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OVERVIEW OF MONONUCLEAR CELL (MNC) COLLECTION PROCEDURES

COBE SPECTRA® APHERESIS SYSTEM
Part 1 of 3
Getting Around

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Presentation Overview

- Review of hematopoiesis
- Indications and rationale for MNC procedures
- General procedural elements
Presentation Objectives

Participants will be able to:

- Briefly describe what factors may influence blood cell development, or hematopoiesis.
- Name the types and functions of cells that form the MNC layer.
- List two indications for performing an MNC procedure.
- Identify three factors affecting collection procedure efficiency.
Hematopoiesis

Hematopoiesis is the process of blood cell production, maturation, and homeostasis, which takes place at various sites in the bone marrow, depending on the age of the individual.
Hematopoiesis (cont)

- All mature blood cells originate from hematopoietic stem cells (HSCs).
- Stem cells are undifferentiated pluripotent “mother cells” capable of unlimited self-renewal and differentiation.
Hematopoiesis (cont)

- Pluripotent stem cell
  - CFU-GEMM
    - BFU-E
    - CFU-Meg
      - CFU-GM
    - CFU-Bas
  - Pre B Cell
  - Prothymocyte

- Lymphoid progenitor cell
  - B cell
  - T cell

- Myeloid progenitor cell
  - RBC
  - Neutrophil
  - Monoc.
  - Eosinophil
  - Basophil
  - Plasma cell
  - Macrophage
  - Mast cell

- Stem cell
- Progenitor cell
- Colony-forming unit
- Intermediate stage
- Mature stage
- Blast cells
Hematopoiesis (cont)

- Lymphoid progenitor cells divide into two cell lines:
  - T-lymphocytes
  - B-lymphocytes
Hematopoiesis (cont)

- Myeloid progenitor cells divide into three main cell lines:
  - Erythroid cell line, which generates red blood cells.
  - Megakaryocytic cell line, which generates megakaryocytes and platelets.
  - Granulocytic/monocytic cell line, which generates granulocytes and monocytes.
Mononuclear cells (MNC), which have a single round nucleus, include:
- Lymphocytes
  - T-cell
  - B-cell
- Monocytes

Polymorphonuclear cells (PMN), which have a nucleus with a multilobed appearance include:
- Granulocytes
- Neutrophils
- Basophils
- Eosinophils
Lymphocytes play an important role in our immune system. Their functions include:

- Recognition of self and non-self cells
  - Foreign invaders have markers called antigens on their cell walls, which lymphocytes recognize.
- Antibody production
- Memory
  - This ability helps the body to react more efficiently to a re-infection by the same foreign substance.
Monocytes spend little time in the peripheral blood. Their functions include:

- **Destruction of bacteria**
  - Monocytes enter the tissues where they mature into macrophages, which engulf and destroy bacteria.

- **Presentation of foreign antigen to lymphocytes**
  - As dendritic cells, monocytes present foreign antigen to lymphocytes to trigger an immune response.
Functions of White Blood Cells (cont)

Granulocytes either circulate in the blood or sit in a marginating pool, ready for release in times of stress (surgery, collection procedures, exercise).

Their functions include:

- Destruction and digestion of invading bacteria.
White blood cells are the soldiers of our body. They protect us from invasion by foreign substances.
Questions?
White Blood Cells as Therapy

- Hematopoietic stem cell transplant (HSCT)
- Donor lymphocyte infusion (DLI)
- Tumor vaccination using dendritic cells
HSCT

HSCT is the intravenous infusion of HSCs collected from bone marrow, peripheral blood, or umbilical cord blood, which is used to re-establish hematopoietic function following the ablation of a patient’s bone marrow and immune system by chemotherapy and/or radiotherapy, or to replace damaged or defective bone marrow.
Indications for HSCT

- **Malignant diseases:**
  - For some types of cancer, there is a correlation between the chemotherapy dose and eradication of tumor. Myeloablative chemotherapy increases the chance of tumor eradication.

