The hematopoietic cell-specific ets family transcription factor PU.1 regulates many lymphoid and myeloid genes. We have determined that PU.1 is critical for lineage-specific expression of the tyrosine phosphatase CD45. CD45 is expressed exclusively in hematopoietic cells at all stages of development, except for mature red cells and platelets. Although CD45 is normally expressed in all leukocyte lineages, it is critically regulated by PU.1 only in myeloid cells. Whereas myeloid cells from PU.1 null mice failed to express CD45, lymphoid cells were CD45⁺ by flow cytometry. Additionally, mRNA for CD45 was absent from PU.1-deficient myeloid cells. To understand the molecular basis for these observations, we characterized a transcriptional regulatory region of the murine CD45 gene containing exons 1a, 1b, and 2. Distinct transcriptional initiation sites for CD45 were demonstrated in T and B cells versus myeloid cells. A transcriptional initiation site in exon 1b (P1b) was principally utilized by myeloid cells. A PU.1 binding site was identified upstream of exon 1b by sequence analysis and DNA binding assays. Using this region of the CD45 locus we demonstrated that PU.1 directly transactivated reporter gene expression. Finally, retrovirus-mediated restoration of PU.1 expression to PU.1-deficient myeloid cells resulted in expression of cell surface CD45 and restored phosphatase activity, confirming the role of PU.1 in the positive regulation of this well known signaling molecule. We conclude that CD45 is regulated differentially in myeloid and lymphoid cells and that sequences critical to direct myeloid expression include a PU.1 binding site upstream of the P1b transcriptional initiation site.

The transmembrane tyrosine phosphatase CD45 is one of the most abundant hematopoietic cell surface proteins (1, 2). CD45 is found exclusively on hematopoietic cells, from primitive CD34⁺ cells through mature cells of all leukocyte lineages, except platelets and mature erythrocytes. Expression of different CD45 isoforms is generated by nontranslational means that include alternative usage of a subset of the 32 coding exons and by post-translational modifications. The pattern of isoform expression is highly regulated and varies according to cell type during differentiation and activation (for a review see Ref. 1).

CD45 function has been studied almost exclusively in terms of its role in signal transduction through the lymphoid antigen receptors. Engagement of these receptors results in activation of members of the Src family of tyrosine kinases (SFKs) (3, 4). The activity of these kinases has been shown to be dependent upon their phosphorylation state, and certain family members are regulated by CD45. Gene targeting of CD45 revealed its critical role in T cell development. T cells of CD45 null mice were arrested at the CD4⁺CD8⁻ stage without further maturation. Although the myeloid compartment of the CD45 null mouse initially appeared normal, subsequent studies of CD45 null macrophages have revealed defects in integrin-mediated adhesion. The CD45-deficient macrophages contained hyperphosphorylated SFKs Hck and Lyn, suggesting that these were CD45 substrates in myeloid cells (5). Thus, the regulation of integrin-mediated cell adhesion by CD45 via its regulation of SFK activity appears to be its best documented function in myeloid cells at present. Although it is not the only phosphatase present, the abundance of CD45 in myeloid cells implies its probable importance; in fact, CD45 accounts for 20% of total phosphatase activity in resting neutrophils (6).

Developmental and cell type-specific expression of CD45 is well established, but the transcriptional mechanisms conferring this regulation remain unclear. Studies in T and B cell lines have demonstrated that CD45 transcription can initiate at three mutually exclusive positions in exon 1a (P1a), exon 1b (P1b), and downstream of exon 1b (P2), but all use the same translational start site contained in exon 2 (7–9). A single report has identified a pyrimidine-rich nucleotide cluster just 5' of the P2 initiation site that possibly has a role in both basal and activator functions in B and T cells (10). Whether multiple transcription initiation sites reflect the capacity for lineage and specific cell type regulation and which if any additional DNA elements control transcription of CD45 is not known. We have now identified a required role for the Ets family transcription factor PU.1 in the regulation of CD45 in myeloid cells. PU.1 is a hematopoietic specific transcription factor found in primitive progenitors and in myeloid and B cells (11–13). The Ets family of proteins share a common DNA-binding domain that recognizes purine rich sequences, usually containing a 5'-GGAA-3' core (12, 14). We and others have shown that PU.1 is implicated in the regulation of multiple lymphoid- and myeloid-specific genes and the loss of PU.1 in mice results in a number of myeloid and B cell lineage defects (Refs. 15–18; reviewed in Ref. 14).

