Leukocyte Integrin CD11b Promoter Directs Expression in Lymphocytes and Granulocytes in Transgenic Mice

By Anthony Back, Katrina East, and Dennis Hickstein

The human leukocyte integrin subunit CD11b is expressed predominantly on myelomonocytic cells. To identify CD11b promoter sequences important for myelomonocytic gene expression and to assess the utility of the CD11b promoter for expressing heterologous genes in vivo, we generated transgenic mice with a human CD4 reporter gene driven by CD11b promoter constructs composed of 1.5, 0.3, or 0.1 kb of DNA sequence 5' to the transcription start site. Using flow cytometry to detect the human CD4 reporter on murine leukocytes, two of three 1.5-kb CD11b promoter founder lines showed surface expression of the human CD4 transgene in granulocytes and lymphocytes. The transgene expression observed in lymphocytes was inappropriate relative to the normal pattern of CD11b expression. Of the eight 0.3-kb or 0.1-kb founder lines, only one 0.1-kb founder line showed transgene expression. The overall pattern of transgene expression among the 11 founder lines does not parallel expression of the endogenous CD11b gene. These studies indicate that additional CD11b regulatory elements will be required to express a reporter gene in vivo in a lineage-specific pattern that mimics the endogenous CD11b gene.

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Materials and Methods

Plasmid construction and generation of transgenic mice. The 1.5-, 0.3-, and 0.1-kb CD11b promoter fragments were generated using the polymerase chain reaction (PCR) to amplify the CD11b promoter fragment from a human genomic CD11b clone identified previously.13 This procedure was designed to add a HindIII site to the 5' end of the CD11b promoter and a BamHI site to the 3' end. The human CD4 cDNA was removed as a BamHI fragment from the plasmid pmCV7. The CD11b promoter fragments were ligated to a mutated human CD4 cDNA and cloned into the pOGH plasmid.16 The human CD4 cDNA is mutated at bp 1348 (C → T) to produce a premature termination codon, resulting in truncation of hormone (hGH) minigene. Because CD11b normally requires association with CD18 before surface expression of the CD11b/CD18 heterodimer, human CD4 was chosen as a reporter gene because it can be expressed as a single-cell surface molecule. The three different transgene constructs use either 1.5, 0.3, or 0.1 kb of the CD11b promoter sequence.

The results of these studies indicate that the 1.5-kb CD11b promoter is capable of directing the expression of the reporter gene in lymphocytes of transgenic mice. However, the pattern of transgene expression does not consistently resemble expression of the endogenous CD11b gene. In addition, the reporter gene expression in these CD11b promoter transgenic mice appears to be dependent on integration site and is unrelated to transgene copy number. These findings suggest that sequences in addition to the proximal CD11b promoter will likely be required for lineage-specific expression of reporter genes in myeloid cells.

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Fig. 1. CD11b transgene constructs. Three human CD11b promoter constructs were used for the production of transgenic mice. CD11b promoter fragments of 1.5, 0.3, or 0.1 kb were linked to a mutated version of the human CD4 DNA along with an hGH minigene and used to generate transgenic mice. The constructs were removed from the plasmid with HindIII and Nsi I. The bent arrows denote the transcription start site. The straight arrows represent the locations of PCR primers used for screening mouse pups. The star denotes the point mutation in the human CD4 cDNA creating a premature stop codon. The Sg II and BamHI sites were used to make the Southern blots in Fig. 2.

