Separating the Wheat From the Chaff: Selection of Benign Hematopoietic Cells in Chronic Myeloid Leukemia

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CHRONIC MYELOGENOUS leukemia (CML) is characterized by the proliferation of clonal myeloid cells originating from a neoplastic multilineage progenitor cytogenetically marked by the specific translocation of the abl oncogene on chromosome 9 to the breakpoint cluster region (bcr) on chromosome 22. Experimental studies and clinical observations have suggested that normal early progenitors or stem cells persist in the marrow of CML patients. Marrow from CML patients placed into Dexter long-term bone marrow cultures (LTBMCs) over time produces increasing numbers of Philadelphia chromosome negative (Ph⁻) hematopoietic cells. When cultured autologous CML marrow cells were transplanted back into a small number of patients, reconstitution with Ph⁻ cells occurred: this suggests that restoration of hematopoiesis originating from benign primitive precursors occurred due to a competitive advantage for normal progenitors or stem cells in culture or in vivo posttransplant. Treatment of early stage CML patients with interferon (IFN) led to a return to complete or almost complete Ph⁻ hematopoiesis in approximately 20%. These observations support the theory that neoplastic CML cells suppress but do not eliminate normal hematopoietic stem cells, and that under appropriate conditions, Ph⁻ cells may re-emerge and predominate in vivo.

The conclusion that loss of the Philadelphia chromosome implies a return to benign hematopoiesis is based on the assumption that the Philadelphia chromosome or bcr/abl rearrangement represents an absolute clonal marker of the disease and even its proximate cause. Recent experiments have provided strong support for this hypothesis: the human CML p210 bcr/abl fusion gene was introduced into murine bone marrow cells with a retroviral vector; transduction of transduced marrow into syngeneic recipient animals resulted in a myeloproliferative syndrome followed by acute lymphoid and myeloid leukemias. However, a few observations do suggest that clonal abnormalities may antedate the bcr/abl translocation. Several patients with a clinical syndrome resembling CML were initially Ph⁻ but later had detectable Ph⁺ cells. Fiakow et al. studied blood cells from women with CML heterozygous for the G6PD isoenzyme types A and B. One patient who expressed a predicted 1:1 ratio of enzyme A:B in normal tissues was found to have 71% enzyme B in Ph⁻ B-lymphoid cell lines. Similar results have been reported in T-cell cultures from G6PD heterozygotes. The number of patients studied in these reports is small; pseudoclonality or artificial skewing toward a single enzyme in heterozygotes is a possible explanation. Furthermore, clonality in some tissues has been observed in as many as 23% of normal females by analysis of HPRT or PGK fragment polymorphisms.

Claxton et al. provided evidence for the reemergence of polyclonal cells after IFN-induced remissions in CML. Differential methylation analysis of an X-chromosome BstXI PGK gene polymorphism showed that in all patients, granulocytes were nonclonal during cytogenetic remission. Polyclonal and therefore presumably normal hematopoiesis was reestablished in patients who achieved a complete cytogenetic remission. This technique cannot exclude the existence of small populations (<5% to 20%) of Ph⁻ monoclonal cells; if so, perhaps clonal Ph⁻ cells are less sensitive to the effects of IFN than Ph⁺ cells.

Attempts to restore normal hematopoiesis in CML must take advantage of biologic and phenotypic differences among heterogeneous cell populations. In CML a premature release of Ph⁺ progenitor cells from the bone marrow is characteristic and may be secondary to an abnormal loss of adhesion to stroma. Verfaillie et al. have described diminished Ph⁺ cell adhesion to fibronectin and increased adherence to basement membrane components, laminin and collagen type IV, compared with normal bone marrow. Alterations of the adhesive properties of CML cells after treatment with IFN has been reported. Unlike conventional chemotherapy for CML, IFN produces complete cytogenetic remissions that may be sustained for years in a small cohort of patients. The mechanism of IFN's action is not clear, but does not appear to be a leukemia-specific cytotoxic effect on late progenitor cells.

