Availability of hematopoietic growth factors (GF) has started a new arena of dose-intensification. The use of such growth factors has resulted in faster hematopoietic recovery of cancer patients and now offers several new treatment modifications. These include: (1) dose-intensification without hematopoietic stem cell support, (2) speedier hematopoetic recovery after hematopoietic recovery after hematopoietic ablative therapy and stem cell transplantation (allo- or autologous); (3) use of combination of growth factors, and (4) improvement in the delivery of anti-microbial drugs which are toxic towards hematopoietic cells (Gancyclovir, Bactrim, etc.). The above treatment strategies are under active clinical trials and can provide improved, cost-effective methods of treating patients with cancer.

Key Words: Growth factors, hematopoietic recovery.

INTRODUCTION

Hematopoietic growth factors (GF) are glycoproteins involved in proliferation, differentiation and regulation of hematopoietic progenitor cells. Considering that hematopoietic toxicity is the most common obstacle in delivering higher dosages of most cancer therapies, these GF can help with dose intensification. Significant research has been performed to prove that various methods (GM-CSF, GC-SF, erythropoietin, primed or unprimed peripheral blood stem cells used alone, or in combination, with bone marrow) cause faster hematopoietic recovery and thus save hospitalization costs. However, we still need to learn more about the quality of hematopoietic engraftment and the risk of such therapies, especially to prove that such manipulations (including various methods of purging if used) do not alter the relapse rate or cause patients to develop secondary malignancies or late complications of graft failure. The situation is even more difficult for patients undergoing allogeneic BMT because immunomodulatory dysfunction can cause further problems with the hematopoietic system and can affect relapse rate.

In this regard, other factors including the higher incidence of CMV infection in patients receiving allogeneic BMT can delay hematopoietic engraftment. Several factors are responsible for hematopoietic engraftment and different assays have been evaluated to assess clinical benefit.

STEM CELL ASSAYS

Some of the important assays are briefly detailed below: (1) Quantitation of colony forming units granulocyte macrophage (CFU-GM) dose infused during autologous stem cell transplant used to be considered the best predictor of hematopoietic engraftment, but several new issues have complicated the correlation. Specifically, CFU-GM assay is not standardized from institution to institution. It only measures one or a few limited types of already committed hematopoietic progenitors, it does not predict the time to engraftment in all situations and it requires 10–14 days to obtain results. Assay based on detecting the presence of CD34 antigen on early hematopoietic progenitors in the stem cell pool evaluates the early hematopoietic progenitors in a matter of hours. This assay can be easily
standardized from institution to institution. Unfortunately, CD34 assays are hard to perform on frozen thawed cells. Several groups have examined the correlation between CD34 positive stem cells infused at transplant and subsequent hematopoietic engraftment, and have found that it is a better predictor than of the number of CFU–GM colonies infused during stem cell transplantation. These colony forming cells may be found in peripheral blood leukocytes collected following stem cell mobilization by chemotherapy. In one recent analysis, it appears that a positive correlation exists between CD34 positive cell fraction and CFU-GM colony forming cells in peripheral blood from normal individuals, or from individuals recovering from cyclophosphamide chemotherapy and receiving no concomitant leukocyte stimulation therapy. Poor correlation was observed when similar data from bone marrow transplantation was analyzed. Accordingly, application of this assay as a substitute for the CFU–GM assay needs further investigation. The authors advocate adoption of this assay in parallel to the CFU-GM, in that it may yield complimentary information and it does retain the advantages of rapid result and ease of standardization. (3) Viable cell count by trypan blue is another assay which helps decide the quality of stem cells, especially as it is easy to perform and is useful in deciding the change with time. In our laboratory, the viability drops substantially after 3–4 years of storage in 110 to 135°C electric freezers. (4) Other assays for the primitive stem cells may also help in the prediction of hematopoietic engraftment and are being evaluated. FACTORS EFFECTING HEMATOPOIETIC ENGRAFTMENT

In clinical trials, patients with AML tend to have the slowest hematopoietic engraftment after AUBMT. Some of the factors which effect hematopoietic reconstitution are listed below:

1. Primary disease and its treatment effect subsequent engraftment.
2. Residual toxicity of therapy (acute and chronic) given before bone marrow harvest.
3. Quality and quantity of bone marrow harvested, cell number, viability, CFU-GM, BFU-E dose, etc.
4. Use of hematopoietic growth factors; dose, route of administration.
5. Bone marrow purging, processing (various purging methods can have different effects on the hematopoietic system, storage, thawing and reinfusion
6. Bone marrow cryopreservation, which may be different for malignant cells when compared with normal hematopoietic cells.

(7) Microenvironment conditions during the time of early transplant, i.e., effect of various infections (especially B19 parvovirus, cytomegalovirus, EpsteinBarr virus, and other viruses), various drugs (especially antibiotic), vitamin levels, and release of various growth factors from the microenvironment and host cells.

(8) In patients undergoing allogeneic BMT; additional factors include: GVHD, treatment of GVHD, genetic disparity (nonmajor HLA type), and immunosurveillance dysfunction. Correlation of in vitro assays of stem cells with subsequent engraftment has been attempted. The data suggest that CD34 positivity is probably the most reliable predictor of engraftment of PBSC when previously untreated patients are utilized, but the disagreement persists as to the true benefit of the assay especially in heavily pretreated patients. The correlation of CD34 positivity in bone marrow and subsequent engraftment is not as good as that with PBSC.

CORRELATION OF STEM CELL ASSAYS AND ENGRAFTMENT

The minimal number of PBSC necessary for hematologic recovery was recently evaluated. Although similar doses of MNCs were used, the reviewer found a striking variation of the dosage of CFU–GM infused between centers which was certainly related to the differences in the performance of the colony assay. In spite of the variation in estimating dosage, rapid engraftment was observed in nearly all of the studies reviewed. Analysis of the data from three centers where dosage of PBSC was compared to hematologic recovery, revealed variations in patient populations, mobilization protocols, and the highdose chemotherapy used. Correlation between CFU–GM dose and the rate of hematopoietic reconstitution was observed. Multivariate analysis of 65 patients transplanted with either allogeneic (n = 14) or autologous (n = 13) bone marrow or chemotherapy mobilized PBSC (n = 38) showed that the CFU–GM dose is the only independent variable influencing neutrophil or platelet reconstitution. A further analysis of these data revealed that a threshold effect exists, i.e., an optimal number of CFU–GM is required to ensure rapid hematopoietic reconstitution. These data suggest an optimum number of 20–50 x 10⁴ CFU–GM kg⁻¹ body weight (BW) which is consistent with previous reports. Likewise, 2 x 10⁶ CD34+ cells kg⁻¹ BW represent conservative doses