CCAAT-Enhancer-binding Proteins (C/EBP) Regulate the Tissue Specific Activity of the CD11c Integrin Gene Promoter Through Functional Interactions with Sp1 Proteins*

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The CD11c/CD18 integrin binds lipopolysaccharide, fibrinogen, and heparin, and mediates leukocyte adhesion, spreading and migration. CD11c/CD18 is primarily found on myeloid cells and its expression is regulated during myeloid differentiation by transcriptional mechanisms acting on the CD11c gene promoter. We now describe that CCAAT/enhancer-binding proteins (C/EBP) contribute to the basal, tissue-specific and developmentally regulated activity of the CD11c promoter. A C/EBP-binding site within the CD11c promoter (CEBP-80) is bound by CEBPs in undifferentiated U937 cells and by C/EBPα- and C/EBPβ-containing dimers in phorbol 12-myristate 13-acetate-differentiating cells, and its disruption decreased the CD11c promoter activity in a cell type-dependent manner. C/EBPα transactivated the CD11c promoter through the CEBP-80 element, and C/EBPα transactivation was also dependent on the Sp1-70- and Sp1-120 Sp1-binding sites. The −90−50 fragment from the CD11c promoter, containing the adjacent CEBP-80, Sp1-70, and AP1−60 sites, differentially enhanced the activity of the minimal prolactin promoter in a cell type-dependent manner. C/EBPα disruption decreased the CD11c promoter activity in a cell type-dependent manner and whose disruption preferentially affects the activity of the CD11c promoter through their binding to adjacent cis-acting elements (24).

CCAAT/enhancer-binding protein (C/EBP) family members are basic-leucine-zipper transcription factors which recognize specific DNA sequences as either homodimers or heterodimers (27). The C/EBP family includes, at least, six members (C/EBPα, β, γ, δ, ε, and CHOP-10/GADD153) which dimerize in a tissue-specific manner, and with highly homologous dimerization and DNA contact domains, and similar DNA binding activities. Members of the C/EBP family have been implicated in regulating the differentiation of distinct mammalian cells, including adipocytes, hepatocytes, and myelomonocytes (27). In fact, C/EBPα, β, and δ expression within the hematopoietic system is restricted to myeloid cells. Based on these facts, and considering the preferential expression of the CD11c/CD18 integrin in differentiated myeloid cells, we have analyzed the role of C/EBP factors in the expression of the CD11c integrin gene in myeloid and other cell types. In the present report we describe the structural and functional characterization of a C/EBP-binding site (CEBP-80) within the CD11c promoter whose occupancy is regulated in a cell type- and differentiation-dependent manner and whose disruption preferentially affects the activity of the CD11c promoter in myeloid cells. The positive regulatory effect of C/EBP on the activity of the CD11c promoter is dependent on adjacent cis-acting elements (Sp1−120, Sp1−70, AP−60). Our results demonstrate the contribution of the C/EBP transcription factors to the tissue-restricted and differentiation-regulated expression of the CD11c/CD18 integrin, reveal a functional interplay among C/EBP, Sp1, and AP-1 family members and identify a cell type-dependent enhancer-like element within the proximal regulatory region of the CD11c promoter.

The CD11c/CD18 (p150, 95, CR4, LeuM5) heterodimer of the leukocyte integrin subfamily (1) mediates leukocyte adhesion during immune and inflammatory responses, is a specific receptor for LPS, iC3b, fibrinogen, and heparin, and participates in leukocyte adhesion to and spreading on protein-coated surfaces (1−14). CD11c/CD18 is primarily expressed on myeloid cells (15, 16), although can be induced upon B cell activation (1−14). CD11c/CD18 is primarily expressed on myeloid cells and its expression is regulated during myeloid differentiation by transcriptional mechanisms acting on the CD11c gene promoter. We now describe that CCAAT/enhancer-binding proteins (C/EBP) contribute to the basal, tissue-specific and developmentally regulated activity of the CD11c promoter. A C/EBP-binding site within the CD11c promoter (CEBP-80) is bound by CEBPs in undifferentiated U937 cells and by C/EBPα- and C/EBPβ-containing dimers in phorbol 12-myristate 13-acetate-differentiating cells, and its disruption decreased the CD11c promoter activity in a cell type-dependent manner. C/EBPα transactivated the CD11c promoter through the CEBP-80 element, and C/EBPα transactivation was also dependent on the Sp1-70- and Sp1-120 Sp1-binding sites. The −90−50 fragment from the CD11c promoter, containing the adjacent CEBP-80, Sp1-70, and AP1−60 sites, differentially enhanced the activity of the minimal prolactin promoter in a cell type-dependent manner. C/EBPα disruption decreased the CD11c promoter activity in a cell type-dependent manner and whose disruption preferentially affects the activity of the CD11c promoter through their binding to adjacent cis-acting elements (24).

