Expression of Multiple Isozymes of Granulocyte, Monocyte, and Macrophage Esterases in Polycythemic Friend Erythroleukemia Cells

By John M. Woytowicz, Philip R. Daoust, Janine Andrè-Schwartz, and Stuart B. Levy

We examined the expression of cytochemical markers of myeloid and monocyte-macrophage differentiation in conjunction with ultrastructural studies of different malignant erythroleukemic cells isolated from mice infected with the Friend polycythemic virus complex (FLV-P). The amounts of fluoride-sensitive and resistant nonspecific esterase activity increased with the progression of malignancy. Isoelectric focusing resolved this enzyme activity into 13 isozymes in the most malignant Friend cell type tested. These same isozymes were found in the adherent cell population of normal spleens. Two of these isozymes were shown to have chloroacetate esterase activity characteristic of granulocytes. Despite these myeloid and monocyte characteristics, light and electron microscopy showed no morphological evidence of differentiation in either of these lineages. This study demonstrates that the Friend erythroleukemic cell contains markers of three different hemopoietic cell types. The expression of myeloid, monocytic, and erythroid traits in these erythroleukemic cells can be used to monitor their malignant progression.

MALIGNANT PROGRESSION is a common finding in cancers of man and animals. With time, transformed cells diverge to different malignant potentials, as assayed by rates of growth and metastases. We have described a similar pattern in a murine erythroleukemia initiated by the Friend polycytemia-inducing virus complex (FLV-P). Initially, differentiating proerythroblasts proliferate in the spleen, liver, and bone marrow of the infected mouse. Later, there is a progression of the Friend cell to different increased malignant states: the eventual tumor cell has been proposed to be the result of a multistep event. An anemia-producing Friend virus complex (FLV-A) also causes a rapid leukemia, but unlike FLV-P, it leads to severe anemia. Both complexes consist of related ecotropic, replication-competent virus and a defective spleen focus-forming virus (SFFV). The disease initiated by the two virus complexes can be distinguished on the basis of response of these cells to erythropoietin: FLV-A-induced Friend cells are erythropoietin-dependent, while FLV-P-induced cells are not.

Nondifferentiating tumor cells can be detected in the spleens of mice infected with either virus complex; however, they are seen 3 wk after infection with FLV-P and 8–12 wk after infection with FLV-A. Some tumor cells can be propagated only in vivo, while others grow in vivo and in vitro; the latter are more tumorigenic in vivo. Two kinds of cell lines can be cloned in vitro: one, the most tumorigenic in vivo, will synthesize hemoglobin in response to inducers like dimethylsulfoxide (DMSO), sodium butyrate, hemin, and HMBA, the other cell type is less tumorigenic and does not differentiate in response to these inducers.

While the erythroid nature of Friend disease has been well documented, several investigators have observed myeloid enzyme activities in Friend cells grown under different culture conditions in vivo and in vitro. These findings raise questions about the exact nature of the Friend cell. In this article, we have been able to separate esterase activity present in Friend cells into different isozymes and to show that these are of monocyte-macrophage as well as of myeloid origin. Ultrastructural studies showed no features of myeloid or monocytic lineage. The number and amount of these enzymes differed among morphologically similar, but different, malignant cell types in polycythemic Friend leukemia.

MATERIALS AND METHODS

Mice

DBA/2J mice (6 wk old) were obtained from Jackson Laboratory, Bar Harbor, ME.

Virus Stocks

The FLV-P virus preparation (20% w/v) was prepared from 14–21-day-old leukemic spleens of DBA/2J mice; it was originally obtained from C. Friend and passed through DBA/2J mice for 9 yr in our laboratory. FLV-A (received 2 yr ago from C. Friend) has been prepared similarly in NIH/Swiss mice obtained from the colony of R.S. Schwartz, Tufts Cancer Center. We have previously described the origin of the cloned ecotropic helper virus, II clone.
at 37°C in a moist 5% CO2 chamber and passaged twice each week so that cells remained in the logarithmic growth phase. The origin of the cloned cell lines A6, C7D, A-B6, B-A2, and B-B4 has been reported earlier.4,28

