Regulatory Mechanisms Acting on Hemopoietic Stem Cells

Some Clinical Implications

E. A. McCulloch, MD and J. E. Till, PhD

HEMOPOIETIC STEM CELLS stand at the beginning of processes of exponential growth and cellular differentiation. They provide the origins from which marrow recovers after injury or grows after transplantation. When stem cells are damaged by injury or disease, the effects are felt throughout each "differon" [1] derived from them. These consequences of stem cell function are important in clinical oncology. Marrow reserve is often the dose-limiting factor in chemotherapy and radiotherapy of tumors. The discovery of the Philadelphia chromosome [2] and its identification in erythropoietic and granulopoietic cells [3] provides clear evidence that human chronic granulocytic leukemia originates from pluripotent stem cells; the disordered proliferation and differentiation which are characteristic of that disease are striking examples of the effects of stem cell lesions on myelopoietic function.

Until recently, the use of the Philadelphia chromosome as a stem cell marker provided the only significant application of stem cell studies to human neoplastic disease. Now, however, the development of cell culture methods applicable to the detection, assay and study of a class of human hemopoietic progenitor cells [4–8] has opened a way to translate animal studies into clinically useful methods.

The purpose of this paper is to describe the basis for using colony formation in culture to assess human myelopoiesis. Two avenues of approach will be considered: the first relates to normal cells, the second to cells in leukemia. In both applications, studies in animals provide precedents that aid the interpretation of human data.

From the Institute of Medical Science, University of Toronto, and the Ontario Cancer Institute, Toronto, Canada.

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Address reprint requests to Dr. E. A. McCulloch, Department of Medical Biophysics, University of Toronto, 500 Sherbourne St, Toronto 5, Canada.

Methods of Study

Many assay methods have been developed for the study of hemopoietic progenitor cells in mice (for recent reviews, see Lajtha [9] and McCulloch [10]). Two of these methods depend upon the capacity of progenitor cells to give rise to countable colonies; in the first, the colonies develop in the spleens of suitable recipients and in the second, colony formation occurs in culture. Since, in each system, colonies derive from single cells, their enumeration provides an estimate of the numbers of cells with colony-forming ability, while the cellular composition of the colonies yields information about the properties of their cells of origin.

Methods depending on colony formation are indirect since progenitor cells are detected only by observing the results of their proliferation and differentiation. Accordingly, the term colony-forming unit or CFU has been developed to describe the observed colony formation without implying an interpretation. This functional nomenclature has the advantage that it can be applied to colony formation regardless of the source of the colonies or the location of their growth. Accordingly, both of the colony-type assays applied to hemopoietic cells can be described in parallel terms.

The first colony assay to be applied to hemopoietic tissue was the spleen colony method.[11,12] An appropriate number of nucleated cells from marrow, spleen, fetal liver, peripheral blood or lymph node are injected into heavily irradiated or genetically anemic recipient mice, and discrete colonies appear in the spleens of these animals 9–14 days later. Because of this location, the colony-forming unit giving rise to such colonies is referred to as CFU-S. Since 95–100% of cells in spleen colonies [13,14] belong to the same clone, the cellular composition of the colonies was used to deduce the properties of CFU-S.[15] These included capacity for extensive proliferation, including self-renewal,[15] capacity to give rise to at least erythropoietic and granulopoietic descendants [14,16–19] and capacity to respond appropriately to physiologic stimulation;[20] such properties are those required for progenitor cells to serve as "independent" stem cells [21] in hemopoiesis and they supply the justification for considering CFU-S to be pluripotent stem cells. The existence of an equivalent pluripotent stem cell in man has been deduced from identification of the Philadelphia chromosome [2] in erythropoietic and granulopoietic cells in patients with chronic myelogenous leukemia.[3]

Colonies develop when hemopoietic cells are cultured in viscid medium (agar or methyl cellulose) in the presence of certain stimulating factors (CSA).[22–25] The colony-forming unit responsible is referred to as CFU-C. Colonies in culture are smaller than spleen colonies, containing