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CD11c/CD18 expression is regulated during myeloid differentiation by mechanisms acting at the level of CD11c gene transcription (16). Determination of the activity of the CD11c gene promoter in distinct cell types has evidenced the importance of the Sp1-binding sites Sp1−70 and Sp1−120 for the basal and myeloid-specific transcription of the CD11c gene (22−24), and demonstrated the essential role that members of the AP-1 family play in that the regulated expression of CD11c during myeloid differentiation (24−26). Furthermore, AP-1 and Sp1 family members appear to have a synergistic effect on the activity of the CD11c promoter through their binding to adjacent cis-acting elements (24).

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*This work was supported in part by Grants PB92/0314 and PM95/0101 from the Ministerio de Educación, FIS93/0134 from Fondo de Investigaciones Sanitarias, and 212/92 from Comunidad Autónoma de Madrid (to A. L. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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 EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—The human cell lines HeLa (epithelial carcinoma), JY (lymphoblastoid B), U937 (histiocytic lymphoma), HL-60 (myelomonocytic leukemia), THP-1 (acute monocytic leukemia), and Hep2 (hepatoma), as well as the murine RAW 264.7 macrophage cell line, were cultured in RPMI supplemented with 10% fetal calf serum, 25 mM HEPES, 2 mM glutamine, and 50 μg/ml gentamicin (complete medium), at 37 °C in a humidified atmosphere with 5% CO2. Induction of differentiation of U937 and HL-60 was carried out with PMA at 5 ng/ml for 48 h (HL-60) or 10 ng/ml for 24 h (U937) and at a density of 5 × 10^6 cells/ml (16, 26). Spleen-derived hairy cells from Hairy Cell Leukemia patients (90% CD19 positive cells, 99% CD11c positive cells) were kindly provided by Dr. H. C. Klimu-Nelemans (University Medical Center, Leiden, The Netherlands). Peripheral blood monocytes and B lymphocytes were isolated according to standard procedures and activated with PMA as described (8).

The Drosophila Schneider cell line SL2 was cultured in Schneider’s medium supplemented with 15% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin, and grown in 100-mm culture plates at a density of 20 × 10^6/plate.

U937, HL-60, HeLa, and JY cells were transfected by electroporation or using Lipofectin, as described previously (22, 23, 28). RAW 264.7 and Hep2 cells were transfected using dioloeoxypropyltrimethyloxylamine methylsulfate following the manufacturer’s instructions (Boehringer Mannheim). PMA treatment was always carried out 16–20 h before luciferase determinations. The amount of DNA in each transfection was determined with a Hoechst dye.”

The CD11c promoter-based oligonucleotide probe X used for EMSA was (−94) 5′-CCTCGGATCGTCTGCTCCTGCG-3′ to −70, while those used for competition experiments included CEBP-CNS (consensus binding site for C/EBP proteins), CEBP-CONSmut, 2xMyb (containing two Myb-binding sites)/(30), E4TF1 (including a GABP/E4TF1-binding site) (31), and Box A, a CD11c-derived oligonucleotide including promoters sequences −19−3′ (5′-TCTGCCATCTGCTTC-3′) that contains the E-box sequence CACCTG. For determination of the nucleotides implicated in oligonucleotides, oligonucleotides including distinct mutations on the sequences surrounding CEBP-80 were used, and their relative positions and mutations are shown in Fig. 1A.

