The T-Lineage-affiliated CD2 Gene Lies within an Open Chromatin Environment in Acute Promyelocytic Leukemia Cells

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ABSTRACT

The nature of hemopoietic progenitors subject to leukemic transformation in acute myeloid leukemia (AML) has not been clearly defined. To address this issue, we have used DNase I hypersensitivity assays to study the chromatin structure surrounding the T-lineage-affiliated CD2 gene in the acute promyelocytic subtype of AML (APL). Upstream and downstream flanking regions of CD2 were found to be hypersensitive to DNase I in primary APL blasts, with an identical pattern of hypersensitive sites to those detected in cells of T-lineage. All of the sites were confirmed to be inaccessible to DNase I in B-lineage leukemia cells. The demonstration of T-cell-associated chromatin features in primary APL blasts has implications for the origin of APL that may arise in more primitive progenitors than previously considered to be the case.

INTRODUCTION

APL⁴ is one of the commonest forms of AML and is characterized by the reciprocal chromosomal translocation t(15;17) leading to the formation of the PML-RARα oncoprotein (reviewed in Ref. 1). This subtype of AML is of particular interest, being the first leukemia for which differentiation therapy, in the form of all-trans-retinoic acid, which directly targets the underlying molecular lesion, has been successfully used in clinical practice (1). The nature of the hemopoietic stem cells subject to leukemic transformation in AML has not been firmly established. Studies concerning engraftment characteristics of primary AML blasts in nonobese diabetic/severe combined immunodeficiency mice support the concept of a leukemic stem-cell pool that gives rise to leukemic blasts that retain a limited differentiation capacity (2). However, in contrast to the majority of cases of AML, blasts from patients with APL fail to engraft in NOD/SCID mice, which suggests that, in this particular subgroup, leukemic transformation may occur in committed myeloid progenitors (2). This is in agreement with the conclusions drawn from population-based mathematical modeling of APL development (3) and the finding that the PML-RARα fusion is detected within the CD34+CD38+ bone marrow subset and not within more primitive CD34+CD38– progenitors (4). Intriguingly, given that APL is generally considered one of the most differentiated subsets of AML, a proportion of cases have been reported to express the T-lymphoid affiliated glycoprotein CD2 at the cell surface (5–9).

Mechanisms underlying surface expression of lymphoid-affiliated antigens in AML are currently unknown. There has been a longstanding debate as to whether this phenomenon indicates that such leukemias represent expansions of rare stem cells that coexpress lymphoid and myeloid antigens (“lineage promiscuity”; Ref. 10) or alternatively arise in lineage-committed cells in which the presence of “inappropriate” surface markers reflects aberrant gene expression associated with leukemogenesis (“lineage infidelity”; Ref. 11). However, it is now apparent that there is greater plasticity in hemopoietic stem cells than previously envisaged (reviewed in Ref. 12), raising the possibility of further models that involve “de-differentiation” or “trans-differentiation” of progenitors after the acquisition of leukemogenic oncogenes before leukemic expansion. Such processes could entail reexpression of other lineage-associated genes.

Understanding the molecular basis of expression of lymphoid-affiliated genes in AML may provide insights into the progenitors subject to leukemic transformation. Previous studies of chromatin structure in leukemia have had a potential disadvantage in that they have been entirely restricted to established cell lines and, therefore, may not be fully representative of the regulation of lineage-affiliated genes during normal hemopoietic development or, indeed, in primary leukemic blasts. For these reasons it is critical to examine these phenomena in primary leukemias; therefore to serve as a model, we have studied long-range chromatin structure surrounding the CD2 gene by a DNase I hypersensitivity assay in APL.

In cells of T-cell lineage, hypersensitive sites have been identified in the 5’ and 3’ flanking regions of CD2 corresponding to key regulatory elements (see Fig. 1); whereas the CD2 locus is inaccessible to DNase I in cells of B-cell lineage and nonhemopoietic tissues (13–18). Hypersensitivity in the 3’ flanking region corresponds to the LCR and has been detected in lymphomyeloid progenitors (KG1 cells) and cells of T-lineage (13–15, 17). Functional activity of the LCR in overcoming repressive effects mediated by constitutive heterochromatin has recently been shown to be dependent on the integrity of a binding site for HP1 (19), which is implicated in chromatin remodeling and which also enhances activity of the promoter of the MPO gene (20). Two 5’ hypersensitive sites have been identified in the CD2 locus and have also been reported to be restricted cells of T-lineage. DHS 2 is located ~50 bp upstream from the transcription start site and relates to the minimal promoter (16). DHS 1 is situated 1.8 kb farther toward the 5’ end, related to a region that includes downstream silencer and upstream activator (derepressor) activities (18). In the present study, we demonstrate that the CD2 locus lies within an open chromatin domain in all of the APL cases examined, which suggests that this subtype of AML may arise in more primitive progenitors than previously considered to be the case.
MATERIALS AND METHODS

