Stem Cells in Normal and Leukemic Hemopoiesis
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This paper deals with three themes: (1) the nature of differentiation in normal and leukemic processes, (2) stochastic and deterministic control mechanisms that affect differentiation, and (3) the nature of the events that separate self-renewing stem cells from their committed descendants. These all impinge on both myelopoietic and lymphopoietic leukemias. The view is advanced that differentiation continues in these diseases, but new programs are assembled abnormally but with normal components.

Many different disciplines now meet in the study of hemopoiesis. Developmental biologists, molecular geneticists, and cell physiologists are challenged to explain the processes underlining continuing differentiation. Oncologists, intrigued by a malignancy that retains many features of normal growth, have used leukemia as a model system, yielding data applicable to many tumors. Clinical scientists, encouraged by their ability to induce complete remission in a widespread and rapidly fatal disease, developed methodologies of systematic therapeutic innovation based on well designed clinical trials. Biochemists, immunologists, virologists, and epidemiologists have all found particular challenges and opportunities in the study of hemopoiesis. It is not surprising, therefore, that much information is available; there is even considerable agreement concerning cellular organization, as evidenced by the similarity of lineage diagrams in the publications of diverse authors. However, major problems remain, and their importance is emphasized as they are restated in the context of each advance in knowledge.

This article contains three continuing themes: these are the lineages and differentiation programs that have been identified in normal and leukemic hemopoiesis; the balance between stochastic and deterministic processes in the regulation of normal and leukemic clonal expansion; and the nature of the determination event when pluripotent stem cells enter into a specific lineage.

These themes are illustrated by an examination of normal and leukemic hemopoiesis, the proposal of a model of acute myeloblastic leukemia (AML), and finally, a comparison of determination in myelopoiesis and lymphopoiesis.

Cellular Organization in Hemopoiesis

Myelopoiesis

The myeloid component of hemopoiesis is a typical cell renewal system. It consists of three classes of cells that differ in their functional capacities (Fig. 1). First, pluripotent stem cells are capable of self-renewal and differentiation; in myelopoiesis, at least two alternatives are considered to be closely linked and mutually exclusive. Self-renewal or “birth” permits each stem cell to maintain an independent clone. Differentiation or “death” leads to the generation of cells of the next class, with the capacity to proliferate extensively; but this proliferation is not associated with self-renewal. Rather, the divisions lead to daughters of increased maturity and the generation of the final cell class, functionally effective but incapable of division. Normal stem cells are capable of entering a quiescent state, commonly termed G₀. In normal steady-state human hemopoiesis, the great majority of stem cells...
are quiescent; they serve as a reserve from which the system may be replenished if it is depleted. This reserve is essential for recovery after treatment with chemotherapeutic agents or wide-field irradiation. Usually only a few stem cells are active; at any time, the hemopoietic system contains members of only approximately \(5 \pm 5\) independent clones.4

The transition from pluripotent stem cells to progenitors capable only of maturation is usually called "determination" or "commitment." The term "death" serves to emphasize the limitation in proliferation that is associated with differentiation of stem cells. The controversies that have centered about mechanisms underlying death and birth processes in stem cells have been reviewed extensively elsewhere.1 The alternatives are: first, models that postulate stochastic mechanisms where birth and death occur at random, governed only by definite probabilities;4 or second, deterministic models where regulation is considered to be based on external mechanisms that "instruct" stem cells either to undergo renewal or to differentiate.6 Major support for stochastic models has been obtained from examinations of the distributions of various cell classes among independent myelopoietic clones.7 The very great clone-to-clone variation observed can be simulated accurately by computer programs in which birth and death are assumed to occur at random. Instructive models usually postulate an inductive influence derived from the immediate surroundings of individual stem cells (microenvironments). The influence of microenvironments has been deduced from histologic studies of individual clones expanding in the spleens or bone marrow cavities of mice.8 However, the apparent clonal homogeneity of differentiated populations, as seen when maturing cells are examined morphologically, was not confirmed when functional assays were used as the basis for clonal analysis.9,10 More recently, the finding that multilineage colonies can develop in cell culture in the absence of microenvironments has cast further doubt on instructive models of stem cell regulation.9-11

On the balance, the evidence at present appears to favor stochastic models; but regardless of the mechanism of its generation, the marked interclonal heterogeneity that is observed normally must be considered whenever hemopoietic populations are compared.

