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Immunology

Description of the leukocyte function-associated antigen 1 (LFA-1 or CD11a) promoter

(adherence/receptor/regulation)

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ABSTRACT **The CD11a/CD18 (leukocyte function-associated antigen 1 or LFA-1) leukocyte integrin is expressed at high levels on the cell surface of T lymphocytes and macrophages, where it mediates homotypic and heterotypic adherence between leukocytes and other cell types by binding to intracellular adhesion molecules 1 and 2 on the conjugate cell. To initiate studies of the molecular regulation of expression of the CD11a molecule, we isolated genomic clones corresponding to the 5′-flanking region of CD11a, identified the transcriptional start sites for CD11a, and characterized the CD11a promoter sequence in transient expression assays. The CD11a promoter (1.7 kb) directed functional activity of a heterologous reporter gene in the T-lymphocyte cell line Jurkat and the myeloid cell line HL-60 but did not direct functional activity in three different nonleukocyte cell lines. Deletional analysis of the CD11a promoter sequence indicated the presence of distinct, cell-type-specific regulatory sequences with the region from −40 to −17 relative to the transcription start sites responsible for most of the *in vitro* activity of the CD11a promoter in the Jurkat T-cell line, and the promoter sequence located within the first 17 bp relative to the transcription start sites responsible for CD11a promoter activity in the HL-60 cell line. Identification of the CD11a promoter provides the opportunity to identify unique cis-acting elements and trans-acting factors responsible for the cell-type-specific expression of CD11a in human leukocytes. Further, the CD11a promoter may be useful in transgenic constructs and in retroviral vectors to direct expression of heterologous genes selectively in leukocytes.**

Three structurally and functionally related heterodimeric structures located on the surface of human leukocytes compose a gene family referred to as the leukocyte integrins (for review, see refs. 1 and 2). These integrin heterodimers enable leukocytes to participate in a variety of adherence-related activities. Each heterodimer consists of a common CD18 or β subunit linked in a noncovalent association with an individual α subunit, designated CD11a (leukocyte function-associated antigen; LFA-1), CD11b (Mac-1), and CD11c (p150,95) (3). The CD11 subunits of this family of molecules are expressed on different populations of leukocytes, where they mediate discrete adherence reactions.

The CD11a subunit, expressed on the surface of both lymphocytes and monocyte/macrophages as a CD11a/CD18 heterodimer, mediates homotypic and heterotypic leukocyte adherence of these cells by binding to one of two defined ligands, intracellular adhesion molecule 1 or 2, on the conjugate cell (4–6). Expression of the CD11a subunit is both tissue-specific and developmental-stage-specific; the CD11a subunit is expressed only on lymphoid and myeloid cells, and it is expressed in highest levels on mature leukocytes.

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To investigate the molecular regulation of CD11a expression, we identified the promoter region of the CD11a gene§ and characterized this promoter in transient expression assays. These studies demonstrated that a 1.7-kb fragment of the 5′-flanking sequence of CD11a demonstrated functional activity when transfected into the Jurkat T-lymphocyte cell line and the HL-60 promyelocytic leukemia cell line but did not demonstrate activity when transfected into three different nonleukocyte cell lines.

MATERIALS AND METHODS

Screening Genomic DNA Libraries and Characterization of DNA Clones. A human leukocyte genomic library in EMBL-3 phage (Clontech) and a human chromosome 16 library in λ Charon 4A (ATCC no. 57758) were screened with a ^{32}P-labeled 33-mer oligonucleotide (TGT CTA GGT TGC CAG CAA ATC CCA CGG GCC TCC) corresponding to a region of the 5′-untranslated region of the CD11a cDNA (7). Positive clones were obtained from each library and analyzed by Southern blotting with the ^{32}P-labeled oligonucleotide as a probe. Restriction fragments from both libraries were subcloned into Bluescript II (Stratagene) plasmids and M13 vectors for detailed restriction mapping and DNA sequencing, respectively.

