Atherosclerosis as an autoinflammatory disease

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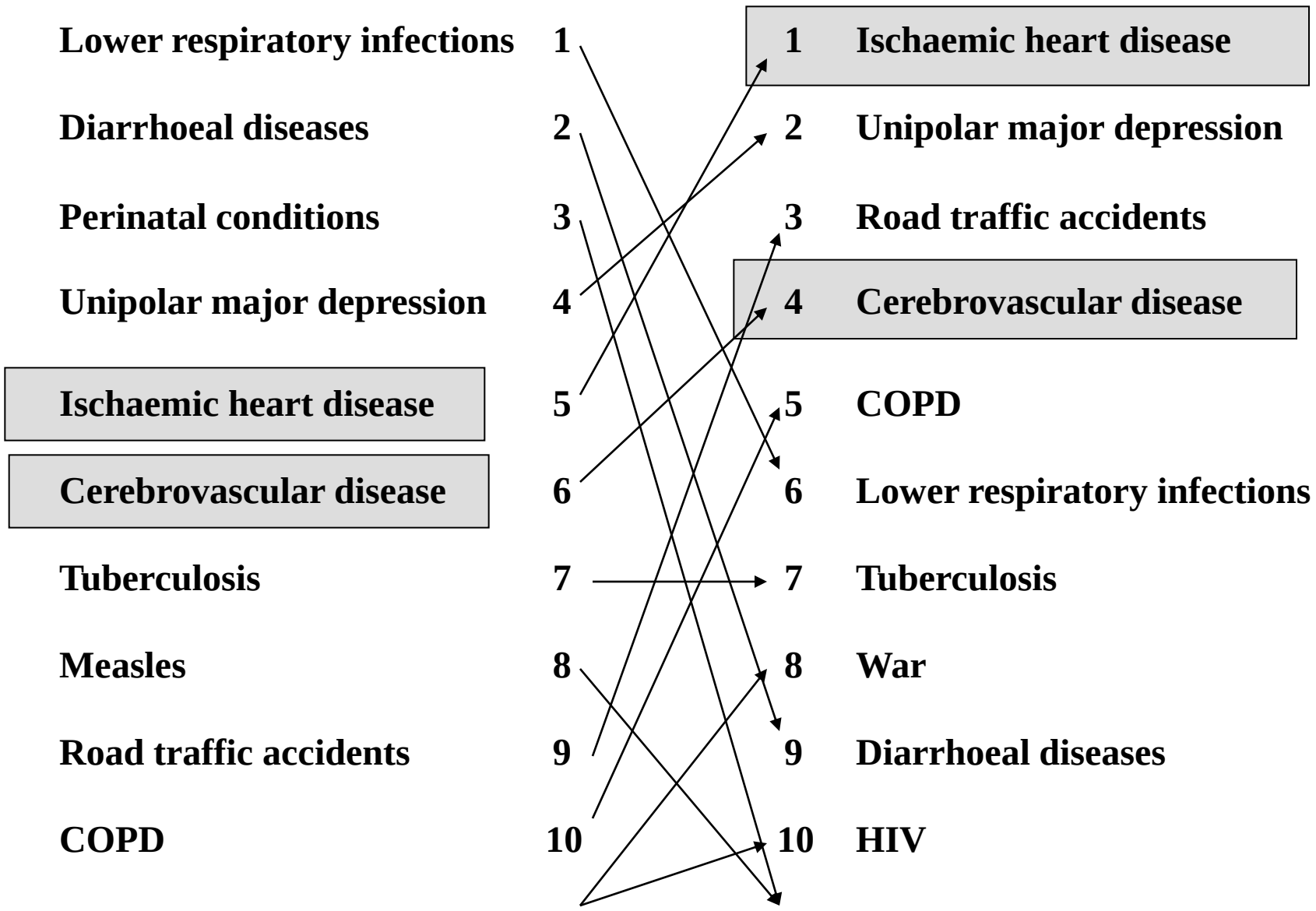
Atherosclerosis – plan of talk/learning objectives

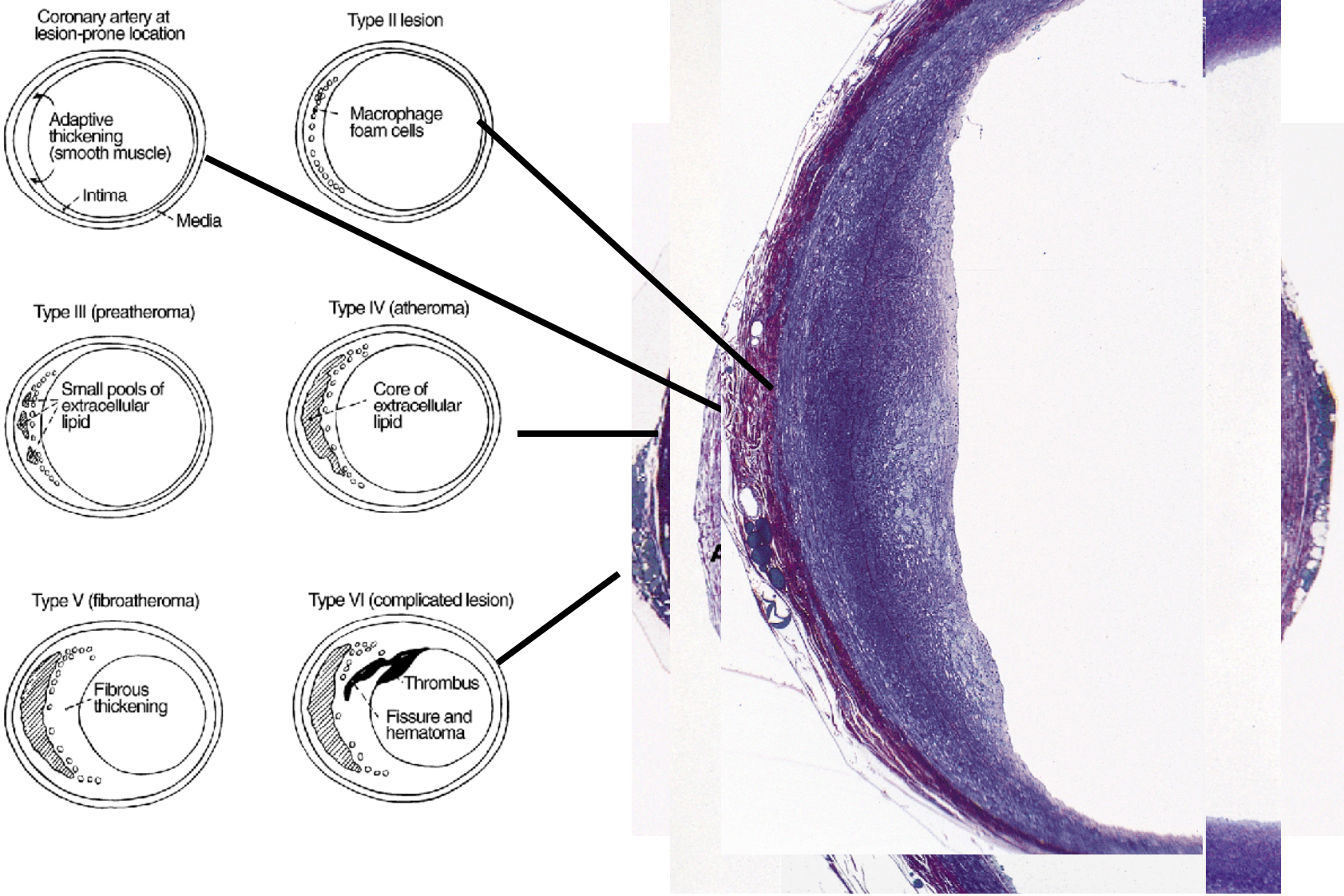
- Cellular pathology of atherosclerosis
- Link between cholesterol and inflammation
- Investigating molecular mechanisms
- Roles of humoral and cellular immunity

World disease burden



Adapted from Lopez and Murray (1998) Nature Med 4: 1241

Type II lesion



Debris (eg modified lipoproteins, apoptotic cells)

