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Large ex vivo expansion and reduced allactivity of umbilical cord T lymphocytes. D. Skea, N. Chang, B. Dobek, R. Hodge and D. Bell. X-Cell Biotech Division, Hemorid 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 standard bone marrow (BM) or peripheral blood (PB) progenitors. For this reason, it has been suggested that such a source would be better suited to non-matched transplantation. However, 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 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 that are comparable to those obtained using unmanipulated BM or PB progenitors. The protocol is based on a combination of unfractionated low density mononuclear cells (LDMC) 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 within two weeks and aged 10% of the cells to proliferate. 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 LDMC 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 selection of a CD4 T lymphocyte subset previously unobtainable with IL-2. 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 leucocyte 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 allreactive umbilical cord blood derived 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. Keever-Taylor, N.H. Collins, S. Curtis, L. Kelley, A. Geo 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 training session, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth. This was supplemented with training sessions, an illustrated manual and the use of a standardized reagent kit for colony growth.

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.

The anti-Fas (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, we have used anti-LFA-1 antibody and anti-CD34 antibody to study the expression of CD34+ lymphocytes with a view to their use in the adoptive immunotherapy of cancer, immune and infectious disease. We have been studying the ex vivo expansion of umbilical cord blood 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 selection of a CD4 T lymphocyte subset previously unobtainable with IL-2. 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 leucocyte 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 allreactive umbilical cord blood derived T lymphocytes could be extremely useful in the development of adoptive immunotherapies focusing on specific functional T lymphocytes subsets.


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