Introduction to the Inflammatory Pathobiology of Atherosclerosis

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Atherosclerosis – plan of talk/learning objectives

- Importance of atherosclerosis to human health
- Cellular pathology of atherosclerosis
- Link between cholesterol and inflammation
- How to investigate molecular mechanisms
- Homeostatic *versus* pathogenic roles of humoral immunity

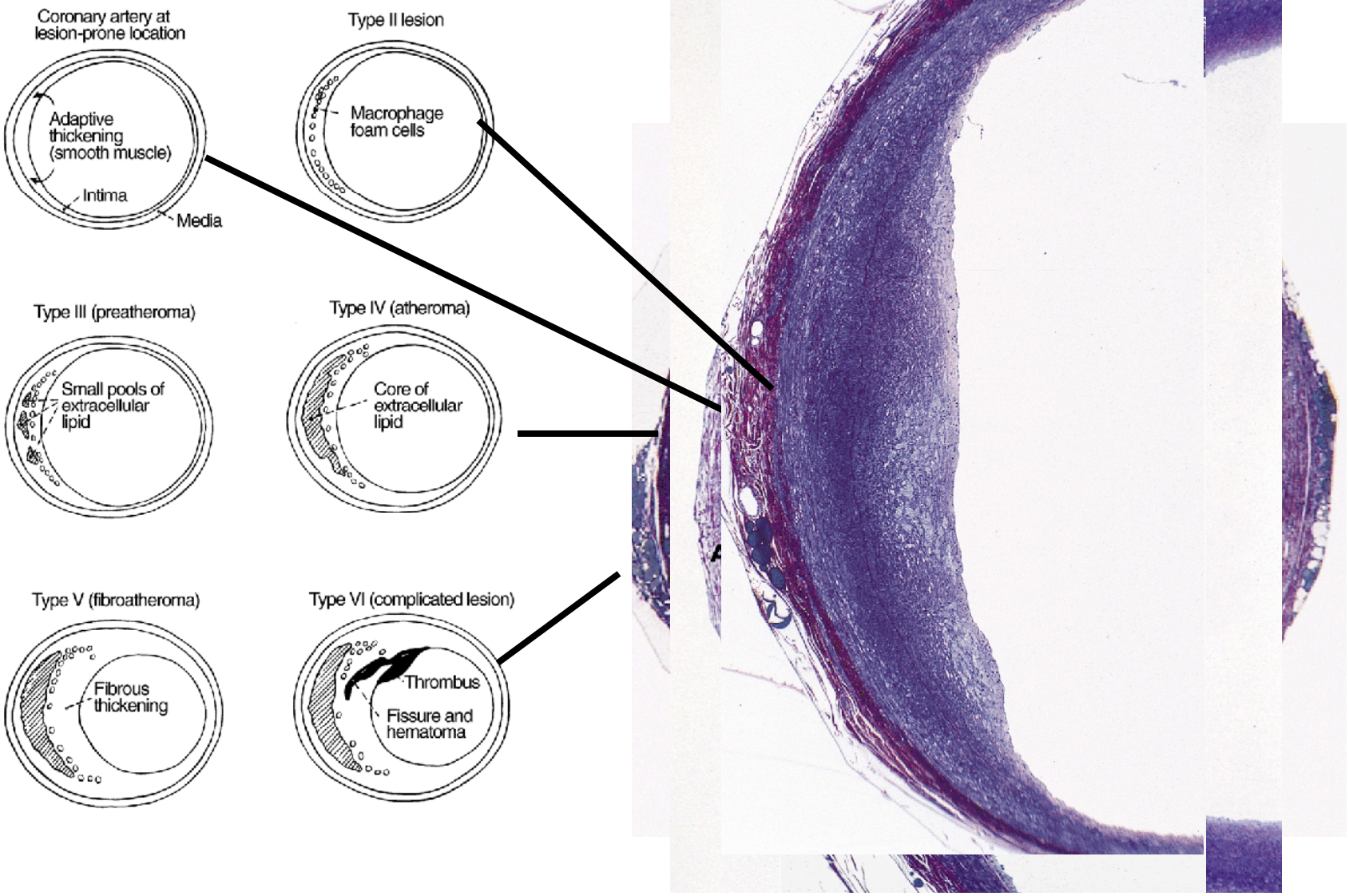
The DEATHS preceding were caused by Diseases and Casualties as follows, viz.