**Cytochemical Assays**

Single-cell suspensions were prepared in BME and centrifuged onto glass slides using a Shandon-Elliott cytocentrifuge at 100 rpm for 10 min. Periodic acid-Schiff staining was performed according to Lille27 and Sudan black B staining by the method of Sheehan.25 Determination of specific and nonspecific esterases followed methods described by Yam29 and Li.30 Slides were read according to intensity of color on a scale of 0-4 +, with 0 being no activity and 4 + being intense filling of the cytoplasm and obscuring of the nucleus. On this scale, the granulocyte read 4 + for specific esterase activity. Estimation of hemoglobin in DMSO-treated cultures was determined by its affinity for benzidine hydrochloride.31

**Extraction of Esterase Activity From Cells**

Esterases were extracted from the cell types described in Table 1 by a modification of the method of Yam.29 Spleens from normal and phenylhydrazine-treated DBA/2J female mice served as controls. Phenylhydrazine is a hemolytic agent that readily induced reactive erythropoiesis and 2-4-fold larger spleens containing erythroid precursor cells.32 In addition to cells in the polycythemic animals, leukemic spleen cells from DBA/2J mice infected with FLV-A and II clone 2 were also examined.

Single-cell suspensions of all cell types were prepared in tissue culture media. To remove adherent cells, i.e., monocytes and macrophages, from tissues, we incubated the cell suspensions for 1 hr at 37°C in tissue culture plates. The nonadherent cells were subsequently washed once in ammonium chloride (0.82% KHCO3, 0.2 M Na2HPO4, pH 7.2) and then stained with 40 mM sodium fluoride (Sigma) in 240 ml of the substrate solution (alpha-naphthyl acetate) described under Cytochemical Assays.

**Electron Microscopy**

DBA/2J mice were sacrificed 14 days after inoculation with FLV-P or FLV-A, 9 mo after infection with II clone 2, and 15 days after passage of a subcutaneous tumor (cell type II). Untreated 7-wk-old DBA/2J mice and mice treated with phenylhydrazine were examined as controls. Cloned cell lines (A6 and C7D) incubated for 5 days with and without 40 mM or 100 mM sodium fluoride (Sigma) in 240 ml of the substrate solution (alpha-naphthyl acetate) described under Cytochemical Assays.

**RESULTS**

Polycythemic Friend leukemia is characterized by increased proliferation of an erythroleukemic cell that retains its ability to differentiate and does not produce tumors or grow in vitro, i.e., cell type I. Two to three weeks after virus inoculation, tumor cell types can be isolated from the leukemic mouse spleen. One cell type

**Table 1. Cytochemical Studies of Esterases in Friend Cells**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>In Vivo Tumorigency (Minimum No. Cells)</th>
<th>Percent Hemoglobin Induction With DMSO</th>
<th>Percent Specific Esterase Activity</th>
<th>Percent Nonspecific Esterase Activity (With Fluoride)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1'</td>
<td>2'</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1'</td>
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<tr>
<td></td>
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<td></td>
<td>2'</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3'</td>
</tr>
<tr>
<td>Phenylhydrazine treated†</td>
<td>---</td>
<td>---</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>I</td>
<td>---</td>
<td>NT</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>+ (10⁶)</td>
<td>&lt;0.5</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>III (A6) + DMSO</td>
<td>+ (10⁶)</td>
<td>&lt;0.5</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>IV (C7D) + DMSO</td>
<td>+ (10⁶)</td>
<td>&lt;0.5</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>SP1</td>
<td>+ (10⁶)</td>
<td>&gt;90</td>
<td>100</td>
<td>&lt;1</td>
</tr>
<tr>
<td>AB6 cloned</td>
<td>+ (10⁶)</td>
<td>30</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>SP2</td>
<td>+ (10⁶)</td>
<td>50</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>BA2 cloned</td>
<td>+ (10⁶)</td>
<td>70</td>
<td>&lt;1</td>
<td>100</td>
</tr>
<tr>
<td>BB4 cloned</td>
<td>+ (10⁶)</td>
<td>72</td>
<td>&lt;1</td>
<td>100</td>
</tr>
</tbody>
</table>

†Erythroid precursor cells only.

‡Frequency depended on relative amounts of very early erythroblasts that contained activity that was partially fluoride-sensitive.
NONERYTHROID ESTERASES IN FRIEND CELLS

(II) will produce subcutaneous tumors \(10^{1.6}\) cells needed), does not differentiate, or readily grow in vitro. Two other tumor cell types arise from type II tumors or can be isolated from the leukemic spleens. Cell type III (e.g., A6) is more tumorigenic (requires \(10^4\) cells) and is unresponsive to DMSO; cell type IV (e.g., C7D) is the most tumorigenic cell, requires as few as 10 cells to produce a subcutaneous tumor, and responds to inducers leading to erythroid maturation and hemoglobin synthesis. In order to understand the origin of the cell types and to find other distinguishable features among them, we examined them for unique ultrastructural differences by electron microscopy and for distinct cytochemical activities.

