IL-1 and IL-6 induce hepatocyte plasminogen activator inhibitor-1 expression through independent signaling pathways converging on C/EBPα

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HepG2 cells (ATCC, Rockville, MD) were grown to 80% confluence with fresh serum-free medium containing 1 ng/ml IL-1β, 1 ng/ml IL-6, and inhibitors of induction identified previously, including (in μmol/l) 10 mevastatin (Sigma, St. Louis, MO), 20 LY-294002, 5 SB-203580, 10 SP-600125, 10 U-0126, 10 JAK inhibitor 1, 18 SN-50, and 20 API-2 (Calbiochem, La Jolla, CA). The inhibitors were used at concentrations that were not cytotoxic (9, 16, 21, 22, 27). Mouse primary hepatocytes were isolated with a two-step collagenase perfusion method (6) from male ICR mice (Hokkaido University Laboratory Animal Center) with procedures conforming to institutional animal study guidelines. Primary hepatocytes were cultured for 48 h in Williams’ medium E containing 10% FBS, 0.1 μmol/l insulin, 1.0 μmol/l dexamethasone, and 20 ng/ml epidermal growth factor. HepG2 cells and mouse hepatocytes were subsequently incubated in serum-free medium for 24 h and stimulated with IL-1β and/or IL-6 for 4 h.

Plasmid constructs. The human PAI-1 promoter region from −829 to +36 bp was amplified with the use of human genomic DNA as template, with upstream (GGGGTACCCGGTTGCTC-CTTGTTTCTTTACCAAGCC) and downstream (CCGGCTGCG-GACAGGCTGTTTGGGCTGGCTGCAAGCC) primers incorporating the restriction sites for Kpn1 and Xho1. After digestion by Kpn1 and Xho1, PCR product was ligated into luciferase reporter plasmid (pGL3-basic; Promega, Madison, WI). The produced plasmid was designated as PAI (−829). Sequence analysis indicated that PAI (−829) contains a 4G/4G polymorphism at −675 bp of the PAI-1 promoter. The same method was used to construct deletion mutant plasmids with the same downstream primer. The upstream primers were as follows: PAI-1 (−663) GGGGTACCTGTAT-CTCGGAGGGGCGGCCCAACA, PAI-1 (−539) GGGGTACCCGTTGGCAGGAGGAGG, PAI-1 (−308) GGGGTACCAAGTCCTGAGGAGGAGGAG, PAI-1 (−239) GGGGTACCAAGGCTATTGGGGTTTGCTCAAAGC, PAI-1 (−210) GGGGTACCAAGGCTATTGGGGTTTGCTCAAAGC, PAI-1 (−121) GGGGTACCAAGGCGGCCGCTGTTTGCAGGAGAGCC.

Mutated plasmids were constructed with the use of an overlap-extension PCR strategy. The mutated primers used were as follows (the mutated site is underlined): C/EBP mutant GGCTATTGGGGTTTGCTCAAAGC, 4G/4G mutant GGCTATTGGGGTTTGCTCAAAGC, and 4G/4G mutant GGCTATTGGGGTTTGCTCAAAGC.

Correct assembly was verified with the use of restriction analysis. Mutated regions generated by PCR were sequenced to identify clones without polymerase reaction errors.

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Transient transfection and luciferase assay. HepG2 cells were seeded at 8 × 10^4/well on six-well plates. After 24 h, 1.5 μg of Firefly luciferase pGL3-basic construct with different length of PAI-1 promoter was cotransfected with 1 μg of Renilla luciferase pRL-TK vector (for transfection efficiency control; Promega) with the use of the DNA-calcium phosphate coprecipitation method. Medium was replaced by serum-free medium 6 h after transfection. The cells were exposed to IL-1β, IL-6, and mevastatin 24 h after transfection and harvested 48 h after transfection. Cell lysate luciferase activity was determined with the use of a Dual-Luciferase Reporter Assay System (Promega).

