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Polyomavirus Enhancer-binding Protein 2/Core Binding Factor/Acute Myeloid Leukemia Factors Contribute to the Cell Type-specific Activity of the CD11a Integrin Gene Promoter*

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The CD11a/CD18 leukocyte integrin (LFA-1; also known as αL/β2) mediates leukocyte transendothelial migration during immune and inflammatory responses and participates in lymphoma metastasis. CD11a/CD18 leukocyte-restricted expression is controlled by the *CD11a* gene promoter, which confers tissue-specific expression to reporter genes *in vitro* and *in vivo*. DNase I protection analysis of the *CD11a* proximal gene promoter revealed DNA-protein interactions centered at position –110 (CD11a-110). Disruption of CD11a-110 reduced *CD11a* promoter activity in a cell type-specific manner, as it reduced its activity by 70% in Jurkat lymphoid cells, whereas the effect was considerably lower in K562 and HepG2 cells. Electrophoretic mobility shift assays showed evidence of cell type-specific differences in CD11a-110 binding and indicated its specific recognition by members of the polyomavirus enhancer-binding protein 2/core binding factor (CBF)/acute myeloid leukemia (AML) family of transcription factors. AML1B/CBFβ transactivated the *CD11a* promoter, with AML1B/CBFβ-mediated transactivation being completely dependent on the integrity of the CD11a-110 element. Therefore, CBF/AML factors play a role in the cell type-restricted transcription of the *CD11a* integrin gene through recognition of CD11a-110. The involvement of CBF/AML factors in CD11a expression raises the possibility that CD11a/CD18 expression might be deregulated in acute myeloid and B-lineage acute lymphoblastic leukemias, thus contributing to their altered adhesion and metastatic potential.

CD11a/CD18 (LFA-1; also known as αL/β2) is a member of the β2 integrin subfamily, the leukocyte-restricted expression of which is developmentally regulated (reviewed in Ref. 1). CD11a/CD18 mediates leukocyte interactions required for immune and inflammatory responses through the recognition of at least one of its three identified counterreceptors, *i.e.* CD50, CD54, and CD102 (1–6). The importance of CD11a/CD18 for leukocyte extravasation is exemplified by the existence of an inherited disease (leukocyte adhesion deficiency) in which leukocytes exhibit a deficient expression of the three leukocyte integrins and the clinical symptoms of which are secondary to the lack of phagocyte migration into inflammatory sites (6, 7). Conversely, under certain circumstances, CD11a/CD18 activity might become detrimental; CD11a/CD18 participates in T cell and lymphoma metastasis (1, 8), and ischemia-reperfusion syndromes, myocardial infarction, and allograft rejection have their origin in an excessive and uncontrolled CD11a/CD18-dependent phagocyte extravasation into the tissues (9, 10). To understand the mechanisms controlling transcription of each leukocyte integrin subunit, the proximal regulatory region of the *CD11a* gene has been isolated (11–13) and shown to confer leukocyte-restricted expression to reporter genes both *in vitro* and *in vivo* (11–14). So far, Sp1- and *ets*-binding sites have been located within the *CD11a* proximal promoter (12, 15), although their functional contribution to the promoter activity remains unknown.

The members of the polyomavirus enhancer-binding protein 2 (PEBP2)[1]/core binding factor (CBF)/acute myeloid leukemia (AML) family of heterodimeric (α/β) transcription factors play important roles in hematopoiesis and osteogenesis (16, 17). To date, three distinct α subunits (AML-1/CBFα-2/PEBP2α-B, AML-2/CBFα-3/PEBP2α-C, and AML-3/CBFα-1/PEBP2α-A) and one β subunit (CBFβ/PEBP2β) have been reported in mammalian cells (16). The α subunits exhibit sequence-specific DNA binding ability, whereas the β subunit does not bind to DNA by itself but interacts with the α subunit and increases its DNA binding affinity (18). The AML-1/CBFβ transcription factor complex is one of the most frequent targets of chromosomal translocations in acute leukemias (17–19), as a high percentage of AML and B-lineage acute lymphoblastic leukemias have altered *AML1* or *CBFβ* alleles. Some of these translocations transform AML-1 into a constitutive transcriptional repressor and disrupt normal hematopoietic cell differentiation (17).