⇩

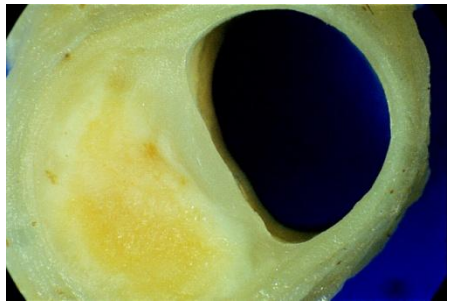
Reversible fatty lesions

⇩

Irreversible remodelling

wound healing

Stable plaques



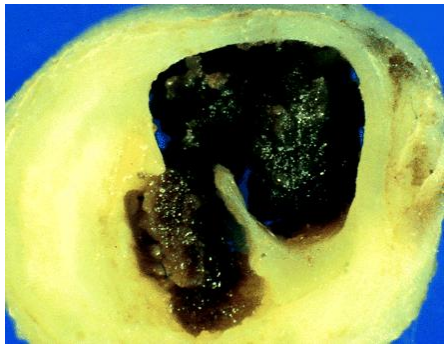
Ischaemia

Angina
Intermittant claudication

⇔

inflammation/thrombosis

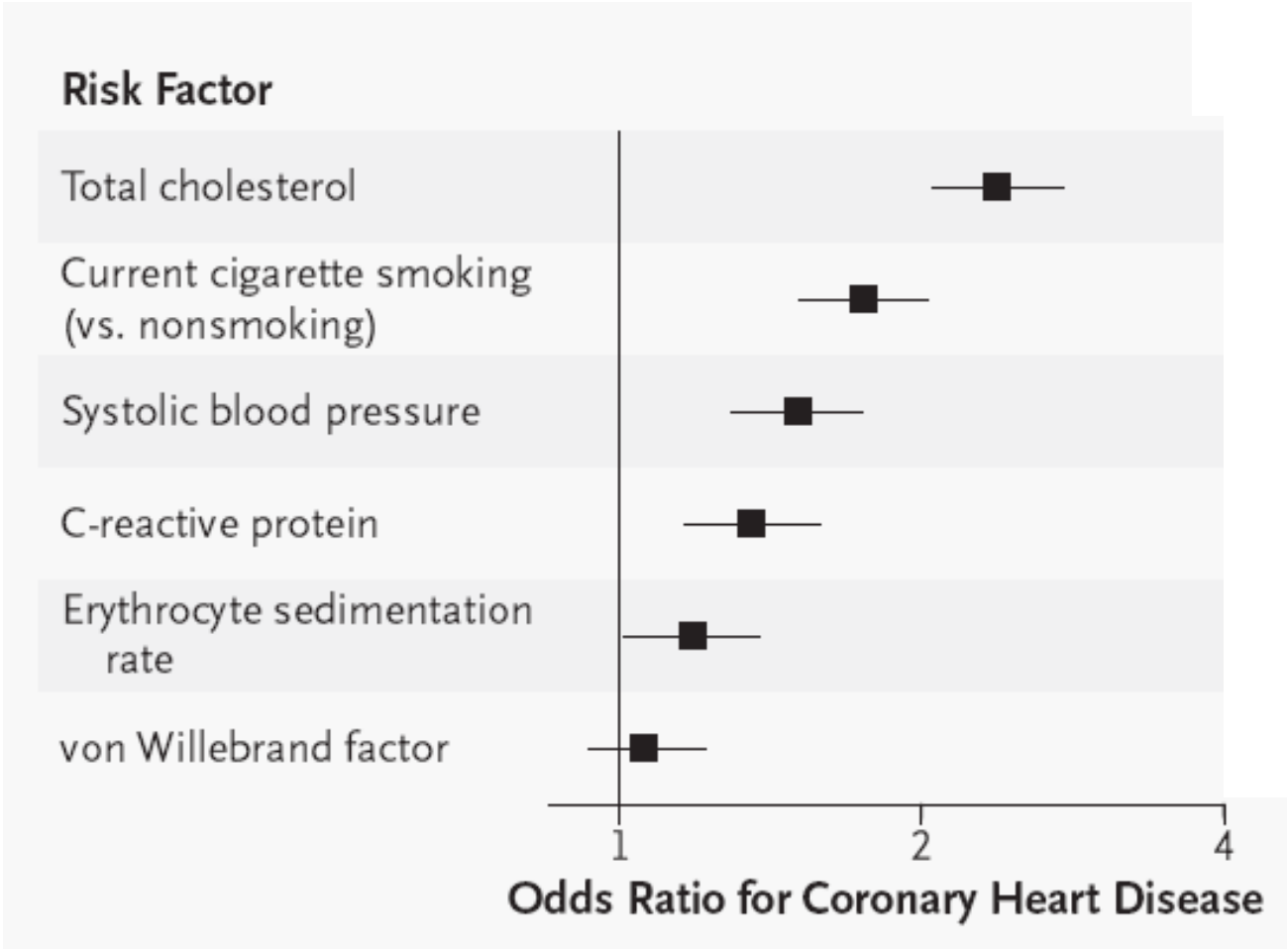
Unstable plaques



Thrombosis and infarction

Acute coronary syndromes
Stroke
Peripheral gangrene

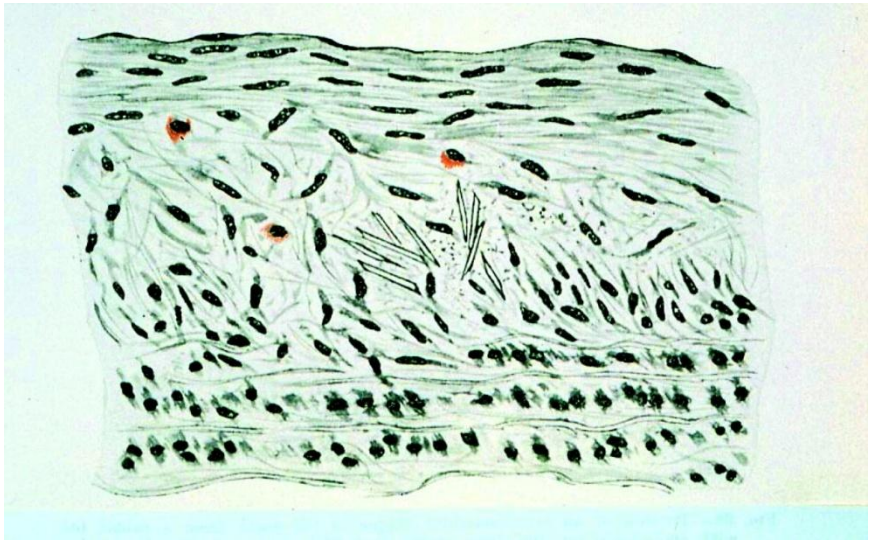
Relative importance of risk factors



Danesh et al 2004 N Engl J Med 350:1387

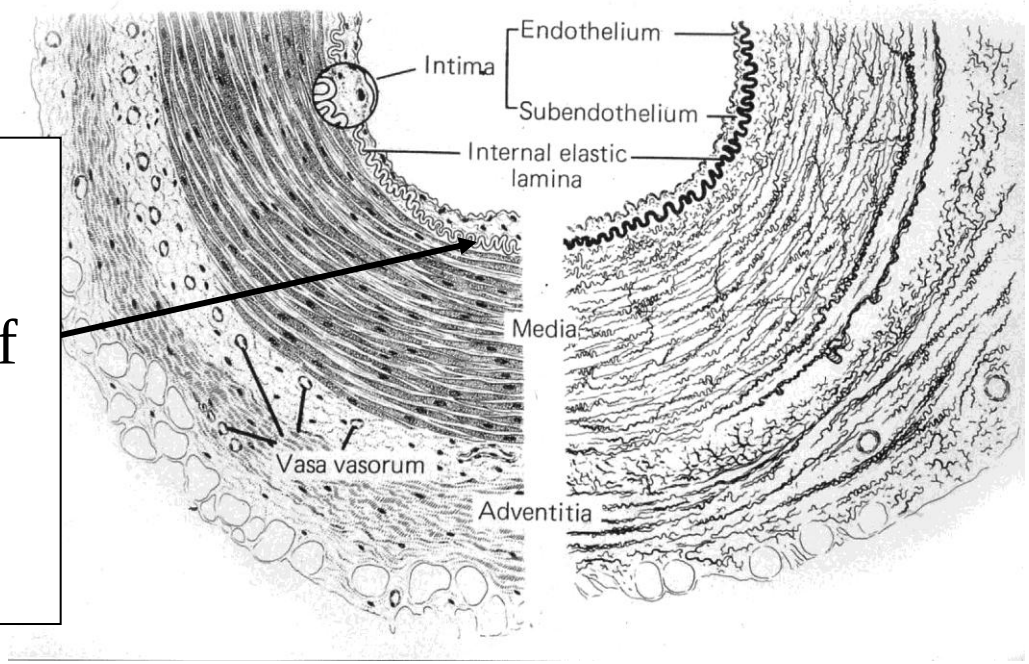
History – the cholesterol hypothesis

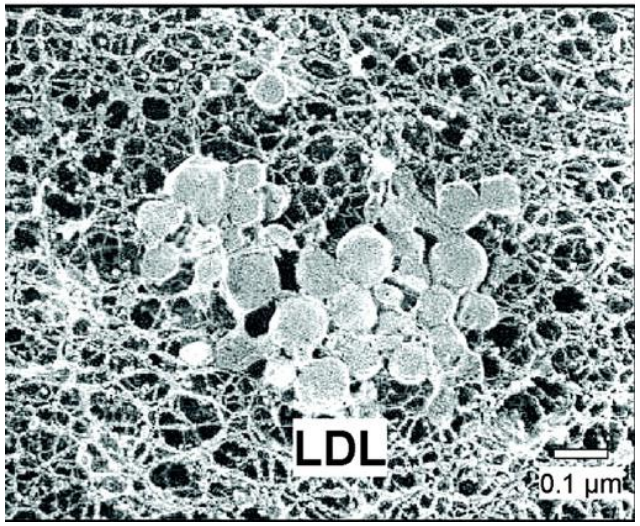
N. N. Anitschkow
Military Medical Academy of St Petersburg
(circa 1904)



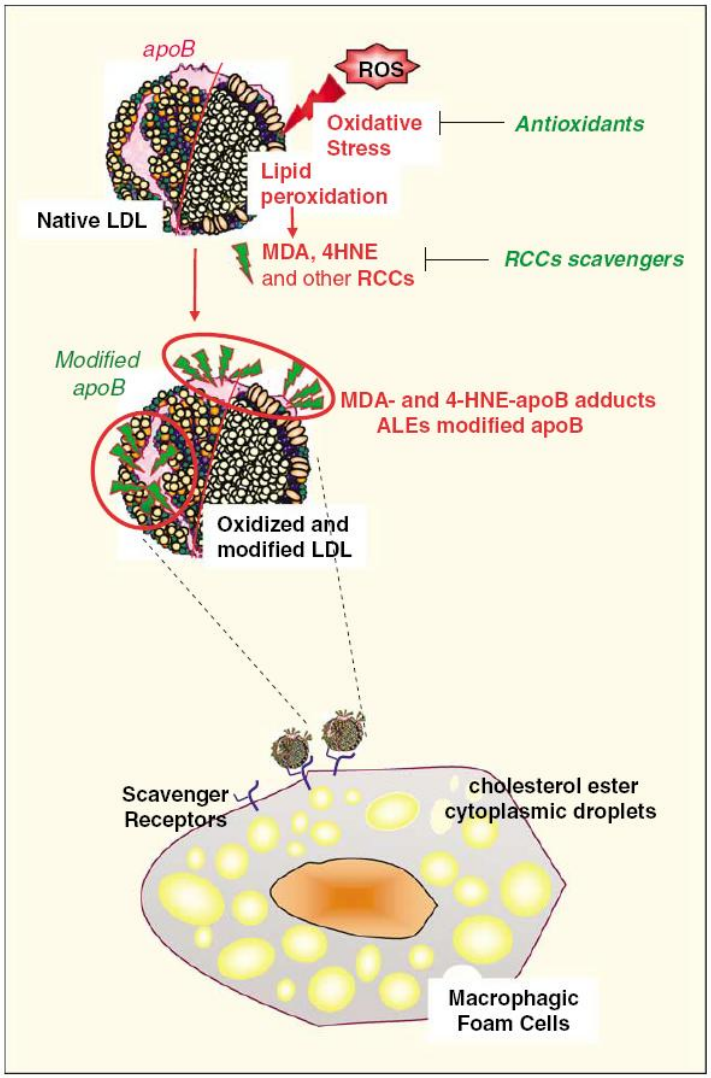
Plaque from a rabbit fed cholesterol for 106 days and then chow for >2 years

Low density lipoproteins (LDL) deposit in the subintimal space at sites of low/complex flow, and bind to matrix proteoglycans

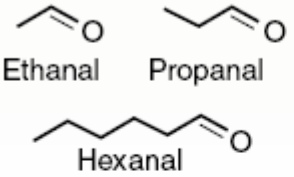




From: Tabas, I. et al. Circulation 2007;116:1832-1844

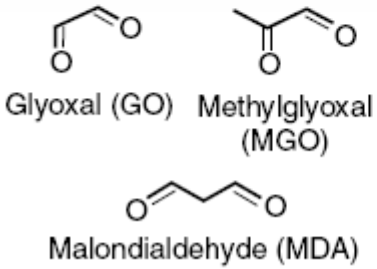


Saturated Aldehydes

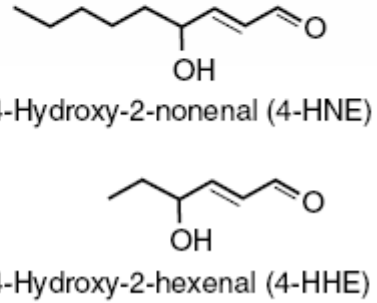


Unsaturated Aldehydes

Dicarbonyls

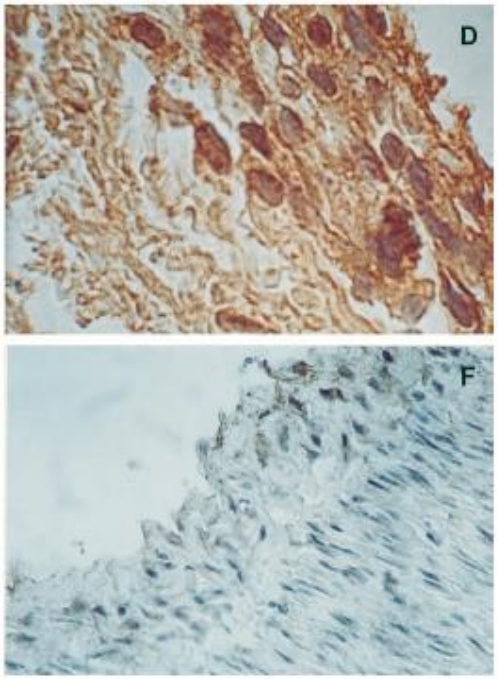


4-Hydroxy-2-alkenals



LDL modification in the arterial wall happens before macrophage infiltration

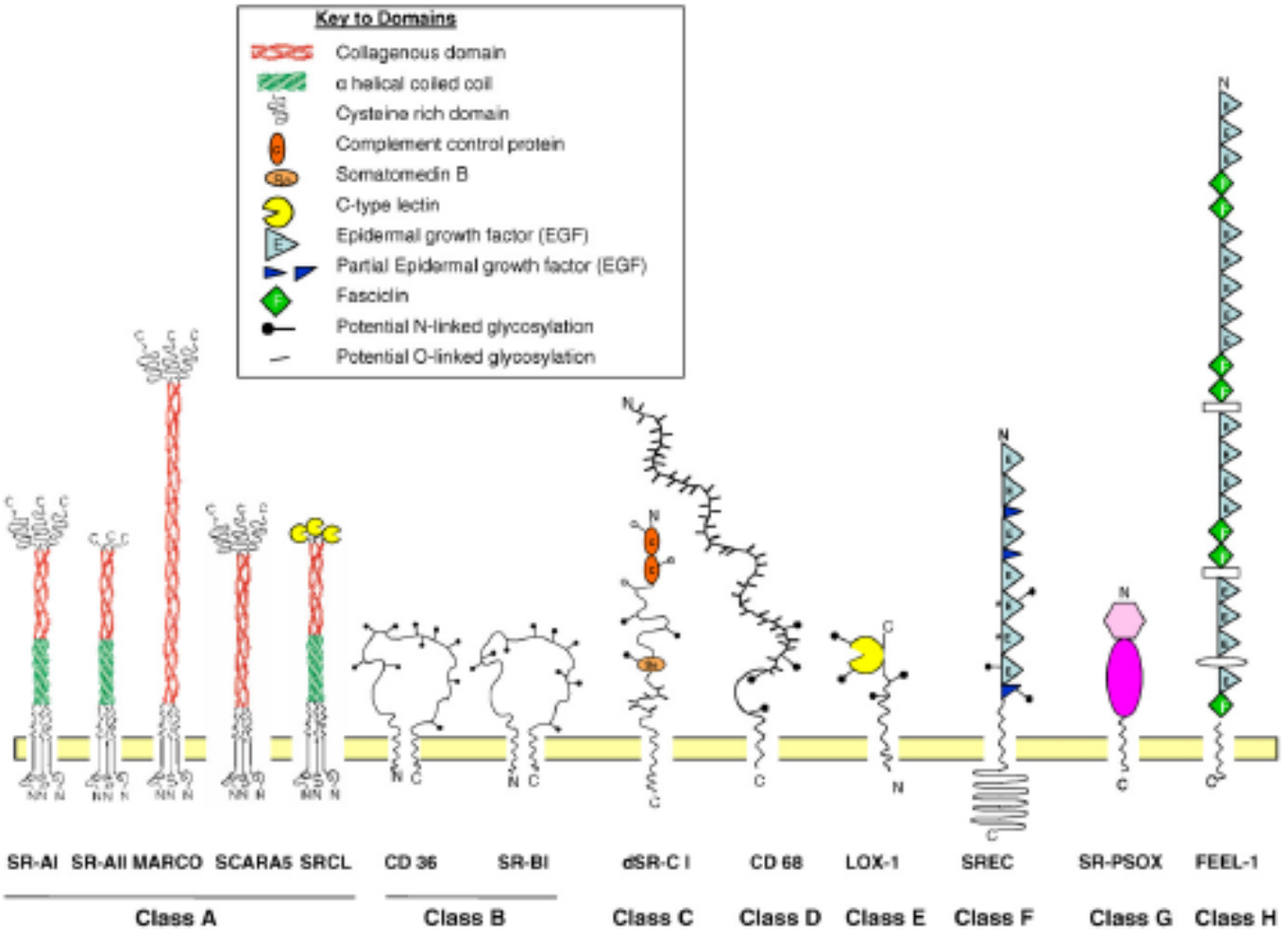
MDA-lysine
(oxidised LDL)



monocyte/møs

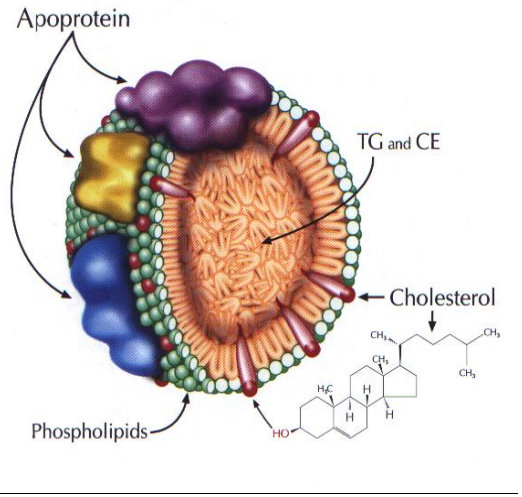
Napoli et al 1997 J Clin Invest 100:2680

