cAMP-induced Interleukin-10 Promoter Activation Depends on CCAAT/Enhancer-binding Protein Expression and Monocytic Differentiation*

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The molecular mechanisms underlying the regulation of interleukin (IL)-10 transcription in monocytic cells by various stimuli during inflammation and the stress reaction are not fully understood. Recently, we provided evidence that stress-induced IL-10 promoter activation in monocytic cells is mediated by catecholamines via a cAMP-dependent signaling pathway including CREB/ATF (cAMP-responsive element binding protein/activating transcription factor) binding to two CRE motifs. However, the mutation of these sites diminished CAMP responsiveness by only 50%, suggesting a role for additional transcription factors and elements in the CAMP-dependent regulation of the human IL-10 promoter. Here, we analyze the functional role of one such factor, C/EBP, in two cell lines of myelomonocytic origin, THP-1 and HL-60, which are known to differ in their differentiation status and C/EBP protein content. We show that the level of basal as well as cAMP-stimulated IL-10 transcription depends on the expression of C/EBPα and β and their binding to three motifs in the promoter/enhancer region. The C/EBP5 motif, which is located between the TATA-box and the translation start point, is essential for the C/EBP-mediated constitutive and most of the CAMP-stimulated expression as its mutation nearly abolished IL-10 promoter activity. Our results suggest a dominant role of C/EBP transcription factors relative to CREB/ATF in tissue-specific and differentiation-dependent IL-10 transcription.

An appropriate balance between pro- and anti-inflammatory cytokines during the immune response is critical in the resolution of many pathological conditions. In this context, interleukin (IL)-10 is of special interest because of its anti-inflammatory and immunosuppressive properties. It is known that IL-10 synthesis in mononuclear cells can be induced or enhanced by various inflammatory stimuli and by sympathetic activation during the stress reaction or injury (1, 2), but the molecular mechanisms underlying the regulation of these processes are not fully understood. Recent reports demonstrate that lipopolysaccharide-induced IL-10 transcription may involve Sp1 activation by p38 mitogen-activated protein kinase (3) or the binding of Stat3 to the 5'-flanking promoter region of IL-10 (4). We showed previously that stress-induced IL-10 expression in monocytic cells is mediated by catecholamines via β2-adrenoceptors linked to a CAMP-dependent signaling pathway (5), which activates CREB/ATF by phosphorylation. These transcription factors act through two CAMP-responsive elements (CRE), CRE1 and CRE4, located within 1308 bp upstream from the translation start codon of the IL-10 gene (6). Yet, site-directed mutagenesis of these two CRE sites resulting in loss of protein binding was not sufficient to abolish CAMP responsiveness with 50% activity remaining. However, we previously described an additional putative CRE site (CRE3) that binds proteins that do not belong to the CREBATF or the activating protein-1 families of transcription factors. Although mutation of this CRE3 motif had no effect on protein binding or on promoter stimulation by cAMP, deletion of CRE3 by 5'-exonuclease digestion resulted in a significant reduction in activity (6). Therefore, we concluded that other CAMP-responsive transcription factors bind to CRE3, even if mutated, or to hitherto unknown regulatory elements in this region during stress/CAMP-dependent activation of the IL-10 promoter. At first, we examined the involvement of three putative activating protein-2 sites exhibiting protein binding, but we were not able to confirm their specificity in supershift assays. We next investigated CCAAT/enhancer-binding proteins (C/EBP), because these transcription factors are shown to be involved in CAMP-dependent gene expression, e.g. of IL-6 and IL-1, as well as of enzymes of catabolic metabolism in the liver (7–9). It is well established that C/EBP proteins mediate CAMP responsiveness by indirect mechanisms, which include their increased expression and translocation into the nucleus in response to elevated CAMP levels. Moreover, they possess domains that contain CAMP-inducible activities that are independent of direct phosphorylation by protein kinase A (PKA) (10, 11). Similar to CREB/ATF, C/EBP proteins belong to the group of basic region/leucine zipper transcription factors that have the potential to form heterodimers with each other and bind to motifs of either specificity (10, 12). C/EBP proteins are known to regulate adipocytic and monocytic differentiation, and their expression patterns vary depending on the state of cellular differentiation (13–15). Recently, we found that promonocytic THP-1 cells

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§ The abbreviations used are: IL, interleukin; C/EBP, CAMP-responsive element binding protein; ATP, activating transcription factor; AU, arbitrary units; CBP, C/EBP-binding protein; C/EBP, CCAAT/enhancer-binding protein; CRE, CAMP responsive element; dbCAMP, N°.2'-O- butyryl-cAMP; EMISA, electrophoretic mobility shift assay; MSV, mouse sarcoma virus; PKA, protein kinase A; TFBS, transcription factor binding site; DBA, DNA block aligner; hs, H. sapiens; mm, M. musculus; ma, M. monax.

