Identification of a Differentiation-specific Cell Surface Antigen on HL60 Cells That Is Associated with Proliferation

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ABSTRACT

We have produced a murine IgM monoclonal antibody (Y201) that recognizes a cell surface antigen present on HL60 cells. Seventy percent of uninduced HL60 cells expressed Y201 antigen, while the remainder did not. There were no morphological differences between HL60 cells that expressed Y201 antigen and cells that did not express Y201 antigen. Cells with the greatest number of antigenic sites were found to have greater proliferative capacity in liquid culture and in soft agar than did HL60 cells deficient in this marker. Expression or lack of expression of the Y201 antigen is not constant over a prolonged period in that both subpopulations ultimately reproduced the original pattern of antigenic expression when grown in liquid culture.

The antigen identified by Y201 was lost with terminal differentiation of HL60 cells using a variety of inducers. Loss of Y201 antigen during differentiation was associated with a decrease in proliferative capacity in soft agar. Loss of Y201 antigen by greater than 95% of differentiated HL60 cells was associated with loss of proliferative capacity. These data suggest that HL60 cells are heterogeneous in regard to proliferative capacity and that this heterogeneity is associated with expression of the cell surface antigen identified by Y201.

INTRODUCTION

Normal hemopoiesis involves both proliferation (self-renewal) and differentiation (maturation). Proliferation is the controlled capacity for infinite cell divisions, and it provides a mechanism for continued maintenance of stem cells. Differentiation occurs among some of the progeny of stem cells resulting in functionally mature but non-proliferating cells. Differentiation and proliferation appear to be associated in normal hematopoiesis, since maturation is eventually associated with loss of regenerative capacity (1-3). In leukemia and possibly other cancers as well, these events are abnormal, and the linkage between proliferation and differentiation less clear. There is evidence that as cells differentiate there is a decrease in proliferative capacity (4-6). However, in some leukemic cell lines differentiation can occur in the complete absence of proliferation (7), indicating a dissociation between the two events. Proliferating leukemic cells appear to be blocked at a particular level of maturation (8). Despite the marked increase in proliferative capacity, it appears that only a portion of cells in a leukemic population has the ability for self-renewal (9).

Studies of differentiation-associated antigens have allowed scrutiny of the relationship between maturation and proliferation. Several studies have identified cell surface antigens present on progenitor cells but lost with maturation. For example, la-like antigens have been detected on human granulocyte/macrophage progenitor cells (10-14) and erythroid progenitor cells (15-17); the common acute lymphoblastic leukemia antigen appears at an early stage of normal lymphocyte development (18, 19), and the transferrin receptor, which is present on a variety of immature cells and lost with differentiation, is related to proliferation (20, 21). A correlation of antigenic changes with differentiation of progenitor cells is emerging. Surface antigens present on immature cells and lost with maturation may identify structures involved in regulating differentiation and/or proliferation.

In the present study, the promyelocytic human leukemia cell line HL60, which can be induced to differentiate with appropriate agents (22), was used as a model for investigating the antigenic and functional changes associated with differentiation. We have identified a cell surface antigen recognized by a monoclonal antibody (Y201) that is lost as cells differentiate and that is associated with loss of cellular proliferation.

MATERIALS AND METHODS

Cells and Cell Culture. HL60 (23), K562 (24), Molt-3 (25), and CCRF-CEM (26) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mm glutamine, 100 units of penicillin, and streptomycin (100 μg/ml). Normal human mononuclear cells were obtained from the peripheral blood of healthy donors and were separated by centrifugation on Ficoll/sodium diatrizoate (Sigma, St. Louis, MO) (27). Polymorphonuclear leukocytes were obtained from the erythrocyte layer of the same gradient by sedimentation of erythrocytes in 3% dextran and removal of residual erythrocytes by hypotonic lysis. Mixed leukocytes were obtained from peripheral blood and separated using 3% dextran sedimentation. Fetal mixed leukocytes were separated from cord blood obtained from healthy neonates using dextran sedimentation and hypotonic lysis of erythrocytes. Bone marrow cells were obtained from patients undergoing diagnostic bone marrow aspirations for purposes other than this study. The mononuclear cells from bone marrow aspirates were purified by Ficoll/sodium diatrizoate gradient centrifugation as described above.

