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Biochemistry

Participation of the transcription factor C/EBPδ in the acute-phase regulation of the human gene for complement component C3

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ABSTRACT **C3, the third component of complement, is critical in the host immune response in that it is involved in both the classical and alternative pathways of complement activation. We have previously shown that a region (bp −127 to −70) within the C3 promoter is indispensable for conferring interleukin 1 (IL-1) responsiveness to this gene. A sequence comparison reveals two CCAAT/enhancer binding protein (C/EBP) consensus sequences, basic DNA binding region and leucine zippers 1 and 2 (bZIP1 and bZIP2), within this region. Site-directed mutagenesis of the more 3′ C/EBP site (bZIP1) in the C3 promoter significantly reduced the basal level of expression and the IL-1 responsiveness of the reporter gene, whereas mutation in the second, more 5′, C/EBP consensus sequence (bZIP2) had a minimal effect on basal expression and IL-1 inducibility. Electrophoretic-mobility-shift assays, with and without antibodies to the different C/EBP proteins that "supershift" protein–DNA complexes, demonstrated that proteins binding at the 3′ C/EBP site formed several complexes. Antibodies to C/EBPα supershifted the majority of complexes formed with extracts from control cells. Antibodies directed against C/EBPδ supershifted the major IL-1-inducible complexes. Western immunoblot analyses showed that the level of C/EBPδ protein was increased dramatically in the nuclei of Hep 3B2 cells after 4 h of IL-1 treatment. When Hep 3B2 cells were cotransfected with a C/EBPδ expression vector and a construct with a C3 promoter and a reporter gene, C/EBPδ was able to trans-activate the C3 promoter in an IL-1-responsive manner. The data strongly suggest that C/EBPδ is the major protein responsible for regulating the acute-phase expression of the human C3 gene.**

The response of hepatocytes to inflammation results in the rapid alteration of the levels of a number of human serum proteins, including C3, the third component of complement, serum amyloid A, α_1-acid glycoprotein, haptoglobin, hemopexin, and C-reactive protein (1, 2). These proteins, which have been termed acute-phase reactants, are synthesized mainly in the liver in response to tissue injury, infection, or other inflammatory stimuli, and the regulation of their expression often involves altered rates of transcription (1–3). A number of cytokines, including interleukin 1 (IL-1) (3–5), interleukin 6 (IL-6) (4, 6), tumor necrosis factor (3, 7, 8), and transforming growth factor β_1 (9), have been shown to be able to play a role in the altered synthesis of the acute-phase reactants.

C3 belongs to a subset of acute-phase reactants whose levels in serum increase moderately during the acute-phase response, from an average concentration of 1.4 ± 0.3 mg/ml to 2.4 ± 0.3 mg/ml (1). The human cultured hepatoma cell line Hep 3B2 has proven to be a useful model system to study the molecular events that occur during the inflammatory response. Although the change in C3 protein levels in the organism is modest, the transcriptional activity of the C3 gene in the cellular *in vitro* model is dramatically altered in response to cytokines. The analysis of C3 gene regulation by nuclear run-on experiments revealed that C3 transcription was increased 24-fold when Hep 3B2 cells were treated with monocyte-conditioned medium, a complex mixture of cytokines (3). C3 falls into the group of acute-phase reactants, including murine serum amyloid A, that are primarily responsive to the cytokine IL-1 (10, 11). Treatment with recombinant IL-1 alone has been found to induce C3 mRNA levels up to 40-fold (3).

We have cloned and characterized the promoter region of the human C3 gene (12). Transient transfection of a luciferase reporter construct linked to a series of 5′ and 3′ deletion mutants demonstrated that a segment between bp −127 and −70 contained a cis-acting element(s) required for both IL-1 and IL-6 inducibility. Examination of the sequence within this region revealed two CCAAT/enhancer binding protein (C/EBP) family consensus sites, CFCS1 and CFCS2 [hereafter referred to as basic DNA binding region and leucine zippers 1 and 2 (bZIP1 and bZIP2, respectively)]. DNase I footprinting analysis revealed that bZIP1 was strongly protected by nuclear extracts prepared from both control cells and cells treated with IL-1 (12). The footprint patterns generated by these extracts were identical.