The abbreviations used are: SFK, Src family kinase; PCR, polymerase chain reaction; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.

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‡ To whom correspondence should be addressed: Deps. of Molecular and Experimental Medicine, MEM55, Scripps Research Inst., 10550 North Torrey Pines Rd., La Jolla, CA 92037. Tel.: 858-784-9123; Fax: 858-784-2121; E-mail: betorbet@scripps.edu.
In this report, we demonstrate that CD45 is transcriptionally regulated by PU.1 in a cell lineage-restricted manner. CD45 expression was detected in T but not myeloid cells obtained from PU.1 null mice. Differential transcriptional start site usage in T and B cells versus myeloid cells was found. Characterization of the region of the CD45 locus containing exons 1a, 1b, and 2 identified exon 1b as the principal CD45 transcriptional start site in myeloid cells. Moreover, our studies showed that PU.1 bound to a specific site upstream of exon 1b and could transactivate reporter gene expression. Finally, retrovirally mediated reintroduction of PU.1 in PU.1 null myeloid cells restored both CD45 expression and activity, confirming the essential role of PU.1 in the regulation of this important signaling molecule.

**EXPERIMENTAL PROCEDURES**

**Mice**—C57BL/6 × 129 PU.1 gene-disrupted mice were produced as previously reported (17).

**Cell Culture and Cell Line 503**—Hematopoietic cells from neonatal liver were isolated and cultured as described (15). Samples labeled "neonatal" were either heterozygous or homozygous for the normal PU.1 allele. The PU.1 null myeloid cell line 503 that was originally isolated from neonatal PU.1 null liver has been described (15, 16, 19). This cell line was utilized for previously described retroviral transduction studies in which PU.1 was restored (19).

**Antibodies and Flow Cytometry**—Cells were prepared and stained for flow cytometric analysis as previously described (17). A CD45 antibody recognizing all isoforms of the molecule was obtained from Pharmingen (clone 30-F11, Pharmingen, San Diego, CA). CD3, CD4, CD8, CD18, Gr-1, and irrelevant isotype control antibodies were obtained from Pharmingen. All flow cytometric analysis was performed using a FACScalibur (Becton-Dickinson, San Jose, CA) and CellQuest software (Becton-Dickinson).

**RNA Isolation and Reverse Transcriptase-PCR for CD45 Expression**—Total cellular RNA was prepared, and 0.5 or 1.0 μg was treated with DNase and subjected to reverse transcription and PCR as previously described (15). Primers used to detect the common 3′ end of CD45 have been previously reported (20). A control reaction for DNA amplification in the absence of reverse transcription was included for each sample and reaction.

**Isolation of B and T Cells from Spleen and Thymus**—Spleen and thymocytes were removed and processed from 6-week-old C57BL/6J mice as previously described (15). To isolate T lymphocytes from the splenocytes, the cells were stained with CD90 (Thy1.2) microbeads (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions, and the CD90-positive cells were isolated using a magnetic column. The remaining CD90-negative cells were then stained with CD19 microbeads (Miltenyi) in a similar fashion to isolate a pure population of B lymphocytes. Cell purity was measured by flow cytometry using B220, CD3, Thy1.2, CD8, and CD4, and the appropriate isotype control antibodies directly conjugated to R-phycoerythrin or fluorescein isothiocyanate (Pharmingen, San Diego, CA).