Preparation of nuclear extract and performance of electrophoretic mobility shift assays. The probe encompassing the C/EBP motif from the PAI-1 promoter was prepared with the use of the following paired complementary oligonucleotides: PAI-1 C/EBP (AGCTATTGGGGTTTGTGTTAGGATTCCTTCAATTTGTTCC) and PAI-1 C/EBP (AGCTAG-GAACATAGTGTCGACAAAACCCCAATA).

Probes were end-labeled with [α-32P]dCTP and purified. DNA binding reactions were performed, and the reactants were incubated for 15 min at room temperature. For electrophoretic mobility shift assays (EMSA), supershift analysis antibodies (Santa Cruz Biotechnology) against C/EBPα (sc-61X), C/EBPβ (sc-150X), and C/EBPδ (sc-636X) were incubated with nuclear extracts for 10 min before addition of the probe. DNA-protein complexes were resolved on 6% polyacrylamide gels, and autoradiography was performed. Images were analyzed by densitometry (ImageJ software; National Institutes of Health, Bethesda, MD).

Western blot analysis of C/EBPδ. Immunological detection of C/EBPδ and PAI-1 was performed with rabbit polyclonal anti-C/EBPδ antibody (sc-636, 1:500 dilutions; Santa Cruz) and anti-PAI-1 antibody (no. 528216, 5,000-fold dilutions; Calbiochem). Images were analyzed with a densitometer. Anti-histone H1 antibody (Santa Cruz) was used to confirm equal loading of sample.

Isolation of RNA and quantitative reverse transcription PCR. Total RNA was extracted and reverse transcribed. PAI-1 and C/EBPδ mRNA were quantified with the use of real-time PCR as described previously (6). The following PCR primers were used: for human PAI-1, upstream (CCATCTCTCAGGCTGTCC) and downstream (AGTCTGCGCTCTGAGTTG) primers; for human C/EBPα, upstream (GACTCAGCAACGACCCATACC) and downstream (GTCCAGTCTTTTCTCTTAT) primers; for human 18S rRNA (an internal control), upstream (AGTCGGAATCAGACGA) and downstream (CGGGTGAGGTTTCCCATATT) primers; for mouse PAI-1, upstream (GACACCTCTAGATGTCAT) and downstream (AGGGTGCAAGAACACCATTTCCAT) primers; and for mouse C/EBPδ, upstream (CTCCGGCACACAACACTAG) and downstream (TTCGGCACAACCCTAAAG) primers; and for mouse β-actin (an internal control), upstream (TGGCTGACATCAAAGAAGA) and downstream (GATGCCACAGGATTCCATA) primers. After a PCR melting curve had been constructed to ensure that nonspecific products had been eliminated, the amount of mRNA was determined by comparison with the standard curve generated from serial dilutions of a T-vector containing cDNA of the gene.

Gene silencing with small interfering RNA. C/EBPδ mRNA silencing was performed with the use of two sequence-specific double-stranded small interfering RNA (siRNAs), C/EBPδ siRNA-a (GAUGCAACGAGAUGUGGGTTU) and C/EBPδ siRNA-b (GUCCUCAGCAAGGCCAUATT) (Ambion, Austin, TX). Double-stranded negative control siRNA and β-actin siRNA (Ambion) were used as negative and positive controls. Transfection of siRNA duplex was performed with TransIT-siQUEST transfection reagent according to the manufacturer’s instructions (Mirus, Madison, WI). In brief, HepG2 cells were transfected with 30 nM siRNA in complete growth medium. Medium was replaced by serum-free medium 24 h after transfection. After serum starvation for 16 h, the cells were stimulated by IL-1β for 4 h for C/EBPδ and 24 h for PAI-1.

Statistical analysis. All experiments were conducted in duplicate with independent separate cultures (n = no. of independent experiments). Data are expressed as means ± SD. Statistical comparison of control and treated groups was performed with Student’s t-tests. The accepted level of significance was P < 0.05.