Abscesses	1	Hernia, or Rupture	3
Aneurism	1	Jaundice	10
Apoplexy	13	Inflammation of the bowels	1
Burns or Scalds	6	——— of the stomach	1
Cancer	5	Killed by lightning	1
Casualties	15	Insanity	1
Childbed	14	Intemperance	2
Cholera Morbus	6	Locked jaw	2
Colic	2	Mortification	11
Consumption	221	Old Age	26
Convulsions	36	Palsy	12
Cramp in the stomach	2	Pleurisy	8
Croup	1	Quinsy	15
Debility	28	Rheumatism	1
Decay	20	Rupture of blood vessels	1
Diarrhœa	15	Small-Pox,(at Rainsford's Island)	2
Drinking cold water	2	Sore throat	1
Dropsy	21	Spasms	2
——— in the head	23	Stillborn	49
Drowned	13	Suicide	1
Dysentery	14	Sudden death	25
Dispepsia or Indigestion	15	Syphilis	12
Fever, bilious	7	Teething	15
——— pulmonic	46	Worms	11
——— inflammatory	24	Whooping Cough	14
——— putrid	6	White swelling	2
——— typhus	33	Diseases not mentioned	48
Flux infantile	57		
Gout	3	Total,	942
Hoemorrhage	4		

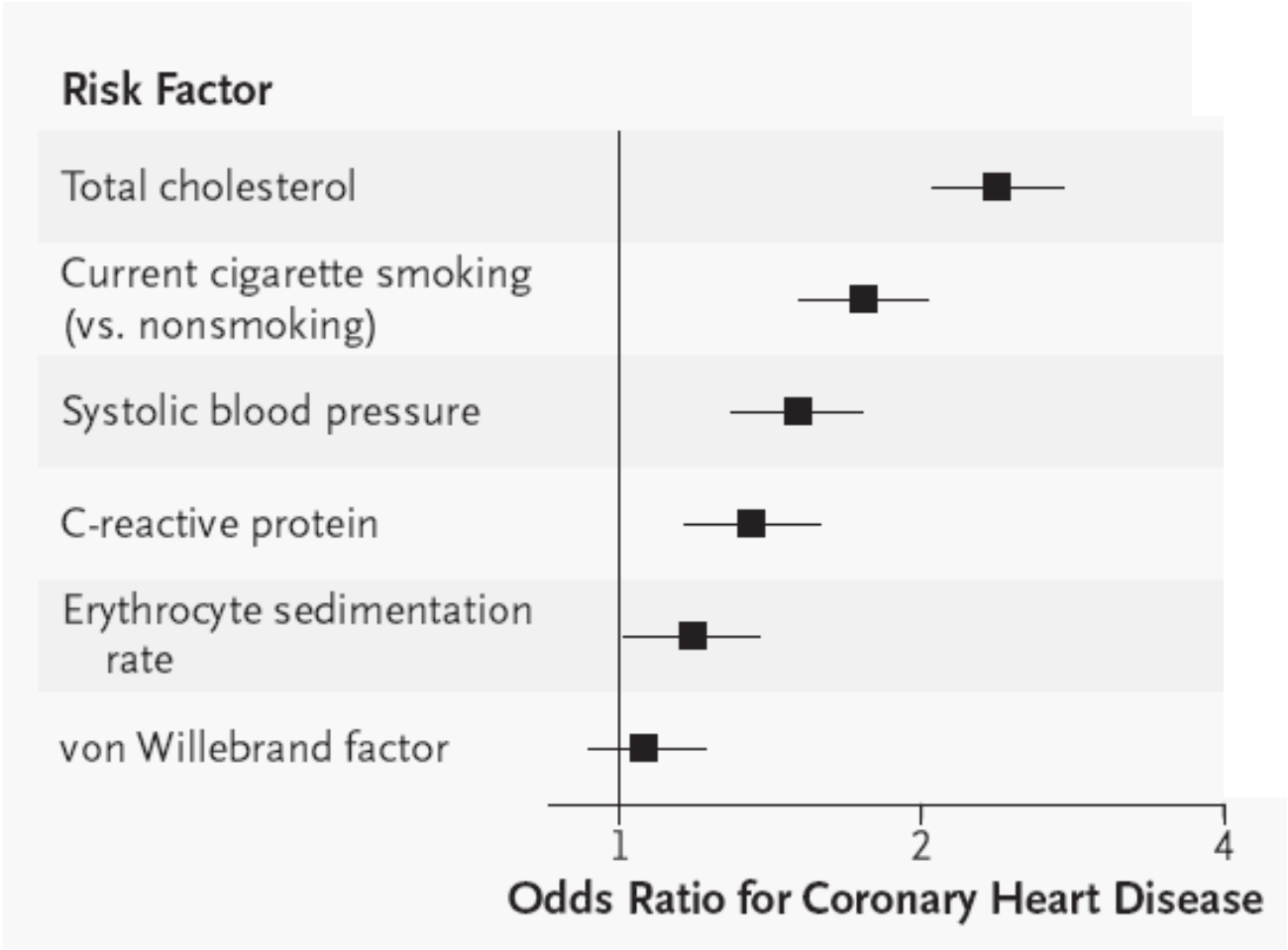
Causes of Death in 1811. Abstract of the Bill of Mortality for the Town of Boston.

