EDITORIAL HYPOTHESIS

The Pathogenesis of Aplastic Anemia: A Defective Pluripotent Hematopoietic Stem Cell With Inappropriate Balance of Differentiation and Self-Replication

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A PLASTIC ANEMIA most commonly is defined as a condition characterized by reduced numbers of red cells, neutrophils, and platelets in the blood in the presence of a hypocellular bone marrow. In most patients it is drug induced. By the above definition, “aplastic anemia” commonly is induced with either chemo- or radiotherapy for neoplastic diseases, but most such patients quickly recover from aplasia. However, occasionally these predictable episodes of aplasia are associated with very prolonged periods of continued pancytopenia after the drug is withdrawn. Other drugs, such as chloramphenicol, which induce aplasia in only an occasional patient, are presumed to do so via some type of hypersensitivity reaction. Aplastic anemia may also occur in association with infectious hepatitis, as an inherited disease, or as an idiopathic disease in which no cause for marrow insult can be identified.

Four basic patterns of outcome can be identified. Complete recovery is somewhat unusual if the form induced by dose-related therapeutic agents is excluded. When complete recovery does occur, it most often is observed early in the course of the disease. The majority of patients with idiopathic or drug hypersensitivity aplasia die within 1 yr of diagnosis unless a successful marrow transplant is accomplished. This group tends to have very severe hypoplasia of the marrow with consequent severe neutopenia, thrombocytopenia, and anemia. However, in our experience, none has complete cessation of production of myeloid elements. A third group has persistent, but milder hypoplasia that may persist for 20 yr or longer. In these patients, the degree of neutropenia and thrombocytopenia usually is modest and, while levels of blood cells may fluctuate somewhat, there is no long-term tendency toward improvement. Finally, certain patients do have improvement in blood values after months or years of disease, but in most of these blood cell recovery is incomplete.

The pathogenesis of the disease is somewhat obscure, particularly the acquired idiopathic and drug-hypersensitivity forms. The primary problem could conceivably be categorized under any one or a combination of the following headings:

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(1) Pluripotent myeloid stem cells
   (a) Reduced number of stem cells
   (b) Defective stem cells

(2) Environment of pluripotent myeloid stem cells
   (a) Structural defects in the supporting microenvironment
   (b) Abnormalities of chemical regulators of myeloid cell growth (cell-cell interaction, short- or long-range humoral factors)
   (c) Inhibitors of cell growth

A defective or hostile environment is unlikely in most patients with aplasia. As others have pointed out, the observation that syngeneic or HLA-matched allogeneic marrow transplants from siblings will grow in a majority of patients with aplastic anemia makes such a consideration unlikely. The only well-defined humoral regulator of hematopoiesis, erythropoietin, has been appropriately increased when measured in patients with aplastic anemia. The presence of an inhibitor of cell growth, such as an antibody also is rendered an unlikely cause of aplasia by the success of marrow transplantation. It could be argued that such an antibody would be affected by irradiation or cyclophosphamide given as preparation for allogeneic transplantation, but syngeneic transplantation in which no such potentially immunosuppressive therapy is employed has been successful in most patients. However, one cannot rule out the possibility that a missing, critical “environmental” cell is transplanted with myeloid stem cells. Furthermore, although occasional failure of marrow transplantation may be blamed on immunologic or technical problems, an abnormal environment cannot be ruled out as a cause of aplasia in a minority of patients.

The only direct assay for possible myeloid stem cells in man is the growth of colonies of neutrophils, eosinophils, and monocytes in vitro in semisolid media from blood or marrow cells. An effective hematopoietic stem cell can be defined as one capable of self-replication and of differentiation. There seems little question that these colonies are clonal, i.e., arise from a single cell; extensive replication and differentiation are implied by their size. Whether or not these cells are pluripotent for erythroid and megakaryocytic tissue has not been absolutely clarified. Most evidence suggests that they are the progeny of the pluripotent cell and are committed to the granulocytic line. However, it is possible that the cultural conditions fail to provide an adequate environment for growth of the other cell lines. Isolation of a human blood cell that might be pluripotent for virtually all myeloid tissue has been reported recently. In any event, the concentration of the cell-forming granulocytic and mononuclear colonies in vitro has been reduced in the marrow of all patients with aplastic anemia studied to date, insofar as we know. Since total marrow cellularity is reduced, this reduced concentration indicates a truly profound depression in total number of these marrow stem cells. Thus, stem cells are reduced severely, at least as measured in this assay.

Can the disease then be considered to represent simply a reduced number of stem cells? One must ask why the stem cell compartment fails to recover. As previously mentioned, aplastic anemia is commonly produced by chemotherapy or radiotherapy used in the treatment of a wide variety of human tumors.
Aplasia caused in this way is rapidly reversed, reflected also by a rising number of stem cells producing granulocytic colonies in vitro,\textsuperscript{11} in the vast majority of such patients. With certain forms of chemotherapy the pluripotent stem cell system might be spared and the hypoplasia simply reflect damage to more mature compartments. A single dose of a cycle-active agent, such as cytosine arabinoside,\textsuperscript{12} has little effect on transplantable murine spleen colony-forming cells, while a non-cycle-dependent drug, such a nitrogen mustard,\textsuperscript{13} has profound effects. However, single doses of drug rarely are used in human chemotherapy, and repeated doses of cycle-dependent agents have profound effects in murine systems.\textsuperscript{14} Assuming that the human stem cell system is similar to that of the mouse, it seems likely that most commonly used chemotherapeutic regimens would severely reduce the size of the pluripotent stem cell compartment. Why does the stem cell compartment regrow in this situation, producing apparent complete hematologic recovery, while such recovery is somewhat unusual in idiopathic aplastic anemia or in that presumably due to idiosyncratic drug reactions? The failure of the stem cell compartment to regrow could reflect “exhaustion” of proliferative ability. Based on studies of cultured, non-hematopoietic cells it has been postulated that the potential number of replicative divisions for any cell is finite.\textsuperscript{15} Certain studies have suggested that the murine hematopoietic stem cell compartment can be exhausted,\textsuperscript{16} but others have suggested that very extensive, perhaps even infinite, self-replication is possible.\textsuperscript{17,18} If stem cell exhaustion is the primary problem in human aplasia, inexorable progression to complete aplasia would be anticipated; but, instead, a number of patients maintain a relatively stable, but reduced, output of cells for years.\textsuperscript{1}