The cytoplasmic domain of the transgenic human CD4, and was a gift of Roger Perlmutter (University of Washington, Seattle, WA). Plasmids containing the transgenic constructs were prepared using cesium chloride equilibrium centrifugation. The CD11b-hCD4-hGH fusion genes were excised from plasmid using HindIII and Nsi I (Fig 1). The transgenic construct DNA was isolated on 0.8% agarose gels and recovered using SpinX (Costar, Cambridge, MA) columns. The resulting DNA was further purified using Qiagen-5 tips (Qiagen, Chatsworth, CA) and resuspended in TE at 100 ng/μL. The microinjection DNA was cut with HindIII and

The 5' primer sequence is based on human CD4 (bp 1517-1541): CGG-ACC-AGA-TGA-ATG-TAG-CAG-ATC-C. The 3' primer sequence was based on human growth hormone (bp 492-859): AAG-CGC-CTG-GTT-TAG-CTG-CTT. A typical 50 μL PCR reaction used 30 ng of tail DNA, 1 μL Vent polymerase (New England Biolabs, Boston, MA), 10 X Vent polymerase buffer supplied by the manufacturer, and 0.2 mmol/L dNTPs. The PCR parameters were 95°C for 10 minutes followed by 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute for 30 cycles. Ambiguous PCR results were resolved by performing DNA slot blots. All analyses were performed using F1 heterozygotic transgenic animals, except for flow cytometry studies of peripheral blood from sterile founder animals.

Assessment of transgene integrity and copy number. Transgene integrity was tested by using genomic DNA in Southern blots. For the 1.5-kb CD11b promoter-hCD4-hGH fusion construct, the DNA was cut with Bgl II to produce a 4.4-kb restriction fragment that included the entire hCD4 cDNA. For 0.3-kb and 0.1-kb CD11b promoter constructs, the DNA was cut with BamHI to produce a 1.7-kb restriction fragment (Fig 1). Blots were performed using ZetaProbe membranes (BioRad, Richmond, CA), hybridized, and washed in sodium phosphate buffer as described by the manufacturer at 65°C. A scanning densitometer (Molecular Dynamics, Sunnyvale, CA) was used to estimate relative copy numbers for each founder line.

Detection of transgene expression in tissue. Transgene expression was assessed using RNA slot blots and Northern blots. RNA was extracted from mouse organs (including spleen, marrow, lymph node, kidney, lung, thymus, muscle, liver, and brain) using a single-step method with Ultraspec (Tel-test, Friendswood, TX). RNA slot blots were made using nitrocellulose membranes (S&S, Keene, NH) following established procedures. The blots were washed at 65°C in 0.1 X SSC, 0.1% sodium dodecyl sulfate (SDS).

Flow cytometry to assess hCD4 reporter gene expression on leukocytes. Peripheral blood was obtained by retroorbital puncture of anesthetized mice. The red blood cells were lysed using Tris-ammonium chloride, washed once, and resuspended in phosphate-buffered saline (PBS). Splenocytes were prepared by teasing cells out of the spleen capsule and washing the cells once in PBS. Peritoneal macrophages were obtained by lavaging the peritoneal cavity with 5 mL of PBS. Human lymphocytes were used as a positive control for human CD4. Murine splenocytes were used as a positive control for CD45R/B220. Murine granulocytes from murine peripheral blood were used as a positive control for antimurine CD11b. Cells were treated with blocking rat IgG for at least 15 minutes before adding specific monoclonal antibodies. Staining was performed at 4°C for at least 30 minutes with saturating concentrations of either antimurine CD11b-fluorescein isothiocyanate (FITC; Serotec, Oxford, UK), antimurine B220-FITC (PharMingen, San Diego, CA), or anti-Gr-1-FITC (PharMingen),4 and antihuman CD4-phycoerythrin (PE) (Becton Dickinson, Mountain View, CA). After staining, the cells were washed twice in PBS and 5% fetal calf serum, and resuspended in 5% fetal calf serum and 7-amino-actinomycin D (7AAD) at 5 μg/mL. The cells were analyzed within 3 hours on a FACScan (Becton Dickinson) using Lysis II acquisition software. Nonviable cells staining positively with 7AAD were identified using FL3 versus forward scatter histograms and excluded from analysis. Data on 50,000 viable cells were collected for each sample. For studies using peripheral blood leukocytes, lymphocytes and granulocytes were identified on histograms displaying forward scatter versus side scatter. Monocytes usually did not appear as a distinct population in these preparations. For studies of peritoneal cells, macrophages were identified on histograms displaying forward scatter versus side scatter. All histograms are 3-decade log/log scales. Lymphocytes, granulocytes, and macrophage populations were confirmed by Wright's staining of sorted cells. Further analysis of list mode data files was performed using ReproMan (True Facts, Seattle, WA).

