Expression of Nucleolar Antigen p145 in Bone Marrow Cells of Patients with Myeloid Leukemias

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ABSTRACT

The expression of a cell cycle-related nucleolar protein (p145) antigen was examined in the bone marrow aspirates of 45 individuals, three of whom had no malignant disease; 30 had a diagnosis of acute myeloid leukemia (AML), and 12 suffered from chronic myeloid leukemia (CML). While no evidence of p145 expression was found in the three normal bone marrow samples, it was noted to be the highest in patients with active leukemia, be they AML or blastic crisis of CML. There was a direct correlation between the percentage of blasts and the percentage of p145-positive cells in all patients. Double labeling with tritiated thymidine and p145 in AML patients with active leukemia showed that the majority of S-phase cells contained p145. Myeloblasts in both chronic phase and blastic crisis of CML expressed p145. Nineteen of 21 AML patients studied during remission had less than 5% p145-positive cells, but three showed 10%, 16%, and 33% positive cells. Since functionally/morphologically, these marrow were normal, the appearance of p145 may indicate a proliferative abnormality preceding maturation arrest and development of relapse. Thus we conclude that p145 is more commonly associated with immature cells and may serve as an early indicator of relapse in AML, but requires further study with larger numbers of patients.

INTRODUCTION

The expression of several nucleolar antigens in malignant cells has been reported in the past (1, 2). One of these nucleolar proteins p145 has been associated with cell proliferation (3). In a previously reported study, p145 was clearly expressed in the proliferating HL60 cells. Its presence, however, could not be detected in cells that were induced to differentiate by retinoic acid stimulation (4). These data suggest a possible use of p145 as a marker of actively cycling cells. Although the importance of measuring the percentage of cycling cells in AML has been clear for many years, this has been impossible to do. In the past, estimation of the "growth fraction" or the percentage of cycling cells in AML was accomplished by continuous i.v. infusions of [3H]dThd for longer than a week (5, 6) and determining the percentage of labeled cells at the end of this period. Besides the many obvious problems of using radioisotopes in vivo, this method cannot differentiate between a cell which is cycling very slowly and one which is truly kinetically quiescent. For example, Clarkson et al. demonstrated that 88 to 93% of cells were labeled with [3H]dThd in AML patients at the end of 8- to 10-day infusions, but whether 7 to 12% of cells were out of cycle or were cycling slower than once every 10 days could not be demonstrated (6). Thus, a continuous infusion of [3H]dThd cannot be used to measure the growth fraction.

Recently, several proteins have been identified which are specific for cycling or quiescent cells. Antibodies to detect such proteins have been produced with the hope that these studies will provide an accurate estimation of the growth fraction in human cancers. These proteins and their antibodies include cyclin (7), statin (8), ki-67 (9), SC2 (10), PAA (11), p145 (4), the HMTNA (12), and the proliferating cell nuclear antigen (13). While statin is specific for cells that have gone out of cycle, all other proteins are cycle specific. Most of the proteins are present in both normal and malignant cycling cells. HMTNA, on the other hand, was felt to be expressed only in cycling malignant cells and not in normal cells (14). Polyclonal rabbit antibodies were used to detect the presence of HMTNA in the past, but now monoclonal antibodies have been developed against several nucleolar proteins with HMTNA-like properties. p145 is one such nucleolar protein antigen.

We have examined the pattern of p145 expression in bone marrow cells obtained from 42 patients with myeloid leukemias and 3 normal individuals in order to address two questions. (a) Is the expression of p145 limited to proliferating cells, and (b) is p145 only detectable in malignant cells? This paper describes our observations on the expression of p145 in cells of patients with acute or chronic myeloid leukemias and the clinical implications of these findings.

MATERIALS AND METHODS

Forty-five individuals are the subject of this report, 42 patients with myeloid leukemias and 3 individuals who did not have any malignant disease but whose "normal" bone marrows were obtained as a control group. Thirty patients had a diagnosis of AML, while 12 patients suffered from CML. Of the 30 AML patients, 6 were studied at the time of initial diagnosis, 12 during CR of their disease, and 12 at the time of relapse or with refractory disease. Of the 12 CML cases, 9 were studied during chronic phase, 2 during blastic crisis, and one during the accelerated phase of CML. BM samples were aspirated into a plastic syringe containing 2 ml of 6% sodium citrate. The specimens were then layered on Ficoll-Hypaque (specific gravity, 1.077) and centrifuged at 1200 x g for 30 min. The light density cells were recovered, washed in PBS, and placed on Alcian blue-coated coverslips at a concentration of 2 x 10^6 cells/ml.

