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Large ex vivo expansion and reduced alloreactivity of umbilical cord blood T lymphocytes. D. Skea, N. Chang, B. Dabek, R. Hedge and D. Bell. *X-Cell Biotech Division, Hemosol Inc., Etobicoke, ON, Canada.*

The use of human umbilical cord blood as a source of transplantable hematopoietic stem cells and progenitor cells may present some advantages over the use of bone marrow. For example, it has been suggested that the degree of HLA-matching may be less stringent and the risk of graft-vs.-host disease (GvHD) may be lower. We have been studying the ex vivo expansion of umbilical cord blood T lymphocytes with a view to their use in the adoptive immunotherapy of cancer, autoimmunity and infectious disease. We have developed a new method, involving the use of a conditioned medium (XLCM™), that consistently results in levels of umbilical cord blood T cell expansion not hitherto possible. Primary cultures of unfractionated low density mononuclear cells (LDMNC) derived from umbilical cord blood treated with 5% XLCM™ routinely show expansions greater than 10,000-fold within a time period of four weeks. By contrast, similar FBS-free cultures treated with IL-2 expand less than 10-fold and not beyond one week, while cultures treated with IL-2 and concanavalin A expand to a maximum of only 300-500-fold over two weeks and again, fail to continue to proliferate thereafter. The monoclonal antibody, OKT3, which, when combined with IL-2 and FBS, is known to stimulate proliferation of adult peripheral blood lymphocytes, permitted only a 17-fold expansion of umbilical cord blood lymphocytes under the same conditions. Thus, XLCM™, which also stimulates adult peripheral blood lymphocyte expansion to levels exceeding 100,000-fold in three to four weeks, is uniquely able to stimulate proliferation of umbilical cord blood lymphocytes to high levels. Cultures of XLCM™-stimulated umbilical cord or adult peripheral blood LDMNC are dominated by $CD4^+$ T lymphocytes for approximately the first two weeks. By four weeks, greater than 80% of the cultured cells bear the $CD8^+$ phenotype. By contrast, umbilical cord blood T lymphocytes cultured in the presence of IL-2 are all predominantly $CD8^+$. Thus XLCM™ not only promotes high levels of umbilical cord blood T lymphocyte expansion not previously possible, but it also permits the selective expansion of different T lymphocyte subsets from a single source. Furthermore, we have shown that umbilical cord blood lymphocytes are both weaker stimulators and poorer responders compared to adult peripheral blood lymphocytes in allogeneic mixed leukocyte reactions. These results suggest that adoptive immunotherapy with umbilical cord blood lymphocytes may be associated with less risk of GvHD. The selective and extensive expansion of subsets of the less alloreactive umbilical cord blood derived T lymphocytes could be extremely useful in the development of adoptive immunotherapies focusing on specific functional T lymphocytes subsets.

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Report on the standardization of clonogeneic hematopoietic precursor assays for the unrelated donor bone marrow transplantation trial. C.A. Keever-Taylor, N.H. Collins, S. Carter, L. Kelley, A. Gee and S. Fuller. *For the National Heart, Lung & Blood Institute, Unrelated Donor Bone Marrow Transplantation Trial Laboratory Committee; Medical College of Wisconsin, Milwaukee, WI.*

