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Review Article

Cell Processing: Current Status and Future Directions

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Specialized clinical cell processing began in the Department of Transfusion Medicine at the National Institutes of Health in 1984. The number and complexity of procedures performed increased quickly and in 1997 a highly specialized cell processing laboratory was opened. The laboratory has approximately 3,000 square feet, specialized air handing, a highly trained staff, and written laboratory procedures. In addition to standard laboratory equipment, the laboratory has numerous cell isolation instruments, flow cytometers, and automated cell counting instruments. The laboratory supports blood and bone marrow transplant protocols by isolating CD34+ stem cells, removing T lymphocytes, culturing lymphocytes to eliminate donor lymphocytes that are reactive with recipient alloantigens, and stimulating lymphocytes to induce Th2 type cells to reduce graft versus host disease. The laboratory has also been preparing dendritic cells to support protocols using immune therapy to treat cancer. In addition, pancreatic islet cells are isolated from organ donors for transplantation to treat type I diabetes mellitus.

Key Words: Cell processing, periphral blood progenitor cells, CD34+ cells, dendritic cells, immune therapy

The Birth of Cell Processing Laboratories

Specialized cell processing laboratories began approximately 20 years ago. Many cell processing laboratories were developed to provide basic procedures to remove plasma and red cell from bone marrow in allogeneic marrow transplants involving ABO incompatible donor-recipient pairs. Because these procedures were similar to those already being performed in blood transfusion laboratories and because blood transfusion services worked closely with bone marrow donor teams, many cell processing laboratories started in hospital blood banks. The National Institutes of Health (NIH), Department of Transfusion Medicine (DTM) began their cell processing laboratory in 1984. The first products that it produced were lymphokine activated killer (LAK) cells to treat melanoma. Over the past 20 years cell processing laboratories have grown and now routinely provide a wide variety of services including cell isolation, cell culture, gene transfection, cryopreservation, dendritic cell preparation, and pancreatic islet cell isolation.

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The Need for Specialized Facilities

The focus of cell processing laboratories was and continues to be to bring procedures developed in research laboratories into clinical practice. This often involves scaling up procedures and converting research procedures from open systems preformed in test tubes and flasks to closed systems involving bags. If the role of cell processing laboratories was limited to converting research procedures from open to closed systems, operating a cell processing laboratory within a transfusion service laboratory would be appropriate. However, soon after cell processing laboratories were started it became apparent that their role was much broader in function and specialized space separate from the blood bank laboratory was needed.

Cell processing laboratories must comply with all aspects of current good laboratory practices (cGLP) and current good manufacturing practices (cGMP).[1] They must write, follow, and maintain

standard operating procedures; maintain records; monitor and control processes and reagents; train staff; and monitor product and procedure quality. In addition to providing products to support clinical patient care, cell processing laboratories must also develop and test new procedures and products. They must also provide research products to the laboratories of clinical investigators. As a result, cell processing laboratories require specialized space to meet their evolving service and research needs and to facilitate compliance with cGMP requirements and evolving standards and regulations.

The DTM Cell Processing Laboratory

The DTM opened its state-of-art cell processing laboratory in 1997. The laboratory boasts 3,000 square feet of office space, general laboratory space, and specialized laboratory space. The special space includes two isolation processing rooms, a cyropreservation and product storage room, and a flow cytometry room. The laboratory has class 10,000 quality single pass air. The air temperature and humidity are tightly controlled at 68 to 72°F and 50% respectively. Airflow in the laboratory is carefully controlled to maintain negative pressure in the isolation rooms. The laboratory's freezers, refrigerators, and incubators are monitored centrally. In addition to basic laboratory equipment such as microscopes, centrifuges, scales, refrigerators and freezers, the laboratory has more specialized equipment including biological hoods, incubators, cell washers, sterile connecting devices, heat sealers, controlled rate freezers, liquid nitrogen storage tanks, cell isolation instruments, and counterflow centrifugal elutriation instruments. The laboratory also has automated cell counters and flow cytometers to measure cell counts and phenotypes during and after processing.

To operate this laboratory, the staff is highly trained and works only in the cell processing laboratory. The laboratory has written instructions for all procedures and important aspects of all procedures are documented in the laboratory's records. The laboratory will soon be documenting its activities using a computer software system designed specifically for cell processing laboratories.

Processing Hematopoietic Progenitor Cells

The DTM cell processing laboratory supports an active blood and marrow transplant program. The laboratory provides a number of graft engineering services. These include CD34+ cell isolation, T-cell depletion, and the modification of donor lymphocytes.