**CD45 Transcript Initiation Analysis**—cDNAs were synthesized using 5 μg of total RNA and a specific murine CD45 exon 2–3 junction primer (spanning a ~50-kilobase intron) as previously described (16). 1–5 μl of a 20-μl reverse transcription reaction was subjected to 25 cycles of PCR using the exon 2–3 primer and a 5′ primer hybridizing to sequences corresponding to previously described potential CD45 transcription initiation sites P1a, P1b, and P2 (10). Location and sequence of primers are shown in Fig. 4. One-fourth to one-third of the PCR reaction was analyzed by agarose gel electrophoresis with ethidium bromide. Controls for DNA contamination and nonspecific amplification contained template without reverse transcription and demonstrated no detectable bands.

**CD45 5′ Regulatory Region Cloning, Mutagenesis, and Transient Transfection Analyses**—A portion of the murine CD45 locus surrounding exons 1a, 1b, and 2 was amplified from T cell line EL-4 genomic DNA and cloned into the plasmid KS+ (Stratagene Inc., San Diego, CA) for sequence analysis. For PCR amplification the following primers were used: 5′ end, 5′-GTCGAGGATTCCTACTGAGCAGAGGAGAGTC-3′; and 3′ end, 5′-GTCGAGACCATGGCTATCTGAGCATC-3′. The CD45 5′ regulatory region was next cloned into the luciferase reporter plasmid pXPF2 (21) using the SalI and XhoI sites. Mutation of the core GGA residues in site 1 (shown in Fig. 4) was performed by overlap extension PCR, as previously described (22).

**RESULTS**

**CD45 Is Not Expressed by PU.1-deficient Myeloid Cells**—We have reported that freshly isolated liver or bone marrow from neonatal PU.1 null mice yields reduced numbers of hematopoietic progenitors and few detectable mature myeloid cells (17). However, when cells isolated from these sources are cultured in interleukin-3-containing medium for 5–14 days, chloroacetate esterase-, Gr-1−, CD18− polymorphonuclear cells (neutrophils) can be identified. Myeloid cells with the same cell surface profile begin to appear by days 3–5 in PU.1 null mice that are kept alive with antibiotics (15–17). Hematopoietic cells freshly isolated from PU.1 null neonatal liver were examined for the expression of the panleukocyte marker CD45 by flow cytometry using an antibody that recognizes all forms of murine CD45 (see “Experimental Procedures”). As might be expected based on the reported paucity of leukocytes, CD45 was not detectable (data not shown). Surprisingly, however, CD45 was not expressed by the myeloid cells cultured in vitro from PU.1 null neonatal liver (Fig. 1A). In contrast, these cells expressed the β2 integrin component CD18 (Fig. 1A), a well known marker of myeloid cells. To determine whether the absence of cell surface CD45 could be explained by the loss of gene transcription, PU.1-deficient myeloid cells were then analyzed for the presence of CD45 mRNA by reverse transcriptase-PCR. We used previously designed primers (20) that amplify a 3′ portion of CD45 sequence known to be present in all forms of the molecule (2). As shown in Fig. 1B, CD45 was undetectable in PU.1-deficient myeloid cells at the mRNA level as well.

**CD45 Transcription Is Differentially Regulated in Lymphoid and Myeloid Cells in PU.1 Null Mice**—We previously demonstrated that the CD45 isoform B220 is expressed on PU.1 null cells committed to B cell development. B lymphopoiesis in these mice is highly aberrant, does not progress beyond a very early stage, and generates few detectable cells in the PU.1 null mouse (17). Detectable thymic development in PU.1 null mice does not occur until 5–8 days after birth, at which time TCRβ+, CD3+, CD4, and CD8 double- and single-positive cells can
consistently be found in numbers about 5–10-fold less than normal littermates (Ref. 17 and Fig. 2). T cells from 11- and 12-day-old PU.1 null mice were analyzed by flow cytometry. As shown, we found that essentially all thymocytes were also CD45+ as well as CD3+ (Fig. 2). Thus, it appeared that CD45 transcription might be differentially regulated in a lineage-specific fashion, because this molecule was expressed by PU.1 null lymphoid cells but not myeloid cells. Intriguingly, although myeloid and B cells normally express PU.1, T cells have not been shown to express PU.1, suggesting that a distinct transcriptional mechanism for CD45 operates in this lineage. Moreover, although PU.1 appeared to be critical for CD45 expression in myeloid cells, it did not appear to be required for B cell CD45 expression.