RESULTS

Generation of transgenic mice carrying CD11b promoter-hCD4-hGH fusion genes. The three CD11b promoter-hCD4-hGH transgenic constructs used to generate transgenic mice are shown (Fig 1). These constructs contain regions of the CD11b promoter extending either 1.5, 0.3, or 0.1 kb 5' from the transcription start site. In all constructs the CD11b promoter drives a reporter gene consisting of a human CD4 cDNA that has been mutated to prevent intracellular signaling, as described in the Materials and Methods. The hGH minigene is located at the 3' end of the transgene to improve transgene RNA stability and potentially enhance transgene expression.19

The different lengths of the CD11b promoter chosen for the transgene constructs were based on results of in vitro transient transfection assays in myeloid cell lines. The 1.5-kb CD11b promoter driving a human growth hormone reporter gene was active in myeloid cells.11,13,14 The 0.3-kb CD11b promoter driving a human growth hormone reporter
gene showed the highest activity of a number of deletion constructs in transient transfection assays (Back, unpublished data). The 0.1-kb CD11b promoter construct was made to test a published finding that 93 bp was sufficient for myeloid-specific promoter activity in transient transfection assays.11

These three CD11b promoter constructs were used to generate transgenic mice by microinjection of the male pronucleus of fertilized mouse eggs. Transgene-positive animals were identified by slot blot hybridization of DNA extracted from the tails of offspring with a 1.7-kb human CD4 cDNA probe. Eleven founder lines were successfully bred to establish heterozygous lines: three lines of CD11b 1.5-kb promoter-hCD4-hGH mice; four lines of CD11b 0.3-kb promoter-hCD4-hGH mice; and four lines of CD11b 0.1-kb promoter-hCD4-hGH mice. Three other founder mice were positive on the initial slot blot analysis but did not pass the transgene to offspring and are presumed to be mosaics. Three additional transgene-positive founder animals (1.5-kb CD11b-hCD4-hGH) were sterile.

Transgene integrity and copy number. To evaluate transgene integrity and estimate copy number, Southern blots were performed (Fig 2). For the 1.5-kb CD11b promoter-hCD4-hGH founders, DNA was digested with Bgl II and the resulting blot was hybridized with the 1.7-kb human CD4 probe, which should identify a 4.4-kb restriction fragment. For the 0.3-kb and 0.1-kb CD11b promoter-hCD4-hGH founders, tail DNA was digested with BamHI and the resulting blot was hybridized with the 1.7-kb human CD4 probe, which should identify a 1.7-kb restriction fragment. These blots verify that the transgenes were integrated into the mouse germline without rearrangement. In all founder lines that were bred, transgenes were integrated intact (Fig 2). This excludes the possibility that lack of transgene expression is caused by disruption of the transgene during or after integration into germline DNA. One of the founders (F49) demonstrated transgene rearrangement and was not analyzed further. Relative transgene copy numbers were estimated to range from 1 to 10 (Fig 2 and Table 1).

