George Lyman Duff Memorial Lecture

The Regression of Atherosclerosis—the journey from the liver to the plaque and back

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Disclosures

• Global Atherosclerosis Advisory Board, Merck
• Medical School Grant, Merck

“One of the benefits of freedom is that people can disagree,” Mr. Bush told a crowd of thousands on a bright Texas day. “It’s fair to say I created plenty of opportunities to exercise that right.”

George W. Bush
April 26, 2013
I. ApoB and VLDL

Diagram showing the pathways of VLDL production, lipolysis, shunt pathway, LDL clearance, and conversion.
Contributors

• Trainees: Ursula Andreo, Pui Butkinaree, Xiaoli Chen, Charles Guo, Vika Gusarova, Emma Kummrow, Ling Li, Vatsala Maitin, Meihui Pan, Raji Pariyarath, Ana Tuyama, Hongxing Wang

• Faculty: Jeff Brodsky, Arthur Cederbaum, Henry Ginsberg, Mahmood Hussain, Peng Li, Julian Marsh, Kathryn Moore, Dan Rader, David Ron, Janet Sparks, Kevin J. Williams, Zemin Yao

• Funding: AHA, Merck, NIH
ApoB Degradation and the Pathway to VLDL

As published in Cell, JBC, JCI, JLR, PLoS One, PNAS

Lipid Unavailability

Metabolic Regulation (e.g., fish oil, insulin)
Niacin

- Vitamin B₃ (niacin and nicotinamide), commonly found in plant and animal foods, is a dietary substrate for NAD/NADP

- Niacin at 2.0 – 3.0 g per day:
  - ↑ HDL-cholesterol (15 – 35%)
  - ↓ LDL-cholesterol (5-25%)
  - ↓ Triglyceride (20 – 50%)
  - ↓ ApoB levels

- Historical mechanism: inhibition of adipocyte lipolysis and lower plasma FFA, which are used for hepatic TG synthesis

- **Hypothesis:** NIACIN has direct effects on VLDL production
Niacin Decreases VLDL-TG and ApoB100 Production Rates *in vivo*

**Graph 1:**
- **X-axis:** Time (hours)
- **Y-axis:** ug/L plasma triglyceride
- **Legend:**
  - Red dots: Control
  - Blue dots: Niacin
- **Statistical Note:** $p < 0.01$

**Bar Graph:**
- **Title:** *In vivo* ApoB-100 secretion
- **Y-axis:** Relative ApoB secretion (Densitometry/TCA)
- **X-axis:** PBS, Niacin
- **Legend:**
  - White bar: Control
  - Black bar: Niacin
  - *: Significant difference

*Charles (Liang) Guo, et al.*
The Effects of Niacin Are Dependent on Metabolic Conversion
NAADP and Autophagy

Niacin → NAADP → Lysosome

Pereira et al., JBC, 2011; Lu et al., JBC 2013
NAADP Regulates the Niacin Effect on ApoB100

NED-19 (antagonist)

TPC2-siRNA

Niacin + DMSO  Niacin + NED-19

Control  Niacin

Control siRNA  Tpcn2 siRNA

*  **
Niacin Effects *in vitro* and *in vivo* Require Autophagy

apoB100 recovery *in vitro*  

VLDL secretion *in vivo*

![Graph showing apoB100 recovery and VLDL secretion](image-url)
II. Atherosclerosis Regression
Contributors

• Trainees: Joe Bass, Robin Choudhury, Emilie Distel, Jonathan Feig, Tadateru Hamada, Bernd Hewing, Jun Kusunoki, Stephanie Mick, Saj Parathath, James Rong, Marie Sanson, Eugene Trogan, Yuliya Vengrenyuk, Chujun Yuan

• Faculty: Jan Breslow, Hayes Dansky, Michael Garabedian, Liz Gold, Ira Goldberg, David Greaves, Stan Hazen, Png Loke, Kathryn Moore, Oscar Puig, Steve Ramsey, Gwen Randolph, Ernane Reis, Larry Rudel, Raanan Shamir, Jonathan Smith, Ira Tabas, Alan Tall, Mark Taubman, Peter Tontonoz, David Williams, Joe Witztum, Steve Young

• Funding: AHA, Astra-Zeneca, Pfizer, Takeda, NIH
ATVB, Cell Metab., Diabetes, Circ., JCI, Nat Immunol., PNAS

Quantity vs. Quality
Simplistic Classification of Macrophages in Tissues, Including Human & Mouse Plaques

**M1**
- Killing of intracellular parasites
- Tumor resistance
- Tissue destruction

- Th1
- IL-12\textsuperscript{high}, IL-23\textsuperscript{high}, IL-10\textsuperscript{low}, ROI; RNI; TNF\textsuperscript{high}, IL-1\textsuperscript{high}, M1 chemokines (e.g. CXCL10).

