DISCOVERING ATCC IMMUNOLOGICAL CELLS - MODEL SYSTEMS TO STUDY THE IMMUNE AND CARDIOVASCULAR SYSTEMS

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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 products and innovative solutions

• Broad range of biomaterials
  – Continuous cell lines, iPSCs, primary cells, and hTERT immortalized cells
  – Bacteria, fungi, yeasts, protists, and viruses
  – Microbial and tumor cell panels
  – Genomic and synthetic nucleic acids
  – Media, sera, and reagents
Outline

Background

CD34+ hematopoietic stem & progenitor cells

Mononuclear cells

CD14+ monocytes
Blood cells

- Blood is comprised of a heterogeneous population of specialized cells
- Three major types
  - **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
- **Intermediates**: Multipotent progenitors (MPPs)
- **Fully differentiated**: Lineage committed progenitors
- **Mature blood cells**: Cell type specific precursors
Hematopoietic cell fate and lineage

HSCs and MPPs

- Common myeloid progenitor
  - Erythrocyte
  - Megakaryocyte
  - Thrombocytes
  - Mast cell
  - Myeloblast
  - Basophil
  - Neutrophil
  - Eosinophil
  - Monocyte
  - Macrophage

- Common lymphoid progenitor
  - Natural killer cell
  - T lymphocyte
  - B lymphocyte
  - Dendritic cell
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

**Primitive HSC**

- **CD34^+**
- **Lin^-**
- **CD90^+**
- **CD38^-**
- **CD45RA^-**
CD34+ cells are a mixed population of stem and progenitor cells
Assays to study hematopoietic stem and progenitor cells

**In vivo**
- Xeno-transplantation
  - CFU-S
  - Serial transplantation
  - Long term reconstitution

**In vitro**
- Semisolid media
  - CFC
  - LTC-IC
- Liquid media
  - Co-culture/3D
  - Differentiation
  - Expansion
Cytokines influence cell fate and lineage *in-vitro*

- **HSCs and MPPs**
  - SCF
  - TPO
  - IL-3
  - M-CSF
  - GM-CSF

- **IL-7**
  - SCF

- **Common myeloid progenitor**
  - IL-3
  - M-CSF
  - GM-CSF

- **Common lymphoid progenitor**
  - IL-7
  - IL-15
  - SCF

- **Erythrocyte**
  - EPO
  - SCF
  - TPO

- **Eosinophil**
  - IL-3
  - IL-5
  - GM-CSF

- **Mast cell**
  - SCF
  - IL-3

- **Myeloblast**
  - IL-3
  - M-CSF
  - GM-CSF

- **Thrombocytes**
  - IL-6
  - IL-11
  - TPO

- **Megakaryocyte**
  - IL-3
  - IL-4
  - CSF
  - G-CSF
  - GM-CSF

- **Neutrophil**
  - IL-6
  - G-CSF
  - GM-CSF

- **Basophil**
  - IL-3
  - IL-4
  - G-CSF

- **Monocyte**
  - IL-6
  - IL-10
  - M-CSF

- **Macrophage**
  - IL-4
  - GM-CSF
  - Flt-3

- **Natural killer cell**
  - IL-2
  - IL-7

- **T lymphocyte**
  - IL-3
  - IL-4
  - IL-7

- **B lymphocyte**
  - IL-2
  - IL-7
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
Outline

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
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+); Normal</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+); Normal</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
General differentiation protocol workflow

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

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

**Analyze**
- Collect cells for analysis

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

- HSCs and MPPs
  - Common myeloid progenitor
    - Erythrocyte
    - Megakaryocyte
      - Thrombocytes
    - Mast cell
    - Myeloblast
      - Neutrophil
      - Eosinophil
    - Basophil
  - Common lymphoid progenitor
    - Natural killer cell
    - T lymphocyte
    - B lymphocyte
    - Macrophage
    - Dendritic cell
Expression of erythroid lineage markers on differentiated BM and CB CD34+ cells

Bone Marrow CD34+ HSPCs

Cord Blood CD34+ HSPCs
Megakaryocyte differentiation and expansion

HSCs and MPPs → Common myeloid progenitor → Erythrocyte, Mast cell, Myeloblast → Megakaryocyte, Thrombocytes, Basophil, Neutrophil, Eosinophil, Monocyte → Macrophage, Dendritic cell, Monocyte → T lymphocyte, B lymphocyte → Natural killer cell → Common lymphoid progenitor
Expression of megakaryocyte lineage markers on differentiated bone marrow CD34+ HPSCs

Day 0

Day 14

Day 28

CD42b vs CD34

CD42b vs CD41a

CD42b vs CD41a

Isotype vs CD34

CD34 vs CD34

CD34 vs CD34

CD34 vs CD34

ATCC
Expression of megakaryocyte lineage markers on differentiated cord blood CD34+ HPSCs
Pan-myeloid differentiation and expansion

- HSCs and MPPs
  - Common myeloid progenitor
    - Erythrocyte
    - Megakaryocyte
    - Thrombocytes
    - Mast cell
    - Myeloblast
    - Basophil
    - Neutrophil
    - Eosinophil
    - Monocyte
    - Macrophage
  - Common lymphoid progenitor
    - Natural killer cell
    - T lymphocyte
    - B lymphocyte

- Dendritic cell
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
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- Mononuclear cells
- CD14+ monocytes
Mononuclear Cells

HSCs and MPPs

Common myeloid progenitor

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Common lymphoid progenitor

- Natural killer cell
- T lymphocyte
- B lymphocyte
- Dendritic cell

Common lymphoid progenitor

- Natural killer cell
- T lymphocyte
- B lymphocyte
- Dendritic cell
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: ≥ 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>≥ 25 x 10^6</td>
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<tr>
<td>PCS-800-013</td>
<td>Bone marrow</td>
<td>Mononuclear cells (BMMCs); Normal, Human</td>
<td>≥ 25 x 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

