Discovering ATCC Primary Immunology Cells - Model Systems to Study the Immune and Cardiovascular Systems

James Clinton, Ph.D.
Scientist, ATCC
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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA.

- World's premiere biological materials resource and standards development organization.

- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions.

- Strong team of 400+ employees; over one-third with advanced degrees.
Outline

- Background
- CD34+ hematopoietic stem & progenitor cells
- Mononuclear cells
- CD14+ monocytes
Blood cells

Blood is comprised of a heterogeneous population of specialized cells

- **Leukocytes**: Acquired and innate immunity
- **Erythrocytes**: Gas transport
- **Thrombocytes**: Wound healing

Millions of blood cells are generated every second, approximately 1 trillion every day

**Hematopoiesis:**

- Dynamic process
- Varies in response to injury or infection
- Individual cells may live for hours to years

**Blood cells arise from hematopoietic stem cells (HSCs)**
Hematopoiesis: A hierarchical system

- Stem cells
- HSCs
- Multipotent progenitors (MPPs)
- Lineage committed progenitors
- Cell type specific precursors
- Mature blood cells
- Fully differentiated
- Intermediates
Hematopoietic cell fate and lineage
Hematopoietic stem cells: Characteristics

- Hematopoietic stem cells (HSCs) are multipotent cells that give rise to all other blood cells.
- HSCs reside primarily in bone marrow (major site of hematopoiesis in adults).
- True hematopoietic stem cells are rare.
- True hematopoietic stem cells can only be confirmed via *in vivo* functional assays.
Hematopoietic stem cells: Markers

- CD34^+ (Lin^-)
- CD90^+ (Lin^-)
- CD38^- (Lin^-)
- CD45RA^- (Lin^-)

Primitive HSC
CD34+ cells are a mixed population of stem and progenitor cells.
Assays to study hematopoietic stem and progenitor cells
Cytokines influence cell fate and lineage *in vitro*
Blood and hematopoiesis summary

- Blood is a heterogeneous tissue - its replacement *in vivo* is a complex process.
- HSCs are responsible for the generation of all other blood cell types.
- Recent advances allow for the identification and isolation of human HSCs as well as other blood cell types.
- This process can be studied *in vitro* though the use of lineage directed differentiation of HSPCs.

*Salmonella* invading an immune cell, photo credit: NIAID
Background

CD34+ hematopoietic stem & progenitor cells

Mononuclear cells

CD14+ monocytes
Primary CD34+ HSPCs

Applications
- *Ex vivo* expansion and differentiation
- Stem cell markers
- Gene transfer
- Cytokine and chemokine expression and regulation
- Receptor expression

Key research areas
- Safely and efficiently expand HSCs *in vitro* or *in vivo* for transplantation
- Immune response
  - Graft-versus-host disease/transplant rejection
- Cancer
- Cell-fate determination

Lymphocytes ingesting bacteria, photo credit: National Institute of Allergy and Infectious Diseases, NIH
Mature lymphocyte generation from CD34+ HSPCs on a 3D matrix

ATCC primary CD34+ HSPCs

- Healthy human volunteer donors; IRB-approved informed consent
- Adult, non-pregnant (excluding cord blood)
- Cryopreserved at P0; Purity: ≥ 90% CD34+
- Age, gender, ethnicity, and blood type on CoA

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Tissue</th>
<th>Type</th>
<th>Size</th>
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<tbody>
<tr>
<td>PCS-800-012™</td>
<td>Bone marrow</td>
<td>Hematopoietic stem/progenitor cells (CD34+)</td>
<td>≥ 0.5 x 10⁶</td>
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<tr>
<td>PCS-800-014™</td>
<td>Cord blood</td>
<td>Hematopoietic stem/progenitor cells (CD34+)</td>
<td>≥ 0.5 x 10⁶</td>
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Bone marrow

Cord blood
CD34+ HSPC lineage directed expansion and differentiation

Goals

- Demonstrate primary CD34+ HSPC capacity for lineage-directed expansion and differentiation *in vitro*
- Confirm multilineage differentiation (erythrocyte, megakaryocyte, and pan-myeloid)
- Compare CD34+ differentiation efficiency from multiple tissues
- Utilize a method amenable to high throughput assays