In the present study, we report the identification of a *cis*-acting element (CD11a-110) specifically recognized by members of the PEBP2/CBF/AML family of transcription factors and implicated in the cell-type restricted activity of the CD11a promoter. Our results indicate the involvement of PEBP2/CBF/

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[1] The abbreviations used are: PEBP2, polyomavirus enhancer-binding protein 2; AML, acute myeloid leukemia; CBF, core binding factor; EMSA, electrophoretic mobility shift assay; CMV, cytomegalovirus.

AML factors in the restricted expression of the CD11a leukocyte integrin and suggest that CD11a/CD18 expression might be deregulated in leukemic cells harboring *AML1* or *CBFβ* gene rearrangements.

EXPERIMENTAL PROCEDURES

Cell Culture—The cell lines HepG2 (hepatoma), HeLa (epithelial carcinoma), Jurkat (T cell lymphoma), JY (lymphoblastoid B), U937 (histiocytic lymphoma), and K562 (chronic myelogenous leukemia) were cultured in RPMI supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin at 37 °C in a humidified atmosphere with 5% CO_2. Induction of differentiation of K562 cells was accomplished in the presence of phorbol myristate acetate at 10 ng/ml for 24 h.

Transfections, Plasmids, and Site-directed Mutagenesis—Transfection in COS-7, HepG2, Jurkat, and K562 cells was performed with Superfect (Qiagen) according to the manufacturer's instructions. Transfections were carried out using 1 μg of reporter plasmid in 24-well plates and with 4×10^4 (COS-7 and HepG2) or $8–15 \times 10^5$ (K562 and Jurkat) cells/well. In all cases, the amount of DNA in each transfection was normalized by using the corresponding insertless expression vectors (CMV-0) as carrier. Each transfection experiment was performed at least three times with different DNA preparations. Transfection efficiencies were normalized by cotransfection with the β-galactosidase expression plasmid pCMV-β-galactosidase, and β-galactosidase levels were determined using the Galacto-Light kit (Tropix).

The CD11a-based reporter gene construct pCD11A170-Luc, in which the expression of the firefly luciferase cDNA is directed by the CD11a promoter region –170/+83, has been previously described (11). The promoterless plasmid pXP2 was used as a control in some transfection experiments. Drs. S. Hiebert (Vanderbilt Cancer Center, Nashville, TN), M. A. Vega (Consejo Superior de Investigaciones Científicas, Madrid, Spain) and Y. Ito (Kyoto University, Kyoto, Japan) generously provided the expression plasmids CMV-AML1B, CDM8-CBFβ1, and pEF-BOS-αA1 (AML3), respectively.

Site-directed mutagenesis was performed on the CD11a promoter construct pCD11A170-Luc using a polymerase chain reaction-based approach. For mutation of the CD11a-110 site, oligonucleotides MS7MUTS ((–120) 5′-CTCCCTGAACCCGAATTCTTTCACAACTCCT-G-3′ (–89)) and MS7MUTAS ((–88) 5′-GCAGGAGTTGTGAAAGAATT-CGGGTTCAGGGA-3′ (–119)) were synthesized substituting the sequence (–111) 5′-CCCCTGCGGTTT-3′ (–100) for the *Eco*RI-containing sequence 5′-CCCgaattcTTT-3′. Polymerase chain reaction was performed on pCD11A170-Luc using either oligonucleotides MS7MUTS and LFA-1α PE#1, which spans +62/+84 (11) and including a naturally occurring *Xho*I site, or MS7MUTAS and LFA-1α170, which contains the promoter region –170/–140 and a *Hin*dIII recognition site at the 5′ end. After *Eco*RI/*Xho*I or *Eco*RI/*Hin*dIII digestion, both polymerase chain reaction products were ligated into *Hin*dIII/*Xho*I-digested pXP2 to yield pCD11A170(–110mut)-Luc. DNA constructs and mutation were confirmed by DNA sequencing.