Studies of recovery of hematopoietic stem cell compartments in irradiated mice may have some bearing on the question of hematopoietic recovery in man. If the stem cell compartment, which produces endogenous spleen colonies in the sublethally irradiated mouse, is reduced below approximately 10\textsuperscript{3\%} of its normal size, differentiation becomes blocked and only self-replication occurs.\textsuperscript{19,20} Self-replication results in replenishment of the compartment to above that threshold, and at that time differentiation, in addition to self-replication, can occur. A similar pattern appears to be true for self-replication and differentiation of transplanted cells producing murine spleen colonies.\textsuperscript{21} This block to differentiation can be considered a protective mechanism for the depleted stem cell compartment in that differentiation is equivalent to death with respect to stem cells. The number of cells that are self-replicating continues to exceed the number that differentiate into mature compartments even after differentiation begins, since the size of the stem cell compartment continues to increase during recovery from irradiation as the number of differentiated cells increases. As measured by endogenous spleen colonies, the compartment size increases steadily until supranormal levels are reached,\textsuperscript{18} while, as measured with the transplanted colony system, the increase ceases at or near normal levels.\textsuperscript{22} If the rate of self-proliferation exceeds the rate of differentiation, the stem cell compartment grows; if the two are equal, the compartment remains unchanged; but if the rate of differentiation exceeds the rate of self-replication, the compartment shrinks. A shrinking compartment, presumably due to continued differentiation, can be demonstrated with endogenous spleen colonies when rather
small doses of irradiation are given. In these studies, when the endogenous compartment reaches a nadir of approximately 10% of normal, self-replication again begins to exceed differentiation as judged by regrowth of the endogenous colony compartment.

Assuming that stem cell recovery in man ordinarily follows the same pattern demonstrated in the above studies in mice, prompt recovery from chemotherapy-induced aplasia would be expected. The rarity of complete recovery from some other forms of aplasia might reflect an abnormality of the stem cell. This abnormality would reflect an undesirable ability to continue to differentiate even though the compartment is severely reduced and at the expense of compartment growth due to self-replication. Such a defect could explain the rather peculiar clinical patterns often seen with this disease, such as the long-term production of reduced numbers of neutrophils, platelets, and red cells. Rates of production of mature cells seem to fluctuate from time to time in some patients, and one would assume that demand for differentiation would not always be the same. However, it should be emphasized that we have no evidence that the strength of differentiating signals in the irradiated mouse is equivalent to those present in human aplasia. It is possible that very strong and long-standing differentiating stimuli might overcome the block to differentiation present in the depleted murine stem cell compartment.

There is no clearly defined animal model for this postulated defect. The W/WV mouse has a hereditary abnormality of hematopoietic and other stem cell compartments, but the balance of proliferation–differentiation has not been studied. Mice treated with x-ray or various chemotherapeutic agents have hematopoietic stem cells that are transiently defective in erythroid differentiating ability, and Morley et al. have shown that a single large dose of busulfan leads to prolonged abnormality of stem cells with prolonged but moderate hypoplasia. The exact nature of this stem cell defect has not been defined, but their data suggest that the damage reduces both self-proliferative and differentiative potential. The nature of the control system in the mouse, which regulates the self-replication–differentiation relationship, is unknown, so it is idle to speculate about the nature of such a theoretic defect in man. However, some regulatory process is implied by the observation that some hours are required before stem cell compartment damage is recognized and for it to decrease sharply (or stop) responding to differentiative signals. Alternatively, induction of differentiation shortly after irradiation might be from “committed” stem cells rather than from the pluripotent compartment. If such a defect results in aplastic anemia of man, the aforementioned response to marrow transplantation implies that the defect lies in the cell, not in the environment.

If this hypothesis should be applicable to human aplasia, then the means by which the stem cell became abnormal must be explained. The simplest explanation would be to suggest that the event which leads to the initial reduction in stem cells also produces the cellular abnormality in balance of self-replication versus differentiation. The mechanism whereby the stem cell compartment is depleted in idiopathic or idiosyncratic drug reaction-induced aplasia is unknown. Alternatively, one might suggest that such patients have congenitally abnormal stem cells and that the abnormality becomes pheno-
typically expressed only when the size of the compartment is depressed severely by external influences.

In summary, we would suggest that pancytopenia and hypoplasia of the marrow which persists for prolonged periods following withdrawal of offending agents may be due to an abnormality of pluripotent stem cells as well as to an initial reduction in their number. This abnormality is one in which inappropriate differentiation of pluripotent cells into mature myeloid lines occurs. For the pluripotent compartment to regenerate to normal size, the proportion of stem cells which self-replicate must exceed the proportion which differentiate. In the mouse, there is a poorly defined control system which leads to self-replication far in excess of differentiation when the stem cell compartment is severely reduced. We suggest that stem cells may be damaged in certain cases of human aplasia so that the rate of self-replication fails to exceed the rate of differentiation and, as a consequence, the hypoplasia persists.

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