Although IL-1 is a major regulator of inflammatory processes, surprisingly little is known about the molecular mechanism of IL-1 action in target gene regulation. Akira *et al.* (13) demonstrated that IL-1 regulates IL-6 gene expression through a C/EBP family protein, NF-IL6/C/EBPβ (hereafter referred to as C/EBPβ). Although C/EBPβ has been reported to be involved in the regulation of several acute-phase genes (14–16), it is not clear that this transcription factor is involved in the action of IL-1 on these genes. The complement C3 gene, which is prominently regulated by IL-1, serves as a paradigm to study the mechanism of target gene regulation by IL-1. In this report we show that C/EBPδ is the mediator of the IL-1 response of the C3 gene. This surprising finding suggests the possibility that C/EBPδ may have wide relevance for IL-1 action for a large number of its target genes.

MATERIALS AND METHODS

Materials. Restriction and DNA modification enzymes were purchased from BRL, New England Biolabs, or Boehringer Mannheim. Two oligonucleotides were prepared for use in site-directed mutagenesis: 5′-GGAAATGGTATTGTGTCGACTGGGGCAGC-3′ for mutant C3luc199mutSal and 5′-GACTGAAAAGCTTATCGCGAGGTATTGAGAAA-3′ for mutant C3luc199mutNru. Underlined nucleotides represent the site of mutation. Two complementary oligonucleo-

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Abbreviations: IL-1, interleukin 1; IL-6, interleukin 6; bZIP, basic DNA binding region and leucine zipper; C/EBP, CCAAT/enhancer binding protein; EMSA, electrophoretic-mobility-shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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tides representing the bZIP1 site in the C3 promoter, bZIP-A (5′-CATGGATGGTATTGAGAAATCTG-3′) and bZIP-B (5′-GATCCAGATTTCTCAATACCATC-3′), were also employed in electrophoretic-mobility-shift assay (EMSA) studies. These oligonucleotides were synthesized by the Nucleic Acids Core in the Institute for Molecular Genetics at Baylor College of Medicine. Recombinant IL-1β was a gift from Biogen (Geneva). Anti-C/EBPα (C14), anti-C/EBPβ (C76), and anti-C/EBPδ (C150) antibodies and the C/EBP family protein expression vectors MSV-C/EBPα, MSV-C/EBPβ, and MSV-C/EBPδ were kindly provided by S. McKnight (Tularik, Inc., South San Francisco) (17, 18). Another C/EBPβ expression vector, pSCT-LAP, was kindly provided by U. Schibler (University of Geneva) (15).

Cell Culture, Transfection Analysis, and Preparation of Nuclear Extract. Hep 3B2 cells were maintained as described (12). Cells used to prepare nuclear extracts were fed fresh medium or fresh medium containing IL-1β (20 ng/ml) 2, 4, 8, 12, 16, or 24 h prior to harvest. Transfection was carried out using calcium phosphate-mediated gene transfer as described (12), with the exception that cells were harvested by Triton lysis in Gly-Gly buffer (25 mM glycyl-glycine, pH 7.8/15 mM $MgSO_4$/4 mM EGTA/1 mM dithiothreitol) containing 1% Triton X-100 instead of three freeze–thaw cycles. For transactivation experiments, a total of 10 μg of plasmid was transfected per 60-mm dish. Each dish received 5 μg of C3luc114 as reporter gene plasmid, 0–5 μg of expression vector, and sufficient pBluescript II KS− (Stratagene) to bring the total amount of DNA to 10 μg. Nuclear extracts were prepared by the method of Baeuerle and Baltimore (19) except that extracts were dialyzed against solution E (12). After dialysis, extracts were assayed for protein concentration as described (12), divided into small samples, quick-frozen in liquid nitrogen, and stored at −70°C.

Site-Directed Mutagenesis. Site-directed mutants were produced by the procedure of Kunkel (20) with the modifications as described (12). Mutations were identified by digesting mutants with restriction enzymes that recognized the sequences we substituted. The mutated *Nco* I–*Nco* I fragment of each resulting phagemid was inserted into the unique *Nco* I site of plasmid C3luc199ΔN/N (12) (which has an 82-bp deletion in the wild-type promoter). Each one of these reporter constructs was further analyzed by diagnostic restriction digestion and by DNA sequencing to verify the presence of the mutation. Mutant C3luc114 was generated by digesting mutant C3luc199mutNru with *Nru* I and *Bam*HI and religating. It contains 114 bp of the C3 promoter from bp −114 to bp +1.

