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The two C/EBP isoforms, IL-6DBP/NF-IL6 and C/EBPδ/NF-IL6β, are induced by IL-6 to promote acute phase gene transcription via different mechanisms

D.P.Ramji, A.Vitelli, F.Tronche[1], R.Cortese and G.Ciliberto*
Istituto di Ricerche di Biologia Molecolare (IRBM) P.Angeletti, Via Pontina Km 30.600, 00040 Pomezia, Rome, Italy and [1]Department of Biotechnology, Pasteur Institute, 25 rue du Dr Roux, 75724 Paris cedex 15, France

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ABSTRACT

The promoter regions of three IL-6 inducible genes, hemopexin (Hpx), haptoglobin (Hp) and C-reactive protein (CRP) contain *cis*-acting IL-6 responsive elements (IL-6REs) which are necessary and sufficient to induce IL-6 transcription activation. Transcription factors of the C/EBP family interact with IL-6REs. Among these, IL-6DBP/NF-IL6 plays a key role in IL-6 signal transduction because its *trans*-activation potential is induced by IL-6 in the human hepatoma cell line Hep3B. We show here that a different C/EBP-related factor, C/EBPδ/NF-IL6β, is the major IL-6 induced protein interacting with IL-6REs in the nuclei of Hep3B cells. In contrast to IL-6DBP/NF-IL6, whose activity in Hep3B cells is modulated by IL-6 via a post-translational mechanism, C/EBPδ/NF-IL6β is transcriptionally induced by IL-6. Another contrasting feature is that the C/EBPδ cDNA transfected in Hep3B cells activates transcription from an IL-6RE synthetic promoter in a constitutive manner which is not further enhanced by IL-6. Therefore, in Hep3B cells, two distinct members of the C/EBP family are recruited in the IL-6 signal transduction pathway via different mechanisms.

INTRODUCTION

The acute-phase (AP) response is characterized by changes in the blood level of several plasma proteins in response to infection, inflammation or other pathological conditions (see 1–2 for reviews). Two classes of AP proteins can be distinguished: the so called positive AP reactants (e.g. haptoglobin, hemopexin, C-reactive protein) whose plasma concentration increases during inflammation, and the negative AP reactants, including albumin and transferrin, whose levels decrease. Changes in the levels of AP reactants are correlated with changes in the transcription rate of the corresponding genes.

Several cytokines have been shown to play an important role in the regulation of AP response. These include interleukin 1 (3–4); interleukin 6 (IL-6; 5–6); leukemia inhibitory factor (7); glucocorticoids (8–9); interleukin 11 (10), transforming growth factor beta (11) and very recently also Oncostatin M (12). Amongst these, IL-6 is the most important mediator of transcriptional activation for the majority of AP genes.

IL-6 is involved in several biological responses (for reviews, see 13–14). Although substantial information has recently accumulated on how IL-6 interacts with two distinct receptor molecules on the surface of target cells (15–16), as yet very little is understood about the signal transduction pathway which mediates its effects. We have addressed this problem by directly analysing the nuclear events following IL-6 interaction with target cells, with the aim of identifying the molecular processes involved in the signalling pathway.

The human hepatoma cell lines Hep3B and HepG2 have proven to be a good model for the study of IL-6 induced gene expression (17–18). Transfection experiments have led to the identification of *cis*-acting IL-6 responsive elements (IL-6REs) in the promoter of three prototypical AP responsive genes (those encoding C-reactive protein (CRP), hemopexin (Hpx) and haptoglobin (Hp) (19–21). These IL-6REs are both necessary and sufficient for IL-6 induced transcription activation of reporter genes.