Electrophoretic Mobility Shift Assays (EMSAs) and DNase I Protection Analysis—EMSAs were performed as described (20). Briefly, 50 ng of double-stranded oligonucleotides were labeled at specific activities of 10^8 cpm/μg using AMV reverse transcriptase and 50 μCi of [^{32}P]dCTP. The probe (0.5 ng with approximately 50,000 cpm) was incubated at 4 °C with 2–5 μg of nuclear extract (or 1–3 μl of transfected COS-7 cells) 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. Unlabeled competitor oligonucleotides were added to the nuclear extracts at a 100-fold molar excess and incubated at 4 °C for 15 min before the addition of the radioactive probe. For antibody inhibition/supershift experiments, 0.5 μl of R-3034 (polyclonal antiserum against the DNA-binding domain of AML-1, generously provided by Dr. N. A. Speck) or α-AML1 (polyclonal antiserum against the N-terminal region of AML-1) was incubated with the nuclear extracts at 4 °C for 30 min before the addition of the probe. The binding reaction was then carried out for 20 min at 4 °C and 1.5 μl of a 10× loading buffer (10 mM Hepes, 10% glycerol, 0.01% bromphenol blue) was added to the reaction. Samples (12 μl) were analyzed by electrophoresis at 15 V/cm and 4 °C on 4–5% polyacrylamide gels containing 0.4× TBE (45 mM Tris base, 45 mM boric acid, 1 mM EDTA). Nuclear extracts were prepared according to Schreiber *et al.* (21) in the presence of protease inhibitors (aprotinin, antipain, leupeptin, pepstatin, and Pefabloc). Extracts from 5×10^4 AML1B-, AML3-, or CBFβ-transfected COS-7 cells were prepared in 250 μl of 50 mM Hepes, pH 7.5, 250 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 0.5 mM dithiothreitol, and protein inhibitors. Extracts from CMV-0-transfected COS-7 cells were used to normalize the amount of extract in each EMSA binding reaction.

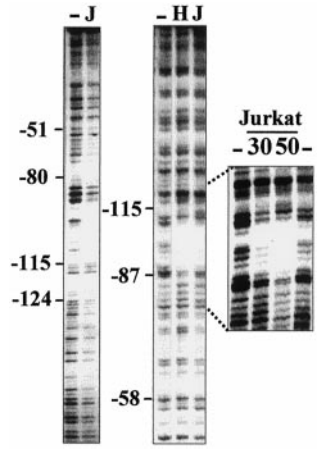

FIG. 1. **DNase I protection analysis of the proximal region (–170/+43) of the CD11a promoter.** Probe was incubated with nuclear extracts from Jurkat (*J*) or HeLa (*H*) (50 μg in the *left panel* and 30 or 50 μg in the *right panel*). Lanes marked – contained 50 μg of bovine serum albumin. After digestion with DNase I, samples were analyzed on denaturing polyacrylamide gels in parallel with G + A sequence ladders generated with the same probe to precisely map each band. Panels show the footprints obtained in the sense (*left panel*) and antisense (*middle* and *right panels*) strands.

The *CD11a* promoter-based oligonucleotides MS4-MS9 and their relative positions are indicated in Table I. Additional double-stranded oligonucleotides used as competitors and/or probes included NFkB-CONS (5′-AGTTGAGGGGACTTTCCCAGGC-3′), which contains the NFκB consensus binding site, AP2CONS (5′-GATCGAACTGACCGC-CCGCGGCCCGT-3′), which contains the human metallothionein IIa promoter AP-2-binding site (22), and AML1CONS (5′-GGATATTT-GCGGTTAGCA-3′) (23). DNase I protection was performed as described (24), except that cells were lysed in 10 mM Hepes, pH 7.6, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.75 mM spermidine, 0.1 mM spermine, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 10 mM Na_2MoO_4. Samples were subjected to electrophoresis on 6% denaturing polyacrylamide gels in parallel with a G + A sequence ladder generated with the same probe.

RESULTS

DNA-Protein Interactions at the CD11a Gene Proximal Promoter—Functional analysis of 5′ deletion mutants of the *CD11a* promoter in distinct hematopoietic cell lines has previously revealed that the –170/+43 fragment retains most of the basal and tissue-specific activity (11). To locate *cis*-acting elements in the *CD11a* proximal promoter, DNase I protection experiments were performed on the –170/+43 DNA fragment using Jurkat ($CD11a^+$), K562 ($CD11a^-$), and HeLa ($CD11a^-$) nuclear extracts. Several areas of DNA-protein interaction were identified, the boundaries and intensities of which differed among the cell lines tested. Protected sequences included the major transcriptional start site, a consensus *ets*-binding sequence GGAA, and a previously identified Sp1-binding site at –70 (data not shown). The most prominent footprint, spanning from –90 to –115, could be detected in both strands and was always stronger in Jurkat and HeLa than in K562 cells (Fig. 1). Increasing amounts of Jurkat nuclear extracts were used to determine the boundaries of this footprint (–90/–115) (Fig. 1), further demonstrating the existence of DNA-protein interactions around position –110 within the CD11a integrin promoter.