Much of myelopoietic proliferation is postdeterministic, originating in progenitors committed to one of the three lineages: erythropoiesis, granulopoiesis, and megakaryocytopoiesis. The organization has the advantage of providing a basis for independent lineage regulation since, after determination, committed progenitors acquire sensitivity to lineage-specific regulators.12

The third subclass—granulocytes, erythroblasts, and mature megakaryocytes undergoing their last terminal divisions and their proliferatively inert end stages—are beyond the scope of this article. It is noteworthy, however, that these are the cells that are seen by the clinical hematologist when a diagnosis is made on morphological grounds; because of the nature of exponential expansion, their precursors are hidden effectively and might not be recognized even if they were morphologically distinct.

**Lymphopoiesis**

The description of myelopoietic organization included the assumption that, for stem cells, renewal and differentiation are incompatible alternatives. This assumption does not appear to hold for lymphopoiesis. Cells phenotypically recognized as T lymphocytes can be maintained in long-term culture, providing T-cell growth factor is included in the medium;13 and individual T-cell colonies have been recloned successfully. Thus, cells entering the T-cell lineage retain renewal capacity.14 Stages in B-lymphocyte development have been defined in molecular terms (Fig. 2).15 In the most primitive B-lymphocyte precursors, genes coding for immunoglobulins are in a germ-line configuration; as an early step in differentiation, immunoglobulin gene rearrangement occurs, yielding cells that are genetically different from their ancestors but not yet identified as B lymphocytes by the production of immunoglobulin. At the next stage, heavy chains are
synthesized in the cytoplasm, providing the phenotypic manifestation of pre-B cells. Then, immunoglobulin appears on the cell surface; clonal expansion occurs when these cells encounter antigens of appropriate specificity. While colony assays are not available for each of these stages, it seems probable that self-renewal is maintained, even during antigen-stimulated B-cell clonal expansion, self-renewal may occur with generation of memory cells. Perhaps only the most mature plasma cells, with immunoglobulin as the dominant cytoplasmic protein, are incapable of self-renewal.

A minimum conclusion to be reached from a comparison of myelopoiesis and lymphopoiesis is that a firm linkage between differentiation and loss of proliferative capacity is not a general characteristic of hemopoiesis. The implication is that separate genetic mechanisms may exist for the regulation of the two processes.

**CLONES, LINEAGES, AND PROGRAMS**

**Clonal Analysis**

Early events in hemopoiesis have been studied using the techniques of clonal analysis. These depend on the identification of cell populations as descendants of a single progenitor. Where putative clonal populations are dispersed, genetic methods based either on the distributions of the isoenzymes of glucose-6-phosphate-dehydrogenase (G6PD) or chromosome markers have been used to establish clonality. When clonal populations remain localized, they may be identified as colonies, either in vivo or in culture. Colony methods are among the principal tools of experimental hematology; the techniques are most useful when the single cells of origin of each colony can be demonstrated, using the same genetic approaches that are applied to dispersed clones. When proof of clonality is available, important properties of early progenitors can be deduced from analysis of the cellular composition of clones. For example, the number of different lineages identified within a clone provides a minimal estimate of the differentiation capacity of the cell of origin. The existence of pluripotent hemopoietic stem cells was first demonstrated by this approach. When it is feasible to test clonal populations for new cells that are capable of generating clones, capacity for self-renewal can be assigned to the original progenitor; then, that progenitor may be properly termed a stem cell.

Recent studies of the distributions of isoenzymes of G6PD among hemopoietic colonies in culture have provided evidence that these are clones only when the cultures are initiated with very dilute cell suspensions, yielding few colonies in each plate. This finding underscores a requirement for caution in determining progenitor properties from analysis of primary colonies. Often, a linear relationship between number of cells plated and colony formation is observed even when the number of cells plated makes it unlikely that each colony is a clone. Such linear relationships have been used as a basis for assessing the frequency of cells with colony-forming potential (colony-forming units or CFU) in cell suspensions and the tissues from which they are derived. The validity of this approach is increased when corrections are made for overlapping; nonetheless, primary plating efficiencies are affected by many factors that are not easily controlled, such as cell damage during manipulation. Therefore, numerical estimates of cell frequency based on such numbers have limited usefulness. However, linearity of a colony assay makes it possible to determine other progenitor properties; for example, cell populations may be exposed to increasing amounts of cytotoxic agents (ionizing radiation or chemotherapeutic drugs), and survival curves may be constructed by observing reduction in plating efficiency with increasing dose. When such curves are single negative exponentials, their slopes (often expressed as the dose required to reduce survival to 10% or D_{10}) reflect progenitor properties. Ionizing radiations are particularly useful since the proliferative capacity of mammalian cells exhibit variation in radiation sensitivity over a very narrow range; a finding of radiation sensitivity within this range provides evidence that most colonies originated in single cells and that cell clumping, occurring immediately before or after plating, was not contributing a significant artifact.