Mapping the 5′ End by Primer Extension. Primer extension analysis was done according to described methods (8). In brief, a 30-mer oligonucleotide primer complementary to the region of CD11a mRNA located 8–38 bp 3′ of the adenine of the initiation ATG and a 30-mer oligonucleotide primer complementary to a region of the CD11a mRNA located 20–50 bp 3′ of the initiation ATG were labeled by using T4 polynucleotide kinase (Promega). Approximately 5 ng of each labeled primer was hybridized to 3 μg of poly(A)-selected RNA from induced HL-60 cells (48-hr treatment with phorbol 12-myristate 13-acetate at 10 ng/ml) at 37°C overnight. The extension reaction was done with 20 units of Superscript reverse transcriptase (BRL) for 2 hr at 42°C in the recommended buffer. The extension products were then sized by electrophoresis on a denaturing 6% acrylamide gel and compared to the sequencing products obtained by using M13 template DNA primed with a −40 primer.

Mapping the 5′ End by RNase Protection Assay. RNA was extracted from human fibroblasts and from HL-60 promyelocytic leukemia cells treated with phorbol 12-myristate 13-acetate at 10 ng/ml for 48 hr. To generate the RNA probe, a 906-bp region of the CD11a genomic sequence spanning −856 bp to +50 bp relative to the initiation ATG of the signal

Abbreviation: CMV, cytomegalovirus.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M87662).

peptide was amplified by using the PCR and subcloned into Bluescript II (Stratagene). Approximately 500 ng of plasmid DNA was digested with *Dde* I, and the RNA probe was synthesized by using [^{32}P]CTP and T3 RNA polymerase (Stratagene). The resultant 390-bp-labeled RNA probe was hybridized to 20 μg of total RNA from each cell type at 42°C overnight. RNase digestion products were generated by using RNase A/RNase T1 (Ambion, Austin, TX) and analyzed on a denaturing 6% acrylamide gel followed by autoradiography. The digested products were run alongside a DNA sequencing reaction from an M13 template primed with a −40 sequencing primer.

Construction of CD11a Promoter/Growth Hormone Reporter Plasmids. Reporter constructs were created by using 1700-, 800-, 400-, 240-, 120-, 60-, 40-, and 20-bp subfragments of the CD11a promoter sequence generated by PCR. The 5′ primers were as follows: 5′-TTG CAC AGC AGT CAC GCC AT-3′ hybridized ≈1700 bp 5′ to the major transcription start sites of CD11a, 5′-GAC AGA GTA AGA CCT TGT CT-3′ hybridized 800 bp upstream, 5′-TCA AGG TCC AGA GAA AGC TC-3′ hybridized 400 bp upstream, 5′-TTG GAT GTT AGT GAG AAC CA-3′ hybridized 240 bp upstream, 5′-TGA ACC CTG CGG TTT CAC AA-3′ hybridized 120 bp upstream, 5′-CAG TGT CAC CAG CCT GTT GCC-3′ hybridized 60 bp upstream, 5′-TGT GAG AAA GTA CCA CTG-3′ hybridized 40 bp upstream, 5′-GGC CAA AGG GCA TGA TCA TTT TC-3′ hybridized 17 bp upstream. The 3′ primer used in the deletions in Fig. 5 5′-TTG CTG GCA ACC TAG ACA GG hybridized to DNA sequences from +19 to +39 relative to the more 5′ major transcriptional start site. The 3′ primer +16 (5′-GTG AAA GAG GAA AAT GAT CA-3′), 3′ primer +2 (5′-TGA TCA TGC CCT TTG GCC TC-3′), and 3′ primer −6 (5′-TGC CCT TTG GCC TCT TAC AG-3′) were used to create the CD11a promoter constructs in Fig. 6. A *Hin*dIII site was added to the 5′ primers during synthesis, and a *Bam*HI site was added to the 3′ primers to facilitate forced orientation cloning into the pØGH vector. The promoterless pØGH plasmid served as a negative control. The positive control plasmid was obtained by ligating the 1.1-kb cytomegalovirus (CMV) immediate/early gene promoter into pØGH, as described (9).

