Haematology and Blood Transfusion Vol. 26
Modern Trends in Human Leukemia IV
Edited by Neth, Gallo, Graf, Mannweiler, Winkler

Assessment of Human Pluripotent Hemopoietic Progenitors and Leukemic Blast-Forming Cells in Culture*

H. A. Messner, A. A. Fauser, R. Buick, L. J-A. Chang, J. Lepine, J. C. Curtis, J. Senn, and E. A. McCulloch

A. Introduction

Myeloproliferative diseases such as acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and polycythemia vera (PV) are now generally considered to be clonal disorders originating in abnormal stem cells (Wiggans et al. 1978; Fialkow et al. 1967, 1977; Adamson et al. 1976; McCulloch and Till 1977; McCulloch 1979). A growth advantage typically displayed by the abnormal clone appears to be responsible for the increased production of phenotypically normal or abnormal cells. The underlying mechanisms for the altered growth rate are presently not understood. Further investigations of this phenomenon are dependent upon the development of assays that facilitate assessment of normal human pluripotent stem cells and permit identification of members of the abnormal clone present in these disorders.

We have recently described such an assay for human pluripotent progenitors (Messner and Fauser 1978; Fauser and Messner 1978, 1979a). They can be readily identified in culture by their ability to form mixed hemopoietic colonies that contain components of all myeloid lineages. Blast-forming cells in patients with acute myeloid leukemia are also accessible for studies in culture (Dicke et al. 1976; Park et al. 1977; Buick et al. 1977). They give rise to colonies of cells with leukemic phenotype. In addition, blast colonies from patients with cytogenetic markers displayed the same chromosomal abnormality as that observed for primary blast cell populations (Izaguirre and McCulloch 1978). These assays permitted investigations directed to examine mechanisms of increased proliferation by utilizing the following principle approaches: The proliferative state of clonogenic cells can be directly assessed by comparing the plating efficiency in controls with that observed after short-term exposure to H_3TdR. Recloning experiments of colonies are instrumental in examining the proliferative potential of cells with respect to self-replication.

It is the purpose of the present communication to review the currently available information about proliferative parameters of human pluripotent progenitors and leukemic blast colony-forming cells and to demonstrate their potential use for the assessment of individual patients.

B. Methodology

I. Culture Conditions for CFU-GEMM and Blast Colony-Forming Cells

The culture assays for CFU-GEMM and blast colony forming cells followed well established principles for hemopoietic progenitors. CFU-GEMM in bone marrow and peripheral blood specimens of normal individuals (Messner and Fauser 1978; Fauser and Messner 1978, 1979a) and patients with leukemia or PV were grown with α-medium or modified Dulbecco's MEM, fetal calf serum, erythropoietin, and medium conditioned by leukocytes in the presence of phytohemagglutinin (PHA-LCM) (Aye et al. 1974). After 12 to 14 days of culture in humidified atmosphere supplemented with 5% CO_2, colonies of 500 to 10,000

* Supported by the Medical Research Council of Canada and the Leukemic Research Foundation, Toronto

cells could be identified that contained granulocytes, erythroblasts, megakaryocytes, and macrophages.

Identical culture conditions led to the development of blast cell colonies in peripheral blood specimens of patients with acute myeloid leukemia (Buick et al. 1977). These colonies develop within 5 to 7 days of culture and grow to a size of 20 to 200 cells with blast cell morphology. Numerical assessment of colonies in primary cultures yielded the primary plating efficiency (PE1) (Buick et al. 1979).

II. Cycle State Analysis

The proliferative state of CFU-GEMM and blast colony-forming cells was examined as previously described (Becker et al. 1965; Minden et al. 1978).

III. Assessment of Self-Renewal

Individual mixed hemopoietic colonies were sufficiently large to be removed from the cultures by micropipette for redispersion into a single cell suspension (Messner and Fauser 1979) and subsequent replating in Linbro micro titer wells. Some colonies were seeded under conditions identical to those employed for primary colonies; others were, in addition, exposed to the feeder effect of 2×10^4 irradiated mononuclear peripheral blood cells from normal individuals. Secondary colonies were scored after 12 to 14 days of culture.

