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# The differentiated phenotype of the transformed cell in leukemias

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**Abstract**

Data are reviewed from several types of leukemias to show that:
1) Leukemic cells share with normal hemopoietic precursor cells differentiation markers with a high degree of lineage fidelity.
2) When leukemic cells can be induced to differentiate, they mature into essentially normal blood cells of the corresponding lineage.
3) When a direct biochemical and molecular analysis of leukemic cells and of pure populations of hemopoietic precursor cells is possible, few, if any, differences are found in the expression of differentiated functions.
These results support a model of leukemogenesis whereby cell transformation affects primarily the mechanisms controling proliferation of normal hemopoietic precursor cells, without altering significantly the expression of differentiated functions. A consequence of the model is that a relatively limited number of changes in the pattern of gene expression, most likely affecting only the hormonal control of growth, is enough to account for most, if not all, of the malignant properties of leukemic cells. The model has important implications for leukemia therapy and for understanding the functional significance of the recently defined oncogenes.

**Zusammenfassung**

Jüngste Erkenntnisse, die man über verschiedene Typen leukämischer Zellen gewonnen hat, zeigen:
1) Die Differenzierungsmerkmale der leukämischen Zellen stimmen mit jenen der entsprechenden hämopoietischen Vorläuferzellen weitgehend überein.
2) Wenn leukämische Zellen veranlasst werden, sich zu differenzieren, entstehen im wesentlichen normale Blutzellen.
3) Wenn eine biochemische und molekulare Analyse von leukämischen Zellen und von reinen Populationen hämopoietischer Vorläuferzellen möglich ist, werden, wenn überhaupt, nur geringe Unterschiede in den differenzierten Funktionen gefunden.
Diese Ergebnisse stützen ein Modell von Leukämogenese, wobei Zelltransformation in erster Linie jene Mechanismen beeinflusst, welche die Proliferation von normalen hämopoietischen Vorläuferzellen kontrollieren, ohne auf den Ausdruck der differenzierten Funktionen einzuwirken. Eine Folge des Modells ist, dass eine relativ begrenzte Zahl von Änderungen in der Genaktivität, wahrscheinlich nur die hormonale Kontrolle des Wachstums berührend, für die meisten, wenn nicht für alle, bösartigen Eigenschaften der leukämischen Zellen verantwortlich ist. Das Modell hat wichtige Auswirkungen auf die Therapie der Leukämie und hilft, die funktionale Bedeutung der kürzlich definierten Oncogene besser zu verstehen.

Transformed cells, such as those isolated from leukemias, often express differentiated functions (Wigley, 1975). For example, many plasmocytomas produce immunoglobulins, Friend erythroleukemic cells can be induced to make hemoglobin, and many lines of basophilic leukemia cells contain histamine. The expression of tissue-specific traits has been an invaluable help in classifying the many forms of leukemias and has also led to the use of leukemia cell lines as *in vitro* models for normal hemopoietic cell differentiation.
Nevertheless, leukemic cells obviously differ in many respects from normal blood cells. Not all functions of the mature cells are found in transformed cells, and those that are expressed usually exhibit levels different