Expression of CD11b promoter-hCD4-hGH transgene RNA. RNA slot blots were made and probed with the 1.7-kb human CD4 reporter transgene to assess RNA expression in different organs of transgenic mice from different founder lines. If the CD11b promoter were sufficient to direct the tissue-specific expression of CD11b, the CD11b promoter-hCD4-hGH transgenes would be expressed predominantly in myeloid cells and could be detected easily in bone marrow-derived RNA on slot blots or Northern blots. This tissue-specific pattern of CD11b expression has been demonstrated in humans14,20 and in mice.21

Slot blots from three founder lines of the 1.5-kb CD11b promoter-hCD4-hGH transgenic mice are shown in Fig 3. In the nontransgenic control mouse (column 1), no human CD4 reporter transgene RNA is detected in any organ. SupT1 denotes RNA isolated from a human lymphocyte cell line that expresses human CD4 and was used as a positive control. Of the three 1.5-kb CD11b promoter-hCD4-hGH founder lines, one founder line (F84) expresses transgene RNA easily detected after an overnight exposure in the spleen, thymus, and bone marrow. Another founder line (F88) expresses similar levels of transgene RNA in spleen and lymph node, and lower levels in bone marrow and liver.

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**Fig 2.** Southern blot of tail DNA. (A) Standards for transgene copy number. Purified plasmid containing the 1.5-kb CD11b promoter construct was digested with Bgl II, electrophoresed, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. The amount of plasmid DNA in each lane was calculated assuming a haploid mouse genome of 3 x 10^9 bp and comparison to Southern blots was made with 10 μg total genomic DNA per lane. (B) Tail DNA from 1.5-kb CD11b promoter-hCD4-hGH founder lines (10 μg tail DNA per lane) digested with BglII, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. Relative copy numbers are F90 = 10; and F60 = 3. (C) Tail DNA from 0.3-kb CD11b promoter-hCD4-hGH founder lines (10 μg tail DNA per lane) digested with BamHI, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. Relative copy numbers are F98 = 10; and F60 = 3. (D) Tail DNA from 0.1-kb CD11b promoter-hCD4-hGH founder lines (10 μg tail DNA per lane) digested with BamHI, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. Relative copy numbers are F84 = 1; F88 = 10; and F60 = 3. (C) Tail DNA from 0.3-kb CD11b promoter-hCD4-hGH founder lines (10 μg tail DNA per lane) digested with BamHI, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. Relative copy numbers are F90 = 10; and F60 = 3. (D) Tail DNA from 0.1-kb CD11b promoter-hCD4-hGH founder lines (10 μg tail DNA per lane) digested with BamHI, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. Relative copy numbers are F84 = 1; F88 = 10; and F60 = 3. (C) Tail DNA from 0.3-kb CD11b promoter-hCD4-hGH founder lines (10 μg tail DNA per lane) digested with BamHI, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. Relative copy numbers are F84 = 1; F88 = 10; and F60 = 3. (D) Tail DNA from 0.1-kb CD11b promoter-hCD4-hGH founder lines (10 μg tail DNA per lane) digested with BamHI, blotted onto a Zetaprobe membrane, and probed with the mutated 1.7-kb human CD4 cDNA. Relative copy numbers are F84 = 1; F88 = 10; and F60 = 3.
### Table 1. Transgene RNA and Surface Protein Expression in CD11b Promoter-hCD4-hGH Transgenic Mice

<table>
<thead>
<tr>
<th>Construct</th>
<th>Founder Line</th>
<th>Copy No.</th>
<th>RNA Slot Blot Positives</th>
<th>Granulocytes</th>
<th>Lymphocytes</th>
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<tr>
<td>1.5 kb</td>
<td>88</td>
<td>10</td>
<td>Spleen, node, thymus, marrow</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>1</td>
<td>Spleen, thymus, marrow</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3</td>
<td>None</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.3 kb</td>
<td>119</td>
<td>4</td>
<td>Thymus</td>
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<td>1</td>
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<tr>
<td></td>
<td>111</td>
<td>2</td>
<td>None</td>
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<td>2</td>
</tr>
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<td></td>
<td>90</td>
<td>3</td>
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<td>0.1 kb</td>
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<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>Marrow</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>Marrow (trace)</td>
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<tr>
<td>Nontransgenic</td>
<td></td>
<td>None</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
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</table>

The RNA positives include any organ giving a signal greater than background. The FACS %hCD4' cells is the percentage of hCD4-positive cells in peripheral blood granulocytes (from the Gr-1/hCD4 studies) or lymphocytes (from the B220/hCD4 studies). For founder lines F88, F84, and F3, these percentages equal the sum of the upper two quadrants from the corresponding histogram in Fig 4.