**Colony Assays and Lineage Diagrams**

A conventional depiction of hemato poiesis is in the form of lineage diagrams based on colony assays (Fig. 3). The first of these, the spleen colony technique, detects a population of pluripotent murine stem cells incapable of clonal growth in the spleens of irradiated or genetically anemic mice (CFU-S). A number of cell culture methods have been described that permit the development of multilineage colonies from putative stem cells in both rodents and man. Early progenitors of granulopoiesis, erythropoiesis, and megakaryocytopoiesis can form colonies in culture under appropriate conditions that always include the addition of plasma clot, agar, or methylcellulose to prevent cell dispersion. These methods, and references to them, are provided in the legend to Fig. 3. The progenitors of the various classes of colonies are ordered in these lineage diagrams on the basis of their properties as determined by the nature of their progeny. For example, CFU-C and
BFU-E are considered committed progenitors restricted to granulopoiesis and erythropoiesis, respectively, because the colonies derived from them contain only a single lineage and self-renewal cannot be demonstrated by replating experiments. The lineage diagram has also been deduced from studies of the distributions of various cell classes among a large number of individual clones; a statistical procedure was developed based on the stochastic model of clonal expansion. It was assumed that growth, determination, and maturation involve opportunities for randomization to occur; therefore, a relationship exists between the number of differentiation events separating two cell classes and the extent of randomization. Randomization may be estimated by measuring the correlations between different cell classes in a large number of individual clones. If the correlation is found to be tight, the two cell classes are considered to be closely related; thus, in Fig. 3, BFU-E is shown closer to the stem population than CFU-C because measurements of BFU-E correlate better with CFU-S than measurements of CFU-C.

Erythropoietic progenitors are recognized in culture by their characteristic red appearance and by the multilobulated nature of colonies derived from BFU-E; multilineage colonies are identified by the heterogeneity of their cellular composition. In general, two methods are used to obtain colonial specificity: these are, first, the source of the cells used to initiate cultures and, second, the culture conditions, particularly the presence in the media of certain stimulatory preparations. For example, hemoglobinization in culture usually requires erythropoietin as well as cells with erythropoietic potential. Colonial specificity is particularly important when culture methodology is applied to abnormal cell populations. Figure 4 is a diagram of a technique developed to obtain colony formation from blast cells from patients with acute myeloblastic leukemia (AML). Peripheral blood was chosen rather than bone marrow because, in the latter, several cell classes with colony-forming capacity are present; in contrast, blast progenitors and T lymphocytes are the predominant proliferative population in AML blood. T lymphocytes may be removed efficiently by forming sheep erythrocyte rosettes and removing these by density centrifugation. The second source of specificity is contained in medium conditioned by normal leukocytes in the presence of phytohemagglutinin (PHA-LCM). Medium conditioned by leukocytes without the lectin is an effective stimulator of granulopoietic colony formation; but PHA-LCM is required for early events in the formation of multilineage colonies and for blast colony formation. In practice, cultures prepared as illustrated in Fig. 4 contained colonies of cells that morphologically resembled the blasts in the patients from which they were derived and, when chromosomal abnormalities were found in the patient's cells, these were also identified in metaphases obtained from colonies. A similar assay is available for the blast cells of ALL but, in this instance, to obtain specificity, the blood must come from ALL patients and the stimulation must be provided by purified T lymphocytes cultured in the presence of PHA (PHA-TCM). It is evident that the choice of an appropriate cell source, its careful preparation by T-lymphocyte depletion, and the use of an appropriate stimulator are not by themselves sufficient to prove specificity. In every experiment, the blast nature of the colonies must be confirmed in order to exclude overgrowth of the plates by T cells. Similar verification of the nature of...
individual mixed colonies, granulopoietic or erythropoietic colonies is required in every experiment where their specificity is crucial to the interpretation of the results.

**Differentiation Programs**

The dependence of lineage diagrams (Fig. 3) on colony assays forces such diagrams into an appearance of order that almost certainly represents an oversimplification. When populations identified by their capacity to form colonies under certain conditions are examined by other means, striking heterogeneities are uncovered. Cells similar in their colony-forming capacity may vary widely in size, density, sensitivity to stimulator and cycle state. It may be more realistic to consider that the life history of each cell begins in a pluripotent ancestor and terminates as a functional entity. Many stages may intervene between the beginning and the end; these may be considered as components of differentiation programs, each representing gene activation or inactivation events modified by epigenetic or environmental influences. Diversity may be increased at each cell division, since thereafter, two daughters need not follow identical programs. Considerable latitude might be allowed in the timing of programmatic components and even in their ordering. If this latitude has a stochastic basis certain programs would be more common than others. Program segments with very similar frequency in timing would then serve to identify apparently discrete stages in differentiation. Certain program segments might have characteristics that are compatible with proliferation and differentiation in cell culture in the presence of selective conditions. Other program segments might be recognized by the appearance of macromolecules; monoclonal or highly purified heteroantisera might recognize such molecules as antigens; these would then be considered differentiation markers with or without lineage specificity.