EMSA and Supershift Assays. A double-stranded oligonucleotide probe containing the bZIP1 sequence bZIP-C3 was produced by annealing the two complementary single-stranded oligonucleotides previously described and end-labeling with the Klenow fragment of DNA polymerase I. Binding reaction mixtures contained 10 μg of nuclear extract, 2.5 μg of poly(dI-dC)·poly(dI-dC), 10,000–20,000 cpm of radioactively labeled probe (≈35 fmol), 30 mM Tris·HCl (pH 8.0), 5 mM Hepes (pH 7.9), 0.66 mM EDTA, 7.5 mM $MgCl_2$, 60 mM KCl, 1.2 mM dithiothreitol, and 14% (vol/vol) glycerol. For competition, a 100-fold molar excess of unlabeled competitor was preincubated with extracts at 30°C for 10 min before adding radioactive probes. For supershift assays, antibodies against C/EBPα, C/EBPβ, or C/EBPδ were also preincubated with extracts for 10 min before the addition of radioactive probes. Incubation of extracts and radioactive probes was performed at 30°C for 30 min, and reaction products were analyzed on high-ionic-strength 5% polyacrylamide gels with TG buffer (50 mM Tris·HCl, pH 7.6/375 mM glycine) (21).

Western Immunoblot Analysis. Extracted protein (30 μg) was separated on SDS/10% polyacrylamide gels at 50 mA for 2 h and transferred to nitrocellulose membranes by the method of Towbin *et al.* (22) at 20 mA overnight. The membrane was then blocked in T-TBS buffer (20 mM Tris·HCl, pH 7.6/137 mM NaCl/0.1% Tween 20) supplemented with 5% (wt/vol) nonfat dried milk at room temperature for 1 h and washed with T-TBS buffer. For detection of bZIP proteins, the dilution factor for each primary antibody was 1:2500 (anti-C/EBPα), 1:2000 (anti-C/EBPβ), and 1:3300 (anti-C/EBPδ). We followed the protocol supplied with the enhanced chemiluminescence (ECL) Western immunoblot detection system (Amersham). The protein was visualized by exposing the membrane to x-ray film for 30–90 sec.

Northern Blot Analysis. Probe fragments for Northern blot analyses were isolated from plasmids for C/EBPα, C/EBPδ, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (23). Northern blots were prepared by standard techniques (24). Hybridizations using the bZIP probes were incubated and washed at high stringency [hybridization at 55°C in 50% formamide/5× SSPE (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA)/0.1% polyvinylpyrrolidone/0.1% Ficoll/±0.1% bovine serum albumin/sheared denatured salmon sperm DNA (50 μg/ml)/0.5% SDS]. The GAPDH probe was hybridized at 42°C. Autoradiographs were quantitated by densitometry; hybridizations were normalized to the GAPDH signal for comparison.

RESULTS

The Consensus Sequence bZIP1, but not bZIP2, Is Required for the IL-1-Inducible Activity of the Human C3 Promoter. Our previous work (12) showed that an IL-1-responsive element of the human C3 promoter was within a 58-bp region between bp −127 and −70. This region contains two bZIP consensus sequences (Fig. 1*A*), one of which was protected in footprinting assays by nuclear extracts prepared from Hep 3B2 cells with or without IL-1 treatment (12). Site-directed mutagenesis of this well-protected bZIP consensus sequence, bZIP1, abrogated the IL-1 responsiveness of the human C3 promoter (12) (Table 1). Mutation of the upstream bZIP consensus sequence bZIP2, however, had little effect on IL-1 inducibility. Furthermore, transfection analysis of C3luc114, a 5′ deletion mutant in which bZIP2 has been eliminated, has further delineated the functional domain to be within a 45-bp region from bp −114 to −71. Thus, bZIP1 is essential for IL-1 inducibility, whereas the bZIP2 consensus sequence at bp −115 to −123 can be deleted without substantially altering the basic IL-1 response of the C3 gene. Furthermore, the bZIP2 site in its normal position did not substitute for a mutant bZIP1 element.