Electron Microscopic Analysis of Leukemic Tumor Cell Types From Polycythemic Forms of Friend Leukemia

Large distinct clusters of erythroblasts and proerythroblasts were observed in the spleens of polycythemic mice 14 days after virus inoculation and in subcutaneous tumors derived from leukemic spleens. Early proerythroblasts were more predominant in tumors than in leukemic spleens and at times were difficult to distinguish from undifferentiated blasts (Fig. 1A). These primitive proerythroblasts exhibited irregularly shaped nuclei with large nucleoli. Unlike leukemic spleens, where an abundance of C-type viral particles were budding from the cells (our findings), there was a striking paucity of virions in the cells making up the subcutaneous tumors. No hematopoietic differentiation other than erythroid was detected in the spleen or tumor.

Although cell types III (A6) and IV (C7D) contained fewer virions than cell types I and II, they otherwise resembled them morphologically. Following incubation with DMSO, both in vitro clones exhibited an increase in cytoplasmic channels and in virion content. These changes, which were discrete in cell type III, were dramatic in cell type IV, as has been described before with similar type cells. As noted in cell types I and II, there was no sign of granulopoiesis evident in these cells; all of them shared morphological features typical of proerythroblasts.

Examination of the spleen of an anemic mouse infected with the cloned Friend helper virus II clone 2

![Fig. 1. (A) Electron micrograph of cells in subcutaneous tumor \((x \times 900)\). Three proerythroblasts (PE) are seen, one of them in mitosis (PE-M). A large blast (B), probably an early proerythroblast, is also shown. (B) Electron micrograph of leukemic spleen cells from a mouse infected with helper virus, II clone 2 \((x \times 900)\). Three proerythroblasts are shown. Two of them, \((PE_1\) and \(PE_2\)) exhibit signs of maturity: clumped nuclear chromatin, abundant cytoplasmic ribosomes, and channels. A third cell \((PE_3)\) appears younger with high N/P, regularly scattered nuclear chromatin, nucleolus, and reduced cytoplasmic area packed with ribosomes. A lymphocyte \((L)\) is also visible. Inset shows C-type viruses budding from the cell membrane \((x \times 17,000)\).]
showed no remarkable difference in cell morphology as compared to leukemic spleens of polycythemic animals, including abundant virion production (Fig. 1B).

**Cytochemical Analysis of Tumor Cell Types**

Cytochemical studies on representative Friend cell types were performed in order to determine if the malignant cell types could be distinguished on the basis of known enzymatic activities. Different cytochemical markers were tested: Sudan black B for lipid, usually indicative of granulocytes; periodic acid-Schiff (PAS) for glycogen; fluoride-resistant nonspecific esterase for granulocytes and macrophages; and fluoride-sensitive esterases for monocytes. The specific esterase, chloroacetate esterase, is particular to myeloid cells.

None of the cells showed PAS positivity. Sudan black B revealed weak granular staining located diffusely in the cytoplasm of all leukemic cell types; prominent staining of neutrophils in the same preparations provided a convenient internal control. Distinct localized staining in vacuoles was more prevalent in cell types III and IV than in cell types I and II. Specific esterase activity was not detected in the reactive erythroid cells (phenylhydrazine-induced) but was found weakly in type I Friend cells. Consistently greater (2+, or more) activity was noted in cell types II, III, and IV. Incubation of cell types III and IV with 1.5% DMSO reduced this enzyme activity to 1+ levels (Table 1).