Monoclonal Antibodies. BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were immunized with HL60 cells intraperitoneally and subcutaneously. Spleen cells from immunized mice were fused with the non-immunoglobulin-secreting, 8-saguanine-resistant myeloma cell line X53-Ag 8.633 (28) by the method originally outlined by Kohler and Milstein (29), with modifications according to Lerner (30). Fusion products were grown in hypoxanthine-aminopterin-thymine selective media (31), and the supernates were screened for antibody binding to fetal mixed leukocytes using an indirect enzyme-linked immunosorbent assay (Bethesda Research Laboratories, Gaithersburg, MD). Forty-six of 1500 clones produced antibodies that bound to cord blood leukocytes. These were further screened for binding to HL60 cells and mixed leukocytes from adults. Strong positive binding was considered an absorbancy of...
at least 21% times background on enzyme-linked immunosorbent assay. Seven clones were found to produce antibody which demonstrated strong binding to HL60 cells and weak or no binding to adult mixed leukocytes. One clone, Y201, was chosen for lack of binding to adult mixed leukocytes and was subcloned by limiting dilution (30). Experiments were conducted using antibody obtained from culture media of growing hybridomas or from ascites of BALB/c mice given injections of hybridomas. Antibody from ascites was titrated by immunofluorescence labeling. Determination of immunoglobulin subclass was done by Ouchterlony analysis (32) using subclass-specific rabbit anti-mouse immunoglobulin and Y201 antibody concentrated about 20 times from supernates of growing hybridoma cultures.

**Induction of Differentiation of HL60 Cells.** Cells were suspended at 0.5 x 10^6 per ml in culture medium containing an inducing agent and allowed to differentiate for 3–6 days. For induction of cells with characteristics of myeloid cells, the medium contained either 1.25% DMSO (22) (Fisher Scientific Co., Fairlawn, NJ), 10^-6 M RA (33) (Sigma) or 5 x 10^-4 M dbcAMP (34). For differentiation of HL60 cells with characteristics of monocytes/macrophages the medium contained 10^-4 M TPA (35).

**Differentiation was documented by examining changes in morphology (decreased size, increased nuclear lobulation), nitroblue tetrazolium reduction (36), and adherence to substrate (35). In addition, we examined expression of nonspecific esterase activity (37), appearance of formyl peptide chemotactic receptor (34, 38), and changes in membrane gangliosides (39, 40) by methods described previously.**

**Immunofluorescence Labeling and Cell Sorting.** The binding of Y201 to cells was assessed by indirect immunofluorescence. Cells were washed once with buffered saline containing 3% bovine serum albumin and 20% normal rabbit serum. The cells were then incubated in hybridoma culture media or ascites containing antibody for 20 min at 4°C. Unbound antibody was removed by washing twice in buffered saline. Tetrathymidylated DNA was isolated (Research Organizations Inc., Cleveland, OH) and used as substrate for the monoclonal antibody-treated cells. The mixture was incubated at 4°C for 20 min and then washed with buffered saline. Non-specific antibodies of the same subclass or culture supernatants from a non-immunoglobulin-secreting mouse myeloma cell line were used as controls.

In all experiments cells were directly observed (Zeiss microscope) using phase optics to examine morphology and fluorescence optics to evaluate antibody binding. Cells were also analyzed using a Becton-Dickinson FACS IV to quantify antibody binding (41). Positive fluorescence as assessed by FACS was defined as an intensity of fluorescence which exceeded 99% of the controls. To determine the relationship between antigen expression and proliferation, labeled HL60 cells were sorted under sterile conditions, and the separated subpopulations were regrown in liquid culture or assayed in a colony-forming assay as described below. The separated populations were also examined for differences in morphology following Giemsa-Wright staining.

**Clonal Growth.** The ability of HL60 cells to form colonies was assayed by culture in double layer semisolid agar using a modification of the method of Pike and Robinson (42). Briefly, the bottom layer consisted of 1 ml of McCoy's 5-A medium containing 20% fetal bovine serum and 0.5% agar (Bacto-agar, Difco, Detroit, MI). The overlayer contained 2 x 10^5 cells in 1 ml of McCoy's 5-A medium containing 20% fetal bovine serum and 0.5% agar. The cultures were incubated for 10–14 days. Discrete aggregates of 40 or more cells were scored as colonies using a Zeiss inverted microscope.

