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Structural and Functional Characterization of the Human CD36 Gene Promoter

IDENTIFICATION OF A PROXIMAL PEBP2/CBF SITE*

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Angel L. Armesilla‡§, Dominica Calvo‡§, and Miguel A. Vega¶

From the ‡Hospital de la Princesa, C/Diego de León 62, Madrid 28006, Spain and ¶Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas, C/Ventanilla 11, Granada 18001, Spain

CD36 is a cell surface glycoprotein composed of a single polypeptide chain, which interacts with thrombospondin, collagens type I and IV, oxidized low density lipoprotein, fatty acids, anionic phospholipids, and erythrocytes parasitized with *Plasmodium falciparum*. Its expression is restricted to a few cell types, including monocyte/macrophages. In these cells, CD36 is involved in phagocytosis of apoptotic cells, and foam cell formation by uptake of oxidized low density lipoprotein. To study the molecular mechanisms that control the transcription of the CD36 gene in monocytic cells we have isolated and analyzed the CD36 promoter. Transient expression experiments of 5′-deletion fragments of the CD36 promoter coupled to luciferase demonstrated that as few as 158 base pairs upstream from the transcription initiation site were sufficient to direct the monocyte-specific transcription of the reporter gene. Within the above region, the fragment spanning nucleotides −158 to −90 was required for optimal transcription in monocytic cells. Biochemical analysis of the region −158/−90 revealed a binding site for transcription factors of the polyomavirus enhancer-binding protein 2/core-binding factor (PEBP2/CBF) family at position −103. Disruption of the PEBP2/CBF site markedly diminished the CD36 promoter activity, indicating an essential role of the PEBP2/CBF factors in the constitutive transcription of the CD36 gene. The involvement of members of the PEBP2/CBF family in chromosome translocations associated with acute myeloid leukemia, and in the transcriptional regulation of the myeloid-specific genes encoding for myeloperoxidase, elastase, and the colony-stimulating factor receptor, highlights the relevance of the regulation of the CD36 gene promoter in monocytic cells by members of the PEBP2/CBF family.

CD36 is a plasma membrane glycoprotein constituted by a single 88-kDa polypeptide chain (Greenwalt *et al.*, 1992). It is a member of a family of proteins, which includes CLA-1 and LIMPII (Vega *et al.*, 1991; Calvo and Vega, 1993; Calvo *et al.* 1995). CD36 binds to a large variety of ligands: thrombospondin (Ash *et al.*, 1987; Silverstein *et al.*, 1992), collagens type I (Tandon *et al.*, 1989) and IV (Ash *et al.*, 1993), fatty acids (Abumrad *et al.*, 1993), oxidized low density lipoprotein (Endemann *et al.*, 1993), anionic phospholipids (Rigotti *et al.*, 1995), and *Plasmodium falciparum*-infected erythrocytes (Barnwell *et al.*, 1985; Oquendo *et al.*, 1989). CD36 is present on monocyte/macrophages, platelets, microvascular endothelium, adipocytes, mammary epithelial cells, and erythroblasts (Barnwell *et al.*, 1985; Oquendo *et al.*, 1989; Kieffer *et al.*, 1989; Greenwalt *et al.*, 1992; van Schravendijk *et al.*, 1992; Swerlick *et al.*, 1992; Abumrad *et al.*, 1993; Greenwalt *et al.*, 1995).

On the basis of its broad ligand-binding specificity, CD36 is considered as a scavenger receptor (Endemann *et al.* 1993; Acton *et al.*, 1994; Nicholson *et al.*, 1995). Scavenger receptors are primarily expressed on macrophages and participate in cell clearance of damaged cellular components and cells and foreign substances such as chemical compounds and pathogens (Krieger and Herz, 1994). In this respect, CD36 expressed in monocyte/macrophage cells cooperates with the $\alpha_v\beta_3$ vitronectin receptor in the recognition and subsequent phagocytic clearance of apoptotic cells (including neutrophils, T lymphocytes, and eosinophils) migrated to inflamed areas (Savill *et al.*, 1992; Ren *et al.*, 1995). In addition, CD36 expressed on macrophages infiltrated in damaged endothelium participates in the macrophage uptake of locally formed oxidized low density lipoprotein, thus contributing to foam cell formation and atherosclerosis development (Endemann *et al.*, 1993; Nicholson *et al.*, 1995).

In vivo regulation of CD36 expression in monocytes may be a complex process resulting from the coordinated interplay between multiple soluble factors and cell surface adhesion molecules. Thus, CD36 expression is dramatically increased on monocytes upon their interaction with activated endothelium and by treatment of monocytes with macrophage colony-stimulating factor or interleukin-4 (Huh *et al.*, 1995). Moreover, CD36 expression varies in some myeloproliferative disorders (Clezardin *et al.*, 1985) and is induced during the differentiation of promonocytes to monocytes and macrophages (Edelman *et al.*, 1986). Up-regulation of CD36 may increase macrophage clearance of apoptotic cells and facilitate monocyte migration through the endothelial barrier, enhancing oxidized low density lipoprotein uptake and monocyte-extracellular matrix interactions. On the contrary, treatment with lipopolysaccharide or γ-interferon results in down-regulation of CD36 mRNA (Huh *et al.*, 1995).