Distinct CD45 Transcriptional Initiation Sites Are Utilized by Lymphoid Cells and Myeloid Cells—

Three distinct CD45 transcriptional initiation sites have been identified in the 5′-untranslated region of this gene and are designated P1a, P1b, and P2. P1a and P1b are located in exon 1a and 1b, respectively, and P2 is found just 5′ of exon 2 (Refs. 9 and 24 and shown in Fig. 3A). It has been shown by primer extension analysis and S1 nuclease mapping that usage of exons 1a and 1b is mutually exclusive and is the result of alternative initiation sites. A schematic drawing of the CD45 5′ regulatory region depicts the previously identified transcriptional initiation sites P1a, P1b, and P2. All transcripts utilize a single ATG in exon 2 for translational initiation. The intron between exons 2 and 3 is ~50 kilobases. B, cDNA was prepared using an exon 2–3 junction-spanning primer and subjected to PCR amplification using this primer and a specific 5′ primer designed to anneal to sequence in each of the initiation regions P1a, P1b, and P2. Predicted PCR products of 247, 235, and 180 base pairs indicating initiation from all three sites were seen in splenic CD19+ B cells and splenic Thy1.2+ and thymic T cells. In contrast, myeloid cells demonstrated initiation primarily from the P1b site with some P2 initiation as well. No PCR products were amplified from PU.1 null myeloid cells that do not express CD45. Controls containing template without reverse transcription were negative (lanes labeled No RT). The far right-hand lane (MW) contains a 100-base pair DNA ladder.

**FIG. 1.** CD45 is not expressed by PU.1-deficient myeloid cells. A, CD18+ myeloid cells cultured from the liver of neonatal PU.1 null mice fail to express CD45 as detected by a monoclonal antibody that recognizes all forms of the molecule. Ig controls (IgG) consisted of irrelevant isotype-matched antibodies. B, reverse transcriptase-PCR was performed using primers designed to amplify the 3′ portion of CD45 that is common to all isoforms. CD45 mRNA was not detectable in PU.1 null myeloid cells (PU.1 Null Myeloid) but was present in normal (homo- or heterozygous for normal PU.1 allele) myeloid cells (Normal Myeloid) cultured in an identical manner and in the T cell line BW5147. PCR amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was included to verify cDNA sample integrity and equivalence. Sizes of bands are given in base pairs (bp). Controls for DNA amplification in the absence of reverse transcription were negative (lanes labeled No RT). The far right-hand lane (MW) contains a 100-base pair DNA ladder.

**FIG. 2.** Thymocytes from PU.1 null mice express CD45. Thymocytes were isolated from 10–12-day-old PU.1 null mice and their normal (homo- or heterozygous for normal PU.1 allele) littermates and were analyzed as described under “Experimental Procedures.” CD45+ and CD8+ cells as well as CD3+CD45− cells were consistently present in the thymus of PU.1 null mice over 8 days of age. Flow cytometry data are presented from a representative PU.1 null and normal individual; at least 12 PU.1 null mice have been examined to date. Ig controls consisting of irrelevant isotype-matched antibodies were negative and are not shown.