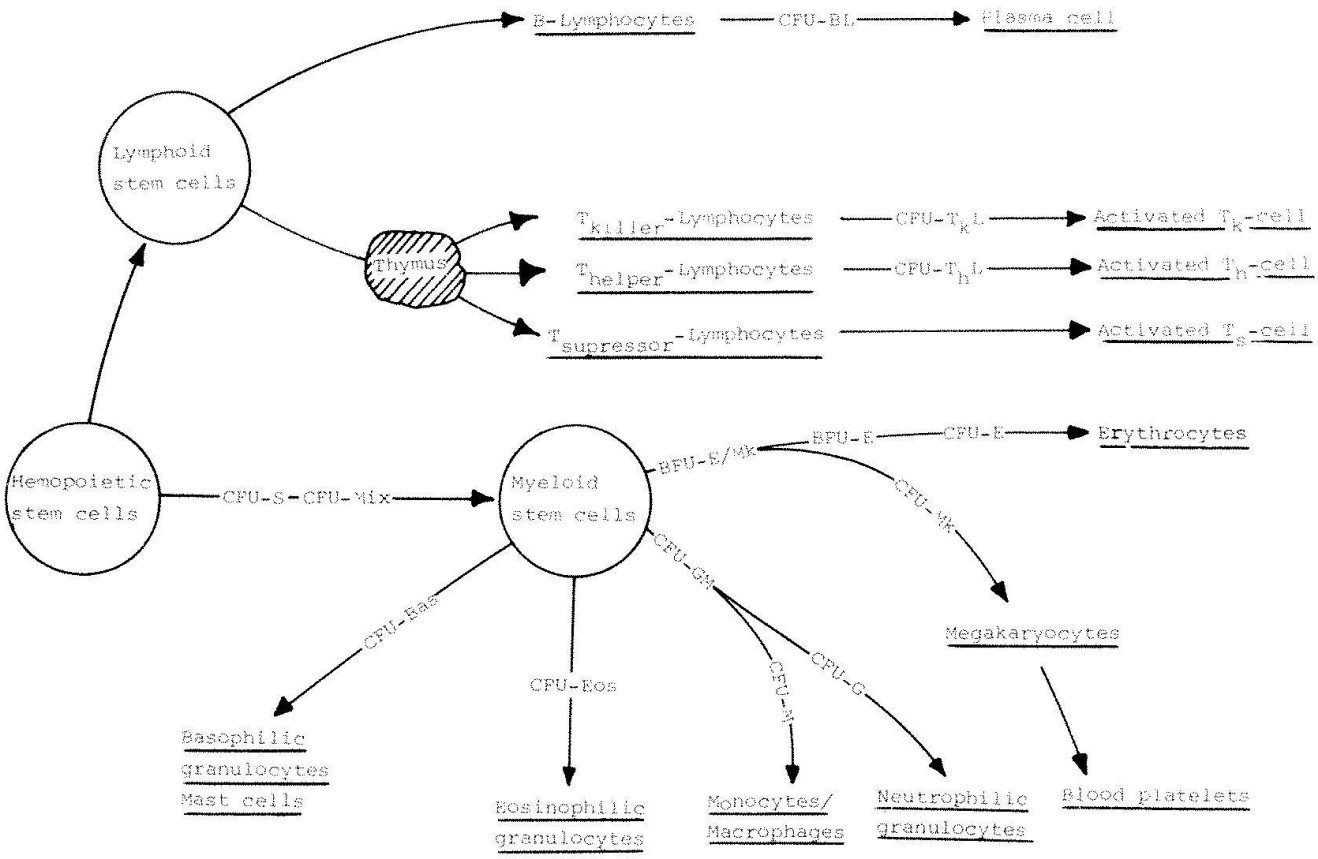


*Fig. 1. Normal hemopoiesis.* Underlined names designate mature, end-stage cells which are morphologically identifiable. Colony-forming units (CFU's) and burst-forming units (BFU's) represent, in the various lineages, proliferating precursor cells which can be assayed functionally (E, erythroid; Mk, megakaryocytic; M, monocytic; G, neutrophilic; Eos, eosinophilic; Bas, basophilic; BL, B-lymphocytic; TL, T-lymphocytic; CFU-S, pluripotent stem cell as assayed in vivo; CFU-Mix, pluripotent stem cell as assayed in vitro).

from those of mature blood cells. Furthermore, leukemic cells can express functions which are not found in their normal counterparts. These "extra" functions often involve the coexpression of markers typical of more than one hemopoietic lineage or the expression of molecules found in embryonic cells. Finally, leukemic cells proliferate *in vivo* and *in vitro,* a property that mature blood cells have either lost altogether or retained under very tight hormonal control. At a time when little was known about normal hemopoiesis, these observations led to the conclusion that leukemic cell transformation was a highly pleiotropic process affecting a whole range of functions related both to cell growth and to cell differentiation, and resulting in a very abnormal, dedifferentiated cell that bears little relationship to normal cells (Wigley, 1975; Ibsen and Fishman, 1979).