Jones et al 2012 NEJM 366:2333

Type II lesion

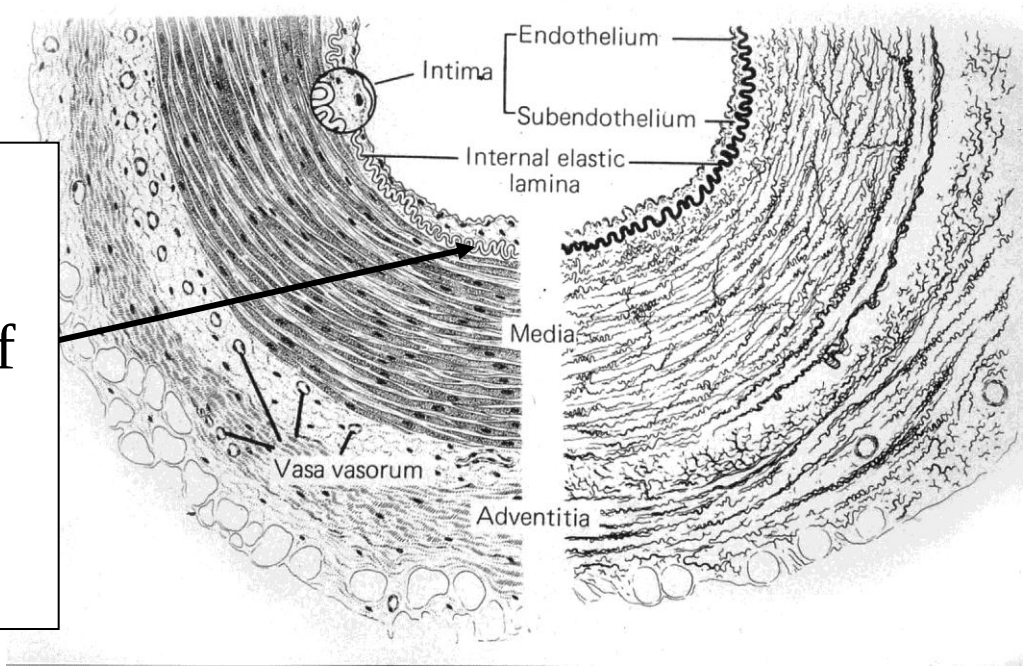


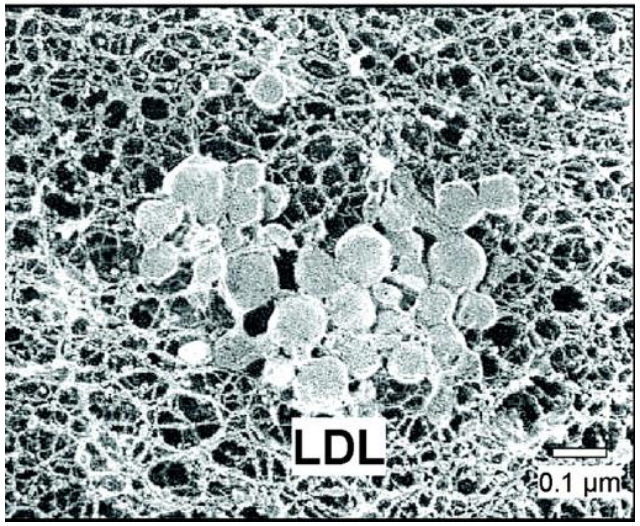
Relative importance of risk factors



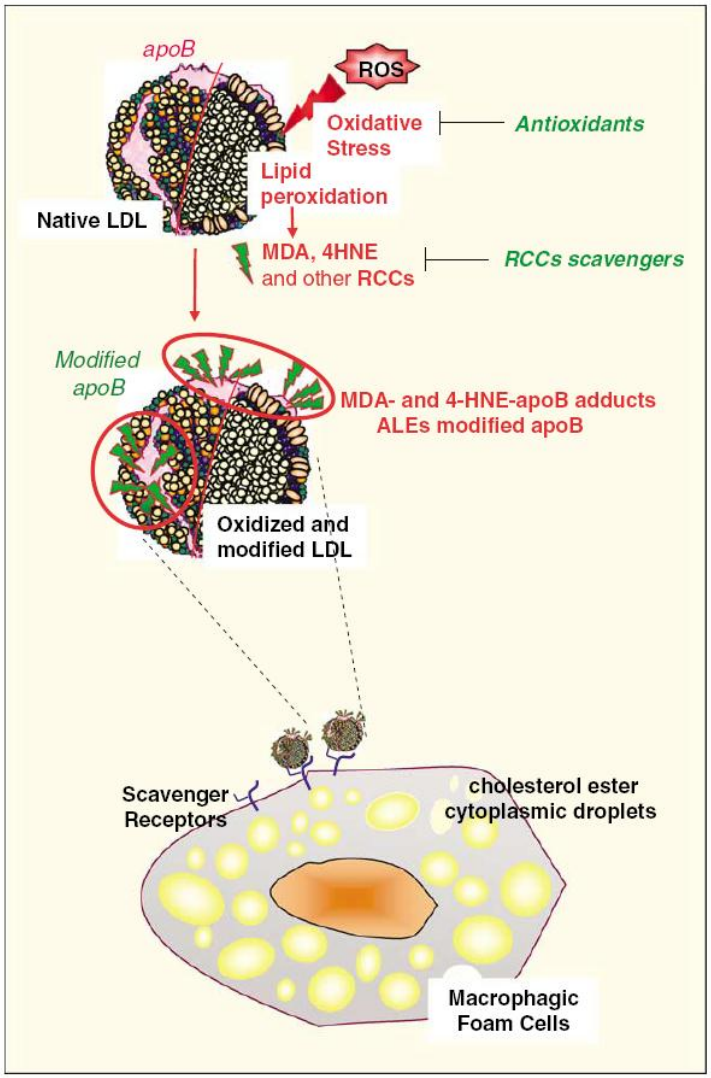
Danesh et al 2004 N Engl J Med 350:1387

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

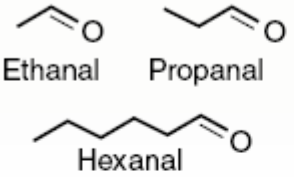