RESULTS

Identification of a C/EBP-binding Site within the CD11c Gene Promoter.—We have previously demonstrated that recombinant c-Myb binds the Myb-85 element within the CD11c promoter (25). Since Myb-85 (−86 CAGTTGC −80) overlaps an E-box sequence (−86 CANNTTG −81) and a sequence closely conforming to the consensus C/EBP-binding site (32) (−84 GTGCGTA −77) (Fig. 1A), an oligonucleotide spanning from −94 to −70 (Box D) was subjected to EMSA to determine the pattern of protein binding to this region of the CD11c promoter. As shown in Fig. 1B, myeloid U937 nuclear extracts produced specific retarded complexes on Box D (marked CEBP) whose formation was prevented by a 100-fold molar excess of cold oligonucleotide probe and whose intensity and mobility differed among cell types (Fig. 2 and data not shown).

To determine whether the formation of the retarded species was dependent on either E-box or Myb-binding site, inhibitory experiments were performed with consensus and mutated oligonucleotides (Fig. 1B). The specific complexes were not inhibited by a 100-fold molar excess of a Myb-binding site (25, 30), by an oligonucleotide from the CD11c promoter containing a distinct E-box (CAGTTG) or by an additional unrelated sequence containing a GABP/E4TF1-binding site (31) (Fig. 1). On the other hand, an oligonucleotide containing a consensus C/EBP-binding site (CEBP-CNS) completely prevented the formation of the E-box bound species at −86 CANNTTG, while the consensus C/EBP site is disrupted, had no effect on complex formation (Fig. 1B). In addition, while mutations at positions flanking the putative C/EBP-binding site abolished complex formation (Box Dmut1, Box Dmut 3), mutations that completely prevent c-Myb binding and eliminate the E-box sequence (Box Dmut1/25) only partially affected CEBP complex formation (Fig. 1A and 1B). By contrast, Box Dmut4 oligonucleotide did not inhibit complex formation (Fig. 1A).
These results indicated that the sequence −83 TTGCGTA −77 (hereafter termed CEBP-80) overlaps the Myb-85 element but is bound by proteins with a DNA-binding specificity distinct from c-Myb. Instead, the CEBP-80 element is bound by factors which recognize high affinity C/EBP-binding sites and require an intact TTGCGTA sequence.

The identity of the involved factors was unambiguously determined through the use of polyclonal antisera against members of the C/EBP protein family. As shown in Fig. 2, the specific interactions in U937 were either supershifted or completely prevented by polyclonal antisera against C/EBPα, while certain anti-CEBPβ antisera weakly affected complex formation and anti-C/EBPδ antisera had no effect. Therefore, the CD11c promoter CEBP-80 element is specifically recognized by transcription factors of the C/EBP family which, in the case of undifferentiated U937 cells, are predominantly C/EBPα-homo- and with a minor proportion of C/EBPβ-containing dimers (Fig. 2). The pattern of C/EBP-80-bound proteins differed among cell types, even within the same cell lineage (Figs. 2 and 3, and data not shown). Nuclear extracts from activated B cells and hairy cell leukemia cells exhibited a single and faint retarded complex, while those seen in monocytes were more heterogeneous and with a higher mobility (Fig. 2). Within the myeloid lineage, the level of CEBP-80-bound complexes, the relative proportion of C/EBPβ and C/EBPα, and the changes in CEBP-80-bound factors associated with myeloid differentiation also differed among the HL-60, U937, and THP-1 cell lines, with C/EBPδ-containing complexes only seen in THP-1 cells (Fig. 3). Monocytic differentiation of HL-60 cells greatly increased CEBP-80-bound species while PMA-mediated differentiation of U937 dramatically reduced the levels of CEBP-80-bound C/EBP factors (Fig. 2). Kinetic studies revealed the appearance of C/EBPβ- and C/EBPδ-containing dimers along U937 differentiation and indicated that CEBP-80-bound factors are undetectable 48 h after PMA addition in U937 cells (Fig. 4). As a whole, the structural analysis revealed that C/EBPα-, C/EBPβ-, and C/EBPδ-containing dimers bind specifically to the CEBP-80 element in the CD11c gene promoter and in a cell type- and differentiation-dependent manner.