Various proteins of the C/EBP family bind to IL-6REs, yielding a complex profile in electrophoretic mobility shift assays (EMSA) and show identical footprinting and methylation interference patterns (19–21). Initial attempts to identify which C/EBP members are involved in IL-6 signal transduction led to the isolation of the transcription factor IL-6DBP (22). This protein has also been cloned independently in other laboratories from human, mouse and rat and named NF-IL6 (23); LAP (24); AGP/EBP (25); CRP2 (26); and C/EBPβ (27). The role of this protein in IL-6-dependent AP gene transcription might be important, as its transactivation potential is post-translationally activated by the cytokine (22). In contrast to this, C/EBPα activity is not enhanced by IL-6.

We show here that another member of the same family, namely C/EBPδ (also called NF-IL6β, CELF, CRP3) (26–29), is activated by IL-6. Unlike IL-6DBP, its activity is modulated at the transcriptional level in Hep3B cells.

* To whom correspondence should be addressed

MATERIALS AND METHODS

Recombinant Plasmids, transient transfections and CAT assays

The cDNA clone for C/EBPδ in an eukaryotic expression vector, under the control of the murine sarcoma virus long terminal repeat, was a generous gift from Dr. McKnight. The EcoRI/BamHI insert from this clone was 'filled-in' with Klenow polymerase and cloned into the SmaI site of PhD vector, under the control of the SV40 enhancer (30).

Monolayers of human hepatoma cell line Hep3B were maintained in Dulbecco modified Eagle's medium supplemented with 10% fetal calf serum. Human IL-6 was obtained from a recombinant vaccinia virus as described previously (19) and used at a concentration of 1000U/ml. DNA transfections were performed according to the calcium phosphate method (31). For CAT assays, the precipitate contained 12μg of reporter (4xα CRP; 21), 5μg of recombinant plasmid and 1μg of Rous Sarcoma Virus-luciferase plasmid (32) as an internal control for transfection efficiency. The precipitate was divided into two 5 cm plates and left for 16h. The precipitate was removed, the cells were washed with phosphate-buffered saline (PBS) and fed with 6 ml of culture medium, with one plate being treated with IL-6. Cells were harvested 48h after transfection and analysed for both CAT (33) and luciferase activity (32). The CAT activity was normalized to the luciferase value, and each transfection was repeated at least three times.

Preparation of nuclear extracts and EMSA

Nuclear extracts from Hep3B cells (either untreated or stimulated with 1000U/ml IL-6) were prepared essentially as described (34) except that the buffers contained the phosphatase inhibitors, sodium fluoride (50mM) and sodium pyrophosphate (10mM). For cycloheximide (CHX) inhibition experiments, the cells were pre-treated with CHX (10μg/ml) for 2h, and then stimulated in its presence with IL-6 for various time intervals (0, 20min, 40min, 1h, 5h). The maximal time of CHX treatment was 7h (5h stimulation with IL-6). In these conditions, the inhibition of protein synthesis and cell-death was 91% and 24% respectively as judged by metabolic labelling with ^{35}S methionine and ^{3}H-uridine, followed by scintillation counting of TCA precipitated total cell lysates.

For EMSA, nuclear extracts (4μg made to 13μl with 40mM KCl, 0.1mM EDTA) were preincubated in a 20μl reaction volume containing 34mM KCl, 5mM $MgCl_2$, 3μg poly (dI-dC), 2.5mM DTT. After 10min on ice, ^{32}P labelled probes were added and the incubation continued for 30 min at room temperature. Free and DNA-protein complexes were resolved on 6% polyacrylamide gels in 0.25× TBE (45mM Tris base, 45mM boric acid, 2mM EDTA). When antisera were added to the EMSA, nuclear extracts and antisera (1:50 dilution) were incubated for 30 minutes on ice, after the addition of the labelled probe.