Factor Binding to bZIP1 Changes from C/EBPα to C/EBPδ in Response to IL-1 Stimulation. A DNase I footprinting analysis (12) of the bZIP1 site showed that the footprint obtained at the bZIP1 site was identical to nuclear extracts from either control or IL-1-stimulated cells. However, EMSA studies of a double-stranded oligonucleotide spanning bZIP1 (Fig. 1*A*) showed major differences between the complexes formed using extracts prepared from control and IL-1-stimulated cells (Fig. 1*B*, lanes 2 and 3). Two complexes (C1 and C2) were formed with the control extracts; these complexes were greatly reduced in intensity when extracts from IL-1-stimulated cells were used. Instead, two major cytokine-induced complexes (S1 and S2) and one minor cytokine-induced complex (S3) were formed. Antibodies specific to individual bZIP proteins were used in supershift analysis to determine which bZIP protein(s) were involved in complex formation. Both control complexes were supershifted by antibodies specific to C/EBPα (Fig. 1*B*, lane 4). The S1 complex was also supershifted by the anti-C/EBPα antisera (Fig. 1*B*, lane 5). IL-1-inducible complexes

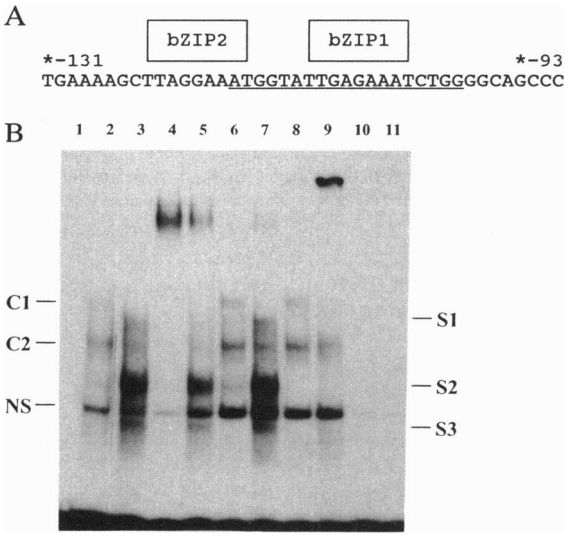

FIG. 1. Analysis of the Hep 3B2 nuclear proteins binding to the bZIP1 site of the human C3 promoter. (*A*) A schematic diagram of the human complement C3 gene promoter from positions −131 to −90 relative to the transcription start site. C/EBP consensus sequences, bZIP1 and bZIP2, are indicated. Underlined sequences are those encoded in the bZIP1 oligonucleotide used in the EMSA studies. (*B*) Nuclear extracts (10 μg) from cells without (lanes 2, 4, 6, 8, and 10) or with (lanes 3, 5, 7, 9, and 11) 24 h of IL-1 treatment are shown. The oligonucleotides representing bZIP1 sequences, bZIP-C3, of the human C3 promoter were labeled and analyzed. Complexes formed are as follows: C1 and C2, extract without IL-1 treatment; S1, S2, and S3, extract with IL-1 treatment; NS, a complex that is not consistently supershifted with any antisera in either extract. Antisera used in supershift assays are as follows. Lanes: 4 and 5, anti-C/EBPα; 6 and 7, anti-C/EBPβ; 8 and 9, anti-C/EBPδ. A 100-fold molar excess of nonradioactive bZIP-C3 oligonucleotide was added as competitor in lanes 10 and 11. Lane 1 contained neither extract nor antiserum. Lanes 2, 3, 10, and 11 contained no antiserum.

S1, S2, and S3 were neutralized completely by antibody prepared to C/EBPδ (Fig. 1*B*, lane 9), but neither C1 nor C2 was altered by anti-C/EBPδ. IL-1 treatment of the Hep 3B2 cells, therefore, caused a change in factor binding to the functional element bZIP1 from C/EBPα to C/EBPδ. Because the S1 complex produced with IL-1 extracts was decreased by both anti-C/EBPα and anti-C/EBPδ, it was likely to represent a heteromeric complex of C/EBPα and C/EBPδ.

An antibody to C/EBPβ did not reduce the intensity of any of the major complexes in either extract although a small amount of protein was supershifted in the IL-1-treated extract (Fig. 1*B*, lanes 6 and 7).