Recent reports propose that IFN-α may overcome the...
defective adherence of CML blast-CFC to stromal cells by changing the neuraminic acid composition of the marrow stromal layer, or by increasing the expression of a surface glycoadhesion molecule, lymphocyte function-associated antigen (LFA3), which is found at low levels in CML. Because IFN-α also increases the proportion of long-term culture initiating cells attached to stromal cells, enhanced proliferation of normal cells may result in competitive suppression of the leukemic clone. The only therapy proven to be curative for CML, allogeneic bone marrow transplantation, is available to a minority of those with the disease. In most centers allografting is limited to patients younger than 50 with a compatible related or unrelated donor. Autologous transplantation circumvents the disadvantages of histocompatibility barriers but leukemic cell contamination of the graft is of obvious concern. Approaches that select for and exploit divergent cell growth characteristics and cell surface characteristics may offer the best chance of isolating benign cells for use in autologous transplantation.

Occasionally, autografting with unpurged grossly Ph+ marrow results in complete sustained clinical and cytogenetic remission. Possible explanations for the inability of nonpurged infused leukemic marrow cells to reconstitute Ph+ disease include terminal differentiation of leukemic progenitor cells, a failure of leukemic cells to “home” normally to the marrow microenvironment, differential sensitivity of leukemic versus normal progenitors to the marrow storage process, or suppression of the leukemic clone by activated T lymphocytes.

Despite these isolated reports, most investigators believe that any benefit of autologous marrow transplantation in CML may be eventually negated by the nearly certain reinfection of large numbers of clonogenic Ph+ progenitor cells. Hence, the design and testing of strategies to eliminate these cells from the autograft may be advantageous. Recent reports suggest that benign and malignant progenitors in CML may be distinguished by the presence or absence of certain cell surface antigens. Verfaillie et al reported that the CD34+/DR- cell fraction in CML marrow contains primitive progenitors capable of initiating LTBM. As early as 1 week after culture initiation all CFU-C in six of seven patients studied lacked the Philadelphia chromosome or bcrl abl rearrangement. The effect did not appear to be related to the LTBM environment, because colony-forming cells grown from early liquid culture also showed no detectable bcrl abl rearrangements. Conversely, even after prolonged culture, the CD34+/DR- fraction produced almost exclusively Ph+ cells. As opposed to CD34+/DR+ fractions from normal marrows, CD34+/DR+ cells from CML patients were able to initiate LTBM and sustain it for up to 8 weeks.

The investigators did not perform analysis for bcrl abl after selection and immediately before culture, thus some selective effect of in vitro culture, as previously reported by Turham et al cannot be ruled out. Differences in antigenic specificity and density between patients and according to disease activity may also limit the efficacy of positive marrow stem cell selection. CD34 expression on CML progenitors varies with the phase of disease. CD34+/DR- CML marrow fractions may not be uniform from patient to patient and may vary over time in an individual patient to exclude populations of pluripotential hematopoietic cells or include leukemic cells. One patient in the report by Verfaillie et al retained Ph+ cells in the CD34+/DR- cell fraction. Another potential problem in using this type of selection clinically was the decrease in the absolute number of CD34+/DR- cells in the CML as opposed to control marrows. Despite these caveats, this approach for selecting benign progenitors from CML marrow autografts is promising and will continue to be evaluated.

Differential manipulation of benign versus CML cells based on disparity in functional growth factor receptor or other antigen expression, response to inhibitory factors such as leukemia inhibitory factor (LIF) or interleukin-4, or bcrl abl expression may provide opportunities for therapeutic intervention. In a recent issue of this journal, Wognum et al reported that the erythropoietin receptor (EpR) is present on 31% to 43% of CD34+ CML cells, but absent or barely detectable on normal CD34+ marrow cells. However, the CD34+/EpR+ CML cells were not evaluated with respect to bcrl abl or Ph+. At present, the technique used to select EpR+ cells results in poor viability that would limit the clinical application of this selective stratagem. These studies suggest that a selective priming approach with administration of erythropoietin before treatment with cycle-specific chemotherapy might cycle CML progenitors and increase tumor killing. The finding of aberrant EpR expression on CML cells should prompt evaluation of the expression of other growth factor receptors to help understand the differential growth characteristics of these cells compared with normal progenitors.

The most specific negative selection method currently under evaluation involves inhibiting the expression of the bcrl abl encoded aberrant p210 protein with antisense bcrl abl oligonucleotides. The growth of blast cell colonies from CML patients in blast crisis was specifically inhibited in vitro by antisense oligonucleotides each patient’s cloned bcrl abl junction. None of these oligonucleotides inhibited the growth of CFU-GM from normal bone marrows. Further investigation of this technique is ongoing, and may eventually provide practical method for negative selection of autograft marrow, or even in vivo gene-targeted antileukemic therapy.