20–5000 cells, compared to in excess of 10^6 cells in spleen colonies. Only granulocytes and large mononuclear cells have been identified in culture, which is a more limited spectrum than the erythroblasts, granulocytes and the megakaryocytes of spleen colonies. However, analysis of the cellular composition of colonies in culture provides only a minimal estimate of the potentials of CFU-C; capacities possessed by a progenitor might not be manifest in culture because specific environmental or nutritional requirements were not met. Accordingly, it was not possible to use these data to conclude that CFU-C is a different cell from CFU-S or restricted in capacity for proliferation and differentiation. However, it was of great importance to establish the relationship between CFU-S and CFU-C since the cell culture method used to detect CFU-C has been applied successfully to human marrow.[4–8]

Interpreting results obtained in man depended on whether the assay procedure was detecting a stem cell analogous to CFU-C in the mouse or some other entity with different properties. The problem was approached by comparing CFU-S and CFU-C in the mouse where both methods could be used and applying criteria other than the cellular composition of colonies to both classes of CFU.

A Comparison of CFU-S and CFU-C in the Mouse

CFU-S and CFU-C are present in marrow, spleen, fetal liver and peripheral blood as similar proportions of the nucleated cells. This numerical coincidence is more striking when individual spleen colonies are examined;[26] cell suspensions prepared from colonies vary in their content of CFU over a 1000-fold range. Nonetheless, for individual colonies the two assays yielded similar relative numbers. Thus, during the growth of spleen colonies from single cells the production of CFU-S and CFU-C remains closely linked, although extreme intercolony cellular heterogeneity is generated. A close relationship must exist between CFU-S and CFU-C and it seemed possible that the two assays were measuring the same cell. Accordingly, it was necessary to compare them in a number of ways in an attempt to establish or disprove identity. These methods included physical separation, response to physiologic manipulation and effect of mutation in significant loci.

Analysis by Methods of Physical Separation

Separation on the Basis of Density

Populations of hemopoietic cells have been separated on the basis of density by centrifuging to equilibrium in either continuous or discontinuous gradients.[27–31] Considerable variation has been reported both

from experiment to experiment and from laboratory to laboratory. In part, the variation may result from problems in controlling osmolarity in the gradients; such problems are particularly severe when proteins such as bovine serum albumin are used as gradient material. However, the fact of separation does not depend on its physical basis and Worton *et al* found CFU-C activity at slightly greater apparent densities than CFU-S.[24]

Separation by Velocity Sedimentation

More reproducible results have been obtained using velocity sedimentation in the "Staput" apparatus designed by Miller and Phillips.[32] In this apparatus, cells sediment under unit gravity through a shallow density gradient designed to prevent convection; since sedimentation velocity is a function of the radius squared, separation is achieved primarily on the basis of size. Under these conditions, CFU-C regularly sediment faster than CFU-S. In a recent series of experiments, Sutherland *et al*[33] have demonstrated this separation under conditions where the peak sedimentation velocity of CFU-C was altered by treating donor animals with vinblastine or colcemid. However, even under these conditions, complete separation of CFU-C from CFU-S was not achieved; rather, significant overlap of the distributions always remained and only rarely were fractions obtained that yielded colonies in one assay and not in the other.

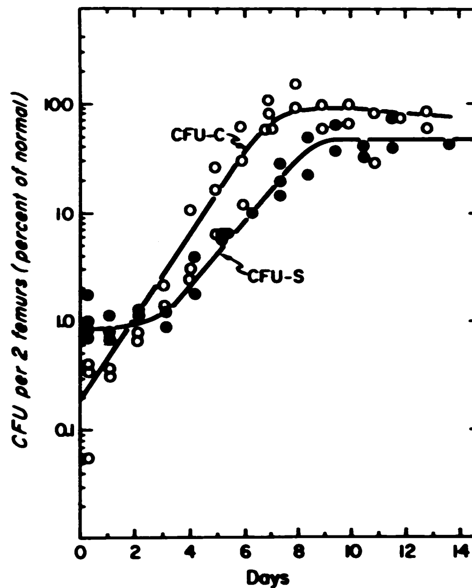
Thus, methods of physical separation provided convincing evidence that CFU-S and CFU-C do not belong to identical populations. However, the data do not exclude the existence of an overlapping population of cells capable of forming colonies both *in vivo* and in culture.

Response to Physiologic Manipulation

If CFU-S and CFU-C represent separate populations of progenitor cells, these might be expected to respond differently to physiologic changes. Information is available from two types of study. First, growth kinetics of CFU-S and CFU-C have been compared after marrow was transplanted into heavily irradiated mice. Secondly, numbers of cells in the DNA synthetic (S) phase of the cell cycle have been measured in resting populations of CFU-S and CFU-C and changes observed during exponential growth.