Functional Relevance of the CEBP-80 Element—The differential occupancy of the CEBP-80 element suggested that it might contribute to the tissue specific activity of the CD11c promoter. The functional effect of mutations abolishing C/EBP binding to CEBP-80 were determined in transient transfection assays and indicated that CEBP-80 disruption greatly de-
The constitutive expression of C/EBPα in undifferentiated myeloid cells (33) suggested that C/EBPα might be responsible for most of the positive regulatory effect of CEBP-80 on the activity of the CD11c promoter and, consequently, transactivation experiments were performed in HeLa cells. Expression of C/EBPα significantly augmented the activity of the CD11c promoter (2-fold increase), an effect which was absolutely dependent on the integrity of the CEBP-80 element (Fig. 6). On the other hand, transfection of expression plasmids for C/EBPδ under similar conditions had no effect on the activity of the CD11c promoter (Fig. 6), while the activity of the CD11a promoter increased upon transfection of C/EBPα, C/EBPβ, or C/EBPδ (Fig. 6). Therefore, C/EBPα contributes to the activity of the CD11c promoter through recognition of the CEBP-80 element.

Functional Interplay between C/EBP and Sp1 Family Factors on the CD11c Promoter—C/EBP and Sp1 factors can recognize their respective binding sites within the CD11c promoter independently of one another (Ref. 23 and this paper): C/EBP factors bind the Box D oligonucleotide, which does not include any Sp1-binding site, and Sp1 interacts with Sp1–70 or Sp1–120 in the absence of any C/EBP-binding site (23). However, the Sp1 contribution to the tissue specific activity of the CD11c promoter (23), the cell type-dependent influence of CEBP-80 on the CD11c promoter activity, and the proximity of the Sp1–70, Sp1–120, and CEBP-80 elements prompted us to analyze whether C/EBP and Sp1 factors were functionally collaborating for the tissue-restricted expression of CD11c. The effect of CEBP-80 disruption on the Sp1 transcriptional activity was evaluated in transactivation experiments in Drosophila SL2 cells, which are devoid of Sp1, and revealed that mutation of CEBP-80 led to a consistent increase in the CD11c promoter transactivation by Sp1 (2–3-fold), indicating that occupancy of CEBP-80 influences the positive transcriptional activity of Sp1 on the CD11c promoter (data not shown). To determine whether integrity of Sp1-binding sites is required for the C/EBPα transcriptional activity on the CD11c promoter, the inverse set of experiments was performed. As expected, C/EBPα transactivation totally depended on CEBP-80 (Fig. 7). However, mutation of either Sp1–70 or Sp1–120 completely abolished the capacity of C/EBPα to transactivate the CD11c promoter (Fig. 7), thus demonstrating that C/EBPα binding is required but is not sufficient for transactivation of the CD11c promoter and indicating that the positive transcriptional effect of C/EBPα on the CD11c promoter is dependent on the integrity of the adjacent Sp1-binding sites Sp1–70 and Sp1–120. Furthermore, mutation of the adjacent AP1–60 also partially inhibited the C/EBPα transactivation (Fig. 7), in agreement with the reported AP1–Sp1 collaboration on the proximal CD11c promoter (24). By contrast, elimination of the binding sites for Myb, PU.1, or GABP had no effect on the ability of C/EBPα to transcriptionally activate the CD11c promoter (Fig. 7). Altogether, these results demonstrate that the positive regulatory activity of C/EBPα is dependent on the adjacent Sp1–70, Sp1–120, and AP1–60 sites within the CD11c promoter. Recent studies on the rat CYP2D5 gene have shown that Sp1 proteins synergize with C/EBPβ at the transcriptional level and facilitate their recognition of DNA elements greatly differing from canonical C/EBP-binding sites (34, 35). In fact, C/EBPβ was not capable of stably interacting with the CYP2D5 cryptic C/EBP-binding site unless Sp1 was bound at a closely juxtaposed site (34, 35). This does not appear to be the case in the CD11c promoter as C/EBP proteins can recognize the CEBP-80 element in the absence of the adjacent Sp1–70 or Sp1–120 site (Figs. 1–4), and anti-Sp1 antibodies or Sp1 consensus oligonucleotides do not affect CEBP-80 recognition (data not shown).