A programmatic concept of differentiation, while somewhat more flexible than that expressed as a lineage diagram, presents a number of problems. Among these is the definition of normal programs. Elsewhere it has been proposed that any program might be considered normal provided it terminated in a functionally effective cell. Such a definition would require the additional postulate that unusual or inefficient programs will be assembled with low probability. Thus, these programs would not be detected, particularly in the context of polyclonal normal hemopoiesis. A similar averaging mechanism has been proposed to explain the apparently orderly recovery of hemopoiesis after injury, even though stochastic processes at the level of determination lead to marked variation among individual clones. In placing great significance on determination in lineage diagrams, it is implied that once a cell has been committed to a specific myelopoietic pathway, its descendants follow that pathway faithfully. Thus, certain markers can be identified with each lineage; examples are spectrin and glycoporphin for erythropoiesis, certain esterases for granulocytes and macrophages, factor VIII for megakaryocytes and platelets, immunoglobulin for B lymphocytes, and receptors for sheep erythrocytes for T lymphocytes. The rapidly developing application of monoclonal antibody technology to hemopoiesis has increased greatly the number of markers; many of these have been assigned lineage specificity empirically on the basis of their distributions among normal hemopoietic populations as defined by other criteria. In some instances, monoclonal antibodies with great lineage specificity within hemopoiesis have been found to identify determinants on cells of other tissues. Common antigens on lymphoid and brain cells is an early example of this phenomenon; reagents, including monoclonal reagents, that identify carcinoembryonic antigen (CEA) on epithelial tumors and embryonic cells also have specificity for granulopoietic cells in the hemopoietic system. Thus, in addition to the usual technical requirements for the demonstration of specificity, many immunologic reagents are useful as lineage markers only in the context of the hemopoietic system.

The concept of lineage fidelity for normal hemopoietic cells appears soundly based for the postdeterministic segments of differentiation programs. Uncertainty exists about earlier events. Macromolecules without lineage specificity, such as HLA-DR and Ia, have been identified on the progenitors of multilineage and single lineage colonies. Colony formation by the earliest cells also appears to depend on lineage-independent stimulators. These, for example burst-promoting activity (BPA), might be expected to bind receptors that lack lineage specificity. In B-lymphocyte development, immunoglobulin gene rearrangement precedes the synthesis of immunoglobulin. Thus, B-lymphocyte precursors may be null in respect to the lineage-specific markers. Myelopoietic progenitors may have similar phenotypes.

An alternative model has been proposed by Till; he suggested that the pluripotent nature of stem cells might be reflected by the expression, perhaps at a very low level, of markers specific for each of the lineages within their capacity for differentiation. Determination and maturation would then be associated with amplification of certain lineage-specific markers and repression of others. A test of this hypothesis would require both the availability of highly purified popula-
tions of pluripotent stem cells and very sensitive methods for detecting either genotypic or phenotypic characteristics associated with pluripotency.

ACUTE MYELOBLASTIC LEUKEMIA

Models of hemopoiesis and the methods on which they are based may be tested as they are applied to the study of AML in adults. Several of its features make this disease a useful subject for concentration: therapeutic progress has been slow and therefore any advance will be welcome; it is sufficiently common that studies are feasible, but AML does not occur so frequently that the priorities of service overwhelm research objectives; and biological questions, particularly the place in hemopoiesis of the blast cells that characterize the disease, are important and challenging.

Clinical

In common with many cancers, controversy exists between those who believe that therapy of AML should be studied in large randomized clinical trials and others who favor a sequential approach in which each new management plan is assessed on the basis of historical controls. Randomized trials are, of course, the best source of convincing statistics; but for a disease with the low prevalence of AML, such trials usually demand institutional collaboration, and in that setting, obtaining fresh specimens for studies that require a research laboratory is often difficult. At the Ontario Cancer Institute, the decision was made to conduct sequential clinical trials in order to promote the best possible integration between clinical and laboratory research.