Table 1. Site-directed mutagenesis studies of the human C3 promoter

Construct	Fraction of wild-type C3luc199 activity	Fold induction by IL-1
C3luc199	1	72.3 ± 15.7
C3luc199mutSal (bZIP1)	0.3 ± 0.2	4.0 ± 1.4
C3luc199mutNru (bZIP2)	1.3 ± 0.1	41.5 ± 15.0
C3luc114	0.6 ± 0.1	38.3 ± 13.4

Values presented are the mean ± SEM of three sets of experiments except for C3luc114, which are the mean of two sets of experiments. Sites of mutation are indicated in parentheses beside the construct name. The fraction of wild-type C3luc199 activity is the level of the luciferase expression promoted by a given construct in unstimulated cells relative to that promoted by C3luc199 (also in unstimulated cells). The fold induction by IL-1 is the increase in luciferase expression promoted by a given construct after IL-1 treatment relative to the level of expression of that construct in unstimulated cells.

The complex labeled NS (Fig. 1*B*) was not completely supershifted by any of the three antisera. In other experiments, the intensity of the NS band was identical in both control and IL-1 extracts whether or not any of the three antibody preparations was included (data not shown). The observation that formation of this complex was inhibited by a 100-fold molar excess of two oligonucleotides unrelated to bZIP1 (NF-κB-like and HNF-1; data not shown) suggested that this complex was not bZIP1-sequence-specific.

C/EBPδ Is Induced in Nuclear Extracts in Response to IL-1 Stimulation. Nuclear extracts prepared from unstimulated (control) and IL-1-treated Hep 3B2 cells were analyzed for C/EBPα, C/EBPβ, and C/EBPδ on Western immunoblots. Anti-C/EBPδ antisera recognized a 35-kDa band on Western immunoblots (Fig. 2). Protein bands larger than 46 kDa were observed when secondary antibodies alone were used and were, therefore, not C/EBP-specific. The amount of C/EBPδ in the nucleus was dramatically increased in response to IL-1 treatment and peaked at 4 h (10-fold, Fig. 2). Its level decreased gradually after 4 h (Fig. 2, lanes 4–7) but remained elevated at 24 h of IL-1 treatment compared to the control extract (Fig. 2, compare lanes 1 and 7). Interestingly, we noticed two immunoreactive bands in the control extract (Fig. 2, lane 1), both of which ran more slowly than the strong immunoreactive band in the IL-1 extracts (Fig. 2, lanes 2–7), suggesting that modification might play a role in the activation of C/EBPδ. We were unable to detect C/EBPα or C/EBPβ in nuclear extracts from either control or IL-1-treated cells by Western immunoblot analysis, suggesting that those two C/EBP family proteins were expressed at lower levels than C/EBPδ. A direct comparison of protein levels was not possible, however, due to differences in antibody titers.

C/EBPδ Trans-Activates the Human C3 Promoter. Expression vectors for the bZIP factors MSV-C/EBPα and MSV-C/EBPδ (18) were cotransfected with the C3 reporter gene construct C3luc114 into Hep 3B2 (Fig. 3). Transfected cells were then treated with or without IL-1 for 20 h. Expression vectors for both C/EBPα and C/EBPδ trans-activated reporter gene expression 5.3- and 15.7-fold, respectively, in unstimulated Hep 3B2 cells, at an expression vector/reporter plasmid ratio of 1:2. The level of reporter gene activity declined at higher concentrations of expression vector, presumably due to squelching (25). In IL-1-treated cells, those transfected with C/EBPδ, but not C/EBPα, showed 3- to 5-fold greater reporter gene activity than cells transfected with the pBluescript vector, suggesting that the trans-activating capability of C/EBPδ, but not C/EBPα, was IL-1-inducible. MSV-C/EBPβ (18), which was also tested for its ability to trans-activate the C3-promoted reporter gene construct, did not significantly alter reporter gene expression (data not shown).

C/EBPδ RNA Is Increased in Hep 3B2 in Response to IL-1 Stimulation. Northern blots were probed sequentially for C/EBPδ, C/EBPα, and GAPDH (Fig. 4). Signal intensities for the bZIP probes were normalized to that of GAPDH for comparison. In two experiments, C/EBPδ RNA was in-

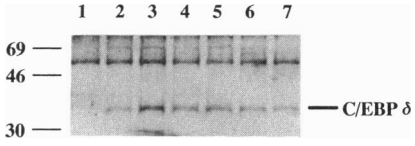

FIG. 2. Kinetic studies of the level of C/EBPδ in nuclei. Nuclear extracts were collected after 0, 2, 4, 8, 12, 16, or 24 h (lanes 1–7, respectively) of IL-1 treatment. Proteins were analyzed by SDS/PAGE on 10% gels, and C/EBPδ was detected by Western immunoblot analysis. Protein molecular mass markers are indicated (in kDa) at the left.