Despite the existence of a substantial amount of data regarding CD36 expression, little information is available on the mechanisms underlying its transcriptional regulation. We have recently delineated the structural organization of the CD36 gene, which revealed the presence of a TATA box located 28 base pairs upstream from the transcription initiation site (Armesilla and Vega, 1994). To define the CD36 gene regions, and to identify the transcription factors important for the ex-

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¶ To whom correspondence should be addressed. Fax: 34-58-203323; E-mail: mavega@samba.cnb.uam.es.

pression of CD36 in monocyte/macrophage cells, we have isolated and characterized the 5′-flanking region of the human CD36 gene. Our data demonstrate that the transcription of the CD36 gene in monocytic cell lines is mainly controlled by its proximal promoter region and that transcription factors belonging to the polyomavirus enhancer-binding protein 2/core-binding factor (PEBP2/CBF)[1] family play a major role in the transcriptional regulation of the CD36 gene.

EXPERIMENTAL PROCEDURES

Cell Culture and Transient Transfections—The cell lines used in this study, U937, Mono Mac 6, and THP-1 (monocytic), Jurkat (T cell leukemia), K562 (erytholeukemic cell line), JY (B lymphoblastoid cell line), and HeLa (epitheloid carcinoma) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, and 2 mM L-glutamine. Peripheral blood monocytes were isolated from blood obtained from healthy volunteer donors by centrifugation on Ficoll-Hypaque and subsequent adhesion to tissue culture dishes.

U937, Mono Mac 6, Jurkat, and K562 cells were transfected by electroporation. 20×10^6 U937 or Mono Mac 6 cells were electroporated in 500 μl of RPMI 1640 medium. Electroporation parameters were set at 2950 microfarads, 100 V, and a resistance of 186 ohms. 20×10^6 K562 cells were electroporated like U937 cells with the electric parameters set up to 400 V, 600 microfarads, and 13 ohms. Jurkat cells were electroporated in 250 μl of OPTI-MEM medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum with the following electroporation parameters: 1700 microfarads, 126 V, and a resistance of 72 ohms. For all electroporation experiments, the cells were incubated a 4 °C for 20 min prior to and after the electric shock. For each electroporation experiment 40 μg of the luciferase-reporter vector and 15 μg of the β-galactosidase reference plasmid pCMV-βgal were used.

HeLa cells were transfected by lipofection as follows. 5×10^5 cells were incubated in 60-mm tissue culture plates with 3 ml of OPTI-MEM medium containing 15 μg of Lipofectin (Life Technologies, Inc.), 5 μg of luciferase reporter vector, and 1.5 μg of pCMV-βgal vector. After 16 h, the transfection mixture was replaced by culture medium, where cells were maintained for 36–48 h.

Luciferase and β-galactosidase activities were measured 15 h after transfection for all cells except HeLa, according to Pahl *et al.* (1991) and Promega published procedures. For HeLa cells those activities were determined 36–48 h posttransfection. Luciferase activities were normalized for transfection efficiency to the β-galactosidase levels provided by the cotransfected internal standard vector pCMV-βgal. Reported data represented the mean from several independent experiments.

Deletion Constructs of the CD36 Promoter Region—An *Eco*RI-*Pst*I fragment of the CD36 gene that contains the TATA box and approximately 2.8 kilobase pairs upstream from it, was obtained from CD36 genomic phage λCh21ACD36.1 (described by Armesilla and Vega (1994)). This fragment was ligated to a pair of partially complementary oligonucleotides (GTGTAGGACTTTCCTGCA and AGCTTGCAGGAAAGTCCTACACTGCA, derived from the promoter region +11/+28 and displaying *Pst*I and *Hin*dIII sites when annealed), and to *Eco*RI-*Hin*dIII-digested vector pUCBM21. Fragment *Eco*RI-*Hin*dIII was blunt-ended with Klenow and subcloned into the blunt-ended *Bgl*II site of the luciferase reporter vector pGL2-Basic (Promega) to yield the plasmid p2.8KbCD36-luc, which included −2.8 kilobase pairs to +28 bp of the CD36 promoter region. Plasmid p2.8KbCD36-luc was used as the starting material to generate the progressive 5′ end deletion constructs p2.5KbCD36-luc to p0.6KbCD36-luc using the Erase a Base kit (Promega). Plasmids of the series p2.8KbCD36-luc to p0.6KbCD36-luc contained the CD36 promoter region comprised between nucleotides $-X$ and +28. Plasmids p267CD36-luc to p38CD36-luc were generated by PCR using the DNA isolated from phage λCh21ACD36.1 as template and pairs of oligonucleotides containing sites for restriction enzymes *Kpn*I and *Xho*I. The resulting PCR fragments were ligated to *Kpn*I-*Xho*I-digested vector pGL2-Basic. The structure of all of the PCR-generated constructs was confirmed by DNA sequencing. Plasmids of the series p267CD36-luc to p38CD36-luc contained the CD36 promoter region comprised between nucleotides $-X$ and +43.

