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Large ex vivo expansion and reduced alloactivity of umbilical cord blood T lymphocytes. D. Skea, N. Chang, B. Debek, R. Hedge and D. Bell. X-Cell Biotech Division, Hemotec 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 or adult peripheral blood. In particular, the absence 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 unmitigated cord blood T cell expansion that are significantly higher than those obtained using unfractured 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 the weeks and age. One of the main mechanisms to proliferate these cells is 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+.

Furthermore, we have shown that umbilical cord blood lymphocytes are two weaker stimulators and poorer responders compared to adult peripheral blood lymphocytes in allogeneic mixed leukocyte reactions. These results suggest that the adoptive immunotherapy with umbilical cord blood lymphocytes may be associated with less risk of GVHD. The selective and extensive expansion of subsets of the alloreactive 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 clonalogenic hematopoietic progenitor assays for the unrelated donor bone marrow transplantation trial. C.A. Keever-Taylor, N.H. Collin, 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 set of reagents and conditions for the enumeration of CFUs in PBPC harvests collected after mobilization. The uniformity in methodology of a number of clonogenic assays in PBPC harvests collected after mobilization of peripheral blood showed that there was considerable variability in the cellular/molecular components of PBPC harvests collected after mobilization of peripheral blood. The variability seen was due in part to the use of different growth factors, with the use of GM-CSF leading to the largest degree of variability in the number of CFUs and BFUs. The variability also reflected differences in the scoring of colonies and in the type of cytotoxic drug used for mobilization. The use of GM-CSF resulted in a 5- to 10-fold increase in the number of CFUs and BFUs when compared to chemotherapy alone. The use of GM-CSF resulted in a 5- to 10-fold increase in the number of CFUs and BFUs when compared to chemotherapy alone. However, the use of GM-CSF resulted in a 5- to 10-fold increase in the number of CFUs and BFUs when compared to chemotherapy alone.