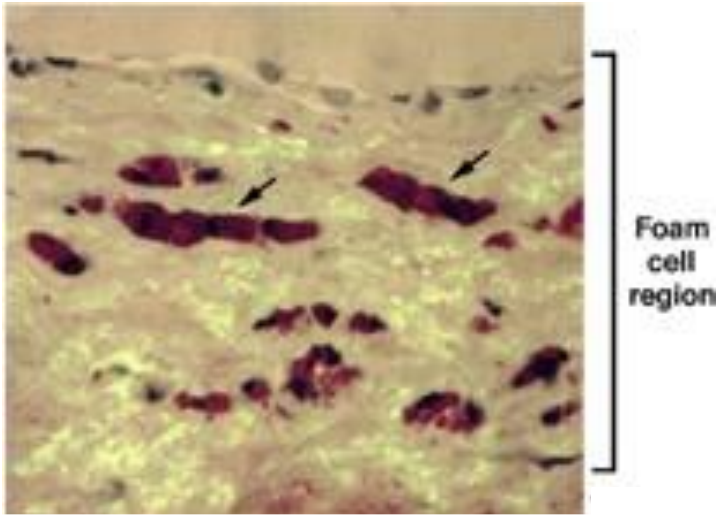
Macrophage scavenger receptors



from Pluddemann et al (2007) Methods 43:207

Foam cells

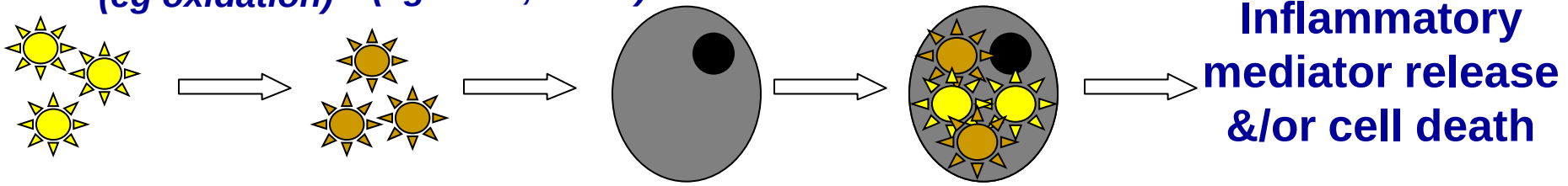




from Dr Howard K

lipoprotein modification (eg oxidation)

via scavenger receptors (eg SR-A, CD36)

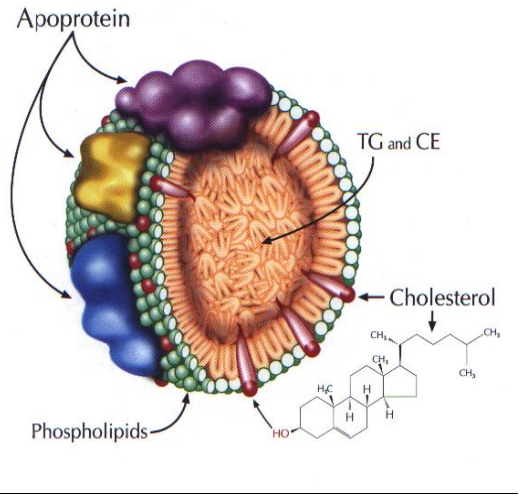


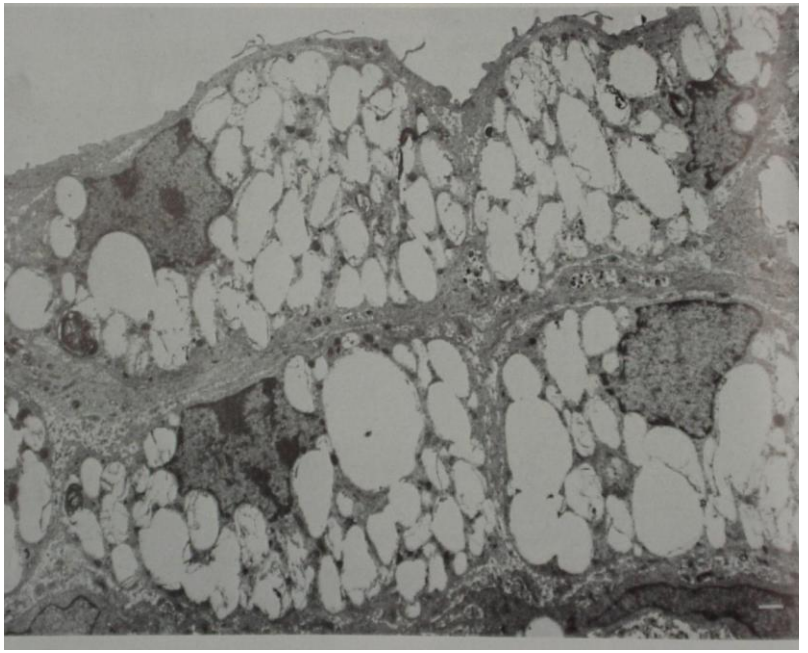
Inflammatory mediator release &/or cell death

Macrophage

"foam cells"

Foam cells

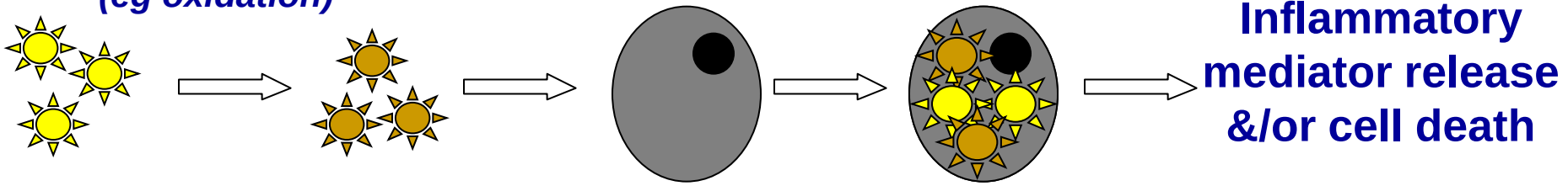




Faggiotto et al 1984 Arteriosclerosis 4:323

lipoprotein modification (eg oxidation)

via scavenger receptors (eg SR-A, CD36)

