20.380
Biological Engineering
Design
Inflammation and Cardiovascular Disease

John Essigmann
February 11, 2010
Human history at a glance ...

We were strong and fast ... even the wolves hung out with us.
As Forest said last time, we consume more Calories than we burn off ... and the Resultant obesity is associated with about 24 Million Americans developing diabetes.

Obesity is also a risk factor for Coronary disease and stroke ... and Inflammation plays a role in the disease.
Obesity Trends* Among U.S. Adults
BRFSS, 1985
(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” woman)

John Groopman, Johns Hopkins
School of Public Health

Public domain map created using data from www.cdc.gov/BRFSS.
Obesity Trends* Among U.S. Adults

BRFSS, 1989

(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” woman)

Public domain map created using data from www.cdc.gov/BRFSS.
Obesity Trends* Among U.S. Adults

BRFSS, 1992

(*BMI ≥ 30, or ~ 30 lbs overweight for 5’ 4” woman)

Public domain map created using data from www.cdc.gov/BRFSS.
Obesity Trends* Among U.S. Adults
BRFSS, 1994
(*BMI ≥ 30, or ~ 30 lbs overweight for 5’ 4” woman)
Obesity Trends* Among U.S. Adults
BRFSS, 1997
(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” woman)
Obesity Trends* Among U.S. Adults
BRFSS, 2003
(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” person)

Source: Behavioral Risk Factor Surveillance System, CDC
Obesity Trends* Among U.S. Adults

BRFSS, 2007

(*BMI ≥30, or ~ 30 lbs. overweight for 5’ 4” person)

Public domain map created using data from [www.cdc.gov/BRFSS](http://www.cdc.gov/BRFSS).
Obesity Trends* Among U.S. Adults
(*BMI ≥30, or about 30 lbs. overweight for 5’4” person)

Forest showed
This slide on Tuesday

<table>
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<th>10%–14%</th>
<th>15%–19%</th>
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Public domain map created using data from [www.cdc.gov/BRFSS](http://www.cdc.gov/BRFSS).
Age-adjusted Percentage of U.S. Adults Who Were Obese or Who Had Diagnosed Diabetes

Obesity (BMI ≥30 kg/m²)

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Diabetes

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Public domain maps by the Centers for Disease Control.
Cardiovascular Disease Follows Exactly the Same Pattern

Heart Disease (Death Rates) 2000-2004
Adults Ages 35 Years and Older by County

Legend for obesity data

No Data  <10%  10%-14%  15%-19%  20%-24%  25%-29%  ≥30%

Public domain map created using data from www.cdc.gov/BRFSS.

What it Cardiovascular Disease?

- Starting at about your age … Gradual thickening of the arteries, usually at bends or bifurcation points
- LDL and cholesterol seep from blood through endothelial call layer into intima of artery (this is like an extracellular matrix)
- Possibly unfolded protein response is next (response to lipid)
- Eventually monocytes invade and differentiate into M1 and M2 macrophages
  - Role of Th2 (helper T-cells)
What is Cardiovascular Disease?
(Cont’d)

• Depending on the resolution of the inflammatory (M1) and anti-inflammatory (M2) arms of the pathway ... an acute problem may develop in the form of a fragile fibrous cap (which is a point of weakness in the artery)

• Fibrous cap is under stress from above (shear pressure from blood flow)

• And below (macrophages turned into foam cells ... which partially apoptose ... and can result in regions of necrosis)

• Smooth muscle cells and fibroblasts try to fill in the chamber created by necrosis
What it Cardiovascular Disease?
(Cont’d)