RESULTS

Effects of IL-1β and IL-6 on PAI-1 promoter activity. To define the effect of IL-1β on PAI-1 promoter activity and determine whether it exhibited overlapping or nonoverlapping effects with respect to IL-6, the PAI-1 (~829) promoter construct was transfected into HepG2 cells, and luciferase activity was measured. IL-1β increased PAI-1 promoter activity by 3.8 ± 0.2-fold, and IL-6 increased PAI-1 promoter activity by 2.4 ± 0.2-fold. IL-1β plus IL-6 increased PAI-1 promoter activity by 7.1 ± 0.5-fold (Fig. 1A).

Mapping of IL-1β- and IL-6-responsive elements in the PAI-1 promoter. To localize the elements mediating the effects of IL-1β and IL-1β plus IL-6, cells were transfected with eight luciferase reporter vectors containing different deletion length of the PAI-1 promoter regions. PAI-1 promoter activity induced by IL-1β and IL-1β plus IL-6 was not effectively decreased with deletions preceding −239 bp. Deletion of the region from −239 to −210 bp was critical in mediating the IL-1β and IL-6 effects.

Identification of IL-1β- and IL-6-responsive elements in the PAI-1 promoter. To determine whether the C/EBP motif (~239 to −213 bp) that we have shown mediates the IL-6 effect (6) is responsible also for the stimulation exhibited by IL-1β and combined effects elicited by IL-1β plus IL-6, the PAI-1 (~829) plasmid with the C/EBP motif mutated was constructed and transfected into HepG2 cells. IL-1β-inducible PAI-1 promoter activity was decreased by 69%. Furthermore, no effect of IL-1β in combination with IL-6 on PAI-1 promoter activity was evident (Fig. 1B).

With the use of a PAI-1 (~829) plasmid with a mutation in 5G (~675 bp) we constructed, the IL-1β- and IL-6-inducible activity persisted. Although mevastatin did not alter basal PAI-1 promoter activity, it significantly decreased PAI-1 promoter activity induced by IL-1β (43 ± 4%) alone or in combination with IL-6 (48 ± 8%; Fig. 1C).

Effects of IL-1β and IL-6 on C/EBPδ binding activity. To determine whether the response of the PAI-1 promoter to IL-1β is mediated by C/EBPδ and, if so, to determine which member of C/EBP family is involved, IL-1β-induced C/EBPδ binding activity was assessed with EMSAs with antibodies against C/EBPα, C/EBPβ, and C/EBPδ. When antibody against C/EBPδ was used, supershift bands were slightly decreased by exposure of the cells for 4 h to IL-1β (Fig. 2A).

When antibody against C/EBPδ was used, supershift bands were unchanged (Fig. 2B). In contrast to binding of C/EBPα or C/EBPβ, C/EBPδ binding activity was increased by exposure of the cells for 4 h to IL-1β (n = 4, P < 0.05; Fig. 2, C and D) and to IL-1β plus IL-6. IL-1β-inducible C/EBPδ binding activity started to increase within 1 h of exposure and peaked with exposure of 4 h (6.7 ± 0.4-fold). Positive interaction between IL-1β and IL-6 was evident at every time point and
was maximal with exposure of 4 h (10.5 ± 0.6-fold; Fig. 3, A and B).

Mevastatin exerted no effects on binding activities of C/EBPα (Fig. 2A) or C/EBPβ (Fig. 2B) induced by IL-1β and/or IL-6. However, it significantly decreased C/EBPβ binding activity induced by IL-1β and IL-1β plus IL-6 (n = 4, P < 0.05; Fig. 2, C and D). These observations suggest that IL-1β- and IL-1β- plus IL-6-inducible transcription was initiated by increased binding of C/EBPβ protein to the PAI-1 promoter.