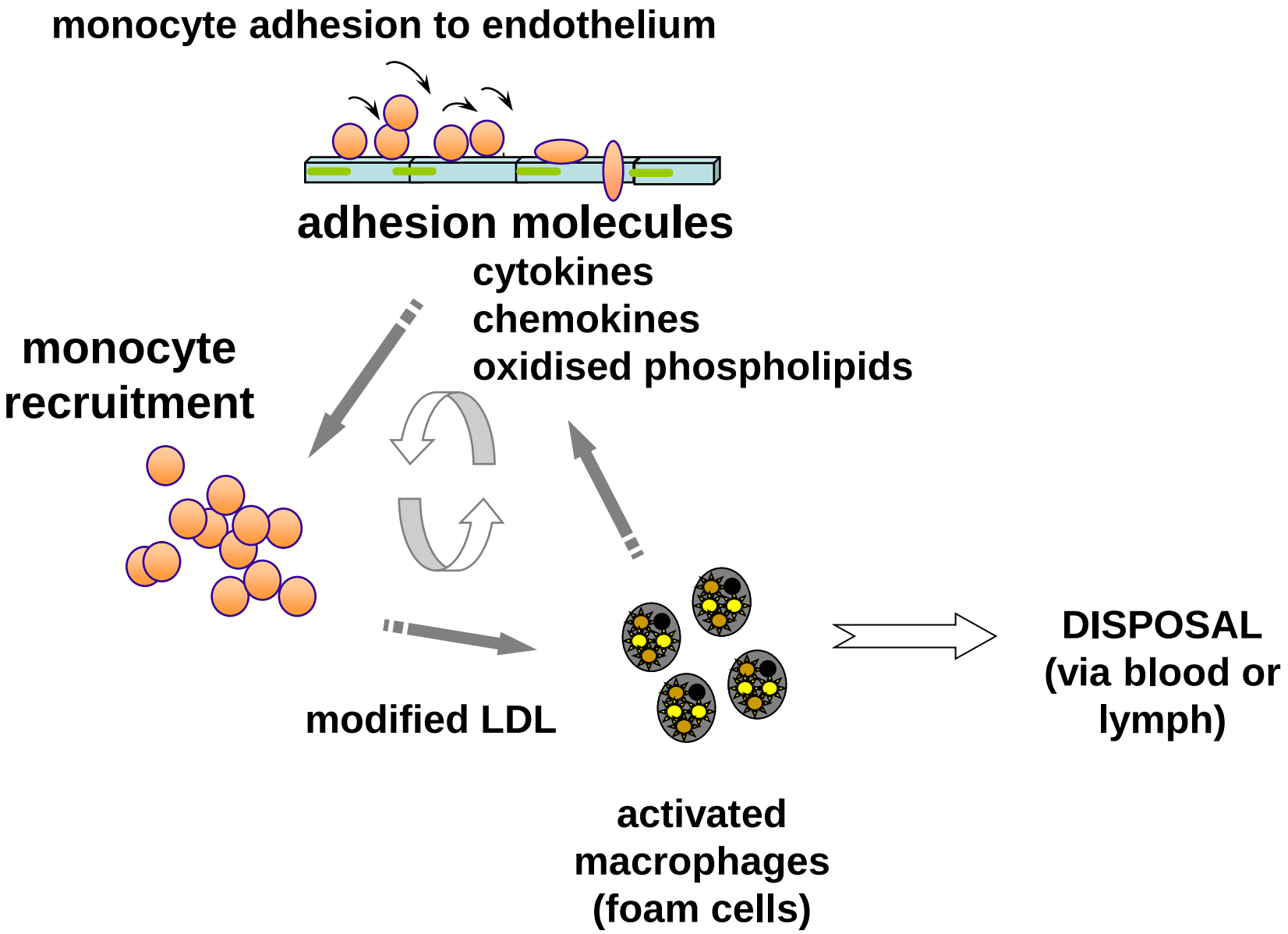


Macrophage

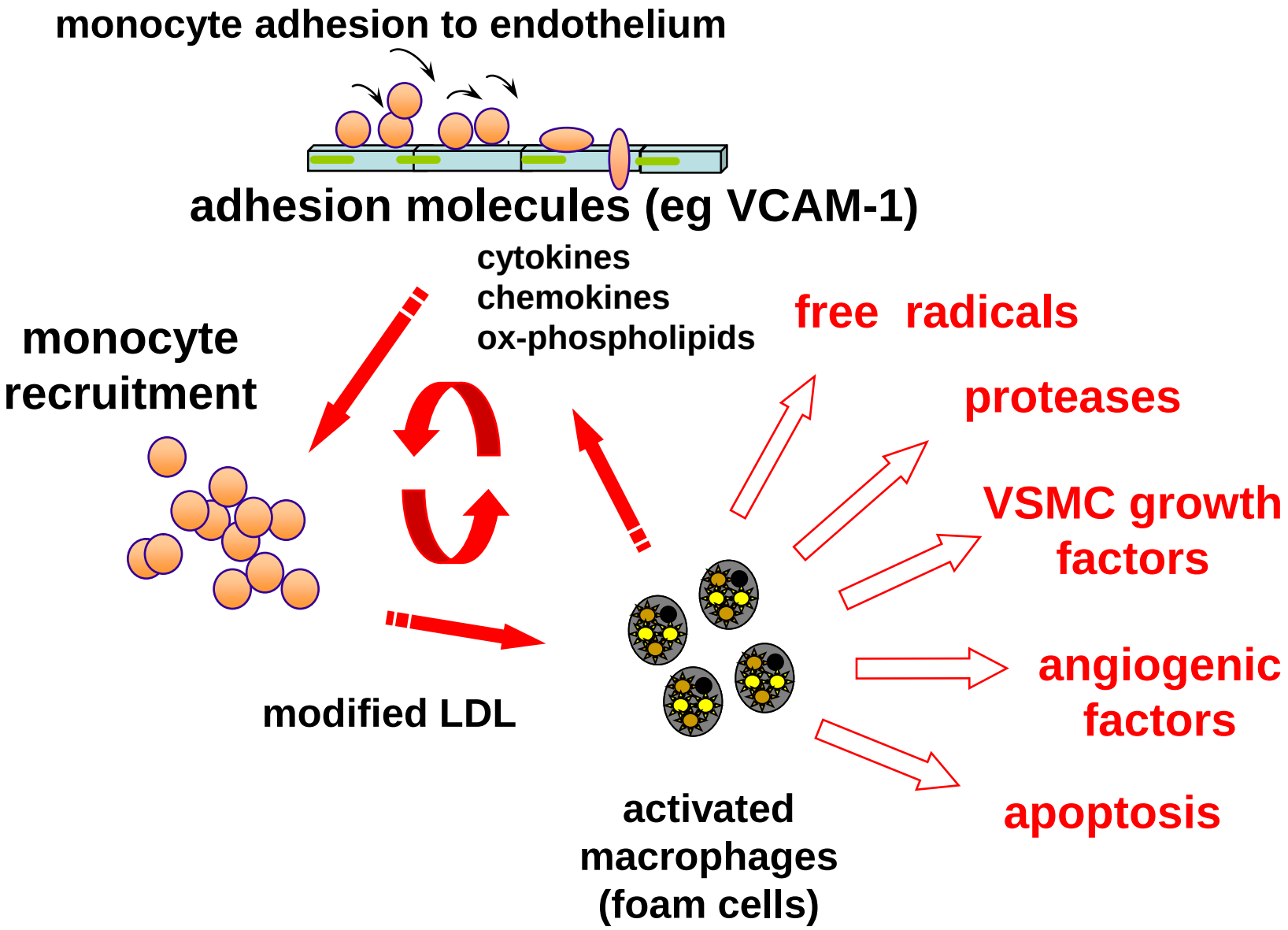
"foam cells"

Inflammatory mediator release &/or cell death

Homeostatic debris disposal



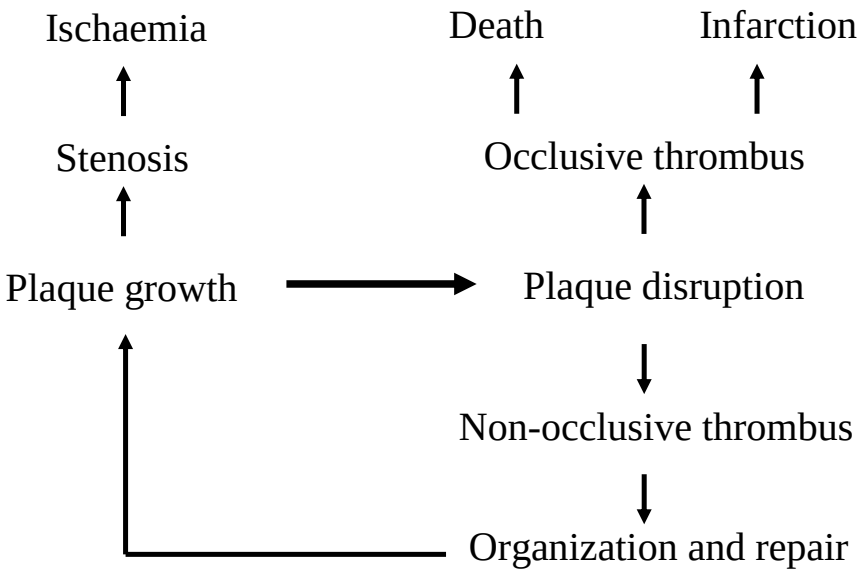
Inflammatory basis of atherosclerosis

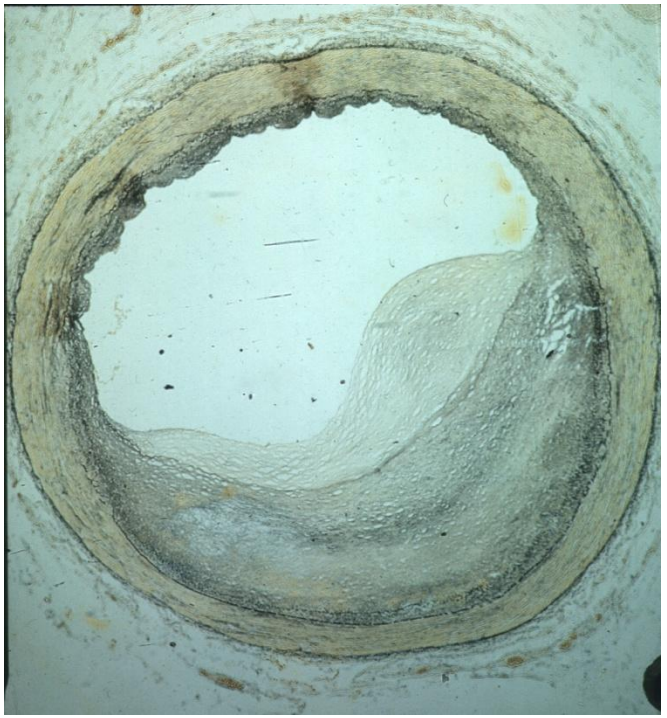


Main cellular players

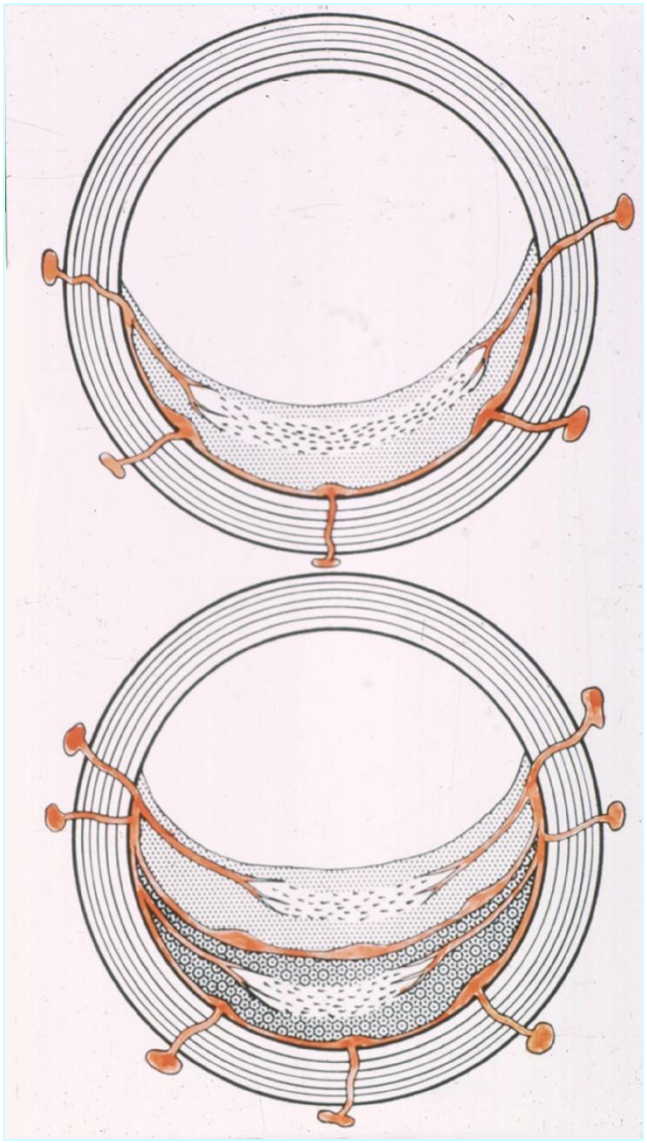
- **Vascular endothelial cells**
  - Barrier function (eg to lipoproteins)
  - Leukocyte recruitment
- **Platelets**
  - Thrombus generation
  - Cytokine and growth factor release
- **Monocyte-macrophages**
  - Foam cell formation
  - Cytokine and growth factor release
  - Major source of free radicals
  - Metalloproteinases
- **Vascular smooth muscle cells**
  - Migration and proliferation
  - Collagen synthesis
  - Remodelling and fibrous cap formation
- **T lymphocytes**
  - Macrophage activation

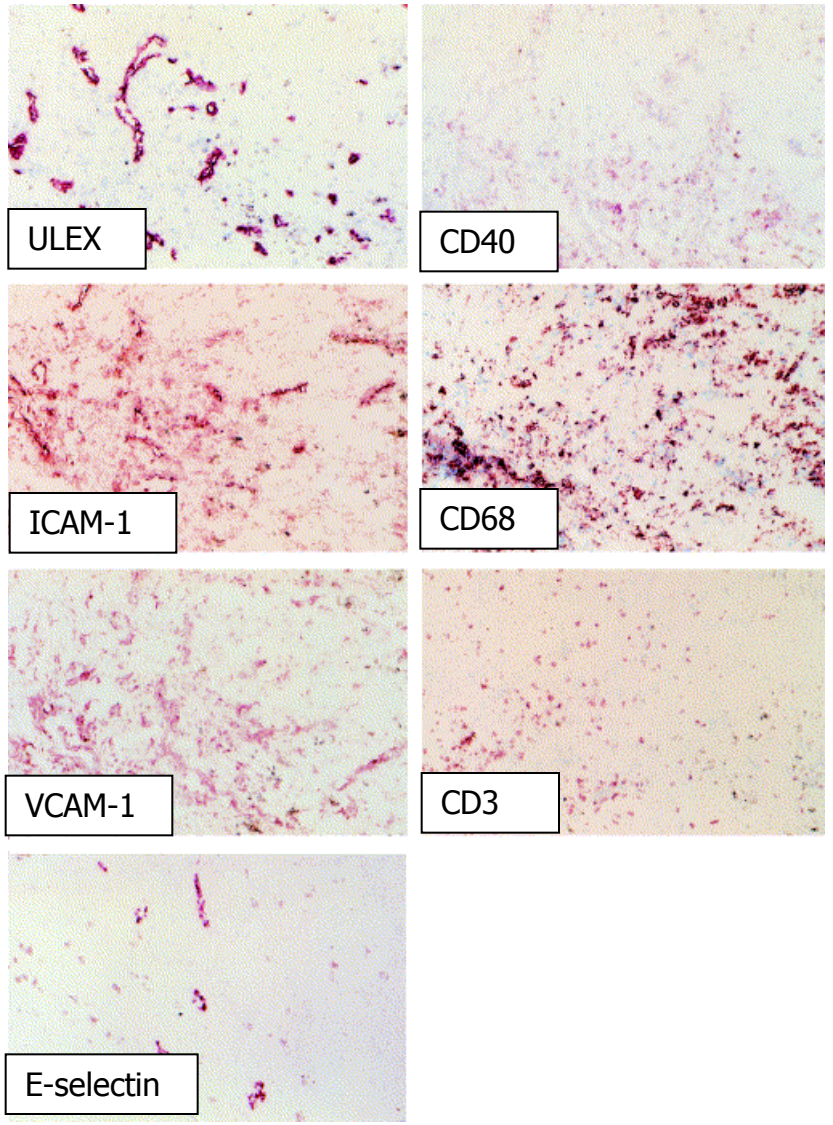
Step-wise progression of atherosclerotic plaques





Vasa vasorum are the back-door for leukocyte recruitment

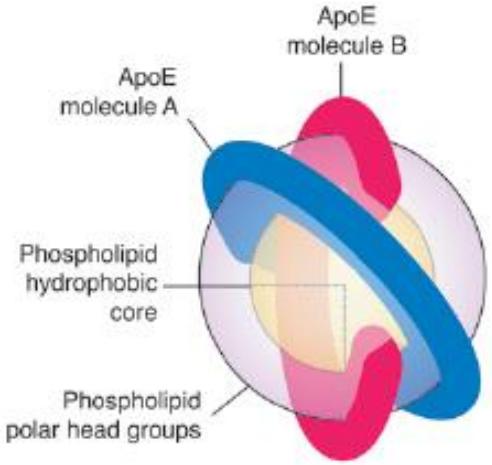




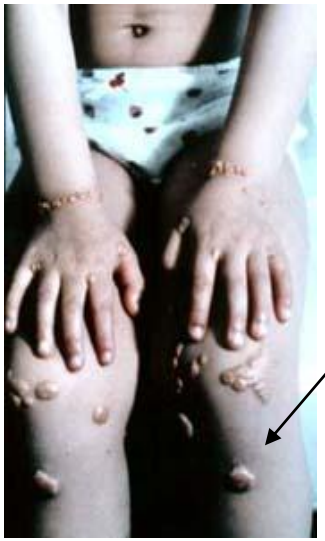
De Boer et al (1999) Cardiovasc Res 41:443.