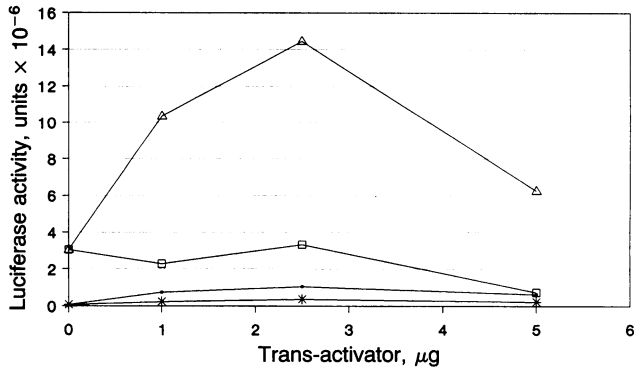

FIG. 3. Trans-activation of C3luc114 by the bZIP factors C/EBPα and C/EBPδ. Data from one representative experiment (performed twice) are shown. Hep 3B2 cells were cotransfected with the reporter gene construct C3luc114 plus increasing amounts of murine sarcoma virus-promoted expression vectors for C/EBPα and C/EBPδ. Cells were incubated with or without IL-1 for 20 h prior to harvest. ■, C/EBPδ; *, C/EBPα; △, C/EBPδ plus IL-1; □, C/EBPα plus IL-1.

creased 14- to 17-fold after 24 h of IL-1 stimulation. In this same time period, C/EBPα RNA decreased by 20–50%.

DISCUSSION

Our analysis of the functionally defined bZIP1 binding site of the human C3 gene has implicated a role for C/EBPδ in the acute-phase regulation of this gene. Using the human hepatoma line Hep 3B2 as an *in vitro* model of the acute-phase response, we have observed several IL-1-induced changes in parameters associated with C/EBPδ that support this role. These include the identification of C/EBPδ as a component of the major IL-1-inducible complexes detectable by EMSA, a dramatic increase in the abundance of C/EBPδ protein in Hep 3B2 nuclear extracts after IL-1 treatment, and IL-1-dependent increases in C/EBPδ mRNA and trans-activating capability.

C/EBPδ was described by Cao *et al.* (18) and Williams *et al.* (26) as a member of the bZIP family of DNA binding proteins. Recently, Kinoshita *et al.* (27) described the cloning of NF-IL6β, the human counterpart of C/EBPδ. They observed that after lipopolysaccharide treatment, C/EBPδ (NF-IL6β) mRNA increased in liver, lung, and kidney tissue. Interestingly, in several cell lines including an osteosarcoma cell line (MG-63), a glioblastoma cell line (SK-MG-4), and two hepatoma cell lines (HepG2 and Hep 3B), C/EBPδ mRNA was not detectable until cells were treated with IL-1

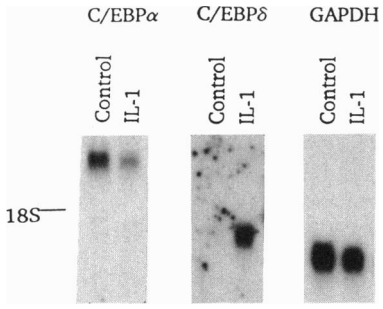

FIG. 4. Northern blot analysis of RNA for C/EBPα and C/EBPδ in Hep 3B2 cells. RNA was isolated from Hep 3B2 cells either unstimulated (Control) or treated with IL-1 (IL-1) for 24 h prior to harvest. Lanes contain 20 μg of total RNA. Probes for the hybridizations are indicated at the top of the figure. Band intensities were normalized to that of GAPDH for comparison.

or IL-6. These findings are consistent with the observed absence of C/EBPδ mRNA in unstimulated cells (Fig. 4).