CD34+ cell isolation

The primary source of hematopoietic stem cells used for transplantation at the NIH is G-CSF-mobilized peripheral blood progenitor cells (PBPCs). In some cases unmanipulated PBSCs are transplanted, but in most cases the PBSC components are enriched for CD34+ cells prior to transplantation. In many cases, CD34+ cells are isolated with the fully automated Isolex Immunomagnetic System. With this system hematopoietic progenitor cells are isolated with the Isolex 300i and CD34 monoclonal antibodies bound to sheep anti-mouse IgG-coated paramagnetic beads (2). The CD34+ cells are released from the paramagnetic beads using peptides that competes with antibody binding to the CD34 antigen. This procedure results in an approximately 75% recovery of CD34+ cells and a 4.1-log reduction of CD3+ cell or T-cells.[2] Further reduction of T-cell is required for some transplant recipients. To further deplete PBPC components of T-cells, anti-CD2, anti-CD6 and anti-CD7 bound to the sheep anti-mouse coated paramagnetic beads is used with the Isolex 300i. These anti-CD2, anti-CD6 and anti-CD7 coated beads are added to Isolex with the CD34+ cell releasing peptide. As CD34+ cells are being released, many of the remaining T-cells are being captured and removed. This so called "positive/negative" selection procedure results in a 4.9-log reduction of CD3+ cell and a 60% recovery of CD34+ cells. This positive/negative selection method for PBPC components results is such low residual T-cell content that T-cells sometime have to be added back to the end product to ensure that engraftment occurs.

Another instrument is also being used to isolate progenitor cells from PBPC components, the Miltenyi CliniMACS immunomagnetic system, which uses a colloidal suspension of superparamagnetic microparticles with diameters of about 50 nm.[2] The microparticles are about the size of a virus. Since this instrument is not yet approved for clinical use in the USA, it is being used for isolating cells for laboratory research. The microparticles used in the Miltenyi system differ from the Isolex beads in that they do not have to be removed from the cells. The microparticles are composed of iron oxide and polysaccharide and they are considered nontoxic and biodegradable. Hematopoietic progenitor cells can be isolated with CD34 or AC133 monoclonal antibodies. Anti- AC133 recognizes a slightly different population of progenitor cells than anti-CD34. CliniMACS isolation of stem cells from PBPC components with anti-CD34 results in a recovery of approximately 60% of CD34+ cells and 5.1 log depletion of CD3+ cells. The DTM cell processing lab is using the CliniMACS with anti-AC133 to isolate hematopoietic progenitor cells from PBPC components for research studies.

Donor lymphocyte infusions

Although T-cell depletion of stem cells reduces graft versus host disease (GVHD), T-cells are needed to reconstitute cell immunity in order to prevent viral infections and leukemia or tumor relapse. As a result, preparation and transfusion of unmanipulated and manipulated donor lymphocytes are important parts of the NIH marrow transplant process.[3] For many transplants at the NIH, lymphocytes from transplant donors are collected prior to the transplant. Unmanipulated donor lymphocytes are transfused periodically after transplant as long as the recipient does not have GVHD.

For some transplant protocols, an attempt is made to deplete donor lymphocytes of T-cells reactive with recipient alloantigens prior to the administration of donor T-cells to the transplant recipient.[4] Recipient peripheral blood monuclear cell (PBMC) components are collected by apheresis. Recipient CD3+ cells are isolated by positive selection with anti-CD3 and magnetic beads. The CD3+ cells are then cultured with IL-2 and anti-CD3 for 10 to 14 days. The expanded recipient cells are irradiated with 2500 cGy and incubated with donor leukocytes. For most protocols donor lymphocytes are obtained from a PBMC apheresis collection prior to the PBPC apheresis collection, but for this protocol the donor leukocytes are prepared from the unabsorbed fraction remaining from G-CSF PBPC components that have undergone combined CD34+ cell positive selection and anti-CD2, anti-CD6, and anti-CD7 negative selection. The donor and recipient cells are then mixed and incubated for 24 hours. During the incubation of donor and recipient cells, the donor lymphocytes that are reactive with recipient alloantigens are stimulated and the donor lymphocytes upregulate CD25 antigen. After the 24 hour incubation anti-CD25 conjugated to ricin A-chain is used as an immunotoxin to kill donor T-cells that are reactive with recipient alloantigens. The immunotoxin treatment is repeated after 24 hours. After another 24-hour incubation the cells are washed. At the time of transplant the recipient is given these donor lymphocytes that lack allo-reactive T-cells.

Some hematopoietic transplant recipients are given CD4+ cells with a Th2 profile to induce a graft versus leukemia (GVL) effect without inducing GVHD.[5] The donor's lymphocytes are treated so they develop a Th2 profile rather than a Th1 profile. Th2 lymphocytes are more likely to produce type 2 cytokines that generally stimulate antibody responses. Type 2 cytokines include IL-4 and IL-10. Th1 cells are more likely to produce type 1 proinflammatory cytokines such as interferon-gamma and tumor necrosis factor. Th2 lymphocytes are more likely to cause a GVL effect rather than GVHD. Th1 cells are thought to be primarily responsible for causing GVHD rather than GVL. To produce donor Th2 cells, a PBMC component is collected from the donor by apheresis. Monocytes are separated from lymphocytes in the PBMC component by elutriation. CD4+ cells are isolated from the lymphocytes by removing CD8+ and CD20+ cells with CD8 and CD20 monoclonal antibodies conjugated to paramagnetic beads. The CD4+ cells are co-stimulated with anti-CD3 and anti-CD28 bound to the same bead and are cultured with IL-2 and IL-4. The Th2

cells are given to the recipient at the time of transplant.