Transient Expression Assays. Electroporation was done by using a Bio-Rad Gene Pulser apparatus (Bio-Rad). The following cell lines were used in transient expression assays: Jurkat T-lymphocyte cell line (10), the HL-60 promyelocytic leukemia cell line (11), the MIA(PaCa-2) human pancreatic carcinoma cell line (ATCC no. CRL 1420), the RD rhabdomyosarcoma cell line (12), the LS180 human colonic adenocarcinoma cell line (13), and the HeLa human epithelial carcinoma cell line (ATCC). All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM)/10% fetal calf serum before transfection. Transfections were done with 1×10^7 cells suspended in 0.5 ml of Hepes buffer, 100 μg of circular plasmid DNA, and control settings of 250 V and 960 μF for all cells, except for 300 V and 960 μF for HL-60 cells and 300 V and 500 μF for Jurkat cells. After electroporation cells were transferred to 5 ml of DMEM and incubated at 37°C in 5% CO_2. Growth hormone levels were assayed after 3 days of incubation by using 100 μl of supernatant and a ^{125}I-labeled anti-growth hormone antibody (Allegro Human Growth Assay Kit, Nichols Institute, San Juan Capistrano, CA).

RESULTS

Characterization of CD11a Genomic Clones. Two human genomic libraries were screened with an oligonucleotide corresponding to the 5′ end of the CD11a cDNA. Approximately 10^6 plaques were screened, and one reactive clone from each library was isolated. Southern blotting indicated that a 1.9-kb *Xho* I fragment from each clone hybridized to the oligonucleotide used to screen the libraries. The 1.9-kb *Xho* I restriction fragments from each library were sequenced and found identical. There was 73-bp overlap between the 3′ end of the *Xho* I restriction fragment and the 5′ end of the cDNA for CD11a (7). A 3.0-kb *Sac I* restriction fragment containing the complete 1.9-kb *Xho I* sequence was subsequently isolated, allowing definition of the exon 1/intron 1 boundary. The first exon of CD11a contains the 5′ untranslated region and 61 bp of the signal sequence.

Localization of the Transcription Start Site for CD11a. Primer extension analysis indicated two major transcriptional start sites, in addition to several minor start sites (Fig. 1). Because the 5′ end of the first oligonucleotide primer used hybridizes to the CD11a mRNA 38 bp downstream from the initiation ATG, the major extension products of 134 and 131 bp in length correspond to transcription start sites located 96 and 93 bp upstream from the initiation ATG. The lengths of the extended products using the second oligonucleotide are staggered by 12 bp compared with those obtained with the first oligonucleotide, as predicted (Fig. 1, compare lanes 1 and 2). Several minor start sites, located upstream from the two major transcription start sites, are present in the primer extension assay and were also consistent with the RNase analysis. Because the migration of the DNA synthesized in the primer extension reaction can be accurately assessed by using acrylamide gel electrophoresis, the lengths observed in the primer extension assay were used to delineate the precise location of the CD11a start sites.

RNase protection analysis confirmed the location of the transcription start sites for CD11a. RNase protection analysis showed the presence of two major protected fragments of ≈155 and 152 bp, in addition to several minor fragments of longer length (Fig. 2). The two major protected fragments were not detected by using human fibroblast RNA and yeast tRNA (data not shown). Because the RNA probe was synthesized with a 50-bp overlap with the signal sequence of the CD11a mRNA, these protected fragments indicate the presence of two transcription start sites located ≈105 and 102 bp upstream from the initiation ATG. These distances are approximate because RNA can migrate up to 10% more slowly than DNA on polyacrylamide gels.