Using the substrate, α-naphthyl acetate, we found that the amount of cytoplasmic nonspecific esterase staining increased in accordance with the increasing malignant potential of the four polycythemic Friend cell types tested (Fig. 2). In contrast, little if any cytoplasmic staining was seen in >90% of cells from phenylhydrazine-induced erythroid spleens. Leukemic cell type I, a differentiating erythroid cell, exhibited faint cytoplasmic staining, i.e., 22% were 1+ reactive and 5% were 2+ reactive. More pronounced activity was seen in the nondifferentiating tumor cell type II, in which 62% were 2+ and 28% were 3+ reactive (Table

![Fig. 2. Nonspecific esterase stain of normal and Friend leukemic cells (alpha-naphthyl acetate, x 1,000). (A) Spleen cells from phenylhydrazine-treated animal: negative late normoblasts, 4+ positive macrophage. (B) Leukemic spleen cells with some 1+ cytoplasmic staining (arrow). (C) Stage II tumor cells: 1+–3+ positive staining. (D) C7D cells: 1+–3+ positive. (E) C7D cells with fluoride inhibition: 0–1+ staining. Fluoride produced dramatic reduction in esterase activity in this tumor cell.](image)
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1). When these cells metastasized to the spleen, they retained this level of nonspecific esterase activity and exhibited specific esterase comparable to cell type II (data not shown). More than 80% of the more malignant cloned cell lines A6 and C7D exhibited levels of 2' activity. A decrease in this esterase activity was noted in type II and C7D cell preparations when 40 mM sodium fluoride was added to the enzyme reaction mixture.

Since esterase activity was not characteristic of mouse erythroid cells, we examined enzyme activity in cells upon induction to erythroid differentiation. Little change in nonspecific esterase activity was observed in both inducible (C7D) and noninducible (A6) cloned cell lines following a 5-day incubation with 1.5% DMSO; at this time, greater than 90% of C7D but less than 1% of A6 showed hemoglobin production. Again, the activity in C7D was more fluoride sensitive than that in A6.

In order to substantiate a general characteristic of Friend tumor cells during malignant progression, we examined five separately isolated new tumor cell lines for their esterase activity (Table 1). Cells in all five tumor types showed 2' or more specific esterase activity, as was seen in the previous tumor cell types II, III, and IV. Nonspecific esterase activity was more varied. Two cell lines, BA2 and BB4, showed 1−2' activity in >85% of the cells, while the other three lines showed 30%−50% 1' activity. Fluoride sensitivity was also different: of the three lines tested, BB4 and AB6 appeared to be somewhat resistant, while BA2 was clearly sensitive.

Separation of Nonspecific Esterase Activity by Isoelectric Focusing

It was not known whether the increase in enzyme activity with tumorigenicity was due to the increased expression of one enzyme or the appearance of new enzyme species, and whether different fluoride-sensitive and resistant enzyme species were being expressed. In order to answer these questions, we examined the nonspecific esterase activity for isoforms by isoelectric focusing. The study revealed an extensive pattern of isoforms that was prominent in the acidic portion of the polyacrylamide gel (Fig. 3). The amount of activity was shown to parallel the increase in nonspecific esterase activity noted by cytochemical analysis. Moreover, changes in the relative intensity of individual isoforms was detectable with this separation technique.

Three isoforms (bands 2, 4, 6 in Fig. 3) appeared to be common in all cell types. They were found in small amounts in the extracts from phenylhydrazine-induced erythroid cells that had been depleted of mature erythrocytes and adherent cells, e.g., monocytes and macrophages. Normal mouse spleen cells (>90% lymphocytes and granulocytes) when depleted of adherent cells exhibited five bands (1, 2, 4, 5, 7) of esterase activity. When adherent cells were not removed, these preparations showed enhanced amounts of these five isoforms and also detectable amounts of eight more acidic isoform species (bands 3, 6, 8, 9, 10, 11, 12, 13; Fig. 3A). When these same isoforms were analyzed by the substrate for specific esterase, bands 2 and 4 were positive for chloroacetate esterase.

![Fig. 3. Isoelectric focusing of isoforms of nonspecific esterases in cell extracts from normal and Friend leukemic cell types. Gel A: Extracts from 2 × 10⁶ cells. (1) Spleen cells from phenylhydrazine-treated mouse; (2) normal spleen cells; (3) normal spleen cells depleted of adherent cells; (4) spleen cells from a leukemic mouse 2 wk after inoculation with Friend virus; (5) cell line A6; (6) cell line C7D; (7) cell line C7D after 5-day incubation with 1.5% DMSO. Gel B: (1) Normal spleen cells; (2) tumor cells (type II); (3) cell line A6; (4) cell line C7D. Gel C: (1) SP2 tumor cells; (2) cloned cell line BB4, derived from tumor SP2. Numbers refer to isof orm bands. (pH Values for areas of the gel tested were: pH 6.7, bands 1, 2; pH 5.8, bands 3, 4; pH 5.2, bands 7-9; pH 4.6, bands 11-13.)](http://www.bloodjournal.org/content/20/6/429/F15)