Growth Kinetics of CFU-S and CFU-C

The kinetics of growth of CFU-S and CFU-C are shown in Text-fig 1. The data are those of Sutherland *et al*[34] and derived from experiments

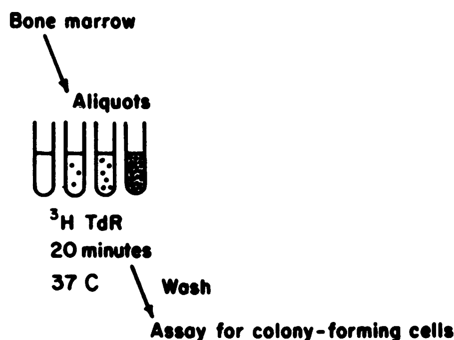


TEXT-FIG 1—A comparison of the growth kinetics of normal CFU-S and CFU-C after 2×10^7 marrow cells were transplanted into irradiated isologous recipients (data of Sutherland *et al*[34]).

in which 2×10^7 nucleated cells from normal donors were transplanted into heavily irradiated recipients; groups of 3–5 of these animals were killed at varying intervals of time and cell suspensions of femoral cells were assayed both *in vivo* and in culture. The growth curve for CFU-S began with a 3-day lag, and then exponential growth occurred with a doubling time of approximately 24 hours. The doubling time for CFU-C was similar (approximately 20 hours), but a lag was not observed. When the same methods were applied to growth in spleen, a difference was also found; however, in this organ both curves were characterized by a 2-day lag phase; the difference lay in the doubling time, 24 hours for CFU-S compared with 13 hours for CFU-C.

Measurement of Cells in DNA Synthesis

Since both CFU-S and CFU-C are present as a very small minority of nucleated cells and since morphologic criteria are not available for their identification, an indirect method was used to determine the fraction of each population in the S phase of the cell cycle.[20] The method is shown in Text-fig 2; aliquots of a suspension being tested are incubated for 20 minutes *in vitro* with increasing concentrations of tritiated thymidine (^{3}HTdR). The cells are washed free of radioisotope and tested for colony-forming ability. Cells in the S phase of the cycle lose colony-forming ability after exposure to concentrations in excess of 10 μCi/ml while cells in other phases are not affected. The specificity of the



TEXT-FIG 2—A diagram of the ^{3}H-thymidine suicide technic for determining the proportion of a cell population in DNA synthesis. Cell kill is observed after exposure to tritiated thymidine (^{3}HTdR).[20,35,53]

inactivation can be controlled by adding "cold" thymidine to the incubation mixture; this will prevent cell kill resulting from incorporation of the nucleotide into DNA but not effects related to external radiation or to nonspecific toxic substances.

When this method was applied to resting mouse marrow, no S phase CFU-S were detected while 20–40% of CFU-C were in DNA synthesis.[35] Thus, under resting conditions, the distribution of CFU-C in the cell cycle is different from that of CFU-S. However, when regenerating marrow was tested, 70–80% of both CFU-S and CFU-C were in the S phase, showing that each class is capable of responding to physiologic stimulus by altering cell cycle parameters. Similar results have been obtained by Rickard *et al*[36] using hydroxyurea, a compound that inactivates cells in DNA synthesis. It seems evident therefore that at least some CFU-C differ from CFU-S in resting marrow.

Effects of Mutation at W and Sl loci on CFU-S and CFU-C

In the mouse, two genetic loci have been identified that regulate colony formation in the spleen. These loci are *W* on chromosome XVII and *Sl* on chromosome IV.[37] The hematologic effect of mutations affecting these loci are recessive and true homozygotes seldom survive; accordingly analysis is made using animals bearing slightly different mutations in both alleles. For the *W* locus, these are mice of genotype W/W^v obtained by crossing B6-$+/W^v$ with WB-$W/+$. For the *Sl* locus, a cross is made between B6-$+/Sl^d$ and WC-$Sl/+$. Mice of genotypes W/W^v and Sl/Sl^d are black-eyed whites with severe macrocytic anemia and increased sensitivity to the lethal effects of ionizing radiation.[38] The hematologic and radiobiologic features of the phenotype arise from defective colony formation, but, as might be anticipated from the genetics, the defect is different in the two genotypes. Mice of genotype W/W^v have defective CFU-S; macroscopic colonies are not formed