The –90/–50 Fragment of the CD11c Promoter Functions as An Enhancer on an Heterologous Promoter—Our results, when considered in conjunction with the reported functional interaction between factors bound at Sp1–70 and AP1–60 (24), suggest that the CEBP-80, Sp1–70, and AP1–60 elements might constitute a functional unit within the CD11c gene promoter whose functional interplay would represent the basis for the tissue-specific and differentiation-regulated expression of the CD11c integrin gene. To determine whether the fragment en-
Compassing the CEBP-80/Sp1–50 region of the CD11c promoter was linked to an heterologous promoter in the sense and antisense orientations, and either as a monomer or a head-to-tail dimer. In myeloid U937 cells, the presence of the −90/−50 region greatly increased the activity of the rat minimal prolactin promoter either in the sense (67-fold) or antisense (147-fold) orientation (Fig. 8). The presence of an additional fragment doubled the enhancing effect in the sense orientation, but provided no additional increase to the enhancing effect of the fragment in the antisense orientation (Fig. 8). The enhancing effect was also observed in epithelial HeLa cells, where the activity of the rat prolactin minimal promoter was increased either 228 times (sense) or 99 times (antisense CEsSpAP) (Fig. 8). Unlike in the case of U937 cells, dimerization of the antisense CEsSpAP oligonucleotide produced a higher enhancing effect than the monomeric antisense fragment (222-versus 147-fold). Therefore, the −90/−50 region of the CD11c promoter is capable of greatly potentiating the transcription from an heterologous TATA-containing promoter, independently on its relative orientation, thus indicating that it acts as an enhancer. Furthermore, the differential potentiating effects of the CEsSpAP oligonucleotide in U937 and HeLa cells indicate that the enhancer activity of the −90/−50 region from the CD11c promoter is cell type-dependent, probably reflecting its recognition by members of the C/EBP and AP-1 transcription factor families.

**DISCUSSION**

We present evidence that C/EBP transcription factors modulate the basal and tissue specific activity of the CD11c integrin gene promoter by recognition of the CEBP-80 element and through functional cooperation with factors interacting with the Sp1–70 and Sp1–120 cis-acting elements. CEBP-80-mediated C/EBPa transactivation of the CD11c promoter is absolutely dependent on the integrity of the Sp1–70 and Sp1–120 Sp1-binding sites, implying that the functional interplay of Sp1-related proteins and C/EBP factors is an important parameter for CD11c/CD18 integrin expression and explaining the involvement of the Sp1–70 and Sp1–120 elements in the tissue specific activity of the CD11c promoter (23). Transcriptional synergy between C/EBP and Sp1 proteins has only been shown on the rat cytochrome CYP2D5 gene (34, 35), where Sp1 also facilitates C/EBPβ binding to a very weak C/EBP-binding site that is not recognized by C/EBPa unless a functional Sp1-binding site is closely juxtaposed (34, 35). Unlike in the case of the CYP2D5 gene, C/EBP factors do recognize the CEBP-80 element in the absence of both Sp1–70 and Sp1–120 elements,
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and Sp1–70 and Sp1–120 occupancy is completely independent on the presence or integrity of the CEBP-80 element (Ref. 23 and this paper). Moreover, anti-Sp1 antiserum or Sp1 consensus binding sites have no effect on the ability of C/EBPs to bind to oligonucleotides including both CEBP-80 and Sp1–70 (data not shown), further demonstrating that C/EBP proteins bind to CEBP-80 independently of adjacent sequence elements, although it is possible that Sp1 might facilitate the C/EBP transcriptional activity on the CD11c promoter and contribute to the distinct transactivation potential of C/EBPα, C/EBPβ, and C/EBPγ. The functional cooperation between C/EBPα and Sp1 on the CD11c promoter is in agreement with previously published data showing that both C/EBPs and C/EBPγ synergize with Sp1 to elicit transcriptional activation on a high-affinity C/EBP site is present, while only C/EBPβ provides transcriptional cooperation on a cryptic C/EBP-binding site (35).