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The leukemic cells (type I) showed a tenfold increased esterase activity over the phenylhydrazine control. Besides the common isozymes nos. 2 and 4, they showed easily detectable esterase activity in isozyme bands corresponding to 5–8 that were found in the adherent cell population. Like the cytochemical results, the profiles of the remaining cell types (II, III, and IV) showed increasing amounts of nonspecific esterase activity in bands corresponding to those seen in the adherent cells; the most noticeable enhancements occurred in bands 7, 8, 9, and 10. In contrast to results of cytochemical staining, the total enzyme activity by this analysis was not as dramatically different between cell type II and cell type I, although differences in isozymes were noted (Fig. 3A and B). There was, however, a marked increase (5–10-fold) in activity in cell types III and IV above levels in cell type I. All the isozyme bands seen in the normal spleen population could be found in clones A6 and C7D, and many of these were also seen in type II tumor cells (Fig. 3). Incubation of clones A6 and C7D with DMSO led to a slight decrease in total nonspecific esterase activity, as was seen in the cytochemical studies. However, coincident with DMSO incubation of clone C7D, band 4 appeared intensified and isozymes 7 and 8 became fainter (Fig. 3A). Fluoride inhibition of enzyme activity in the gels was limited with 40 mM fluoride, but was evident with 100 mM. However, even under these conditions complete inhibition of the more active bands was not seen.

The five newly derived Friend subcutaneous tumor cell lines (Table 1) were examined for esterase activity by gel analysis. Two were uncloned tumor lines (SP1 and SP2) that resembled cell type II when originally isolated. From these tumors, cloned cell lines that were more tumorigenic and DMSO inducible were established in vitro: clone AB6 (derived from tumor SP1 passage 5) and clones BA2 and BB4 (both derived from tumor SP2 passage 5). The expression of different nonspecific esterase isozymes correlated closely with their malignant character. Moreover, the malignant progression of the in vitro clones AB6, BA2, and BB4 from the respective parent tumors, SP1 and SP2, was also reflected in an increase in esterase activity (Fig. 3C).

Since very little is yet known of the origin of the target cell of the anemic form of Friend leukemia, we compared nonspecific esterase activity in spleen cells from the anemic form of Friend leukemia with that of spleen cells in the polycythemic disease. Severe anemia developed in adult DBA/2J mice within 1 mo after inoculation with FLV-A and after 9 months with II clone 2, the ecotropic helper virus. In both cases, the nonspecific esterase profile of the erythroleukemic spleen cells resembled that of the spleen cells (cell type I) in the polycythemic disease.

**DISCUSSION**

We examined morphological and biochemical characteristics of different Friend cell types in polycythemic leukemia in an attempt to define more clearly the nature of the target cell and to monitor tumorigenic progression. We have considered this progression as occurring at the level of the cell; however, the tumorigenic property may be that of the cell population.

By light and electron microscopy, four different Friend cell types were found to resemble early erythroblasts. Nondifferentiating cells were more prominent in tumors than in leukemic spleens, and fewer virus particles were evident in these early tumor cells. This finding confirmed an earlier report that noted a decrease in the production of ecotropic virus from tumor cells.

While this disease has been described as an erythroleukemia, we have demonstrated cytochemical markers of maturing granulocytes, macrophages, and monocytes in Friend cells during malignant progression. Chloroacetate esterase, a marker of granulocytes, was detected in these four cell types. Sudan black B, a lipophilic stain that is also indicative of granulocytes, was detected in all Friend cells. DMSO treatment of both A6 and C7D appeared to decrease both these granulocytic enzyme markers. Changes in these activities may indicate that, as the Friend cell enters a pathway of terminal erythroid differentiation, its commitment allows expression of fewer characteristics of myeloid differentiation. Nonspecific esterase activity, which was largely sensitive to fluoride inhibition, produced distinct granular staining in the cytoplasm of these cells; this activity was only slightly diminished by DMSO. This finding suggests that a monocyte-macrophage component remains present in the differentiated Friend erythroid cell.