In the last two decades, however, considerable progress has been made toward understanding normal hemopoietic cell differentiation (Till and McCulloch, 1980) and this has led to a rethinking about the nature of the leukemic cell. Thanks to the design of novel morphological, cytochemical and immunocytochemical techniques, better identification of blood cells and of their immediate progenitors is now possible. More importantly, the development of colony assays *in vivo* and *in vitro* now provides an indirect functional analysis of several types of precursor cells (Metcalf, 1977). As a result, we know now that all the cellular elements of the blood arise from a limited number of pluripotent stem cells which persist throughout the life of an individual and from which cohorts of proliferating and differentiating cells arise continuously in response to physiological needs. The salient features of this process are outlined in figure 1. Four aspects of the system should be kept in mind. First, stem cells are relatively undifferentiated and specific functions appear progressively dur-

ing differentiation. Second, successive commitment steps restrict progressively the differentiation capabilities of the stem cells, so that, for example, transient, bipotential cells appear (CFU-GM in fig. 1). Third, cell proliferation accompanies differentiation all the way to the mature end-stage cell, in which this property either disappears (e.g. erythrocytes, granulocytes) or is subject to very specific triggers (e.g. macrophages, lymphocytes). Fourth, certain stages of hemopoiesis occur outside the bone marrow in specialized organs such as the thymus, or in the various tissues of the body (e.g. mast cell maturation), implying that certain normal hemopoietic precursor cells have the capacity to migrate and divide outside of their tissues of origin.

*Table 1.* Distribution of MBM-1, a mouse differentiation marker defined by a rat monoclonal antibody, on the surface of hemopoietic cells.

| Cell type | Presence of MBM-1 |
|---|---|
| Pluripotent stem cells | |
| assayed in vitro (CFU-Mix) | all negative |
| assayed in vivo (CFU-S) | all negative |
| Biopotential neutrophil-monocyte precursor cells (CFU-GM) | 40 % positive cells |
| Neutrophil precursor cells (CFU-G) | 40 % positive cells |
| Monocyte precursor cells (CFU-M) | 65 % positive cells |
| Peritoneal macrophages | 8 % positive cells |
| Blood neutrophils | all positive |
| Eosinophil granulocytes | all positive |
| Peritoneal mast cells | all negative |
| Early erythroid precursor cells (BFU-E) | all negative |
| Late erythroid precursor cells (CFU-E) | all negative |
| Erythroblasts, erythrocytes | all negative |
| B-lymphocytes (spleen and lymph nodes) | all negative |
| T-lymphocytes (spleen) | 55 % positive cells |

The data are based on immunofluorescent staining of various hemopoietic organs and analysis of cell fractions obtained after fluorescence-activated, antibody-mediated cell sorting of bone marrow cells, using hematological staining procedures for mature cells and functional assays for progenitors. For details, see Davis et al. (1983).
MBM-1 is present on cells of the myeloid series (neutrophil-monocyte pathway of differentiation) and on a subpopulation of T-lymphocytes. The nature of this antigen is unknown.

With these considerations in mind, it is evident that many properties of leukemic cells, including some that appear to be highly abnormal, are, in fact, properties of normal hemopoietic precursor cells, such as the various CFU's, BFU's and stem cells listed in figure 1. Hence the new model of leukemogenesis postulates that cell transformation uncouples the mechanisms for control of proliferation and differentiation in hemopoietic progenitors (Greaves, 1982; Till, 1982; Warner, 1982). As a result, leukemic cells are generated with a capacity for unlimited self-renewal and a greatly decreased capacity for terminal differentiation. This does not require a major reorganization of the pattern of gene expression in the leukemic cell, which remains largely the same as in the corresponding non-transformed precursor cell. This model leads to the following predictions: (1) Leukemic cells should display differentiation markers of normal hemopoietic precursor cells with a high degree of lineage fidelity; (2) If leukemic cells can be induced to differentiate terminally, they should generate essentially normal end-stage cells; (3) A direct comparison between leukemic cells and their normal counterparts should fail to reveal any significant differences in the expression of differentiated functions. In this report, I shall present recent results from several laboratories, including my own, which are in agreement with these predictions.

Antibodies reacting with specific markers of hemopoietic cell differentiation are becoming available in increasing numbers, thanks to the development of the hybridoma technology (Kennett et al., 1980). In this approach, rats or mice are innoculated with cells from allogeneic hemopoietic organs. Antibodies are thus generated against the many antigenic molecules present on the different cell types and the different maturation stages found in the innoculum. Following fusion of the immunized spleen cells with a myeloma cell line, the resulting hybridomas can be selected and cloned, and the cell specificity of the antibodies that some of them produce can be established with standard im-