• If this cell division and migration process succeeds, the cap is stabilized ... you will die of something else
• If they fail, the cap is sheared off by blood flow, and tissue factor from intima mixes with blood
• Tissue factor activates blood platelets
• Thrombogenesis occurs (blood clot)
• A fibrous clot grows and can occlude the artery
• If it is a coronary artery, downstream necrosis will occur – Heart attack (Myocardial Infarction)
• Even if it is not a coronary artery, the clot can break free and cause distal damage (e.g., stroke)
Video of Man Having a Heart Attack

http://www.youtube.com/watch?v=Qo3Nf_mJjAw
First a Quick Overview

Sequence of Events Leading to Atheromatous Plaque
A large artery consists of three morphologically distinct layers. The intima, the innermost layer, is bounded by a monolayer of endothelial cells on the luminal side and a sheet of elastic fibres, the internal elastic lamina, on the peripheral side. The normal intima is a very thin region (size exaggerated in this figure) and consists of extracellular connective tissue matrix, primarily proteoglycans and collagen. The media, the middle layer, consists of SMCs. The adventitia, the outer layer, consists of connective tissues with interspersed fibroblasts and SMCs.
Initiation of atherosclerosis

Circulating leukocytes

The intima is composed of a single layer of endothelial cells overlying a subendothelial matrix.

Figure removed due to copyright restrictions. See article citation below. The figure shows a cross-section through an artery depicting circulating leukocytes adhering and migrating into the intima, where they divide.

Progression of atherosclerosis

Figure removed due to copyright restrictions.
See Figure 2 from Packard, Rene R.S. and Peter Libby.
"Inflammation in Atherosclerosis: From Vascular Biology
Inflammatory mediators can inhibit collagen synthesis and evoke the expression of collagenases by macrophage foam cells within the intima. This imbalance diminishes the collagen content of the fibrous cap, rendering it weak and rupture-prone.
Now a More Detailed View

Sequence of Events Leading to Atheromatous Plaque

When systems biology was new …

Bob Tepper, founder, former president of Millennium Pharmaceuticals

Tried to take an integrated view of cancer, CVD and diabetes
Lesion initiation

Sites of lesion formation are determined in part by haemodynamic forces acting on endothelial cells. These influence the permeability of the endothelial barrier and expression of endothelial cell (EC) genes such as that for nitric oxide synthase (NOS). An important initiating event is the retention of LDL and other apolipoprotein B (apoB)-containing lipoproteins as a result of interaction with matrix components. The LDL undergoes oxidative modification as a result of interaction with reactive oxygen species (ROS) including products of 12/15 lipoxygenase (12-LO) such as HPETE. Oxidation of LDL is inhibited by HDL, which contains the antioxidant protein serum paraoxonase.
Figure showing lipoprotein transport pathways and fates removed due to copyright restrictions.
Lipoproteins (liver) help create emulsions, but it is a great challenge to carry large volumes of a heterogeneous class of lipids.
Action of bile salts in emulsifying fats in the intestine (these micelles are in the intestine ... those in the next slide are in the blood plasma)

The lipids are then digested and transported through the intestinal mucosa ... and end up in the blood stream

Figure showing the chemical action of bile salts has been removed due to copyright restrictions.
Generalized structure of a plasma lipoprotein

Figures of plasma lipoprotein structure and of a chylomicron binding to lipoprotein lipase have been removed due to copyright restrictions.

Binding of a chylomicron to lipoprotein lipase on the inner surface of a capillary
Moreover … These lipid-glycoprotein conjugates need to be distinguished from sentinels of infections (e.g., LPS) … a hard task!
Minimally oxidized LDL stimulates the overlying endothelial cells to produce adhesion molecules, chemotactic proteins such as monocyte chemotactic protein-1 (MCP-1), and growth factors such as macrophage colony-stimulating factor (M-CSF), resulting in the recruitment of monocytes to the vessel wall. Oxidized LDL has other effects, such as inhibiting the production of NO, an important mediator of vasodilation and expression of endothelial leukocyte adhesion molecules (ELAMs). Among endothelial cell adhesion molecules likely to be important in the recruitment of leukocytes are ICAM-1, P-selectin, E-selectin, PCAM-1 and VCAM-1. Important adhesion molecules on monocytes include β2 integrin, VLA-4, and PCAM-1. Advanced glycosylation endproducts (AGEs) are formed in diabetes and these promote inflammation via specific receptors on endothelial cells.
Foam-cell formation