Others have reported cytochemical evidence of granulocytic differentiation in polycythemic Friend erythroleukemic cells. Specific esterase activity has been reported in Friend cells at different frequencies (1%–80%) depending on the cell line. With some cell lines, the activity appeared to increase in response to DMSO induction of erythroid differentiation. Our data show that uninduced tumor cells have strong specific esterase activity, which in two cell lines decreased with DMSO. Revoltella and associates noted only small amounts of myeloperoxidase activity in a few cells in some uninduced cell lines. We have found no myeloperoxidase activity in our cell lines A6 and C7D (data not presented). They found nonspecific esterase activity that was only partially fluoride sensi-
tive in all Friend cell lines grown in the presence or absence of DMSO. All of these markers suggested a granulocytic nature of the Friend cell. It is obvious from our own studies that some of this activity is related to monocyte-macrophage isozymes. Moreover, our findings and these other studies show that expression of these enzymes is varied in different cloned cell lines.

Of the cytochemical features analyzed, only nonspecific esterase activity distinguished among the cell types. Nonleukemic erythroid cells, i.e., phenylhydrazine-treated spleens, showed little if any activity; cell type I was weakly positive, whereas cell types II through IV exhibited increasing amount of activity. Analysis by isoelectric focusing further resolved this enzyme activity into a profile of 13 isozymes. Each of these was found in a mixture of adherent cells from normal spleens. Three major enzyme species (bands 2, 4, 6) were common to all cell types tested; two of these species were shown to be specific esterase. Other enzymatic bands were also present in adherent-cell-enriched preparations and were presumably from macrophages. In normal marrow and spleen, we found nonspecific esterase activity sensitive to fluoride in monocytes, but resistant in macrophages. The isozymes we have detected were, therefore, consistent with monocytes and macrophages. Though the physiologic role of this class of enzymes remains uncertain, phenotypic changes in the malignant potential of Friend cells in DBA/2J appear related to their expression.

The emergence of malignant cell types and the progression of characteristic isozyme profiles were reproducible findings. Five DBA/2J Friend cell tumors recently isolated presented tumorigenic growth characteristics that corresponded closely with the patterns of nonspecific esterase activity just described. Moreover, the cloned cell lines AB6, BA2, and BB4, which were more tumorigenic than their respective parent tumors, exhibited an isozyme profile characteristic of a more malignant cell type.

Golde et al.21 have suggested that the granulopoiesis they observed in in vitro cultures of leukemic cells in the presence of colony-stimulating activity (CSA) resulted from the presence of a pluripotent stem cell that was not yet committed to the erythroid pathway. We have shown previously that tumor cells introduced into Millipore chambers will differentiate into leukocytes and macrophages when the chambers are placed in the peritoneum of mice.4 These hyperbasophilic cells remain undifferentiated as tumors and retain the capacity for leukocytic differentiation under the appropriate conditions. In fact, some cell lines (e.g., A6, BA2) show macrophage-like characteristics by binding to the surface of the tissue culture plate in response to DMSO (unpublished observation).

Studies on the anemic35 and polycythemic8 forms of Friend disease have shown a marked increase in immature granulocytic cells at 6–8 wk after infection of normal bone marrow cultures in vitro. When these cells were used to reconstitute lethally irradiated syngeneic hosts, an initial episode of granulocyte proliferation was replaced by erythroblasts and the animals eventually died of erythroleukemia. Whereas the disease produced in vivo, following the transfer of pro-myelocytic lines to adult and newborn mice, was phenotypically granulocytic, the disease in vivo, caused by the viruses isolated from these cell lines, was erythroleukemia and, in our studies, all tissues following virus infection were erythroid by morphological features, despite biochemical evidence of monocyte-macrophage and granulocytic markers.

The simultaneous presence of granulocytic and erythroid cytochemical markers, e.g., heme, spectrin, and specific esterase, within single malignant cells in Friend disease resembles Di Guglielmo's syndrome in humans.37 Erythroblasts from these patients express the properties of granulocytes, e.g., myeloperoxidase and specific esterase activity, suggesting that these erythroblasts may have developed from an early stem cell that was capable of granulocytic differentiation. Moreover, acute myelogenous or myelomonocytic leukemia is the eventual outcome.

Our studies have demonstrated simultaneous expression of erythroid, granulocytic, and monocyte-macrophage traits in Friend erythroleukemia tumor cells. Furthermore, the increasing activity of nonspecific esterases represents an increasing number of adherent cell isozymes as detected by isoelectric focusing. This increase in expression occurred concomitantly with the development of increasingly malignant cell types.

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Expression of multiple isozymes of granulocyte, monocyte, and macrophage esterases in polycythemic Friend erythroleukemia cells

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