Andrew D. Leavitt, MD

BIOGRAPHY:

Andrew D. Leavitt, MD is a Professor with appointments in the Department of Laboratory Medicine and the Department of Medicine (Hematology). He received his MD from Harvard Medical School, and completed his internal medicine residency training at The University of Michigan. He then came to UCSF in 1988 to begin his Hematology Fellowship training and was appointed to the UCSF faculty in 1992. Dr. Leavitt is the Medical Director for the Adult Blood and Marrow Transplant (BMT) Laboratory, and Assistant Medical director for the UCSF Blood Bank. He is also Directs the Non-malignant Hematology section within the UCSF Department of Medicine, and is the Medical Director of the UCSF Adult Hemophilia Treatment Center, a federally funded center for patients with congenital bleeding disorders. He sees patients in the UCSF hematology clinic, and provides hematology consultation for patients in the hospital. Among his various teaching responsibilities, he is the director for the UCSF School of Medicine 2nd year Hematology course. His past research has focused on megakaryocyte development and platelet function, and his current laboratory research focuses on improving hematopoietic stem cell options for patients in need of a bone marrow transplant. He has served on the editorial boards for Blood, the leading professional journal in hematology, and the Journal of Clinical Investigation.
Stem Cells, and Blood Cells, and Blood Counts, Oh My!*

a walk down the hematologist’s yellow brick road

Andrew D. Leavitt, MD
UCSF Mini Medical School
June 4, 2013

*With apologies to L. Frank Baum and all the lions and tigers and bears…

Our Road Over the next 90 minutes

- Stem cells – Definitions
  - Types: embryonic, adult, IPS
- Hematopoiesis is THE model for stem cell biology
- Hematopoietic development:
  - Fetal & Adult
  - Location, Potential, & Output
- The Bone Marrow:
  - All about location: The Niche
  - What it looks like
- Bone Marrow Transplant – Stem Cell Sources:
  - Marrow, Peripheral Blood, Umbilical Cord Blood
- The Complete Blood Count (aka: CBC)
- Blood Donations & Transfusions

What Makes a Stem Cell a Stem Cell?

Conception in a Dish

Half of your genes

Provided by CIRM
Embryonic stem cells & Early Development

Comes from undifferentiated cell from the inner cell mass of the early pre-implantation embryo (BLASTOCYST) that has the potential to become any cell type in the body.

1 cell embryo
4 cell embryo
Morula
Developing Blastocyst
8 cell embryo
Trophoblast
Blastocyst
Blastocoel

Human embryonic stem cells (hESCs) can divide indefinitely.

Day 1
Day 2
Day 3
Day 4
Day 5
Day 5

Development Milestones and Terms

Days* 1-7: Zygote to blastocyst...
Days 7-14: Uterine implantation
Day 14: Three distinct layers begin to form
Days 14-21: Beginning of future nervous system
Days 21-24: Beginning of future face, neck, mouth, and nose
Weeks 3-8: Beginning of organ formation
Week ≥8: Called a fetus

Embryonic Development:
~1 day for a Zebrafish (3+ weeks for a human)

Starts at 64 cell stage for the Zebrafish

Keller et al. 2008
Adult Stem Cell

1. UNDIFFERENTIATED cell
2. Located within differentiated tissue
3. Can renew itself indefinitely AND,
4. With certain limitations, give rise to all the specialized cell types of the tissue from which it originated.

**BEST EXAMPLE:** Hematopoietic Stem Cell (HSC)

Tissues thought to contain adult stem cells: Skeletal muscle, Hair follicle, Heart muscle, Dental pulp, Colon, Liver, Prostate, Mammary gland, Fat, Pancreas...

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Stem Cells & Potential...