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harbor significantly more C/EBPs than the myelomonocytic progenitor cell line HL-60 (16) and that THP-1 cells, unlike HL-60 cells, produce high levels of IL-10 protein after cAMP treatment (data not shown). To gain insight into the components involved in cAMP/stress-induced IL-10 expression, we asked whether C/EBP transcription factors contribute to promoter stimulation through the CRE3 site and four putative C/EBP binding sites, which were predicted by computer analysis. Using THP-1 and HL-60 cells, we demonstrate that C/EBPα and β are critical in both basal and cAMP-stress-dependent regulation of IL-10 expression during monocytic differentiation. As targets, we identified three previously unknown C/EBP motifs in the promoter/enhancer of the IL-10 gene, one of which corresponds to the recently described CRE3 site.

EXPERIMENTAL PROCEDURES
Electrophoretic Mobility Shift and Assay (EMSA) and Supershift Assay—Preparation of nuclear extracts and electrophoretic mobility shift assays were performed essentially as described elsewhere (16, 17). Ten μg of nuclear proteins extracted from THP-1 cells were incubated with 1 ng of radiolabeled oligonucleotides in a 20-μl reaction for 20 min at room temperature. The location of C/EBP binding motifs and the sequences of the used oligonucleotides are listed in Table I with the exception of CRE1 and CRE4, which we have described previously (6). For competition or supershifting, 100 ng of unlabeled oligonucleotide or 1.5 μl of the specific antisera were added, respectively. The C/EBPα, β, and δ antisera and oligonucleotides corresponding to C/EBP and CRE consensus binding sites were obtained from Santa Cruz Biotechnology. 

Cell Culture, Transfection, and Luciferase Assay—THP-1 and HL-60 cells were transfected with various expression constructs using a calcium phosphate coprecipitation method as previously described (16, 17). For transfections in THP-1, 106 cells/0.25 ml in 0.4-cm electroporation cuvettes were incubated with a gene plasmid, 5 μg for pGL2-1308 and 1 μg for pMSV/EBPα and pMSV/EBPβ expression plasmids, and 8 μl of a solution containing 0.7 mM CaCl2, 1.5 mM MgCl2, and 0.5 mM glutathione, pH 7.5. Electroporation was accomplished using the Gene Pulser (BioRad, Richmond, CA). 

RESULTS

C/EBPα and β Bind to Three Motifs in the IL-10 Promoter/Enhancer Region—In this study, we analyzed five putative C/EBP sites named C/EBP1 to C/EBP5 according to their position in the enhancer region upstream of the human IL-10 promoter (Table I). Four of these motifs (C/EBP2–5) were newly predicted by the MatInspector Professional program with core similarity of 0.7 and optimized matrix similarity. One