**Treatment with Y201 Antibody and Complement.** Complement-dependent cytotoxicity of Y201 antibody was tested using the assay for clonal growth as described above. HL60 cells were washed in buffered saline containing 3% bovine serum albumin and 20% normal rabbit serum, followed by incubation with monoclonal antibody at 4°C for 20 min. Fresh frozen human serum as a source of complement was then added to a final concentration of 50% for 40 min at 37°C. After incubation, the cells were washed twice in McCoy's 5-A medium and plated for clonal growth. Colony numbers in cultures treated with antibody and complement were compared to control cultures treated with medium alone, antibody alone, or complement alone.

**Correlation of Y201 Expression with Cell Cycle Analysis.** HL60 cells were separated by centrifugal elutriation, using a Beckman J-21, on the basis of velocity sedimentation for populations enriched in specific phases of the cell cycle (43). An unseparated control and subgroups chosen on the basis of cell size and cell number (43) were fixed in 95% ethanol, followed by indirect immunofluorescent labeling with Y201. These same populations were then examined for DNA distribution with mithramycin staining, followed by FACS analysis using a laser wavelength of 457 nm (44, 45).

**RESULTS**

**Induction of Myelocytic or Monocytic Differentiation.** Within 3 days after the addition of DMSO or RA to HL60 cells there was a slight increase in the number of myelocytes compared to uninduced cells. By the sixth day of incubation, cells treated with DMSO were predominantly at the myelocyte stage, with about 10% appearing as banded or segmented neutrophils, while cells treated with RA were more mature, with about 40% banded or segmented neutrophils. Most of the cells induced with dbcAMP did not morphologically mature beyond the metamyelocyte stage, but other evidence of maturation (decrease in cell size and active pseudopodia formation) was visible earlier than with the other myelocytic inducing agents. Adherence to glass, nitroblue tetrazolium dye reduction in response to opsonized yeast, and a decrease in cell size were apparent after 3 days of treatment with all three myelocytic inducers. Formyl peptide chemotactic receptor was detected on HL60 cells 3 days after the addition of DMSO but after only 24 h of dbcAMP induction.

TPA-treated HL60 cells showed morphological features of monocytes and macrophages that were maximum after 3 days of treatment. After 1 day of incubation with TPA the cells were adherent to each other and to the plastic culture flask, and scraping was required to detach them. By 3 days the cells stained strongly for nonspecific esterase. TPA-induced HL60 cells did not express formyl peptide chemotactic receptor at 3 days. TPA-induced HL60 cells but not DMSO-induced cells showed a 10-fold increase in Ga, gangliosides without alteration in other gangliosides. Only a small percentage of TPA-treated cells produced nitroblue tetrazolium even weakly in response to opsonized yeast.

**Characteristics of Y201 Antibody.** The Y201 antibody was shown to be of the IgM subclass in an Ouchterlony immunodiffusion assay and the antibody fixed complement. Treatment of HL60 cells with Y201 antibody and complement together resulted in 14–25% colony formation compared to cells incubated with complement alone. Antibody alone had no effect on colony formation.

Y201 antibody labeled most of the HL60 cells (Chart 1). K562 cells did not express the Y201 antigen, while Molt-3 and CCRF-CEM cells only weakly expressed the antigen (Table 1). Indirect immunofluorescence labeling and FACS analysis confirmed that Y201 did not bind to peripheral blood polymorphonuclear leukocytes or most of the peripheral blood mononuclear cells from adults. Only about 0.25% peripheral blood mononuclear cells
DIFFERENTIATION ANTIGEN ASSOCIATED WITH PROLIFERATION

Chart 1. FACS analysis of monoclonal antibody Y201 reactivity with uninduced HL60 cells. The vertical axis represents the number of cells, and the horizontal axis represents the fluorescence intensity with each subdivision corresponding to a 10-fold increase in fluorescence. The high intensity curve on the right represents uninduced HL60 cells exposed to Y201 and followed by tetramethylrhodamine isothiocyanate-conjugated goat anti-mouse immunoglobulin. The dotted lower intensity fluorescence curve on the left represents the control. The voltage setting on the fluorescence photomultiplier tube was 650 V in this experiment and in those reported in Charts 2 and 3. This setting remained relatively constant and was determined by the fluorescence of the cells treated with control antibody.