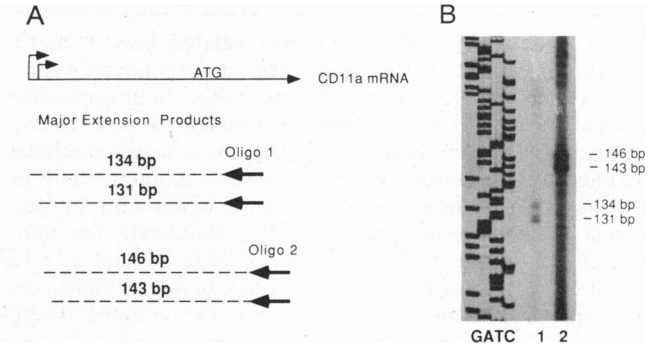

FIG. 1. Primer extension analysis of CD11a. (*A*) Schematic diagram of primer extension and the length of the extended products. Two ^{32}P-labeled 30-mer oligonucleotides located from 8 to 38 bp from the initiation ATG (oligo 1) and 20–50 bp from the initiation ATG (oligo 2), were hybridized to 3.2 μg of phorbol 12-myristate 13-acetate-treated HL-60 poly(A)$^+$ RNA and extended with reverse transcriptase. Location of the two major transcription start sites identified by primer extension appear as right-angle arrows on the mRNA (top line). The dashed lines indicate extension products; lengths of the extension products are listed above the dashed line. (*B*) Locations of the primer extension products on a 6% polyacrylamide sequencing gel. The DNA sequence on the left-hand side used for sizing was derived from sequencing M13 with the −40 primer; the lengths of the major extension products are designated at right.

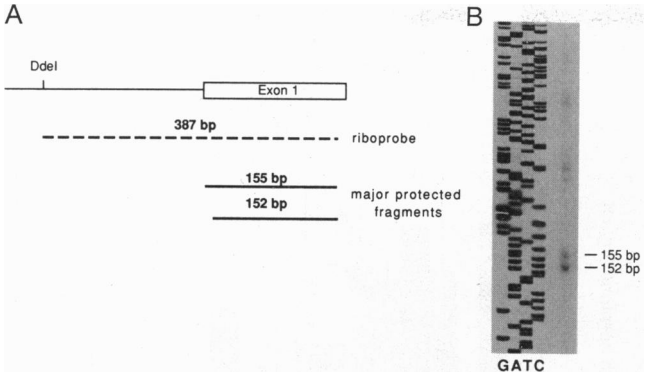

FIG. 2. Mapping of the 5′ end of the *CD11A* gene by RNase protection analysis. (*A*) Schematic diagram of RNase protection analysis. Location of the *CD11A* exon 1 and the *Dde* I restriction site used to generate the RNA probe is shown in the top line. The length of the RNA probe and its relationship to the 5′ end of the *CD11A* gene are represented by the dashed line. The 3′ end of the RNA probe corresponds to 50 nt downstream from the initiation ATG. The bottom two heavy lines represent the two major protected fragments. (*B*) A 387-bp RNA probe was hybridized to 20 μg of total RNA from phorbol 12-myristate 13-acetate-treated HL-60 cells and digested with RNase A and RNase T1; the resultant protected fragments were electrophoresed on a 6% denaturing polyacrylamide gel. A DNA sequencing reaction using a single-stranded M13 template and the −40 primer were used to determine the length in nucleotides (left lanes). The length of the two major bands corresponding to the protected fragments is shown, as well as longer minor protected fragments (right lanes).

DNA Sequence Analysis of CD11a Promoter. The 1.8 kb of DNA upstream from the transcriptional start sites was sequenced and searched for consensus binding sites for transcription factors. Although the *CD11A* gene lacks a TATA or CAAT box typical of eukaryotic class II promoters, it contains several potential binding sites for transcriptional activators. These putative binding sites are identified (Fig. 3). This 1.8 kb of *CD11A*-flanking sequence contains a potential binding site for transcription factors Oct-1 or Oct-2 (14), a potential binding site for PU.1 on the noncoding strand (15), and a potential binding site for Sp1 (16). A large proportion of the 1.8-kb immediate-5′ *CD11A*-flanking region was found to contain *Alu* sequences (Fig. 3) (17).

Transient Expression Assays with the CD11a Promoter Constructs. The functional activity of the 5′ flanking region of *CD11A* was assessed in transient expression assays in both leukocyte and nonleukocyte cell lines (Figs. 4–6). The promoterless pØGH construct was used as a negative control and a growth hormone expression plasmid containing the CMV immediate/early gene promoter was used as a positive control. The 1.7-kb CD11a-promoter construct generated low levels of growth hormone in the MIA (PaCa-2) human pancreatic carcinoma cell line, the RD rhabdomyosarcoma cell line, and the LS180 colonic adenocarcinoma cell line, and intermediate levels of growth hormone in the HeLa cell line (Fig. 4 and data not shown). In contrast, the CD11a-promoter construct produced very high levels of growth hormone in the Jurkat T-cell line and moderately high levels of growth hormone in the myeloid cell line HL-60. The results shown represent four separate experiments.