The following oligonucleotides were used as probes:

αCRP	5′ GGGCATAGTGGCGCAAACTCCCTTACTG 3′
Hp C	5′ GATCCAGGAATTACGAAATGGAGGAG 3′
Hpx A	5′ GGGTATTTGCAGTGATGTAATCAGC 3′

EMSAs relative to the CHX experiments were performed with the following adjustments in order to obtain a better resolution of the various retarded bands: after the incubation with the probe, the samples were centrifuged for 5′ at 4°C in microfuge and electrophoresed on a 30 cm. long 6% polyacrylamide gel in 0.25×TBE at 4°C which was prerun for 30′ at 4°C.

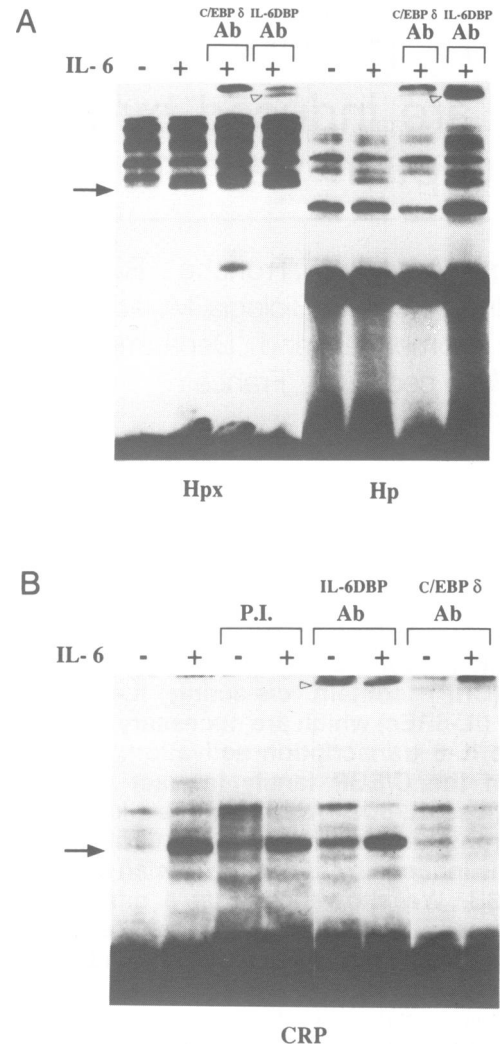

Figure 1. Identification of the IL-6 induced IL-6RE binding complex as C/EBPδ/NF-IL6β. Electrophoretic mobility shift assays (EMSA) were carried out using either the HpX (a site) or the Hp (c site) IL-6 RES as probes (**Panel A**) or the CRP (α site) IL-6REs (**Panel B**) and 4 μg of nuclear extracts from Hep3B cells either uninduced (−) or IL-6 induced (+) for 5 hrs with 1000 U of hIL-6. The IL-6 inducible complex is shown by the big dark arrow. Antisera against C/EBPδ/NF-IL6β and anti-IL6DBP antibodies were added to the binding reaction, as indicated in the Materials and Methods section.

Northern, Western blot and immunofluorescence analysis

Exponentially growing Hep3B cells were treated with IL-6 for several time intervals (0, 1, 2, 4, 6, 12 and 24h) and total RNA was prepared according to the procedures of Chomezynski and Sacchi (35). 15μg of each RNA sample were electrophoresed through denaturing formaldehyde-agarose gels (36), transferred to HyBond-N+ (Amersham), and probed with radiolabelled antisense oligonucleotide corresponding to positions 781 to 741 of the C/EBPδ coding sequence (27).

For preparation of whole cell extracts, Hep3B cells were washed with PBS and scraped off the tissue culture dish with 3ml of TEN (40mM Tris, pH 7.5, 1mM EDTA, 150mM NaCl). Cells were pelleted in a microcentrifuge tube and resuspended

in 6 volumes of high-salt buffer (10mM Hepes, pH 7.9, 400mM NaCl, 0.1mM EDTA, 0.5mM DTT, 5% glycerol, 0.5mM PMSF, 1mM Benzamidine). After 15min on ice, the cells were pelleted again and the supernatant was stored at −80°C. Equal amounts of protein (30μg) were precipitated with trichloroacetic acid (TCA), and electrophoresed through a 12.5% SDS-polyacrylamide gel (37). The proteins were then transferred onto nitrocellulose and probed with a 1:1000 dilution of polyclonal antibodies against C/EBPδ (a gift from S.L.McKnight; 27) or IL-6DBP (22). Antigen-antibody complexes were then detected by use of alkaline-phosphatase coupled goat anti-rabbit antibodies.

