A STUDY OF THE CIRCULATING MYELOID PROGENITOR CELL IN MAN

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CONTENTS

SUMMARY

This thesis examines the circulating myeloid progenitor cell (PB CFU-GM) in normal subjects and in patients with Acute Non-lymphoblastic Leukaemia (ANLL). The aims are, firstly, to develop an accurate assay for PB CFU-GM; secondly, to establish the normal range for PB CFU-GM; thirdly, to study the changes in the levels of PB CFU-GM in normal subjects under various physiological conditions; fourthly, to study the changes in the levels of PB CFU-GM in patients with ANLL; fifthly, to study the collection and cryopreservation of peripheral blood mononuclear cells (PB MNC) from these patients during very early remission and sixthly, to use these cells for autologous stem cell rescue at relapse to test whether they possess haemopoietic reconstitutive capacity and whether longer lasting second remissions may result.

PB CFU-GM were assayed by culturing PB MNC in alpha-modified Eagle's medium with 15% foetal calf serum in 0.3% agar using human placental conditioned medium (HPCM) as a source of Colony Stimulating Activity (CSA). Colonies of >40 cells after 14 days' incubation were scored as CFU-GM. Using this system, studies in normal subjects demonstrated that no linear relationship exists between the number of PB MNC cultured and the number of CFU-GM detected. Further

assays performed with monocyte-depleted PB MNC alone and with the monocytes added back showed that there is a critical number of monocytes in this assay system above and below which CFU-GM growth decreases. When PB MNC were cultured at 0.625 to 10 x 10^5 cells per plate, the highest CFU-GM growth occurred in most subjects at either 1.25 or 2.5 x 10^5 cells per plate but in particular subjects it might occur at any of the five plating numbers used and the optimal plating number varies even in the same individual studied at different times. Thus it is important to perform this assay with several plating numbers in order to measure PB CFU-GM accurately. Because of the larger number of monocytes present, this monocyte effect is much more important in the PB CFU-GM assay system than in the bone marrow CFU-GM assay system where such an effect has already been demonstrated by others. The same monocyte effect was observed when PB MNC from patients with ANLL, acute lymphoblastic leukaemia, myelofibrosis, Hodgkin's Disease and drug induced agranulocytosis were cultured.

The monocyte effect when cryopreserved PB MNC were cultured was different from that when fresh cells were cultured. The highest CFU-GM growth usually occurred at a higher plating number (either 5 or 10 x 10^5 cells per plate) and this was shown to be due to changes in monocytes after the freeze-thaw process thereby affecting the CFU-GM/monocyte interaction. This study also shows that leucocyte feeder layers are a better source of CSA than HPCM when cryopreserved PB MNC are cultured.

In the present study, the normal range of PB CFU-GM was found to be wide and the levels were higher in males

than in females, as found in previous reports. The levels from normal subjects fitted a log-normal distribution. No significant difference was found between levels measured at 9 am and 2 pm or on consecutive days. Repeated measurements in individuals over a two year period showed moderate variation around each individual's own mean. Physical exercise led to a threefold increase in the levels of PB CFU-GM, reflecting the presence of a mobilisable pool. Such a readily mobilisable pool may contribute to the fluctuations noticed in individuals studied at different times. Previously reported lower normal ranges may be explained by a failure to take into account the monocyte effect.

Measurements of PB CFU-GM in 15 ANLL patients showed that a mean increase of 25 times the mean normal level occurred during very early remission, 15 to 29 days after the completion of induction chemotherapy. No such increase was found in patients not entering complete remission. Thus these high levels most probably reflect the intense recovery by normal haemopoietic cells while the patient is entering remission. The high levels lasted for several days while the platelet count rose rapidly. Such findings had not been reported before and raised the possibility of harvesting PB MNC during this phase for later autologous stem cell rescue.

Three or four continuous flow leukaphereses were performed on each of five ANLL patients during very early remission and yielded cells containing a mean of 37 x 10^4 CFU-GM/kg body weight for each patient. This represents five times the average yield of CFU-GM obtained by bone

marrow aspiration under general anaesthesia. There were no significant side-effects. These cells were cryopreserved and the PB CFU-GM remained viable after more than two years storage.

Two of five the patients who have had peripheral blood cells cryopreserved have relapsed. In the first patient, re-induction was attempted with high dose melphalan chemotherapy followed by infusion of his stored cells. Haemopoietic recovery started 11 days after the melphalan infusion, much earlier than the three to four weeks observed in other patients treated with high dose melphalan but not receiving stem cell rescue. However, leukaemic cells regrew quickly so that no definite conclusion could be drawn. The second patient received 1200 rads total body irradiation followed by autologous stem cell infusion containing 23 x 10^4 CFU-GM/kg body weight. Early recovery was again observed but recovery was incomplete. Eight weeks after infusion, the absolute neutrophil count was above 1,000/µl, the lymphocyte count was normal but the platelet count was only 19,000/µl and the patient required red cell transfusion. By thirteen weeks, there was evidence that the stem cell graft function was improving but the leukaemia had also relapsed. This case demonstrated that the harvested cells possess haemopoietic reconstitutive capacity but the incomplete recovery suggests that there are considerably fewer pluripotent stem cells per CFU-GM compared to bone marrow cells collected at stable remission. Whether these cells harvested in very early remission contain fewer leukaemic cells and will thus give rise to longer lasting second remissions requires further clinical investigation.

The use of such cells for haemopoietic reconstitution following supralethal chemoradiotherapy may represent a new therapeutic option for ANLL patients who are ineligible for allogeneic bone marrow transplantation because of age or lack of suitable donors.