In the last decade, three such trials have been conducted under the leadership first of Donald Cowan, then of John Curtis, and always with the cooperation of John Senn of Sunnybrook Medical Centre. Each trial used chemotherapy more aggressive than its predecessor. The first trial included cyclophosphamide, arabinosyl cytosine, and vincristine (CAV); then the anthracycline, adriamycin, was combined with ara-C (AAC); and in the most recent study, thioguanine was added to the combination (ACT). In spite of the increasing intensity of the chemotherapy, all three regimens have yielded very similar results when outcome was assessed in terms of the success of remission induction, the duration of remission and patient survival. The success of remission induction has varied from 50% to 55%; median survival has been about 250 days; and of those patients entering remission, approximately 60% relapsed in the first year; the remaining 40% had a much better experience, with some survivors after 5 yr. These results are not very different from those reported recently by two groups, one in England and the other in the United States, that conducted large multicenter randomized trials.

The conduct of sequential trials requires that particular attention be paid to the assessment of clinical attributes contributing to the variation in outcome. The distribution of these attributes among the patients in each study group is a measure of the value of that group as a control for subsequent trials. The relative importance of host factors (such as age) and disease characteristics (percent marrow blasts) in each patient may be evaluated, as well as the contribution of therapy. We have observed shifts in the relative importance of these attributes. In the first trial, patient age and the peripheral platelet concentration contributed to outcome. With the addition of the anthracycline, age remained important, but the nature of the disease, as indicated by the percentage of blasts in the marrow, emerged as more significant. In the most recent trial using three drugs, only percentage of marrow blasts remained as a significant contributor to the variance. These findings may be interpreted as indicating that improvements in therapy made success more likely for older patients and for those with little residual normal hemopoietic activity; the findings also served as the basis for a hypothesis: that the biologic nature of the disease is, ultimately, the important determinant of outcome.

The Cellular Biology of AML

Fialkow and his collaborators have studied a number of G6PD heterozygous females with AML. They found the expected double enzyme pattern in somatic fibroblasts, but only a single isozyme was present in the blast cells and some of the myeloid lineages. They concluded that, at the time of presentation, each patient harbors only a single malignant clone. Thus, together with such diseases as chronic myeloblastic leukemia (CML), polycythemia vera (P vera), and idiopathic myelofibrosis (IMF), AML may be considered to be a clonal hemopathy. The finding of a dominant clone does not rule out a multicellular origin in these diseases, although transformation in only a single precursor remains an attractive possibility. Regardless, one malignant cell in each patient had properties that permitted it to become dominant over other transformants, if they existed, and to suppress clonal expansion by normal stem cells. One part of the mechanism leading to clonal dominance may relate to the capacity of normal stem cells to enter a G0 state (Fig. 1). Baserga et al. have suggested that G0 may not occur in malignant populations. This generality may be applicable to malignant hemopoietic cells, since CFU-GEMM in CML and blast progenitors in
AML have been found to be in a proliferative rather than a resting state.

The clonal hemopathies in general are considered to originate in pluripotent stem cells, because abnormal clones have been shown to contain cells belonging to the three lineages of myelopoiesis. It is uncertain, however, whether all AML clones are trilineage in composition. Granulopoietic differentiation has long been associated with the disease, because myeloperoxidase can often be demonstrated histochemically in AML blasts. Blackstock and Garson have provided convincing chromosomal evidence for erythropoiesis in AML clones; G6PD studies have demonstrated the coexistence of erythropoiesis and granulopoiesis in the abnormal population in some elderly patients with AML. All these findings support the origin of the disease in pluripotent stem cells. However, Fialkow and colleagues have reported young G6PD heterozygous patients in whom the blast cells and granulopoietic elements were members of the same clone, but where at least some erythropoietic cells belonged to different clones. One interpretation of their finding is that some AML clones may originate in a postdeterministic cells, perhaps CFU-C. Alternatively, as a consequence of leukemic transformation or progression following that event, certain leukemic pluripotent stem cells may have lost the capacity for erythropoietic differentiation. Support for this view may be obtained from examination of the continuous cell line KG1; this was started from cells from a patient originally considered to be erythroleukemic. No evidence of erythropoietic differentiation can now be found in the line; the absence of erythropoiesis cannot be attributed solely to culture conditions, since K562, derived from blast cells from a patient with CML, continues to express both erythropoietic and granulopoietic lineage markers.