Expression of p145 Detected by Single Labeling. The detection of the nucleolar antigen p145 was accomplished by a triple sandwich technique previously described by Freeman et al. (4). After the cells were placed on coverslips, they were fixed for 20 min with 2% paraformaldehyde (methanol free) and washed twice for 10 min in PBS. The cells were then permeabilized in acetone at -20°C for 5 min and washed twice for 10 min in PBS again. The monoclonal antibody specific for p145 was diluted in PBS at a 1:300 dilution, and 140 μl were added to each coverslip and incubated at 37°C in 5% CO2 for 1 h. The coverslips were then washed 3 times for 15 min in PBS, and rabbit anti-mouse immunoglobulin (Dako Corporation) diluted 1 to 100 in PBS-Nonidet P40 was added to the slides and incubated at 37°C in 5% CO2 for 20 min. The coverslips were washed for 5 min in PBS, and 140 μl of FITC-conjugated goat anti-rabbit IgG (Tago) diluted 1 to 20 in PBS were
added to each coverslip and incubated at 37°C in 5% CO₂ for 20 min to detect the rabbit antibody. The coverslips were washed twice for 5 min in PBS, rinsed with distilled water, and mounted with Fluoromount-G (Fisher Scientific). The slides were then examined under a fluorescence microscope. Nucleoli that expressed p145 were identified as being positively labeled if there was clear fluorescence labeling detected. The percentage of cells containing p145 was thus calculated. We have also used immunoperoxidase-labeled secondary antibody with the ABC enhancement to obtain slides which show brown staining overlying the nucleoli that express p145. This ABC technique allows us to examine the slides under a light microscope. It must be remembered that two types of control samples were processed simultaneously. (a) All the steps of the procedure described above were followed precisely in control samples, except no monoclonal antibody was used. These slides were simultaneously assessed with the experimental slides containing the monoclonal antibody. All such control slides were negative. (b) Initial studies used a monoclonal antibody against phthalate. In every instance, both normal and leukemic cells failed to bind this antibody, thereby confirming the specificity of the p145 antibody.

In addition, we wanted to identify the number of cells containing prominent nucleoli by methods other than morphology, since p145 is expressed only in nucleoli. For this purpose we used the monoclonal antibody B23 described earlier (15) which binds to nucleoli of all cells be they cycling or quiescent. By following the exact same procedure described above in using the monoclonal antibody B23, we were therefore able to precisely estimate the percentage of cells containing prominent nucleoli.

### Detection of p145 Expression in S-Phase versus Non-S-Phase Cells by the Double-Labeling Technique

We also combined the immunofluorescence technique of detecting p145 with autoradiography (16) to achieve a double-labeling of cells in the following way. After separation of Ficoll-Hypaque, 5 x 10⁶ cells/ml were suspended in 2 ml of RPMI 1640 and 10% fetal calf serum. Next, 10 µl/ml of [³H]dThd (stock solution, 1 mCi/ml; specific activity, 60 to 90 Ci/mmol; ICN Chemical and Radioisotope Division, Irvine, CA) were added to the suspension and incubated for 1 h at 37°C in 5% CO₂. After incubation was completed, cells were washed 3 times in nonlabeled thymidine and once in PBS. The cells were resuspended in 1 ml of PBS, and 3 to 4 drops of this suspension were placed on Alcan blue-coated coverslips as described before. The cells were fixed in 2% paraformaldehyde and processed by the monoclonal antibody to detect p145 expression using FITC-labeled secondary antibody. Following air drying, the coverslips were coated with NTB 2 (Eastman Kodak, Rochester, NY) nuclear track emulsion and exposed in the dark for 24 h at ~70°C. Kodak D-19 developer was used, and after fixation, coverslips were mounted onto glass slides with Fluoromount-G and examined under a fluorescence microscope. A cell was considered positively labeled for p145 if the nucleoli demonstrated immunofluorescence. The presence of at least 5 silver grains detected over the cell indicated [³H]dThd incorporation in that cell. Double-labeled cells demonstrated both fluorescent nucleoli as well as at least 5 black grains overlying the nucleus. At least 500 cells were counted from each slide to determine the percentage of cells expressing p145 and those in S phase as determined autoradiographically.