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                    10        20        30        40        50        60   Notes for Boxes
                    |         |         |         |         |         |
-1800  TTGAGGCTGCAGTGAGCCGAGATCACACCACTCCAGCCTAGGCAAGAGTGAGACCCTCTC
-1740  TCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAACCATCAAAATGTTTTGCACAGCAGTCA
-1680  CGCCATTTTACATTTCTGCCAGCAATGTGCACCAGGCTTCCAGTTTCTTCACATCTTCAC
-1620  TAACTCTTATTTCCTTTGCTTTAAACTCTAACCATCAAAGTAGGTGTAAAGGGTATCTCA
-1560  CTGTGGTTTCATTTGCATTTCTCTAATGACTAATAGTGTTAAGTATCATTTCATGTGCAT   (1)
-1500  GTTGGCCATTTATATGTCATTGGAGAAATGTCTACTCAAACCGTTTGCTCATTTAGAAAC
-1440  TTAGGTAGGTTGGTCTGAGTGCAGTGGTGTTTAAAACTAATTTTTTTTTGAGACAAAGTC
-1380  TCACTCTGTCGCCCAGGCTGGAATGCAATGGTGAGATCTTGGCTCACTACAACCTCCATC
-1320  TCCTGGGTTCAAGCAATTCTTCTGCCTCAGCCTCTCAAGTAGCTGGGATTACAGGCATGC
-1260  GCCACCACGCCTGGCTAATTTTTGTATTTTTGGTAGAGACGGGGGTTTCTCCATGTTGGC
-1200  CAGGCTGGTCTCGAACTCTTGACCTCAGGTGATCCACCTGCCTCGGCCTCCACAGGGCTA
-1140  GGATTAGAGGTGTGAGCCACCGCACCAGGCCGTTTAAAACTAATGGAGCACAACCAGTTA
-1080  CCAATATCTTTGTTCCTTCTCCACTCCCTCTGCTTCAACTTGACTAGCCTAAAATAAATA
-1020  AATTTAAAAAACTGGGCACAGTGGCTCACACCTGTAATCCCAGCACTTTGGGAGGCCGAG
 -960  GCAGGAGGATTACTTGAGCATAGGAGTTCAAGATCAGCCTGGGCAACTAGTGAAAAACCA
 -900  TCTCAAAAAAGAAAAATTAGCCAGGCATGGTGGCATGCACCTGTGGTTTCAGCTACTTAG
 -840  AGCAGAGGTGGAGGATCGCTTGATTCTGGAGTTCAAGGTTGCATTGAGCTGTGATCGCGC
 -780  CAGTGCACTCTCGCTTGGGTGACAGAGTAAGACCTTGTCTCAAAAAATTTAAAACAAAAC
 -720  AAAAAAAACTGGTTATTTGTCTTTTTATTGGTGAATTATAAGAGTTTTAAAAAATATATT
 -660  CTGGAAACAAATCCCTTATTAGAGATATGATTTGCAAATATTTTCTCCAATTTTTTTTTT
 -600  TTTTAAAGACAAAGTTTCACTTTGTCGCCCAGGCTGGTCTTGATTCCTGGCTTCAAGAGA
 -540  TGCTCTTACCTCCACCTCCTGAAGCCCAAAGGGCTGGAATTACAGCCAGTGAGCCTGCAC
 -480  CCAGCCTCCAATTCTTTAGATTTTACATTTTAGAACCAAAATGGGTTAAATACACTGTTC
 -420  TGTAATCTGCTCTTTTCTTTAATAGTAGTTCATGTACATCTTTCAAGGTCCAGAGAAAGC
 -360  TCTCACTTTCTCCCCGTTTTATTTTTCCTTCCCTCATTCTTTTTCACTGCTGCATAGCAT
 -300  TCCATTGTAATTTTGCCACTGTTTATTAGACCAGTCCTCTGCTGAGCTTTACAGAGCCCT
 -240  TAGTTGGATGTTAGTGAGAACCATGACAGCAGTGAGACTGTCATCTCCCTGACATGCTGT
 -180  CAGCTTTTGGATGATGTGAAAATGCAAGCAGGCACAGGAAATGTCTCTAACTTGCTTACA
 -120  CTTCCTCCCTGAACCCTGCGGTTTCACAACTCCTGCAGGCACACCTCCCTCCCCGCCTGC   (2),(3)
  -60  CAGTGTCACCAGCCTGTTGCCTCTGTGAGAAAGTACCACTGTAAGAGGCCAAAGGGCATG
   +1  ATGATTTTCCTCTTTCACCCTGTCTAGGTTGCCAGCAAATCCCACGGGCCTCCTGACGCT
  +61  GCCCCTGGGGCCACAGGTCCCTCGAGTGCTGGAAGGATGAAGGATTCCTGCATCACTGTG
                                                MetLysAspSerCysIleThrVal
 +121  ATGGCCATGGCGCTGCTGTCTGGGTTCTTTTTCTTCGgtaggcaagggaggaggcagggg
       MetAlaMetAlaLeuLeuSerGlyPhePhePhePhe
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FIG. 3. DNA sequence of the CD11a promoter. The nucleotide sequence for the 5′ end of the *CD11A* gene is displayed. Uppercase letters indicate the promoter and exon 1 sequence; lowercase letters denote the beginning of intron 1. Nucleotide +1 corresponds to the first major transcription start site, and the nucleotides preceding it are represented by negative numbers. The two major transcription start sites are indicated by right angle arrows. The amino acids encoded by exon 1 are displayed underneath the corresponding nucleotide sequence. *Alu* sequences are underlined. Putative binding sites for regulatory elements are boxed, and notes on the boxes are as follows: (1), octamer box = ATTTGCAT; (2), inverse PU site = CTTCCTC; and (3), inverse Sp-1 = CCCCGCCT.