The uncertainty concerning the properties of the cell of origin of certain AML clones underscores a general principle of clonal analysis; the composition of the clone provides evidence about the capacities of its progenitors for differentiation but not about the limits to those capacities. An additional factor affecting the value of clonal analysis is evident in studies of the concentration of committed progenitor cells in the marrow of patients with clonal hemopathies. Figure 5 illustrates the problem; it contains data obtained from patients with AML prior to treatment (Curtis et al., 1975) and ranked in order of increasing frequencies of CFU-C per 10^6 nucleated cells (---). Results for samples obtained from the same patients 8 wk after initiation of treatment are also shown ( ). The cultures contained added colony-stimulating activity. (Reprinted with permission. Copyright 1978 by Cold Spring Harbor Laboratory.)

most individuals yielding few, if any, colonies, while in some instances exuberant growth was seen. This is the pattern that might be generated by stochastic processes during clonal expansion; since AML is clonal, the initial CFU-C distribution might be generated in just that way. The data obtained at the second examination are also shown in the figure as closed points; these were ranked in the same order as the data obtained at presentation. It is apparent that no correlation was observed between the two measurements. This finding is consistent with plating efficiency variation generated by a stochastic process rather than reflecting a primary characteristic of each AML clone. Similar findings were obtained when BFU-E and CFU-E were assayed in leukemic marrow. It was not surprising, therefore, to find that initial values of these progenitors were not significant attributes contributing to the variation in outcome following chemotherapy.

Blast cells, identified morphologically by their lack of differentiated characteristics, are the dominant and diagnostic population in AML clones. The appearance of blast cells in other clonal hemopathies usually heralds an unstable and accelerated phase of the disease. The colony assay for blast progenitors, described earlier, has sufficient specificity to permit its application to the study of their properties. The sensitivity of blast progenitors to the chemotherapeutic agents adriamycin and ara-C was measured with the view to determining whether the D_10 values reflected the efficacy of treatment regimens containing...
these drugs. A wide patient-to-patient variation was observed, ranging from marked sensitivity to complete resistance. In addition, a biologic property of blast progenitors was assayed. This was their capacity for self-renewal as measured by a replating technique (Fig. 6). As is seen in the figure, cells obtained from pooling colonies were replated using the same conditions that yielded primary growth, or alternatively, cells from individual clones were cultured; in the latter case, the addition of irradiated feeder cells was found to be essential.

Primary blast colonies from peripheral blood cells of AML patients contained cells capable of blast colony formation upon replating. However, secondary plating efficiency (PE2) was never great, although like drug sensitivity, it was highly variable from patient to patient. When individual colonies were replated, the distribution of blast progenitors among colonies was found to be heterogeneous; the form of the distribution was similar to that observed when colonies of other classes were examined. The generation of such distributions may be attributed to "birth" and "death" processes occurring at random. Taken together, these data from blast colony replating were interpreted to mean that both self-renewal and determination-like events were occurring during the development of blast colonies. It follows that blast progenitors retain the stem cell properties of their pluripotent ancestors, but they are able to give rise only to descendants that follow programs ending in proliferatively inert cells without the useful characteristics of the normally differentiated elements of the blood.

**Lineage Infidelity in AML**

The analysis of the data from blast colony formation raises, at once, the question of the nature of the cellular programs that follow blast progenitor renewal. Earlier it was argued that differentiation programs might be considered normal provided they terminated in functional cells; by this criterion, the programs of blast cells are abnormal because they end in proliferatively inert cells that retain the morphology of blasts. Postdeter-

![Fig. 6. Blast progenitor renewal. A diagram illustrating the procedure for replating blast cell colonies.](image)
The interpretation of this finding remains complex because of the uncertainty concerning the nature of predeterministic differentiation programs. The finding of doubly marked blast cells might be interpreted as indicating that these cells were arrested at a developmental stage characterized by multiple marker expression. To find a control where single cells might express multiple lineage markers is not easy. Normal marrow, marrow originating after transplantation, and multiple lineage colonies in culture have been searched extensively and unsuccessfully for evidence of lineage infidelity. Nonetheless, as the predeterministic nature of normal hemopoietic colonies remains uncertain, the observation of doubly marked blast cells might be construed as a quantitative amplification of a normal process.

The Clinical Correlates of Blast Cell Properties

Analysis of three sequential chemotherapy trials in AML (see earlier) led to two conclusions: first, that clinical outcome was not strongly dependent on the details of the chemotherapeutic protocol, and second, that the biologic nature of the disease in each patient was the principal determinant of outcome.\(^5\) Characterization of the blast cells in AML provided ways of testing these conclusions since, for each property studied, patient-to-patient variation was observed. Thus, when sensitivity of blast progenitors to ara-C or adriamycin was measured using the culture assay, patients were identified whose cells were highly resistant, had intermediate sensitivity, or were highly sensitive. Adriamycin sensitivity has consistently failed to contribute to variation in either the success of remission induction or the duration of survival. In earlier experiments, ara-C sensitivity was correlated with remission induction but not with survival; with data from a larger number of patients, even the association with initial response has not been maintained. The culture assay used to measure sensitivity does not, of course, detect pharmacokinetic or metabolic contributions to the role of individual agents in therapy. However, the findings in culture support the clinical conclusion in respect to chemotherapy, and at a minimum, suggest that measurement of drug survival curves may not be useful in AML, at least in the setting of multidrug treatment regimens.