From: Tabas, I. et al. Circulation 2007;116:1832-1844

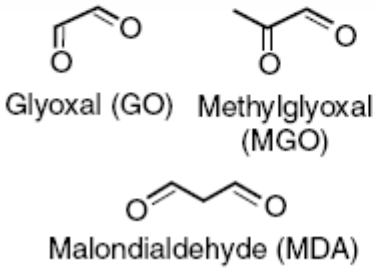


Saturated Aldehydes

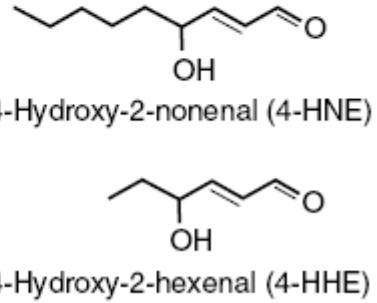


Unsaturated Aldehydes

Dicarbonyls

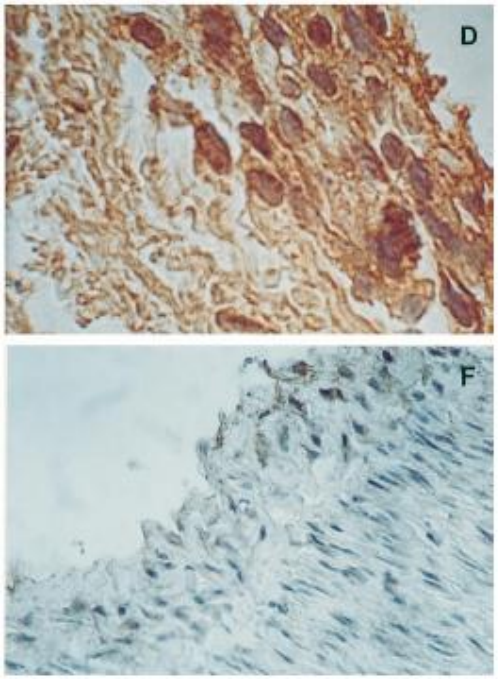


4-Hydroxy-2-alkenals



LDL modification in the arterial wall happens before macrophage infiltration