DNA-Protein Interactions at the CD11a Gene Promoter Element (CD11a-110)—To characterize the factor(s) giving rise to the –90/–115 footprint, EMSA was performed using partly overlapping probes spanning the region between –120 and –79 (Table I). This region is located immediately upstream from the

TABLE I
CD11a promoter-based oligonucleotides and their relative positions

CD11a−140	CTCTAACTTG CTTACACTTC CTCCCTGAAC CCCTGCGGTT TCACAACTCCTGCAGGCACA CCTCCCTCCC CGCCTGCCAG TGTCACCAGC −51
MS9−140	CTCTAACTTG CTTACACTTC CTCCCTGAAC −111
MS8	−130 CTTACACTTC CTCCCTGAAC CCCTGCGGTT TC −99
MS7	−120 CTCCCTGAAC CCCTGCGGTT TCACAACTCCTGC −88
MS6	−110 CCCTGCGGTT TCACAACTCCTGCAGGCACA CC −79
MS5	−95 ACTCC TGCAGGCACA CCTCCCTCCC CGCCTGC −64
MS4	−80 CCTCCCTCCC CGCCTGCCAG TGTCACCAGC −51

Sp1-binding element at –70 (15) and downstream from the sequence recognized by an unidentified and ubiquitous *ets*-related factor (–130) (12). Using the MS7 probe (–120/–88), a low mobility complex, which resolved as a doublet in some experiments, was produced by Jurkat nuclear extracts (indicated by an *asterisk* in Fig. 2 and hereafter termed AML-110). A specific complex of higher mobility was detected with extracts from the B lymphoblastoid cell line JY (Fig. 2*A*). By contrast, no retarded complex was produced by nuclear extracts from K562, whereas phorbol myristate acetate-differentiated K562 cells exhibited a complex similar to that seen in Jurkat extracts (Fig. 2*A*). The specificity of the AML-110 complex was demonstrated as it was completely inhibited in the presence of a 100-fold molar excess of unlabeled MS7, it was partially competed by AP2CONS and unaffected by the unrelated NFkBCONS oligonucleotide (Fig. 2*A*). In addition, formation of the AML-110 complex was prevented by oligonucleotides MS6 (–110/–79) or MS8 (–130/–99) (Fig. 2, *A* and *B*) but was not affected by MS4 (–80/–51), MS5 (–95/–64), or MS9 (–140/–111) (Fig. 2, *B* and *C*), thus demonstrating the dependence of the AML-110 complex on the sequence (–110) 5′-CCCTGCGGTTTC –3′ (–99). In fact, EMSA of MS6 (–110/–79) also yielded the AML-110 complex, and its formation was similarly abrogated by either MS8 (–130/–99), MS7 (–120/–88), or MS6 (–110/–79) but not altered by either MS5 (–95/–64) or MS9 (–140/–111) (data not shown). The involvement of the identified sequence in AML-110 complex formation was further confirmed by the lack of inhibitory activity of a mutated MS7 probe (MS7MUT), in which the core of the sequence (–110) 5′-CCCTGCGGTTTC-3′ (–99) had been replaced by an *Eco*RI site ((–110) 5′-CCgaattcTTTC-3′ (–99)) (Fig. 2*C*).

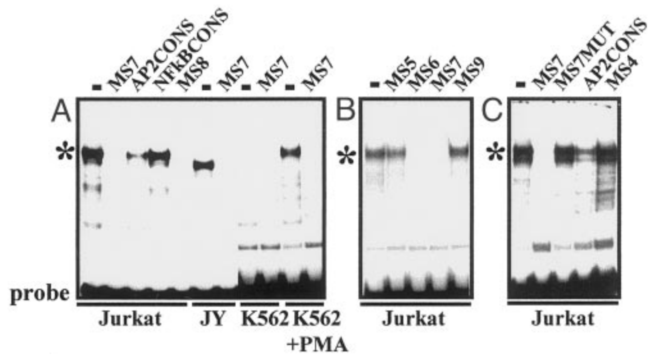

FIG. 2. **Nuclear factors interacting with the MS7 probe and identification of nucleotides involved in recognition of CD11a-110.** All panels show EMSA performed on the MS7 oligonucleotide using nuclear extracts from Jurkat leukemic T cells, B lymphoblastoid JY cells, erythroleukemic K562 cells, and phorbol myristate acetate-differentiated K562 cells, as indicated. Major specific retarded complexes are indicated by an *asterisk* (AML-110). Unlabeled competitor oligonucleotides (MS4, MS6, MS7, MS9, MS7MUT, AP2CONS, and NFkBCONS) were added at 100-fold molar excess.