One 1.5-kb founder line (F60) does not express transgene RNA in any organ. Results for RNA slot blots from other founder lines are summarized in Table 1. Of the four founder lines of mice bearing the 0.3-kb CD11b promoter constructs, transgene RNA expression was detected in only founder line (F119), which was in the thymus. Of the four founder lines bearing the 0.1-kb CD11b promoter construct, one founder line (F3) expressed transgene RNA in bone marrow cells at moderate levels and one founder line (F1) expressed very low levels in marrow cells.

Transgene expression on peripheral blood cells directed by the CD11b Promoter. Two-color cytofluorometric analyses of leukocytes isolated from the peripheral blood of transgenic mice were performed to verify the hematopoietic lineage of cells expressing the human CD4 reporter transgene. Peripheral blood leukocytes were stained simultaneously with pairs of anti-hCD4 and cell lineage markers for B lymphocytes, (murine B220), monocytes, and granulocytes (murine CD11b and murine Gr-1). If the CD11b promoter were sufficient to direct the tissue-specific expression of CD11b, the CD11b promoter-hCD4-hGH transgenes would be expressed predominantly in myeloid cells and the hCD4 reporter gene product would be detectable by flow cytometry on peripheral blood granulocytes as well as a small percentage of B lymphocytes.

Our CD11b promoter transgenic lines did not consistently demonstrate the expected pattern of hCD4 expression on peripheral blood leukocytes (Table 1). The 1.5-kb CD11b promoter founder lines demonstrating the highest levels of transgene RNA expression had the highest levels of surface expression of the human CD4 reporter transgene on peripheral blood leukocytes (Fig 4). One 1.5-kb founder line (F84) expresses human CD4 primarily in granulocytes (Fig 4, row 2). One 1.5-kb founder line (F88) expresses human CD4 in granulocytes and lymphocytes (Fig 4, row 3), but the number of lymphocytes expressing hCD4 is inappropriately high relative to normal CD11b expression. The third 1.5-kb founder line (F60, not shown) did not express the transgene. In addition, three sterile 1.5-kb founder mice showed no hCD4 expression on flow cytometry (data not shown). None of the four lines of 0.3-kb CD11b promoter mice demonstrated surface expression of the hCD4 transgene (data not shown). Only one founder line (F3, row 4, Fig 4) of four 0.1-kb CD11b promoter lines had detectable CD11b expression on granulocytes and lymphocytes, but the number of lymphocytes expressing hCD4 is inappropriately high relative to normal CD11b expression. None of the three remaining founder lines of 0.1-kb CD11b promoter mice demonstrated surface expression of the hCD4 transgene (data not shown).

### Fig 3. RNA slot blots. Fifteen micrograms of total RNA was blotted onto nitrocellulose membranes and probed with the 1.7-kb human CD4 cDNA. Exposure time was 48 hours.
CD11b promoter in transgenic mice

The expression of the human CD4 reporter transgene in lymphocyte subsets (Fig 5). If the CD11b promoter was sufficient to direct the tissue-specific expression of CD11b, the hCD4 reporter gene product would be detectable by flow cytometry only on a small percentage of CD5+ B lymphocytes.

The only two founder lines with transgene expression in lymphoid cells (F88 = 1.5 kb, F3 = 0.1 kb) show that the hCD4 transgene is expressed, inappropriately, in many CD5+ splenocytes (Fig 5, column 4). In founder F88 (1.5 kb), a small population of CD5+ splenocytes expresses the transgene (Fig 5, column 4, row 2), but most of the splenocytes that express hCD4 are CD5+. The transgene expression observed in splenocytes is not parallel to expression of the endogenous murine CD11b gene.

