we show that PU.1 binds to the COX-2 promoter in a stimulus-dependent manner and increases COX-2 expression in macrophages. Moreover, we provide evidence that PU.1 increases acetylation of C/EBP-β elicited by LPS treatment. Our results indicate that PU.1 collaborates with C/EBP-β in inducing COX-2 upon stimulus challenge.

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REFERENCES
Transcriptional Regulation of the Cyclooxygenase-2 Gene in Macrophages by PU.1
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