<table>
<thead>
<tr>
<th>Site</th>
<th>Site position (bp)</th>
<th>Oligonucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/EBP1 (CRE3)</td>
<td>−865/−852</td>
<td>tcggagcttgtaaagacattcttg</td>
</tr>
<tr>
<td>mutC/EBP1</td>
<td>−865/−852</td>
<td>tcggagcttgtaaagacattcttg</td>
</tr>
<tr>
<td>C/EBP2</td>
<td>−584/−574</td>
<td>ggggcttcTTGGGacAIttgaag</td>
</tr>
<tr>
<td>mutC/EBP2</td>
<td>−584/−574</td>
<td>ggggcttcTTGGGacAIttgaag</td>
</tr>
<tr>
<td>C/EBP3</td>
<td>−452/−439</td>
<td>caaTtaTTTTCRATCCcaat</td>
</tr>
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<td>mutC/EBP3</td>
<td>−452/−439</td>
<td>caaTtaTTTTCRATCCcaat</td>
</tr>
<tr>
<td>C/EBP4</td>
<td>−435/−418</td>
<td>tttgatcttcaagTTGCGCTATtcg</td>
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<td>−435/−418</td>
<td>tttgatcttcaagTTGCGCTATtcg</td>
</tr>
<tr>
<td>C/EBP5</td>
<td>−49/−30</td>
<td>cttttctgTGCCATATcagc</td>
</tr>
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<td>cttttctgTGCCATATcagc</td>
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motif, C/EBP1, is identical to the previously analyzed CRE3 site, which was shown not to be involved in CREB/ATF-induced promoter activation (6). Oligonucleotides harboring presumptive C/EBP motifs (Table I) were initially examined for their ability to compete with the consensus sequence using THP-1 nuclear extracts that are known to contain considerable amounts of C/EBP proteins. Unlabeled oligonucleotides consisting of C/EBP1, 3, 4, and 5 competed efficiently with the C/EBP consensus oligonucleotide for C/EBP protein binding (Fig. 1A, lanes 2, 4, 5, and 6), whereas C/EBP2 failed to compete effectively (lane 3). Supershift assays with labeled C/EBP1, 3, and 5 oligonucleotides revealed that DNA-protein complexes contained C/EBPa (Fig. 1B, lane 2, 4, and 9) and C/EBPβ but not C/EBPδ. Data obtained using C/EBP5 oligonucleotide are shown as a representative example for the reaction with antisera against C/EBPβ and δ (lanes 10 and 11). The proteins shifting the C/EBP4 oligonucleotide failed to react with C/EBPα or β antisera (Fig. 1B, lanes 6 and 7). Since we found that the C/EBP motif, which is identical to the previously analyzed CRE3 site binds C/EBPα and β, we wanted to exclude this possibility for the other CRE motifs. Competition experiments revealed that protein binding to labeled C/EBP3 does not interfere with unlabeled CRE consensus, CRE1 or CRE4 oligonucleotides (Fig. 1D, lanes 3, 4, and 6). Supershift analysis confirmed that protein complexes bound to CRE1 and CRE4 oligonucleotides do not contain C/EBPα or β (data not shown). The location of C/EBP binding sites in the 1308-bp IL-10 promoter/enhancer fragment is shown with respect to CRE motifs and the TATA box in Fig. 2.

To under-
C/EBP were obtained by overexpression of C/EBP in the absence or presence of dbcAMP. Positive values indicate fold stimulation of the basal promoter activity obtained with the mock plasmid or the respective concentration of pcDNA/p30 by cAMP. Negative values represent the ratio (inhibition factor) between the basal and the reduced activity obtained with the mock plasmid and pcDNA/p30 in the absence of cAMP. Mean values ± S.D. of three independent experiments are shown.

**TABLE II**

<table>
<thead>
<tr>
<th>Plasmid</th>
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<th>AU cAMP</th>
<th>SF</th>
<th>SF Basal</th>
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<tbody>
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<td>pcDNA3</td>
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<td>1492</td>
<td>12.2</td>
<td>100</td>
</tr>
<tr>
<td>pcDNA3/p30</td>
<td>45</td>
<td>235</td>
<td>5.2</td>
<td>38</td>
</tr>
<tr>
<td>IF</td>
<td>2.7-fold</td>
<td>6.3-fold</td>
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</table>

C/EBPα Influences Constitutive IL-10 Promoter Activity in HL-60 and THP-1 Cells—We recently showed that the promyelomonocytic cell line HL-60 contains significantly less endogenous C/EBPα compared with THP-1 cells (16). To study the influence of C/EBP on basal IL-10 promoter activity, we co-transfected pGL2-1308 along with increasing amounts of pcDNA3/p30 and incubated in the absence or presence of dbcAMP. Positive values indicate fold stimulation of the basal promoter activity obtained with the mock plasmid or the respective concentration of pcDNA/p30 by cAMP. Negative values represent the ratio (inhibition factor) between the basal and the reduced activity obtained with the mock plasmid and pcDNA/p30 in the absence of cAMP. Mean values ± S.D. of three independent experiments are shown.

**Fig. 3.** Influence of p30 overexpression on basal and cAMP-stimulated IL-10 promoter activity in THP-1 cells. Cells were co-transfected with pGL2-1308 along with increasing amounts of pcDNA3/p30 and incubated in the absence or presence of dbcAMP. Positive values indicate fold stimulation of the basal promoter activity obtained with the mock plasmid or the respective concentration of pcDNA/p30 by cAMP. Negative values represent the ratio (inhibition factor) between the basal and the reduced activity obtained with the mock plasmid and pcDNA/p30 in the absence of cAMP. Mean values ± S.D. of three independent experiments are shown.

