Use of Glucose-6-Phosphate Dehydrogenase Markers to Study Human Myeloproliferative Disorders

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In accordance with inactivation of one X-chromosome in each somatic cell, females heterozygous at the X-chromosome linked glucose-6-phosphate dehydrogenase (G-6-PD) locus for the usual B gene (Gd^B) and a common variant allele such as Gd^A have two populations of cells – one producing type B G-6-PD and the other, type A. Thus, normal tissues from a Gd^B/Gd^A heterozygote manifest both B *and* A isoenzymes (a double-enzyme phenotype), but a tumor with a single cell (clonal) origin shows B *or* A G-6-PD (a single-enzyme phenotype). The same rationale allows delineation of stem-cell relationships. If a tumor arises in a multipotent stem cell of type A, all descendants of that stem cell will type as A.

In this communication G-6-PD studies are reviewed that indicate that chronic myelocytic leukemia and related disorders have clonal origin at the time of study and involve stem cells pluripotent for granulocytes, erythrocytes, platelets and monocytes/macrophages. The questions of whether marrow fibroblasts or blood lymphocytes arise from the leukemia progenitor and of whether there are any residual normal stem cells are emphasized.

I. Chronic Myelocytic Leukemia (CML)

A. Clonal Origin in Pluripotent Marrow Stem Cells

Thus far, 12 women with Philadelphia-chromosome (Ph^1)-positive CML and heterozygous at the G-6-PD locus have been studied. Both B and A isoenzymes were found in normal tissues, but only one type of G-6-PD was seen in the CML granulocytic cells (8 patients typed as B and 4 as A) [8,9]. The fact that single-enzyme phenotypes occur in CML granulocytic cells, whereas granulocytes from G-6-PD heterozygotes without hematopoietic diseases have double-enzyme phenotypes [6], strongly favors a clonal origin of CML. This postulate is also supported by studies with other isoenzyme and chromosomal markers (references given in [9]). However, the conclusion that CML has a clonal origin applies only to the stage of the disease at the time of study. Conceivably, at a very early phase many cells may be affected, but by the time CML is evident one clone has evolved. The fact that at the time of diagnosis all CML cells are of clonal origin virtually ex-

cludes any hypothesis of pathogenesis based on continuous recruitment of hitherto normal cells.

In G-6-PD heterozygotes with CML, single-enzyme phenotypes are found in erythrocytes, platelets and blood monocytes/macrophages as well as in granulocytes [8]. Thus, the disease involves a multipotent marrow stem cell, a conclusion supported by studies with other markers (references given in [7]). In contrast to the blood cells, cultured marrow fibroblasts from 3 patients with CML displayed both B and A enzymes, indicating that at least in these cases, these cells do not arise from the CML clone. Similar conclusions were reached using Ph^1 as a marker [5, 11, 14]. One of the patients we studied had myelofibrosis [8]. The facts that marrow fibroblasts grown from this patient lacked Ph^1 and had a normal double-enzyme phenotype suggest that the myelofibrosis is not part of the CML clonal proliferation and is probably a secondary phenomenon.

B. Do Blood Lymphocytes Arise from the CML Stem Cell?

To investigate the origin of different lymphocyte populations we studied three G-6-PD heterozygotes with CML. The CML myelocytic cells in each patient showed a single type: B.

Simple preparative methods all failed to separate lymphocytes from granulocyte precursors and other immature forms. Thus we adopted complex multistaged preparative protocols. These methods and the procedures used to identify T or B lymphocytes are described in detail elsewhere [10]. The results provide evidence for at least two and possibly three lymphocyte populations.

1. T-lymphocytes which do not Arise from CML Stem Cells
In each of the three patients there is a population of E-rosette forming lymphocytes which has a normal double-enzyme phenotype in marked contrast to the single-enzyme phenotypes found in the CML clones. These T cells were most easily demonstrated when the patient was in complete clinical remission.

2. Non-T lymphocytes which Arise from CML Stem Cells
A population of non-E-rosette forming lymphocytes was identified which had the same single-enzyme phenotype as did the CML clone. This population was demonstrated when the patient was in relapse or in clinical remission. These cells had complement receptors and many of them manifested B-lymphocyte characteristics such as cell-surface and intracytoplasmic Ig, and Ig synthesis.

3. T-lymphocytes which may Arise from CML Stem Cells
Preliminary studies suggest that there may be a population of lymphocytes which has a single-enzyme G-6-PD phenotype and T-cell characteristics including lack of cell-surface Ig and formation of E-rosettes. In contrast to the non-clonal T-cells which were most easily demonstrated in clinical remission, these "clonal" "T"-cells have thus far been demonstrated only when the disease is in relapse. However, conclusions based on these find-

ings must be guarded. For example, since the disease was active at the time of study, the difficulties of isolating and characterizing lymphocyte populations were increased. In addition, it cannot be concluded firmly that this clonal subpopulation is analogous to normal T-lymphocytes since it may consist of B-lymphocytes or undifferentiated cells of the leukemic clone which have acquired T-cell markers during evolution of leukemia.