Undifferentiated proliferating myeloid cells abundantly express C/EBPα, while myeloid cell differentiation or activation causes a gradual decrease of C/EBPα and a concomitant induction of C/EBPβ and C/EBPγ (33, 36). All the studies so far reported indicate that C/EBPα might be more important at earlier stages of the myeloid differentiation pathway, while C/EBPβ (and C/EBPγ) would be more relevant in the functional activation of differentiated myeloid cells (33, 37). Our results demonstrate that C/EBPα is the predominant C/EBP factor affecting the activity of the CD11c promoter in undifferentiated U937 and other proliferating myeloid cells, where CEBP-80 disruption greatly affects the promoter activity. The changes in CEBP-80-bound proteins observed by EMSA (increasing presence of C/EBPβ, gradual loss of CEBP-80 retarded complexes) suggest that the contribution of the distinct C/EBP factors to the CD11c gene transcription varies along myeloid differentiation. In this regard, the loss of CEBP-80-bound species along U937 PMA-triggered myeloid differentiation might be explained if only C/EBPα-containing dimers were capable of recognizing CEBP-80 or, alternatively, by the induction of other members of the C/EBP family known to produce non-functional C/EBP dimers (e.g. CHOP 10/GADD153) (38, 39).

C/EBPα transactivation of the CD11c promoter was not only affected by mutations at Sp1-binding sites but also by disrupting the AP1–60 AP-1-binding element. This finding, together with the described interactions between AP-1 and Sp1 family members on AP1–60/Sp1–70 (24), indicates that multidirectional functional interactions take place among the transcription factors bound to the most proximal region of the CD11c promoter. Furthermore, since Sp1 activity appears to be controlled by the retinoblastoma gene product (pRB) (40, 41) and members of the C/EBP family also interact with pRB and pRB-like proteins (42, 43), this multidirectional cooperation might be governed by pRB, thus coupling the CD11c integrin gene expression to the proliferative state of the cell. In this case, the proximal regulatory region of the CD11c promoter spanning from –130 to –50 would confer responsiveness not only to differentiation agents and tissue-specific stimuli but also to proliferative signals. As a preliminary analysis, and to determine whether the transcriptional behavior of the –90/–50 fragment, we have evaluated the effects of the CESpAP sequence on a heterologous promoter and demonstrated that a composite element including CEBP-80, Sp1–70, and AP1–60 is capable of greatly enhancing the activity of the prolactin promoter regardless of its orientation. Therefore, the –90/–50 fragment constitutes a positive regulatory unit within the CD11c gene promoter.

The expression of the CD11c/CD18 integrin is greatly increased upon monocyte extravasation (Ref. 1 and references herein) and we have previously hypothesized that this effect could be mediated by an extracellular matrix (ECM)-responsive element within the regulatory regions of the CD11c gene (44). Analysis of adhesion-generated intracellular signals have demonstrated that ECM recognition by integrins enhances AP-1 transcriptional activity (45, 46) and revealed the importance of a C/EBP-binding site within the β-casein gene ECM-responsive enhancer (47). Therefore, the AP-1- and C/EBP-binding sites within the CD11c promoter could potentially serve as switches for modulation of the CD11c/CD18 integrin expression in response to the state of cellular adhesiveness and depending on the integrins engaged in ECM attachment. Consequently, we are currently determining not only whether the CESpAP sequence is a bona fide tissue-restricted enhancer, but is capacity of conferring ECM-responsiveness. Moreover, the CD11c promoter is responsive to several myeloid differentiation stimuli and the monocytic differentiation-responsiveness precisely maps to the CESpAP region (25, 26). The differentiation-associated changes in C/EBP and AP-1 protein levels and in the occupancy of C/EBP-80 (this paper) and AP1–60 (26) strongly suggest that the differentiation responsiveness of the CD11c promoter relies on the combined action of C/EBP and AP-1 factors, a situation that has also been recently proposed for the transcriptional induction of collagenase-1 during monocytic differentiation (48). Thus, considering the opposite changes in the levels of c-Fos and C/EBPα, it is tempting to speculate that CD11c gene transcription would shift from C/EBP-driven to AP-1-driven during monocytic differentiation: CD11c gene transcription might be mostly CEBP-80-dependent C/EBPα-driven in proliferating undifferentiated cells and the weight of the CEBP-80-dependent transcription would gradually decrease along differentiation, due to lower C/EBPα expression (33) and to increased C/EBPβ, and possibly GADD153, levels. Conversely, the contribution of the AP1–60 element would concomitantly rise along monocytic differentiation as a consequence of the increased expression of c-Fos (49, 50) and, in this manner, the CD11c promoter activity in differentiated myeloid cells would be predominantly AP1–60-dependent and AP-1-driven, in agreement with the greatly decreased differentiation-inducibility seen upon mutation of the AP1–60 site (25, 26). Furthermore, at the light of the combinatorial theory for tissue-specific expression (reviewed in Ref. 51), the pattern of expression of c-Fos and C/EBPα, together with the presence of functional C/EBP- and AP-1-binding sites within the CD11c promoter, might represent an essential parameter for the restricted expression of the CD11c/CD18 integrin.