### Determination of p145 and B23 Expression in “Normal” Bone Marrows by the Double-Labeling Technique

Bone marrow aspirates were obtained from three individuals undergoing surgery for other reasons who did not have any malignant disorder. Following the separation of mononuclear cells on Ficoll Hypaque, these specimens were studied for p145 and B23 expression by the double-labeling technique as described above.

### Determination of p145 Expression of Immature versus Mature Myeloid Cells

In order to examine whether any immature myeloid cells expressed p145, we identified two patients in the chronic phase of CML and separated their peripheral blood cells into two fractions: an immature, blast-enriched fraction and a blast-depleted fraction containing primarily mature myeloid cells. The method used to separate the two fractions has been described in the past (17). The separation procedure was considered effective because the light density blast-enriched fraction contained at least 3 times as many immature cells as the higher density cell population containing the blast-depleted fraction. Expression of p145 and B23 was assessed in each fraction separately.

### Statistics

The relationship between measurements (and measurements expressed as ranks) was assessed by calculation of the Pearson product-moment correlation for pairs of variables (SPSS/PC* for the IBM PC/XT/AT; SPSS, Inc., Michigan).

## RESULTS

Expression of p145 in Patients with AML. Fig. 1A shows a bone marrow sample from an AML patient processed by the immunofluorescence technique to detect the expression of p145. The bright nucleolar fluorescence in the majority of blasts can be clearly seen overlying the cells. Fig. 1B demonstrates double labeling where an S-phase cell (marked by the presence of black grains) clearly shows expression of p145 along with a number of non-S-phase cells also expressing nucleolar p145 reaction. Thirty patients who had a diagnosis of AML were studied for the expression of p145 in their bone marrow cells. These data are presented in Table 1 and Fig. 2. There were 6 previously untreated patients with AML who had a mean blast count of 48% in the BM aspirate. Among these 6 patients, the mean percentage of p145 expression was 67%, while the range is graphically displayed in Fig. 2. Only one patient in this group had less than 60% p145-positive cells (14%). She had MDS-t with 25.6% blasts and 15.8% promyelocytes in the BM. Twelve AML patients were studied during CR, and Fig. 2 graphically demonstrates the distribution of p145-positive cells in these individuals. Nine patients had less than 5% cells that expressed any p145, while three patients had 11%, 16%, and 33% of cells positive for p145. The first of the last three patients relapsed within a few weeks of this examination. The patient with 33% p145-positive cells received a course of consolidation therapy immediately after this examination. Therefore the significance of this rather high percentage of p145-positive cells during CR cannot be clearly determined. The third patient with a high p145 expression during CR has been found to have the same clonal abnormality by cytogenetic study during CR that she had at the time of diagnosis of AML. At the present time this patient’s marrow is hypercellular but contains less than 5% blasts.

Thus it seems that, during remission, most patients do not have more than 5% of cells expressing p145 in their bone marrows in parallel with less than 5% of blasts by morphology. In the 12 patients who were studied either during relapse of leukemia or those with refractory leukemia, it can be seen from Fig. 2 that there is a wider distribution of p145 expression in these individuals, with 0 to 70% of cells in the bone marrow being positive. It is important to note that the three patients with relapsed or refractory AML whose BM showed less than 10% p145-positive cells all had clinically nonprogressive disease. On the other hand, individuals with higher values of p145-positive cells had progressive disease that was almost directly proportional to the percentage of p145 cells; i.e., the higher the percentage, the more rapidly progressive was their leukemia. These observations suggest that a close relationship exists between the expression of p145 antigen by leukemic cells and leukemic cell proliferation.

Expression of p145 in Patients with CML. Fig. 3 demonstrates the distribution of p145 expression in 9 patients studied during the chronic phase of CML, 2 patients studied during blastic crisis, and one patient with accelerated disease. During the chronic phase, the expression of p145 is very low, all nine individuals demonstrating less than 10% p145 positive cells, while during blastic crisis, both patients have higher values...
Fig. 1. A, bone marrow sample from an AML patient processed by the immunofluorescence technique to detect p145 expression. Bright nucleolar fluorescence can be seen overlying the cells. B, double labeling where an S-phase cell (marked by the presence of black grains) clearly shows nucleolar expression of p145 along with a number of non-S-phase p145-positive blasts.