*(more definitions)*

- **Totipotent:** (whole) A cell that can give rise to all the cells of a new individual, including cells of the amniotic sac and the placenta.
  - Zygote (fertilized egg)
- **Pluripotent:** (more) A cell that can give rise to all types of adult tissue cells but not extraembryonic components of the trophoblast or the placenta.
  - Embryonic stem cell
  - Induced pluripotent stem (iPS) cell
- **Multipotent:** (many) A cell that can give rise to several types of mature cells within ONE mature organ, tissue or physiological system.
  - Adult stem cell (hematopoietic stem cell)

(HSCs are often incorrectly called pluripotent)

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The Basic Developmental Paradigm:
**A Unidirectional Path of More Options to Fewer**

Waddington model

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**Induced Pleuripotent Stem (iPS) Cell**

**A Paradigm Shift**

Waddington model
Induced Pleuripotent Stem (iPS) Cell

Some background biology #1

DNA → RNA → Protein

“Transcription Factor”:
- binds to a specific DNA sequence
- regulates gene expression where it binds
- can regulate many (related) genes

Induced Pleuripotent Stem (iPS) Cell

Some background biology #2

- All cells of an organism have the same DNA.
- Cell-to-cell differences reflect different gene expression patterns among cells.
- Transcription factors regulate sets of genes.
- Maybe... just maybe...one could find a set of transcription factors that 'defines' the stem cell state, & maybe that set of transcription factors expressed in a mature cell could **re-establish the stem cell state**.

Induced Pleuripotent Stem (iPS) Cell

* * * * * * * * *

No colonies
At day 10

14 – Oct3/4
15 – Sox2
20 – KIF4
22 – c-Myc

Induced Pleuripotent Stem (iPS) Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Cell 126:663-676, 2006

- Identified 24 candidate transcription factors
- Expressed them in various combinations in fibroblasts
- Identified a set of 4 factors that give a fibroblast many characteristics of an ES cell

Induced Pleuripotent Stem (iPS) Cell

“De-differentiation”
Induced Pluripotent Stem (iPS) Cell

De-, Re-, and Trans-differentiation

So What?

- Basic Research
- Disease-specific research
- Possible therapies
Better Living Through iPSC cells...

6/3/2013

6

Cells, e.g. skin

iPSCs

6

Compatible transplants

7

Lets Shift Gears

World War II

The ‘birth’ of hematopoietic stem cells & Bone marrow transplants

History of Hematopoietic Stem Cell Transplantation

ARCHIVES OF SURGERY

Volume 5

April, 1930

VOLUME 1

EFFECT OF TRANSPLANTATION OF HUMAN MARROW INTO IMMUNIZING ANIMALS

Paul E. Boyer, M.D.

WIlliam Irish, M.D.

Surgical mobilized spleens are blocked from irradiation by lead blocks

History of Hematopoietic Stem Cell Transplantation

J Lab Clin Med 34:1538, 1949

Surgically mobilized spleens are blocked from irradiation by lead blocks
 Bone Marrow Protects Against Radiation Injury

Evidence Favoring the Cellular Theory of BM Protection

The Radiation Sensitivity of Normal Mouse Bone Marrow Cells, Determined by Quantitative Marrow Transplantation into Irradiated Mice

A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells

Allowed them to infer the number of cells that survived a given dose of radiation
One 'Source Cell' (aka, a Stem Cell) for All or Multiple Source Cells?

Therefore, A Hematopoietic Development Tree

The Changing Location of Hematopoiesis During Gestation & Post-partum

The Changing Location of Hematopoiesis in Utero

Terms: Medullary (in the marrow) & Extra-medullary (not in the marrow)
The hematopoietic stem cell niche

(Neutrophil, location, location, hematopoiesis is like real estate)

Niches:
Endosteal, Perivascular
Intercellular:
Matrix, Cells
Growth factors:
Soluble, Cell surface
Points:
Quiescent and dividing HSCs
Symmetric & Asymmetric divisions

Transcription factors & hematopoiesis

SCN - supra-chiasmatic nucleus
NE - norepinephrine
RHT - retinal-hypothalamic tract

Yes, there is a nervous system of the bone marrow!
The Hematopoietic Stem Cell Circulation

*NOTE: This just shows the endosteal niche for simplicity, but there is also a perivascular niche.

Wilson & Trumpp 2006

Circulate

Mobilize/Release

Retain

Lodge

Home

And now…the marrow & the cast of characters

Bone Marrow Biopsy

Bone Marrow Aspirate

Peripheral Blood

Examples of bone marrow biopsies

Normal

Hypocellular

Lymphoma

lymphoid nodule

Bone Marrow - How do we get?