The likely demonstration in CML of clonal lymphocytes suggests that there is a common hematopoietic stem cell for some lymphocytes as well as for myelogenous cells and it is this stem cell which is involved in the leukemia. These data may explain why in some cases of blast crisis, the cells have characteristics which resemble those found in the common type of acute lymphoblastic leukemia (e.g., see Chapters by Boggs and Greaves, this volume). The demonstration of E-rosetting cells from patients with CML in remission that do not arise from the leukemia stem cell may reflect persistence of restricted stem cells committed to differentiate only into T-lymphocytes.

Mitogen-stimulated mitoses within the lymphocytes having single-enzyme phenotypes and thereby presumably arising from the CML stem cells generally lacked Ph^1. One possibility is that the cells in metaphase are not representative of the vast majority of enzyme-producing cells, but a more intriguing possibility is that cells which are clonally derived only acquire Ph^1 at a later stage in leukemogenesis (see below).

C. Are there any Residual Normal Stem Cells in CML?

The fact that during remission in CML, the single-enzyme G-6-PD phenotypes persist provides no evidence for residual normal stem cells [8]. However, it was possible that a minor isoenzyme component had been missed if it had contributed less than 5% of the total G-6-PD activity. To study this problem at a more sensitive level, we analyzed granulocytic colonies grown in semi-solid medium. Such colonies from normal G-6-PD heterozygotes have single-enzyme phenotypes and arise from single cells. Thus, analysis of a single colony is equivalent to study of the one progenitor cell from which it was derived. Of almost 1000 granulocytic colonies studied, one colony had a G-6-PD phenotype different from that observed in the CML blood clone [9]. These data provide no evidence for residual, normal granulocyte colony-forming cells (CFU-C) in patients with CML, a situation which contrasts with that found in polycythemia vera (see below).

On the other hand, some studies using Ph^1 as a marker do suggest persistence of normal stem cells. For example, the presence of some normal stem cells in CML was suggested by the observation of Ph^1-negative granulocytic colonies in 3 of 5 patients in one study [3]. (However, other investigators have found such colonies to be uniformly Ph^1-positive [2,15,17]). Further evidence favoring persistence of some Ph^1-negative cells in CML derives from the appearance of such cells in patients treated with cycle-active intensive therapy [4]. How are these chromosome observations suggesting persistence of normal stem cells in CML reconciled with the failure

using G-6-PD as a marker to detect granulocytic colonies arising from non-CML progenitors? One possibility is that only some patients have Ph^1-negative CFU-C, but not the ones we studied. Alternatively, there may be some Ph^1-negative CFU-C in all patients. If this hypothesis is correct, then our failure using G-6-PD to find evidence of stem cells that do not derive from the CML clone suggests that cells which are clonally derived acquire Ph^1 at a later stage in leukemogenesis. According to this hypothesis, CML would be a multi-staged disease. As indicated above, the possibility that some clonally derived lymphocytes lack Ph^1 would be in accord with this hypothesis.

II. Polycythemia Vera and Myeloid Metaplasia with Myelofibrosis

A. Clonal Origin in Pluripotent Marrow Stem Cells

Studies of two G-6-PD heterozygotes with polycythemia vera [1] and two with agnogenic myeloid metaplasia with myelofibrosis [12, 13] indicate that at least in the patients investigated, the disorders involve multipotent hematopoietic stem cells and suggest that at the time of study, the diseases have a clonal origin. According to some theories of pathogenesis, polycythemia vera and agnogenic myeloid metaplasia result from proliferation of normal stem cells in response to unknown myeloproliferative stimuli. The G-6-PD data do not support these hypotheses and are more compatible with neoplastic origin.

B. Are there any Normal Stem Cells in Polycythemia Vera?

As described in detail elsewhere in this volume (see Chapter by Adamson), in contrast to CML, analyses of granulocytic and erythroid colonies from patients with polycythemia vera indicate that there are stem cells which do not arise from the polycythemia vera clone detected in the blood and therefore are presumably residual normal stem cells [16]. However, these cells are demonstrable in vitro only in the presence of erythrocyte or granulocyte stimulating factors. Thus, although there are normal stem cells in patients with polycythemia vera, their expression is suppressed in vivo. This contrast between polycythemia vera and CML suggests a basic difference in the regulatory abnormalities in the two disorders although they may involve the same or a similar multipotent hematopoietic stem cell.

C. Myelofibrosis in Agnogenic Myeloid Metaplasia

The factors underlying the marrow fibrosis, the predominant clinical feature in this disease, are unknown. Many workers feel that it is part of the same process as that which affects the myeloid cells. Our results suggest that this hypothesis, which predicts finding the same single-enzyme phenotype in the marrow fibroblasts as the one observed in the blood cells, is not correct.