### Table 1 Expression of p145 in AML and CML patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Status</th>
<th>No. of patients</th>
<th>Mean % of blasts in BM</th>
<th>Mean % of B23 in BM</th>
<th>Mean % of p145 in BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML Diagnosis</td>
<td>CR</td>
<td>12</td>
<td>47 (14-91)</td>
<td>67 (14-90)</td>
<td>75 (46-95)</td>
</tr>
<tr>
<td></td>
<td>Relapsed or refractory</td>
<td>12</td>
<td>54 (12-98)</td>
<td>68 (11-100)</td>
<td>67 (14-90)</td>
</tr>
<tr>
<td>CML Chronic phase</td>
<td></td>
<td>9</td>
<td>45 (12-78)</td>
<td>68 (62-74)</td>
<td>45 (32-58)</td>
</tr>
<tr>
<td></td>
<td>Blastic crisis</td>
<td>2</td>
<td>74 (62-85)</td>
<td>68 (62-74)</td>
<td>45 (32-58)</td>
</tr>
<tr>
<td></td>
<td>Accelerated phase</td>
<td>1</td>
<td>13</td>
<td>58</td>
<td>6</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.

similar to AML patients at diagnosis. Table 1 shows the values of mean percentage of blasts as well as the percentage of B23-positive cells.

Double Labeling for p145 and [3H]dThd. In order to compare the expression of p145 in cells actively synthesizing DNA with non-S-phase cells, double labeling with [3H]dThd and the monoclonal antibody to detect p145 was performed in cells obtained from several leukemic patients. We found that most leukemic cells which incorporate [3H]dThd also react with the antibody to p145, thus demonstrating that, at least in the acute leukemic patient with active disease, p145 is consistently expressed during the DNA synthesis phase (Fig. 1B). In these slides, we noticed no difference in the intensity of labeling for p145 in S-phase versus non-S-phase cells. On the other hand, when studied during complete remission the double-labeled technique revealed that only few S-phase cells express p145. In summary therefore, the majority of S-phase leukemic cells were associated with p145 expression, but in patients studied during CR, many S-phase cells did not react with the antibody to p145.

p145 Expression in Immature versus Mature Cells. Peripheral blood cells from 2 patients in the chronic phase of CML were separated into blast-enriched immature and blast-depleted, mature fractions. Expression of p145 and B23 was assessed in each fraction separately. Fig. 4 shows a Wright-Giemsa stain of the two fractions, demonstrating a successful separation with the majority of cells in the high density fraction being primarily maturing myeloid cells (Fig. 4B) and the light density fraction containing primarily blast cells (Fig. 4A). Table 2 shows the expression of B23 and p145 in the two separated fractions. While 98% and 71% cells are positive for p145 in the blast-enriched fraction, 0% and 0.1% cells express p145 in the mature cell population. The expression of B23, on the other hand, is of interest since not only 99% and 96% cells in the blast-enriched fraction are positive, but 22% and 58% cells in the blast-depleted group also express B23. In this latter population, B23 must be marking many more mature myeloid forms such as myelocytes which are obviously not positive for p145. These data once again confirm previous findings that p145 expression appears to be associated with immature cells.

Relationship of B23 and p145 Expression with the Percentage of Blasts in Bone Marrow Aspirates. Correlation of the differ-

Fig. 2. Expression of p145 in AML patients.

Fig. 3. Expression of p145 in CML patients.
Fig. 4. A, Wright-Giemsa stain of blast-enriched fraction from a CML chronic phase patient showing that the majority of cells are immature and contain nucleoli. B, blast-depleted fraction of CML chronic phase cells stained by Wright-Giemsa which shows mostly maturing myeloid cells.

Table 2 Expression of p145 and B23 in blast-enriched and blast-depleted fractions of peripheral blood cells from chronic phase CML patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>% of B23</th>
<th>% of p145</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 3 Statistical analysis of the relationship between expression of p145, B23, and percentage of blasts in bone marrows of AML patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>B23 (% of blasts)</th>
<th>p145 (% of blasts)</th>
<th>% of p145/ % of B23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parametric</td>
<td>0.62&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.73</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ranks</td>
<td>0.58</td>
<td>0.69</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28</td>
<td>30</td>
<td>28</td>
</tr>
</tbody>
</table>

<sup>*</sup> r, P value.

<sup>a</sup> Number of measurements.