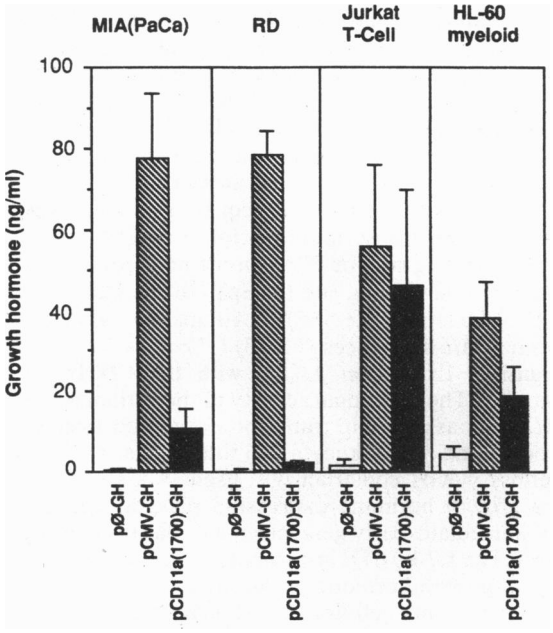

FIG. 4. Transient expression assays of CD11a promoter. Transient expression assays were used to assess the functional activity of the CD11a promoter. Three different growth hormone reporter constructs were used: (*i*) pØGH, the promoterless control plasmid; (*ii*) pCMV-GH containing the immediate/early CMV promoter; and (*iii*) a 1.7-kb CD11a promoter sequence cloned into pØGH designated pCD11a(1700)-GH. Electroporation and subsequent analysis were done as described.

