Atherosclerosis

Joseph J Boyle
joseph.boyle@imperial.ac.uk
Learning Objectives

• know the origins & functions of the main inflammatory cells atherosclerotic plaques
• know the functions of macrophages in atherosclerotic plaques
• understand the differences between stable and unstable atherosclerotic plaques
Atherosclerosis

• Chronic inflammation of large arteries
• Predominantly intimal
• Sites – depends on haemodynamics
  – Branches and bends
  – Coronary arteries
  – Abdominal aorta
  – Carotid bifurcation
Theories Of Atherogenesis

• Insudation Hypothesis (Virchow 1852)
  – Cholesterol leaks into vessel walls

• Thrombogenic Hypothesis (Rokitansky 1852)
  – Blood clots absorbed into vessel walls

• Response To Injury Hypothesis (Ross 1972)
  – Initiated by damage to endothelium
  – Monkeys and rabbits fed cholesterol – endothelium first site of damage
  – Rats given balloon injury – lesions similar to atherosclerosis develop

  – Endothelial Dysfunction Hypothesis (Ross 1980)
    • The response to injury is actually modulated function rather than complete destruction

  – Inflammation Hypothesis (Ross 1999)
    • The response to injury occurs in the form of inflammation
Risk Factors

• Hypertension (high blood pressure)
• Hyperlipidaemia (high cholesterol)
• Diabetes
• Smoking
• Obesity
• Genetic factors
• Renal failure
Lipoproteins

- Low density lipoprotein (LDL)
  - ‘bad guy’ cholesterol rich
  - synthesised in liver and carries cholesterol from liver to rest of body including arteries
  - High density lipoprotein (HDL) ‘good guy’ - reverse cholesterol transport
  - carries cholesterol from rest of body including arteries back to liver

- Triglyceride - mainly in very low density lipoprotein and chylomicrons but also in other forms. Visible ‘fat’ is mainly triglyceride
Low Density Lipoprotein

Figure 1:
Schematic Illustration of a Lipoprotein Particle

- Apoprotein
- TG and CE
- Cholesterol
- Phospholipids
• Nobel Prize for Medicine 1985
• Hunted for gene for familial hypercholesterolaemia (high LDL and severe atherosclerosis)
• Discovered LDL receptor (feedback-regulated by cholesterol)
• Proposed a second LDL receptor - not under feedback control - in atherosclerotic lesions
• Called ‘scavenger’ since it hoovers up extra LDL
• Actually a family of ‘scavenger receptors’ mainly in macrophages
Brown & Goldstein

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Hyperlipidaemia

- Oxidised LDL, modified LDL are highly inflammatory and toxic forms of LDL that are found mainly in vessel walls.
- LDL Oxidised LDL dangerous version
  - inflammation
  - endothelial damage
  - vascular smooth muscle cell death
Oxidised LDL

• Event either in matrix of from processing of LDL by oxidative enzymes of activated macrophages
  – myeloperoxidase,
  – nitric oxide synthase
  – Inducible NADPH oxidase

• Phagocytosed by macrophages via scavenger receptors MSRA, CD36 and others → foam cells

• CD36 → phagocytosis, activates transcription factor PPAR-gamma (via PGJ2) → gene transcription
Principle of Immunohistochemistry

- **Label**
- **Antibody** (body immune defence protein that binds to specific molecules)
- **Many different molecules in section**
- **Tissue Section** (Micrometre thick slice of tissue)
Oxidised LDL
Oxidised LDL
Endothelium

- CD34 Endothelial marker = Brown
- HLA-DR Leukocyte Molecule = Brown
- Adhesion Molecule = Brown
Endothelium

• Lining of blood vessel - not inert
  – Normally an actively anti-thrombogenic surface
  – Portal of LDL emigration
  – Modulated by shear Stress
  – NO, PGI_{2} (vasodilators, antiplatelet)
  – Secretes endothelin (vasoconstrictor)
  – Expresses VWF (coagulant)
  – Expresses adhesion molecules ICAM, Selectin
  – Regulates monocyte adhesion and emigration
Blood monocytes differentiate to tissue macrophages

