IDENTIFICATION OF BCR/ABL-NEGATIVE HEMATOPOIETIC PROGENITOR CELLS WITHIN CHRONIC MYELOID LEUKEMIA MARROW

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ABSTRACT

Chronic myeloid leukemia (CML) is a clonal neoplastic disorder of the hematopoietic stem cell. Despite the stem cell origin, it is believed that normal stem cells co-exist with the malignant stem cells in the bone marrow of patients with chronic phase CML. Immunofluorescence cell sorting and differential stromal adhesion were investigated as possible ways that normal primitive progenitor cells and stem cells could be isolated from within CML marrow for use in autologous transplantation. Initially, $CD34^+DR^-$ and $CD34^+DR^+$ cells were isolated using centrifugal elutriation, monoclonal antibody labeling, and flow cytometric cell sorting. Polymerase chain reaction (PCR) analysis of these $CD34^+$ subpopulations was used to detect the presence of the BCR/ABL translocation characteristic of CML. The $CD34^+DR^+$ subpopulation contained BCR/ABL(+) cells in 13 of 14 marrow specimens, while the $CD34^+DR^-$ subpopulation contained BCR/ABL(+) cells in 7 of 12 CML marrows. Progenitor cell assay results from 8 patients revealed that the $CD34^+DR^-$ subpopulation contained significantly fewer BCR/ABL(+) progenitor cells than either low density bone marrow (LDBM) or the $CD34^+DR^+$ fraction. After 28 days of stromal cell-free long-term culture, 5 of 9 cultures initiated with LDBM, and 4 of 6 cultures initiated with $CD34^+DR^-$ cells produced BCR/ABL(-) cells. Taken together, these results confirm that normal and leukemic progenitor cells co-exist within CML bone marrow. Adherence to selected components of bone marrow stroma was also examined as a potential means of removing BCR/ABL(+) stem cells from CML marrow. There was a marginally significant ($0.05<p<0.1$) decrease in the capacity of CML progenitors to adhere to thrombospondin (TSP), but there was no difference in their capacity to attach to *c-kit*-ligand (KL) or to the combination of TSP and KL. PCR analysis of the myeloid progenitors within the adherent and non-adherent fractions revealed no significant difference in the percentage of BCR/ABL(+) colonies between any of the fractions and the input population.

Although the cytoadhesion assay did not appear to select for normal progenitors, flow cytometric selection for $CD34^{+}DR^{-}$ cells may be an appropriate primary enrichment step for the isolation of normal stem cells for use in autologous transplantation.

Table of Contents