**Effects of IL-1β and IL-6 on C/EBPβ protein in nuclear extracts.** To determine whether mevastatin altered the concentrations of C/EBPβ protein induced by IL-1β and IL-1β plus IL-6, Western blot analysis was performed on nuclear extracts. IL-1β increased the concentration of C/EBPβ protein by 6.7 ± 0.7-fold, and IL-1β plus IL-6 increased the concentration of C/EBPβ protein by 8.9 ± 1.6-fold (Fig. 4, A and B). Mevastatin decreased IL-1β-inducible C/EBPβ by 37 ± 6% and IL-1β plus IL-6-inducible C/EBPβ by 39 ± 5% (n = 3, P < 0.05).

**Fig. 1.** A: identification of responsive elements for IL-1β and/or IL-6 on the plasminogen activator inhibitor-1 (PAI-1) promoter. HepG2 cells were cotransfected with PAI-1 (−829) or a series of deletion mutant or pGL3-basic plasmid. pRL-TK plasmid was used as an internal control. The absolute luciferase activity was measured by dividing the firefly luciferase activity with the internal control Renilla luciferase activity. The relative luciferase activity was then measured by normalizing absolute luciferase activity with respect to control activity in each plasmid group. Values are means ± SD (n = 6). *P < 0.05 vs. PAI-1 (−829) without agent (*) and vs. PAI-1 (−829) luciferase activity induced by IL-1β and IL-1β + IL-6 (#). B: effects of mutations in the 5G and C/EBP motif on the PAI-1 promoter activity induced by IL-1β and/or IL-6. Wild type indicates the PAI-1 (−829) construct. 5G mutant indicates a PAI-1 (−829) with a mutation at −675 bp. C/EBP mutant indicates a PAI-1 (−829) with the C/EBP motif (−226 to −213 bp) mutated. The relative luciferase activity was measured by normalizing absolute luciferase activity with respect to control activity in each plasmid group. Values are means ± SD (n = 6). *P < 0.05 vs. PAI-1 (−829) luciferase activity induced by IL-1β and IL-1β + IL-6. C: effects of mevastatin (Mev) on the PAI-1 promoter activity induced by IL-1β and/or IL-6. HepG2 cells were incubated with IL-1β, IL-6, and/or mevastatin. Values are means ± SD of the degree of increase compared with controls without agents (n = 4). P < 0.05 vs. PAI-1 (−829) without agent (*) and vs. PAI-1 (−829) luciferase activity induced by IL-1β and/or IL-6 (#).

**Fig. 2.** Effects of IL-1β, IL-6, and mevastatin on C/EBP binding to the PAI-1 promoter. HepG2 cells were exposed to mevastatin for 1 h and then to IL-1β and/or IL-6 for 4 h. The nuclear extracts were collected, and electrophoretic mobility shift assays (EMSA) were performed. Complex 1 (C1) consisted of CCAAT-enhancer binding protein (C/EBP) α and C/EBPβ, complex 2 (C2) of C/EBPβ and C/EBPδ, and complex 3 (C3) of C/EBPβ (6). A: supershift experiment with antibody against C/EBPα. B: supershift experiment with antibody against C/EBPβ. C: supershift experiment with antibody against C/EBPδ. D: C/EBPδ supershift bands shown in C were analyzed by densitometry. Values are means ± SD of the degree of increase compared with controls without agents. The upper halves of the whole images are shown. P < 0.05 vs. control ($) and vs. C/EBPδ binding activity induced by IL-1β and/or IL-6 (#).
although it did not affect accumulation of C/EBPβ protein in the absence of the cytokines.