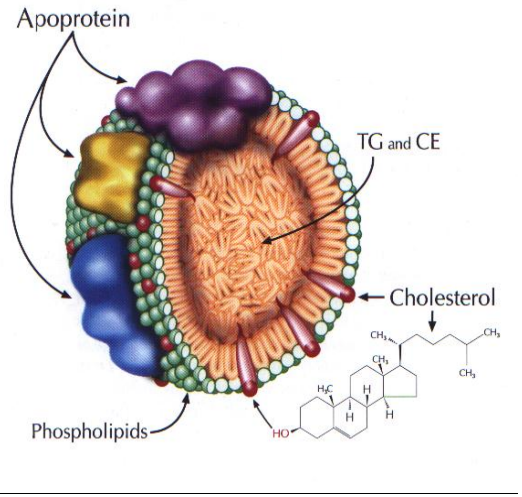
MDA-lysine
(oxidised LDL)

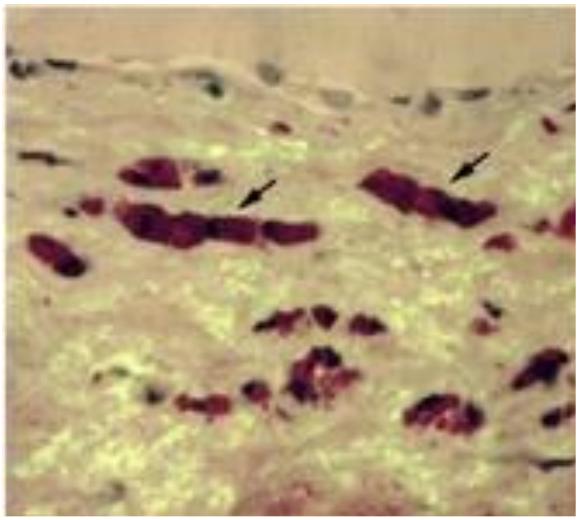


monocyte/møs

Napoli et al 1997 J Clin Invest 100:2680

Foam cells

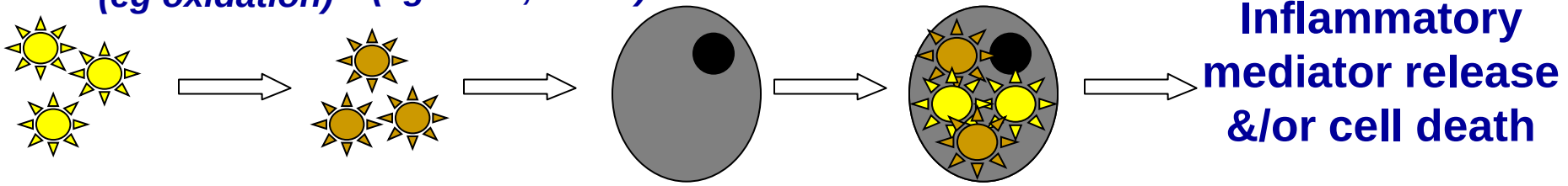




Dr Howard Kruth

lipoprotein modification (eg oxidation)

via scavenger receptors (eg SR-A, CD36)