munological techniques and antibody-mediated cell sorting. Using such a strategy, we have isolated rat monoclonal antibodies reacting with various subpopulations of mouse bone marrow cells, and one of these reagents has been characterized more fully (Davis et al., 1983). The surface antigen it recognizes, MBM-1, is found on a number of normal hemopoietic cells, as summarized in table 1. When a series of cell lines, established from different types of leukemias, are screened with the same antibody, the results given in table 2 are obtained. In all cases, the expression of MBM-1 in leukemic cells is consistent with lineage fidelity and no evidence for an aberrant expression can be found. The results of more extensive studies of the same type (Greaves, 1982; Warner, 1982), using human and murine leukemic cells or cell lines and a large panel of monoclonal antibodies directed against lymphocyte differentiation markers, are entirely consistent with the model of a precursor cell origin of leukemias and fail to support the idea of dedifferentiation or gross aberrant gene expression.

Leukemic cells, under certain circumstances, are capable of generating normal mature cells. This has now been shown in several types of leukemias. Friend erythroleukemia cells in culture express a set of differentiated functions that place them around the CFU-E stage (see fig. 1) of erythroid maturation (Marks and Rifkind, 1978). When treated with various chemicals, such as dimethylsulfoxide or sodium butyrate, these cells undergo a number of changes. Using several enzymatic markers of erythroid differentiation, whose changes *in vivo* have been documented (Denton et al., 1975; Setchenska and Arnstein, 1978), it is possible to show that Friend cell differentiation follows essentially a normal pathway, as summarized in figure 2 (Conscience and Meier, 1980; Conscience and Meier, 1980). Although established Friend cell lines do not enucleate well *in vitro,* one often finds among newly isolated Friend cell lines (Conscience et al., 1982) clones containing a relatively large proportion of enucleating cells, following chemical induction of differentiation. An analysis of these enucleating cells (Deslex, 1982), using single cell immunostaining procedures, shows that differentiated Friend cells undergo membrane rearrangements similar to those occuring *in vivo* during enucleation of the orthochromatic erythroblast (Geiduschek and Singer, 1979). These findings are shown in figure 3. Moreover, in improved culture conditions, established Friend cell clones also appear to enucleate normally (Volloch and Housman, 1982). Certain murine myeloid and lymphoid leukemia cell lines are similarly capable of undergoing normal terminal differentiation, when treated with various effectors (Sachs, 1978; Sachs, 1980; Ralph et al., 1982). Finally, the most convincing evidence available today showing that leukemic cells can mature into normal end-stage cells comes from work done with a temperature-sensitive mutant of avian erythroblastosis virus (AEV) (Beug et al., 1982). When grown at a temperature at which the virally coded transforming protein is functional, AEV-transformed cells display properties of immature chicken erythroblasts. If the temperature is raised to 42° C, the transforming protein becomes inactive and the cells differentiate irreversibly within a few cell divisions over a few days into perfectly normal chicken erythrocytes.

A direct biochemical and molecular comparison between transformed leukemic cells and the corresponding normal precursor

*Table 2.* Distribution of MBM-1, a mouse hemopoietic differentiation marker defined by a rat monoclonal antibody, in several leukemia cell lines.

| Leukemia cell line | Lineage and /or cell phenotype | Presence of MBM-1 |
|---|---|---|
| Friend cells (F46N) | erythroid | — |
| 426C | myelomonocytic | + |
| 427E | myelomonocytic | + |
| WEHI-3 | myelomonocytic | + |
| P3.X63.Ag8.6.5.3 | myeloma | — |
| WEHI-231 | B-lymphoma | — |
| WEHI-279 | B-lymphoma | — |
| 707/3 | pre B-cell | — |
| 18.81 | pre B-cell | — |
| 136.5 | thymoma | + |
| PB-1 | mast cell | — |

Comparison with the data shown in Table 1 reveals that expression of MBM-1 is restricted to leukemic cells related to lineages where the antigen is normally expressed. For the origin of the lines listed here and the indirect immunofluorescent technique used, see Davis et al. (1983).