when marrow from these animals is transplanted into heavily irradiated recipients. However, normal marrow cells will form colonies in spleens of mice of genotype W/W^v even if these recipients are not irradiated.[12] In contrast, hemopoietic cells from mice of genotype Sl/Sl^d from macroscopic spleen colonies in normal hosts but +/+ cells grow very poorly after they are transferred into irradiated Sl/Sl^d recipients.[39] These data are interpreted to mean that the *W* locus regulates a function intrinsic to CFU-S that is required for normal colony formation, while the *Sl* locus directs an environmental factor extrinsic to CFU-S. The activity of the *Sl* gene product cannot be demonstrated in parabiosis experiments [39] and has been transferred from animal to animal only by splenic transplantation.[40] It is probable, therefore, that the *Sl* gene product acts at short range, possibly through cellular interaction.[41]

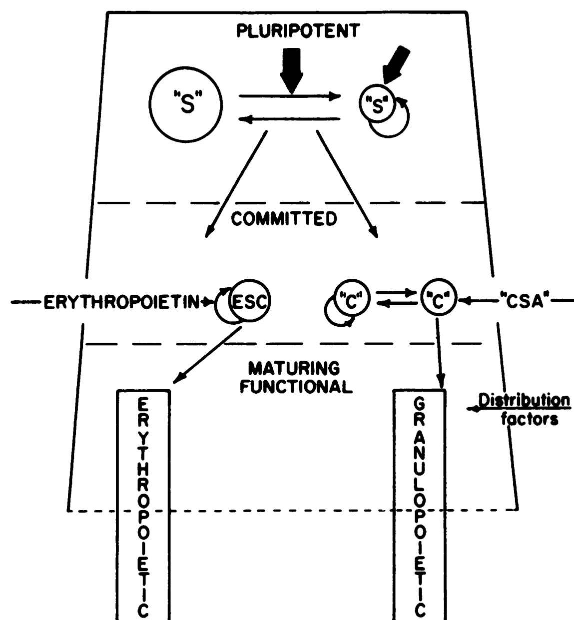
In contrast with the pronounced effect of mutation at *W* and *Sl* on CFU-S, CFU-C are little affected. Normal numbers of CFU-C are present in both types of genetically anemic mice,[34,42] and the culture colonies are indistinguishable from normal ones. Thus, the most clear-cut distinction between CFU-S and CFU-C was found in the genetically anemic mice. The possibility was considered that the requirements of the assays might be responsible for the observed difference; *W* and *Sl* function might be essential for the 20 generations required for spleen colony formation but not for the five generations needed for colonies to form in culture. However, this technicologic explanation was made improbable by extending the growth of CFU-C by transplanting cells from mice of genotype W/W^v into irradiated normal or Sl/Sl^d recipients.[34] Cells from the genetically anemic donors proliferated with doubling times of 29 hours, compared with 20 hours for cells of normal origin in both +/+ and Sl/Sl^d irradiated recipients. Thus, the intrinsic defect affecting CFU-S in mice of genotype W/W^v had only a modest effect on the growth of CFU-C and this prolongation of the doubling time could readily be explained if CFU-S, a pluripotent stem cell, is required for the generation of committed stem cells detected by the cell culture assay.

A Minimal Model of Myelopoiesis

The physical, physiologic and genetic evidence summarized in the preceding section can most readily be interpreted as indicating that CFU-S and CFU-C are distinct populations of progenitor cells, although the possibility is not excluded that some overlap exists between them. This conclusion, with the evidence upon which it is based and data from other sources, was used to construct the model of myelopoiesis pre-

sented in Text-fig 3. The outline of the figure is intended to represent the limits of a hemopoietic organ. Its expanding margins are symbolic of the exponential expansion that occurs during proliferation and differentiation. The organ is divided into three major compartments, although the interrupted lines separating these divisions are intended to suggest the possibility of overlap between them. At the top of the diagram, pluripotent stem cells are shown in two physiologic states: the majority are at rest, but able to undergo a reversible transition with a small population of proliferating cells. Self-renewal in this population is symbolized by a circular arrow. The margins of the hemopoietic organ surrounding pluripotent cells are shown as solid lines indicating that this compartment is relatively isolated from regulatory influences arising elsewhere in the body. Rather, control is achieved through short-term regulators, shown as arrows in the figure and acting either to affect the partition of cells between resting and cycling states or upon the self-renewing component. Pluripotent stem cells are designated in the diagram by the letter S relating them to the appropriate spleen colony assay.