**FIG. 3.** Distinct CD45 transcriptional initiation sites are utilized in lymphoid cells and myeloid cells. A, a schematic drawing of the CD45 5′ regulatory region depicts the previously identified transcriptional initiation sites P1a, P1b, and P2. All transcripts utilize a single ATG in exon 2 for translational initiation. The intron between exons 2 and 3 is ~50 kilobases. B, cDNA was prepared using an exon 2–3 junction-spanning primer and subjected to PCR amplification using this primer and a specific 5′ primer designed to anneal to sequence in each of the initiation regions P1a, P1b, and P2. Predicted PCR products of 247, 235, and 180 base pairs indicating initiation from all three sites were seen in splenic CD19+ B cells and splenic Thy1.2+ and thymic T cells. In contrast, myeloid cells demonstrated initiation primarily from the P1b site with some P2 initiation as well. No PCR products were amplified from PU.1 null myeloid cells that do not express CD45. Controls containing template without reverse transcription contained no bands (data not shown).
tion of transcription. However, all CD45 transcripts utilize the same ATG codon present in exon 2 to begin translation (9). Using a PCR-based assay previously described by DiMartino et al. (10), we identified CD45 transcriptional initiation sites in primary B cells, T cells, and myeloid cells. Thymic T cells derived from either wild type or PU.1 null mice as well as wild type splenic Thy1.2+ T cells demonstrated initiation from all three potential start sites (Fig. 3B). Similarly, wild type CD19+ splenic B cells were able to initiate transcription from P1a, P1b, and P2 (Fig. 3B). In contrast, wild type neutrophils and macrophages did not utilize the P1a site but did use the P1b and, inefficiently, the P2 site for initiation (Fig. 3B). As expected, PU.1 null myeloid cells demonstrated no PCR products, consistent with their failure to express CD45 (Fig. 3B). Thus, in contrast to the three potential start sites for transcriptional initiation that can be utilized by T and B cells, myeloid cells have only two defined start sites.

**PU.1 Binds Upstream of Exon 1b and Regulates Reporter Gene Expression**—To determine whether PU.1 directly regulated the murine CD45 gene, we cloned and analyzed the region of the CD45 genomic locus including exons 1a, 1b, and 2 (9, 24). Within the first 140 base pairs upstream of the P1b major murine transcriptional start site and downstream of the P1a transcriptional start site, we identified by sequence analysis two potential PU.1 binding sites, designated as sites 1 and 2 (Fig. 4A). Both sites contained the critical core residues, 5′-GGAAATG-3′ (12), and these potential PU.1 binding sequences are conserved between the murine and human CD45 genomic locus (24).

To determine whether PU.1 bound to either of these sites, oligonucleotides for each site were generated, and band shift analyses were performed using in vitro translated PU.1 as well as nuclear extracts from macrophages that express PU.1. These data showed that PU.1 had high binding affinity for site 1 (−124 to −107; Fig. 4A) and very weak affinity for site 2 (−68 to −51; Fig. 4A) (data not shown). To demonstrate specific binding to site 1, competition analyses were performed using site 1, mutant site 1, and site 2 oligonucleotides as cold competitors (Fig. 4B). As shown, the site 1 oligonucleotide efficiently competed with the labeled site 1 probe, but an oligonucleotide with a mutation of the core GGA residues of site 1 was unable to compete. Site 2 oligonucleotides were able to compete efficiently only at 500-fold molar excess, further confirming the lower affinity of PU.1 for this sequence as compared with site 1.

To further delineate the binding of PU.1 to site 1, we performed methylation protection analysis and observed that both core GG residues were protected from methylation (data not shown), which is consistent with our previous studies on how
Restoration of PU.1 expression in the PU.1-deficient myeloid cell line 503 restores CD45 expression and activity. PU.1 was reintroduced to PU.1-deficient 503 cells by retroviral transduction as previously described (503-PU). A, cell surface expression of CD45 protein was restored in 503-PU cells. The solid black area represents cells stained with irrelevant isotype-matched antibody, whereas the heavy black line represents staining with an CD45 monoclonal antibody. B, CD45 was immunoprecipitated from cells and its phosphatase activity was measured at 94 and 80% of wild type neutrophil activity level in two different PU.1-restored 503 samples (503-PU1 and 503-PU2). In contrast, CD45 activity in PU.1-deficient 503 cells (503) was 8% of wild type neutrophil activity.

