Clonal Diseases of the Myeloid Stem Cell Systems

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The structure and kinetics of the hematopoietic stem cell compartment have long been the subject of considerable speculation. Based on morphologic observations of normal and abnormal human marrow and of a perturbed system in experimental animals, primarily the rabbit, in 1938, Downey [1] concluded there was a stem cell capable of giving rise to all hematopoietic tissue. He believed this cell in turn gave rise to a lymphoid stem cell and to a myeloid stem cell. The myeloid stem cell could give rise directly to erythroid, megakaryocytic and monocytic cell lines and in turn produced a tertiary stem cell which could generate neutrophils, eosinophils and basophils. With the development of functional assays for clonal cell growth in vivo and in vitro and through the use of chromosome marked clones this suggested structure has proved to be correct in substance although certain minor variations are indicated. Most definitive studies of the structure of the stem cell compartment are in mice [2] but, in general, the data generated in human diseases suggest that the human stem cell structure is the same as that of the mouse. In Fig. 1, one current "best guess" is shown. There seems little doubt that at least 3 concatenated precursor compartments exist for all myeloid cells. Whether there are still more intermediate stages and whether or not most cells forming colonies in vitro are stem cells (i.e. capable of self-replication) remain open question.

Studies from Phillips Laboratory [2] using irradiation induced chromosomally marked clones injected into W/W^v mice have shown the presence of an hematopoietic stem cell which is totipotent for all hematopoietic cells, lymphoid as well as myeloid (THSC) and distinct from a cell pluripotent for myeloid tissue (PMSC). This latter cell produces spleen colonies in irradiated recipients and is, therefore, also known as a colony forming unit cell (CFU-S), but whether other classes of cells will also produce spleen colonies is unknown. The structure of the lymphoid compartment as derived from the THSC will not be discussed.

A cell which is restricted to the production of neutrophil-monocyte colonies in vitro, colony forming unit – culture, (CFU-C) has characteristics distinguishing it from the CFU-S. Among a variety of differences including a much higher percent in DNA synthesis [3], perhaps the most convincing is the presence of normal CFU-C in the W/W^v/mouse in the face of very abnormal behaving CFU-S [4]. Cells apparently restricted to production of erythroid (burst forming unit, BFU-E) [5] megakaryocytic (CFU-meg) [6] and eosinophilic (CFU-Eos) [7] are also demonstrable by in vitro analysis.

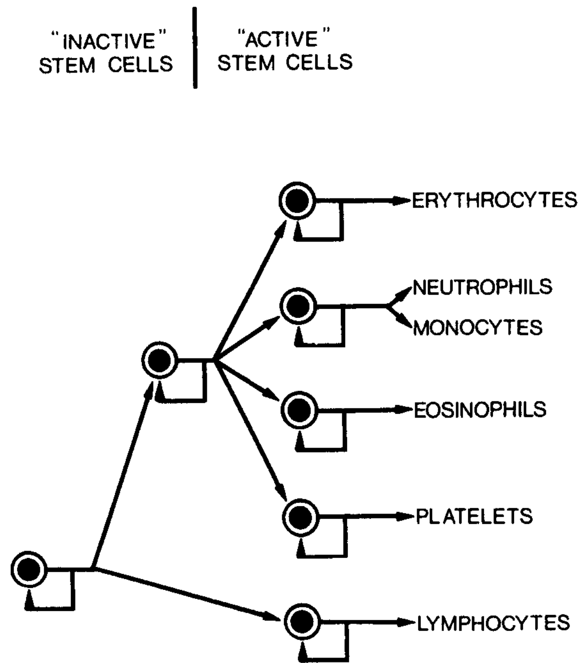

Fig. 1. A model of the Hematopoietic stem cell system. Three concatenated cell systems are presented. "Active" refers to the fact that a high percentage of cells forming colonies in vitro are in DNA synthesis while the more pluripotent spleen colony forming cell compartment has few cells in DNA synthesis

At the present time, human diseases of the stem cell system appear to involve either the THSC or the PMSC (CFU-S) compartment, although specific defects in more committed compartments may possibly explain diseases of a single cell line such as Diamond-Blackfan anemia or certain forms of congenital neutropenia. However, no clonal markers have been identified in these conditions.