Primary blast cell colonies within individual plates were pooled and redispersed. These cells were counted prior to replating with 2×10^4 irradiated normal peripheral blood cells (Buick et al. 1979). The results were expressed as secondary plating efficiency (PE2).

IV. Separation of PHA-LCM

Isoelectric focusing in a density gradient was used to separate PHA-LCM (Scandurra et al. 1969; Fauser and Messner 1979b). Each fraction was dialyzed, reconstituted with DMEM, and examined for stimulatory activity for CFU-GEMM, BFU-E, CFU-C in normal individuals and blast colonyforming cells in patients with acute leukemia.

C. Results

I. Comparison of the Culture Conditions for CFU-GEMM and Blast Colony Forming Cells

Almost identical culture conditions are employed for both clonal assays. The addition of PHA-LCM is essential for the development of mixed hemopoietic colonies and blast cell colonies in the majority of the examined specimens; only occasionally can mixed colonies (Messner et al., to be published) or blast cell colonies be observed without the addition of this stimulatory material. With the exception of PV, erythropoietin is required for the maturation of erythroid cells within mixed colonies. Attempts were not successful to alter the cellular composition of blast cell colonies and induce erythroid differentiation with erythropoietin.

Some information is now available that the stimulatory activity for both colony types present in PHA-LCM may be associated with different molecules (Fauser and Messner 1979b). Separation of PHA-LCM by isoelectric focusing yielded the stimulatory activity for CFU-GEMM, BFU-E, and CFU-C in fractions of Ph 5 to 6.5. In contrast, stimulatory activity for leukemic blast cells was observed in fractions of Ph 5.5 to 7.5. This information suggests that molecules responsible for the stimulation of CFU-GEMM and blast colony forming cells are not completely identical.

II. Assessment of the Proliferative State of CFU-GEMM and Blast Colony Forming Cells

The proliferative state of CFU-GEMM was assessed by short term exposure to H_3TdR for patients with different clinical conditions: normal individuals in steady state and during bone marrow regeneration and patients with various clonal hemopathies such as PV, CML AML and acute myelofibrosis. CFU-GEMM were found to be quiescent under steady state conditions. They proliferate actively during bone marrow regeneration, for instance after bone marrow transplantation (Fauser and Messner 1979c). A reduction of the plating efficiency to 50% of control values was regularly observed during the early phase of engraftment. CFU-GEMM in patients with

clonal hemopathies generally displayed a similar increase in proliferative activity. Data are available for six patients with PV (Fauser and Messner 1979d), seven patients with CML (Messner et al. 1980; Lepine and Messner, unpublished work), one patient with AML and acute myeolofibrosis (Messner et al. 1980) that demonstrate a reduction of the plating efficiency ranging from 20% to 60%. The results for patients with PV suggest that the proliferative state of individual patients was independant of the clinical condition or therapeutic modulation. Similar examinations are in progress for patients with CML and AML.

The cycle state of blast colony forming cells was assessed for patients with acute myeloid leukemia and patients with preleukemia that contained blast colony forming cells in their peripheral circulation (Senn et al. 1979). Minden et al. (1978) demonstrated that blast colony forming cells in all examined patients with acute myeloid leukemia were found to be in cycle as documented by a 50% reduction of their plating efficiency with short-term exposure to H_3TdR. This observation was confirmed for three of four patients with preleukemia (Senn et al. 1979). Studies are in progress on patients with long-lasting remissions that have not been subjected to recent chemotherapeutic interventions.,

III. Recloning of CFU-GEMM and Blast Colony Forming Cells

Mixed hemopoietic colonies were removed from the cultures by micropipette, redispersed to yield a single cell suspension, and replated in Linbro micro titer plates. These experiments were performed using mixed colonies grown from bone marrow samples of four normal individuals. Of the 107 mixed colonies 26 gave rise to 13 mixed, 192 pure granulocytic, and 195 erythroid secondary colonies. This observation supports the view that clones derived from various pluripotent progenitors are heterogeneous. While 75% of primary mixed colonies contained only mature elements, some primitive progenitors were identified in 25%.

