EDITORIAL

Hematopoietic Stem Cell Theory in Relation to Possible Lymphoblastic Conversion of Chronic Myeloid Leukemia

By Dane R. Boggs

CURRENT EVIDENCE SUGGESTS that chronic myeloid leukemia (CML) must be considered a clonal disease of a pluripotent hematopoietic stem cell compartment. The Philadelphia chromosome (Ph') abnormality (translocation of a portion of the long arms of chromosome 22 onto chromosome 9') is found not only in neutrophil precursors, but in precursors of red cells, eosinophils, and probably platelets as well as monocytes. These observations plus the failure to find the Ph' in skin cells or lymphocytes from patients with CML or in bone marrow cells from their nonleukemic identical twins indicate involvement of selected cellular systems. The clonal nature of the disease is further suggested by studies of affected patients who had mosaicism of sex chromosomes or were heterozygous for certain isozymes. As CML developed, nonmyeloid cells remained mosaic or heterozygous for isozymes, but myeloid cells were uniform in respect to sex chromosomes or isozymes. Thus, the simplest explanation is that the disease begins in a single stem cell, pluripotent for neutrophils, monocytes, eosinophils, red cells, megakaryocytes, and probably basophils. The affected cell has a growth advantage over normal stem cells so that eventually virtually all active stem cells bear the Ph'.

Normal stem cells may still be detected in vitro and further, if intensive chemotherapy is given, occasional patients recover with a normal appearing marrow and lose the Ph' defect for some months at least. The stem cell defect is not identical in all patients in that the degree of feedout into various cellular paths varies from patient to patient, at least as reflected in blood levels of the various myeloid leukocytes, of platelets, and of red cells.

Blastic crisis, the most common cause of death in patients with CML, is characterized by increasing failure of maturation with continued overproduction of immature cells, most commonly myeloblasts and promyelocytes, but underproduction of mature neutrophils, platelets, and red cells. Based on chromosome studies, blast crisis appears to be due to acquisition of yet another clone of abnormal stem cells in addition to the Ph' clone. Patients going into blast crisis have been observed to acquire a second Ph' or other abnormal...
chromosome, lose the second with a return to a CML-like phase following therapy, and reacquire it as blastic crisis recurs.

In acute myeloid leukemia (AML) chromosomal abnormalities also involve red cell precursors and may disappear with remission and reappear with relapse. In this respect AML and blastic crisis of CML are rather similar.

The morphologic expression of AML is quite variable, and the predominant cell may be a myeloblast, promyelocyte, eosinophilic promyelocyte, basophilic promyelocyte, monocyte, "myelomonocyte," or proerythroblast. Megakaryocytic types are omitted from this list, but recognition of very immature megakaryocytes is difficult, if not impossible, but current techniques, and fairly marked megakaryocyte morphologic abnormality may be observed in AML. Since there are only minor differences in the clinical expression of these morphologically diverse syndromes, they can be thought of as representing slightly different expressions of a clonal disease of the stem cell system. Thus, one might anticipate that blastic crisis of CML would not always be expressed morphologically in myeloblastosis, and indeed it is not. Promyelocytes may predominate, as may myelomonocytes. I have seen one patient in whom the predominate cell became a "Schilling type" monocyte, and occasionally proerythroblasts become the predominate cell. All of the above makes some sense within the aforementioned concept of CML being a disease of the pluripotent stem cell system. I want to raise the suggestion that still another form of blastic conversion may occur, one that does not seem to make sense at first glance, namely, lymphoblastic conversion in CML.

I have seen slides from three patients with preexisting Ph$^+$ positive CML who converted to a blastic phase in which the blasts had the morphologic characteristics of lymphoblasts in Wright’s-stained smears. These smears were coded, mixed with smears from other patients with AML or ALL, and repeatedly called lymphoblastic by experienced observers. Such morphologic identification is in no sense proof that they were lymphoblasts. However, ALL usually responds to steroid therapy, while AML does not. It may be of significance that steroid response or lack of response in acute leukemia can be predicted with a probability exceeding 0.9 by such morphologic study. One of these patients was given prednisone, 60 mg/day, for 1 wk during which blasts in the blood declined from $80 \times 10^9$/liter to virtually 0, but she died of infection. The second developed complete remission with prednisone, vincristine, and cyclophosphamide, and the third remitted on a complex protocol of multidrug therapy which allows no implications for cell type. The response of a number of patients with CML in blast crisis to prednisone and vincristine, agents with little effect in AML, is of some interest.

If they were lymphoblastic we might consider it chance development of ALL in a patient with CML, but alternatively, there is reason to believe that lymphoblastic conversion of CML might occur from consideration of current evidence about the structure of the hematopoietic stem cell system.

