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Procurement of Stem Cells by Continuous-Flow Centrifugation

To the Editor:

The report by Richman et al.,1 of an increase in circulating granulocytic stem cells (CFU) in peripheral blood following chemotherapy has far-reaching implications. We are interested in the concept of harvesting circulating stem cells from the peripheral blood of patients recovering from chemotherapy as a means of improving the treatment for childhood cancer. Cure rates for many pediatric neoplasms have increased substantially with the introduction of intensive combination chemotherapy, but efforts in this direction are currently limited, primarily by the problems of profound myelosuppression. The ability to harvest significant quantities of stem cells easily, to store them in liquid nitrogen, and then to return them to the patient after ablative doses of chemotherapy might considerably increase the therapeutic ratio of a variety of treatment regimens and significantly increase the percentage of cures obtained.

During investigations into the optimal conditions for procuring circulating stem cells by continuous-flow centrifugation using the Aminco celltrifuge, we have varied such factors as the speed of centrifugation and the site of the collection port in relation to the buffy coat. Our preliminary results with 27 experiments on normal donors have shown an increase in the concentration of CFUs/ml with a range of from 7.5 to 6697, with a median of 534. A 3.5-hr leukapheresis procedure collecting 200 ml of cells could therefore yield 10^5 CFUs. When the optimal conditions for collecting CFUs are established, it is likely that the range in normals will be of the order of 2-5 × 10^5 per collection. If we extrapolate from the findings...
of Richman et al. of a median fourfold rise in circulating CFUs after chemotherapy, with values rising as much as 20-fold, it should prove possible to harvest routinely $10^6$-$10^7$ CFUs in a single 3.5-hr leukapheresis procedure. This quantity is of the same order of magnitude as that obtained in a bone marrow transplantation harvest. Although circulating CFUs may not be qualitatively as effective as bone marrow colony-forming units in reconstituting the bone marrow, and thereafter the peripheral leukocyte count, leukapheresis followed by reconstitution with autologous CFUs post chemotherapy may prove to be a major new procedure in enhancing the efficacy of intensive care chemotherapy.

MARTIN G. MOTT
ELLEN GILKERSO
Children's Hospital at Stanford
Children's Oncology Program
520 Willow Rd.
Palo Alto, Calif. 94304

REFERENCE

Procurement of Stem Cells by Continuous-Flow Centrifugation: Reply

To the Editor:

The observations of Mott and Gilkerson provide additional documentation of the ability to obtain high concentrations of stem cells from the peripheral blood by continuous- or semi-continuous-flow centrifugation. Further investigations of optimum methods for augmenting the number of circulating stem cells are certainly in order. It is of interest that Ross et al., in canine studies, have demonstrated a rebound increase in stem cells on the day following pheresis, suggesting a sensitive regulatory mechanism. In our studies, however, we have not observed a significant change in circulating CFU-C concentrations either immediately following or 24 or 48 hr after a 3-liter pheresis. These conflicting results may be explained by differences in methodology and particularly in the amount of blood pheresed.

In addition to pheresis itself, other agents which may stimulate an outpouring of stem cells into the circulation, such as etiocholanolone, endotoxin, steroids, lithium, and adrenalin, should be explored. Ross et al. have recently demonstrated increased circulating stem cells in dogs treated with dextran sulfate prior to pheresis. As we have indicated, the numbers of circulating stem cells increase following chemotherapy in most, but not all, patients. With improved pheresis techniques combined with the effect of chemotherapeutic and other agents on the concentration of circulating stem cells, it may indeed be easily possible to obtain a bone marrow transplant dose of stem cells from the peripheral blood for studies of autologous stem cell reconstitution.

Demonstration of the efficacy of reconstitution with autologous bone marrow stem cells has been difficult. In pilot studies, we have been unable to show a clearly beneficial effect of autologous bone marrow infusion when used with high doses of cytoxan and adriamycin. Better drug models to test the efficacy of autologous reconstitution are needed, especially using agents which produce delayed or prolonged periods of suppression (e.g., nitroso-ureas, melphalan, myleran). Autologous stem cell reconstitution may prove useful in patients who have undergone multiple courses of chemotherapy over many years and in whom tolerance to chemotherapy is decreased. Autologous stem cells harvested prior to any therapy might be used to restore the bone marrow to pretreatment status, thus allowing continuation of effective therapy and prolongation of disease-free survival.

CAROL RICHMAN
ROY S. WEINER
RONALD A. YANKEE
University of Chicago
Section of Hematology/Oncology
950 E. 59th St.
Chicago, Ill. 60637

University of Florida
College of Medicine
Section of Medical Oncology
J. Hillis Miller Health Center
Gainesville, Fla. 32610

Sidney Farber Cancer Center
35 Binney St.
Boston, Mass. 02115
Procurement of stem cells by continuous-flow centrifugation [letter]

MG Mott and E Gilkerson