A very different conclusion was reached from assessment of the results of procedures designed to disclose biologic properties of blast cells. First, PE2, the measure of renewal capacity of blast progenitors, has consistently been associated with both remission induction and survival; in multivariate analysis, PE2 was shown to be a more significant prognostic attribute than either patient age or the percentage of blast cells in the marrow.\(^6\) In a recent series of 45 patients treated at the Ontario Cancer Institute, the highly significant contribution of PE2 has been maintained.\(^50\)

We have suggested that the finding of lineage infidelity in AML blasts is a manifestation of abnormal differentiation program assembly and possibly abnormal gene expression. Our ability to detect blast cells with markers of two different lineages is constrained both by the number of reagents available and by their capacity to detect cytoplasmic or surface markers. We would anticipate, therefore, that only extreme deviation from the normal would be recognized, and this should exist in highly malignant clones. Data on 26 patients with AML and 15 patients with ALL are given in Table 1. It may be seen that 10 of the 26 AML patients had some blasts that exhibited lineage infidelity, of these patients, only 1 achieved remission, while 15 of the 17 AML patients in whom lineage infidelity was not identified responded to chemotherapy. This difference is highly significant \((p = 0.002)\). In contrast, while lineage infidelity was observed in patients with ALL, a correlation with successful remission induction was not seen; it is possible that this negative finding reflects the greater ease with which remission is achieved in ALL than in AML. Regardless, the data support both the biologic significance of the finding of doubly marked cells and the concept that a correlation exists between deviation from normal differentiation programs and resistance to treatment.

**DETERMINATION AND GENE REARRANGEMENTS**

Figure 7 is composite from Figs. 1 and 2, designed to permit comparison between myelopoiesis and lymphopoiesis.
poiesis. Both begin in stem cells, and as stated earlier, some stem cells may be common to both. In each case, an early event has been identified as the beginning of differentiation. In myelopoiesis, this step is determination; in lymphopoiesis, rearrangements of immunoglobulin genes (Ig) is, at present, the earliest detectable manifestation of B-lymphocyte differentiation. The consequences of determination and Ig gene rearrangements are similar: both lead to the generation of diversity. In the case of myelopoiesis, this is intercellular diversity, manifest as heterogeneous populations of mature and maturing blood cells. In B-lymphocyte differentiation, Ig gene rearrangements are an important part of the mechanism that leads to the intermolecular diversity of antibodies. Stochastic events appear to be important in both myelopoietic determination and Ig gene rearrangements.

Much is known about Ig gene rearrangements; they follow a hierarchy, with μ gene being rearranged first, followed by κ and λ. However, since these events do not appear to be rigidly controlled, aberrant rearrangements happen, yielding genes that fail to be correctly processed. Indeed, aberrant gene rearrangement is one of the mechanisms leading to allelic exclusion in normal B lymphocytes. Less is known about myelopoietic determination. Even its stochastic nature has been deduced by statistical methods applied to the analysis of individual clones. The available probes that might be equivalent to cloned immunoglobulin genes are those for globins; detailed studies of abnormal hemoglobins have not provided evidence for gene rearrangement. Nor are examples of mechanisms available from other eukaryotic systems that resemble the molecular details of Ig gene rearrangement. However, both determination and Ig gene rearrangements appear to be early steps in differentiation, steps that begin in stem cells. Both processes end in the generation of diversity, although diversities of different kinds. It is unlikely that myelopoietic determination mimics Ig gene rearrangement precisely. Nonetheless, two biologic processes with a similar cellular point of origin, both including stochastic processes and each leading to diversity, might have features in common; especially, pathologic lesions affecting such mechanisms might have similar consequences, even though the mechanisms themselves might be quite different.

Evidence for a stem cell origin of some myelopoietic leukemias is convincing. ALL may also have a stem cell origin. The finding of Ig rearrangement in the blast cells of non-B non-T ALL patients is compatible with leukemic events first occurring in stem cells and then finding their initial expression in a broadened range of Ig rearrangements; the probability of aberrant rearrangements also might become greater than normal. Korsmeyer et al. have recently reported a series of 25 cases of non-B non-T ALL; of these, only 5 had cytoplasmic μ chains and 2 additional cases had cytoplasmic κ and λ chains. All 25 showed heavy chain gene rearrangement. In addition, light chain rearrangements were seen in 7 cases, although only 2 were Ig producers. Kappa light chain gene deletions were also seen.