Pluripotent stem cells are capable of irreversible transitions to committed progenitors of erythropoiesis or granulopoiesis. The latter are shown in the diagram as populations labeled *C*, indicating their



TEXT-FIG 3—A minimal model of myelopoiesis (see text).

capacity for colony formation in culture. This population is evenly partitioned between resting and proliferating cells. Indirect evidence is available that committed precursors of erythropoiesis exist (for a review, see McCulloch [10]); such cells are responsive to the hormone erythropoietin and are designated in the diagram as *ESC* or erythropoietin-sensitive cells. Experiments by Hodgson,[43] using vinblastine, indicate that ESC proliferate rapidly even when erythropoietin is not present. The margins surrounding the committed compartment are shown as open; regulators derived from other parts of the animal enter this compartment to affect CFU-C and ESC. For erythropoiesis, the regulator is erythropoietin whose primary site of production is believed to be the kidney. For granulopoiesis, colony-stimulating activity or CSA is shown in a position analogous to that of erythropoietin. While CSA is required for colony formation in culture, its physiologic role *in vivo* remains conjectural; however, alterations in levels observed during variations in peripheral granulocytes [44] are compatible with the view that this material or substances closely related to it play a role in regulation.

The last compartment contains the maturing, morphologically recognizable erythropoietic and granulopoietic cells. This compartment is shown closely related to the peripheral blood; mature forms are outside the hemopoietic organ and, at least for the polymorphonuclear leukocytes, regulatory mechanisms exist that influence the anatomic distribution of functional cells.

The different control mechanisms acting at three levels in myelopoiesis have survival value. Pluripotent stem cells are essential for continuing hemopoietic function. They require protection from external influences that might lead to their depletion; they respond, instead, to local influences regulating population size.[41] Committed progenitors form the basis for independent regulation of separate cell lineages derived from a common progenitor; therefore, these need to respond to external influences bearing information from other organ systems.

Both long- and short-range regulators acting on progenitors have numerically great effects upon the delivery of functional cells because of the amplification inherent in exponential growth. However, such effects are delayed by the time required for proliferation and differentiation. Accordingly, a final class of regulator is available to mobilize granulocyte reserves from marrow or marginal pools [45] to meet immediately the requirements imposed by bacterial invasion or trauma.

The model in Text-fig 3 is labeled a minimal model; other pathways of differentiation and other mechanisms of regulation undoubtedly exist.

However, the features displayed in the figure are needed to explain the data and provide a useful basis for interpreting clinical information.