Acknowledgments—We gratefully acknowledge Drs. H. C. Kluin-
REFERENCES

1. Corbi, A. L. (1996) in Leukocyte Integrins: Structure, Expression and Function, R. G Landes Biomedical Publishers, Austin, TX

2. Bilsland, C. A., Diamond, M. S., and Springer, T. A. (1994) J. Immunol. 152, 4582–4589

3. Ingalls, R. R., and Golenbock, D. T. (1995) J. Exp. Med. 181, 1473–1479

4. Stacker, S., and Springer, T. A. (1991) J. Immunol. 146, 648–655

5. Keizer, G. D., te Velde, A. A., Schwarting, R., Figdor, C. G., and de Vries, J. (1987) Eur. J. Immunol. 17, 1317–1322

6. Keizer, G. D., Borst, J., Visser, W., Schwarting, R., de Vries, J. E., and Figdor, C. G. (1987) J. Immunol. 138, 3330–3336

7. Estera, L., O’Reilly, K. L., and Splittor, G. A. (1991) Vet. Immunol. Immunopathol. 20, 213–227

8. Postigo, A. A., Corbi, A. L., Sanchez Madrid, F., and De Landazuri, M. O. (1991) J. Exp. Med. 174, 1313–1322

9. Loike, J. D., Silverstein, S. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1044–1048

10. Diamond, M. S., Alon, R., Parkos, C. A., Quinn, M. T., and Springer, T. A. (1995) J. Cell Biol. 130, 1473–1482

11. Blackford, J., Reid, H. W., Pappin, D. J. C., Bowers, F. S., and Wilkinson, J. M. (1989) Eur. J. Immunol. 19, 2940–2950

12. Anderson, D. C., Miller, L. J., Schmalstieg, F. C., Rothlein, R., and Springer, T. A. (1986) J. Immunol. 137, 15–27

13. te Velde, A. A., Keizer, G. D., and Figdor, C. G. (1987) Immunology 61, 261–267

14. Nueda, A., Lopez-Rodriguez, C., Rubio, M., Sotillos, M., Postigo, A., del Pozo, M. A., Vega, M., and Corbi, A. L. (1995) J. Immunol. 154, 3113–3117

15. Hogg, N., Takacs, L., Palmer, D. G., Selvendran, Y., and Allen, C. (1986) Eur. J. Immunol. 16, 240–248

16. Bellion, T., Lopez-Rodriguez, C., Vara, A., Rochems, G., Bernabeu, C., and Corbi, A. L. (1994) J. Immunol. 24, 41–47

17. de la Hera, A., Alvarez-Mon, M., Sanchez-Madrid, F., Martinez, C., and Durantez, A. (1988) Eur. J. Immunol. 18, 1131–1134

18. Chadburn, A., Inghirami, G., and Knowles, D. M. (1992) Hematol. Pathol. 6, 193–202

19. Huieatt, J. W., and Lefrancois, L. (1995) J. Immunol. 154, 5684–5693

20. Schwartz, R. D., Stein, H., and Wang, C. Y. (1985) Blood 65, 974–983

21. Hansson, C. A., Gribbin, T. E., Schnitzer, B., Schlegelmilch, J. A., Mitchell, B. S., and Stoolman, L. M. (1990) Blood 76, 2360–2367

22. López-Calderon, M., Nueda, A., Vara, A., Garcia-Aguilar, J., Tugores, A., and Corbi, A. L. (1993) J. Biol. Chem. 268, 1187–1193