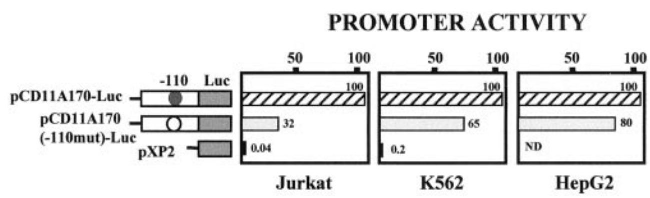

FIG. 3. **The CD11a-110 element affects the CD11a promoter activity in a cell type-specific manner.** Jurkat, K562, and HepG2 cells were transfected with pCD11A170-Luc, the corresponding mutant at the CD11a-110 element (pCD11A170(–110mut)-Luc), or the promoterless vector pXP2, and luciferase activity was determined. Each experiment was performed at least three times using distinct DNA preparations, and a representative experiment is shown. Promoter activity is expressed relative to the activity produced by the wild-type pCD11A170-Luc reporter plasmid in each transfected cell line, after normalization for transfection efficiency. Wild-type and mutant CD11a-110 sites are depicted as *filled* and *open circles,* respectively.

Contribution of the CD11a-110 Element to the Cell Type-specific Activity of the CD11a Gene Promoter—To find out the functional contribution of the CD11a-110 element to the whole *CD11a* promoter activity, the CD11a-110 sequence CTGCGG was replaced by GAATTC in the context of the CD11a promoter region –170/+83, as this mutation had been shown to prevent AML-110 complex formation (Fig. 2). Mutation of the CD11a-110 element reduced the activity of the *CD11a* promoter to 30% of the wild-type value in Jurkat cells (Fig. 3), demonstrating that the CD11a-110 element greatly contributes to the *CD11a* integrin gene transcription. More importantly, the role of CD11a-110 was cell type-dependent because its disruption had a lower effect in K562 (reduced to 65%) and HepG2 cells (reduced to 80%) (Fig. 3). Therefore, the CD11a-110 element is required for an optimal activity of the CD11a gene promoter and participates in the cell type-dependent activity of the CD11a promoter. This result suggests that the factors involved in AML-110 complex formation contribute to the restricted expression of the CD11a/CD18 integrin by recognition of the CD11a-110 element.

Members of the PEBP2/CBF/AML Family of Transcription Factors Recognize the CD11a-110 Element—Comparison of the pattern of EMSA complexes among distinct hematopoietic cell lines indicated that recognition of CD11a-110 was cell type-specific. Search for cell type-specific transcription factors of which the cognate sequences were homologous to CD11a-110 suggested the involvement of the CBF/AML family of transcription factors (17, 18), a hypothesis further supported by the weak expression of CBF/AML factors in K562 cells (25), in which no AML-110 complex was observed (Fig. 2*A*). Moreover, the CD11a-110 element was identical to one of the consensus CBF-binding sequences (23), and therefore, we tested whether CBF/AML proteins contribute to formation of the AML-110 complex. As shown in Fig. 4, formation of the retarded complexes on the MS7 probe with Jurkat or JY nuclear extracts was completely abrogated in the presence of the AML1CONS oligonucleotide, which contains a consensus binding site for members of the CBF/AML transcription factor family. In addition, the MS7 probe prevented recognition of the consensus CBF/AML-binding sequence (AML1CONS) by CBF factors, whereas MS7MUT had no effect, thus suggesting that CBF/AML factors recognize the sequence CTGCGG (Fig. 4). The binding of CBF/AML-related factors to the CD11a-110 element was further demonstrated using polyclonal antibodies against