PU.1 and other Ets domain proteins interact with DNA (12). Because we verified that PU.1 could bind to sites in the amplified CD45 5‘-untranslated region, we next tested whether PU.1 could directly regulate reporter gene activity through site 1. A wild type and site 1 mutated CD45 5‘ regulatory region were each cloned into the luciferase reporter gene plasmid, pXP2. HeLa cells, which express neither CD45 nor PU.1, were cotransfected with these amplified CD45 5‘ regulatory region plasmids with and without the PU.1 expression plasmid PU.1P6 (12). As shown, cotransfection of the wild type amplified CD45 5‘ regulatory region with PU.1 resulted in an 8–9-fold increase in luciferase activity (Fig. 4C). Cotransfection of the mutant CD45 reporter plasmid with PU.1 resulted in only a 2-fold increase over background (Fig. 4C). Thus, these studies demonstrate that PU.1 can bind to and transactivate through site 1 (−124 to −107; Fig. 4A), which is located upstream of the major point of transcriptional initiation for the CD45 gene in myeloid cells.

Restoration of PU.1 Is Sufficient to Restore Expression of CD45 in PU.1-deficient Myeloid Cells—To elucidate the role of PU.1 in CD45 expression and myeloid development, we reintroduced PU.1 by retroviral transduction into the PU.1 null myeloid cell line 503 (12, 15, 16). 503 is an interleukin-3-dependent PU.1 null neonatal liver-derived myeloid cell line that expresses Gr-1 (40–50%), chloroacetate esterase (>90%), and CD18 (80%). 503 cells have phenotypic and functional characteristics consistent with aberrantly or incompletely matured neutrophils (16). Similar to the myeloid cells that can be generated in short term interleukin-3 cultures of PU.1 null liver (Fig. 1), 503 cells do not express CD45 (Fig. 5A). However, in further support for the role of PU.1 in the regulation of CD45 transcription in myeloid cells, we observed both CD45 message (data not shown) and surface protein expression following PU.1 restoration to 503 cells (503-PU; Fig. 5A). Finally, phosphatase activity of immunoprecipitated CD45 was restored in two PU.1-restored 503 clones at 94 and 80% of normal neutrophil activity, whereas PU.1-deficient 503 cells exhibited only 8% of normal activity (Fig. 5B). Our combined results provide clear evidence that PU.1 is directly and critically involved in the expression of CD45 specifically in myeloid cells.

DISCUSSION

In this study we have investigated the role of PU.1 in regulating transcription of the transmembrane tyrosine phosphatase CD45 in myeloid and lymphoid lineages. Gene disruption of PU.1 in mice resulted in the loss of CD45 expression on myeloid cells but not lymphoid cells. Of the three transcriptional start sites initially described in T and B cells (8, 9, 10), termed P1a, P1b, and P2, myeloid lineages primarily utilized P1b, and to a lesser extent P2, for transcriptional initiation. We have identified two potential PU.1 binding sites 5‘ of the P1b and P2 CD45 transcriptional start sites, of which one appears critical for directing CD45 gene expression in myeloid cells. Moreover, mutation of the strongest PU.1 binding site in the CD45 5‘ regulatory region abolished transactivation of a luciferase reporter in transfection assays. These results indicate that the expression of CD45, which is ubiquitously expressed on hematopoietic cells, is differentially controlled in myeloid and lymphoid cells and demonstrate that PU.1 is necessary for CD45 expression in myeloid cells.