Thus, molecular events usually associated with the beginning of B-cell differentiation led to leukemic blast cell populations that did not follow usual B-lymphocyte differentiation programs; rather, their programs ended in cells that were morphologically blasts and often did not bear any phenotypic markers of either B- or T-cell lineages. A colony assay exists for ALL blasts cells, indicating that they retain proliferative capacity, but when colonies obtained using this assay were replated, only a minority were capable of secondary colony formation. It is probable, therefore, that the programs of abnormal ALL blasts include “death” components analogous to those found in programs of AML blasts. If, as Baserga et al. suggest, malignant cells cannot exist in a G0 state, leukemic transformation of lymphoid stem cells must introduce a “death” probability. Without the possibility of either “death” or “rest,” malignant B lymphocytes would multiply so rapidly that every patient with ALL would die before reaching a treatment center.

Earlier, the proposition was advanced that AML blasts followed programs in which genes were expressed abnormally. Aberrant rearrangements in ALL usually lead to failure of immunoglobulin expression, but may be compatible with cytoplasmic μ chain synthesis. Occasionally, AML patients are encountered who are effectively “null” in respect to known lineage markers. Such AML blasts would then be analogous to those of non-B non-T ALL with rearranged immunoglobulin genes but lacking cytoplasmic μ chains. However, the aberrant genetic events leading to AML blasts might be compatible with the cellular expression of some lineage markers; such blasts would be analogous to ALL blasts with cytoplasmic μ chains.

It is proposed, then, that leukemic transformation occurs in stem cells; an early consequence is associated with an increase in the range stochastic events at the first differentiation step that are compatible with cell survival. When these events extend beyond the boundaries of the normal, they initiate and specify the differentiation programs of leukemic blasts, whether these be lymphopoietic or myelopoietic. Further, in these abnormally assembled programs, components of different lineages, including myelopoietic and lympho-
Conclusions

Three themes run through this article. First, concepts of hematopoietic lineages and programs required consideration whenever normal or leukemic hematopoiesis were discussed. The central importance of pluripotent stem cells emerged repeatedly. Only cells with the stem property of self-renewal can originate and maintain independent hematopoietic clones. Such clones are marked by the extreme cellular diversity introduced as stem cells undergo differentiation, giving rise to the multiple lineages of myelopoiesis and lymphopoiesis. In considering this diversity, it became apparent that it was not easy to find a clear boundary between the normal and the malignant; the need for such a definition is pressing. Perhaps it would be met when genes coding for lineage-specific markers are available as molecular probes. The precedent provided by the use of cloned immunoglobulins in defining B-cell maturation makes this approach attractive.

Lineages and programs are important in understanding malignant clones. Because of the uncertainty of the boundaries of the normal, only extreme deviations could be considered abnormal; thus, the proliferation of blast cells in acute leukemia was accepted as abnormal because such proliferation did not yield functional cells and blast cell growth was sometimes accompanied by lineage infidelity. Perhaps the most striking finding derived from the analysis of leukemic lineages, however, was the observation that the biologic characteristics of abnormal clones were more important as determinants of clinical outcome than either host characteristics or the details of chemotherapeutic programs.

The second theme was the significance of stochastic processes in hematopoietic differentiation. The view was advanced that, particularly for early events, these are more important in determining the cellular composition of clones than are regulatory signals originating in either the immediate or distant environment. The importance of such stochastic processes was evident in analyzing cellular data from patients with leukemia. The hematopoietic system in these individuals is predominantly clonal. When these abnormal clones are characterized, marked heterogeneity is uncovered; this type of patient-to-patient variation must be distinguished from interclonal differences that reflect primary pathologic events. It is suggested that the distinction may be made most readily by distinguishing characteristics that are stable with time from those that vary.

The third theme was determination—the qualitative change that separates stem cells from their immediate descendants committed to specific differentiation lineages. A comparison between determination, as it is observed in myelopoiesis, and the rearrangements of immunoglobulin genes that occur as the first step in B-cell differentiation disclosed many similarities. This comparison supported a model in which it was proposed that the origins of intercellular diversity in myelopoiesis might be similar to the origins of intermolecular diversity in lymphopoiesis. Once again, the need to develop appropriate molecular probes was evident.

Finally, continuing progress in the understanding of normal and malignant hematopoiesis will, in the future, as in the past, depend on interactions of many different disciplines and the cooperation of individuals with many different skills.

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References

11. Nakahata T, Ogawa M: Identification in culture of a class of hematopoietic colony forming unit with extensive capability to self-
renew and generate multipotential hemopoietic colonies. Proc Natl Acad Sci USA 79:3843, 1982
STEM CELLS IN HEMOPOIESIS

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Stem cells in normal and leukemic hemopoiesis (Henry Stratton Lecture, 1982)

EA McCulloch