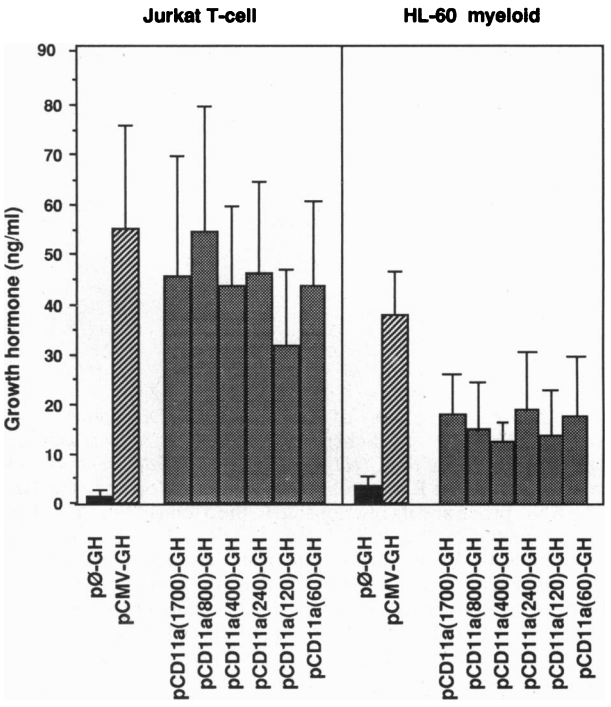

FIG. 5. Deletional analysis of the CD11a promoter. Transient expression assays were used to assess the functional activity of a series of CD11a promoter constructs in which the 5′ end of the CD11a promoter sequence was progressively deleted. pCD11a(1700)–GH, pCD11a(800)–GH, pCD11a(400)–GH, pCD11a(240)–GH, pCD11a(120)–GH designate growth hormone reporter constructs containing either 1700, 800, 400, 240, 120, and 60 bp of CD11a promoter sequence, respectively. pØGH designates the promoterless control plasmid, and pCMV-GH designates the CMV promoter containing growth hormone construct used for comparison.

Transient expression assays were performed by using growth hormone constructs containing CD11a promoter sequences that were progressively deleted from the 5′ and 3′ ends to further delineate the portion of the CD11a upstream sequences that contain functional activity *in vitro*. Constructs containing 1700, 800, 400, 240, 120, 60, and 40 bp of the immediate 5′ sequence of *CD11A* all produced similar levels of growth hormone in both the Jurkat T-cell line and the HL-60 myeloid cell line (Fig. 5). Reduction of CD11a promoter sequence from 40 to 17 bp resulted in a marked decrease in growth hormone levels in Jurkat T cells, indicating that important elements for CD11a expression are contained within the 23 bp deleted (Fig. 6). In contrast, sequences located within the first 17 bp upstream from the transcription start sites were responsible for constituitive CD11a promoter activity in the HL-60 cell line. Because the CD11a promoter possessed a consensus sequence for an "initiator" of transcription at the start site (5′-YYCAYY YYY-3′ XXY) (18), we constructed 3′ deletions to progressively mutate this site (Fig. 6). There was no effect from these deletions of the initiator sequence; however, these deletions did indicate that the sequence from +16 to +39 from the transcription start (i.e., 5′-untranslated region sequence) reduced expression from the CD11a promoter, indicating the presence of inhibitory or repressor elements in this region (Fig. 6).

DISCUSSION

We have identified the transcription start site and characterized the promoter region of the *CD11A* gene. Using a combination of primer extension and RNase protection assays, we demonstrated the presence of two major transcription start sites along with several minor transcription start sites located 5′ to the major start sites. The genomic DNA upstream from the start site cluster does not contain a canonical CAAT or TATA box but does have consensus sequences for the transcription factors Sp1 (16) and PU.1 (15). Transient expression assays indicated that a 1.7-kb fragment of the 5′-flanking region of *CD11A* conferred functional activity upon a heterologous reporter gene in the Jurkat T-cell line and the HL-60 myeloid leukemia cell line but did not confer activity in three different nonleukocyte cell lines. Deletional studies of the CD11a promoter sequence indicated the presence of distinct regulatory sequences in different cell types with the region from −40 to −17 relative to the transcription start sites responsible for most of the *in vitro* activity of the CD11a promoter in the Jurkat T-cell line, and the promoter sequence located within the first 17 bp relative to the transcription start site responsible for constitutive CD11a promoter activity in the HL-60 cell line. In addition, sequences located within the 5′-untranslated region of the CD11a cDNA inhibited CD11a expression.

Promoters for TATA-less genes fall into two broad categories. The first group consists of G + C-rich promoters found primarily in housekeeping genes (19). These promoters usually contain binding sites for the transcription factor Sp1. The second group consists of promoters that direct expression in a tissue or stage-specific manner but which are not G + C-rich; these promoters initiate transcription at one or a few tightly clustered transcription start sites (19). An example of the latter is the terminal deoxynucleotidyltransferase (TdT) (20). The CD11a promoter fits into this second class of TATA-less, tissue-specific promoters.