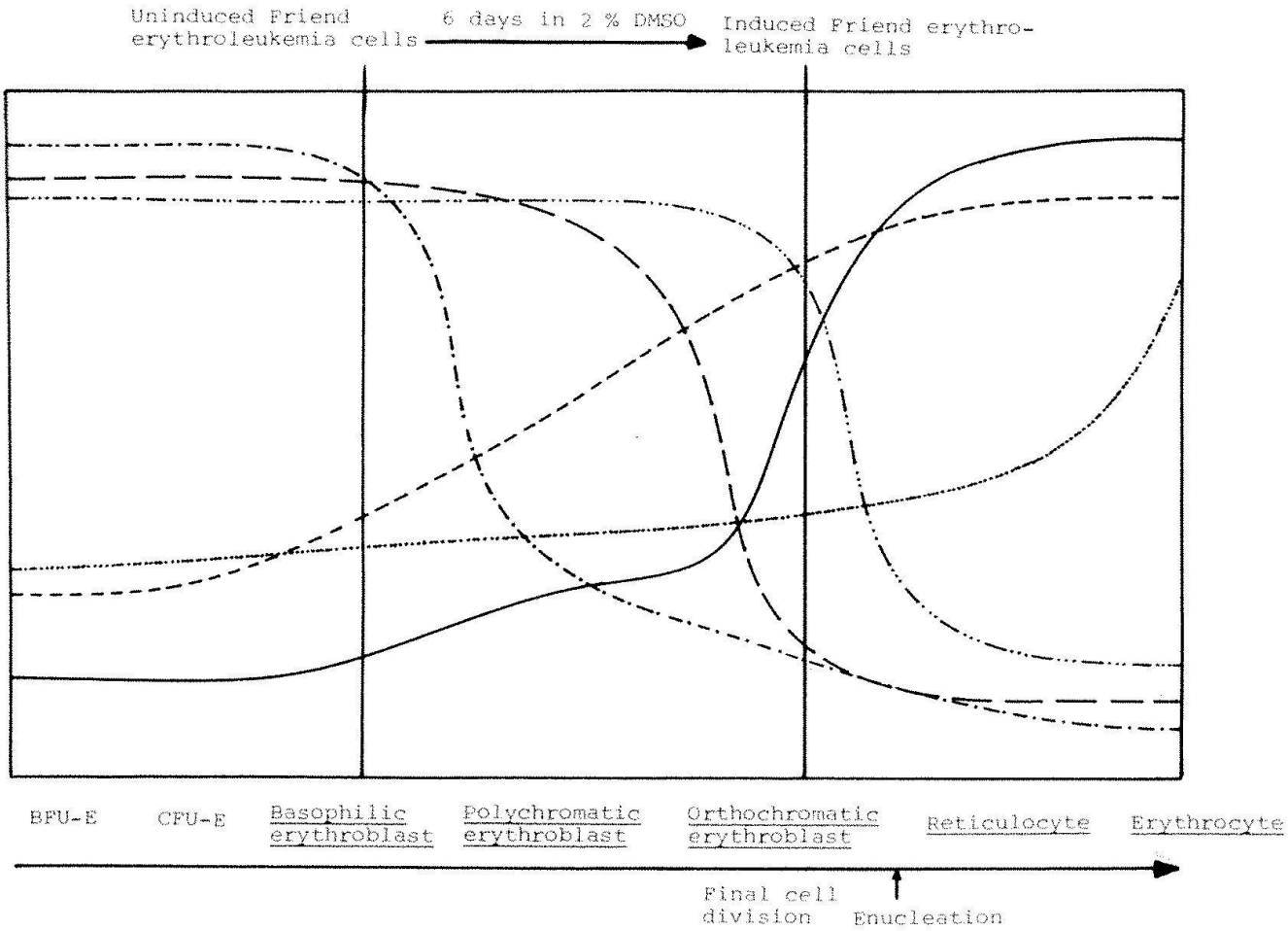


*Fig. 2. Comparison between normal erythroid differentiation in vivo and chemically-induced Friend erythroleukemia cell differentiation in vitro.* The plots (——— hemoglobin; · · · catalase; ——— 6-phosphogluconate dehydrogenase; --- carbonic anhydrase; ·-·-· lactate dehydrogenase; ·-·-·-·-· glucose-6-phosphate dehydrogenase) illustrate the changes in specific activities (ordinate, arbitrary scale) that take place in vivo during late erythroid differentiation. They have been compiled from the data of Denton et al. (1975) and Setchenska and Arnstein (1978). At the bottom of the figure are listed a few intermediate cell types of erythropoiesis. As in Fig. 1, underlined names designate morphologically identifiable cells, whereas CFU-E (colony-forming unit-erythroid) and BFU-E (burst-forming unit-erythroid) represent late and early erythroid progenitors, respectively, that can be assayed functionally.
When the same markers are followed in Friend erythroleukemia cells during chemically-induced differentiation, as indicated at the top of the figure, the same pattern of changes is observed: hemoglobin and carbonic anhydrase increase, lactate dehydrogenase and 6-phosphogluconate dehydrogenase decrease, and glucose-6-phosphate dehydrogenase and catalase do not change (for details, see Conscience and Meier [1980] and Conscience and Meier [1980]). This is taken as evidence for the view that Friend cells, upon induction, resume an essentially normal program of erythroid differentiation.