Table 1

<table>
<thead>
<tr>
<th>Cells</th>
<th>% Y201-positive*</th>
</tr>
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<tbody>
<tr>
<td>HL-60 (promyelocytic leukemia)</td>
<td>71 (62–85)*</td>
</tr>
<tr>
<td>K562 (erythromyeloid leukemia)</td>
<td>0</td>
</tr>
<tr>
<td>Molt-3 (acute T-cell lymphoblastic leukemia)</td>
<td>1.2 (0–2.3)</td>
</tr>
<tr>
<td>CCRF-CEM (T-cell lymphoblastoid)</td>
<td>2.3 (0–4.7)</td>
</tr>
<tr>
<td>Neutrophils (peripheral blood)</td>
<td>0</td>
</tr>
<tr>
<td>Mononuclear cells (peripheral blood)</td>
<td>0.25 (0–0.5)</td>
</tr>
<tr>
<td>Mononuclear cells (bone marrow)</td>
<td>2.5 (1–5)</td>
</tr>
</tbody>
</table>

*Percentage of Y201 antigen-positive cells.

and 1–5% mononuclear cells from seven bone marrow aspirations were strongly labeled by Y201 antibody (Table 1). The Y201 positive marrow cells did not appear to be promyelocytes. They were small cells with large nuclei and scanty eosinophilic cytoplasm when Giemsa stained after sorting by FACS.

Relationship of Proliferation and Expression of Y201 Antigen. Uninduced HL60 cells were separated into Y201 positive (most fluorescent 5% of population) and Y201 negative fractions (least fluorescent 5% of population) and were characterized for differences in proliferation. Controls were labeled HL60 cells that were processed through the FACS without separation into subpopulations. Y201 positive and negative HL60 cells were of equal size, had identical morphology and viability (trypan blue exclusion), and were indistinguishable from controls. The subpopulations and controls were assayed for colony formation in soft agar, while growth curves were obtained from cells placed in liquid culture. The Y201 positive fraction produced 3½ times more colonies than did the Y201 negative fraction. There was an increased number of colonies from the Y201 positive cells compared to that of the unseparated HL60 cells (298% at day 14, as shown in Table 2). The Y201 negative HL60 cells (298% at day 14, as shown in Table 2). The Y201 negative HL60 cells had a slight decrease in colony-forming ability compared to controls (85% at day 14).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>No. of colonies (14 days)</th>
</tr>
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<tbody>
<tr>
<td>Unseparated HL-60 cells</td>
<td>141</td>
</tr>
<tr>
<td>Y201-positive cells</td>
<td>420</td>
</tr>
<tr>
<td>Y201-negative cells</td>
<td>121</td>
</tr>
</tbody>
</table>

Chart 2. FACS analysis of the reactivity of Y201 antibody on subpopulations of HL60 cells. HL60 cells were initially separated by FACS into Y201 positive and negative fractions (5% most and least fluorescent). Two weeks later the subpopulations were relabeled with Y201 and re-analyzed with FACS. The curve on the right is the same subset of cells exposed only to control antibody. B shows Y201 reactivity with the fraction that 14 days previously had not expressed the Y201 antibody (right curve) as compared to control antibody.

Equal numbers of Y201 positive and Y201 negative cells were cultured in liquid media for 2 weeks. The doubling time of the Y201 positive culture was 30 h, which was identical to that of unseparated HL60 cells. HL60 cells that did not bind Y201 antibody had a slower initial growth rate, with a doubling time of 42 h. During prolonged culture the doubling time of the Y201 negative cells eventually decreased to 30 h. Two weeks after sorting the two populations were reevaluated for expression of the Y201 antigen, and both subpopulations were found to contain Y201 antigen positive and negative cells, with a distribution and intensity of labeling similar to unsorted HL60 cells (Chart 2). HL60 cells were elutriated into subgroups that were enriched for G1, S, and G2-M. There was no difference in the distribution of Y201 antigen between the different populations.