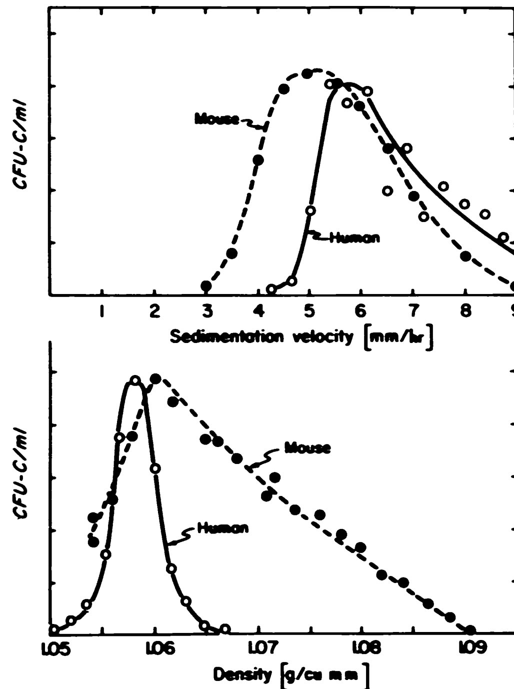
Significance of Normal Human CFU-C

Colony formation may be obtained in culture from cells of normal human marrow or peripheral blood using conditions very similar to those developed for mouse marrow.[4–8] Two critical requirements for culture are a viscid suspending material and colony-stimulating activity (CSA). In our laboratory, the suspending material is methyl cellulose and is assumed to prevent cell dispersion, allowing formation of discrete colonies. CSA is a cell product either elaborated in culture or obtained from body fluids. For the mouse, CSA has been obtained from a variety of cultured cells,[7,47] serum of leukemic mice[46] and concentrates of human urine[25]; partial purification has been achieved and the activity has tentatively been identified with a glycoprotein of molecular weight 45,000–190,000.[25,47] For human cells, the most reliable source of CSA is human peripheral leukocytes.[7,8] Supernatants of cultures of these cells have been found to be active by Chervenick *et al*,[7] and Iscove *et al*[8] have reported potent CSA activity developing in culture fluid layered over normal leukocytes immobilized in agar. CSA that is active for human cells also stimulates colony formation by mouse cells. However, the reverse is not true; CSA derived from mouse cells will not improve colony formation by human marrow. Human cells will form small colonies even without the addition of CSA. Since such colonies, which contain 10–50 cells, appear only with cell inocula in excess of 5×10^4 cells per dish, it is probable that their development depends on small amounts of CSA produced by cells added to the assay plates.

If human CFU-C are to provide a clinically useful indicator of marrow function, it is necessary to know whether or not these cells occupy a place in myelopoiesis equivalent to that of mouse CFU-C. In an attempt to answer this question, physical and physiologic properties of human CFU-C were compared with available data from the mouse.

Comparison of Physical Properties of Human and Murine CFU-C

On the basis of density and velocity measurements, mouse CFU-C are known to be larger and lighter than the majority of nucleated cells in marrow. The same methods of cell separation were applied to marrow specimens obtained from patients without hematologic disease. A comparison of the results obtained with the mouse data is presented in Text-fig 4. The lower panel of the figure is obtained by centrifugation to equilibrium in gradients of Ficoll[48] while the upper panel is derived



TEXT-FIG 4—A comparison of murine and human CFU-C by velocity sedimentation (*top*) and equilibrium density centrifugation in Ficoll (*bottom*).

from sedimentation at unit gravity in a Staput apparatus.[49] It is apparent that human cells are both slightly lighter and slightly larger than those of mice; however, in each instance, human CFU-C occupy positions very similar to mouse CFU-C relative to the profile of nucleated cells.

The data from velocity sedimentation of human cells has already been applied to a specific human problem, that of marrow transplantation.[50] In the mouse, cells responsible for graft-versus-host disease sediment more slowly than CFU-C. A direct measurement of graft-versus-host activity is not available for human marrow, but cells responding to homologous peripheral blood leukocytes in the mixed leukocyte reaction peak at lower values than CFU-C.[51] On this basis, marrow to be used for transplantation was separated in a large Staput vessel. A pool of fractions containing rapidly sedimenting cells was transplanted into a leukemic patient after preparation according to the method of Santos.[52] As judged by blood group markers, a successful engraftment was achieved which persisted for approximately 75 days. Graft-versus-host disease was mild, consisting of recurrent episodes of desquamating skin rash and mild diarrhea; the patient died on the eighty-first day after transplant because of graft failure. The separation procedure used was based on

assays of CFU-C and provides an example of their use as the guide to stem cells in clinical experimentation.

Cell Cycle Studies of Human CFU-C

Under resting conditions, approximately 40% of mouse CFU-C are in the DNA synthetic phase of the cycle; this proportion increases to 80% during marrow regeneration.[35] Since the ^{3}HTdR suicide assay is performed *in vitro*, it can be applied directly to human cells.[53] In a series of 6 patients without evidence of marrow stimulation, values ranging from 10 to 30% kill have been obtained. During growth of a marrow transplant (see above) the kill was 37%. It was also possible to make measurements on serial marrow aspirations from a patient recovering after 225 rads of whole-body radiation. Before irradiation, the kill was 19%, while during the recovery phase values of 43 and 48% were obtained. The number of observations are few; nonetheless, the results are consistent with the view that, like the mouse, human CFU-C change their cell cycle parameters in response to stimulus for rapid growth.