It has been previously recognized that clonongeneic assays for committed progenitors are poorly reproducible between laboratories. This has largely been ascribed to the use of locally prepared materials and reagents and to differences in criteria for colony scoring. The Laboratory Committee for the Unrelated Donor Trial was charged to standardize this assay for the monitoring of progenitor cell content in infused marrow. To this end a written protocol was developed requiring a commercially available reagent kit utilizing recombinant cytokines to stimulate colony growth. This was supplemented with training sessions, an illustrated manual for scoring and a series of cell exchanges with follow-up conference calls to discuss results. A total of 12-14 labs participated in 4 separate cell exchanges in which 2 samples of marrow were tested at 2 plating concentrations for BFU-e and CFU-GM content. The source laboratory prepared and shipped identical marrow samples via overnight carrier. All exchanges included nonmanipulated marrow. The first 3 exchanges included marrow T cell depleted (TCD) by counterflow centrifugal elutriation and the 4th exchange included marrow TCD by complement-mediated lysis with a T-cell specific moAb. Exchange #1 required on site preparation of mononuclear cells from the unmanipulated marrow, while subsequent exchanges used whole marrow. Plating concentrations included 2.5×10^4/mL in all exchanges, 5×10^4/mL in Exchange #1 and 1×10^4/mL in Exchanges 2-4. One mL cultures were plated in triplicate in 35 mm culture plates. Incubation was at 37°C for 14 days in a well-humidified atmosphere of 5% CO_2. Colonies per 10^5 cells were calculated for each plate in which the total colonies did not exceed 100. Viability was ≥85% for all shipped samples with the exception of the sample TCD by moAb that had to be dropped from analysis due to poor viability. There was considerable variability within a lab, as determined by assessment of the coefficient of variation between the two plating concentrations and among the replicate cultures. Variation between labs was even higher as assessed from data expressed as the average of both concentrations to the 10^5 cells plated. The coefficient of variation for the 4 exchanges ranged from 29.4% to 86% and did not improve over time. There was greater variation in samples with the lowest colony growth. Eleven of the 14 participating labs reported data >1 SD from the mean on one or more occasions. Variability was similar for CFU-GM and BFU-e. We conclude that variability in the CFU assay is not due solely to differences in methodology or scoring criteria, but may additionally be affected by shipping, conditions of incubation or technical skill. Alternative methods for measuring stem cell content that are more amendable to standardization, (e.g., Assessment of CD34 content), may be a preferred measure of stem cell content.

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No expression of LFA-1 on murine hematopoietic progenitor cells with colony-forming or radioprotective capacity. J.F.M. Pruijt, C.G. Figdor, Y. van Kooyk, R. Willemze and W.E. Fibbe. *Laboratory of Experimental Hematology, Leiden University Medical Center and Laboratory of Tumor Immunology, University Hospital Nijmegen, The Netherlands.*

The β_2-integrin LFA-1 (CDT11a) is expressed in-vitro on human committed hematopoietic progenitor cells (HPC) (Blood 80: 429, 1992 and 87: 4120, 1996). Recently, we have demonstrated that anti-LFA-1 blocking antibodies completely prevent the rapid mobilization of HPC with colony-forming and radioprotective capacity induced by IL-8 in mice (Blood 86: 1886a, 1996 (Abstr.)). We therefore, studied the expression and functional role of LFA-1 on HPC in-vitro and in-vivo. First, bone marrow (BM)-derived mononuclear cells (MNC) from BALB/c mice were incubated with anti-LFA-1 antibody (H154.163) and Goat-anti-Rat-Pe (GaRa-Pe). In the BM ± 50% of the MNC were $LFA\text{-}1^{neg}$. Cultures supplemented with G(M)-CSF/IL-1/IL-3/IL-6/SCF and EPO of 7500 sorted cells indicated that the $LFA\text{-}1^{neg}$ fraction contained the majority of the colony forming cells (CFU) ($LFA\text{-}1^{neg}$ 154 ± 64 v. $LFA\text{-}1^{pos}$ 22 ± 13, mean ± SD, n = 5). To assess the radioprotective capacity, lethally-irradiated recipient mice were transplanted with increasing numbers of BM-derived $LFA\text{-}1^{neg}$ or $LFA\text{-}1^{pos}$ MNC. The radioprotective capacity resided almost entirely in the $LFA\text{-}1^{neg}$ cell fraction, the radioprotection rate after transplantation of 10^3, 3×10^3, 10^4 and 3×10^4 cells being 80, 80, 100 and 100% respectively. In contrast, after transplantation of 3×10^3, 10^4 and 3×10^4 $LFA\text{-}1^{pos}$ cells, a radioprotection rate of 11, 0 and 30% was obtained. Subsequently, BM-derived sorted Wheat-germ-agglutinin $(WGA)^{pos}/Lin^{neg}$ cells were stained with rhodamine (Rho) (100 ng/ml, 20'37°C), followed by incubation in Rho free medium (20'37°C). Rho^{neg} cells were isolated and incubated with anti-LFA-1 antibody and GaRa-Pe.