Changes in Y201 Antigen Expression during Differentiation. Differentiation of HL60 cells with all inducing agents examined resulted in loss of the Y201 antigen. The number of days required to reduce antigenic expression to less than 5% of the

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Differential Antigen Associated with Proliferation

Table 3
Reactivity of monoclonal antibody Y201 with differentiated HL60 cells

<table>
<thead>
<tr>
<th>Inducing agent</th>
<th>Day 3</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>23-24%</td>
<td>2-3</td>
</tr>
<tr>
<td>RA</td>
<td>3-4</td>
<td>1-3</td>
</tr>
<tr>
<td>dbcAMP</td>
<td>0.8-1</td>
<td>4</td>
</tr>
<tr>
<td>TPA</td>
<td>8-10</td>
<td>4</td>
</tr>
</tbody>
</table>

*Values represent ranges of four separate experiments.

Table 4
Effect of differentiation on colony formation of HL60 cells

<table>
<thead>
<tr>
<th>Inducing agent</th>
<th>No. of colonies</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1148</td>
<td>100%</td>
</tr>
<tr>
<td>3-day induction/DMSO</td>
<td>257</td>
<td>22.4%</td>
</tr>
<tr>
<td>6-day induction/DMSO</td>
<td>58</td>
<td>5.5%</td>
</tr>
<tr>
<td>3-day induction/TPA</td>
<td>63</td>
<td>5%</td>
</tr>
<tr>
<td>6-day induction/TPA</td>
<td>2</td>
<td>0.2%</td>
</tr>
<tr>
<td>3-day induction/dbcAMP</td>
<td>18</td>
<td>1.6%</td>
</tr>
<tr>
<td>3-day induction/RA</td>
<td>5</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

Some progenitor cell surface antigens change with differentiation (10-21). While such markers may identify structures involved in regulating maturation and/or proliferation, they are also useful if they correspond to a functional state of the cell with regard to proliferation. In our present study we have used a monoclonal antibody, Y201, to identify an antigen on HL60 cells which is lost in the process of differentiation and is associated with loss of proliferation.

Numerous cell surface antigens on HL60 cells are decreased but not completely lost with differentiation (46-49). We have identified an antigen which is undetectable on almost all terminally differentiated HL60 cells. Transferrin receptor is also lost with differentiation of HL60 cells (20, 21). The Y201 antigen is different from the transferrin receptor in that the transferrin receptor is present on most cultured human hematopoietic tumor cell lines, including Molt-3, CEM, and K562 (20, 21), while the Y201 antigen is not expressed on these cells. While we and others have been able to immunoprecipitate transferrin receptor from radiolabeled HL60 cells with OKT9 (21), Y201 does not immunoprecipitate any material under the same conditions. Furthermore, Y201 does not identify any bands after Western blot of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of HL60 cell membranes using conditions used by us previously for other studies (50).

Recently two antigens, identified by monoclonal antibodies, that are lost from the surface of HL60 cells differentiated to monocytic cells have been described (51). Six normal bone marrow cell preparations were examined, and it was found that one antibody reacted with 25 to 40% of the bone marrow cells and the other reacted with 15 to 25%. This is unlike the antigen identified by Y201, which is expressed on only 1 to 5% of mononuclear cells from bone marrow (Table 1).

The antigen identified by Y201 antibody is not present on all uninduced HL60 cells (Chart 1). This heterogeneous binding of Y201 to uninduced HL60 cells is confirmed by cytotoxic assays involving complement binding. Cells incubated with Y201 antibody and complement formed 14-25% of the number of colonies formed from cells incubated with complement alone. This corresponds to the approximate percentage of HL60 cells not expressing the Y201 antigen (Table 1).

Our data, like that of other investigators (52), indicate that there is heterogeneity of clonogenic potential among HL60 cells. This heterogeneity is associated with the heterogeneity of expression of the Y201 antigen. Since cells which are initially deficient or rich in Y201 antigen both reproduce the antigenic spectrum and growth pattern of the parent HL60 population after a period of growth in liquid medium (Chart 2), the heterogeneity is not due to distinct clonal subsets of HL60 cells. The distribution of Y201 antigen among uninduced HL60 cells does not appear to be due to the stage of the cell cycle, nor does it correlate with visible evidence of differentiation.
Since HL60 cells that are rich in Y201 antigen have increased clonogenic potential (Table 2) yet are capable of giving rise to Y201 negative cells (Chart 2), it is possible to speculate that highly proliferative cells (Y201 positive) may enter a state in which they are less proliferative (Y201 negative). HL60 cell populations in exponential phase of growth maintain a constant distribution of Y201 antigen-rich and Y201 antigen-deficient cells (Chart 1). It is likely that in the absence of an inducing agent the transition from Y201 antigen rich to Y201 antigen poor may be thought of as an early, reversible step toward differentiation. With continued differentiation in the presence of an inducing agent the process becomes irreversible, and at some point cellular ability for dedifferentiation, proliferation, and antigenic expression of Y201 is permanently lost (Chart 3, Table 3).