Characterization of Leukemic Samples. APL samples (n = 11) used for this study were derived from the United Kingdom Medical Research Council all-trans-retinoic acid trial; central morphological review was provided, as reported previously (21). Two morphological subgroups of APL are distinguished by FAB classification of AML (22, 23), namely the M3 and M3v forms. Nested reverse transcriptase-PCR to confirm the presence of the PML-RARα fusion gene and distinguish 5′ (ber 3) and 3′ (ber 1, ber 2) PML breakpoint patterns was performed as described previously (24). In addition, two cases of AML FAB type M1 and a case of B-lineage leukemia, B-PLL, were studied, after informed patient consent.

Immunophenotypic Analysis. Immunophenotyping was performed as described previously (25). All flow cytometry measurements were performed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA) using the Cell Quest software for data acquisition and analysis. A gate was set on the FSC/SSC plot to analyze data on the blast gate. Double labeling was performed using FITC-conjugated anti-CD2 (Caltag Laboratories, Burlingame, CA) in combination with phycoerythrin-conjugated antibodies against CD3 (T-lineage), CD34 (hemopoietic stem cell), CD117 (c-kit), B-lineage marker CD19 (Caltag; Coulter), or early myeloid markers CD13 and CD33 (Coulter, Hialeah, FL). The after combinations of antibodies were selected to establish CD2 expression in APL and other AML samples (CD2/CD34, CD2/CD13, CD2/CD33, and CD2/CD117), to determine the degree of T-cell contamination in clinical samples used for DNase I hypersensitivity assays (CD2/CD3), and to characterize B-lymphoid leukemias used as negative controls in this assay (CD2/CD19). CD2 positivity was defined on the basis of the antigen being detectable at the surface of at least 20% leukemic cells. Relative expression of CD2 on the cell surface of APL blasts, T-lymphoid cell lines, and normal T-lymphocytes was determined by fluorescence quantification using the Quantum Simply Cellular (QSC) Microbeads kit (Sigma, St. Louis, MO). This system converts the peak channel reading relating to positive cells into a measure of ABC, thereby providing an indication of the relative number of molecules expressed on the cell surface, as described previously (25, 26). For clinical samples and cell lines, at least 5000 events were acquired using identical instrumental settings for FL1 and FL2 to those used for microbead analysis.

RQ-PCR. CD2 and HBPI mRNA expression levels in cell lines, primary APL blasts, and normal peripheral T-cells were determined by RQ-PCR, using Taqman technology and an ABI 7700 (Applied Biosystems, Foster City, CA). Primer/probe sets to detect CD2 and HBPI cDNA were designed using the Primer Express software (Applied Biosystems), in accordance with the manufacturer’s instructions: CD2, forward primer, 5′-ACAGAGGTGGAGAATGAGTG-3′, and reverse primer, 5′-CAGGTGTTGAGGAGGATG-3′, and Probe, 5′-FAM-TCAACCTCCTCACGCACATCTCC-TAMRA-3′; and HBPI, forward primer, 5′-GAAGGCTGATAGAGGAAGATC-3′, and reverse primer, 5′-TACATTTTCTTCTGGGAAACAACCTTGA3′; and Probe: 5′FAM-CAGAACCATGCCCCTTTGTGAATGCGCA-TAMRA-3′.

Expression of CD2 and HBPI was compared with two different endogenous controls: ABL and β2-microglobulin (β2M), using published primers and probe sets (27) for which comparable expression has been documented between normal hemopoietic cells and acute and chronic leukemias (28). All of the RQ-PCR assays were confirmed to specifically amplify cDNA. Primer and probe concentrations were optimized according to the manufacturer’s protocol. PCR reactions were performed in a 25-μl volume, containing 2× PCR universal mastermix (Applied Biosystems), 300 nM primers, 200 nM probe, together with 100 ng of cDNA as template. cDNA synthesis and the thermocycling conditions for PCR were as described (29); water controls were included for both the reverse transcriptase and PCR steps. Reactions were performed in triplicate, and gene expression level was derived from the respective standard curve run in parallel. Plasmid standards for each target gene were generated and serially diluted to encompass the range of expression levels encountered in hemopoietic cells (29). To control for variations in RNA quality or quantity and efficiency of the reverse transcriptase step, expression of CD2 and HBPI were normalized for that of the control genes.