Highly oxidized aggregated LDL is formed in the vessel as a result of the action of reactive oxygen species (ROS) and the enzymes sphingomyelinase (SMase), secretory phospholipase 2 (sPLA₂), other lipases, and myeloperoxidase (MPO). The oxidized aggregated LDL is recognized by macrophage scavenger receptors such as SR-A, CD36 and CD68. Scavenger receptor expression is mediated by cytokines such as tumour necrosis factor-a (TNF-a) and interferon-g (IFN-g). Foam cells secrete apolipoprotein E (apoE), which may facilitate removal of excess cellular cholesterol via HDLs. The death of foam cells leaves behind a growing mass of extracellular lipids and other cell debris. – Probably contributes to Unfolded Protein Response

Figure removed due to copyright restrictions.
See Figure 5 from Lusis, Aldons J. "Atherosclerosis." Nature 407 (2000).
Formation of fibrous plaques

Figure removed due to copyright restrictions.
See Figure 6 from Lusis, Aldons J. "Atherosclerosis." Nature 407 (2000).
Complex lesions and thrombosis

Figure removed due to copyright restrictions.
See Figure 7 from Lusis, Aldons J. "Atherosclerosis." *Nature* 407 (2000).
Complex lesions and thrombosis

Complex lesions and thrombosis

- It is like the bursting of an abscess
- And it leaves behind scarring
- And non-elastic, thickened tissue

Hansson et al. *Nature Reviews Immunology* 6, 508-519 (July 2006) | doi:10.1038/nri1882

Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology.
Stages in the development of atherosclerotic plaques

Figure removed due to copyright restrictions.
See Figure 2 from Lusis, Aldons J. "Atherosclerosis." Nature 407 (2000).
Stages in the development of atherosclerotic plaques

Monocyte transmigration.
The thin-section electron micrograph of a cross-section of the aorta of a 9-weekold apoE-deficient mouse shows a monocyte (arrow) moving between two endothelial cells (arrowheads) to enter the intima (int). The asterisk denotes a cluster of lipid underneath the endothelial cell.

Stages in the development of atherosclerotic plaques

**Foam-cell Formation.** Freezeetch electron micrograph of the cytoplasm of a macrophage foam cell in the intima of a rabbit fed a high-fat diet for two weeks. Large lipid droplets with the onion skin configuration typical of cholesterol esters (ce) as well as LDL cholesterol esters (ce) as well as other lipid-filled compartments (arrows) can be recognized. Some compartments contain large aggregated LDL particles (asterisk) resembling those in previous figure.

CE = Cholesterol esters

Cytoplasm of a foam cell

LDL

Stages in the development of atherosclerotic plaques

Fibrous lesion. Light micrograph (2400x) of a section of an advanced human coronary atherosclerotic lesion that has been immunostained for the acrophage specific antigen EMB-11 (red). A, adventitia; I, intima; IEL, internal elastic lamina; M, media

Section of a human coronary artery

Three Possible Resolutions

- Stabilized Cap
- Thickened Wall with fibrosis
- Myocardial Infarction


Immune Cell Involvement

In Atherosclerosis
Immune Cell Infiltration and Balance

Early atherosclerosis

Advanced atherosclerosis

Efferocytosis = Successful clearance of apoptotic macrophages = good; when this fails, the system tips toward fibrous cap formation, and necrosis – necrosis draws in more inflammatory cells

Ira Tabas, Nature Reviews Immunology 10, 36-46 (January 2010)

Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology.