Mouse models of atherosclerosis

- **ApoE-/-**
  - 34kd component of VLDL and chylomicrons
  - ligand for LDL receptor

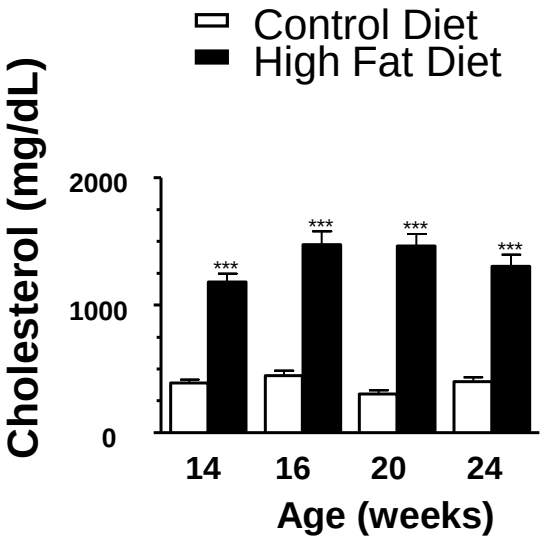


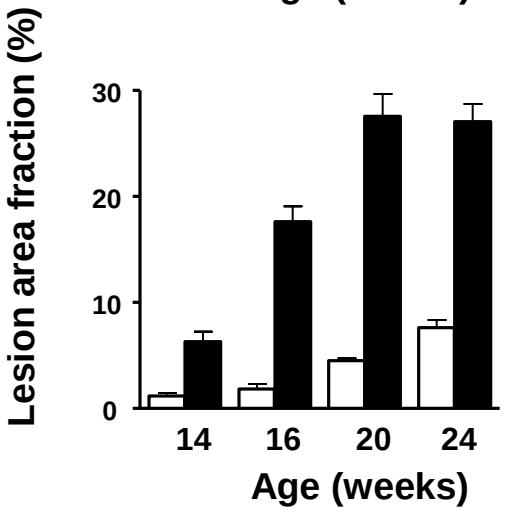
- **LDL receptor -/-**
  - Mutations in familial hypercholesterolaemia

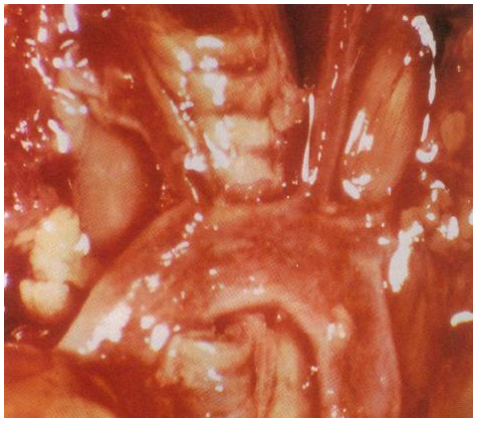


xanthoma

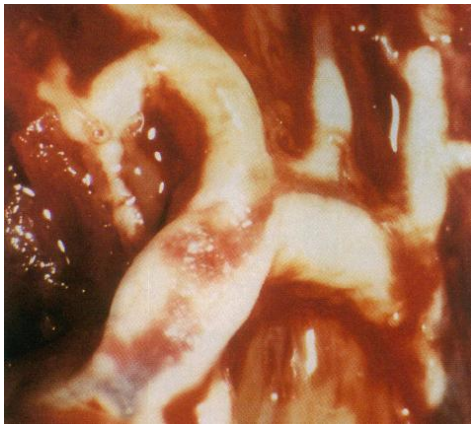
Atherosclerosis in *Ldlr*$^{-/-}$ mice





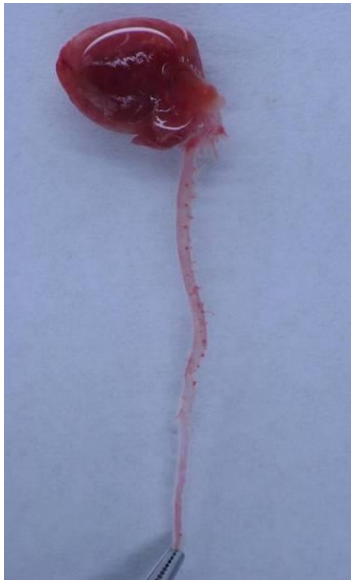


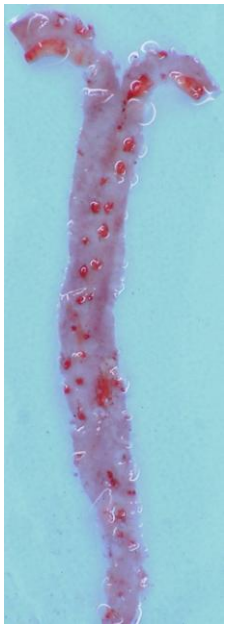
wild-type

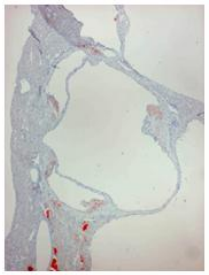
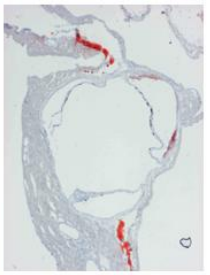
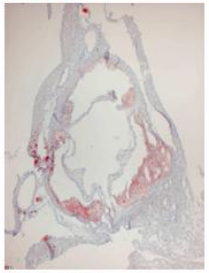
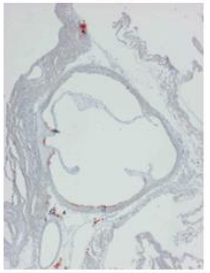
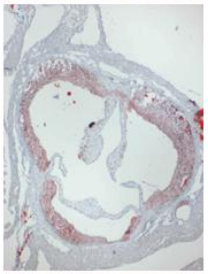
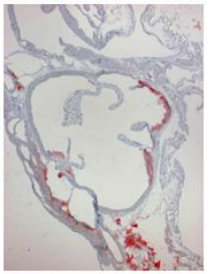
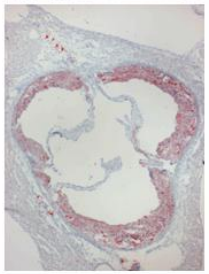
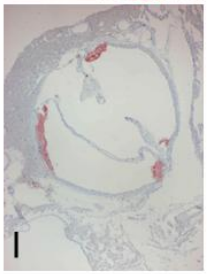


Ldlr-/- high fat 13 mo

Ishibashi et al 1994 JCI 93:1885





	high fat	chow
14		
16		
20		
24		

weeks

Aortic valve

Influence of adhesion molecules, chemokines and cytokines on mouse atherosclerosis

Accelerators

Adhesion molecules
P-selectin
E-selectin
ICAM-1
VCAM-1

Chemokines & receptors
MCP-1
CCR2
CXCR2
CX3CR1

Cytokines
IL-1
TNFα
IL-4
IL-6
IL-12
IL-18
IFNγ

Decelerators

IL1RA
TGFb
IL-10

Debris (eg modified lipoproteins, apoptotic cells)

Reversible fatty lesions

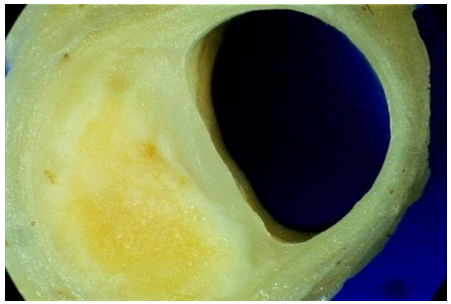
inflammatory drive + - *homeostatic innate immunity*

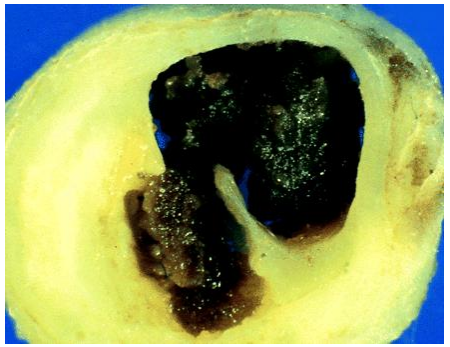
Irreversible remodelling

wound healing

environmental and genetic influences

inflammation



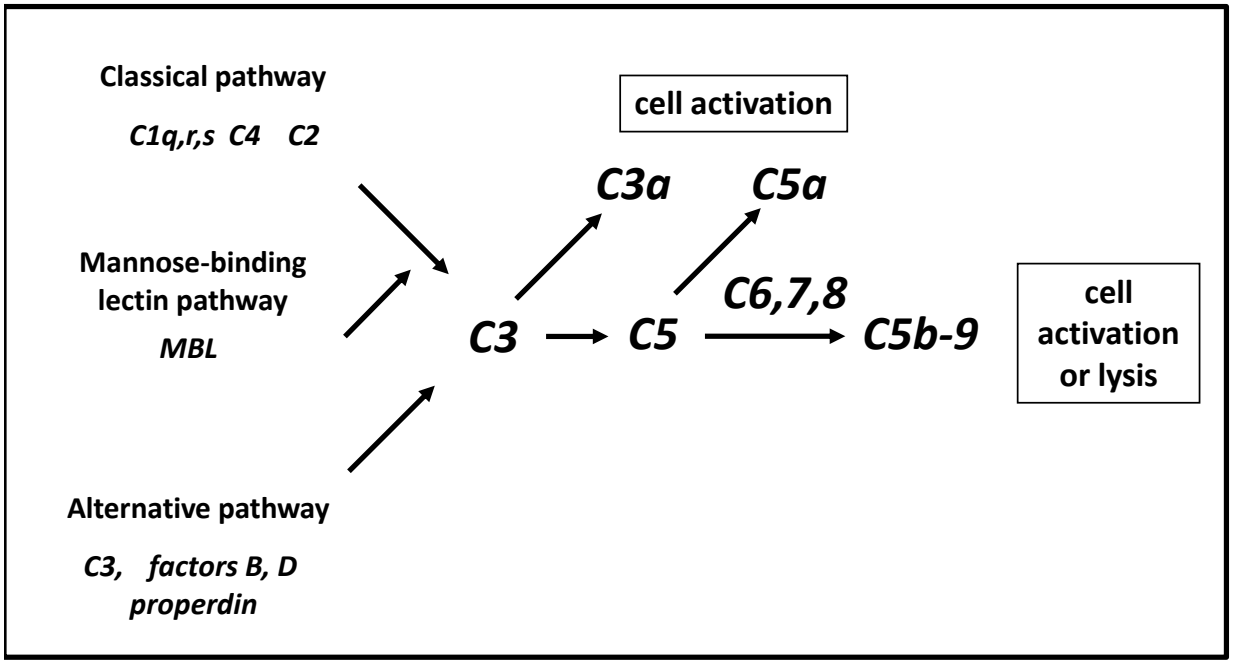


Stable plaques

Unstable plaques

Role of complement in atherosclerosis

Apoptotic cells
Cholesterol crystals
Denatured LDL
Microparticles
Immune-complexes
Endotoxin

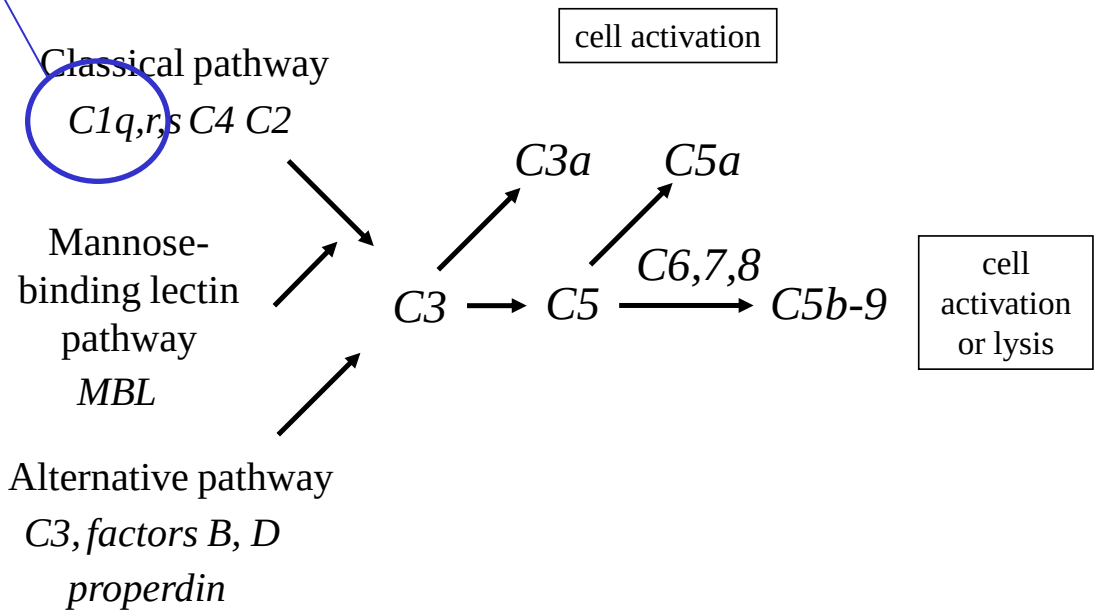


Immunohistological evidence for complement activation in human atherosclerosis

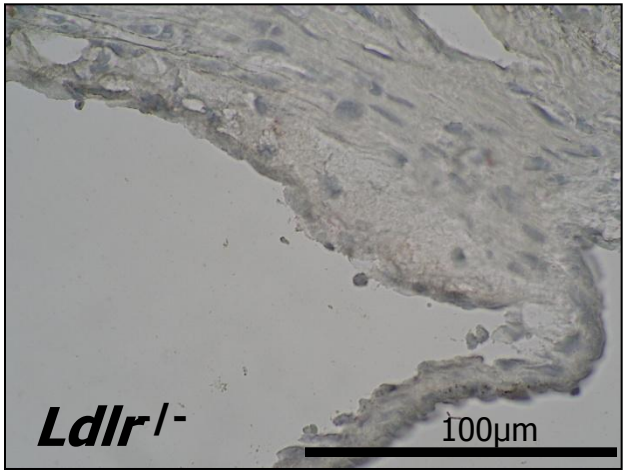
C6 deficient rabbits on a high fat diet are protected (*Geertinger & Sørensen 1977; Schmiedt et al 1998*)