Stem cell → Monoblast → Monocyte → Macrophage

Tissues: Microglia (CNS), Kupffer cells (liver), Alveolar macrophages (lung), Osteoclasts (bone)

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Monocyte-macrophage differentiation occurs *after* transendothelial migration

The macrophage foam cell as a target for therapeutic intervention
Cartoon of macrophages in atherosclerosis

P. Libby, Nature 2002
Origins of macrophages in atherosclerotic plaques

- Endothelium a portal of entry for monocytes
- Mice with reduced VCAM-1 have reduced atherosclerosis and reduced macrophages in atherosclerotic plaques
- Mice with knockouts for monocyte chemoattractant protein-1 (MCP-1) or MCP-1 receptor have reduced atherosclerosis and reduced plaque macrophages
- Early plaques – arterial endothelium
- Advanced plaques – capillary endothelium
- Monocytes differentiate to macrophages
Functions of Macrophages

• Monocyte differentiate to macrophages and foam cells
  – oxidise / modify lipoproteins
  – phagocytose modified lipoproteins
  – activated by modified lipoproteins
  – may help export lipoproteins back out of the plaque

• Degrade collagen

• die by apoptosis

• secrete growth factors

• secrete inflammatory mediators

• secrete pro-apoptotic molecules
Conceptual overview of macrophages
Macrophages have oxidative enzymes

• **NADPH Oxidase**
  – superoxide O$_2^-$
  – Other (ROS) derived from superoxide
  – $\rightarrow$ hydrogen peroxide H$_2$O$_2$

• **Inducible nitric oxide synthase (iNOS)**
  – High concentrations of free radical NO nitric oxide

• **Myeloperoxidase**
  – HOCl hypochlorous acid (bleach) from ROS + Cl$^-$
  – HONO$\cdot$ Peroxynitrite (NO-adduct) from ROS + NO
Macrophages - Clearance

- Pathogens
  - Pathogen pattern recognition receptors
- Oxidised lipoproteins
  - Macrophage scavenger receptor A
  - CD36
- Apoptotic (dead) cells
  - Phosphatidylserine receptor
- Haemoglobin
  - CD163
Macrophage Scavenger Receptors

- Bind to oxidised forms of LDL (the form found in plaques)
- May stimulate macrophages
- High uptake pathway
- Leads to foam cells
- Bind modified forms of LDL e.g. Oxidised LDL
- Also bind LDL modified by several other forms of modification –
  - NO-modification
  - HOCl-modification,
  - enzymatic removal of sugars
  - partial proteolytic digestion
Plaque monocytes eat cholesterol and become macrophages and foam cells

All - CD68 monocyte macrophage marker
Macrophages surround areas of free cholesterol in atherosclerosis
Plaque macrophages express inflammatory mediators

- Tumour Necrosis Factor -α
- CD68 (Macrophage molecule)
Plaque macrophages make multiple mediators
Macrophages - Tissue Repair

• Platelet derived growth factor
  – Vascular smooth muscle cell survival and cell division

• Fibroblast growth factor
  – Smooth muscle cells survival, cell division

• Transforming growth factor beta
  – Increased collagen synthesis

• Vascular endothelial growth factor
  – New capillary formation
PDGF

- Platelet derived growth factor
- PDGF AA, AB, BB dimers
- Secreted by macrophages
- Macrophages secrete IL-1 which triggers PDGF release from VSMCs
- PDGF activates transmembrane tyrosyl kinase receptors (classical growth factor receptor) activating phospholipase-C gamma and phosphoinositide-3-kinase
- Potent chemoattractant for VSMCs
- Mitogen, stimulates matrix synthesis, survival factor,
Macrophage functions

- Differentiate to macrophages and foam cells
- Oxidise / modify lipoproteins
- Phagocytose modified lipoproteins
- Activated by modified lipoproteins
- Die by apoptosis
- May help export lipoproteins back out of the plaque
- Secrete growth factors
- Secrete inflammatory mediators
- Secrete pro-apoptotic molecules
Macrophage Collagenolysis