In the G-6-PD heterozygote with agnogenic myeloid metaplasia who had equal amounts of B and A isoenzymes in cultured skin fibroblasts and only type A in blood cells, both B and A isoenzymes in equal proportions were found in cultured marrow fibroblasts [12]. This patient also had a distinctive chromosome abnormality (47, XX, +8) in the blood cells which was not detected in a single marrow fibroblast. These G-6-PD and cytogenetic findings strongly suggest that the marrow fibrosis in this patient was not part of the basic process which led to clonal proliferation of hematopoietic stem cells and that the myelofibrosis was a secondary abnormality. Similar conclusions were reached from chromosome studies of a patient with *acute* myelofibrosis [18].

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References

1. Adamson, J.W., Fialkow, P.J., Murphy, S., Prchal, J.F., Steinmann, L.: Polycythemia vera: Stem-cell and probable clonal origin of the disease, New Engl. J. Med. **295,** 913–916 (1976)
2. Aye, M.T., Till, J.E., McCulloch, E.A.: Cytological studies of granulopoietic colonies from two patients with chronic myelogenous leukemia. Exp. Hematol. **1,** 115–118 (1973)
3. Chervenick, P.A., Ellis, L.D., Pan, S.F., Lawson, A.L.: Human leukemic cells: In vitro growth of colonies containing the Philadelphia (Ph^1) chromosome. Science **174,** 1134–1136 (1971)
4. Clarkson, B.D., Dowling, M.D., Gee, T.S., Cunningham, I., Hopfan, S., Knapper, W.H., Vaartaja, T., Haghbin, M.: Abstracts of XV. Congress of the International Society of Hematology, P. 136. Jerusalem 1974
5. De La Chapelle, A., Vuopio, P., Borgstrom, G.H.: The origin of bone marrow fibroblasts. Blood **41,** 783 (1973)
6. Fialkow, P.J.: Primordial cell pool size and lineage relationships of five human cell types. Ann. Hum. Genet. **37,** 39–48 (1973)
7. Fialkow, P.J.: Clonal origin of human tumors. Biochim. Biophys. Acta **458,** 283–321 (1976)
8. Fialkow, P.J., Jacobson, R.J., Papayannopoulou, T.: Chronic myelocytic leukemia: Clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am. J. Med. **63,** 125–130 (1977)
9. Fialkow, P.J., Denman, A.M., Singer, J., Jacobson, R.J., Lowenthal, M.N.: Human myeloproliferative disorders: Clonal origin in pluripotent stem cells. In: Differentiation of Normal and Neoplastic Hematopoietic Cells. Clarkson, B., Marks, P.A., Till, J.E., Eds. N.Y. Laboratory, Cold Spring Harbor, pp. 131–144 (1978)
10. Fialkow, P.J., Denman, A.M., Jacobson, R.J., Lowenthal, M.N.: Chronic myelocytic leukemia: Origin of some lymphocytes from leukemic stem cells. J. Clin. Invest. 62:815–823 (1978)
11. Greenberg, B.R., Wilson, F.D., Woo, L., Jenks, H.M.: Cytogenetics of fibroblastic colonies in Ph^1-positive chronic myelogenous leukemia. Blood **51,** 1039–1044 (1978)
12. Jacobson, R.J., Salo, A., Fialkow, P.J.: Agnogenic myeloid metaplasia: A clonal proliferation of hematopoietic stem cells with secondary myelofibrosis. Blood **51,** 189–194 (1978)
13. Kahn, A., Bernard, J.F., Cottreau, D., Marie, J., Boivin, P.: Gd(–) Abrami: A deficient G-6-PD variant with hemizygous expression in blood cells of a woman with primary myelofibrosis. Hum. Genet. **30,** 41 (1975)

14. Maniatis, A.K., Amsel, S., Mitus, W.J., Coleman, N.: Chromosome pattern of bone marrow fibroblasts in patients with chronic granulocytic leukaemia. Nature **222,** 1278 (1969)
15. Moore, M.A.S., Metcalf, D.: Cytogenetic analysis of human acute and chronic myeloid leukemic cells cloned in agar culture. Int. J. Cancer **11,** 143 (1973)
16. Prchal, J.F., Adamson, J.W., Murphy, S., Steinmann, L., Fialkow, P.J.: Polycythemia vera: The in vitro response of normal and abnormal stem cell lines to erythropoietin. J. Clin. Invest. **61,** 1044–1047 (1978)
17. Shadduck, R.K., Nankin, H.R.: Cellular origin of granulocytic colonies in chronic myeloid leukaemia. Lancet **1971 II,** 1097–1098
18. Van Slyck, E.J., Weiss, L., Dully, M.: Chromosomal evidence for the secondary role of fibroblastic proliferation in acute myelofibrosis. Blood **36,** 729–735 (1970)