Site-directed Mutagenesis—Mutation in the PEBP2 site at position −103 of the CD36 promoter was performed by polymerase chain reaction. Plasmid p158CD36-luc was used as a template. Primer A (GTTGGTACCTCAGTAATGTGCTGTGT) and primer B (GCGAAGCTTTTGTTGCCAGAGGAATTGAAAG) were used to generate fragment 1. Primers C (GCGAAGCTTCACTGGGATCTGACACTGTAG) and D (GGTCTCGAGGATCAAATGGTATTCTGCAGG) were used to generate fragment 2. Primers B and C were partially complementary and carried the point mutations for the PEBP2 site. PCR fragment 1 was digested with *Kpn*I and *Hin*dIII. PCR fragment 2 was digested with *Hin*dIII and *Xho*I. Both digested fragments were ligated to *Kpn*I-*Xho*I vector pGL2-Basic to generate the pCD36–158(m−102/−98)-luc plasmid. Nucleotide sequence of the mutant was confirmed by DNA sequencing.

Nuclear Extracts and EMSA—Nuclear extracts were prepared as described by Schreiber *et al.* (1989), with the inclusion of the phosphatase inhibitor sodium orthovanadate at 10 mM, the protease inhibitors aprotinin, leupeptin, and pepstatin at 1 μg/μl, and phenylmethylsulfonyl fluoride at 2 mM.

For EMSA experiments double-stranded oligonucleotides were ^{32}P-labeled using avian myeloblastosis virus reverse transcriptase. 0.5 ng of probe at a specific activity of about 10^8 cpm/μg were incubated for 15 min at 4 °C with 2–6 μg of nuclear extracts in 20 μl containing 28 mM EDTA, 15 mM KCl, 6 mM $MgCl_2$, 7 mM HEPES (pH 7.9 at 4 °C), 7% glycerol, 1 mM dithiothreitol, 2.5 μg of poly(dI-dC), and 1 μg of acetylated DNase-free bovine serum albumin. For competition assays, unlabeled oligonucleotides were added to the nuclear extracts at a 50-fold molar excess 15 min before the addition of the radiolabeled probe. When required, antibodies were added to the nuclear extracts 15 min prior to the addition of the radioactive probe. Two μl and 1 μl of the antibodies α-AML1 (Meyers *et al.*, 1993) and R3034 (kindly provided by Dr. N. A. Speck) were used, respectively. Binding reactions were electrophoresed at 15 V/cm on 4–5% polyacrylamide gels in 0.4 × TBE (45 mM Tris base, 45 mM boric acid, 1 mM EDTA) at 4 °C. Gels were dried and exposed to Kodak XAR film. The sequence of the oligonucleotides used for EMSA is shown in the figure legends.

RESULTS

A 158-bp Fragment from the 5′ Region of the CD36 Gene Controls Gene Expression in Monocytic Cell Lines—The involvement of CD36 expressed on monocyte/macrophage cells in atherosclerosis development and phagocytosis of apoptotic cells prompted us to investigate the transcriptional regulation of the CD36 gene in this cell type. As cell models we have used the widely characterized monocytic cell lines Mono Mac 6, U937, and THP-1 (Ziegler-Heitbrock *et al.*, 1988; Lübbert *et al.*, 1991). All of them displayed CD36 on the cell surface as determined by staining with fluoresceinated CD36-specific antibodies.[2] By contrast, Jurkat, K562, and HeLa cells were used as nonexpressing CD36 cell lines, as evidenced by the absence of CD36-specific cell surface staining and by Northern blot analysis.[2]

To examine the promoter activity of the 5′-flanking region of the CD36 gene and to identify potential cis-acting regulatory elements essential for its constitutive transcriptional activity, a series of deletion fragments of the region 5′-upstream from the TATA box of the CD36 gene (in the range from −2.8 kilobase pairs to −38 bp) was generated and coupled to the luciferase reporter vector pGL2-Basic. Plasmids were transfected in several cell lines, and the luciferase activity directed by each construct was measured as described under "Experimental Procedures."

CD36 deletion promoter constructs yielded between 40- and 80-fold higher luciferase activities than the pGL2-Basic promoterless construct in Mono Mac 6 and U937 cells (Figs. 1, *A* and *C*), while they only reached a maximum of 14 times in Jurkat and K562 cells (Fig. 1, *B* and *D*), demonstrating that 1) the 5′-upstream region of the CD36 gene possesses promoter activity and 2) the reporter luciferase gene under the control of the CD36 promoter is more efficiently transcribed in the CD36-expressing monocytic cell lines Mono Mac 6 and U937 than in the non-CD36-expressing cell lines Jurkat and K562. In this regard, the promoter activities of the CD36 constructs in Mono

[1] The abbreviations used are: PEBP2/CBF, polyomavirus enhancer-binding protein 2/core-binding factor; EMSA, electrophoretic mobility shift assay; PCR, polymerase chain reaction; bp, base pair(s).