Fig. 5. A, expression of B23 versus percentage of blasts in bone marrow aspirates of AML patients. B, percentage of p145-positive cells versus percentage of blasts in bone marrow aspirates of AML patients.

Fig. 6. Relationship between the expression of p145 nucleolar antigen and percentage of cells reactive with the monoclonal antibody B23 in AML patients.

Expression of B23 and p145 in “Normal” Bone Marrows. Table 4 demonstrates these data for normal marrows from three individuals. No p145-positive cell was detected in any of these specimens. However, almost one-third of the cells in these samples demonstrated bright nucleolar positivity for B23. The labeling indices in two of these normal specimens were 7% and 13%.

Table 4 Expression of p145 and B23 in normal bone marrows

<table>
<thead>
<tr>
<th>Patient</th>
<th>% of B23</th>
<th>% of p145</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>58</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Figure 5A. Twelve of the patients in this figure were studied during remission of AML, and less than 5% myeloblasts were present in their aspirates. Yet, the expression of B23 in these 12 patients ranged between 15% and 76%. Figure 5B demonstrates similar correlations between the percentage of blasts in the aspirates with the number of cells labeled by antibody to p145. Nine of 12 CR patients had no p145 expression, while patients with a higher percentage of blasts in their bone marrow aspirates also had higher numbers of p145-positive cells. Although these data suggest that, while B23 is not specific for myeloblasts, p145 expression appears to be so, yet the relationship between the latter is not linear. In other words, not all immature cells contain p145. Finally, Fig. 6 shows the relationship between B23 and p145 expression in AML patients. While there is a good correlation between the two results (r = 0.77 by parametric analysis and r = 0.83 by rank; Table 3), regression analysis demonstrates that the y-axis is intercepted at 42.5%, thereby implying that many B23-positive cells do not express p145 and that the converse is not true.
DISCUSSION

We have examined bone marrow specimens of 45 individuals in order to determine whether the expression of p145 is limited to malignant cells in cycle. Three of these 45 individuals had entirely normal bone marrows since they did not have any malignant disease. Interestingly, none of these three normal samples demonstrated evidence of p145 expression. We used the monoclonal antibody B23 as a positive control for nucleolar recognition in parallel with measurement of p145 expression. The antibody B23 recognizes a nucleolar phosphoprotein more abundant in tumor and proliferating cells than in normal resting cells (18, 19). However, this nucleolar protein can be detected in normal quiescent cells in lower amounts, and therefore, B23 serves as a useful marker for identifying the percentage of cells which have nucleoli not evident by morphology alone. As shown in Table 4, 28 to 84% of cells in the three normal specimens were found to be brightly marked by the B23 antibody while being completely negative for p145 expression. Thus the first important observation reported in this paper is that the nucleolar protein antigen p145 was not present in normal marrow specimens in detectable amounts at least by the methods used in this paper.

Forty-two patients with myeloid leukemias were studied, 30 with AML and 12 with CML. Of the 30 AML patients, 18 were studied with active disease and 12 during complete remission of their illness. The expression of p145 was found to be higher in the former group of patients as compared to those in remission (Fig. 2). While the percentage of p145-positive cells in all 30 AML patients appeared to be correlated well with the percentage of myeloblasts in the BM aspirates of 28 AML patients. These patients include individuals with active leukemia as well as those whose marrows were in complete remission at the time of study. In the 12 CR patients who had less than 5% blasts by morphology (Fig. 5A), the percentage of cells expressing B23 ranged from 15% to 76%. Since similar high reactivity was also found in normal marrows (Table 4), these B23-positive cells probably represent many normal nucleoli-containing cells such as erythroid precursors or maturing myeloid cells including promyelocytes, myelocytes, etc. In general, while there appears to be a good correlation between B23- and p145-positive cells, Fig. 6 clearly shows that many B23-positive cells do not express p145. However, the converse is not true.

In order to determine whether expression of p145 was more commonly associated with immature myeloid cells, two CML patients were studied whose chronic phase cells were separated into blast-enriched and blast-depleted fractions. Ninety-nine% and 96% of cells of the blast-enriched fractions, respectively, were positive for B23, and 98% and 71% of cells expressed p145. On the other hand, almost no p145 was detected in the blast-depleted fraction, while 22% and 58% of the cells, respectively, were positive for B23 (Table 2). These observations suggest that, while the B23 antibody reacts with possibly all cells and 96% cells of the blast-enriched fractions, respectively, were positive for B23, and 98% and 71% of cells expressed p145. On the other hand, almost no p145 was detected in the blast-depleted fraction, while 22% and 58% of the cells, respectively, were positive for B23 (Table 2). These observations suggest that, while the B23 antibody reacts with possibly all cells containing nucleoli, the p145 nucleolar protein is detectable only in immature cells. These observations are compatible with those of Freeman et al. (4) who demonstrated the disappearance of the p145 antigen as HL60 cells differentiated.