Transgene expression on peritoneal macrophages directed by the CD11b promoter. A two-color cytofluorometric analysis of peritoneal macrophages was performed to evaluate expression of the human CD4 reporter gene in myeloid cells outside of peripheral blood (Fig 6). Peritoneal macrophages were used to examine transgene expression in myeloid cells other than granulocytes. If the CD11b promoter were sufficient to direct tissue-specific regulation of CD11b, the hCD4 reporter gene product should be detectable on peritoneal macrophages from the CD11b promoter transgenic mice.

Peritoneal macrophages were stained simultaneously with pairs of anti-hCD4 and either murine CD11b or murine Gr-1. The founder lines that express the hCD4 transgene in peripheral blood granulocytes also express the hCD4 transgene in peritoneal macrophages. Data from 1.5-kb founder lines are shown (F88) demonstrating appropriate expression of the hCD4 transgene in peritoneal macrophages.

DISCUSSION

This study indicates that the CD11b proximal promoter directs expression of a human CD4 reporter gene in the leukocytes of transgenic mice, although transgene expression appears to be dependent on integration site and is not related to transgene copy number. Three of eleven founder lines demonstrated easily detectable levels of transgene-derived human CD4 on the surface of peripheral blood leukocytes. In these three founder lines, transgene expression was documented in granulocytes and peritoneal macrophages, as would be expected from the normal pattern of CD11b expression, but, in addition, transgene expression in two founder lines was documented in lymphocytes in a pattern not characteristic of normal CD11b expression. These results suggest that distant DNA regulatory sequences not included in the 1.5-kb CD11b proximal promoter are required to direct gene expression in hematopoietic cells in a pattern paralleling the endogenous CD11b gene.

CD11b expression in human and murine hematopoietic cells is largely restricted to terminally differentiated myeloid cells. In the CD11b promoter transgenic founder lines that expressed the human CD4 reporter gene, tissue specificity for myeloid cells was not observed. Two of the three founder lines (F88 = 1.5 kb, F3 = 0.1 kb) with the highest levels of expression showed human CD4 surface expression on lymphocytes, as well as in granulocytes. Although transgene expression in CD5+ B lymphocytes would be consistent with the tissue distribution of the endogenous CD11b gene, most of the CD5+ splenocytes did not express the human CD4 reporter transgene. The transgene expression observed in lymphocytes cannot be explained by an expanded CD4+ or CD8+ compartment, in which small subsets of CD11b+ cells are normally present. These results suggest that the complete set of regulatory sequences controlling lineage-specific expression of CD11b in granulocytes or lymphocytes is not contained within the 1.5-kb proximal CD11b promoter.

However, some of the sequences important for CD11b expression may be present within the 1.5-kb CD11b promoter, compared with the shorter 0.3-kb and 0.1-kb constructs. Two of three founder lines of 1.5-kb CD11b promoter transgenic mice expressed the transgene on FACS. By comparison, only one founder line of the eight 0.3-kb or 0.1-kb lines expressed the transgene on FACS. This finding suggests that a cis-active regulatory element important for expression in vivo is present only in the 1.5-kb promoter construct. Additional 1.5-kb founder lines might confirm the presence of sequences important in CD11b expression. Our
Nontransgenic

1.5 kb (F88)

0.1 kb (F3)

B220 mCD4 mCD8 mCD5 mCD11b

Fig 5. Splenocyte flow cytometry. Splenocytes were stained with the monoclonal antibodies indicated and analyzed on a FACScan. The numbers indicate the percentage of cells in each quadrant.

studies initially identified nine transgene-positive 1.5-kb founders. Three of these animals were sterile. Of the remaining six animals that were bred, two failed to produce any transgene-positive offspring, and one demonstrated a rearranged transgene on Southern blots. Thus, three lines of 1.5-kb mice were available for complete analysis. The three sterile 1.5-kb founder animals were analyzed for transgene expression in peripheral blood leukocytes; none showed any transgene expression. Taken together, these results suggest that, although the CD11b proximal promoter contains some of the sequences required for hematopoietic tissue specificity, it does not contain all of the sequences required for regulated expression of CD11b in myeloid cells.