> 95% of the Rho^{neg} cells were $LFA\text{-}1^{neg}$. Cultures of 750 sorted cells showed that the $LFA\text{-}1^{neg}$ fraction contained all CFU (247 v. 1, mean, n = 4). Transplantation of 150 Rho^{neg} $LFA\text{-}1^{neg}$ or up to 600 Rho^{neg} $LFA\text{-}1^{pos}$ cells protected 100 and 0% of lethally-irradiated recipient mice, respectively. These results show that HPC with colony-forming or radioprotective capacity in steady-state BM do not express LFA-1.

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Expression of APO-1/FAS antigen (CD95) in peripheral blood progenitor cells (PBPC) is influenced by the mobilisation regimen used. A.C. Parker, J.I.O. Craig and R.S. Anthony. *University of Edinburgh, John Hughes Bennett Laboratory, Western General Hospital, Edinburgh, Scotland, UK.*

Fas antigen (CD95) is a transmembrane protein belonging to the TNF superfamily. Activation by its ligand results in cell death through apoptosis. Fas is expressed at low levels on unstimulated $CD34^+$ bone marrow (BM) cells but increases following exposure to growth factors including G-CSF. The majority of PBPC are mobilised with a combination of chemotherapy + G-CSF. The effects of mobilisation on Fas expression are unknown. Using dual colour flow cytometry, Fas expression was studied on CD34 + ve progenitor cells from PBPC harvests collected after mobilisation with either cyclophosphamide (Cy) + G-CSF (300 μg/kg/day for 8 days) or standard chemotherapy + G-CSF. Thirty nine PBPC harvests from patients with acute leukaemia, CML, lymphoma and 5 normal bone marrows were analysed. The CD95 FITC fluorescence channel on the cytometer was calibrated with commercial microbeads to quantify Fas antigen expression as molecules/cell. A minor population of $CD34^+$ cells in PBPC (22.3%) and BM (23.2%) were Fas+. There was no evidence for a higher percentage of $CD34^+$ cells expressing Fas antigen in PBPC mobilised with G-CSF. However, there was a significant increase ($p = 0.0003$) in the receptor density when the number of molecules on the cell surface were analysed. The cell surface density of Fas increased from 2.2×10^3 molecules/cell in BM to 6.2×10^3 molecules/cell in PBPC. Mobilisation with Cy increased the average yield of $CD34^+$ progenitor cells from 2.47×10^6/kg with standard chemotherapy to 4.48×10^6/kg. The use of Cy significantly decreased the number of $CD34^+$ cells that were Fas+ (Cy 15.0%, standard chemotherapy 42.4%; $p = 0.0018$) and the density of Fas molecule expression on progenitor cells (Cy 4.5×10^3 molecules/cell, standard chemotherapy 11.1×10^3 molecules/cell; $p = 0.039$). In conclusion, the type of cytotoxic drug used for mobilisation appears to influence the level of Fas expression in PBPC. The use of haematopoietic growth factor for mobilisation does not increase Fas expression in PBPC when compared to unstimulated BM progenitor cells. As there is differential expression of Fas during haematopoietic cell maturation, the lower levels of Fas expression found on Cy mobilised PBPC may indicate the presence of a more primitive progenitor cell population in these harvests.

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Phenotypic and functional analysis of $CD34^+$ L-selectin subsets from human bone marrow, mobilized peripheral blood and umbilical cord blood. B. Bielorai, I. Kashiwakura, D. Sotiropoulos, G. Debnath, P.J. Hendrikx and J.W.M. Visser. *Lindsley F. Kimbal Research Institute, New York Blood Center, New York, NY.*

Interactions between hematopoietic stem cells (HSC) and the bone marrow (BM) microenvironment involve various adhesion molecules. L-selectin is one of the adhesion molecules expressed on HSC. Recent studies of clinical stem cell transplantation indicated that the number of $CD34^+$ cells in mobilized peripheral