The double-labeling experiment in patients with active leukemia also provided data to support the hypothesis that p145 expression is associated with S-phase cells. With the currently available techniques, the only interphase cells we can definitely identify as being in cycle are those marked as being in S phase by some specific probe such as triitated thymidine. Therefore, using simultaneous labeling of cells by \(^{3}H\)dThd and the monoclonal antibody against p145, we were able to demonstrate that, at least in patients with active leukemia, the majority of S-phase cells expressed p145 (Fig. 1B). In samples obtained during complete remission of AML, however, only a few S-phase cells were positive for p145. Since G1 or G2 cells cannot be recognized by light microscopy, all one can conclude from these data is that p145 is expressed by leukemic cells in S phase.

The observation that many S-phase cells in the marrows of CR patients were negative for p145 could mean one of two things. Either S-phase cells in the CR marrows were not myeloblasts (they could be erythroid precursors, promyelocytes, etc.) and p145 is expressed only in myeloblasts, or if they were myeloblasts and did not express p145 during S phase, then

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Table 4 Expression of p145 and B23 in "normal" bone marrows processed by double labeling

<table>
<thead>
<tr>
<th>Marrow</th>
<th>% of B23</th>
<th>% of p145</th>
<th>% of LI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.5</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>84</td>
<td>0</td>
<td>ND</td>
</tr>
</tbody>
</table>

* LI, Labeling index or the percentage of S-phase; ND, not done.
perhaps these myeloblasts are at a more mature level than are leukemic myeloblasts found in AML patients with active disease. The former does not appear to be true, since the 3 normal specimens in S phase did not demonstrate any p145 reactivity. If the latter is true, then p145 expression is not only associated with relative immaturity, but also with some property more commonly found in malignant cells because leukemic myeloblasts in S phase express it consistently, while S-phase myeloblasts in CR and normal BM specimens do not. Since p145 is expressed by most S-phase leukemic cells, this property may be cell cycle related. The observations that p145 expression is present in rapidly dividing normal tissue, such as hypertrophied prostate or testes (3), suggest that expression of p145 may be dependent upon the proliferative rate. Taken together, these observations suggest that the presence of p145 in cells is an indication of cell immaturity and possibly of high proliferative rate. Whether all cells which express this antigen are in cycle is suggested but not proven by these data. One property of immature cells that distinguishes them from their more mature counterparts is a higher "proliferative potential" or the ability to undergo many cycles. p145 expression may be indicative of the proliferative potential of cells. We should be able to answer this question by double labeling cells with the monoclonal antibody against p145 and the polyclonal antibody we are currently using to determine the expression of c-myc protein in leukemic cells. Since p145 is nucleolar and c-myc protein is nuclear and always absent in nucleoli, we should be able to detect the two simultaneously. These experiments are now under way.

In summary therefore, we have examined the expression of a cell cycle-related protein antigen p145 in patients with myeloid leukemias and 3 normal individuals. It appears that, while this protein is absent in the three normal marrows examined as well as the majority of CR marrows, it is more commonly expressed by immature myeloid cells in the leukemic patients. There was a direct correlation between the percentage of myeloblasts in the BM aspirate and the number of p145-positive cells in AML patients, although every blast did not express p145. In CML patients, both chronic and blastic phase myeloblasts contain the p145 protein. Furthermore, double-labeling with [3H]TdR and the monoclonal antibody against p145 showed that, in patients with active leukemia, the majority of S-phase cells were positive for p145, but during complete remission, most S-phase cells were negative for p145 expression. While p145 is not a tumor-specific antigen, it does appear to identify some cell cycle properties which are more commonly found in very immature and/or malignant cells. Whether these represent a rapid cycling ability or a high proliferative potential is unknown at present. We expect that similar data collected on a population of patients serially from the time of diagnosis, through remission, to relapse of AML will provide more insight into the proliferative kinetics of leukemias.

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REFERENCES

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