Recloning experiments of blast cell colonies derived from various patients with acute myeloid leukemia demonstrated that secondary colonies of blast cell phenotype can be observed (Buick et al. 1979). The frequency may vary greatly. Morphologically, the secondary colonies appear to be rather homogeneous. However, functional assessment of pooled blast colonies as feeder cells indicated that cells within blast cell colonies may elaborate stimulators that promote growth of blast cell colonies (Taetle, personal communication).

1. Clinical Correlations

It was attempted to assess the clinical value of these culture studies by correlating the recloning efficiency of primary blast cells with the response of patients to chemotherapy. In 44 patients, a significant association was found between low PE2 values and successful remission induction (Buick et al. 1979; Buick et al. 1981).

2. Modulation of Cloning Efficiency In Vitro

Since the recloning efficiency may represent an important prognostic parameter to predict the responsiveness of patients to chemotherapy, the question of whether the recloning efficiency represents an invariable determinant for each patient or whether it can be modulated was asked. This was tested in three different experimental approaches: primary blast colonies were grown after exposure to adriamycin and cytosine aribinoside (ARA-C) and surviving colonies replated. While dose-dependent reduction of PEl was regularly observed for both drugs, PE2 was not influenced by adriamycin but was decreased for ARA-C. In addition, tumor promotors such as TPA enhanced the recloning efficiency (PE2) (Chang and McCulloch 1979). Taetle and McCulloch (1979) reported that interferon was capable of inhibiting PE2.

D. Conclusions

Assays are now available that permit the identification of human pluripotent hemopoietic progenitors in culture and facilitate assessment of leukemic cell populations that form colonies of blast cell phenotype. Both colonies develop under almost identical culture conditions and require stimulatory molecules that are provided by PHA-LCM. Preliminary evidence suggests that these molecules may not be identical and may be separable by procedures such as isoelectric focusing. CFU-GEMM of

normal individuals under steady state conditions appear to be quiescent. This contrasts with results obtained for the proliferative state of CFU-GEMM during regeneration and for patients with various clonal hemopathies. It was feasible to study patients with PV and CML prior to and subsequent to therapeutic interventions without demonstrating changes in the proliferative state. The increased proliferative rate of CFU-GEMM may adequately explain the increased number of cells observed in these disorders. The active proliferation of leukemic blast colony forming cells is consistent with the interpretation of a growth advantage of blast cells observed under these clinical conditions. This was further documented by assessing the recloning ability of blast colony forming cells. In addition, it becomes apparent that chemotherapeutic intervention in patients with acute myeloid leukemia is complex and may include, beside cytoreduction, changes in the recloning ability of blast colony forming cells. It is thus conceivable to select drugs with differing biological effects for patients with specific cell culture prognostics.

Our studies thus far have not succeeded in identifying the relationship of pluripotent hemopoietic progenitors and blast-forming cells. One of the main difficulties is related to the low frequency of mixed colonies observed during phases of relapse and the greatly reduced number of blast cell colonies at the time of remission. The observation that CFU-GEMM as well as blast cell colonies can be recloned may provide a powerful tool to be employed in the future, particularly if specific fractions of PHA-LCM become available to facilitate selective cloning.