Methods

- Cryopreserved CD34+ primary cells from bone marrow and cord blood
- Serum-free liquid culture
- Analysis of phenotype by surface marker expression
- Commercially available cytokine cocktails

Killer T Cells, photo credit: Alex Ritter
General differentiation protocol workflow

1. Thaw & seed:
   - Thaw vial of CD34+ cells
   - Seed directly into differentiation media
   - Basal media: SFEM II
   - Supplement with cytokines

2. Maintenance:
   - Add fresh media every ~2 days
   - Re-suspend in fresh media every 4-7 days
   - Maintain concentration below 1x10^6/mL

3. Analyze:
   - Collect cells for analysis

For detailed differentiation protocols see the ATCC website
Erythroid differentiation and expansion

Diagram showing the differentiation and expansion of erythrocytes into various cell types, including Mast cell, Myeloblast, Megakaryocyte, Thrombocytes, Basophil, Monocyte, Macrophage, and Dendritic cell.
Expression of erythroid lineage markers on differentiated BM and CB CD34+ cells

- Bone Marrow CD34+ HSPCs
  - Day 0
  - Day 7

- Cord Blood CD34+ HSPCs
  - Day 7
Megakaryocyte differentiation and expansion
Expression of megakaryocyte lineage markers on differentiated bone marrow CD34+ HPSCs
Expression of megakaryocyte lineage markers on differentiated cord blood CD34+ HPSCs
Pan-myeloid differentiation and expansion
Expression of pan-myeloid lineage markers on differentiated BM and CB CD34+ HPSCs

Bone Marrow CD34+ HSPCs

Cord Blood CD34+ HSPCs
Specificity of pan-myeloid-directed differentiation of CD34+ HPSCs

- **Bone Marrow CD34+ HSPCs**

- **Cord Blood CD34+ HSPCs**
ATCC primary CD34+ HSPCs: Summary

- **High purity**
  - Single donor
  - ≥ 90% CD34+

- **Multiple tissues**
  - Bone marrow
  - Cord blood

- **Multipotent**
  - Erythroid
  - Megakaryocyte
  - Pan-myeloid
Outline

Background

CD34+ hematopoietic stem & progenitor cells

Mononuclear cells

CD14+ monocytes
Mononuclear cells
Primary mononuclear cells

Applications
• Isolation and study of cell subpopulations
• Molecular expression profiling

Key research areas
• Infectious disease
• Blood pathologies
• Immunology
• Vaccine development
• Toxicology
• Regenerative medicine

Monocyte and erythrocytes
# Primary mononuclear cells

- Healthy human volunteer donors; IRB-approved informed consent
- Adult, non-pregnant
- Cryopreserved at P0; Purity: $\geq 90\%$ CD45+
- Age, gender, ethnicity, and blood type on CoA

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<td>PCS-800-011™</td>
<td>Peripheral blood</td>
<td>Mononuclear cells (PBMCs); Normal, Human</td>
<td>$\geq 25 \times 10^6$</td>
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<td>PCS-800-013™</td>
<td>Bone marrow</td>
<td>Mononuclear cells (BMMCs); Normal, Human</td>
<td>$\geq 25 \times 10^6$</td>
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- Peripheral blood mononuclear cells
- Bone marrow mononuclear cells
Primary mononuclear cells

Reported on CoA (lot specific)

Specific marker expression

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<tr>
<th>Peripheral Blood</th>
<th>Bone Marrow</th>
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<tbody>
<tr>
<td>CD45+ (%)</td>
<td>CD45+ (%)</td>
</tr>
<tr>
<td>CD3+ (%)</td>
<td>CD3+ (%)</td>
</tr>
<tr>
<td>CD8+ (%)</td>
<td>CD8+ (%)</td>
</tr>
<tr>
<td>CD14+ (%)</td>
<td>CD14+ (%)</td>
</tr>
<tr>
<td>CD19+ (%)</td>
<td>CD19+ (%)</td>
</tr>
<tr>
<td>CD56+ (%)</td>
<td>CD34+ (%)</td>
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<td>CD56+ (%)</td>
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Non-viral method increases natural killer cells’ anti-cancer cell cytotoxicity