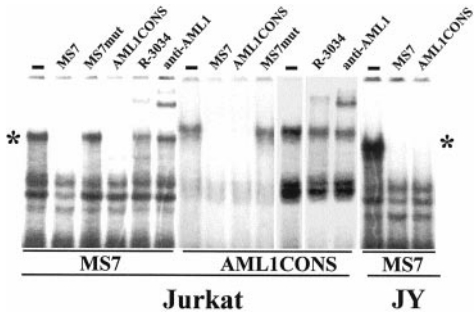

FIG. 4. **Members of the AML/PEBP2/CBF family of transcription factors recognize the CD11a-110 element.** EMSA was performed on the MS7 and AML1CONS oligonucleotide probes using nuclear extracts from Jurkat leukemic T cells or B lymphoblastoid JY cells in the absence (–) or presence of unlabeled competitor oligonucleotides (MS7, MS7MUT, and AML1CONS) or polyclonal antisera against AML proteins (R-3034 and anti-AML1). Unlabeled competitor oligonucleotides were added at a 100-fold molar excess.

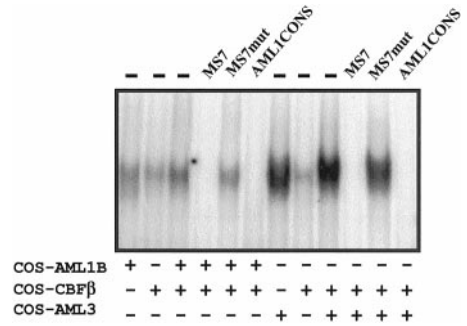

FIG. 5. **Recognition of CD11a-110 by AML1, AML3, and CBFβ-enriched cell extracts.** EMSA performed on the MS7 oligonucleotide probe using cellular extracts from COS-7 cells transfected with either CMV-0, CMV-AML1B, CMV-AML3, or CMV-CBFβ. Binding reactions were performed in the absence (–) or presence of unlabeled competitor oligonucleotides (MS7, MS7MUT, or AML1CONS) at a 100-fold molar excess.

AML1; a polyclonal antiserum against the N-terminal region of AML1B produced a supershift, and the R-3034 polyclonal antiserum, which recognizes the AML1B DNA-binding domain, also inhibited complex formation (Fig. 4). Therefore, proteins structurally related to the PEBP2/CBF/AML family of transcription factors specifically bind the CD11a-110 element and give rise to the AML-110 complex.

To further demonstrate recognition of the CD11a-110 element by CBF/AML factors, AML1B, AML3, and CBFβ were overexpressed in COS-7 cells, and the resulting extracts were assayed for binding to MS7. MS7 was recognized by either AML1 or AML3 alone, and coincubation with CBFβ-containing extracts produced a strong retarded complex (Fig. 5). The intensity of the AML3-containing complex was considerably higher, an effect that might reflect differences between the levels of expression obtained from pEF-BOS and pCDM8 plasmids. More importantly, AML1/CBFβ or AML3/CBFβ recognition of CD11a-110 was competed by either MS7 or AML1CONS, but unaffected by the MS7MUT oligonucleotide. Therefore, CBF/AML factors recognize the CD11a-110 element within the *CD11a* promoter and participate in formation of the AML-110 complex.

Transactivation of the CD11a Integrin Promoter by CBF/AML Factors—Because mutation of CD11a-110 has a cell type-dependent effect on the activity of the CD11a promoter, we tested the functional effect of overexpressing CBF/AML factors in K562 cells, which express extremely low levels of CBF/AML (23). Expression of AML1B alone had a minimal effect on the *CD11a* promoter activity, whereas expression of CBFβ always produced a considerable reduction in the activity of the promoter (Fig. 6). However, co-expression of both AML1B and CBFβ produced a considerable increase (12–16-fold) in the activity of the *CD11a* promoter (Fig. 6). The CD11a promoter transactivation was observed at distinct reporter:vector ratios (Fig. 6), and its dependence on the co-expression of AML1B and CBFβ was in agreement with the structural data shown in Fig. 5 and the known functional activities of CBF/AML factors (16–19). Therefore, CBF/AML factors directly contribute to the activity of the *CD11a* integrin gene promoter.