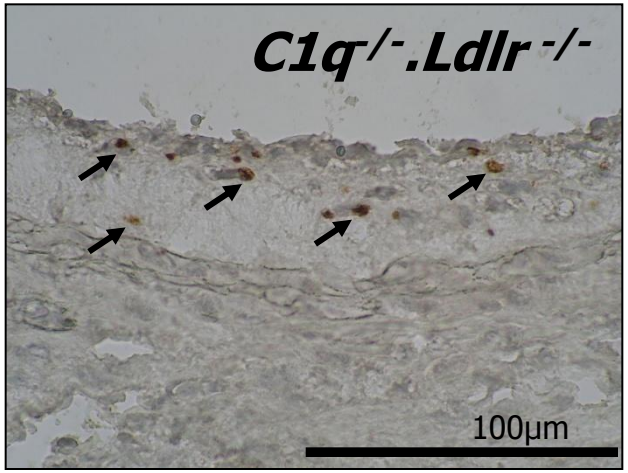
$C3^{-/-}$ mice show impaired progression to complex lesions *(Buono et al, 2002)*

C1q deletion accelerates atherosclerosis on a low fat diet (*Bhatia et al 2007 Am J Pathol*)

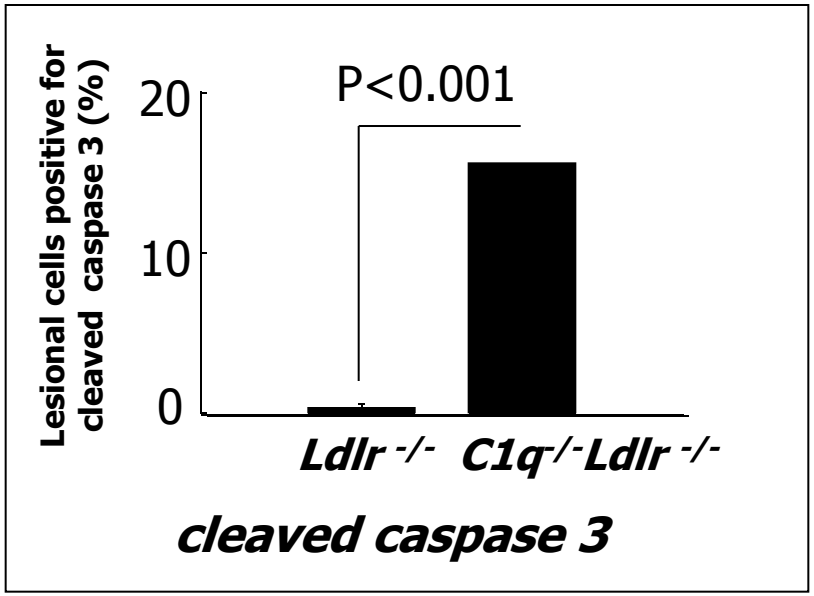


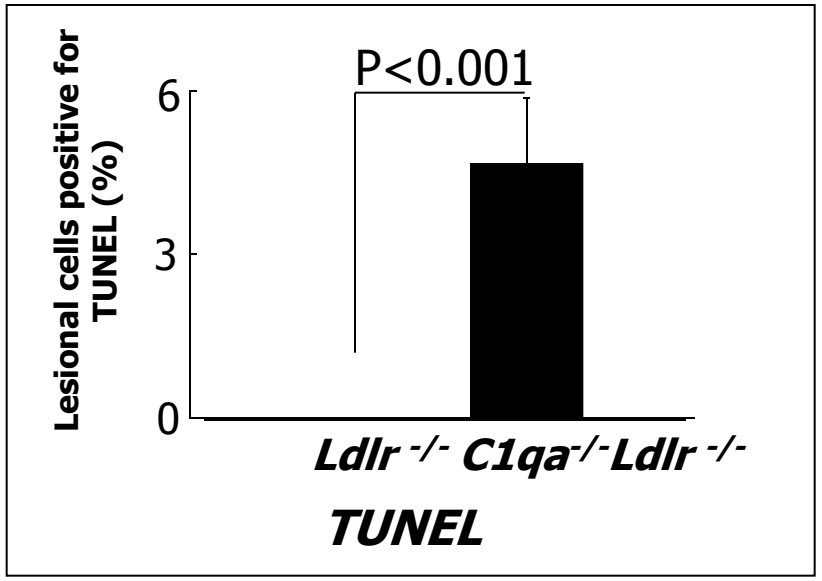
C1qa$^{-/-}$*Ldlr*$^{-/-}$ mice show impaired apoptotic cell clearance





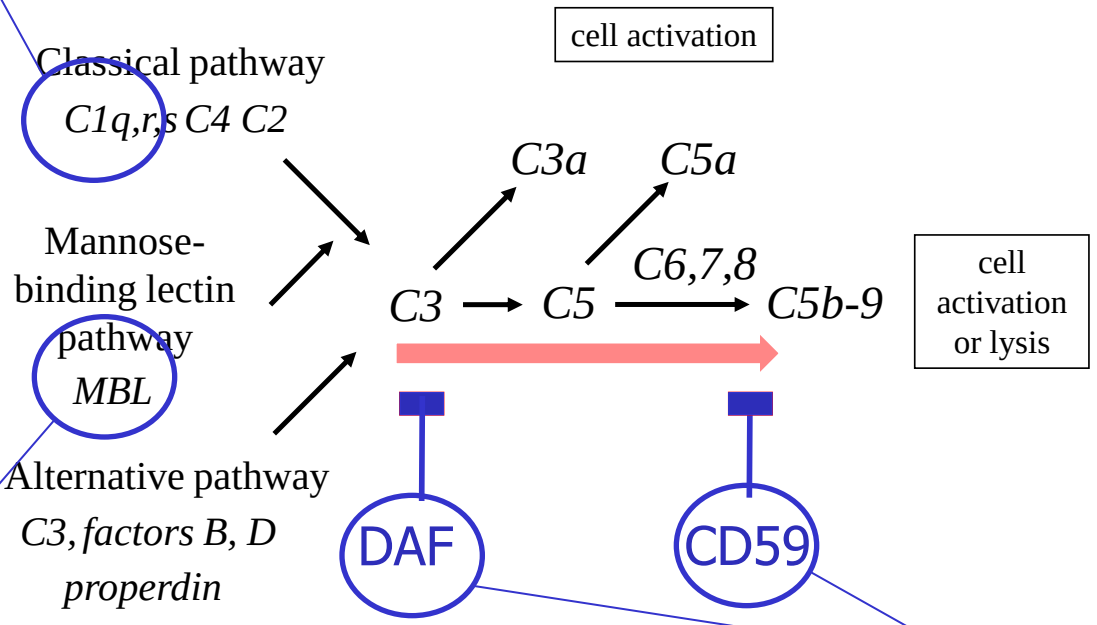
cleaved caspase 3





Bhatia et al (2007) Am J Pathol 170:416

C1q deletion accelerates atherosclerosis on a low fat diet (*Bhatia et al 2007 Am J Pathol*)



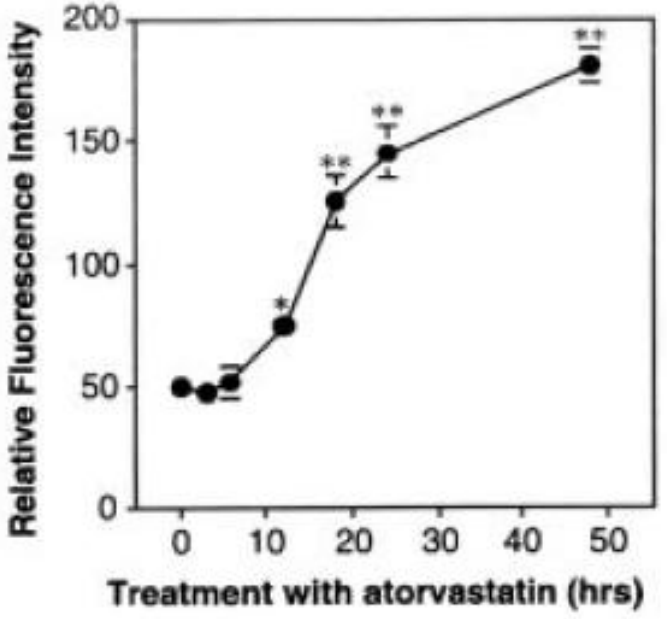
Accelerated atherosclerosis in mice with MBL-A and MBL-C deficient bone marrow (*Matthijsen et al 2009 Circulation*)

Acceleration of lesions in mice with DAF or CD59 deficiency (*Yun et al 2008 ATVB, Leung et al 2009 Am J Pathol*)

Induction of DAF and CD59 in endothelial cells

DAF is induced by TNFα, IFNγ, thrombin, VEGF, bFGF, C5b-9 and statins

Mason et al (1999) Blood 94:1673
Lidington et al (2000) Blood 96:2784
Mason et al (2001) Arthritis Rheum 44:138
Mason et al (2002) Circulation Research 91:696
Mason et al (2002) Am J Physiol 282:C578
Ahmad et al (2003) Immunology 110:258
Mason et al (2004) J Biol Chem 279:41611

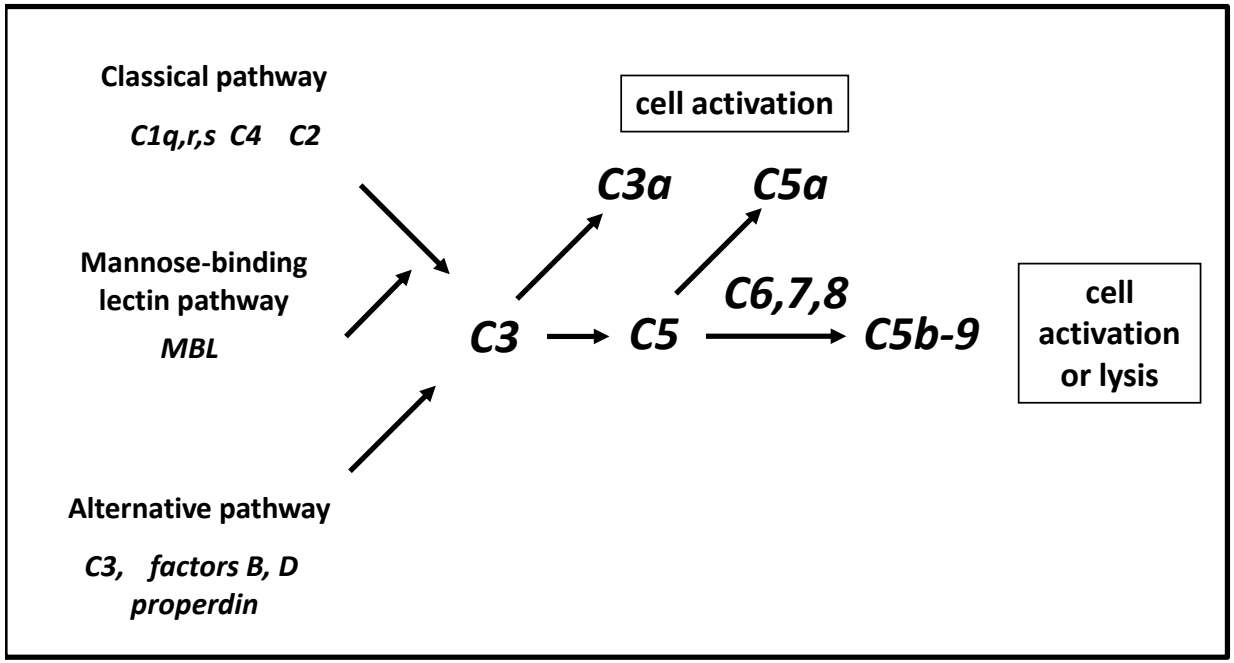


CD59 is upregulated by hypoxia and shear-stress

Kinderlerer et al (2006) Arthritis Res Therapy 8:R130
Kinderlerer et al (2008) J Biol Chem 283:14636

Role of complement in atherosclerosis

Apoptotic cells
Cholesterol crystals
Denatured LDL
Microparticles
Immune-complexes
Endotoxin



What is the role of IgM natural antibodies in the system?