Immunosuppressive assay using PBMCs

MSCs

Mitomycin C

24h

CD3/CD28 beads

+ PBMCs

24h

BrdU

48h

Analyze by flow cytometry

18h

For detailed differentiation protocols see the ATCC website
MSCs suppress activated T-cell proliferation
ATCC Mesenchymal Stem Cells

The complete study, presented at ISSCR 2014, is available on the ATCC website:

Comparative analysis of cell proliferation, immunosuppressive action, and multi-lineage differentiation of immortalized MSC and MSC from bone marrow, adipose tissue, and umbilical cord blood

Dezhong Yin, Ph.D., Joy A. Wells, James Clinton, Ph.D. and Chaozhong Zou, Ph.D.
ATCC Cell Systems, 22 Firstfield Rd, Suite 180, Gaithersburg, MD 20878, USA
ISSCR Poster #: F-3115

For more information on our MSC products: www.atcc.org/stemcells

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<td>PCS-500-010™</td>
<td>Primary</td>
<td>Umbilical Cord-derived Mesenchymal Stem Cells</td>
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<tr>
<td>PCS-500-011™</td>
<td>Primary</td>
<td>Adipose-derived Mesenchymal Stem Cells</td>
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<tr>
<td>PCS-500-012™</td>
<td>Primary</td>
<td>Bone Marrow-derived Mesenchymal Stem Cells</td>
</tr>
<tr>
<td>SCRC-4000™</td>
<td>Immortalized</td>
<td>hTERT Immortalized Adipose-derived Mesenchymal Stem Cells</td>
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Outline

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- CD34+ hematopoietic stem & progenitor cells
- Mononuclear cells
- CD14+ monocytes
Primary monocytes (CD14+)
Primary CD14+ monocytes

**Applications**
- Isolation and study of monocyte subtypes
- Differentiation
- Phagocytosis
- Chemotaxis/migration assays

**Key research areas**
- Immunology
- Monocyte polarization
- Inflammation associated pathologies
- Infectious disease
- Cytokine release

Bone cancer cell, photo credit: Burnett Lippincott-Schwartz, National Cancer Institute
Primary monocyte-derived macrophages are suitable for studying *Lm*, cell lines are not.

ATCC primary CD14+ monocytes

- Healthy human volunteer donors; IRB-approved informed consent
- Adult, non-pregnant
- Cryopreserved at P0; Purity: ≥ 90% CD14+
- Age, gender, ethnicity, and blood type on CoA

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<td>Peripheral blood</td>
<td>Monocytes (CD14+)</td>
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Macrophage differentiation protocol

Monocytes

Wash to remove loosely attached and floating cells

3-4x

1.5 h

72 h

Media change

Media change

24-72 h

Swirl plate

Analyze by flow cytometry

For detailed differentiation protocols see the ATCC website
Generation of CD68+ macrophages from monocytes

- Macrophages differentiated from CD14+ monocytes for 9 days
- Morphology characteristic of type M1-polarized macrophages
- > 80% of cells were CD68+
Dendritic cell differentiation protocol

Monocytes

Media change. Retain non-adherent cells.

Media change. Retain non-adherent cells.

Media change. Retain non-adherent cells.

24 h

72 h

72 h

24-72 h

Analyze by flow cytometry

For detailed differentiation protocols see the ATCC website
Generation of CD83+ DCs from monocytes

- After 8 days differentiation
  - 95% of non-adherent cells were CD14-
  - >70% of cells were CD83+
Summary

ATCC offers a variety of well-characterized and functionally validated primary hematopoietic cell types

- CD34+ HSPCs
- BMMCs and PBMCs
- CD14+ monocytes

ATCC provides hematopoietic lineage-specific differentiation protocols

- Erythroid
- Megakaryocyte
- Non-specific myeloid
- Dendritic
- Macrophage

ATCC hematopoietic cells are useful in numerous areas of research
Thank you for joining today!

Register for more ATCC “Excellence in Research” webinars, or watch recorded webinars, at www.atcc.org/webinars

- July 28, 2016
  12:00 PM EST
  Brian A. Shapiro, Ph.D., Technical Writer, ATCC
  Neural Progenitor Cells – Toxicological Models for the 21st Century

Please email additional questions to: tech@atcc.org