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**Fig. 2.** 1308-bp fragment of the IL-10 promoter/enhancer region as cloned in pGL2-basic. The CRE and C/EBP motifs within the IL-10 promoter/enhancer region are delineated in relation to the TATA box (black box) and the IL-10 translation start (arrow) point. Gray circles and squares represent CRE and C/EBP motifs, respectively.
Efficient IL-10 Promoter Activation by cAMP Depends on C/EBPα—From p30 overexpression experiments and functional analysis of C/EBP site mutations, we know that C/EBPα has a significant effect on cAMP stimulation in THP-1 cells. We now wanted to investigate whether promoter responsiveness is impaired in HL-60 cells because of the low C/EBP protein content in this cell line. In fact, comparing the cAMP responsiveness of the IL-10 promoter in both cell lines, we found that the elevation of intracellular cAMP levels had only a small effect in HL-60 cells, whereas in THP-1 cells, elevated cAMP levels induced a steep rise in promoter activity (Fig. 9). As already shown in Fig. 6, promoter activity was substantially increased, supplying HL-60 cells with exogenous C/EBPα. Starting from a higher level of constitutive promoter activity, it could be further stimulated by cAMP in HL-60/C/EBPα cells up to the same level as that achieved in THP-1 cells expressing endogenous C/EBPα (Fig. 9). Even though the cAMP stimulation rate remains nearly unchanged in HL-60 and HL-60/C/EBPα cells, together, our data indicate that for maximal promoter activity, both the presence of C/EBP protein and the phosphorylation of CREB are necessary.

Comparative Analysis of the IL-10 Promoter/Enhancer Region—The transcription start site of the human IL-10 gene has not yet been identified experimentally, but a human EST and 5’ ends from mRNA of other species (Sus scrofa L20001, Ovis aries Z29362, and Trichosurus vulpecula AF026277) suggest that the transcription start site lies at position −59. Three IL-10 sequences were analyzed: human (hs), murine (mm), and the M. monax (ma) promoter/enhancer. All of the three sequences contain a TATA-box located at similar distances from the ATG: hs −89; mm −87; and ma −86. Pairwise comparison with DBA revealed two blocks of similarity that appeared in all of the three pairs: Block A at approximately −500 to −300 bp and Block B from −100 bp to the ATG start codon containing
the TATA-box. These conserved sequences were analyzed by the MatInspector Professional program (core similarity 0.95, optimized matrix). Block A in human, mouse, and *M. marmota* contains one C/EBP binding site (binding site matrix CEBP.01 in *hs* at -439 to -452, in *mm* at -450 to -463, and in *ma* at -422 to -435) and one CREB binding site (binding site matrix ATF.01 in *hs* at -401 to -414, CREB.01 in *mm* at -415 to -422, and CREB.04 in *ma* at -385 to -396), corresponding to the C/EBP3 and CRE4 motifs analyzed in the human IL-10 promoter (Fig. 10). In Block B, another C/EPB site (C/EBP5 in the human sequence) was identified: in *hs* at -30 to -43 and in *ma* at -39 to -52 (both matched the C/EPB.01 matrix). Initially, in the murine sequence, this matrix was not detected, but after lowering the search parameters, the equivalent sequence was found at positions -39 to -52 (Fig. 10). The quality of local alignments (data not shown) and TFBS matrix similarity suggest a closer relationship of the human and the *M. monax* IL-10 gene versus human and mouse or mouse and *M. monax*.

**DISCUSSION**

With this study, we add new results to our previous findings concerning the important role of cAMP and cAMP-inducing...
agents in the trans-activation of the human IL-10 gene in monocytic cells. Here, we show that the binding of the transcription factors C/EBPα and β to three motifs in the IL-10 promoter/enhancer region contributes to basal activity of the IL-10 promoter and is essential for maximal cAMP stimulation in differentiated monocytic cells. Each of the individual C/EBP motifs appeared to have a different impact on the trans-activation of the IL-10 gene. The most important effector site seems to be C/EBP5. Because of the proximity of C/EBP5 to the TATA box, an interaction of C/EBPα bound to this motif with the basal transcription-initiation complex can be envisaged (24, 25). Conversely, this model explains the comparatively high trans-activating capacity of the 376-bp fragment lacking CRE1, CRE4, C/EBP1, and C/EBP3 sites but retaining C/EBP5. The C/EBP3 motif, close to the main CRE4 site, had a moderate influence, whereas C/EBP1 seems to be less important. C/EBP1 coincides with the CRE3 site that failed to bind CREB/ATF (6) despite its greater sequence similarity to the CRE rather than to the C/EBP consensus motif. The binding of C/EBP to non-consensus CRE sites and the functional substitution of CREB by C/EBP family members have been described previously (7, 26). It is not surprising that the functionally relevant motifs C/EBP3, CRE4, and C/EBP5 fall into regions that are evolutionarily conserved between human, mouse, and M. monax, while the less important C/EBP1 and CRE1 sites are not conserved. These findings together with the fact that plasmids with single site mutation of C/EBP3 and CRE4 (6) retained similar cAMP-responsive activity as pGL2-376, bearing only the intact C/EBP5 motif hint at C/EBP-CREB interaction at multiple sites in a hierarchical and cooperative manner. We assume that the function of C/EBP3 requires prior occupation of the downstream C/EBP5 motif and the proximal CRE4 site, which implicates potential DNA looping and/or co-activator protein binding (19, 25, 27).