DNase I Hypersensitivity Assays. Assays were performed, based on the method of Wotton et al., (13) using a variety of hemopoietic cell lines, peripheral blood T-lymphocytes (cultured in the presence of 2 μg/ml PHA, 20 ng/ml IL-2 (courtesy of V. Morales, A. Lawrence, and D. Cantrell, Imperial Cancer Research Fund, London), and cells derived from patients with newly diagnosed (untreated) acute or chronic leukemia. Typically, 2–7 × 10⁶ cells were used for each assay. Blast percentages exceeded 90% of the mononuclear cell fraction in all of the primary leukemia cases examined (median, 98%; range, 91–99%). DNA was prepared from nuclei that were digested with a series of DNase I concentrations, including an untreated control; then it was digested with an appropriate restriction enzyme (BamHI for 5′ sites, DHS 1 and 2, and EcoRI for analysis of DHS 3). Positions of the cDNA probes, relative to the hypersensitive sites are shown in Fig. 1. Probe A, which detects a 9-kb genomic fragment containing the first and second exons, was used to identify 5′ hypersensitive sites; the presence of additional bands of 2.6 and 0.8 kb in digests of DNA prepared from DNase I-treated nuclei was indicative of the presence of DHS 1 and DHS 2, respectively. Probe B, which detects a 20-kb genomic fragment including the fourth and fifth exons, was used to identify DHS 3, which was indicated by an additional 3.6-kb band in digests of DNA prepared from DNase I-treated nuclei. DNA 3 corresponds to the strong tissue-specific enhancer in the region of the LCR (13–15). In samples lacking detectable hypersensitive sites, efficacy of DNase I and restriction enzyme digestion was confirmed by rehybridization of filters to probes L1 and da (gift from Prof. D. R. Higgs, Institute of Molecular Medicine, Oxford) designed to detect constitutive hypersensitive sites within the α-globin promoter and the upstream regulatory element, HS-40 (30).

RESULTS

Characterization of Leukemic Samples Subject to DNase I Hypersensitivity Assays. Among the 11 primary APL cases evaluated (10 M3v, 1 M3), immunophenotype analysis revealed that all of them expressed CD13 (median, 95%; range, 92–99%) and CD33 (median, 99%; range, 93–99%), whereas CD117 was detected in 4 of 10 that were tested (median, 90%; range, 57–97%). Surface expression of CD2 was detected in 5 of 11 cases, all with M3v morphology (Fig. 2; Table 1), of which 3 coexpressed CD34 and 1, CD19. CD2 expression was confirmed in PHA/IL-2-stimulated T-lymphocytes and the mature Jurkat T-cell line; whereas primary blasts from two patients with AML M1 and the remaining cell lines studied were CD2 negative (Table 1). Quantitation of the surface expression of CD2 by flow cytometry revealed comparable levels in the five positive APL cases (median ABC, 22 × 10⁹; range, 11–69 × 10⁹) to the Jurkat T-cell line (ABC, 66 × 10⁹) and unstimulated T-lymphocytes derived from five normal donors (median ABC, 56 × 10⁹; range, 4731

Fig. 1. Position of DNase I hypersensitive sites (DHS) relative to the genomic structure of the CD2 gene as defined in peripheral blood T-lymphocytes and Jurkat cells (13). Letters above the structural line: B, BamHI restriction sites; R, EcoRI restriction sites. Black boxes, exons. Gray boxes, the position of the cDNA probes A and B, used for hybridization to Southern blots, in relation to the genomic structure.

REGULATION OF CD2 EXPRESSION IN APL

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9–128 \times 10^3). However, a dramatic increase in surface expression of CD2 was detected in donor T-lymphocytes cultured in the presence of IL-2 and PHA (ABC, 1.3 \times 10^3), in accordance with a previous study (31).

RQ-PCR confirmed that detection of CD2 by flow cytometry in APL is correlated with higher mRNA expression (Table 1). High-level overexpression of HBP1 mRNA, and no correlation between CD2 and HBP1 mRNA expression was observed in the lymphoid and myeloid cell lines evaluated (Table 1).