Immunofluorescence staining was done by use of cells grown on coverslips. Cells were fixed in 5% acetic acid in ethanol for 5min at −20°C, washed twice for 5min each with solution A (PBS, 0.2% gelatine), and then blocked with solution B (PBS containing 3% bovine serum albumin, 20mM $MgCl_2$, 0.3% Tween 20). Probing with primary and secondary (rhodamine conjugated antirabbit or antimouse) antibodies were performed in PBS for 1h in a humid chamber. The slides were washed twice with solution A containing 1% Triton X100 after incubation with each antibody, mounted using paraffin and analysed by microscopy.

RESULTS

C/EBPδ represents the IL-6 induced complex that interacts with IL-6RE

We have previously shown that upon incubation with human hepatoma nuclear extracts from Hep3B cells, DNA segments carrying IL-6REs form multiple DNA−protein complexes which yield a complex pattern in EMSA (19−21). Whilst the exact pattern of retarded bands varies depending upon the IL-6REs used, a common prominent feature is that a new DNA−protein complex appears in nuclear extracts derived from IL-6 treated cells, which is not present in those that are untreated. As shown in Figure 1, the IL-6 inducible complex (indicated by a dark arrow) is reproducibly observed in EMSA using IL-6REs from the promoter of Hpx (A site), Hp (C site) (panel A) and from the CRP promoter (α site) (panel B). The appearance of this complex was correlated to induction of acute phase gene transcription (19−21).

The majority of the other complexes involves the presence of nuclear proteins with DNA binding specificity and dimerization properties identical to C/EBP (22). In the light of the recent finding that both IL-6DBP/NF-IL6 and C/EBPδ/NF-IL6β mRNA are induced by IL-6 *in vivo* in mouse liver (23, 28), we tried to determine their involvement in the induction of acute phase gene expression in Hep3B cells.

In a first set of experiments we used polyclonal antibodies raised against rat IL-6DBP or C/EBPδ to identify if the IL-6 induced DNA−protein complex can be attributed to one of these two proteins. In a preliminary set of experiments we were able to see that these antibodies are specific to each protein. In fact, antibodies raised against IL-6DBP (22) are able to immunoprecipitate both *in vitro* synthesized rat IL-6DBP and the human counterpart NF-IL6 but not rat C/EBPδ and the human counterpart NF-IL6β. The opposite behaviour is shown by antibodies raised against rat C/EBPδ (27) (data not shown). As can be seen in Figure 1, the inducible complex obtained with each of the three probes (shown by the dark arrow) cannot be 'supershifted' or inhibited by using anti-IL-6DBP antibodies. It is interesting to observe that the use of our anti IL-6DBP antibody

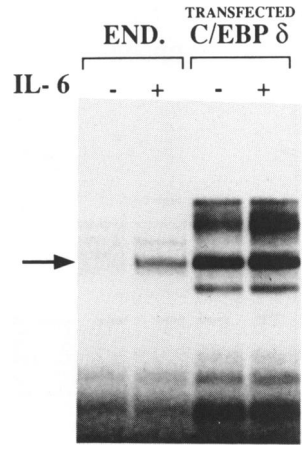

Figure 2. The transfected C/EBPδ cDNA gives rise to a major complex which comigrates with the IL-6 induced complex. EMSAs were carried out on nuclear extracts of non transfected Hep3B cells (END) in the presence (+) or absence (−) of IL-6, and on nuclear extracts of uninduced Hep3B cells transfected with C/EBPδ. The CRP (α site) was used as a probe. The arrow indicates the IL-6 induced protein-DNA complex.