A striking difference in the electrophoretic-mobility-shift pattern was observed when nuclear extracts from Hep 3B2 cells treated with IL-1 were compared with those from control cells. Supershift analysis using antibodies to the C/EBPα and C/EBPδ showed that C/EBPα bound the bZIP oligomer in the control extracts whereas C/EBPδ bound the oligomer in the IL-1-stimulated extracts. In Western immunoblot analysis, the level of C/EBPδ in nuclear extracts increased dramatically after 4 h of IL-1 treatment, suggesting that increased expression, activation, or mobilization of C/EBPδ occurred to increase its level in the nucleus. Since the immunoreactive species found in control extracts had slightly different electrophoretic mobilities, modification of C/EBPδ might be the initial IL-1-inducible event. It has been reported that modification of C/EBPβ, specifically phosphorylation, plays an important role in regulating the mobilization (28) or the activation (29) of C/EBPβ. Modification of C/EBPδ remains to be examined in our acute-phase system. However, it is clear that the level of C/EBPδ is greater in cells stimulated with IL-1. Perhaps sustained activity of C/EBPδ during the acute-phase response also relies on increased amounts of the δ protein.

C/EBPα also trans-activates C3 expression, but in an IL-1-independent manner. This fact, coupled with the binding of C/EBPα to the functional bZIP1 site using extracts from unstimulated cells, suggests that baseline expression of the C3 gene involves C/EBPα activity. Once cells were stimulated with IL-1, however, α binding activity decreased as δ binding activity increased with a concomitant increase in C3-promoted transcription. We have not yet determined whether the reduced binding of C/EBPα in IL-1-stimulated extracts is due to differences in the relative binding efficiencies of C/EBPα and C/EBPδ or to a decrease in the level of C/EBPα protein.

C/EBPβ has been implicated in the regulation of a number of acute-phase genes, including α_1-acid glycoprotein (14, 30, 31) and C-reactive protein (16). The role of C/EBPβ in C3 gene regulation is unclear. The construct MSV-C/EBPβ contains the entire β coding region but did not trans-activate the C3 promoter. However, pSCT-LAP, the liver-enriched transcriptional activator protein (LAP) vector constructed by Descombes *et al.* (15), which lacks the first AUG of the β coding region, acted as a potent IL-1-inducible trans-activator of C3 reporter gene expression (data not shown). This result parallels observations described by Descombes and Schibler (32) in the analysis of the albumin promoter. They found that the full-length LAP construct encoded three proteins, one from each of the in-frame AUGs. Two of these proteins served as trans-activators for albumin-promoted gene expression; the third, liver-enriched inhibitory protein (LIP), served as an inhibitor of trans-activation. It is possible that the difference in trans-activation we obtained with the two C/EBPβ vectors reflects the relative ratio of LAP to LIP produced from them. We were unable, however, to detect any appreciable quantities of C/EBPβ in nuclear extracts prepared from either unstimulated or IL-1-stimulated Hep 3B2 cells by either EMSA or Western immunoblot analysis that would support a major role for C/EBPβ in the acute-phase regulation of the C3 gene. The major IL-1-inducible complexes observed by EMSA were not attributed to C/EBPβ binding since β antibodies had little effect on the overall banding pattern. Thus, although the effect of IL-1 on expression of some genes, such as the IL-6 gene (13), is mediated by C/EBPβ, this factor cannot be the sole mediator of IL-1 responsiveness for all the inflammation-controlled genes. Our observation suggests, therefore, that there may be at least two general mechanisms, one involving C/EBPδ and

the other involving C/EBPβ, that can trigger and regulate expression of the inflammatory genes by IL-1.

Isshiki *et al.* (33) have described the reciprocal expression of α and β RNA in mouse liver in response to IL-6 stimulation. Alam *et al.* (34) further found not only that the steady-state level of C/EBPβ mRNA increased up to 4-fold in mouse liver in response to lipopolysaccharide injection but also that the level of C/EBPδ mRNA increased 70-fold within 8 h of lipopolysaccharide treatment and that these changes were likely to be predominantly posttranscriptionally regulated. We have identified a specific promoter sequence in the human C3 gene that is activated by C/EBPδ. Our observation that C/EBPα and C/EBPδ had reciprocal binding activity in response to IL-1 in the cultured cell line Hep 3B2 suggests that this *in vitro* model system will have great utility in analyzing the mechanism(s) associated with the C/EBPα to C/EBPδ transition during the acute-phase reaction. This observation increases our understanding of the mechanism of the inflammatory mediator IL-1 and the mechanism of acute-phase regulation of complement C3, a protein involved in the response to tissue damage and infection.

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