In summary, consistent evidence is available that human CFU-C are physically and functionally similar to murine CFU-C. If this conclusion is valid, the culture procedure does not measure pluripotent stem cells but rather a descendant committed to granulopoiesis. However, the close association of mouse CFU-S and CFU-C provides assurance that CFU-C may be used as a guide to the position of pluripotent stem cells in separation procedures and during recovery after injury. Moreover, the cell cycle experiments provide direct evidence that a regulatory mechanism known to exist in the mouse also operates in human myelopoiesis. This mechanism, which affects the cell cycle parameters of progenitor cells, is believed to be important in protecting normal marrow function in patients treated with chemotherapy. Many chemotherapeutic drugs act at specific phases in the cell cycle and exhibit greater toxicity for tumor cells than for normal cells because the former have a greater probability than the latter of being in a sensitive phase of the cycle. Thus, if human pluripotent stem cells, like their committed descendants and their murine equivalents, undergo reversible transitions between states of rapid proliferation and states of rest, the cellular basis for high-dose intermittent chemotherapy suggested for the mouse by Bruce and his collaborators [54] may also apply to man.

CFU-C in Human Leukemia

In the preceding section, evidence was presented to validate the use of CFU-C as a measurement of normal marrow function. In this

section, preliminary findings in human leukemia will be summarized and the hypothesis advanced that the culture method may provide a useful tool for the investigation of the disordered proliferation and differentiation characteristic of this disease.

Typical Findings in Adult Acute Myelogenous Leukemia

Marrow from patients with acute myelogenous leukemia (AML) in relapse is defective in its capacity to form colonies in culture.[4,5,8] In the absence of CSA, only very occasional small colonies are found even at high cell inocula. When CSA is added, the number of colonies increases and it is possible to obtain a linear relationship between cells plated and colonies observed. However, such colonies are smaller than normal and the efficiency of formation is much less than that for normal marrow. Like normal marrow, nonetheless, many cells are mature granulocytes in colonies derived from leukemic patients.

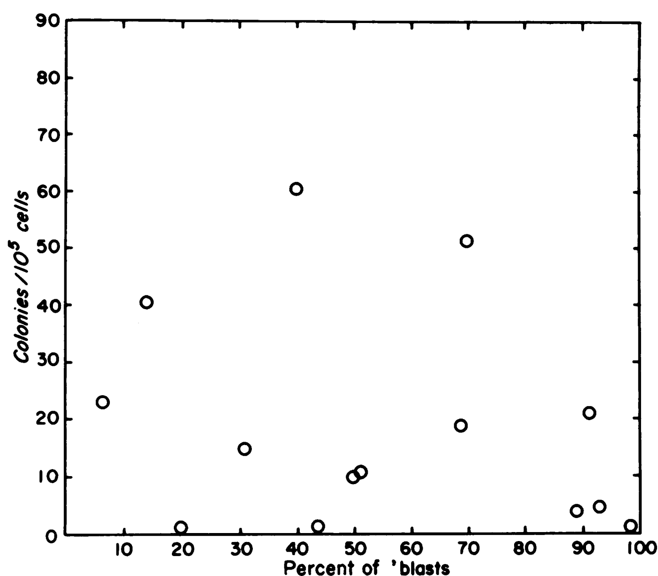
Since relapse is characterized by a high proportion of leukemic blast cells in the marrow, it appeared possible that defective colony formation might reflect dilution of normal elements by malignant cells. Such an explanation would lead to the prediction that a close correlation should be found between the number of marrow blasts and the efficiency of colony formation. A test of this hypothesis is presented in Text-fig 5; in the figure, colony-forming efficiency (CFU-C/10^5 nucleated cells plated with CSA) is plotted against the percent of blasts in the marrow for 14 patients with AML. In every case, the efficiency of colony formation was much less than the 80–120 CFU-C/10^5 cells found for normal marrow. However, a close correlation with percentage of blasts was not found.

