Large ex vivo expansion and reduced alloreactivity of umbilical cord blood T lymphocytes. D. Skea, N. Chang, B. Dobek, R. Hedges and D. Bell. X-Cell Biotech Division, Hemotec Inc., Exboro, ON, Canada.

The use of human umbilical cord blood as a source of transplantable hematopoietic stem cells and progenitor cells may present certain advantages over the use of adult bone marrow. Furthermore, it has been suggested that 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, autoimmune and infectious diseases. 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 in primary cultures of unmanipulated umbilical cord blood. This medium is able to stimulate unmanipulated cord blood T cells to proliferate, whereas IL-2 and FBS, when used together, were shown 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 umbilical cord blood T cell expansion not hitherto possible. Primary cultures of umbilical cord blood are incubated with XLCM™ and IL-2 at 37°C for 14 days in a lymphocytes subsets. This method of expansion does not exceed 100. Viability was >85% for all 1639 458-m colonies in which the total colonies did not exceed 100. 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 immunoreactivity.

The CD95 FITC fluorescence channel on the cytometer was calibrated with a 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 umbilical cord blood T cell expansion not hitherto possible. Primary cultures of umbilical cord blood are incubated with XLCM™ and IL-2 at 37°C for 14 days in a lymphocytes subsets. This method of expansion does not exceed 100. 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 T lymphocytes could be extremely useful in the development of adoptive immunotherapies focusing on specific functional T lymphocytes subsets.

Report on the standardization of clonogenic hematopoietic progenitor assays for the unrelated donor bone marrow transplantation trial. C.A. Weaver-Taylor, N.H. Collinger, 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 clonogenic 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 common method of assay and a common scoring system for examining marrow for 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 monoclonal antibody. Exchange 1 required on site preparation of mononuclear cells from the nonmanipulated marrow, while subsequent exchanges used whole marrow. Plating concentrations included 2.5 x 10^5/ml in all exchanges, 5 x 10^5/ml for exchange 2 and 10 x 10^5/ml for exchange 3. Each sample was plated in triplicate in 35 mm culture plates. Incubation was at 37°C for 14 days in a well-humidified atmosphere of 5% CO2. Colonies per 10^3 cells were calculated for each plate in which the total colonies did not exceed 100. Viability was 6% 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 and between laboratories in the assessment of colony morphology due to the presence of 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 10^3 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 colony counts for both 10^3 and 10^4 cells plated. 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 amenable to standardization, (e.g., Assessment of CD34 content), may be a preferred measure of stem cell content.

No expression of LFA-1 on murine hematopoietic progenitor cells with embryonic and fetal liver origin. I.F.M. Priumi, C.G. Figdor, van Kooij, W. Willenre and W.E. Fibbe. Laboratory of Experimental Hematology, Leiden University Medical Center and Laboratory of Tumor Immunology, University Hospital Nijmegen, The Netherlands.

The β-integrin LFA-1 (CD11a) 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 prevented the rapid mobilization of HPC with colony-forming and radioprotective capacity induced by IL-3 in mice (Blood 86: 1886a, 1996). 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 (Goat-PE). In the BM ± 50% of the MNC were LFA-1+, Cultures supplemented with GMP-CSF/IL-7/11-3/IL-CSF and anti-LFA-1 antibody of sorted MNC indicated that the LFA-1+/11 fraction of the colony forming cells (CFU) (LFA-1+/11 154 ± 64 v. LFA-1+ 22 ± 13, mean ± SD, n = 5). To assess the radioprotective capacity, lethally-irradiated recipient mice were transplanted with increasing numbers of BM-derived LFA-1+/11 or LFA-1- MNC. The radioprotective capacity resides almost entirely in the LFA-1+/11 cell fraction, the radioprotection rate after transplantation of 10^3, 10^4 and 10^5 cells being 80, 110 and 100%, respectively. In contrast, after transplantation of 3 x 10^3, 10^4 and 10^5 LFA-1- cells, a radioprotection rate of 11, 0 and 30% was obtained. Subsequently, BM-derived sorted Wheat-germ-agglutinin (WGA)+(Li+) cells were stained with rhodamine (Rh) (100 ng/ml, 20°C), followed by incubation in Rhio medium (20°C). Rhio cells were isolated and incubated with anti-LFA-1 antibody and GMP-CSF.

> 95% of the Rho+ cells were LFA-1+. Cultures of 750 sorted cells showed that the LFA-1+ fraction contained all CD4 (247 v. 1, mean, n = 4). Transplantation of 150 Rho+ LFA-1- or up to 600 Rho+ LFA-1+ 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.

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

Antigen CD95 (Fas) is a transmembrane protein belonging to the TNF superfamily. Activation by its ligands 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+ 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 marrow samples were analysed. The CD95 FITC fluorescence channel on the cytometer was calibrated with commercially available monoclonal antibodies directed against Fas. 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 x 10^5 molecules/cell in BM to 6.2 x 10^5 molecules/cell in PBPC. Mobilisation with Cy increased the average yield of CD34+ progenitor cells from 2.47 x 10^7 kg with standard chemotherapy to 4.84 x 10^7/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 x 10^5 molecules/cell, standard chemotherapy 11.1 x 10^5 molecules/cell; p = 0.033). In conclusion, the type of cytotoxic drug used for mobilisation appears to influence the 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.