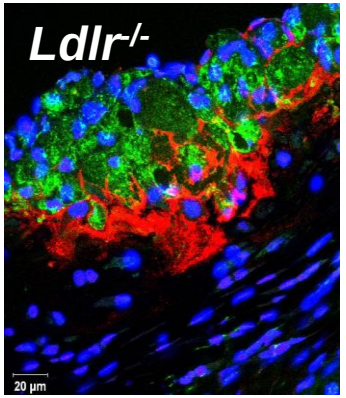
Natural IgM antibodies

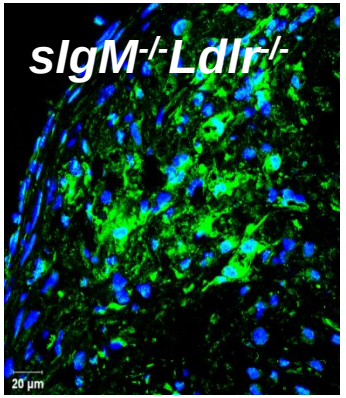
- germline encoded
- secreted largely by B1 cells
- scavenger functions (bacteria, oxLDL, apoptotic cells)
- Bind ubiquitous epitopes (eg phosphorylcholine)
- immunization studies suggest atheroprotective effects
- can be explored directly using secretory $IgM^{-/-}$ mice, generated by mutation of the $C\mu$ gene

Green –CD68 (møs)

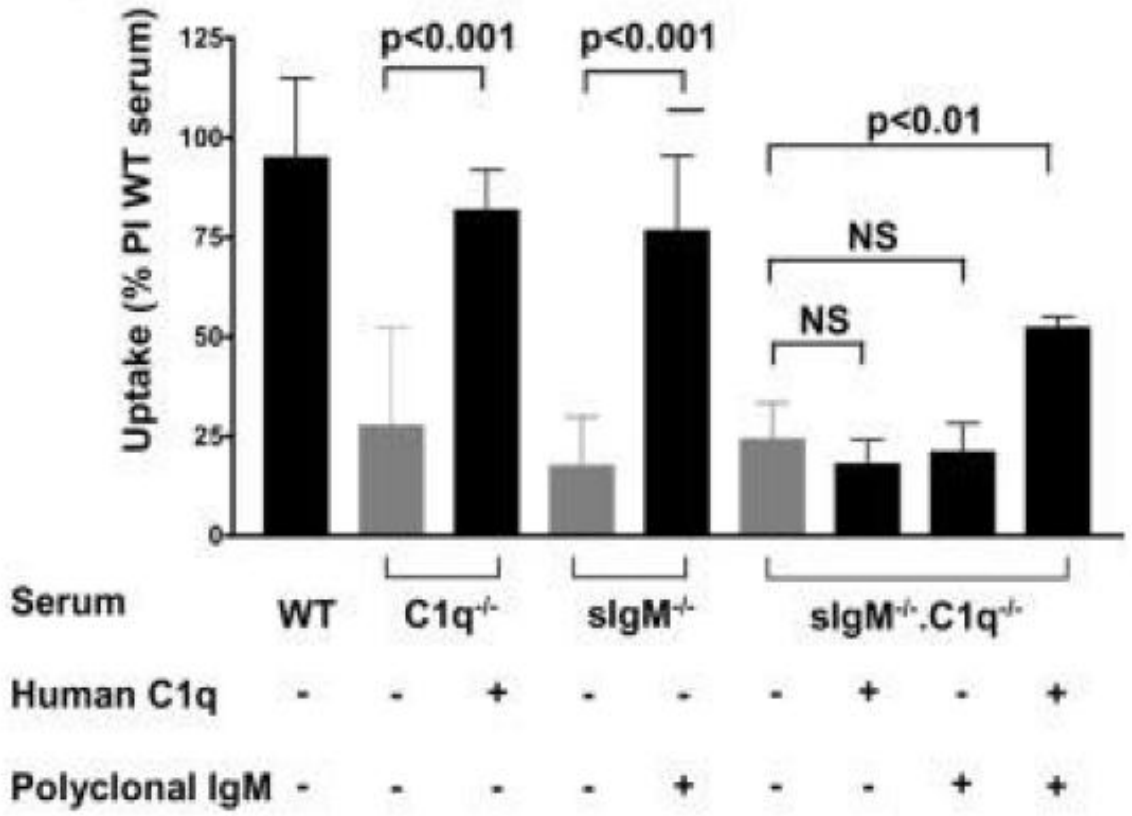
Red –IgM

Blue –nuclei



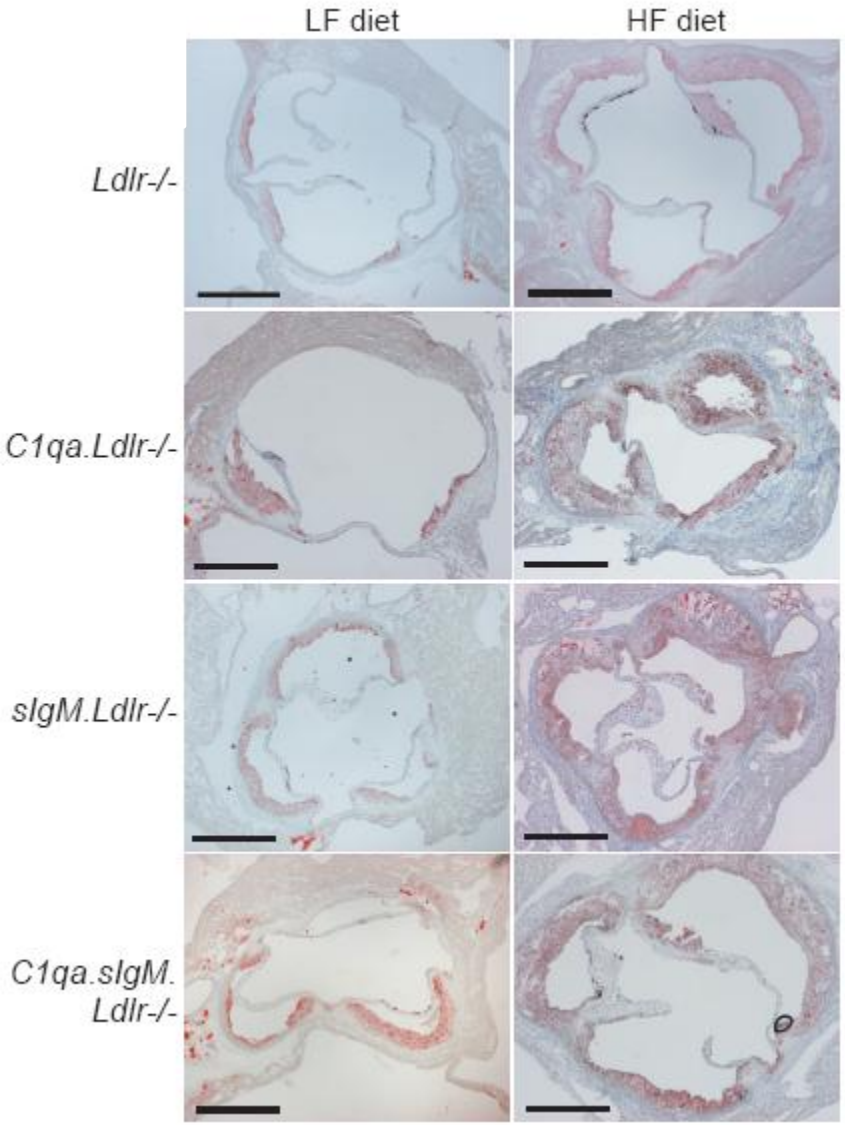


IgM and C1q are required for uptake of apoptotic cells *in vitro*

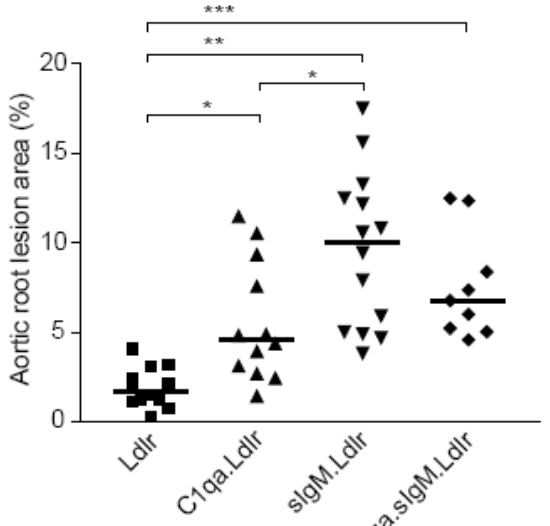


Quartier et al 2004 Eur J Immunol 35:252

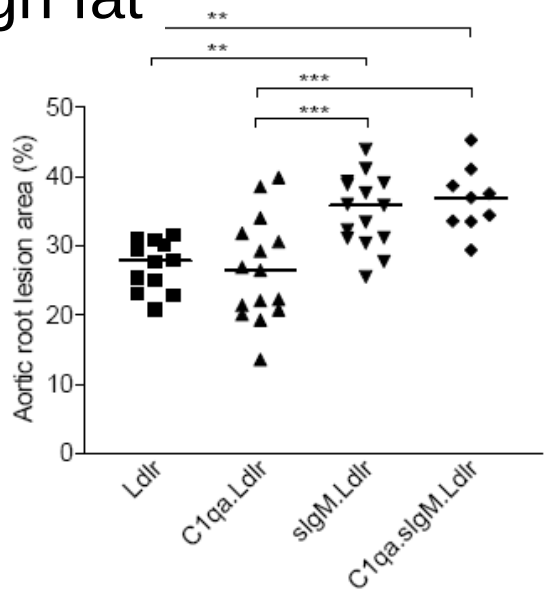
IgM deficiency accelerates atherosclerosis