Effects of IL-1β and IL-6 on expression of PAI-1 mRNA and C/EBPβ mRNA in HepG2 cells and mouse primary hepatocytes. Results obtained with quantitative real-time reverse transcription PCR showed that IL-1β and IL-6 increased expressions of both PAI-1 mRNA and C/EBPβ mRNA (n = 3, P < 0.05) in HepG2 cells (Fig. 4C) and in mouse primary hepatocytes (Fig. 4D). PAI-1 mRNA peaked with 4 h of exposure, and C/EBPβ mRNA peaked with 2 h of exposure (data not shown). Mevastatin significantly decreased PAI-1 mRNA and C/EBPβ mRNA induction by IL-1β and/or IL-6 in HepG2 cells (n = 3, P < 0.05) consistent with C/EBPβ being an essential mediator of induction by both cytokines.

Effects of siRNA-mediated downregulation of C/EBPβ on IL-1β-inducible PAI-1 expression. To further determine whether C/EBPβ is responsible for upregulation of PAI-1 induced by IL-1β, C/EBPβ siRNA transfection experiments were performed. C/EBPβ and PAI-1 protein were analyzed with Western blots. C/EBPβ expression was decreased by exposure of HepG2 cells to C/EBPβ siRNA. PAI-1 protein secretion induced by IL-1β was significantly decreased as well (n = 3, P < 0.05; Fig. 5, A and B). β-Actin expression was decreased by 72.4% with exposure of HepG2 cells to β-actin siRNA (n = 3, P < 0.05; Fig. 5C). β-Actin siRNA or negative control siRNA exerted no effects on PAI-1 and C/EBPβ expression.

Fig. 4. Effects of IL-1β, IL-6, and mevastatin on concentrations of C/EBPβ protein and PAI-1 mRNA in HepG2 cells and mouse primary hepatocytes. A: HepG2 cells were exposed to mevastatin for 1 h and then to IL-1β and/or IL-6 for 4 h. The nuclear extracts were collected, and Western blots were performed. Histone H1 was used as a loading control. B: C/EBPβ bands shown in A were analyzed by densitometry. Values are means ± SD of the degree of increase compared with controls without agents at time 0 (n = 3).
3-kinase, LY-294002, an inhibitor of p38/mitogen-activated protein kinase (MAPK), SB-203580, an inhibitor of c-Jun N-terminal kinase (JNK)/MAPK, SP-600125, an inhibitor of extracellular signal-regulated kinase (ERK)/MAPK, U-0126, a potent inhibitor of JAKs, JAK inhibitor 1, an inhibitor of nuclear factor-κB (NF-κB), SN-50, and an Akt/protein kinase B (PKB) signaling inhibitor, API-2. Under basal (unstimulated) conditions, LY-294002 decreased baseline C/EBP mRNA expression, and protein and API-2 increased PAI-1 mRNA expression and PAI-1 protein (Figs. 6A and 7A). The changes in absolute amounts of C/EBP mRNA and PAI-1 mRNA induced by IL-1β or IL-6 (Fig. 6B and D) were accompanied by directionally similar changes in C/EBP protein and PAI-1 protein (Fig. 7B and C). As was evident after normalization for basal mRNA levels (Fig. 6A), induction of relative increases in C/EBP mRNA and PAI-1 mRNA by IL-1β was significantly increased by LY-294002 and decreased by SB-203580, SP-600125, and U-0126 (Fig. 6C). Induction of relative increases in C/EBP mRNA and PAI-1 mRNA by IL-6 was significantly decreased by JAK inhibitor 1 (Fig. 6E). SN-50 and API-2 exerted no effects on PAI-1 and C/EBP expression induced by IL-1 or IL-6. These results