**Table 1** Results of DNase I hypersensitivity assays at the CD2 locus in relation to CD2 expression status in primary leukemias, peripheral T-lymphocytes, and hematopoietic cell lines.

<table>
<thead>
<tr>
<th>Type of leukemia</th>
<th>CD2 status flow cytometry (％ positive blasts)</th>
<th>Normalized mRNA expression level (per 10^4 copies ABL)^a</th>
<th>5'</th>
<th>3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>AML M3v</td>
<td>+ (95%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P2</td>
<td>AML M3v</td>
<td>+ (89%)</td>
<td>3.89 \times 10^4</td>
<td>4.98 \times 10^4</td>
</tr>
<tr>
<td>P3</td>
<td>AML M3v</td>
<td>+ (92%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P4</td>
<td>AML M3v</td>
<td>+ (95%)</td>
<td>1.04 \times 10^5</td>
<td>4.59 \times 10^4</td>
</tr>
<tr>
<td>P5</td>
<td>AML M3v</td>
<td>+ (98%)</td>
<td>3.12 \times 10^4</td>
<td>8.03 \times 10^4</td>
</tr>
<tr>
<td>P6</td>
<td>AML M3v</td>
<td>− (2%)</td>
<td>1.53 \times 10^5</td>
<td>2.22 \times 10^5</td>
</tr>
<tr>
<td>P7</td>
<td>AML M3v</td>
<td>− (0.5%)</td>
<td>1.24 \times 10^4</td>
<td>1.13 \times 10^5</td>
</tr>
<tr>
<td>P8</td>
<td>AML M3v</td>
<td>− (2%)</td>
<td>1.94 \times 10^3</td>
<td>3.64 \times 10^4</td>
</tr>
<tr>
<td>P9</td>
<td>AML M3v</td>
<td>− (0%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P10</td>
<td>AML M3v</td>
<td>− (4%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P11</td>
<td>AML M3</td>
<td>− (2%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P12</td>
<td>AML M1</td>
<td>− (12%)</td>
<td>3.27 \times 10^5</td>
<td>1.68 \times 10^5</td>
</tr>
<tr>
<td>P13</td>
<td>AML M1</td>
<td>− (0%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P14</td>
<td>B-PLL</td>
<td>− (0.5%)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Jurkat T-cell</td>
<td>T-cell</td>
<td>+ (99%)</td>
<td>8.59 \times 10^6</td>
<td>1.01 \times 10^4</td>
</tr>
<tr>
<td>Peripheral T cells</td>
<td>T-cell</td>
<td>+ (81%)</td>
<td>9.18 \times 10^5</td>
<td>2.27 \times 10^5</td>
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<tr>
<td>NB4</td>
<td>AML M3</td>
<td>− (0%)</td>
<td>3.06 \times 10^5</td>
<td>2.74 \times 10^5</td>
</tr>
<tr>
<td>HL60</td>
<td>AML M2</td>
<td>− (0%)</td>
<td>3.97 \times 10^6</td>
<td>6.39 \times 10^4</td>
</tr>
<tr>
<td>HEL</td>
<td>Erythroleukemia</td>
<td>− (0%)</td>
<td>6.83 \times 10^5</td>
<td>1.29 \times 10^5</td>
</tr>
<tr>
<td>K562</td>
<td>CML</td>
<td>− (0%)</td>
<td>41</td>
<td>1.58 \times 10^5</td>
</tr>
<tr>
<td>U937</td>
<td>Promonocyte</td>
<td>− (0%)</td>
<td>1.62 \times 10^5</td>
<td>3.20 \times 10^5</td>
</tr>
<tr>
<td>HEL</td>
<td>Erythroleukemia</td>
<td>− (0%)</td>
<td>3.97 \times 10^6</td>
<td>4.65 \times 10^4</td>
</tr>
</tbody>
</table>

^a Relative expression levels of CD2 and HBP1 in primary patient samples, normal T cells and cell lines were comparable when data were normalized to β2M as the control gene rather than to ABL.

^b ++/−, the band associated with a particular hypersensitive site appeared weak.

^c Denotes the presence of an underlying bcr3 PML breakpoint; all remaining cases of APL were confirmed to have a bcr1 PML breakpoint.