Peripheral Blood
- CD45+ (%)
- CD3+ (%)
- CD8+ (%)
- CD14+ (%)
- CD19+ (%)
- CD56+ (%)

Bone Marrow
- CD45+ (%)
- CD3+ (%)
- CD8+ (%)
- CD14+ (%)
- CD19+ (%)
- CD34+ (%)
- CD56+ (%)
Non-viral method increases natural killer cells’ anti-cancer cell cytotoxicity

Immunosuppressive assay using PBMCs

MSCs

Mitomycin

24h

CD3/CD28 beads + PBMCs

24h

BrdU

48h

18h

Analyze by flow cytometry

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

Proliferation (% CD45+BrdU+ cells)

CD3/CD28 Activation

-  +  +  +  +  +  +

PBMC alone
PBMC alone
BM-MSC PBMC (1:5)
AT-MSC PBMC (1:5)
UCB-MSC PBMC (1:5)
hTERT-MSC PBMC (1:5)
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

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

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<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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<td>PCS-500-012</td>
<td>Primary</td>
<td>Bone Marrow-derived Mesenchymal Stem Cells</td>
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<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+)

HSCs and MPPs

Common myeloid progenitor

Erythrocyte

Mast cell

Myeloblast

Common lymphoid progenitor

Natural killer cell

T lymphocyte

B lymphocyte

Megakaryocyte

Thrombocytes

Basophil

Neutrophil

Eosinophil

Monocyte

Macrophage

Dendritic cell
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, courtesy of Burnett Lippincott-Schwartz, National Cancer Institute
Primary monocyte-derived macrophages are suitable for studying Lm, cell lines are not suitable.

CD14-Dependent Monocyte Isolation Enhances Phagocytosis of Listeria monocytogenes by Proinflammatory, GM-CSF-Derived Macrophages

Caroline Neu1, Anne Sedlaj1, Carina Bayer1, Sabine Förster1, Peter Crusweil1, Jan-Hendrik Niess1

1 Institute of Molecular and Biophysics, University of Ulm, Ulm, Germany; 2 Institute of Biochemistry, University of Ulm, Ulm, Germany; 3 Institute of Experimental Pathology, University of Ulm, Ulm, Germany; 4 Institute of Medicine, University of Ulm, Ulm, Germany; 5 Institute of Virology and Immunology, University of Ulm, Ulm, Germany; 6 Department of Medical Genetics and Medicine, University of Ulm, Ulm, Germany

Abstract

Macrophages are an important line of defense against invading pathogens. Human monocytes are derived by different methods, and they are used as models to study various infections. In contrast, in vivo studies focus on primary macrophages. The use of cell lines is a well-known method to study certain aspects of Lm infection. While cell lines are suitable for studying Lm, primary macrophages are not suitable for studying Lm, because Lm can only be cultured in primary macrophages that have been isolated from the mouse bone marrow. In this study, we isolated primary macrophages from healthy human volunteers and used them to study the phagocytosis of Lm. The results show that primary macrophages from healthy human volunteers are suitable for studying Lm. However, the use of cell lines is not suitable for studying Lm, because Lm can only be cultured in primary macrophages that have been isolated from the mouse bone marrow. In conclusion, primary macrophages are suitable for studying Lm, and cell lines are not suitable for studying Lm.

Introduction

Listeria monocytogenes is a food-borne Gram-positive obligate intracellular pathogen that is able to cause a wide range of clinical infections and is thus found in a variety of habitats [1]. In humans, the disease caused by Lm is termed listeriosis and is associated with immunocompromised individuals, pregnant women, newborns, and elderly patients with a mortality of 30-35% in these at-risk groups [2]. Infections with Lm are usually acquired upon consumption of contaminated food products and thus are the most common routes of infection in the gastrointestinal tract [1]. Lm is able to cross the intestinal epithelial barrier, and it is able to enter the host cell through the transcytotic tract [1]. Once inside the cell, Lm multiplies, expands, and finally escapes and spreads within the host cell, where it can be transferred to another host cell by budding the host cell actin cytoskeleton [1].

Macrophages play a central role in activating and balancing the pro- and anti-inflammatory pathways of the host immune system to ensure efficient host responses against invading pathogens. In vivo, macrophage differentiation is driven by GM-CSF and IL-4. High levels of GM-CSF induce a pro-inflammatory phenotype resulting in high IL-12 secretion. These pro-inflammatory cells are also termed M1 macrophages. By contrast, M-CSF polarizes macrophages to an anti-inflammatory phenotype characterized by low IL-10 secretion, which is referred to

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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<tr>
<td>PCS-800-010</td>
<td>Peripheral blood</td>
<td>Monocytes (CD14+)</td>
<td>≥ 50 x 10^6</td>
</tr>
</tbody>
</table>
Macrophage differentiation protocol

Monocytes

1.5 h

Wash to remove loosely attached and floating cells

3-4x

Media change

72 h

Media change

72 h

24-72 h

Swirl plate

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

Day 1
Monocytes

Day 6
Immature DCs

Day 8
Mature DCs

- 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!

Register for more webinars in the ATCC “Excellence in Research” webinar series at www.atcc.org/webinars.

Thank you for joining today!

Please send additional questions to tech@atcc.org

March 5, 2015
10:00 AM, 3:00 PM ET
David Clawson, Lead Biologist, ATCC
Enhancing vector-borne research with biological and molecular standards

March 19, 2015
10:00 AM, 3:00 PM ET
Scott Sutton, Ph.D., Principal, Microbiology Network and Tracy Vandenbroek, Product Line Business Manager, ATCC
Microbiology quality control as described in the Compendia