[2] A. L. Armesilla, D. Calvo, and M. A. Vega, unpublished observations.

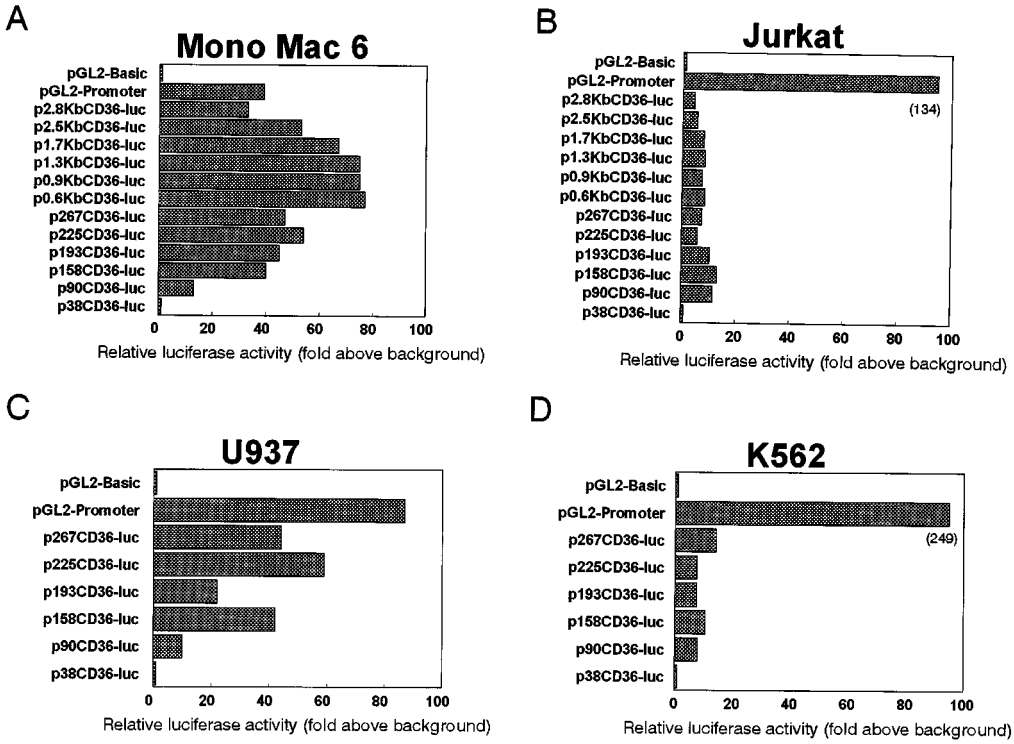

FIG. 1. **Deletion analysis of CD36 promoter.** A panel of CD36 promoter deletion constructs coupled to the reported luciferase gene were transiently transfected into the cell lines Mono Mac 6, U937, Jurkat, and K562, as described under "Experimental Procedures." Promoter activity of each construct was expressed as -fold activity above the background activity conferred by the promoterless control plasmid pGL2-Basic, corrected for transfection efficiency (Böttinger *et al.*, 1994). *Number* enclosed in *parenthesis* in *charts B* and *D* denote the relative luciferase activities yielded by the pGL2-promoter construct (which contains the SV40 promoter).

Mac 6 and U937 cells were higher than or comparable with the activity directed by vector pGL2-Promoter (Promega) (which contains the SV40 promoter), while they were significantly lower in the cell lines Jurkat and K562 (Fig. 1, *A–D*). Taken together, these observations indicate that the activity of the CD36 promoter correlates with the expression levels of CD36 and suggests that the promoter contains regulatory elements that contribute to the tissue-specific expression of this gene in monocytic cell lines.

As shown in Fig. 1*A*, comparable luciferase activities were obtained after transfection of constructs having 5′ ends ranging from −2.8 kilobase pairs to −158 bp in Mono Mac 6 cells, indicating that the region −158/+43 retains most of the promoter activity (Fig. 1*A*). Data obtained with U937 cells further supported this finding (Fig. 1*C*).[2] Nevertheless, our experiments did not rule out the possibility that other regions located upstream from position −158 may play important positive or negative regulatory roles, which might have remained hidden by mutual compensatory effects. A further deletion extending to −90 bp resulted in a 70% reduction of the basal promoter activity in both Mono Mac 6 and U937 cells, dropping the activity to the levels found out in the CD36-negative cell lines Jurkat and K562 (Fig. 1, *A–D*). These results point out the presence of strong positive regulatory elements within the region −158/−90, which are required for the efficient transcription of the CD36 gene in monocytic cell lines. Deletion to −38 bp (a construct that still preserved an intact TATA box) abrogated promoter activity, indicating the presence of regulatory elements in the region −90/−38 necessary for the basal transcription of the CD36 gene.

The Region Comprising the Nucleotides −102/−98 Is Required for Optimal Transcription of the CD36 Gene—A search within the −158/−90 region for nucleotide sequences corresponding to binding sites for known transcription factors revealed the sequence ACCACA at position −103, which conforms to the core site for the binding of the transcription factors belonging to the PEBP2/CBF family (Meyers *et al.*, 1993; Ogawa *et al.*, 1993b), also referred as the AML1 family.