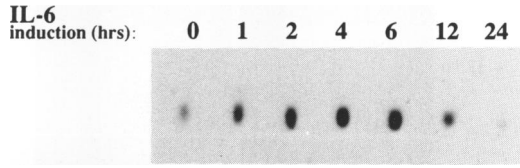

Figure 3. C/EBPδ/NF-IL6β mRNA is induced by IL-6 in a time-dependent manner. Northern blot analysis on total RNA prepared from Hep3B cells at various time intervals after IL-6 stimulation (see Materials and Methods).

preparation, while not leading to reduction or elimination of the IL-6 induced band, often gives rise to the appearance of a slow migrating band on the top of the gel (shown by an open arrow). This new complex is specifically induced by the immune antisera since it is not present when the pre-immune serum is used (Figure 1, panel B). Furthermore, as shown in the experiment with the CRP probe, it can be detected both in uninduced and in IL-6 induced extracts. This finding can be interpreted as an antibody-triggered induction or stabilization of IL-6DBP/NF-IL6 binding to the IL-6RE.

In contrast, antibodies against C/EBPδ (27) completely abolish the IL-6 induced complex suggesting that it contains C/EBPδ/NF-IL6β. This is further substantiated by EMSAs performed with extracts from cells transfected with a C/EBPδ expression vector and using the CRP (α site) IL-6RE as a probe (Figure 2). These extracts show several new complexes which are presumably all due to heterodimeric combinations of the newly synthesized recombinant C/EBPδ protein. The most abundant species, which we interpret as the C/EBPδ homodimer, co-migrates with the IL-6 induced complex seen in untransfected cells.

The mRNA and protein levels of C/EBPδ and not IL-6DBP are induced in Hep3B cells by IL-6

We have previously produced some evidence that the IL-6 dependent complex is at least partially independent of *de novo* protein synthesis because its induction is also observed when cells

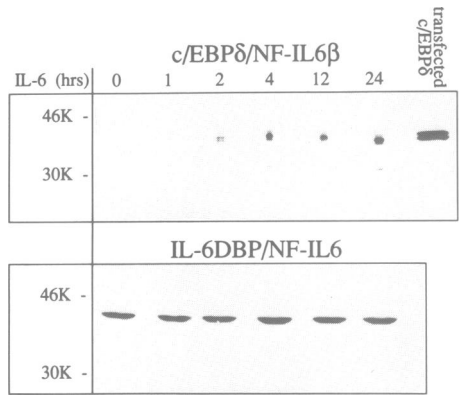

Figure 4. C/EBPδ/NF-IL6β synthesis is induced by IL-6 in Hep3B cells. Hep3B total cell lysates were prepared at various time intervals after IL-6 stimulation. 30 μg were loaded for each lane. After a 12.5% SDS PAGE, proteins were transferred to nylon membranes and revealed by antibodies to C/EBPδ/NF-IL6β (gift of Dr. McKnight) or to IL-6-DBP/NF-IL6β (22). The larger size of the bands in the control lane in the upper panel is due to the use of a larger slot former for the loading of proteins extracted from C/EBPδ transfected cells.

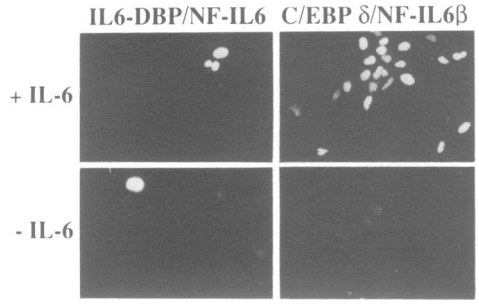

Figure 5. IL-6-DBP/NF-IL6 and C/EBPδ/NF-IL6β proteins are both nuclear proteins. Immunofluorescence analysis of Hep3B cells: Cells transfected with IL-6DBP-TAG and either treated (+) or not treated (−) with IL-6, were fixed in acetic acid and probed with a monoclonal anti-TAG antibody. Polyclonal anti-C/EBPδ antibodies were used on untransfected Hep3B cells in the presence (+) or absence (−) of IL-6. The signal was revealed in both cases by rhodamine conjugated secondary antibodies.