23g needle; ¾”

Biopsy

Aspirate

Hoffbrand et al Essential Hematology 2001
The Hematopoietic Development Tree

LYMPHOID (CLP)

MYELOID (CMP)

Stem Cells

Multipotent Progenitor Cells

Committed Precursor Cells

Mature Cells

Identify by functional assay

Identify by routine marrow staining

Aspirate Gives the Best Cell Definition

Bone Marrow Aspirate - Myelopoiesis

The Hematopoietic Development Tree & Disease

Normal

Acute Leukemia

Chronic Leukemia
Hematopoietic Stem Cell Transplantation - The Start of Human Studies

- Mid 1950s: realized that BM transplant could be used to treat diseases of the marrow and not just to provide radiation protection
- Mid 1950s - mid 1960s: Lots of failed attempts for allo transplant – interest drops
- 1968: First successful transplant to treat an inherited immunodeficiency using HLA matched sibling
- 1975: NEJM paper by Thomas* et al reviewed the state of BMT, and helped define critical issues in the field, including HLA matching, infectious complications, GVHD, etc. and showed clearly that one could use this therapy to cure people with leukemia.
- 1977: Thomas et al report on 100 patients with MDS leukemias treated with HSC transplantation, with 13 long term survivors.
- 1979: Better results if treat earlier, i.e., in first remission.

*E. Donnall Thomas awarded the Nobel Prize in 1990 for BMT.

How Far We Have Come: Timeline for Blood and Marrow Transplantation

Diseases Commonly Treated with HSC Transplantation

<table>
<thead>
<tr>
<th>AUTOLOGOUS*</th>
<th>ALLOGENIC**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancers</td>
<td>Cancers</td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Myelodysplastic syndromes</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Myeloproliferative disorders</td>
</tr>
<tr>
<td>Germ-cell tumors</td>
<td>Lymphoma (Non-HD &amp; HD)</td>
</tr>
</tbody>
</table>

Other Diseases

<table>
<thead>
<tr>
<th>AUTOLOGOUS*</th>
<th>ALLOGENIC**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune disorders</td>
<td>Aplastic anemia</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>Sickle cell anemia</td>
</tr>
</tbody>
</table>

HSC sources for clinical application - Bone Marrow

1. General anesthesia in operating room
2. 200-300 entries into posterior iliac crest yielding >1,000 ml
3. Overnight stay (or more)
4. Sore for a while
5. Net loss of a couple units of blood
6. Dose: ≥ 2 x 10⁶ nucleated cells/Kg
HSC sources for clinical application: Peripheral Blood Stem Cells

1. Mobilization - G-CSF; CXCR-4 antagonist; Rx
2. Two IVs & a cell separator
3. 3-4 hours
4. Cytokine can give flu-like symptoms
5. Product is ~250-300 ml
6. “Dose”: 2-5 x 10^6 CD34+ cells/Kg

HSC sources for clinical application: Cord Blood

1. No pain
2. No cytokines
3. <100 ml
4. Dose: >2 x 10^7 nucleated cells
5. Permits greater immune (HLA) mismatch

Clinical limitations: Supply-Demand

- 80% of patients lack a related, HLA matched donor
- Data from NMDP suggests: 10-50% of patients needing a donor remain without one
- Highly pronounced in certain ethnic groups:
  - African American (50%)
  - Asian American (35%)
  - Hispanic ancestry (35%)

So, we think….

Better in the bag than in the trash…

…and lets expand!!
Bone marrow is a very active place

~5,000,000 RBC’s/µL
~5 L of blood (70 Kg person) = 5 x 10^6 µL
So, ~ 25 x 10^12 RBC in body

If RBC life span = 120 days...
then we each make ~2 x 10^11 RBC’s/day
Or
~8,000,000,000 RBC’s/hour!
(Yes, 8 billion!)
That is a lot of cell divisions...

As if that is not impressive enough...

~8,000,000,000 RBC’s/hour
RBC is ~8 microns in diameter
= ~64,000,000,000 microns
= ~40 miles if placed side-by-side

~25 x 10^12 RBC in body
RBC is ~8 microns in diameter
~2 x 10^14 microns = ~124,000 miles!

But, we are visual animals:
124,274 miles means...

Earth’s circumference = 24,901 miles
So, total body RBC side-by-side?
CIRCLE THE EARTH 5 times

Red Blood Cell (RBC)

Delivers O₂ from your lungs to all your tissues
Takes CO₂ from your tissues to your lungs
Also ~ binds NO (a vasodilator)
Their size: ~8 µ (capillaries have ~3 µM diameter)
Their life-span: 120 days
Mature RBCs have no nucleus
Too few RBCs = anemia
What tells your body to make RBCs?