Healthy Response to CVD Would Shift from Left to Right

Ira Tabas, Nature Reviews Immunology 10, 36-46 (January 2010)

Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology.
• LDL diffuses from the blood
• LDL particles associate with proteoglycans of the extracellular matrix
• LDL modified by enzymes and oxygen radicals $\rightarrow$ oxLDL
• Biologically active lipids are released and induce endothelial cells to express leukocyte adhesion molecules
• Monocytes and T cells bind to VCAM1-expressing endothelial cells through very late antigen 4 (VLA4)
• Monoc. and T cells respond to locally produced chemokines by migrating into the arterial tissue

Hansson et al. Nature Reviews Immunology 6, 508-519 (July 2006)

b. Monocytes differentiate into macrophages in response to local macrophage colony-stimulating factor (M-CSF)

- Expression of many pattern-recognition receptors increases, including scavenger receptors and Toll-like receptors (TLRs)

- Scavenger receptors mediate macrophage uptake of oxLDL particles, which leads to intracellular cholesterol accumulation and the formation of foam cells

- TLRs bind LPS, heat-shock protein 60 (HSP60), oxLDL and other ligands, which instigates production of many pro-inflammatory molecules by macrophages

Hansson et al. Nature Reviews Immunology 6, 508-519 (July 2006)

Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology.
T cells undergo activation after interacting with APCs, such as macrophages or dendritic cells.

APCs process and present local antigens including oxLDL, HSP60 and possibly components of local microorganisms.

A T helper 1 (Th1)-cell-dominated response ensues, possibly owing to the local production of interleukin-12 (IL-12), IL-18 and other cytokines.

Antigen presentation and TH1-cell differentiation might also occur in regional lymph nodes.
Th1 cells produce inflammatory cytokines including IFN-g and TNF and express CD40 ligand (CD40L).

These messengers prompt macrophage activation, production of proteases and other pro-inflammatory mediators, activate endothelial cells, increase adhesion-molecule expression and the propensity for thrombus formation, and inhibit smooth-muscle-cell proliferation and collagen production.
- Response is shut down thanks to Th2 cells
- Plaque inflammation attenuated in response to the anti-inflammatory cytokines IL-10 and TGF-β
- These are produced by several cell types including regulatory T cells, macrophages and, for TGF-β, also vascular cells and platelets. TCR, T-cell receptor
Figure removed due to copyright restrictions.
See Figure 1 from Libby, Peter, et al. "Inflammation in Atherosclerosis: From Pathophysiology to Practice."
*Journal of the American College of Cardiology* 54 (2009).
Cells Involved in Atherosclerosis Express Pattern-Recognition Receptors Involved in Innate Immunity

While we usually think of these “receptors” in the context of response to a bacterial infection -- in the arterial wall they respond to LDL, Apolipopop. and other agents to trigger inflammagion

Libby, 2009
Cells Involved in Adaptive Immunity and Their Effect on Arterial Lesions

Five classes of lymphocytes

Figure removed due to copyright restrictions.
See Figure 3 from Libby, Peter, et al. "Inflammation in Atherosclerosis: From Pathophysiology to Practice." Journal of the American College of Cardiology 54 (2009).
C-Reactive Protein
A new diagnostic marker of inflammation

Binds to phosphocholine expressed on the surface of dead or dying cells

Three-dimensional model of c-reactive protein made using PyMOL by user Skolstoe on Wikimedia Commons.
Inflammation is Sensed in Many Organs
That information is transmitted to the liver

Figure removed due to copyright restrictions.
Inflammation sensed by the heart, blood vessel wall, macrophages, and adipose tissue leads to the release of cytokines that transmit this information to the liver. See Figure 1 from Rader, Daniel, J. "Inflammatory Markers of Coronary Risk." New England Journal of Medicine 343 (2000).
Role of C-Reactive Protein in CVD

Verma et al., 2005

Role of C-Reactive Protein in CVD

Role of C-Reactive Protein in CVD

Treatment of CVD

Treatments that address the immune mediated component of the disease
Addressing the disease

- Diet, exercise … still the best (stop smoking = inflammatory)
- Statins, anti-hypertensives, platlet-directed anti-inflammatory and anticoagulative agents, and anything that reduces insulin resistance
- Omega-3 FAs are precursors of protectins … effective
- Future
  - Agents that shift the macrophage M1 – M2 balance (Omega-3 FA and drug S1P lipid) might do this by binding to MPhage)
  - Activators of PPARs
  - Inducers of IL-10 and TGF-beta (if local) retard plaque progression (note = Protein drugs present delivery obstacles)
  - Immunize high risk people with apoptotic cells → increase IgM to apoptotic cell surface proteins
A Few Project Ideas