are treated with IL-6 in the presence of cycloheximide (20−21). Therefore we have investigated the properties of C/EBPδ/NF-IL6β biosynthesis in Hep3B cells by Northern blot, Western blot and Immunofluorescence analysis. RNA and total cell extracts were prepared from Hep3B cells treated with IL-6 for various times. As shown in Figure 3, C/EBPδ/NF-IL6β mRNA is present in trace amounts in untreated cells, and is induced by IL-6 in a time-dependent manner. These results are also mirrored by Western blot analysis using antibodies against C/EBPδ. Figure 4 shows a time course expression of C/EBPδ/NF-IL6β after Hep3B stimulation with IL-6. Two major protein species are detected which are probably due to post-translational modifications. The same two forms are also obtained after transfection of the C/EBPδ cDNA in uninduced cells (Fig. 4, upper panel, right most lane). In contrast, IL-6DBP/NF-IL6 is present constitutively in Hep3B cells, and its levels do not change upon IL-6 treatment (Fig. 4).

Recently, Metz and Ziff (38) have shown that in pheochromocytoma cells PC12 , cAMP causes IL-6DBP to *trans-*

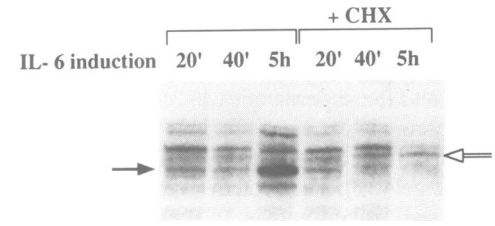

Figure 6. C/EBPδ/NF-IL6β induction is inhibited by cycloheximide. EMSA were carried out on nuclear extracts from Hep3B cells stimulated for various time intervals with IL-6 in the absence (−) or presence (+) of 10 μg/ml. of cycloheximide (CHX). The DNA used as a probe is the CRP (α site). The dark arrow indicates the IL-6 inducible complex corresponding to C/EBPδ/NF-IL6β. The open arrow indicates a slower-migrating cycloheximide-insensitive complex.

Table 1. The transcriptional activity of the transfected C/EBPδ is not modulated by IL-6.

IL-6 induction	% of chloramphenicol conversion −	+	fold of induction
phD	1.99	3.67	1.84
c/EBP α	53.63	58.99	1.1
c/EBP δ	19.55	24.17	1.24

Transient transfection experiments in Hep3B cells: the reporter plasmid contained four copies of the CRP (α site) fused to the CAT gene. Both C/EBPδ and C/EBPα cDNA were cloned in the phD eukaryotic expression vector under the control of the SV40 enhancer- early promoter.

locate to the nucleus. To understand whether this is the mechanism by which IL-6 induces the activity of IL-6DBP/NF-IL6 in Hep3B cells, we performed immunofluorescence analysis of cells transfected with a IL-6 DBP-TAG cDNA which codes for a full-length IL-6DBP protein fused at the carboxy-terminus to a 14 aminoacid peptide (TAG) which is specifically recognized by a monoclonal anti-peptide antibody (39). Our results show that immunofluorescence due to anti-TAG reactivity is always localized exclusively in the nuclear compartment both in cells which were not subjected to IL-6 stimulation (−IL-6) and in stimulated cells (+IL-6). The percentage of positive staining cells was about 10% and was not changed by IL-6 treatment. In contrast, C/EBPδ/NF-IL6b is not detectable in untreated cells and is present exclusively in the nucleus of cells treated with IL-6 (Fig. 5), again supporting the conclusion that C/EBPδ/NF-IL6β is induced by IL-6 only through *de novo* gene transcription and protein synthesis, and not by nuclear translocation of pre-existing cytoplasmic protein. In this last case, since we are detecting endogenous C/EBPδ/NF-IL6β production, virtually all Hep3B cells became immunostained after IL-6 treatment.