In our studies we examined the pattern of Y201 binding on HL60 cells during induction of differentiation with various chemical inducers. Terminal differentiation was measured by loss of clonogenic potential in soft-gel agar, as well as functional and morphological evidence of differentiation. We found differences dependent upon the agent used in the rate of loss of Y201 antigen (Chart 3) and the timing of loss of proliferative capacity (Table 4).

Loss of clonogenic capacity of induced HL60 cells correlated with loss of Y201 antigen with all inducing agents examined. At day 3 of induction with DMSO, two distinct population peaks, Y201 positive (23.5%) and Y201 negative (66.5%), were evident by FACS analysis. At this time the DMSO-induced HL60 cells had diminished proliferative capacity (22.4% of control; see Table 4). At 6 days of induction only 3.5% of the induced HL60 cells express Y201 antigen, and proliferative capacity was 5% of control uninduced HL60 cells. This indicates that during induction with DMSO prior to terminal differentiation proliferative capacity decreases parallel to a decrease in Y201 antigen expression.

At 3 days of TPA induction Y201 antigen continued to be expressed by 8–10% of the induced HL60 cells (Table 3), and clonogenicity was 8% of control uninduced HL60 cells (Table 4). Both the expression of Y201 antigen and proliferative ability were substantially decreased by 6 days of treatment. RA and dbcAMP both resulted in marked loss of Y201 antigen and clonogenic capacity after 3 days of treatment. With all inducers studied, complete loss of proliferative capacity did not occur until there was loss of Y201 antigen by greater than 95% of cells in the induced population. Thus, Y201 antigen loss appears to correspond closely to loss of proliferative capacity.

Some investigators have examined the clonal ability of HL60 cells grown in soft-gel agar cultures containing the inducing agent (52–54). We exposed HL60 cells to inducing agents in liquid medium for a period of time and then cloned the cells in soft-gel agar without inducing agent in order to determine the number of cells which retain proliferative capacity (Table 4). This allowed an evaluation of the period of exposure to an inducing agent necessary to eliminate the capacity for self-renewal. Fibach et al. (55), using a similar method, reported that up to 18 h of incubation with 1.5% DMSO in liquid medium followed by cloning in 0.83% (w/v) methylcellulose resulted in no reduction of cloning capacity but that after 24 h cloning capacity is reduced to 30% of controls. He concluded that the loss of self-renewal is one of the first properties to be altered irreversibly during differentiation. Our data suggest that complete loss of colony-forming capacity does not occur until functional and morphological differentiation is already evident in most of the cells.

It would seem paradoxical that Y201 antibody, which was made against and binds tightly to the human promyelocytic cell line HL60, does not bind to promyelocytes in normal bone marrow. However, the antigen appears to be expressed on cells with two characteristics: (a) cells that are increased in proliferative capacity; and (b) cells that are blocked at a stage prior to terminal differentiation. Both of these characteristics are present in cells of the HL60 cell line, while normal promyelocytes have lost self-renewal capacity and are not blocked in the progression to terminal maturation.

Y201 antibody provides a marker for terminal differentiation, as defined by loss of colony-forming ability in HL60 cells induced with a variety of agents. The suggestion that the human acute myeloid leukemias may involve a block in differentiation (8) has led to the screening of a variety of potential agents. Markers, such as the Y201 antigen, are becoming increasingly important in this screening process.

Biochemical analysis of membrane changes in HL60 cells undergoing differentiation (56) suggests that such changes may be related to the process of maturation and loss of self renewal. The Y201 antigen may provide an additional probe to analyze the molecular events occurring at the cell surface membrane in the process of differentiation. In addition, the small subset of Y201-positive mononuclear cells present in peripheral blood and bone marrow may be an important subset in terms of proliferation. Further work to identify the biochemical nature of the antigen identified by Y201 and the subset of Y201-positive cells from bone marrow is currently under way.

REFERENCES

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