Lineage-specific mechanisms of transcriptional regulation of the same gene are rarely reported but not unique. Very recently, characterization of the promoter of the RAG2 gene has shown that different transcriptional factors are responsible for B cell-specific versus T cell-specific expression of this lineage- and developmental stage-specifically regulated gene. Whereas a similar upstream region was required for promoter activity in both B and T cells, mutations of this region affected transcriptional activity differentially. Furthermore, B cell-restricted BSAP was critical for RAG2 promoter activity in B lineage cells (25, 26). We found that freshly isolated primary B and T cells initiate transcription from three different sites in the CD45 genomic locus containing exons 1a, 1b, and 2, consistent with previous data from T and B cell tumor lines (10). However, in primary neutrophils and macrophages, only the P1b site and to a lesser extent the P2 site appear to be utilized for transcriptional initiation. To date, other regulatory elements in the CD45 genomic locus, such as TATA- and CCAAT-like sequences, have not been identified. A single report suggests that a pyrimidine-rich nucleotide cluster just 5‘ of the P2 initiation site might have a role in both basal and activator functions in B and T cells (10). Whether similar pyrimidine-rich sequences have a role for transcriptional initiation of the more prominent start sites located at P1a and P1b is unclear. Additionally, our results do not exclude the possibility that more distal regulatory elements exist that have not yet been described.

Our previous studies of PU.1 null mice have demonstrated that CD45 was expressed on cells committed to the B cell lineage (17). This observation might reflect the preferential use of the P1a start site in cells committed to the B cell lineage in the absence of PU.1. Alternatively, given our demonstration of the use of the P1b and P2 start sites by B cells, it is tempting to speculate that other Ets family members may be involved in CD45 expression in lymphoid cells. Spi-B is most closely related to PU.1, sharing a 67% amino acid homology in the DNA-binding domain. This protein is expressed in both T and B cells (27, 28), unlike PU.1, which is not detectably expressed in committed T cells (17). It has been documented that Spi-B null mice express CD45 (B220) and do not have T cell abnor-
PU.1 Is a Lineage-specific Regulator of CD45

malaries as do CD45 null mice (29, 30), suggesting that their expression of CD45 is normal (28). This outcome might be expected if both Spi-B and PU.1 could be used by B cells for CD45 expression. It is also possible that other PU.1-related Ets proteins may be responsible in these lineages, such as the recently described Spi-C, which is expressed in B cells (31, 32) or other as yet undiscovered PU.1-like Ets proteins. Alternatively, it may be that an entirely different class of transcription factors regulates CD45 expression in B and T cells, similar to the example of RAG2 gene regulation in B versus T cells cited above (25, 26). In this regard, it will be important to determine whether the PU.1 site(s) are critical for CD45 expression from P1b in B and T cells.

The role of CD45 in hematopoietic cells is best understood in T and B cell lineages. CD45 has been shown to regulate SFK-mediated antigen receptor signaling in T cells and B cells (reviewed in Refs. 4 and 33), and hyperphosphorylated SFKs Lck and Fyn were observed in T cells from CD45 null mice (34). It was previously believed that dephosphorylation of SFKs by CD45 was always a kinase-activating event that counterbalanced the phosphorylation-induced inactivation by Csk; however, this idea has recently been disputed (33). For example, macrophages derived from CD45-deficient mice contained hyperphosphorylated and hyperactivated Hck and Lyn, indicating that these SFKs were substrates that were normally negatively regulated by CD45 in myeloid cells. Defects in integrin-mediated adhesion, which is known to be critical for functions associated with normal innate immunity, were demonstrated in CD45-deficient macrophages (5). We have identified many functional deficits that exist in PU.1-deficient myeloid cells, including abnormal adherence and chemotaxis, phagocytosis, and killing of microbes (16). Exactly how the absence of CD45 relates to these abnormalities is not yet clear.

With this report, we have extended the list of PU.1-regulated genes beyond myeloid growth- and function-specific genes, such as CD11b, gp91phox, and myeloid CSF receptors, to include signal transducing molecules such as the tyrosine phosphatase CD45. Tyrosine kinases and phosphatases have been implicated in influencing cellular events ranging from proliferation to motility to generation of antimicrobial activity (reviewed in Refs. 35 and 36). We have documented direct transcriptional regulation of CD45 by PU.1, and significantly, this regulation occurs exclusively in myeloid cells. Understanding the means by which this lineage-restricted gene regulation is accomplished may provide critical insight into the mechanisms that control the defining events of lineage development and confer normal myeloid function.

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