A variety of techniques are now available which allow measurement of changes in murine stem cells. Response of plethoric mice to erythropoietin is a relatively direct measure of stem cells feeding the erythroid compartment. In vitro culture of cells in semisolid media measures a cell pluripotent for
neutrophils, monocytes, and eosinophils, and liquid culture and culture of cells in diffusion chambers implanted in animals probably measure growth and the progeny of the same cell (these in vitro techniques are the only stem cell assays which have also been applied to man). Transplantation of mouse marrow cells, spleen cells, nucleated blood cells, peritoneal cells, or exudate cells into lethally irradiated mice leads to formation of spleen colonies. These colonies, shown to be clonal under certain circumstances, contain neutrophils, monocytes, eosinophils, red cells, and megakaryocytes as well as unidentified medium-sized round cells in either "pure" or mixed populations. There is cytogenetic evidence in mice and in rats that spleen colonies and myeloid tissue can be repopulated by cells which share common ancestry with cells repopulating lymphoid tissue. Whether the spleen colony-forming cell is capable of lymphoid differentiation under the extreme circumstances of these experiments or whether there is activation of a still more primitive stem cell pluripotent for colony-forming cells and lymphoid tissue has not been determined (Fig. 1). Endogenous (as opposed to transplanted) stem cells can also be assayed by the spleen colony technique in sublethally irradiated mice or in heavily irradiated mice in which marrow is shielded. From these studies it appears that there is a murine stem cell which is pluripotent for all blood cells, including the lymphocyte.

However, the long-standing debate concerning a single versus multiple individual stem cells (unitarian, neonunitarian, and polyphyletic theories) is not entirely settled. The various murine stem cell assays rarely are in agreement as regards changes in compartment size. This has led to the hypothesis that there are concatenated stem cell compartments of increasing differentiation (Fig. 1). Part of the evidence for this consists of observations that the compartment producing transplanted spleen colonies is either primarily in G0 or in a very long G1, while a much greater proportion of compartments measured by other techniques is in DNA synthesis. The exact structure of concatenation of compartments is far from clear, and an alternate, unitarian theory in which there is only one stem cell which differentiates into a given cell type according to a probability function related to the stage of the cell cycle has not been con-

Fig. 1. A possible model of the hematopoietic stem cell system. Each cell portrayed is a stem cell, i.e., capable of self-replication as well as differentiation. The myeloid system is shown as normally maintained by three specialized stem cells. There is a cell pluripotent for lymphocytes and myelocytes, but its exact relation to the cell pluripotent for all myeloid tissue is uncertain. As discussed in the text, the exact structure of the entire system is unclear.
clusively disproved. For example, Goldwasser has data suggesting that the erythropoietin-sensitive cell is sensitive primarily during $G_2$.\(^{22}\) Lajtha\(^{23}\) made a statement which seems very appropriate concerning the current state of knowledge of murine stem cell compartment structure: "We have reached a fairly complex state of ignorance."

Independent of the exact structure of the murine stem cell pool, there is fairly compelling evidence\(^{17}\) that at some point in the system there is a cell which is pluripotent for both myeloid and lymphoid tissue. If there is a similar cell in man and if CML is a disease of that cell, lymphoblastic conversion of CML would be a reasonable expectation.

Superficially, it would seem that the failure to find the Ph\(^1\) defect in lymphocytes exposed to phytohemagglutinin (PHA) from patients with CML would negate the concept that CML is a disease involving a stem cell which is pluripotent for myeloid and lymphoid tissue. Lymphocytes which enter DNA synthesis and undergo mitosis when exposed to PHA are thought to be thymus dependent or "T" lymphocytes. However, even if CML is due to an abnormal cell, pluripotent for lymphocytes as well as myeloid cells, replacement of the normal T compartment by leukemic T cells would not be anticipated for years or perhaps not at all. Kinetic studies of T lymphocytes suggest that they have an extraordinarily long intermitotic survival time, perhaps averaging many years.\(^{24}\) Furthermore, the lymphocyte may act as its own stem cell in the mature animal; the "mature" lymphocyte which is induced to divide may reproduce itself. Thus, even if CML is a disease of a stem cell capable of undergoing lymphocytic differentiation, it might take many years before the defect appeared in an appreciable number of PHA-stimulated lymphocytes. Most reported studies of chromosomes in PHA-stimulated lymphocytes from patients with CML are from patients in whom the diagnosis was made shortly before the study was performed,\(^{4}\) and similar studies in the occasional long-term survivors with CML would be of interest.

The hypothesis that lymphoblastic conversion of CML may occur is based on what many will consider to be highly questionable data, appearance of blasts in Wright's-stained smears. However, this hypothesis can be tested, and it is hoped that this editorial will stimulate an examination of such measures as "B" and "T" surface characteristics in the occasional blastic conversion in CML in which the immature cells are not obviously of myeloid origin.

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REFERENCES

7. Clarkson B: Personal communication
22. Goldwasser E: Personal communication
23. Lajtha LG: Address given before the American Federation of Clinical Research, Atlantic City, N.J., 1973
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