There is evidence that the myeloid leukemias (ML) acute (A) and chronic (C) involve a wide spectrum of hematopoietic tissue. In the case of AML the most common morphologic expression is by an increase in myeloblasts. However, the cells often have some monocytic features as well and any of the myeloid cell lines may appear as the predominant morphologic expression in AML (Table 1). The very frequent myeloblast-monocyte morphologic mix-

Table 1. Morphologic expressions of myeloid leukemia

AML	CML	in blast crisis
√	√	Myeloblastic
		Maturation
√	√	Promyelocytic
√	√	Promyelocytic (Eosinophil)
√	√	Promyelocytic (Basophil)
√	√	Monoblastic – monocytic
√	√	Myelomonocytic
√	√	Erythroblastic
√	√	Megakaryoblastic
√	√	Any mixture of above
(X)	√	Lymphoblastic
X	√	Any mixture of above

ture may reflect the immediate common origin of these cells. To date there has been no means of separating individual precursors for these cell lines in the in vitro clonal assays. When colonies of human or murine cells are grown in semi-solid media in the presence of colony stimulating factor, mixed colonies of neutrophils and monocytes occur [7]. Is AML one basic disease involving a pluripotent myeloid stem cell or a series of diseases involving the specific progenitors; such as, CFU-C or BFU-E, etc? Put another way, if these are induced by an oncornavirus, what is the primary target cell?

There are a number of fairly simple clinical observations which bear on this question. First of all, there is almost never a shifting myeloid-lymphoid picture in AML and a mixed myeloid-lymphoid presentation of AML is not recognized. AML is defined arbitrarily in this paper as excluding any patient in whom the Ph^1 chromosome is present for I have observed an apparently mixed myeloid-lymphoid blastic pattern in patients presenting with Ph^1 positive acute leukemia. Such patients are defined, again somewhat arbitrarily, as presenting in the blastic those of CML (see below). Thus, the THSC does not appear to be involved in AML. However, a shifting morphologic expression within myeloid cell lines does occur. Perhaps the most commonly recorgnized shift is in the patient who presents with a predominantly erythroblastic picture but proceeds to develop an increasing predominance of myeloblasts or myelomonoblasts. Even when the predominant cell is a myeloblast at the time of diagnosis, megaloblastic erythroid precursors and abnormally small megakaryocytes are often present if the marrow smear is searched with diligence. This suggests that more than one myeloid cell line is involved in the AML process and suggests that the target cell is the PMSC rather than the more differentiated CFU-C systems. This is supported by chromosomal studies in which marker chromosomes in the myeloblastic cells have also been found in erythroid cells [8].

Clinical observations in CML suggest the target cell may be the THSC. During the chronic phase, abnormalities of all of the myeloid cell series may be observed suggesting that the leukemic clone is at least feeding through the PMSC. During acute transformation, all of the morphologic spectrum seen in AML may appear and, furthermore, a lymphoblastic or even a mixed lymphoblastic-AML picture may develop [9]. The Philadelphia chromosome is found in erythroid precursors, megakaryocytes, monocytes and eosinophils as well as in neutrophil precursors [10]. Uniformity of G-6 PD isozymes in the myeloid series of patients whose non-hematopoietic cells are heterozygous confirms the clonality of the disease and again indicates involvement of more than one cell in the myeloid series [11]. Furthermore, such heterozygotes may also have certain lymphocyte populations homozygous for the isoenzymes, strongly suggesting that the THSC is the target cell [12].

Polycythemia Rubra Vera (PRV) and idiopathic myelofibrosis (IMF) are also diseases in which there is clinical evidence for disturbance in cell production of all of the myeloid cell systems. Analysis of G-6 PD isozyme data is compatible with the concept that these diseases are also clonal diseases of myeloid stem cells [11]. In paroxysmal nocturnal hemoglobinuria there is evidence for abnormality of neutrophils and platelets as well as for red blood

cells suggesting that this also might be a disease of the pluripotent myeloid stem cell [13]. As yet, there is little data which will allow one to make a guess as to whether these diseases are at the level of the THSC or the PMSC. However, the report of the development of acute lymphoblastic leukemia in a patient with PRV [14] favors the THSC rather than the PMSC being the affected cell.

Still other diseases, such as aplastic anemia and cyclic neutropenia are diseases which appear to involve myeloid stem cells, although it seems unlikely that they are clonal.

How does a single stem cell take over the entire production of the myeloid system? In most patients, chromosomal and isozyme data indicate that all cell production is from the clone and in vivo evidence for persistent growth of normal stem cells is lacking. Evidence relative to the question of whether normal cells are still present is discussed below. As a generality, when we observe a clone of cells which is growing with seeming inappropriateness and eventually leading to death we make a diagnosis of a malignant neoplasm. For this reason most now consider PRV and IMF as well as AML and CML to be malignant neoplasms. The "neoplastic" cell, in this case a neoplastic THSC or PMSC, must have some form of relative growth advantage as compared to the normal cells and secondly, its growth must in turn somehow be suppressive for growth of the comparable compartment of normal cells. Theoretically, these two characteristics could be independent phenomenon or might be mediated by the same mechanism.

In any system which I've been able to envision which would allow the neoplastic cell to take over the myeloid system, there must be an abnormality in that cell with respect to its response to normal, physiologic factors regulating the system. This abnormality could range from complete autonomy of growth (a cell which would continue to grow without regard to the presence or absence of physiologic regulators) to subtle defects; such as, one in which the neoplastic cell was simply more sensitive to growth stimulators or less sensitive to growth inhibitors than is the normal cell. In either event, the normal cells could become repressed by a variety of mechanisms. As the neoplastic clone expanded the normal control system might recognize the expanded neoplastic stem cell system and repress the normal one or the neoplastic clone could even produce inhibitors of the normal.