To evaluate the functional significance of the putative PEBP2 site, the core PEBP2 binding site within the pCD36–158-luc construct was mutated from ACCACA to AAGCTT (originating the construct pCD36–158(m−102/−98)-luc). When these constructs were transfected in Mono Mac 6 and U937 cells, the promoter activity directed by the mutant construct pCD36–158(m−102/−98)-luc was 30% of the activity obtained by the wild type construct pCD36–158-luc (Fig. 2). By contrast, comparable activities were yielded by both the mutant and wild type constructs in HeLa cells, an observation consistent with the low levels of PEBP2/CBF factors detected in this cell line (Fig. 3). In Jurkat cells, the mutant also decreased the low promoter activity directed by the wild type construct. This observation, in agreement with the presence of PEBP2/CBF factors in this cell line (Figs. 3 and 4), suggests that the PEBP2/CBF site by itself does not confer the tissue specificity of the CD36 gene in monocytic cells. Interestingly, the level of reduction in promoter activity in monocytic cells when the PEBP2/CBF site was mutated was similar to the decrease in promoter activity observed when the activity directed by the pCD36–90-luc construct (which does not contain the PEBP2 site) was compared with the activity yielded by the pCD36–158-luc construct (which does contain the PEBP2 site) (Fig. 1, *A* and *C*). These results demonstrate the importance of the region −102/−98 for the constitutive transcription of the CD36 gene.

Transcription Factors of the PEBP2 Family Bind to the −102/−98 Region—To investigate the trans-acting factors that account for the promoter activity ascribed to the −102/−98 region, a double-stranded oligonucleotide spanning the −110/−91 region and comprising the PEBP2 core sequence ACCACA, was radiolabeled and used as a probe in EMSA with

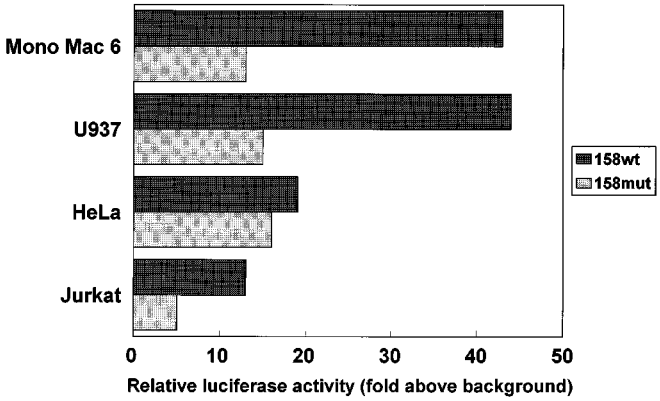

FIG. 2. **Mutation of site −102/−98 severely impairs the activity of the CD36 promoter.** CD36 promoter wild type construct pCD36–158-luc (represented as *158wt*) and mutant construct pCD36–158(m−102/−98)-luc (represented as *158mut*) were transiently transfected in Mono Mac 6, U937, Jurkat, and HeLa cells. For each cell line assayed, promoter activities were represented as indicated for Fig. 1.

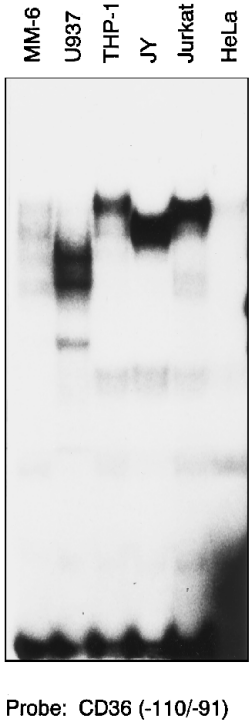

FIG. 3. **Binding of CD36 promoter region −110/−91 to nuclear extracts from several cell lines.** Radiolabeled double-stranded CD36 promoter −110/−91 oligonucleotide, GCAACAAACCACACACTGGG, was incubated with nuclear extracts from cell lines lines of different origin (Mono Mac 6, U937, and THP-1, monocytic; JY, B lymphoblastoid; Jurkat, T cell leukemia; HeLa, epitheloid carcinoma).

nuclear extracts derived from several cell lines, including the monocytic cell lines Mono Mac 6, U937, and THP-1 (Fig. 3). Major low mobility complexes were observed for all of them, although practically undetectable levels were found in nuclear extracts from HeLa cells. The specificity of the complexes was verified by competition with an excess of the unlabeled oligonucleotide.[2] The multiplicity, distinct relative mobilities, and wide cellular distribution of the complexes bound to oligonucleotide −110/−91 was consistent with the EMSA pattern for transcription factors of the PEPB2/CBF family (Fig. 3) (Meyers *et al.*, 1993, 1995; Ogawa *et al.*, 1993b; Nuchprayoon *et al.*, 1994). The dissimilarity between the retardation complexes detected in the monocytic cell lines analyzed (Figs. 3 and 4*A*) may be the result of partial proteolytic degradation of the nuclear extracts obtained from cell lines Mono Mac 6 and U937. In fact, high levels of protease activity have been reported in nuclear extracts derived from monocytic cell lines, making isolation of nuclear extracts without proteolytic degradation extremely difficult (Galson and Housman, 1988; Pahl *et al.* 1993). Particularly, susceptibility to proteolytic cleavage of PEBP2/CBF factors in nuclear extracts from some monocytic cell lines has been documented (Nuchprayoon *et al.*, 1994). Moreover, the relative intensities of the complexes derived from Mono Mac 6 showed significant variations depending on the preparation and age of the extract and on the numbers of freezing and thawing processes undergone by the extract.[2] For the above reasons, only the experiments performed with nuclear extracts from THP-1 are shown, although similar conclusions were reached when nuclear extracts from Mono Mac 6 and U937 cells were used.[2]

