21 PROPERTIES OF CORD BLOOD CD34+ CELLS IN LONG TERM CULTURE INITIATING CELLS AND HPP-Q (High Proliferative Potential-Quiscent) ASSAYS

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Cord blood is an alternative to bone marrow and peripheral blood as a source of transplantable hematopoietic tissue. However, because of reduced volume, the stem cell content is limited; its use as a graft for adult patients might require ex vivo expansions. We have investigated the behavior of cord blood CD34+ cells in two different culture systems: LTC-IC and HPP-Q assays. LTC-IC (Long Term CultureIniating Cells) are the hematopoietic progenitors able to generate colony forming unit cells (CFU) after 5-8 weeks (35-60 days) of culture on bone marrow stroma and represent the most primitive progenitors currently detectable in vitro. HPP-Q assay is a relatively new, rapid and simple assay to detect the High Proliferative PotentialQuiescent Cells, it can be completed within two weeks. Data from previous research had demonstrated that massive stem cell expansion in cord blood can be obtained in well defined culture conditions. In our experiments; after delivery of the baby with the placenta still in utero, cord blood was collected into PBS-EDTA containing syringes; flow cytometric analysis (CD34, Rhodamine123, HLA-DR, CD71, CDw90, FLT3) was made, CD34+ cord blood cells were selected with MACS Multi Sort CD34 magnetic beads, CD34 and Rhodamine123 expression of these cells were analyzed with flow cytometry; and the cells were cultured in LTC-IC and HPP-Q cuiture systems. Seven cord blood samples were evaluated. The purity of selected CD34+ cord blood cells were 74.2 ± 8.4. Expansion rate in LTC-IC was higher when compared to HPP-Q, two-tofour fold LTC-IC were detected. A positive correlation of Rhodamine123^{dull} CD34+ cord blood cells and CAFC (Cobblestone Area Forming Cells) in LTC-IC was observed. This shows that CD34+Rhodamine123^{dull} cell population belong to earlier stages of stem cell ontogeny and may be used as a predictive value for engraftment. Our research is aimed to represent an initial step towards cord blood transplantation and larger scale cultures for transplantation in our university hospital. This ongoing research is supported by TÜBİTAK project number SBAG 1980.

22 LONG TERM CULTURE-INITIATING CELL ASSAY BY USING LIMITING DILUTION

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Even though much progress has been made in defining primitive hematologic cell phenotypes by using flow cytometry and clonogenic methods, the direct method for study of marrow repopulating cells still remains to be elusive. Long Term Culture-Initiating Cells (LTC-IC) are the most primitive human hematopoietic cells detectable by in vitro functional assays. We have used murine bone marrow stromal cell line M2-10B4 (ATCC) as feeder layer which is known to release IL-3 and G-CSF, and a good alternative to human bone marrow stromal cellls. After reaching to confluent level of M2-10B4 in RPMI 1640 in approximately one week, cells were irradiated with 15 cGy from a $137C_s$ source, tripsinized and then the cells were plated to 96 well micro plates in IMDM (Iscove's Modified Dulbecco's Medium). Plates could be maintained for 1-5 weeks by exchanging the half of medium. CD34+ peripheral stem cells or cord blood cells selected with MACS magnetic beads were overlaid on M2-10B4 in six dilutions with 16 wells per dilution. Cultures were fed by half medium change weekly. Plates were kept at incubator with 5% C02. LTC-IC was scored with Cobblestone Area Forming Cells (CAFC) by week 5-8. This LTC-IC assay with limiting dilution is easier than the other LTC-IC assays since ready made murine bone marrow stromal cell line plates can be used in five weeks and to count CAFC is less time consuming. This method is used as an "ex vivo expansion" assay for CD34+ cells.