Undoubtedly spurred on by the observation that most megaloblastic anemias, once widely thought to be closely allied to leukemia, were due to vitamin deficiency, a long standing hypothesis has been held by many that a least certain "leukemias" may represent faulty regulatory systems rather than intrinsic neoplastic abnormality of the cell identified as "leukemic". In my opinion, the demonstration that these are clonal diseases, coupled with the demonstration that the normal counterpart cells are either absent or repressed rules out this hypothesis as a primary cause of the disease. There may be abnormalities of the regulatory system as well, but I think these must be considered secondary to the primary neoplastic process rather than as playing a causative role.

Just as the primary direct evidence for clonality of disease comes from

chromosome and isozyme data, so does the evidence for the presence of some residual normal stem cells.

Is a chromosome abnormality an accurate marker as to whether or not a cell is part of the clone of human leukemia? This question cannot be answered with certainty, but there is growing evidence, if of an inferential nature only, that it does not. I think all would agree that all cells bearing the Ph^1 abnormality are part of the clone in CML and that cells bearing a consistent chromosome abnormality in AML, PRV or IMF are part of that clone. It is the converse situation where serious questions must be raised; it is not clear that a cell not bearing the chromosome abnormality is not part of the clone. A number of pieces of evidence suggest that only a portion of the clone carries the chromosome abnormality.

Perhaps the strongest evidence suggesting that this is true are the somewhat discrepant findings with respect to chromosome abnormalities and isozyme studies in PRV and IMF [11, 15]. The discrepancies may be due to the fact that both studies have been done in a very limited number of patients and parallel studies have not been done in the same patient, but discrepancies are there none-the-less. All isozyme studies to date in patients with active PRV and IMF have indicated that all myeloid cells analyzed from the patients are part of the clone. However, in those patients in whom a chromosome abnormality has been found it often is present in only a portion of the analyzed myeloid tissue [16]. Similarly, when a chromosome defect is present in AML it often is not present in all analyzed myeloid tissue, even when virtually 100% of the myeloid cells appears to be leukemic on stained smears. This is also true for changes other than the Ph^1 in blastic crisis of CML [17] and quite discordant changes in chromosome defects and morphology may be observed in blastic crisis [18].

Although most patients with CML have the Ph^1 chromosome in all analyzed myeloid metaphases, some do not. The general assumption is that the latter patients are chimeric, i.e. have persistence of both normal and leukemic cells, an assumption which may or may not be true. When chromosome analysis was carried out on granulocyte-macrophage colonies grown in vitro, Ph^1 negative colonies were found in some patients in whom all direct metaphases had been positive [15]. This suggested that normal stem cells were still present, but that they were dormant in vivo. However, when Fialkow and coworkers (see Fialkow's paper in this symposium) analyzed G-6 PD isozymes in individual G-M colonies from patients with CML, no colonies were found which were not part of the clone. Resulution of this seeming discrepancy will require further studies in which both chromosomes and isozymes are analyzed in colonies from the same patient.

For the above reasons, the use of the lack of chromosome markers to prove the persistence of normal stem cells in these clonal diseases may be questioned. Keeping that in mind, there is none-the less fairly strong evidence that normal stem cells persist in these diseases but probably in a quiescent state. First, and perhaps foremost, with respect to the strength of the evidence is the development of remission in AML. Suffice it to say that virtually all current evidence points to remission in AML representing the re-

growth of the normal myeloid system while the clone has been reduced and held in check by therapy. Evidence is also quite strong for the persistence of normal PMSC in PRV. All myeloid tissue taken directly from the patient for isozyme analysis has apparently been part of the clone. However, when colonies of erythroid tissue have been grown from the same patient, some have been isozymically heterozygous (see Adamson's paper in this symposium). This is compatible with the previously expressed concept that the expanding neoplastic clone induces repression of normal cells in vivo, but that they are still present. As noted above the situation is not so clear in CML.

In summary, there is strong evidence that CML, AML, PRV and IMF are clonal diseases of a pluriopotent hematopoietic stem cell and suggestive evidence that PNH is such a disease. There is strong evidence that CML is a disease of the THSC and suggestive evidence that PRV may involve this cell; AML more likely is a disease of the PMSC and IMF is a clonal disease of one of these two cells. The nature of the growth advantage enjoyed by the abnormal cell as compared to the normal cell is unknown. However, the data in hand strongly suggest that normal stem cells, while still clearly present in some of the diseases, are quiescent and not producing mature cells in most patients. Existing data appears to rule out the possibility that any of these diseases is due to faulty regulation by factors external to the stem cell itself.

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