Binding of radiolabeled oligonucleotide −110/−91 to nuclear extracts from THP-1 and Jurkat cells gave rise to several complexes whose formation was completely prevented by a 50-fold excess of the same unlabeled oligonucleotide, demonstrating their specificity (Fig. 4*A*). An oligonucleotide containing a consensus core for the PEBP2/CBF site (designated as AML1 cons.) and derived from the Moloney virus enhancer (Wang and Speck, 1992) competed the binding of all complexes to the oligonucleotide −110/−91, indicating that the sequence ACCACA was responsible for the appearance of the observed complexes (Fig. 4*A*). This observation was confirmed by the absence of inhibition by a mutant oligonucleotide (−110/−91 mut), which spanned the −110/−91 region and contained a cluster of mutations identical to those generated in the mutant construct pCD36–158(m−102/−98)-luc (Fig. 4*A*). These data indicate that the decrease of transcriptional activity in this mutant is due to the loss of its capability to interact with the factors bound to the PEBP2/CBF site (nucleotides −103/−98).

To identify the molecular nature of the complexes observed, nuclear extracts obtained from THP-1 and Jurkat were independently incubated with two distinct antibodies specific for AML1 factors before the addition of the labeled oligonucleotide −110/−91. As shown in Fig. 4*B*, the α-AML1 antisera, raised against the N-terminal region of the AML1 α subunit (Meyers *et al.*, 1993), induced specific supershift in only a fraction of the complexes (as negative control see *last lane* showing the binding of the probe to the antibody). Identical results have been obtained by several investigators (Meyers *et al.*, 1993; Nuchprayoon *et al.*, 1994; Zhang *et al.*, 1994). Moreover, the rabbit polyclonal antibody R3034 raised against the DNA-binding domain of AML1, but not a preimmune rabbit serum, prevented the formation of most of the complexes. Given the high degree of amino acid sequence similarity within the regions used to generate both the α-AML1 and the R3034 antisera, between the different members constituting the PEBP2/CBF family, it is conceivable that both antisera react with several members of the PEBP2/CBF transcription family (Levanon *et al.*, 1994). Finally, a similar set of experiments was carried out using extracts isolated from peripheral blood monocytes (Fig. 5). As expected, PEBP2/CBF transcription factors present in monocytes bind *in vitro* to the CD36 promoter, therefore allowing us to extend the conclusions to normal (nontransformed) monocytes. Altogether, these findings demonstrate that factors belonging to the PEBP2/CBF family bind to the region −103/−98 of the CD36 promoter and regulate its functional activity .

DISCUSSION

In this report we have examined the transcriptional regulation of the CD36 gene in monocytic cell lines. Deletion analysis has revealed 1) that the CD36 promoter contributes to its