cells is only possible when the latter can be cultured as pure populations and when malignant cells arise in such cultures by cell transformation. Such a situation has recently been observed in our laboratory (Ball et al., 1983). Like others (Galli et al., 1982), we have been able to grow lines of basophil-/mast cell precursors, by exposing mouse bone marrow cells to the appropriate growth factors (contained in media conditioned either by pokeweed mitogen-stimulated spleen cells or by the WEHI-3 myelomonocytic leukemia cell line). Unexpectedly, nine months after its isolation, one of these lines transformed spontaneously. As shown in table 3, non-transformed basophil/mast cell lines require the continuous presence of growth factors for proliferation *in vitro* and for cloning. The transformed cells do not. In addition, the normal lines fail to give rise to tumors *in vivo,* while the transformed cells are highly tumorigenic and invasive in syngeneic animals. In spite of these dramatic differences in growth properties, the trans-

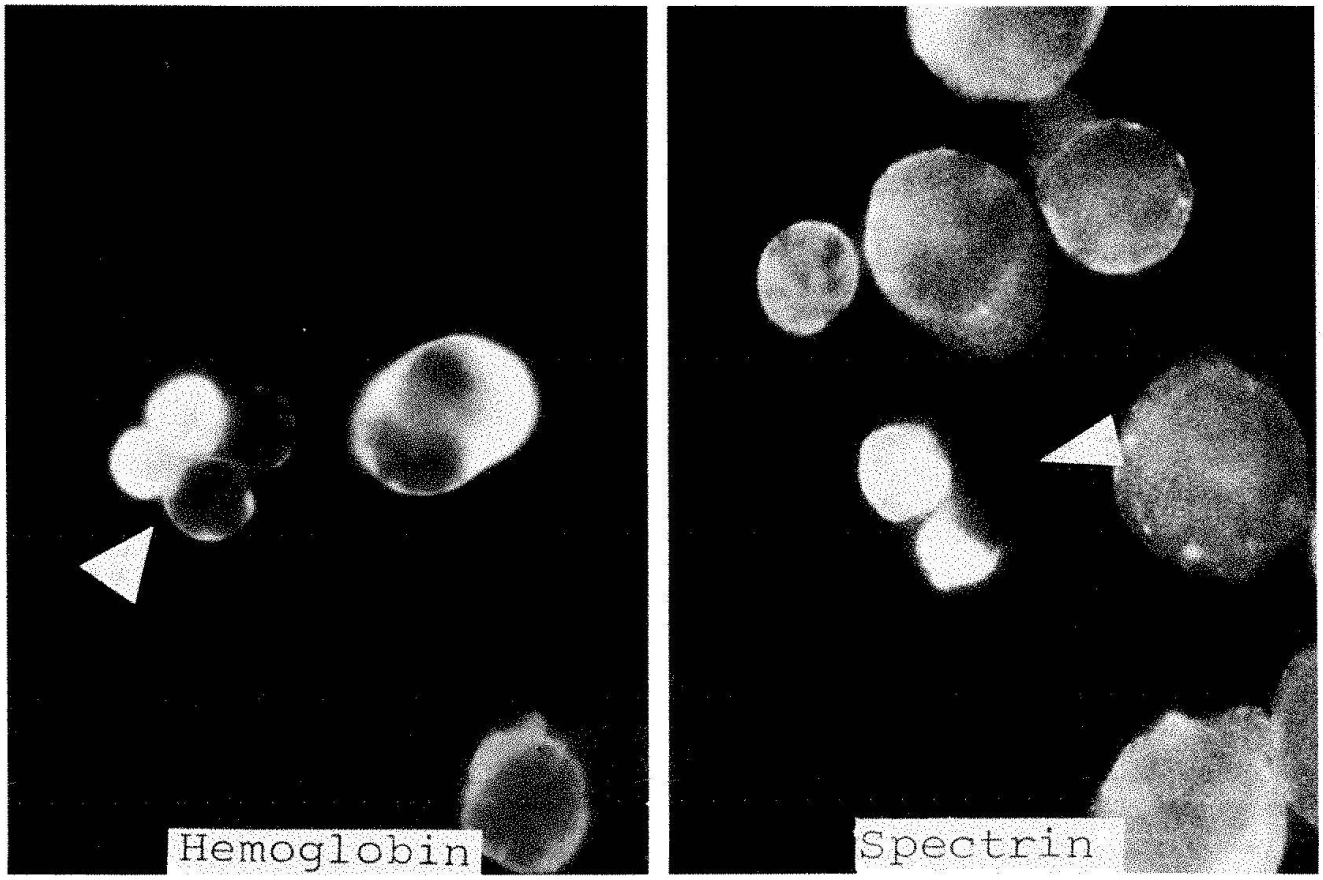


*Fig. 3. Enucleating erythroblast-like cells in a newly-transformed Friend erythroleukemia cell line, after 4 days in 1.3 % dimethylsulfoxide.* Staining was done by an indirect immunofluorescence technique, using rabbit anti-mouse hemoglobin and anti-mouse spectrin antisera, as described in Conscience et al. (1982).
After staining for hemoglobin, two enucleating cells (arrowhead) display a bright cytoplasmic half (the future reticulocyte) and a dark nuclear vesicle, surrounded by a thin ring of fluorescence. The latter stems from the small amount of hemoglobin-containing cytoplasm, trapped between the nucleus and the section of the plasma membrane that surrounds it.
After staining for spectrin, the nuclear side of the enucleating cells (arrowhead) remains totally unstained, indicating that all the spectrin molecules in the plasma membrane have been translocated into the cytoplasmic side of the cells.
A similar rearrangement is known to occur in normal enucleating erythroblasts (Geiduschek and Singer, 1979) and is taken here as additional evidence for the apparent normalcy of Friend cell differentiation in vitro.

*Table 3.* Growth properties of transformed and non-transformed basophil/mast cell precursors grown in vitro with and without the addition of growth factors.