CFU-C in Myelomonocytic or Promyelocytic Leukemia

Senn [55] has studied marrow cultures obtained from 4 patients considered to have myelomonocytic or promyelocytic leukemia. All of the patients were elderly and 3 had elevated levels of urinary muramidase. In all 4 cases, efficiency of marrow colony formation was markedly increased; values ranged from 150 to in excess of 300 CFU-C/10^5 cells. Further, similar efficiencies were obtained even in cultures without CSA. Elevated numbers of CFU-C have been reported by Pike and Robinson in the marrow of some of their patients with acute leukemia.[6]

The Effect of Remission-Induction on CFU-C in Acute Myelogenous Leukemia

Harris and Freireich have reported improvement in efficiency of colony formation after remission-induction in acute leukemia.[5] We have confirmed this finding in 8 patients who achieved hematologic remission



TEXT-FIG 5—Marrow findings in 14 patients with acute myelogenous leukemia before treatment. The number of CFU-C per 10^5 nucleated cells is shown as a function of the percentage blast cells in the marrow. The cultures were prepared with colony-stimulating factor from human leukocytes.[8]

in AML with intermittent combination chemotherapy (vincristine, cytosine arabinoside and cyclophosphamide). Remission was usually associated with greatly increased efficiency of colony formation although, after some courses, few colonies were formed when there were less than 5% blast forms in the marrow. In many instances, colony-forming efficiency was in excess of three times normal values. In most instances, however, the colonies were smaller than those seen in normal cultures and usually colony-forming efficiency fell either above or below the normal range. In the absence of control data showing the effect of chemotherapy on normal marrow, detailed conclusions cannot be reached. However, changes with therapy support the hypothesis that the culture method yields data relevant to cellular mechanisms in leukemia.

Unanswered Questions

An unassailable explanation for the culture findings in human leukemia is not available; nonetheless, the data may be examined in light of current models both of leukemia and of myelopoiesis.

The most straightforward explanation of the data is that CFU-C in leukemic marrow are derived from normal hematopoietic progenitor cells coexisting with one or more leukemic populations. After treatment with

cytotoxic chemotherapeutic agents, the load of leukemic cells is reduced and marrow function is restored by the proliferation and differentiation of surviving normal stem cells. The variation in numbers of CFU-C found in patients in remission or about to enter remission might reflect different points on kinetic curves for marrow recovery.

If the CFU-C found in patients in leukemia in relapse can be interpreted to mean that an equivalent number of pluripotent stem cells are also present, it is necessary to conclude that at least in some instances many, perhaps normal, numbers of stem cells coexist with leukemic cells. The failure of such cells to maintain a normal supply of functional cells would then constitute an important part of the cullular defect in acute leukemia and might provide a target for therapy.

A somewhat different explanation of the data may be considered in light of the model of myelopoiesis presented in Text-fig 3. An essential feature of the model is that most pluripotent stem cells do not form colonies under the conditions used to detect CFU-C. If leukemic transformation of CFU-S altered the mechanism that regulates the transition to CFU-C, the proportion of cells capable of colony formation in culture might be reduced. A decreased rate of production of CFU-C might account for at least part of the defective myelopoiesis in leukemia. Similar reasoning provides an explanation of the excess of CFU-C found in myelomonocytic leukemia. This form might be considered to be the consequence of lesions affecting other specific regulatory mechanisms controlling proliferation or differentiation. An important feature of the model presented in Text-fig 3 is that such regulatory mechanisms are multiple; indeed it is reasonable to assume that many more exist than have been discovered. It is thus reasonable to consider that lesions affecting specific mechanisms would lead to characteristic clinical pictures. The delineation of such pictures depends upon the availability of discriminatory assays. If the culture method detects committed progenitor cells independently of their pluripotent progenitors, it may provide an example of such an assay.

The second explanation of cellular events in leukemia has important implications for therapy. If CFU-C in patients with leukemia are derived from leukemic pluripotent stem cells, strategies designed to eliminate such cell cells are unlikely to be successful. Rather, remissions as presently achieved with chemotherapy may be the result of temporary corrections of regulatory failure.

Conclusion

The hemopoietic system plays a central role in many problems that

confront the clinical oncologist. The treatment of nonhematologic malignancies is often limited by damage to bone marrow. If better methods were available to quantitate this damage and study the kinetics of repair, the range of radiation or chemotherapeutic dosage might be extended or safer regimens of treatment devised. The availability of a culture method for measuring a class of hemopoietic progenitors closely related to pluripotent stem cells opens the door for such studies directly in man. Human hematologic malignancies, the leukemias, present an even greater opportunity for the experimental hematologist. Uncovering the specific sites of lesions leading to disordered hematopoiesis may be expected to increase our knowledge of normal regulation. Perhaps such studies may even yield novel approaches to therapy.

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