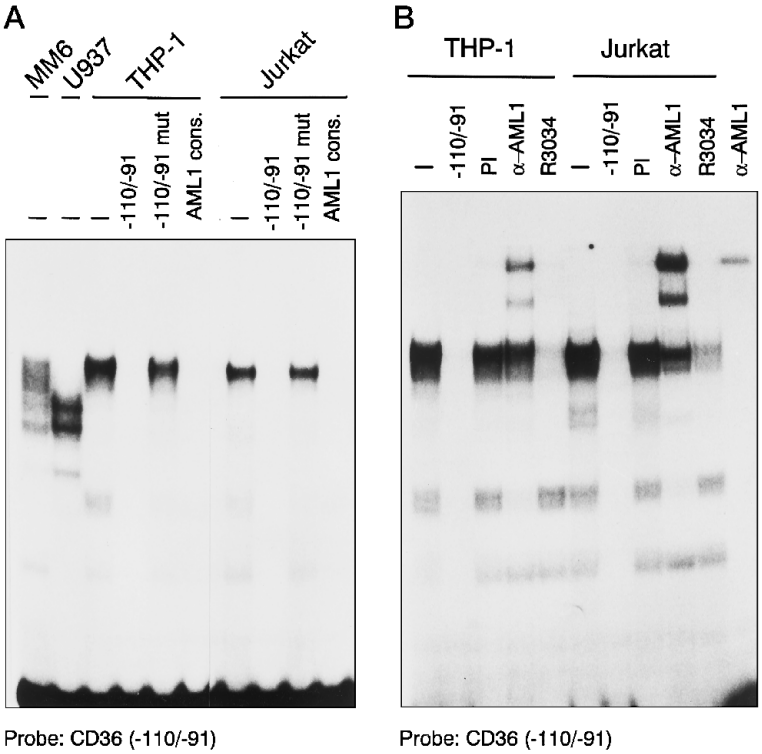

FIG. 4. **CD36 promoter contains a PEBP2/CBF site at region −103/−98.** *A*, radiolabeled double-stranded CD36 promoter −110/−91 oligonucleotide was incubated with nuclear extracts from cell lines THP-1 and Jurkat. Prior to adding the radiolabeled oligonucleotide, nuclear extracts were incubated with a 50-fold molar excess of the following cold double-stranded oligonucleotides: −110/−91, to demonstrate specificity of complexes; −110/−91 mut (GCAACAAAAGCTTCACTGGG), to assay the effect of positions −102/−98 in the binding; and AML1 cons. (GGATATCTGTGGTAAGCA), an oligonucletide containing a PEBP2/CBF consensus site from the Moloney murine leukemia virus enhancer, to assay the molecular nature of the complexes bound to oligonucleotide −110/−91. −, no inhibitor added. *B*, nuclear extracts from cell lines THP-1 and Jurkat were incubated with radiolabeled double-stranded oligonucleotide −110/−91, without (−) or with a prior incubation with a 50-fold molar excess of cold oligonucleotide −110/−91, rabbit preimmune sera (*PI*), or AML1-specific antisera α-AML1 or R3034. The *last lane* shows the effect of the antisera α-AML1 on the labeled double-stranded oligonucleotide −110/−91 in the absence of nuclear extract.

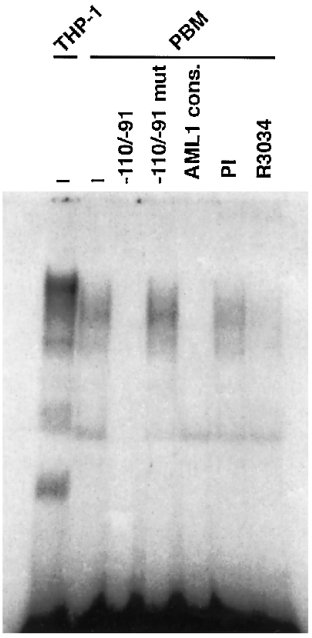

FIG. 5. **Nuclear extracts from peripheral blood monocytes contain PEBP2/CBF factors that bind to the CD36 promoter PEBP2/CBF site.** Radiolabeled double-stranded CD36 promoter −110/−91 oligonucleotide was incubated with nuclear extracts from THP-1 cells and from peripheral blood monocytes. Prior to adding the radiolabeled oligonucleotide, nuclear extracts from monocytes were incubated with a 50-fold molar excess of the cold double-stranded oligonucleotides −110/−91, −110/−91 mut, and AML1 cons. (see Fig. 4 legend for details) or with rabbit preimmune sera (*PI*) or AML1-specific antisera R3034. −, no inhibitor added.

monocytic-specific expression, and 2) that most of the promoter activity is contained within the −158/+43 region, which encompasses the subregion −158/−90 required for the efficient transcription of the gene in monocytic cells. We have also shown that within the above region members of the PEBP2/CBF family of transcription factors bind to nucleotides −103/−98. Moreover, mutation of the PEBP2 site severely impaired the expression of a reporter gene under the control of the CD36 promoter, outlining the significant contribution of the PEBP2/CBF site to the transcriptional activity of the CD36 gene.

PEBP2/CBF are heterodimeric DNA-binding proteins. They were initially identified as factors interacting with the polyomavirus enhancer core site (Wang and Speck, 1992). They are constituted by an α subunit, which binds to the DNA consensus sequence ACCACA (Meyers *et al.*, 1993) and harbors a transactivation domain, and a β subunit, which enhances the DNA-binding affinity of the α subunit through heterodimer formation (Ogawa *et al.*, 1993a; Wang *et al.*, 1993). While the β subunit is encoded by a single gene, the α subunits are encoded by three genes, designated as PEBP2αA, PEBP2αB, and PEBP2αC in mouse and AML3, AML1, and AML2 in human, respectively (Bae *et al.*, 1994, 1995; Levanon *et al.*, 1994). The α subunits share a 128-amino acid region known as the runt domain, first found (and hence its name) in the *Drosophila melanogaster* segmentation gene, *runt* (Kania *et al.*, 1990; Kagoshima *et al.*, 1993). This domain is required for DNA binding and heterodimerization (Ogawa *et al.*, 1993b). Alternatively spliced forms for the α subunits (Ogawa *et al.*, 1993b, Bae *et al.*, 1994, Levanon *et al.*, 1994) and the β subunit (Ogawa *et al.* 1993a, Wang *et al.*, 1993) have been described. The relevance in gene regulation of the different protein forms is at present unknown (Bae *et al.*, 1994).