**M2s**
- Angiogenesis
- Immunoregulation
- Tissue remodeling
- Parasite encapsulation
- Tumor promotion

- IL-12\textsuperscript{low}, IL-23\textsuperscript{low}, IL-10\textsuperscript{high}, arginase-1; TNF\textsuperscript{low}, IL-1ra\textsuperscript{high}, decoy IL-1 RII\textsuperscript{high}, scavenger, mannose, galactose receptor\textsuperscript{high}, M2 chemokines (e.g. CCL22).
Convergent Biology: Plaque Regression and M2 Macrophages

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Method</th>
<th>Lipid Δ</th>
<th>M2 ↑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversa (LDLr-/-)</td>
<td>Genetic switch</td>
<td>Lower LDL</td>
<td>Y</td>
</tr>
<tr>
<td>LDLr-/-</td>
<td>Diet change</td>
<td>Lower LDL</td>
<td>Y</td>
</tr>
<tr>
<td>LDLr-/-</td>
<td>LDLr adenovirus</td>
<td>Lower LDL</td>
<td>Y</td>
</tr>
<tr>
<td>LDLr-/-</td>
<td>Anti-miR33</td>
<td>Raise HDL</td>
<td>Y</td>
</tr>
<tr>
<td>ApoE-/-</td>
<td>Infuse HDL</td>
<td>Raise HDL</td>
<td>Y</td>
</tr>
<tr>
<td>ApoE-/-</td>
<td>Aortic Transplant</td>
<td>Raise HDL</td>
<td>Y</td>
</tr>
</tbody>
</table>

*Cell Metabolism, Circulation, Diabetes, JCI, PNAS*
Plaque Regression Changes the Balance

Killing of intracellular parasites

Tumor resistance → Tissue destruction

Th1

M1

IL-12\textsuperscript{high}; IL-23\textsuperscript{high}; IL-10\textsuperscript{low}; ROI; RNI; TNF\textsuperscript{high}; IL-1\textsuperscript{high}; M1 chemokines (e.g. CXCL10).

Angiogenesis

Immunoregulation

Parasite encapsulation

Tissue remodeling

Tumor promotion

M2s

IL-12\textsuperscript{low}; IL-23\textsuperscript{low}; IL-10\textsuperscript{high}; arginase-1; TNF\textsuperscript{low}; IL-1ra\textsuperscript{high}; decoy IL-1 RII\textsuperscript{high}; scavenger, mannos, galactose receptor\textsuperscript{high}; M2 chemokines (e.g. CCL22).
Models of the M1/M2 Seesaw
Ongoing Recruitment of Monocytes During Plaque Regression

newly recruited cells

Llodra et al., PNAS 2004
Fates of High and Low Monocytes in Tissues

Gr1−/Ly6C<sub>low</sub>
- Inflammatory response
- Phagocytosis
- Wound repair (e.g. VEGF)
- Tissue remodeling
- Chemokines (e.g. CXCL9/10)
- Mediate formation of foam cells in response to lipids?

Gr1<sup>+</sup>/Ly6C<sub>high</sub>
- Tip-DC / M1-type or classically activated macrophage
- Phagocytosis
- Bacterial clearance (e.g. iNOS, ROS)
- Inflammation (e.g. TNF)
- Proteolysis
- Mediate formation of foam cells in response to lipids?

Blood

Tissue

Woollard & Geissmann, Nature Reviews Cardiology, 2010
The 2 major monocyte subsets in human (top) and mouse (bottom) blood

**HUMAN**
- Classical: 85 - 95%
- Nonclassical: 5 - 15%
- CD14^+ CD16^-
- CD115^+ Gr-1^hi (Ly6C^hi) 50%

**MOUSE**
- CD14^{lo} CD16^{+}
- CD115^{+} CD11c^{+} Gr-1^{lo} (Ly6C^{lo}) 50%
- CCR2

Mouse Model of Regression: The Rapid Normalization of Plasma Lipid Profile

Chow: TC 500; HDL 30
WD: TC 1500; HDL 30
(mg/dL)

APO E -/-

WT

TC 100
HDL 65
Deficient in CCR2
Poor Regression and Very Few M2 Macrophages Without Newly Recruited Ly6Chi Monocytes

Yuliya Vengreynyuk, et al.
Fates of High and Low Monocytes in Tissues: Revised

Woollard & Geissmann, Nature Reviews Cardiology, 2010
III. Atherosclerosis “Nano-Theranostics”

“The answer is Nanotechnology. What is the question?”
Contributors

• Trainees: Alessandra Barazza, Robin Choudhury, Maria Coimbra, Yanqing Ma, Orli Even-Or, Stewart Russell

• Faculty: David Cormode, Omid Farokhzad, Zahi Fayad, Valentin Fuster, Willem Mulder, Kevin J. Williams

• Funding: NIH
The Beginning....
Building an HDL Diagnostic (Plaque Imaging) Agent

Greatly Enhanced Plaque Imaging in Mice with Gd-HDL

Pre-contrast 1 hr post-contrast 24 hr post-contrast 48 hours post-contrast

The Versatility of HDL as a Diagnostic and Therapeutic:

As reported in ACS Nano, JACC, NanoLetters, Nat Med, etc.
Liver-X-receptor agonists

- Challenge: Fatty liver
Chemical structure of GW3965-containing NP’s. The particle consists of an outer PEG surface, and a biodegradable polymer matrix loaded with hydrophobic GW3965.
LXR-NPs induce plaque macrophage gene expression

*Ldlr*−/− mice fed western diet X16 weeks, injected with NPs or free drug 3X/wk for 2 weeks

*Orli Even-Or, et al.*
LXR-NPs delay atherosclerosis progression without making the liver steatotic
Special Thanks To:
WIKIPEDIA: The blessing of *Shehecheyanu* is recited in thanks or commemoration of doing or experiencing something that occurs infrequently and from which one derives pleasure or benefit.

שֶּׁחֶכֶּהַ יָנָנֻ הָאֵינוּ בֹּזְמֵן לָזַמְמוֹ הָוה

I am thankful for being able to reach this occasion.