23. Lopez-Rodriguez, C., Chen, H., Tenen, D. G., and Corbi, A. L. (1995) Eur. J. Immunol. 25, 3498–3503

24. Neti, J. D., Reineimann, B. C., and Petrus, M. N. (1996) Mol. Cell. Biol. 16, 2940–2950

25. Rubio, M. A., Lopez-Rodriguez, C., Nueda, A., Aller, P., Armesilla, A. L., Vegam, M. A., and Corbi, A. L. (1995) Blood 86, 3715–3724

26. Lopez-Rodriguez, C., Kluin-Nelemans, H. C., and Corbi, A. L. (1996) J. Immunol. 156, 3780–3787

27. McKnight, S. (1992) Transcriptional Regulation, pp. 771–795, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

28. Nueda, A., Lopez-Calderon, M., Vara, A., and Corbi, A. L. (1993) J. Biol. Chem. 268, 19095–19111

29. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419

30. Hernández-Munain, C., and Krangel, M. S. (1994) Mol. Cell. Biol. 14, 473–483

31. Watanabe, H., Wada, T., and Handa, H. (1990) EMBO J. 9, 841–847

32. Osada, S., Yamamoto, H., Nishihara, T., and Imagawa, M. (1996) J. Biol. Chem. 271, 3891–3896

33. Scott, L. M., Civen, C. L., Rorth, P., and Friedman, A. D. (1992) Blood 80, 1725–1735

34. Lee, Y.-H., Yano, M., Liu, S.-Y., Matsuura, E., Johnson, P. F., and Gonzalez, F. J. (1994) Mol. Cell. Biol. 14, 1338–1349

35. Lee, Y.-H., Williams, S. C., Baez, M., Sterneck, E., Gonzalez, F. J., and Johnson, P. F. (1997) Mol. Cell. Biol. 17, 2083–2047

36. Freitag S. O., and Geddes, T. J. (1992) Science 256, 379–382

37. Zhang, D.-E., Hetherington, C. J., Meyers, S., Rhaides, K. L., Larson, C. J., Chen, H. M., Hierbert, S. W., and Tenen, D. G. (1996) Mol. Cell. Biol. 16, 1231–1240

38. Ron, D., and Habener, J. F. (1992) Genes Dev. 6, 439–453

39. Fawcett, T. W., Eastman, H. B., Martindale, J. L., and Holbrook, N. J. (1996) J. Biol. Chem. 271, 14283–14289

40. Udvadia, A. J., Templeton, D. J., and Horowitz, J. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3953–3957

41. Chen, L. I., Nishimaka, T., Kawan, K., Kitabayashi, Y., Kusuma, K., Fu, Y., Grunwald, S., and Chiu, R. (1984) Mol. Cell. Biol. 14, 4380–4389

42. Kramer, A., Carstensen, C.-P., and Fahl, W. E. (1996) J. Biol. Chem. 271, 6579–6582

43. Chen, P.-L., Riley, D. J., Chen-Kiang, S., and Lee, W.-H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 465–469

44. Corbi, A. L., and Lopez-Rodriguez, C. (1997) Leuk. Lymph. 25, 415–425

45. Tremble, P., Damsky, C. H., and Werb, Z. (1995) J. Immunol. 154, 1707–1720

46. Yamada, A., Nikaido, T., Nojima, Y., Schlossman, S. F., and Morimoto, C. (1991) J. Immunol. 146, 53–56

47. Roskelley, C. D., Srebrow, A., and Bissell, M. J. (1995) Curr. Opin. Cell. Biol. 7, 736–747

48. Deyle, G. A. R., Pierce, R. A., and Parks, W. C. (1997) J. Biol. Chem. 272, 11840–11849

49. Sariban, E., Mitchell, T., Rambaldi, A., and Kufe, D. W. (1988) Blood 71, 488–493

50. Mavilio, F., Testa, U., Spotti, N. M., Petrin, M., Peloiz, E., Bordignon, C., Amadori, S., Mandelli, F., and Peschle, C. (1987) Blood 69, 160–164

51. Ernst, P., and Smale, S. T. (1995) Immunity 2, 311–319
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J. Biol. Chem. 1997, 272:29120-29126.
doi: 10.1074/jbc.272.46.29120

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