- **Non-malignant diseases:**
  - For some hereditary disorders affecting the hematopoietic system, transplantation of non-defective donor cells following myeloablative chemotherapy can improve symptoms or halt disease progression.
Indications for HSCT (cont)

Because myeloablative chemotherapy destroys the bone marrow and immune system, pluripotent stem cell *rescue by* transplantation is required to *restore* hematological and immune function.
DLI is the infusion of lymphocytes from the donor of a stem cell graft to the graft recipient for the purpose of combating or preventing relapse of the cancer the patient is receiving treatment for.
Malignancy Relapse

- DLI induces a graft-vs.-tumor (GVT) or graft-vs.-leukemia effect (GVL).
- GVT effect is an anti-tumor immune response caused by donor cells fighting patient tumor cells.
Non-Ablative Allogeneic SCT

- Non-ablative conditioning regimens permit stem cell engraftment with limited regimen-related toxicity.
- DLI is used to induce GVT effects and chimerism.* Chimerism is thought to be necessary to induce GVT effects.

*Presence of hematopoietic donor cells
Full chimerism = 100% donor cells
Mixed chimerism = Certain percentage of donor cells and certain percentage of host cells.
Targeted Cytotoxic T-Lymphocytes (CTLs)

CTLs are produced by ex-vivo incubation of donor T cells with antigen-treated APCs.*

In the patient’s body, CTLs bind specifically to cells with antigen they recognize and instantly kill the targeted cell.

*Antigen presenting cells (e.g. dendritic cells)
Tumor Vaccination Using Dendritic Cells (DCs) (Research)

DCs are antigen-presenting cells. The goal of tumor vaccination is to have DCs present tumor antigen to the T-lymphocytes, thereby triggering an immune response.
DCs
How DCs Influence Immune Response
Dendritic cells are antigen-presenting cells (APCs) that:

1. Capture antigen.
2. Migrate to draining lymphoid organs.
3. After a process of maturation, select antigen-specific lymphocytes to which they present the processed antigen, thereby inducing immune response.
Rationale for DC Tumor Immunotherapy

DCs in cancer patients fail to recognize danger, or the tumor down-regulates DC differentiation and activation. Removing DCs from the patient, manipulating them in vitro, and providing them with tumor antigen results in DCs capable of activating T-lymphocytes to fight the cancer.
Cellular Immunotherapy for Tumor Vaccination Using DCs Generated From Enriched Monocytes
Indications* for DC Tumor Immunotherapy

- Melanoma
- Prostate cancer
- Multiple myeloma
- Small cell lung cancer
- Renal cell carcinoma
- Breast cancer

*Protocols for different applications are under investigation or in clinical trials.
*Currently, mostly heavily pretreated patients with advanced cancer are selected for immunotherapy.
General Procedural Elements

- Separation of blood components
- Vascular access
- Anticoagulation
- Collection procedure considerations
Separation of Blood Components

Centrifugal force separates cells based on their specific gravity.
Separation of Blood Components (cont)
Effects of G-Force on Separation
Effects of G-Force on Separation

Centrifuge speed
Inlet pump flow rate

Separation Factor

Centrifuge Speed

Flow
Vascular Access

Obtaining adequate vascular access is a critical and key element when performing an apheresis procedure.

- Anticubital/peripheral venipuncture
- Femoral catheter
- Subclavian catheter
- Jugular access
- Ports
- Arteriovenous fistula or graft
Anticoagulation

- ACD-A
- Heparin
- Combination of ACD-A and heparin
Clotting Cascade

Heparin: Thrombin has been inactivated, preventing thrombus formation

Citrate binds free ionized calcium to prevent blood from clotting
Collection Procedure Considerations

Several factors impact the outcome of each collection procedure, including:

- Collection timing
- Equipment performance
- Operator technique
HSC Collection Timing

The best criteria for determining the timing of the first collection procedure is the pre-collection peripheral CD34+ cell count. There is a very good correlation between the pre-CD34+ cell count and the CD34+ cell yield of the collected product.
Equipment and Operator Technique

The type of equipment and operator technique influence collection outcomes, product quality, and ultimately the outcome of the transplantation process.
Operator Technique

- Accurate and timely data input
- Proper anticoagulation
- Adequate amount of blood volume processed
- Procedure optimization
- Alarm prevention and troubleshooting
Overview of MNC Procedures

References


Dendritic Cell Therapy References


Donor Lymphocyte Infusion References