To study the role of C/EBPα in basal and cAMP-stimulated promoter activation, we used the truncated 30-kDa C/EBPα isoform (p30), which bears a conserved binding domain but lacks N-terminal regions required for its trans-activation and differentiation potential (19–21). Overexpressed p30 reduced the cAMP stimulation rate beyond the inhibition of basal activity and abolished the remaining promoter responsiveness of the double CRE mutant by competition with endogenous p42 C/EBPα in THP-1 cells. Data obtained from structure/function analysis of C/EBPα provide evidence that the mechanisms of constitutive and cAMP-inducible trans-activation are different and involve distinct albeit overlapping domains in the N-terminal portion of the protein (amino acids 55–86 and 52–124, respectively), which are both deleted in p30 (9, 19, 28, 29). The cAMP-mediated promoter stimulation was consistently stronger in THP-1 cells than in HL-60 cells, in agreement with their higher differentiation status and endogenous C/EBPα/β expression levels. When supplementing HL-60 cells with exogenous C/EBPα, we found that basal/constitutive promoter activity rose in a concentration-dependent manner and the cAMP response in HL-60/EBPα cells reached similar levels as in THP-1 cells. However, given the substantially higher basal level, the cAMP stimulation rate seems to be independent of C/EBPα overexpression. The reason for this remains unclear. We suggest that the increasing stimulation of the basal activity in THP-1 cells in response to rising cAMP concentrations involves enhanced expression, trans-location, and the binding of C/EBPα/β as well as phosphorylation of CREB, C/EBP and cofactors, and their interaction (11, 30–32). The model of transient overexpression of C/EBPα in HL-60 cells can only partially reproduce these processes. Co-transfection experiments with the dominant negative inhibitor of CREB in HL-60/EBPα cells revealed that trans-activation by C/EBPα to some extent depends on phosphorylated CREB being constitutively present.
in low amounts. Beyond this, C/EBPa overexpression seems to up-regulate the IL-10 promoter activity by itself. Taken together, our data from the mutation and co-expression experiments with p30 and CREB133 indicate a co-operative interaction of CREB and C/EBP transcription factors both in basal and in cAMP-stimulated promoter activation. In contrast to CREB, the cAMP-inducible domain of C/EBPa lacks a PKA phosphorylation site; thus, the mechanism of its activation by cAMP is yet unclear (33). It has been suggested that a co-activator could interact with the CAMP-inducible domain of C/EBP transcription factors only after it itself has been phosphorylated by PKA. This model is similar to that of CREB activation recruiting CREB-binding protein (CBP)/p300 after PKA phosphorylation with the exception that phosphorylation of the co-activator rather than the DNA-binding protein would regulate the interaction (9, 28, 31). In addition, CBP/p300 is a possible mediator between CREB and C/EBP. Indeed, CBP/p300 comprises a binding domain for C/EBPa/β (amino acids 1752–1859), which is distinct from its CREB binding domain (amino acids 552–660) (34). On the basis of our experimental data, we conclude that CREB/ATF mediates a relatively low activating potential in the absence of C/EBPa/β proteins, which are necessary for maximal IL-10 promoter activation. In our view, multiple enhancer binding proteins need to be available simultaneously in differentiated monocytes to achieve stable binding site occupancy required for maximally activated IL-10 transcription. This suggestion is in agreement with the observation that C/EBP expression is tissue-specific and varies during differentiation, while CREB/ATF is ubiquitously expressed (35, 36). Therefore, C/EBP-mediated transcription of a gene, in particular IL-10, is regulated in a tissue-specific manner and depends on the state of differentiation of a given cell. Experimental data from a mouse model, which revealed immediate IL-10 production in response to lipopolysaccharide and CAMP-elevating agents by hepatocytes known to contain high amounts of C/EBPα, support this hypothesis (37, 38).

In contrast to pro-inflammatory cytokines, which are mainly regulated by transcription factors such as NFkB and activating protein-1, the transcription of the prototypic anti-inflammatory cytokine IL-10 in monocytes, as shown here, is activated by cAMP or CAMP-inducing agents, e.g. catecholamines during sympathetic activation through transcription factors C/EBP and CREB. The mechanism of their interaction remains to be investigated.

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REFERENCES