| | Non-transformed cells | Transformed cells |
|---|---|---|
| Population doubling time without factors | die | 28 hours |
| Population doubling time with factors | 40 hours | 23 hours |
| Cloning efficiency without factors | 0 % | 6 % |
| Cloning efficiency with factors | 4 % | 80 % |
| Tumorigenicity and invasiveness in vivo | no | yes |

For details, see Ball et al. (1983).

formed cells cannot be distinguished from their normal counterparts with respect to the expression of differentiated functions: both cell types express the same markers, to a similar extent, and look like immature mast cells (table 4). Thus, in this instance, transformation clearly does not shift the cell population toward a different state of differentiation. There is also no evidence for a complete maturational block in the transformed cells. These, like their untransformed counterparts, display a striking cellular heterogeneity in the degree with which basophil/mast cell traits are expressed. The same heterogeneity persists after cloning of both types of cell lines. For example, the number of Toluidine Blue-positive granules per cell varies considerably, as well as the de-

gree of metachromasia of each granule. This suggests that cells at different stages of maturation are present and that a reduced capacity to undergo normal terminal differentiation persists in these cultures. This has been recently established in the case of the non-transformed cells (Galli et al., 1982). Interestingly enough, the transformed cells remain diploid, so that tumor formation in this system is not associated with the emergence of gross chromosomal abnormalities, and, moreover, they still respond to the addition of growth factors by an increase in growth rate and cloning efficiency (tab. 3).

In summary, the only significant cellular change that can be correlated with malignancy in the transformed basophil/mast cell cultures is their loss of requirement for added growth factors in vitro. This observation supports a classical hypothesis (Holley, 1972) relating the abnormal growth properties of malignant cells to a decreased sensitivity toward natural regulators. It is obviously unclear, at this stage, whether the growth factor independence *in vitro* reflects a similarly altered response towards endogeneous growth promoting and inhibiting substances.