Because CD11a-110 mutation prevented its recognition by CBF/AML factors, we analyzed the effect of disrupting CD11a-110 on the CBF/AML-mediated transactivation of the CD11a promoter. As shown in Fig. 7, mutation of CD11a-110 greatly reduced the AML1B/CBFβ-mediated transactivation of the *CD11a* promoter. On average, disruption of CD11a-110 reduced the transactivation to 25% of the level observed on the wild-type promoter (Fig. 7). In addition, AML3 was also capable of transactivating the *CD11a* promoter in a CD11a-110-dependent manner, although its level of transactivation was always lower than that produced by AML1 (data not shown). Therefore, CBF/AML transactivation depends on the integrity of CD11a-110, and CBF/AML factors contribute to *CD11a* promoter activity by recognizing the CD11a-110 element.

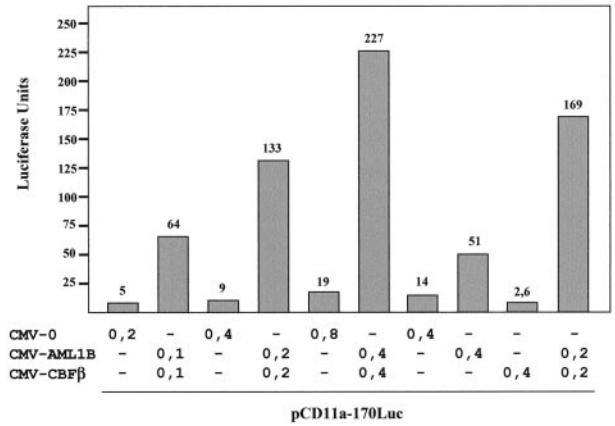

FIG. 6. **AML/CBF factors transactivate the CD11a integrin gene promoter.** K562 cells were transfected with 1 μg of pCD11A170-Luc together with the indicated amounts (in μg) of CMV-0, CMV-AML1B, or CMV-CBFβ expression plasmids. The luciferase units produced by each transfection experiment are indicated. In all cases, total DNA was kept constant. Each experiment was repeated at least four times, and a representative experiment is shown.

DISCUSSION

The CD11a/CD18 integrin mediates essential adhesive interactions during leukocyte transendothelial migration, in CTL- and NK-mediated killing, in antigen presentation, and in T cell hybridoma and lymphoma metastasis (reviewed in Ref. 1). CD11a/CD18 expression is leukocyte-restricted by transcriptional mechanisms acting on the regulatory regions of the *CD11a* and *CD18* genes (11–14, 26–28). We have previously shown that deletion of the –170/–100 fragment greatly reduces the basal activity of the *CD11a* promoter (11). In the present report, we demonstrate the presence of a CBF/AML-binding site at –110 (CD11a-110) that is specifically recognized by members of the CBF/AML family of transcription factors and contributes to the cell type-specific activity of the *CD11a* promoter. Consequently, CBF/AML factors appear as essential players in the control of the cell type-specific transcription of the *CD11a* gene through recognition of the CD11a-110 element. CD11a-110 is the first functionally characterized *cis*-acting element within the *CD11a* gene promoter of which the contribution to the cell type-specific activity of the *CD11a* promoter

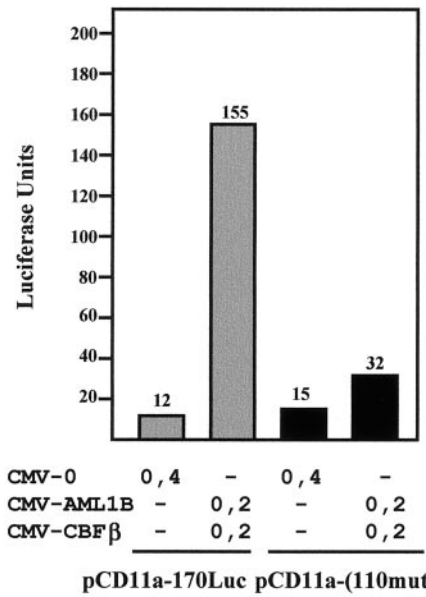

FIG. 7. **AML/CBF transactivation of the CD11a promoter is dependent on the integrity of the CD11a-110 element.** K562 cells were transfected with 1 μg of pCD11A170-Luc or pCD11A-(110mut)-Luc together with the indicated amounts (in μg) of CMV-0, CMV-AML1B, or CMV-CBFβ expression plasmids. The luciferase units produced by each transfection experiment are indicated. In all cases, total DNA was kept constant. Each experiment was repeated at least four times with two distinct DNA preparations, and a representative experiment is shown.

resembles that of Sp1-binding sites within the *CD11b* and CD11c promoters (15, 29).