Fig. 6. Effects of the inhibitors of signaling pathways on PAI-1 and C/EBP mRNA induced by IL-1 or IL-6. HepG2 cells were treated with LY-294002 (LY, 20 μmol/l), SB-203580 (SB, 5 μmol/l), SP-600125 (SP, 10 μmol/l), U-0126 (U, 10 μmol/l), JAK inhibitor 1 (JAK1, 10 μmol/l), SN-50 (SN, 18 μmol/l), or API-2 (20 μmol/l) for 1 h. They were then exposed to IL-1 or IL-6 for 4 h. Total RNA was isolated, and real-time PCR was performed. 18S rRNA was used as an internal control. A, B, and D: absolute amounts of mRNA. Values are means ± SD of the degree of increase over controls (without stimulating agents). C and E: relative mRNA levels were determined by normalizing PAI-1 or C/EBP mRNA levels after induction by IL-1β (B) or IL-6 (D) with respect to control mRNA levels in cells treated with each inhibitor without stimulating cytokines (A). *P < 0.05 vs. PAI-1 mRNA induced by IL-1β (*), vs. C/EBP mRNA induced by IL-1β control (†), vs. PAI-1 control (§), vs. IL-1β-inducible C/EBP mRNA in negative control group (‡), and vs. IL-1β-inducible PAI-1 in negative control (†). P < 0.05 vs. IL-1β-induced PAI-1 in negative control (†). C: HepG2 cells were transfected with negative control siRNA and β-actin siRNA. After 4 h, total mRNA was extracted. β-Actin, PAI-1, and C/EBP mRNA were measured with the use of real-time PCR. *P < 0.05 vs. β-actin transfected by negative control siRNA.
In this study, a specific region of the PAI-1 promoter required for IL-1β induction of PAI-1 expression was delineated. The deletion of the elements from −239 to −210 bp containing a C/EBP motif was found to decrease IL-1β-inducible promoter activity. Mutation of the C/EBP motif decreased IL-1β-inducible PAI-1 promoter activity and abolished induction by of IL-1 plus IL-6 combined on promoter activity. Thus the C/EBP motif appears to be critical in mediation of the IL-1β response.

An insertion/deletion (4G/5G) polymorphism at −675 bp may alter PAI-1 gene expression induced by cytokines (4, 7, 17, 18). In the present study, no difference was found between 4G/4G and 5G/5G type in terms of basal and IL-1β-inducible PAI-1 promoter activity. The binding activity of C/EBPβ, but not C/EBPα or C/EBPδ, was increased by IL-1β. This was accompanied by an increase in the amount of C/EBPβ mRNA and C/EBPδ protein. Downregulation of C/EBPδ in response to siRNA reduced expression of PAI-1 induced by IL-1β. Thus C/EBPδ appears to be responsible for IL-1β-inducible PAI-1 expression.

Results in previous studies showed that IL-1β and IL-6 increase C/EBPδ expression in hepatocytes (15, 19). Results in our study extend these observations by demonstrating that IL-1β and IL-6 exert combined effects on expression of C/EBPδ mRNA and C/EBPδ protein. We found analogous results in primary mouse hepatocytes, increasing the likelihood that the results obtained with HepG2 cells can be extrapolated to human liver in vivo.

Several members of the C/EBP family, including C/EBPα, C/EBPβ, and C/EBPδ participate in mediating acute-phase responses (15, 19). The binding activity of C/EBPs to PAI-1 promoter was decreased and that of C/EBPβ was not altered by exposure of HepG2 cells to the inflammatory cytokines we studied. By contrast, concentrations of C/EBPβ mRNA and protein were low at baseline and were markedly increased by IL-1β. Therefore, it is likely that, following stimulation by IL-1β, C/EBPα bound to the PAI-1 promoter is replaced by C/EBPδ that consequently activates transcription of PAI-1.

In contrast to C/EBPβ, activated both early and late, C/EBPδ is likely to act late in altered gene expression associated with acute-phase reactions (19).