Low fat

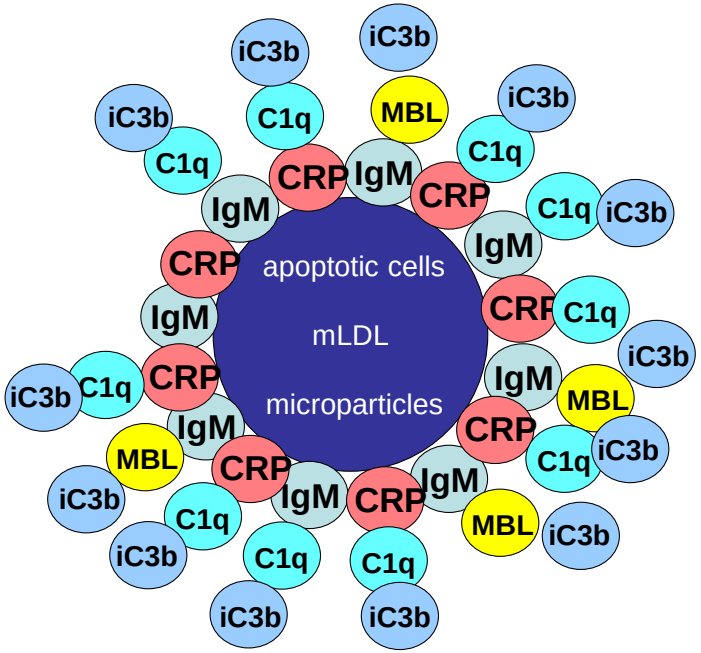


High fat



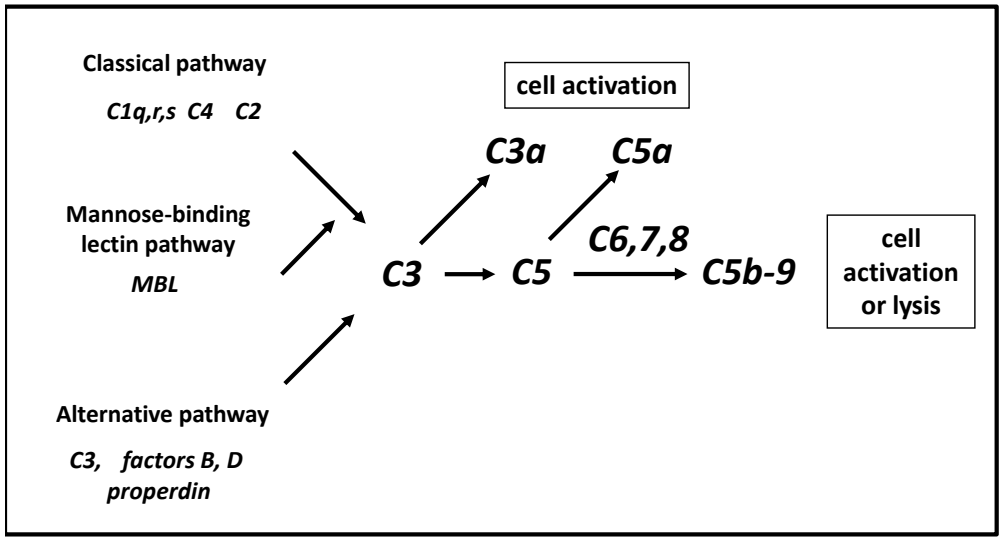
Lewis et al 2009 Circulation 120:417

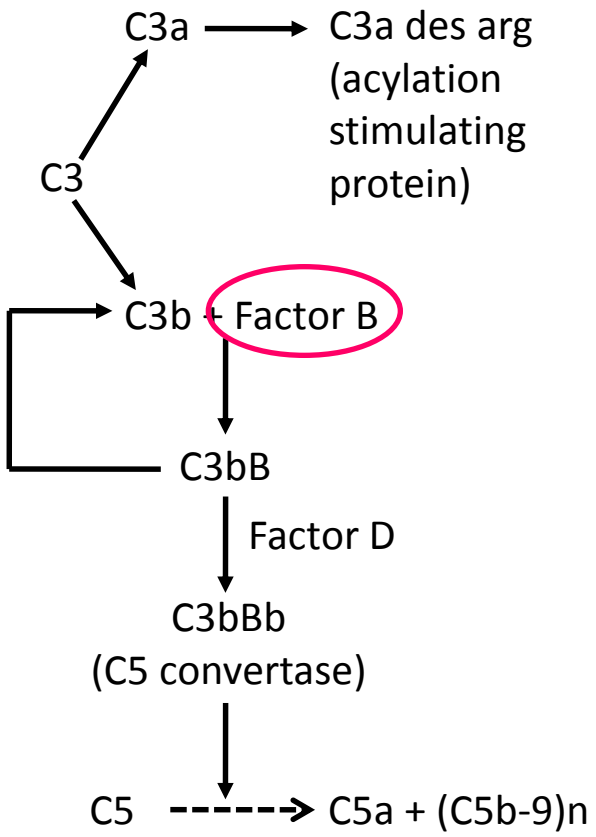
Hypothetical model of the role of complement and IgM natural antibodies in atherosclerosis



Homeostatic clearance

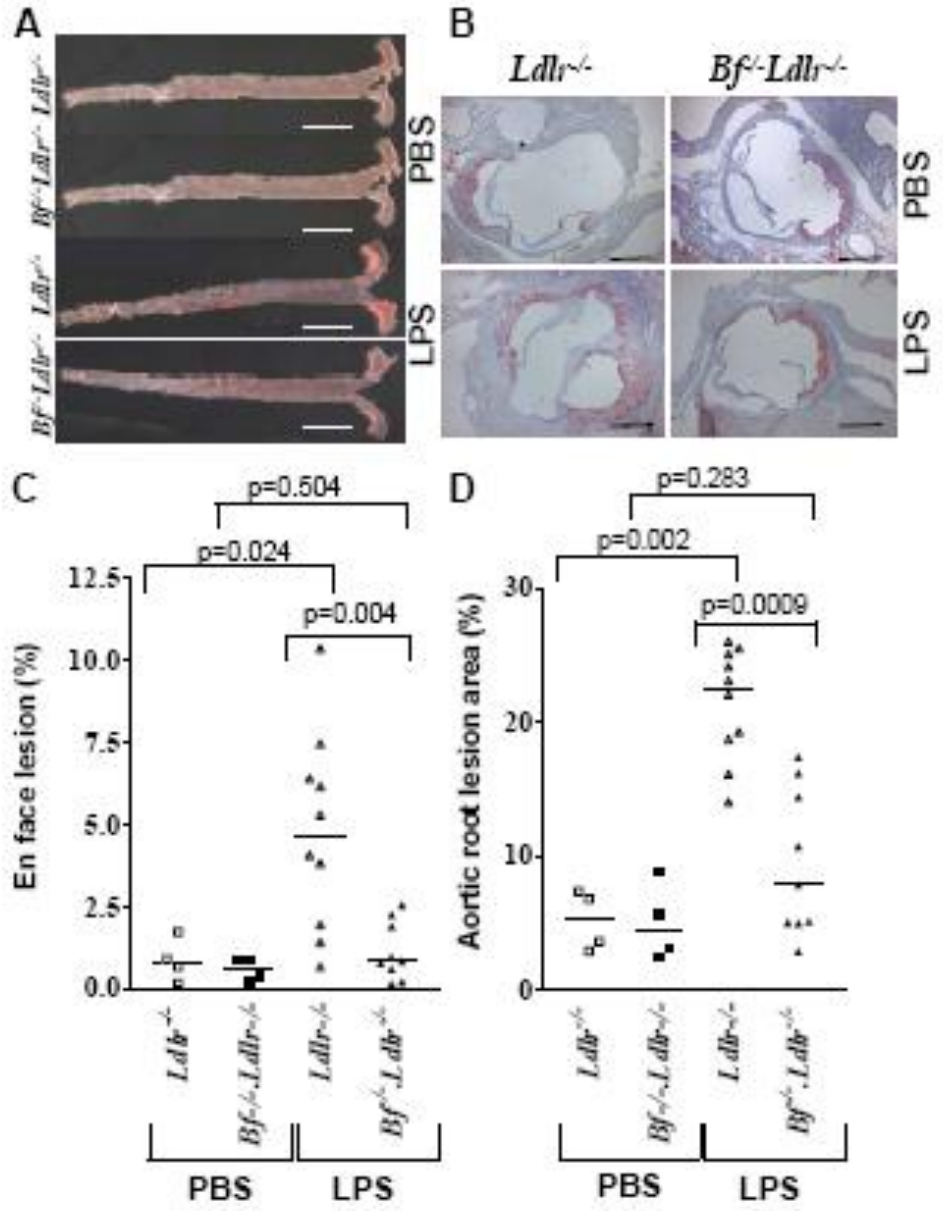
Alternative pathway



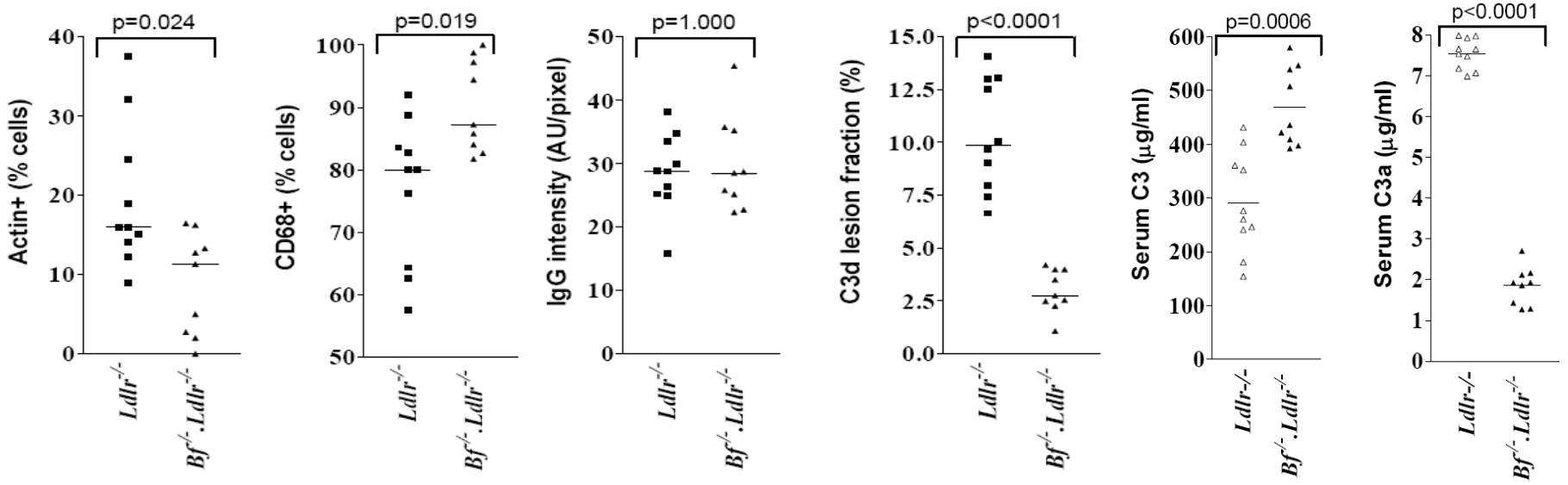


Hypothesis Activation of the alternative pathway stimulates proinflammatory downstream complement activation by over-riding complement inhibitors and is pro-atherogenic

Factor B deficiency protects *Ldlr*$^{-/-}$ mice from atheroslerosis accelerated by endotoxin



Reduced local complement activation in endotoxin-treated Factor B deficient *Ldlr*$^{-/-}$ mice



Malik et al (2010) Circulation 122:1948

Complement, antibodies and the homeostatic balance in atherosclerosis

- Natural IgM antibodies and upstream complement activity (classical and lectin pathways) are homeostatic, probably through enhancing debris clearance.

- Activation of downstream pro-inflammatory effects are prevented under homeostatic conditions by complement regulators (eg DAF, CD59).

- Over-activation of the system via the alternative pathway overrides this regulation and is proatherogenic.

Role of T lymphocytes in atherosclerosis

- Atherosclerotic plaques contain MHC Class II positive dendritic cells and T lymphocytes at all stages

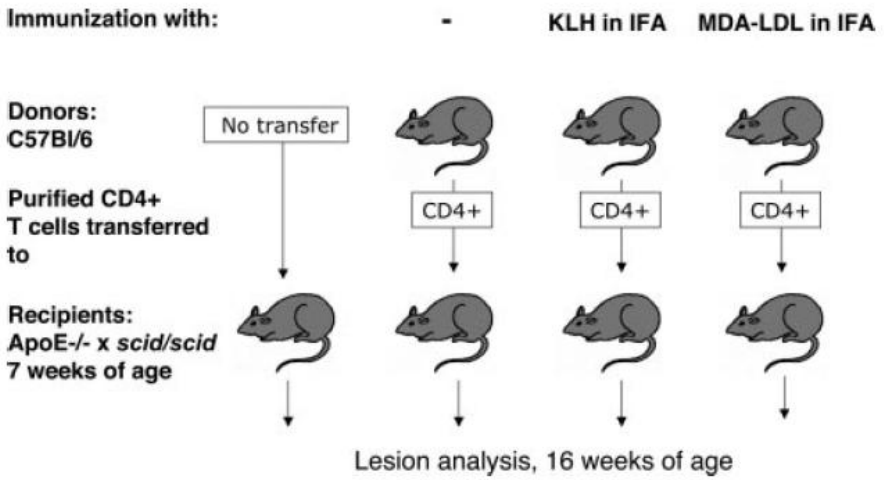
- Plaque T cells show evidence of activation (eg HLA-DR, IFNγ)

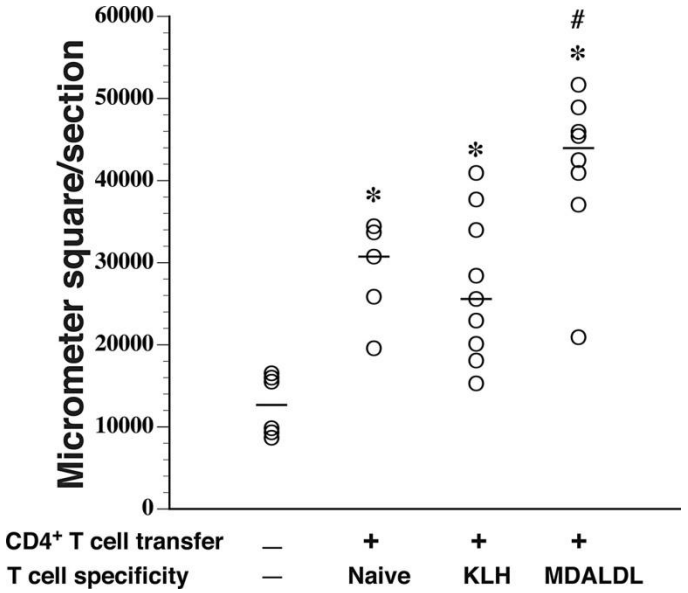
- Plaque T cells are oligoclonal and ~10% react with oxidised LDL

- T cells may activate macrophages and VSMC (eg via CD40L-CD40 contact interactions)

- Activated T cells are present in the circulation during acute coronary syndromes (ie plaque instability)

Effects of CD4 T cell transfer





Zhou et al (2006) ATVB 26:864

Summary

• Atherosclerosis can be viewed as a dynamic chronic inflammatory disease of arteries

• The innate immune system (macrophages, complement, natural IgM antibodies) regulate the safe disposal of lipoproteins and other debris from the arterial wall and is intrinsically protective

• Overdrive of the innate immune system leads to irreversible remodelling, and this may be accelerated by adaptive T cell-dependent immune mechanisms