Although the CD11a promoter lacks a typical CAAT or TATA box, it does contain potential binding sites for transcription factors that may be responsible for CD11a expression during development. The transcription factor Sp1, for which there is a potential binding site located ≈70 bp upstream of the major transcription start site, has been shown

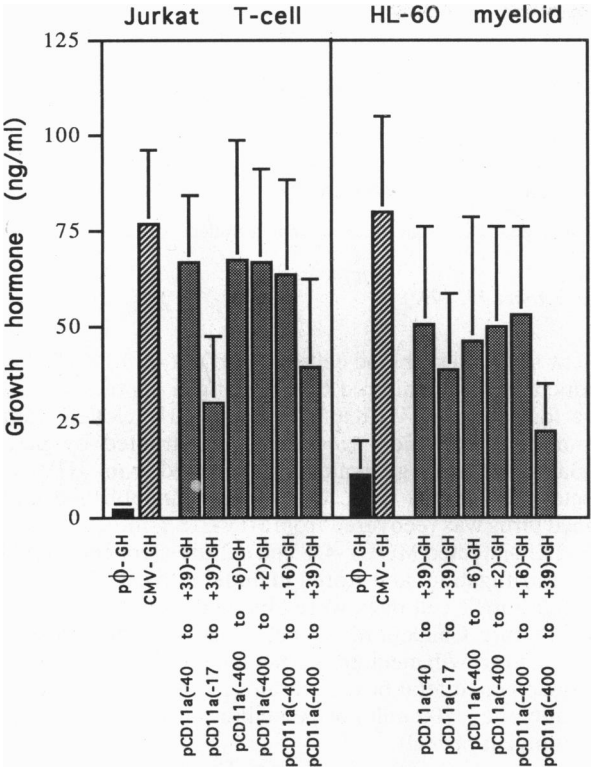

FIG. 6. Deletions flanking the *CD11A* transcriptional start sites. The effect of deletions surrounding the transcriptional start sites of *CD11A* were analyzed in transient transfection assays. The −40 and −17 constructs contained DNA sequence upstream from the start sites. The remaining constructs extend from −400 to the designated distance 3′ to the start sites. The mean and SD for four experiments is shown.

to play a primary role in mediating TATA-less transcription initiation by interacting with a tethering factor that binds TFIID in the absence of a TATA box, thus anchoring the transcription complex to the promoter (21). In addition to the Sp1-binding site, the CD11a promoter possesses a PU box. PU boxes have been identified previously as lymphoid and myeloid-specific enhancers that bind the transcriptional activator PU.1 (15). Finally, the CD11a promoter sequence possesses a potential binding site for the ubiquitous transcription factor Oct-1 (14). These sequences did not appear to play a significant role in modulating the constitutive expression of the *CD11A* gene in the *in vitro* transient expression studies we performed because deletional constructs of the CD11a promoter lacking these sites possessed similar functional activity compared to the nondeleted construct. However, these sequences may be important in inducible CD11a expression during hematopoietic cell differentiation.

The genes encoding the CD11a, CD11b, and CD11c members of the leukocyte integrin gene family are located in a cluster on chromosome 16p11–p13.1 (22). Because the individual *CD11* genes are expressed in a lineage- and cell-type-specific manner, understanding how these molecules are expressed may provide insight into lineage-specific gene expression during leukocyte differentiation. Comparison of the promoter structure of the CD11a subunit to that of CD11b indicates the presence of a number of shared as well as specific features (23). Both lack consensus CAAT or TATA boxes, both contain two major start sites of transcription, both possess an Sp1 site, and both possess PU boxes. In addition, both possess large *Alu* sequences within the immediate 2 kb of 5′-flanking sequence.

Analysis of the promoter region of the leukocyte integrins, including CD11a, may help identify genetic elements critical for the control of gene expression in leukocytes. Identification of these elements may lead to the isolation of tissue-specific trans-acting factors that are responsible for leukocyte gene expression. In addition, the CD11a promoter may prove useful in transgenic constructs or retroviral vectors to direct the expression of genes in leukocytes.

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