*Table 4.* Differentiated traits expressed in culture by transformed and non-transformed basophil/mast cell precursors grown in vitro.

| Differentiated traits | Non-transformed cells | Transformed cells |
|---|---|---|
| Basophilic granules with May-Grünwald-Giemsa | + | + |
| Metachromatic granules with Toluidine Blue | + | + |
| Astra Blue staining | + | + |
| Staining for chloro-acetate-esterase | + | + |
| Staining for aminopeptidase | + | + |
| Histamine content (pg/cell) | 0.022 ±0.002 | 0.15 ±0.02 |
| Number of IgE receptor sites per cell | $1.2 \times 10^5$ | $2.3 \times 10^5$ |

The staining reactions of both cell types are less intense than those of mature mast cells and display heterogeneity from cell to cell. Transformed cells consistently exhibit higher amounts of histamine, but both values are much lower than those of mature mast cells. For details, see Ball et al. (1983).

If so, however, it might be a sufficient alteration to explain the tumoral growth of these cells *in vivo,* since normal basophil/mast cell precursors are known to leave the bone marrow and "invade" the tissues, where they undergo terminal differentiation and limited proliferation (Matsuda et al., 1981).

The data reviewed here support the hypothesis that leukemic cells are functionally equivalent to normal hemopoietic precursor cells with an altered regulation of cell division. It therefore appears that few cellular changes are required to effect transformation of the normal precursor cells. While the nature of these changes is still obscure, the results obtained by comparing directly normal and transformed basophil/mast cell precursors suggest clearly that cell transformation affects primarily the hormonal controls of cell proliferation and leaves intact the expression of differentiated functions.

Such a model of leukemogenesis presents some important implications. The similarity of leukemic cells to normal hemopoietic progenitors provides a rationale for their use as models for studying certain aspects of normal development. In particular, they can be used as immunogens with a reasonable hope of obtaining lineage-specific and/or differentiation stage-specific monoclonal antibodies. These, in turn, can be used to better characterize and purify the corresponding normal precursor cell, which, in most cases, still remains an elusive entity. Such an approach could be used therapeutically to enrich for certain cell types and/or delete others in the bone marrow cell populations prepared for transplantation into radiation-treated leukemic patients, thus decreasing the immunological hazards currently associated with the procedure. Since leukemic cells *in vitro* can often be induced to differentiate terminally into normal, non-malignant mature cells, another therapeutic application could consists in trying to achieve the same results *in vivo.* Preliminary attempts along these lines have been disappointing so far, but the use of specific monoclonal antibodies might allow for a better targeting of agents known to promote such differentiation (Moore and Sheridan, 1982).

A better characterization of the functional changes leading to a leukemic cell goes to-

gether with a better understanding of the genetics of the process. As reviewed elsewhere in this volume, oncogenes that are either abnormal or abnormally expressed have been found associated with a number of tumors and leukemias. Several of them have been cloned and sequenced. Thus, the genetics of these entities is now far advanced, but their physiological significance to the normal and the transformed cells is still obscure. I hope that model systems like the transformed and non-transformed basophil-/mast cell lines described here, where a direct biochemical and molecular comparison of the two cell types is possible, will prove useful in narrowing down the possibilities and in providing for adequate experimental verification of hypotheses.

## Updating note

Using the basophil/mast cell lines described above, we have obtained now results that support many of the ideas expressed in the preceeding review:

1. The factor required for the proliferation of the non-malignant cells in interleukin-3 (IL-3; Conscience and Iscove, unpublished results).
2. Factor-independent proliferation in vitro and tumorigenicity in vivo remain associated in somatic cell hybrids between malignant and non-malignant cells, in agreement with the model that the two phenotypes are causally related (Conscience and Fischer, *Differentiation,* in press).
3. The lesion leading to factor-independent proliferation in the malignant cells seems to be located at the level of signal transduction between the IL-3 receptor and the cellular and nuclear targets of hormone action; it could involve an altered protein kinase C and/or phosphatidyl-inositol-diphosphate metabolism (Mazurek and Conscience, manuscript in preparation).
4. Expression of the c-*mic* and c-*fos* proto-oncogenes is IL-3 dependent in the non-malignant cells and has become constitutive in the transformed cells, thus indicating an essential role for these two genes in the regulation of cell proliferation, survival and differentiation in the basophil/mast cell lineage and, possibly, in other IL-3 regulated hemopoietic lineages (Conscience and Martin, manuscript in preparation).
5. Monoclonal antibodies directed against the transformed, as well as the non-transformed cell lines have been isolated; all of them cross-react extensively with both cell types, in support of the idea that the transformation event was not accompanied by a major shift in differentiated states. (Conscience et al., manuscript in preparation).

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