The evidence presented above that the IL-6 induced DNA-protein complex is due to C/EBPδ/NF-IL6β and that the biosynthesis of this protein is induced at the transcriptional level in Hep3B cells, is in contradiction with our previous findings (21−22), and so we sought an explanation for this discrepancy. Hep3B cells were first pre-treated for 2h with CHX (10μg/ml) and then stimulated in the presence of CHX with IL-6 for various time intervals (Fig. 6). Nuclear extracts were used in a high resolution EMSA using the CRP (α site) IL-6RE as a probe. In these improved conditions (see Materials and Methods section)

it is evident that the major IL-6 dependent complex (attributable to C/EBPδ/NF-IL6β) is indeed affected by CHX but, in addition to this, a slower migrating complex can be detected which is unaffected by CHX, but also by IL-6. In the experiment shown in Figure 6 this complex (shown by an open arrow) is weaker than the IL-6 induced complex identified as C/EBPδ/NF-IL6β in the previous paragraphs (shown by a dark arrow). Substantial variations in the relative intensity of these two retarded species are observed in different experiements, depending on variations in cell responsiveness to IL-6, cell density, etc. Most likely in the previous set of experiments we used conditions which were not able to resolve these two complexes and therefore reached a mistaken conclusion.

C/EBPδ activates transcription of a promoter containing IL-6REs from an acute phase gene

In order to investigate the role of C/EBPδ in IL-6 induced transcription activation, we performed transient expression experiments in Hep3B cells. In this assay system, the ability of C/EBP-related transcription factors produced by eukaryotic expression vectors to stimulate transcription of an IL-6RE-CAT reporter gene construct in the presence or absence of IL-6 can be investigated. The reporter plasmid used contains four copies in tandem of the IL-6RE of the CRP gene fused to its own TATA box and transcriptional initiation site (21). In these conditions, we have already shown that the activity of the transfected IL-6DBP is very low in the absence of IL-6 and is increased several-fold by IL-6 stimulation (22). As shown in Table 1, when C/EBPα was constitutively expressed under the control of the SV40 enhancer/early promoter, we obtained a strong transactivation of our reporter gene which, however, was not further enhanced by IL-6. The same behaviour was also observed when C/EBPδ was expressed under the control of the same promoter.

DISCUSSION

A family of C/EBP-related factors interacts with IL-6REs in the promoter regions of several acute-phase genes (Hpx, Hp, CRP) (19−21). Several members of this family have recently been cloned and it has been shown that they are capable of dimerizing in all intrafamiliar combinations (22,26,27). These include: C/EBP (C/EBPα; 40); IL-6DBP (also called LAP, NF-IL6, AGP/EBP, CRP2, C/EBPβ; 22−24); CRP1 (26); Ig/EBP (also named C/EBPγ; 41,27); CHOP (42) and C/EBPδ (CRP3, CELF, NF-IL6β; 26,27,29).

IL-6 regulates the intracellular concentration and/or the activity of the members of this family. C/EBPα mRNA levels are down-regulated in mice by IL-6 (43) and since it has been demonstrated that this protein interacts with the albumin promoter, a negative AP gene, (44) this could explain the IL-6 dependent down-regulation of the albumin protein levels. In contrast to C/EBPα, the level of IL-6DBP/NF-IL6 mRNA in mice is increased by IL-6 (20, 43). More recently also C/EBPδ/NF-IL6β has been shown to be transcriptionally regulated by LPS (and presumably by IL-6) in mice (28). These findings imply that these two factors are involved in regulating positive AP reactants. However, in light of the findings of Descombes et al. (24) and Williams et al. (26) that IL-6DBP mRNA levels do not correlate with the protein levels, it is clearly essential to obtain other data on the changes in the protein level *in vivo*.