EPO (erythropoietin):
1. Increases # of E-committed progenitors
2. Increases GATA1 and FOG expression
3. Enhances anti-apoptotic gene expression
4. Increases transferrin receptor expression

Hemoglobin

Hemoglobin (Hgb) in the RBC carries O₂, which is poorly soluble in water, to tissues & CO₂ from tissues to lungs.

~640,000,000 Hemoglobin molecules/RBC

Colors of a bruise are globin breakdown products

Erythropoiesis: Hemoglobin Switching

(ALWAYS: 2 alpha & 2 "beta-like" (3' 'p' options?)

Megakaryocytopoiesis – making platelets
Megakaryocyte undergoing proplatelet formation
560 images @ 10 minute intervals

A quantitative perspective of blood

Normal Values for Peripheral Blood Counts (CBC)

<table>
<thead>
<tr>
<th>Category</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cells</td>
<td>4.4 - 5.0 x 10^6/ul</td>
</tr>
<tr>
<td>White Blood Cells</td>
<td>3.4 - 10.0 x 10^3/ul</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>150 - 400 x 10^3/ul</td>
</tr>
<tr>
<td>&gt; 21 yrs old: Neutrophils</td>
<td>1.8 - 6.8 x 10^3/ul</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.0 - 3.4 x 10^3/ul</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.2 - 0.4 x 10^3/ul</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.0 - 0.4 x 10^3/ul</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.0 - 0.1 x 10^3/ul</td>
</tr>
</tbody>
</table>

70 Kg adult has ~ 5 L blood volume

~ 0.8 x 10^10 RBCs/hr
~ 1 x 10^9 Neuts/hr
~ 0.5 - 1 x 10^11 platelets/hr

Peripheral blood-100x
Marrow cells in the peripheral blood
A 42 year old man is brought to the ER by his wife because he is acting strange, has a fever. You get a CBC that shows Hgb 9.9, Platelet count 37K, and WBC 9.7.

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**Blood Donations: Infectious Disease Safety**

A. Donor Intake: Plays a major role in maintaining a safe blood supply
B. Unit Testing: Tests performed on all units collected in the United States

1. VDRL (antibody) 1947
2. HBsAg (viral protein) 1971
3. HIV 1 (antibody) 1985
4. ALT (enzyme) 1986
5. HBsAb (antibody) 1986
6. HTLV (antibody) 1988
7. HCV (antibody) 1990
8. HIV 2 (antibody) 1992
9. HIV-1 p24 Ag* (antigen) 1996
10. NAT for HIV (DNA) 1999/2002
11. NAT for HCV (DNA) 1999/2002
12. NAT for West Nile (DNA) 2003
13. Bacteria (growth) 2003/4
14. Trypanosoma cruzi** (antibody) 2007

*Blue = discontinued; **Chagas disease

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Consent

Platelets

Pheresis machine
Human Immunodeficiency Virus (HIV) and Hepatitis B (HBV) and Hepatitis C (HCV) Transfusion Transmission Risk

DISEASE TRANSMISSION: What is the risk?

<table>
<thead>
<tr>
<th>Disease</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>1: 220,000</td>
</tr>
<tr>
<td>HCV</td>
<td>1: 1,800,000</td>
</tr>
<tr>
<td>HIV</td>
<td>1: 2,300,000</td>
</tr>
<tr>
<td>HTLV I(II)</td>
<td>1: 2,998,000</td>
</tr>
</tbody>
</table>

Bacteria*

- sepsis: 1: 74,807
- septic death: 1: 498,711

*ARC Eder et al. Transfusion 2007; 47: 1134

U.S. Blood Collections & Transfusions*

Collections:
- 10,877,000 allogeneic (WB/RBC) donors: 29% first time; 71% repeat donors
- ~5/1,000 people age 16-64
- 17,286,000 units WB/RBC collected
- 1,352,000 platelet pheresis collections

Transfusions:
- 15,014,000 whole blood/Red blood cell transfusions
- 2,021,000 platelet transfusions
- 23,668,000 total transfusions

PLEASE

Roll up your sleeve and become a blood donor if you can!
Thank you...Questions Please!