Previous studies using transient transfections have shown that Sp1 and PU.1 are important for the expression of CD11b in myeloid cells and have suggested that the CD11b promoter is myeloid specific. However, transgenic animals represent a more stringent test of tissue specific expression than transfection of cell lines in vitro. The transgene expression mediated by the CD11b promoter described in this report indicates that the 1.5-kb CD11b promoter is not sufficient for high-level gene expression in myeloid cells. A transgenic construct using the entire CD11b gene, including 5′, 3′, exon, and intron sequences, would be extremely valuable in light of these results, because fully regulated expression of CD11b may require a number of disparately located elements. Because the CD11b gene spans more than 50 kb, this type of transgenic construct would require use of large genomic sequences, requiring P1s or yeast artificial chromosomes.

Two features of the transgene constructs could have an effect on the reporter gene expression mediated by the CD11b promoter. First, the human CD4 reporter gene could have some unanticipated effect on transgene expression. Although the human CD4 cDNA used in these transgenes was mutated by truncating the cytoplasmic domain, to minimize the effect on the cells expressing the transgene, it might be possible for the transgene to be toxic to the cells expressing the transgene. However, among the founder lines, transgene RNA expression paralleled surface expression of human CD4 in the tissues expressing the highest levels of hCD4 RNA. Thus, an hCD4 effect on transgene expression seems unlikely, which indicates that the hCD4 reporter gene may be useful in other transgenic studies involving murine hematopoietic cells. Second, the hGH minigene located at the 3′ end of the transgenic constructs could have affected
The molecular mechanisms that control human myeloid tissue specificity in gene expression have not been well defined using in vivo experimental systems. Studies of genes expressed in myeloid cells have not identified discrete sequences responsible for myeloid tissue specificity in transgenic mice. The human c-fps fes gene encodes a tyrosine kinase expressed in immature and mature myeloid cells and, when a 13-kb genomic fragment was used to make transgenic mice, RNA expression was demonstrated in bone marrow, bone marrow-derived macrophages, and spleen, although not directly in granulocytes. The human gp-91phox promoter, normally driving the gp91phox gene expressed in granulocytes, has also been used in transgenic mice, but unexpectedly yielded reporter gene expression only in monocytes and macrophages—not in granulocytes. Myeloid-specific genes in nonhuman species have also been studied. Tissue-specific expression in chicken macrophages has been extensively studied using the chicken lysozyme gene. These studies indicate that enhancer sequences located as far as 6.1 kb from the coding sequence are important for myeloid-specific expression of chicken lysozyme. Mouse MRP8 and MRP14 are calcium-binding proteins expressed in myeloid cells. The MRP8 promoter has been used to express bcl-2 in granulocytes of transgenic mice, but tissue specificity directed by this promoter was not well described. The evidence to date suggests that selective gene expression in myeloid cells is controlled by disparately located regulatory sequences.

Regulatory sequences that direct myeloid gene expression might be useful to express biologically important molecules selectively in myeloid cells of transgenic mice or to target genes to myeloid cells for retroviral gene therapy. Further study of the regulatory sequences controlling the expression of leukocyte integrin CD11b should provide insight into the molecular mechanisms of myeloid gene expression.

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REFERENCES


6. Kasaian MT, Ikematsu H, Casali P: Identification and analysis


21. Pytel R: Amino acid sequence of the murine Mac-1a chain reveals homology with the integrin family and an additional domain related to von Willebrand factor. EMBO J 7:1371, 1988


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