^d ND, not determined; B-PLL, B-prolymphocytic leukemia; CML, chronic myeloid leukemia.
chromatin in T-lineage cells (Table 1; Fig. 3A), whereas 5' and 3' flanking regions were inaccessible to DNase I in Daudi cells (Table 1; Fig. 3C), in accordance with previous studies (13). Analysis of leukemic blasts derived from five patients with CD2-positive APL, revealed an identical pattern of hypersensitive sites to that detected in normal T-lymphocytes and Jurkat cells (Table 1; Fig. 3, E and F). Interestingly, all three sites were also detected in each of the six cases of APL studied that were found to lack surface expression of CD2.

Fig. 3. Detection of DNase I hypersensitive sites in the 5' and 3' flanking regions of the CD2 gene in CD2-positive and -negative cases of hypogranular variant APL. Hypersensitivity to DNase I was detected in the 5' (upper panels) and in the 3' (lower panels) flanking regions of the CD2 locus in CD2-positive normal peripheral T cells (A), in cases of CD2 positive APL [e.g., cases P2 (E) and P3 (F)], as well as in cases of CD2-negative APL [e.g., cases P8 (G) and P9 (H)] and CD2-negative myeloid cell lines such as U937 (B). In the analyses of the chromatin structure surrounding the 5' flanking region of the CD2 locus of peripheral T cells and APL patient P9 shown, the concentrations of DNase I favored the detection of DHS 2 over DHS 1. 5' (upper panels) and 3' (lower panels) flanking regions of CD2 were inaccessible to DNase I in cells of B-lineage: Daudi (C) and primary PLL cells (D). In each experiment, increasing amounts of DNase I are denoted by the triangle above the autoradiograph, with the restricted no-DNase-I control loaded to the left-hand lane (labeled 0). Arrowheads, bands indicating the presence of hypersensitive sites.
REGULATION OF CD2 EXPRESSION IN APL

In all of these cases, immunophenotype analysis of mononuclear cells used for DNase I hypersensitivity assays revealed that T-lymphocytes comprised <2%, such that it can be assumed that the hypersensitive sites that were detected were a true reflection of the chromatin structure surrounding CD2 in the leukemic population, rather than an effect caused by contaminating lymphocytes. This was confirmed by an analysis of a chronic B-cell neoplasm (PLL), revealing a closed chromatin pattern (Fig. 3C) identical to that detected in the Daudi cell line (Fig. 3C).

DNase I hypersensitivity assays were then extended to include primary cases of AML and a series of myeloid cell lines, which lacked CD2 expression by immunophenotype analysis (Table 1). Hypersensitivity in 5’ and 3’ flanking regions was detected in both of the primary AML M1 cases examined. Furthermore, DHS 1, 2, and 3 were present in NB4, HL60, and U937 cells (Fig. 3B), although bands associated with DHS 1 and DHS 2 were weak in NB4. In HEL, only DHS 3 and a weak band corresponding to DHS 2 were detected; whereas all of the regions of CD2 were found to be inaccessible to DNase I in K562 cells.

DISCUSSION

Characterization of the mechanisms by which expression of lineage-specific and lineage-restricted genes are coordinated during hematopoietic development may provide insights into the processes underlying leukemogenesis and the progenitors subject to leukemic transformation. Previous investigations of the expression of the T-lineage-associated glycoprotein CD2, have essentially focused on its regulation during lymphopoesis. However, in this study, which to our knowledge is the first to investigate chromatin structure in primary leukemic blasts, we have shown that CD2 lies within an open chromatin domain in the APL subtype of AML, with a pattern of hypersensitive sites that are identical to those detected in cells of T-lineage. Furthermore, upstream and downstream regions of CD2 were accessible to DNase I in both of the cases of CD2-negative AML analyzed, as well as in NB4 (APL), HL60 (derived from AML FAB M2), and U937 (promonocyte) cell lines, which all lack detectable (by flow cytometry) expression of CD2 at the cell surface. This was an unexpected finding, in the light of previous studies performed in transformed cell lines and normal peripheral T-lymphocytes in which the presence of upstream hypersensitive sites was strictly correlated with CD2 expression among cells of T-lineage (13). However, these original studies did not include an analysis of CD2 regulation in myeloid cells.