From studies on living organisms it is impossible to learn details of the molecular events in IL-6 induced gene expression. This is mainly due to the complexity of the AP response, which involves several tissues with possible feed-back loops. The hepatoma cell line Hep3B is a good experimental system for the investigation of the role of IL-6 and C/EBP-related factors in the activation of AP gene transcription (7, 8, 17).

Using this system, we have previously shown that IL-6DBP activity is post-translationally modulated by IL-6 (20). We confirm here that IL-6DBP is expressed constitutively in Hep3B, but we also show that it is not present in the major IL-6 induced complex protein DNA. This result is in contrast with what has been observed by Isshiki et. al. (43) in HepG2 cells, where immune serum against NF-IL6 is able to inhibit the IL-6 induced complex in gel retardation experiments. The same antiserum (generous gift of Dr T.Kishimoto) in our conditions does not show any detectable inhibition using extracts from either untreated or IL-6 treated Hep3B cells (data not shown). In these cells the IL-6 induced complex contains C/EBPδ/NF-IL6β as shown by competition with specific antibodies. Furthermore, both the mRNA and protein levels of C/EBPδ/NF-IL6β, are induced by IL-6 in a time-dependent manner.

In Hep3B cells the activity of IL-6DBP/NF-IL6 is enhanced by IL-6 through a modulation of pre-existing nuclear protein whereas C/EBPδ/NF-IL6β participates in AP gene regulation through *de novo* protein synthesis. C/EBPδ/NF-IL6β is able to trans-activate AP gene expression as shown by cDNA transient transfection experiments, in which the level of activation is not altered by IL-6.

The findings of this paper provide a possible model for events of AP gene transcription in response to IL-6. During the early phase, IL-6 induces the activation potential of pre-existing nuclear IL-6DBP/NF-IL6 by a post-translational mechanism. However, this event may not be enough for full induction of AP genes. Indeed, Natsuka et al. (45) have recently shown that in Hep3B cells stably expressing exogenous copies of NF-IL6, no IL-6-dependent activation of fibrinogen, another AP gene, can be observed. In addition, the average induction of Hp production in these cells is substantially lower than in non-transfected cells, suggesting that another factor would be required. It is conceivable, therefore, that both these C/EBP family members participate in the regulation of AP gene transcription with different kinetics, a fast one for IL-6DBP/NF-IL6 and a slow one for C/EBPδ/NF-IL6β. Given the extreme complexity of the system, it is obvious that only genetic approaches (antisense oligonucleotides, ribozymes or gene targeting) aimed at reducing the quantity of a specific C/EBP-related protein will allow the role of individual factors in AP gene expression to be clearly defined.

As the activities of IL-6DBP/NF-IL6 and C/EBPδ/NF-IL6β are induced by different mechanisms (post-translational for IL-6DBP/NF-IL6 and transcriptional for C/EBPδ/NF-IL6β), IL-6DBP/NF-IL6 may be involved in the transcriptional activation of C/EBPδ/NF-IL6β. However, several lines of evidence suggest that the situation may be more complex. In fact, since C/EBPα can also act as a repressor on some promoters or enhancers (46−47), it is possible that down-regulation of C/EBPα by IL-6 may be responsible for the induction of C/EBPδ/NF-IL6β. Alternatively, transcription of C/EBPδ/NF-IL6β may be induced by other factors that do not belong to the C/EBP-family. For example, IL-6 has been shown to transiently induce JunB transcription (48). These speculations must clearly await the characterization of the C/EBPδ/NF-IL6β promoter.

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