Thoughts I had while preparing this lecture
Project Ideas

- Tetrathiomolybdate has been tried (successfully) to combat CVD – Tom Maciag paper
- Use a phage display library to find plaques. Deliver a payload (drug) or image the lesion
  - Renata Pasqualini paper
- Blood vessels become “leaky” in the vicinity of an infection. Design a nanoparticle to squeeze through the space to deliver a drug – Shiladit Sengupta
- National Geographic approach to ID novel therapeutic targets
  - Search for genes at intersection of the CVD, Diabetes and Cancer ven diagrams
Do we have a chance to conquer complex diseases?

Scan of article from National Geographic (January 2010) removed due to copyright restrictions.
Read the article from National Geographic.
Or We Could Throw in the Towel and Do Something Useful With Tools Available Today

Adiponectin and an Ob/Ob Background

BE Design: We could grow healthy sumo wrestlers
Extra Slides Dealing With Pathways

May be useful for your presentations
Regulation of liver glucose metabolism by substrates and energy status
Regulation of glucose, FA and TG metabolism in adipose tissue
Lipids circulation and usage in fasted state

- VLDL
- IDL
- LDL
- HDL
- Albumin
Glucose, FA and energy metabolism in cardiac muscle

Major Pathway (70%)
Minor Pathways (30%)
AMPK in energy homeostasis

Exercise Hypoxia

ATP → ADP

Other stimuli, PKCζ

AMP

AMPKK (LKB1)

AMPKα AMPKβ

CaMKKβ

Glucose uptake and metabolism

GS (Glycogen synthase)

GLUT4 (Glucose transporter 4)

PFK (Phosphofructokinase)

eNOS (Nitric oxide synthase)

AMPK activation

Triglycerides and Malonyl-CoA

MCD (Malonyl-CoA carboxylase)

GPAT (Glucero-3-phosphate acyltransferase)

ACC2 (Acetyl-CoA carboxylase)

PFK (Phosphofructokinase)

eNOS (Nitric oxide synthase)

IRS1 (Insulin Receptor substrate 1)

mTOR

Protein synthesis and growth

Gene expression

Leptin Adiponectin

PPARγ

PGC-1α (PPARγ coactivator)

Rheb

TSC1/2

mTOR

CFTR

IRIS1

(eNOS substrate 1)
Regulation of glucose, FA and TG metabolism in adipose tissue

- Adrenaline
- Glucagon
- Leptin
- Autocrine
- Insulin
- PI3K
- JAK
- IRS1
- AC
- PDK
- PKB
- mTOR
- Ras
- Raf
- MEK1/2
- MEK1/2
- ERK1/2
- PDK
- PKB
- Desnutrin
- Hormone sensitive lipase (HSL)
- Monoglyceride hydrolase
- Acyltransferases
- Adipocyte Fat Store
- Palmitoyl-CoA
- 3-Glycerophosphate
- Leptin mRNA
- SREBP-1c
- PPAR
- Foxa1
- Foxo1
- STAT 3
- AP-1
- GR
- GR
- PPAR
- PPAR
- SREBP-1c
- PPAR
- PPAR
- PPAR
- PPAR
- PPAR
- PPAR
- PPAR
- PPAR
- PPAR
- ATP-citrate Lyase
- PEPCK
- Adiponectin mRNAs
- LpL, ACC, GLUT4, FAS, PEPCK
- PPAR
- Foxa1
- Foxo1
- STAT 3
- AP-1
- Citrate
- 3-Glycerophosphate
- Glycogenolytic pathway
- Glycolysis
- Glyceroneogenesis
Inflammation links classic risk factors to altered cellular behavior within the arterial wall and secretion of inflammatory markers in the circulation.

Figure removed due to copyright restrictions.
See Figure 4 from Packard, Rene R.S. and Peter Libby. "Inflammation in Atherosclerosis: From Vascular Biology to Biomarker Discovery and Risk Prediction." *Clinical Chemistry* 54 (2008).