These approaches to separating the “wheat from the chaff” in CML raise a number of questions. Can subpopulations of CD34+ cells reconstitute the human hematopoietic system after transplant? Berenson et al have developed an immunoadsorption technique using the anti-CD34 antibody 12.8 and solid-phase avidin-biotin to positively select human CD34+ cells. Compared with unselected fractions, CFU-GM generated from long-term marrow cultures of CD34+ cells were enriched 63-fold. Nine patients have been transplanted with the CD34+ fraction obtained using this technique, and acceptable hematologic recovery was achieved. Baboon marrow depleted of CD34+...
cells using this technique failed to reconstitute ablated recipients. Therefore, cell populations containing CD34+ progenitors appear to be responsible for at least short-term hematopoietic reconstitution. Although CD34+ cells support human LTBMCS (H-LTBMCS), it will be difficult to prove that the CD34+ population contains the in vivo repopulating stem cell. It may be argued that H-LTBMCS do not accurately reflect in vivo hematopoiesis because their 8- to 12-week span is in fact relatively short compared with in vivo reconstitution. Furthermore, the H-LTBMCS system itself is technically challenging and very difficult to maintain for prolonged periods of time. Although the studies of Berenson et al. suggest that multilineage reconstitution occurs with CD34+ enriched fractions, the conditioning regimens used in these patients were not always completely myeloablative and the mean purity of reinfused CD34+ populations of cells was only 65%. Therefore, non-CD34+ populations of cells present in the graft, or endogenous stem cells that survived the conditioning regimen, could be responsible for long-term in vivo reconstitution. Because positive selection of CD34+ cells may result in the elimination of T lymphocytes and other effector cells, immune status after transplant must be carefully studied, particularly with more ablative conditioning regimens.

Theoretically, marrow cell selection/purging should be of substantial benefit to CML patients in whom considerable numbers of Ph+ cells contaminate harvested bone marrow. Large, prospective controlled studies would be required to determine definitively the benefit of purging based on clinical outcome alone. Proof of efficacy is desirable because these methods can damage normal marrow cells and delay hematopoietic recovery. In CML, a number of small phase I/II studies of in vitro chemotherapy/biologicals or long-term culture of harvested marrow have been completed or are ongoing to try to eliminate leukemic cells from the autograft and improve survival.

If relapse after autografting is related primarily to endogenous leukemia cells that survive the conditioning regimen, the application of cell selection approaches to CML may be premature. In humans with CML it is impossible to distinguish autografted leukemia cells from the endogenous leukemia cells that persist in vivo after intensive therapy. The use of gene transfer techniques to mark cells from the marrow or peripheral blood stem cell graft may permit a more complete understanding about the origin of relapse after autologous transplant and the efficacy of cell selective techniques.

REFERENCES

6. Barnett MJ, Eaves CJ, Phillips GL, Hogge DE, Humphries RK, Klingemann H-G, Lansdorp PM, Reece DE, Shepard JD, Eaves AC: Autografting with curative intent for patients with chronic myeloid leukemia, in Dicke K, Armitage JO, Dicke-Eaves AC: Autografting with curative intent for patients with chronic myelogenous leukemia in mice, could be responsible for long-term in vivo reconstitution of human hematopoietic reconstitution. It may be argued that H-LTBMCS do not accurately reflect in vivo hematopoiesis because their 8- to 12-week span is in fact relatively short compared with in vivo reconstitution. Furthermore, the H-LTBMCS system itself is technically challenging and very difficult to maintain for prolonged periods of time. Although the studies of Berenson et al. suggest that multilineage reconstitution occurs with CD34+ enriched fractions, the conditioning regimens used in these patients were not always completely myeloablative and the mean purity of reinfused CD34+ populations of cells was only 65%. Therefore, non-CD34+ populations of cells present in the graft, or endogenous stem cells that survived the conditioning regimen, could be responsible for long-term in vivo reconstitution. Because positive selection of CD34+ cells may result in the elimination of T lymphocytes and other effector cells, immune status after transplant must be carefully studied, particularly with more ablative conditioning regimens.

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