The presence of hypersensitivity in the 3’ regulatory region of CD2 has previously been correlated with T-lineage potential (13). This region contains a LCR that includes enhancer elements that together are able to confer high-level tissue-specific expression of a CD2 transgene, independent of the site of integration (14, 32). Hence, it was of interest to detect hypersensitivity in this region in myeloid leukemia cells and primary APL blasts, because a variety of experimental data have suggested that this latter form of leukemia arises from committed myeloid progenitors (2–4). Recently, evidence has emerged supporting a role for HBP1 in the functional integrity of the LCR, although other factors binding in this region may also be implicated (19). In the present study, we found no correlation between HBP1 mRNA expression and the presence of DHS 3. Furthermore, there was no relationship between HBP1 expression and that of CD2 mRNA or protein. Therefore, transcriptional regulation of HBP1 is unlikely to account for lineage-associated differences in CD2 expression, which may depend on tissue-specific factors interacting with 5’ and/or 3’ regulatory elements (17, 33).

There are currently no data regarding the chromatin configuration of lineage-affiliated genes in human bone marrow progenitors. However, DNase I hypersensitivity assays performed in multipotent, mouse bone-marrow progenitor cell lines have demonstrated that the regulatory regions of a number of genes the products of which have been considered lineage restricted, including β-globin, CD36, IgH, and MPO, lie within open chromatin before lineage commitment (34–36). Interestingly, the CD36 enhancer was found to remain accessible to DNase I after the differentiation of FDCP cells into mature granulocytes (34); the possibility that regulatory regions of the CD2 locus are accessible to DNase I in the same experimental system remains to be addressed. Single-cell reverse transcriptase-PCR approaches have also established that multiple lineage-affiliated gene products are coexpressed in multipotential hematopoetic progenitors derived from human or mouse bone marrow (37, 38). There are a number of ways in which our data can be interpreted. However, the identification of T-lineage chromatin features in APL, taken together with the experimental evidence suggesting that multiple lineage-affiliated genes are poised for transcription in early progenitors, is in accordance with the “lineage promiscuity” model to account for the inappropriate lineage-marker expression in acute leukemia (10). This hypothesis suggests that such leukemias originate in hematopoietic stem cell progenitors that coexpress various lineage-associated antigens (10). Further support for this view is derived from the identification of rare stem cells coexpressing CD2 and myeloid-associated antigens in normal marrow (39), and from studies correlating CD2 expression in APL with the presence of the B-lineage surface marker CD19 (7, 8). Taken together, these data would imply that APL may arise in more primitive progenitors than previously considered to be the case (2–4). Indeed, a recent study in which highly purified human bone marrow progenitors were transduced with retroviral vectors carrying the PML-RARα fusion lends support to such a concept, being consistent with the possibility that expression of the fusion protein in early progenitors could induce the APL phenotype (40).

An additional key question relates to the reason why approximately one-fourth of APL cases express detectable levels of cell-surface CD2, whereas the remainder do not (7). In this regard, it is of interest that CD2 expression is closely correlated with hypogranular variant morphology and expression of the stem cell marker CD34; this contrasts with the classical hypergranular form of APL, which typically expresses neither surface marker (6–9). This raises the possibility that these morphological subsets of APL, which are both characterized by the PML-RARα fusion, could arise from different hematopoietic progenitor populations. Therefore, the potential for CD2 expression in APL could be determined by the nature of the progenitor subject to leukemic transformation, particularly its stage along the myeloid differentiation pathway. According to this scheme, CD2+CD34+ APL variant cases would arise from the most primitive precursors, whereas CD2−CD34− classical cases are derived from the least primitive progenitors. If this is shown to be the case, it could reconcile contradictory data as to whether PML-RARα expression is detectable in multipotential progenitors (4, 41) and also account for differences in sensitivity of hypergranular M3 and M3v cases to ATRA in vitro (42). It would, therefore, be of interest to determine the chromatin environment surrounding the CD2 locus in a larger series of primary cases of hypergranular APL; however, this would be technically difficult because such cases typically present with pancytopenia, which limits the possibility of obtaining sufficient blasts to perform DNase I hypersensitivity assays. Nevertheless, in agreement with the findings in primary M3v, 5’ and 3’ flanking regions of CD2 were accessible to DNase I in the single case of primary classical M3 examined and in the NB4 cell line that was originally derived from a patient with hypergranular APL (43).

It would also be interesting to determine to what extent other epigenetic mechanisms, particularly subnuclear location and relationship to pericentric heterochromatin (44, 45), play a role in CD2 regulation in normal hemopoiesis in humans and whether this is disturbed during leukemogenesis. It is hoped that addressing these possibilities will not only establish mechanisms accounting for the detection of lymphoid markers...
protein that enhances myeloperoxidase (MPO) promoter activity. Leukemia (Balti-

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