To determine which signaling pathways are involved in the IL-1- or IL-6-induced increased PAI-1 expression mediated by C/EBPδ, several relatively selective inhibitors of the signaling pathways were used. These inhibitors were used at concentrations that do not lead to apparent cytotoxicity (9, 16, 21, 22, 27). In HepG2 cells, IL-6 activates JAK, ERK, p38, and the PI 3-kinase/Akt pathway but does not activate the JNK signaling pathway (21, 22). When we examined upstream transduction pathways, we found that the JAK pathway was critical for IL-6-inducible C/EBPδ and subsequent PAI-1 production. Whether IL-6 induces C/EBPδ through STAT3 is not clear. STAT3 contributes to greater induction of C/EBPδ gene transcription by IL-6, and cooperative interaction of STAT3 with Sp1 is essential for the induction of C/EBPδ (2). However, overall induction of C/EBPδ is only minimally defective in STAT3-deficient mice (1).

IL-1β activates C/EBPδ through the ERK pathway in human enterocytes and p38/MAPK pathway in epithelial cells (11, 24). In HepG2 cells, IL-1β activates PI 3-kinase and p38, ERK, and JNK signal pathways (20, 22). In the present study, all three MAPK pathways were shown to be involved in the induction of C/EBPδ and PAI-1 mRNA by IL-1β, and PI 3-kinase signaling negatively influenced IL-1β induction. Akt/PKB is a downstream component of the PI 3-kinase signaling pathway. API-2 selectively inhibits the cellular phosphorylation/activation of all three homologous Akt isoforms (27). In the present study, API-2 increased PAI-1 expression induced by IL-1 and IL-6. However, increased basal PAI-1 expression by Akt inhibition may have contributed to the apparent IL-1- and IL-6-inducible PAI-1 increase by Akt inhibition. Hence it is unclear to what extent Akt regulates IL-1- and IL-6-mediated PAI-1 expression. Apart from the C/EBP family, NF-κB is another important transcription factor mediating IL-1 effects during acute-phase reactions in liver (8). However, our results suggest that NF-κB is not involved in upregulation of PAI-1 mediated by IL-1.

Mevastatin decreased expression of PAI-1 and inhibited increases of C/EBPδ mRNA, C/EBPδ protein, and C/EBPβ binding to the PAI-1 promoter induced by IL-1 and IL-6. Thus mevastatin exerted inhibitory effects downstream of C/EBPδ. Most of the pleiotropic properties of statins are attributable to inhibition of intracellular isoprenoids, important elements of

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**Fig. 7.** Effects of the inhibitors of signaling pathways on PAI-1 and C/EBPδ protein induced by IL-1β or IL-6. HepG2 cells were treated with the indicated inhibitors for 1 h. They were then exposed to IL-1β or IL-6. After 4 h, nuclear protein was extracted for assay of C/EBPδ protein by Western blot. After 24 h, medium was collected for assay of PAI-1 protein. A: effects of inhibitors on basal PAI-1 and C/EBPδ protein levels. B: effect of inhibitors on PAI-1 and C/EBPδ protein levels induced by IL-1β. C: effect of inhibitors on PAI-1 and C/EBPδ protein levels induced by IL-6. The results are representative of those obtained in 3 independent experiments.

**DISCUSSION**

In this study, a specific region of the PAI-1 promoter that consequently activates transcription of PAI-1. The JAK signaling pathway is known to be critical for IL-6-induced effects.

**Inhibitors**

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**A**

- C/EBPδ
- PAI-1

**B**

- C/EBPδ
- PAI-1

**C**

- C/EBPδ
- PAI-1

**Inhibitors**

- Con
- LY
- SB
- SP
- U
- JAK
- SN
- API-2

**IL-1**

**IL-6**

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the small G protein of the membranous signaling pathway (25). Because the kinase inhibitors used can potentially cross inhibit other kinases, further investigation of upstream signaling pathways of IL-1β and IL-6 will be necessary to identify distinct signaling pathways and additional targets that can be influenced by statins.

In conclusion, our results indicate that IL-1 and IL-6 exert combined, directionally similar effects on expression of PAI-1. However, these effects are mediated by distinct signaling pathways that converge on a final, common mediator, C/EBPβ.

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