On the basis of the cellular distribution, one should expect to find transcripts corresponding to all of the three AML α subunits in monocytic cell lines (Levanon *et al.*, 1994). Nevertheless, identification of the α subunits, which interact with the CD36 promoter must await generation of specific reagents for each subunit.

So far, only a few genes have been shown to be regulated by the PEBP2/CBF transcription factors. Gene expression of the myeloid genes encoding murine neutrophil elastase, myeloperoxidase (Suzow and Friedman, 1993; Nuchprayoon *et al.*, 1994), and human colony-stimulating factor receptor (Zhang *et al.*, 1994) is controlled by PEBP2/CBF transcription factors. Functional sites for PEBP2/CBF factors have been also described in the enhancers of T cell receptor genes (Gottschalk and Leiden,

1990; Redondo *et al.*, 1992) and in the enhancers of murine leukemia viruses (Wang and Speck, 1992).

Like the murine myeloid genes neutrophil elastase, myeloperoxidase (Nuchprayoon *et al.*, 1994), and the T cell receptor α enhancer (Giese *et al.*, 1995), trans-activation experiments carried out in HeLa cells with vector pEF-BOSαB1 (which drives the expression of the αB chain) did not enhance the activity of the pCD36–158-luc construct,[2] suggesting the requirement of other factors for the transcriptional activation mediated by PEBP2/CBF. In this respect, reconstitution of the T cell receptor α enhancer activity in nonlymphoid cells required the assembly of a stereospecific complex constituted by the transcription factors PEBP2/CBF, Ets-1, and the lymphoid-specific high-mobility group protein LEF-1 (Giese *et al.*, 1995). That the mutation of the PEBP2 site abolished most of the transcriptional activity does not imply that other sites for transcription factors might not be required for efficient transcription of the CD36 gene. Transcription factors Ets-1, Ets-2 (Wotton *et al.*, 1994), and Myb (Hernández-Munain and Krangel, 1994, 1995) are known to synergize with PEBP2/CBF factors. Meyer *et al.* (1995), on the basis of the functional studies of PEBP2/CBF sites in several promoters, have outlined the conclusion that the PEBP2/CBF factors are necessary but not sufficient for tissue-specific activity. Our data on the PEBP2/CBF site in the CD36 promoter support this statement.

The human AML1 gene is involved in translocations t(8;21)(q22;q22) and t(3;21) (q26,q22) (reviewed by Nucifora and Rowley (1995)) and accounts for 12% of all forms of acute myeloid leukemia (Miyoshi *et al.*, 1993). In addition, the human PEBP2/CBF β gene undergoes an inversion (inv(16)(p13q22)), also associated with a particular subtype of acute myeloid leukemia (Liu *et al.*, 1993). The short transcript form of AML1 (designated as AML1a), which lacks the transactivation domain, as well as the fusion proteins resulting as a consequence of the translocations dominantly suppress transcriptional activation by presumably interfering with binding of transactivating PEBP2/CBF forms (Meyers *et al.*, 1995). Through this mechanism the PEBP2/CBF forms lacking transactivation capacity inhibit myeloid differentiation and may cause leukemia (Tanaka *et al.*, 1995). All of these studies highlight the importance of the AML1 gene in myeloid cell growth and/or differentiation, and strengthen its proposed role in the regulation of CD36 gene expression in monocytic cell lines.

Little information is available on the factors and molecular events that regulate the expression and functional activity of the PEBP2/CBF genes. Dr. Ito and co-workers (Lu *et al.*, 1995) have recently discovered that the β subunit of PEBP2/CBF is mainly found in the cytoplasm, from where it translocates to the nucleus and binds to the α chain, thus increasing binding affinity of the α subunit for the DNA. Molecular events that dictate such a unique regulatory mechansim are so far unknown, but they are likely to be crucial in regulating the transcriptional activity of the PEBP2 transcription factors, and the genes under their control. It would therefore be rational to investigate whether changes in the expression and in the transcriptional activity of PEBP2 factors occur under conditions that modulate the RNA expression levels of CD36, such as interaction of monocytes with E-selectin, interleukin-4, lipopolysaccharide, and γ-interferon (Huh *et al.*, 1995).

Interestingly, the high levels of CD36 expression found in immature erythrocytes (Edelman *et al.*, 1986) may be a consequence of the expression of PEBP2/CBF αB chain detected in this type of cells (Satake *et al.*, 1995). Nevertheless, the expression of CD36 in a variety of different tissues makes conceivable the existence of distinct regulatory mechanisms on each tissue type. In this respect, expression of CD36 in murine B cells (although so far not detected in human B cells) has been shown to be dependent on the transcription factor Oct-2 (König *et al.*, 1995), a factor not found in all cell types where CD36 is expressed.

We are currently analyzing the transcription factors governing the basal promoter activity found downstream from position −90. Preliminary experiments indicate that a Sp1 site close to the TATA box could possibly account for this activity.

The characterization of the CD36 gene promoter provided in this report establishes the molecular basis to further dissect the promoter in activating conditions, such as those existing in the microenvironments where monocytes are found when participating in foam cell formation and clearance of apoptotic cells.

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