The AML-1/CBFβ transcription factor complex is one of the most frequent targets of chromosomal translocations in acute leukemias (17–19). Both genes are affected by as many as 11 chromosomal translocations in either AML or B-lineage acute lymphoblastic leukemia, and some of these rearrangements transform AML-1 or CBFβ into constitutive transcriptional repressors (*e.g.* AML1/ETO and CBFβ/MYH11) that disrupt normal hematopoietic cell differentiation (17, 30). AML1/ETO can act as a dominant negative inhibitor of AML1 transactivation (31), although AML1/ETO and AML1 can also synergistically transactivate the macrophage colony-stimulating factor receptor promoter (32). Thus, the involvement of AML1 and CBFβ in the *CD11a* gene transcription raises the possibility that CD11a/CD18 integrin expression might be altered in some of these lymphoproliferative disorders. In this regard, diminished or absent CD11a/CD18 expression has been noted in cases of B-lineage acute lymphoblastic leukemia (33), and the expression of CD11a/CD18 significantly correlates with splenomegaly, resistance to induction chemotherapies and short survival periods in AML patients (34). Therefore, it will be of interest to determine whether an association exists between *AML1* or *CBFβ* chromosomal translocations and the expression of the CD11a/CD18 integrin, a task we are currently undertaking.

AML1 products are expressed in most tissues and at high levels in hematopoietic cells (16–19, 30), in which they collaborate in the organization of promoters prior to transcriptional activation, and are transcriptional activators of myeloid and lymphoid-specific genes, including T cell receptor subunits, (35), myeloperoxidase (36), interleukin-3 (37), and neutrophil elastase (38). However, CBF/AML proteins are relatively weak transcriptional activators in isolation, and they potently enhance transcription rates in cooperation with several factors (*e.g.* Ets-1, PU.1, c-Myb, and CCAAT/enhancer-binding protein α) via cooperative DNA binding or interactions with co-activators (17, 39). In fact, CBF/AML-binding sites are usually flanked by sites for CCAAT/enhancer-binding protein, Myb, or Ets factors (16–19). In the case of the *CD11a* promoter, the CD11a-110 element is adjacent to an Ets-binding element and to putative CCAAT/enhancer-binding protein- and Myb-binding sites, suggesting that some of these interactions may participate in the transactivation of the CD11a promoter by CBF/AML proteins and contributing to the cell type-specific expression of the CD11a integrin. On the other hand, a different type of interaction might also affect the involvement of CBF/AML factors in the transcriptional activity of the CD11a promoter. Thus, in agreement with the partial inhibitory effect of AP2CONS on AML-110 complex formation (shown in Fig. 2), we have obtained evidence that AP-2α and AP-2β can transactivate the *CD11a* promoter and that recombinant AP-2 factors bind the CD11a-110 element *in vitro,* although with lower affinity than CBF/AML factors (data not shown). Because AP-2 factors are capable of preventing the binding of other transcription factors to overlapping or adjacent *cis*-acting sequences (NF1, AP-3, and NFκB) (40–42), it is conceivable that AP-2 could also be regulating the access of CBF/AML factors to the CD11a gene promoter in certain cell types and thus regulating CD11a integrin expression.

The CD11a/CD18 integrin plays a key role in the triggering of immune and inflammatory responses (1). However, under certain circumstances (*e.g.* lymphoma metastasis and ischemia-reperfusion injuries), the functional activity of CD11a/CD18 and related integrins becomes detrimental to the host, and in fact, anti-CD11a antibodies can inhibit these processes (8–10). The involvement of CBF/AML factors in the transcription of the *CD11a* gene implies that CD11a/CD18 expression might be deregulated (either positively or negatively) in leukemic cells with chromosomal translocations affecting either *AML1* or *CBFβ* alleles (AML M2 and B-lineage acute lymphoblastic leukemia), thus contributing to altered adhesive and/or metastatic phenotypes. The identification of CBF/AML factors as key players in the transcription of the *CD11a* gene will allow the dissection of the signaling pathways that regulate CD11a/CD18 integrin expression and, subsequently, the development of strategies to modulate the adhesive and migratory capabilities of leukocytes and tumor cells.

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Polyomavirus Enhancer-binding Protein 2/Core Binding Factor/Acute Myeloid Leukemia Factors Contribute to the Cell Type-specific Activity of the CD11a Integrin Gene Promoter

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