Van der Wal et al 1999 Cardiovascular Research 41:334
Macrophage Proteases

- Matrix metalloproteases
- Serine proteases
  - Neutrophil Elastase
- Aspartate proteases
  - Cathepsin D (intracellular, not collagenolytic)
MMPs

• Family of proteases - catalytic site contains Zn
  – MMP-1
  – MMP-2
  – MMP-3
  – MMP-9
  – MMP-14

• MMP-1, MMP-2, MMP-9 cleave collagen
• Inhibited by TIMPs
• TIMP-1 overexpression reduces atherosclerotic tissue weakening
• Activate each other by proteolysis
• MT-MMPs are transmembrane proteins that activate other MMPs
Plaque macrophages express coagulation-regulating proteins

- Macrophages are a rich source of tissue factor, which activates the intrinsic pathway
- Tissue factor activates thrombin via tissue factor pathway
- Upregulated by CD40 and apoptosis

Moreno et al. Circulation 1996
Macrophages die in plaques by a regulated process called apoptosis.

Blue = nuclei  Green = dead cells with cleaved DNA
Vascular Smooth Muscle Cells

- ‘Good Guy’ vs ‘Bad guy’
- 2 phenotypes - contractile vs synthetic
  - PDGF & ROS switch between

- Synthetic
  - Synthesise Collagen
  - Migrate from media or adventitia
  - In some experiments – may be derived from circulating bone marrow stem cells
  - Mass effect

- Not proliferative except in rodent experiments
- Prone to apoptosis
VSMC phenotypes

**Contractile**
- ↑ Myofilaments
- ↑ α-actin
- ↑ Smoothelin
- ↑ Calponin

**Synthetic**
- Endoplasmic Reticulum
- ↑ Matrix genes
- ↑ Elastin
- ↑ Collagen
- ↑ Proteoglycans
- ↓ α-actin
- ↓ Smoothelin
- ↓ Calponin

235 genes changed
VSMC phenotypes

Contractile

Angiotensin II
Platelet derived growth factor (PDGF)
Reactive oxygen species (ROS)

Synthetic
Arterial remodelling
Smooth muscle cells

Nuclei = Blue

$\alpha$-actin (smooth muscle protein) - Brown
Stable vs Unstable Plaques

• **Unstable plaques**
  – Acute coronary syndromes (ACS)
    • Myocardial infarction
    • Unstable angina
    • Chest pain, acute ECG changes and troponin-T elevation

• **Stable plaques**
  – cause *chronic stable angina* by progressive stenosis
  – extreme (critical, >95%) narrowing, shear may provoke de-endothelialisation and acute coronary syndrome.

• **Unstable plaques have more inflammation**
Characteristics of vulnerable and stable plaques
Plaque Rupture

- Cause thrombosis
- Exposes blood to macrophage debris in plaque centre
- Macrophages rich in tissue factor - procoagulant
- Collagen exposed
**Unstable plaques**

- **Acute coronary syndromes**
  - myocardial infarction
  - unstable angina

- **Structure**
  - large lipid cores
  - thin fibrous cap
  - Few vascular smooth muscle cells
  - prominent inflammatory infiltrate with many macrophages
Fatal Coronary Plaque Ruptures (unstable plaques) are associated with Macrophage Inflammation.

J. Pathol. 1997
Ruptured coronary plaques have more inflammation than controls.

![Bar chart showing percentage of plaques with inflammation of superficial cap / shoulder by source of plaque: Coronary thrombosis (autopsy), Control (autopsy), Severe IHD (Cardiac explants). The chart indicates a significant difference (P<0.05).]
Coronary Plaque Macrophages Have Digestive Enzymes

H&E – General stain

Cathepsin-D Macrophage digestive enzyme

Fibrous Cap

Lipid Core

Thrombus

Fibrous Cap

Thrombus

Lipid Core
Summary

1. Macrophages in atherosclerotic plaques are from monocytes via endothelium.

2. Macrophages in atherosclerotic plaques lyse matrix via MMPs, secrete free radicals, oxidise LDL, are activated by oxidised LDL, phagocytose, release growth factors, express tissue factor and kill other cells.

3. Unstable atherosclerotic plaques are rupture-prone and contain increased macrophages.