Immune Therapy for Cancer

Immune therapy is being used to treat many different types of cancers. The DTM cell processing laboratory is preparing dendritic cells to process and present peptide antigens to treat Ewing sarcoma, rhabdomyosacroma, colon cancer, and other solid tumors.[6] PBMC components are collected from the patient by apheresis. Counterflow centrifugal elutriation is used to isolated monocytes from PBMCs. The monocytes are then incubated with IL-4, GM-CSF and CD40 ligand to produce mature dendritic cells.[7,8] The dendritic cells are then incubated with tumor-specific peptide and administered to patients.

Pancreatic Islet Cell Transplantation

Pancreatic islet cell transplants are being used to treat type I diabetes mellitus.[9-11] To support these transplants at the NIH Clinical Center, the DTM cell processing laboratory is isolating pancreatic islet cells from pancreases obtained from multiorgan donors with beating hearts who have been declared brain dead. Pancreatic islet cell isolation involves dissection of the pancreas, digestion of the pancreas, concentration of digestate by centrifugation and purification of islet cells by density gradient centrifugation. The pancreatic islet cells are administered via the portal vein and have been used to effectively treat diabetes.

Summary

The DTM cell processing laboratory has changed and grown with each passing year. Throughout the laboratory's life it has supported blood and bone marrow transplantation, gene therapy, and immune therapy patients. In addition, the laboratory expects to soon support new protocols to treat patients with cardiac disorders. The DTM cell processing laboratory will soon be remodeled to accommodate new protocols. Continued growth is expected for many years for the DTM cell processing laboratory.

REFERENCES

1. Read EJ. Quality assurance for cell processing: no more blind faith. Transfusion 1996;36:1-4.
2. Read EJ, Carter CS. T-Cell depletion of Hematopoietic progenitor cell products for allogeneic tranplantation. In Sacher RA, editor. Cellular Therapy: New Frontiers in Transfusion Medicine. Bethesda, Maryland: American Association of Blood Banks; 2002. p.29-44.
3. Barrett AJ, Mavroudis D, Tisdale J, Molldrem J, Clave E, Dunbar C, et al. T cell-depleted bone marrow transplantation and delayed T cell add-back to control acute GVHD and conserve a graft-versus-leukemia effect. Bone Marrow Transplant 1998;21:543-51.
4. Solomon SR, Tran T, Carter CS, Donnelly S, Hensel N, Schindler J, et al. Optimized clinical-scale culture conditions for *ex vivo* selective depletion of host-reactive donor lymphocytes: a strategy for GvHD prophylaxis in allogeneic PBSC transplantation. Cytotherapy 2002;4:395-406.
5. Fowler D, Hou J, Foley J, Hakim F, Odom J, Castro K, et al. Phase I clinical trial of donor T-helper type-2 cells after immunoablative, reduced intensity allogeneic PBSC transplant. Cytotherapy 2002;4:429-30.
6. Dagher R, Long LM, Read EJ, Leitman SF, Carter CS, Tsokos M, et al. Pilot trial of tumor-specific peptide vaccination and continuous infusion interleukin-2 in patients with recurrent Ewing sarcoma and alveolar rhabdomyosarcoma: an inter-institute NIH study. Med Pediatr Oncol 2002;38:158-64.
7. Wong EC, Maher VE, Hines K, Lee J, Carter CS, Goletz T, et al. Development of a clinical-scale method for generation of dendritic cells from PBMC for use in cancer immunotherapy. Cytotherapy 2001;3:19-29.
8. Wong EC, Lee SM, Hines K, Lee J, Carter CS, Kopp W, et al. Development of a closed-system process for clinical-scale generation of DCs: evaluation of two monocyte-enrichment methods and two culture containers. Cytotherapy 2002;4:65-76.
9. Hirshberg B, Rother KI, Digon BJ 3rd, Lee J, Gaglia JL, Hines K, et al. Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. Diabetes Care 2003;26:3288-95.
10. Read EJ. Going where the action is: cellular therapy for diabetes mellitus. Cytotherapy 2003;5:241-2.
11. Read EJ, Lee J, Harlan DM. Isolation of pancreatic islets for transplantation in type I diabetes. In: Sacher RA, editor. Cellular Therapy: New Frontiers in Transfusion Medicine. Bethesda, Maryland: American Association of Blood Banks; 2002. p.45-76.