References

Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann E (1976) Polycythemia vera: Stem cell and probable clonal origin of the disease. N Engl J Med 295:913–916 – Aye MT, Niho Y, Till JE, McCulloch EA (1974) Studies of leukemic cell populations in culture. Blood 2/205:219 – Becker AJ, McCulloch EA, Siminovitch L, Till JE (1965) The effect of differing demands for blood cell production on DNA synthesis by hemopoietic colony forming cells of mice. Blood 26:296–308 – Buick RN, Chang LJ-A, Messner HA, Curtis JE, McCulloch (1981) Self-renewal capacity of leukemia blast progenitor cells. Cancer, in press – Buick RN, Till JE, McCulloch EA (1977) Colony assay for proliferative blast cells circulating in myeloblastic leukemia. Lancet I:862–863 – Buick RN, Minden MD, McCulloch EA (1979) Self renewal in culture of proliferative blast progenitor cells in acute myeloblastic leukemia. Blood 54:95–104 – Chang LJ-A, McCulloch EA (1979) A tumor promotor increases the self renewal of blast progenitors in acute myeloblastic leukemia. Blood [Supp I] 54/5: p 169a – Dicke KA, Spitzer G, Ahearn MJ (1976) Colony formation in vitro by leukemic cells in acute myelogenous leukaemia with phytohaemagglutinin as stimulating factor. Nature 259:129–130 – Fauser AA, Messner HA (1978) Granuloerythropoietic colonies in human bone marrow, peripheral blood and cord blood. Blood 52:1243 – Fauser AA, Messner HA (1979a) Identification of megakaryocytes, macrophages and eosinophils in colonies of human bone marrow containing neutrophilic granulocytes and erythroblasts. Blood 53:1023 – Fauser AA, Messner HA (1979b) Separation of stimulating activity in PHA-LCM by isoelectric focusing for leukemic blast cell colonies and colonies of normal hemopoietic progenitors. Blood [Supp I] 54: p 107 – Fauser AA, Messner HA (1979c) Proliferative state of human pluripotent hemopoietic progenitors (CFU-GEMM) in normal individuals and under regenerative conditions after bone marrow transplantation. Blood 54/5:1197 – Fauser AA, Messner HA (1979d) Pluripotent hemopoietic progenitors in peripheral blood of patients with polycythemia rubra vera. Blood [Supp I] 54: p 138 – Fauser AA, Messner HA (submitted to Blood) Pluripotent hemopoietic progenitors (CFU-GEMM) in polycythemia vera: Analysis of erythropoietin requirement and proliferative activity – Fialkow PJ, Gartler SM, Yoshida A (1967) Clonal origin of chronic myelogenous leukemia in man. Proc Natl Acad Sci USA 58:1468–1471 – Fialkow PJ, Jacobson RJ, Papayannopoulou TH (1977) Chronic myelocytic leukemia: Clonal origin in a stem cell common to the granulocyte erythrocyte, platelet and monocyte/macrophage. Am J Med 63:125–130 – Izaguirre CA, McCulloch EA (1978) Cytogenetic analysis of leukemic clones. Blood [Supp 1] 52:287 – McCulloch EA (1979) Abnormal myelopoietic clones in man. J Natl Cancer Inst 63:883–891 – McCulloch EA, Till JE (1977) Stem cells in normal early hemopoiesis and certain clonal hemopathies. In: Hoffbrant AV, Brain M, Hersh J (eds) Recent advances in hematology, vol 2. Churchill-Livingston, London, pp 85–110 – Messner HA, Fauser AA (1978) Distribution of fetal hemoglobin in individual colonies by RIA: A lead towards human stem cells. In: Stamatoyannopoulos, Nienhuis (eds) Cellular and molecular regulation of hemoglobin switching. Grune & Stratton, pp 379 – Messner HA, Fauser AA (1979) Human pluripotent hemopoietic progenitors (CFU-GEMM) in culture. In: NIH Conference on Aplastic Anemia, San Francisco. – Messner HA, Fauser AA, Lepine J, Martin M (1980) Properties of human pluripotent hemopoietic proge-

nitors. Blood Cells 6:595–607 – Minden MD, Till JE, McCulloch EA (1978) Proliferative state of blast cell progenitors in acute myeloblastic leukemia. Blood 52:592–600 – Park C, Savin MA, Hoogstraten B, et al. (1977) Improved growth of the in vitro colonies in human acute leukemia with the feeding culture method. Cancer Res 37:4594–4601 – Scandurra R, Cannella C, Elli R (1969) Use of isoelectric focusing in the purification of L-glutamate – phenylpyruvate aminotransferase. Sci Tools 16:17 – Senn JS, Pinkerton PH, Messner HA, McCulloch EA (1979) Detection of blast cell colonies in preleukemic states. Blood [Suppl I] 54/5: p 175a – Taetle R, Buick RN, McCulloch EA (1980) Effect of Interferon on colony formation in culture by blast cell progenitors in acute myeloblastic leukemia. Blood 56:549–552 – Wiggans RG, Jacobson AJ, Fialkow PJ, et al. (1978) Probable clonal origin of acute myeloblastic leukemia following radiation and chemotherapy of colon cancer. Blood 52:659–663