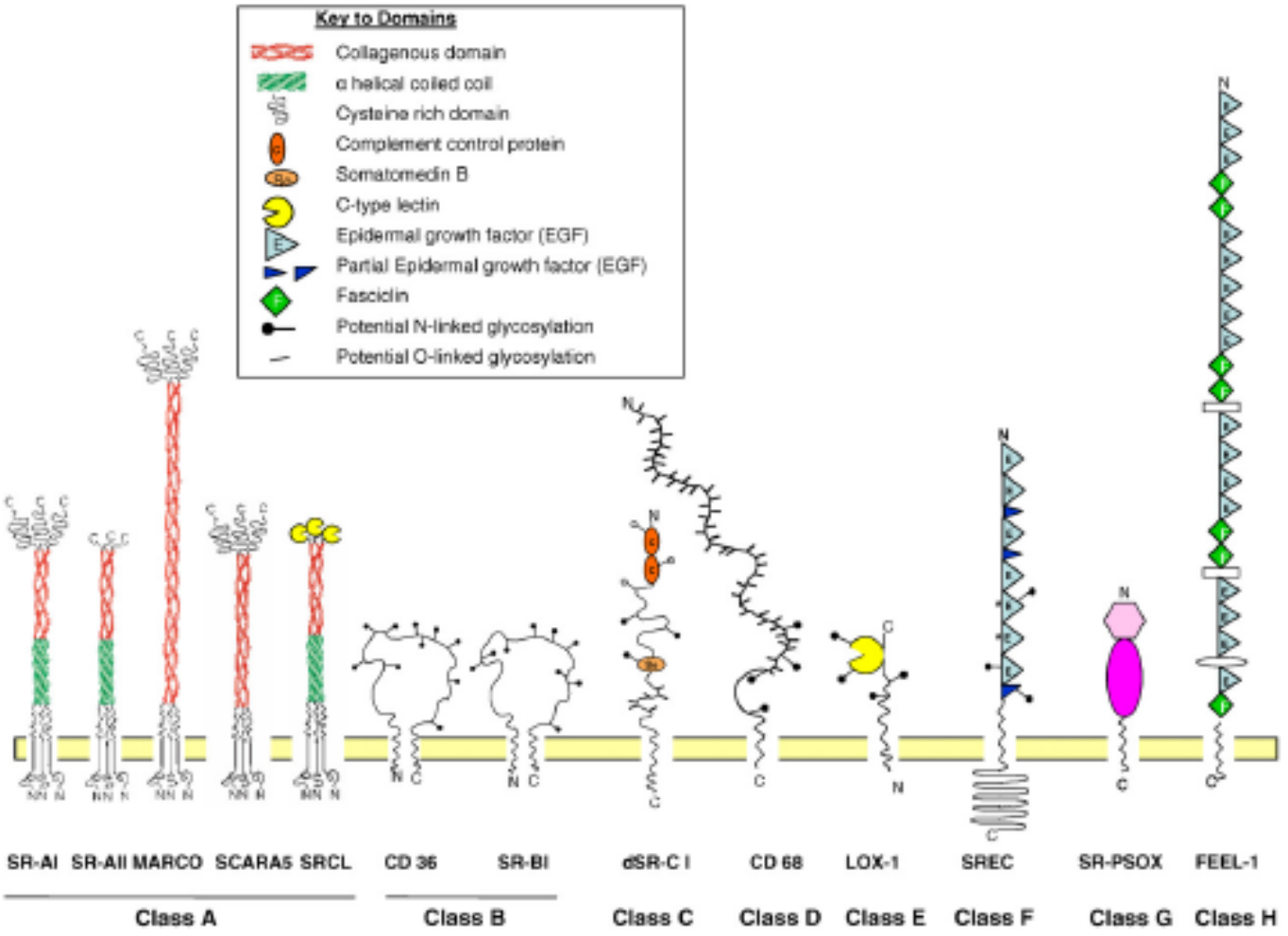


Macrophage

"foam cells"

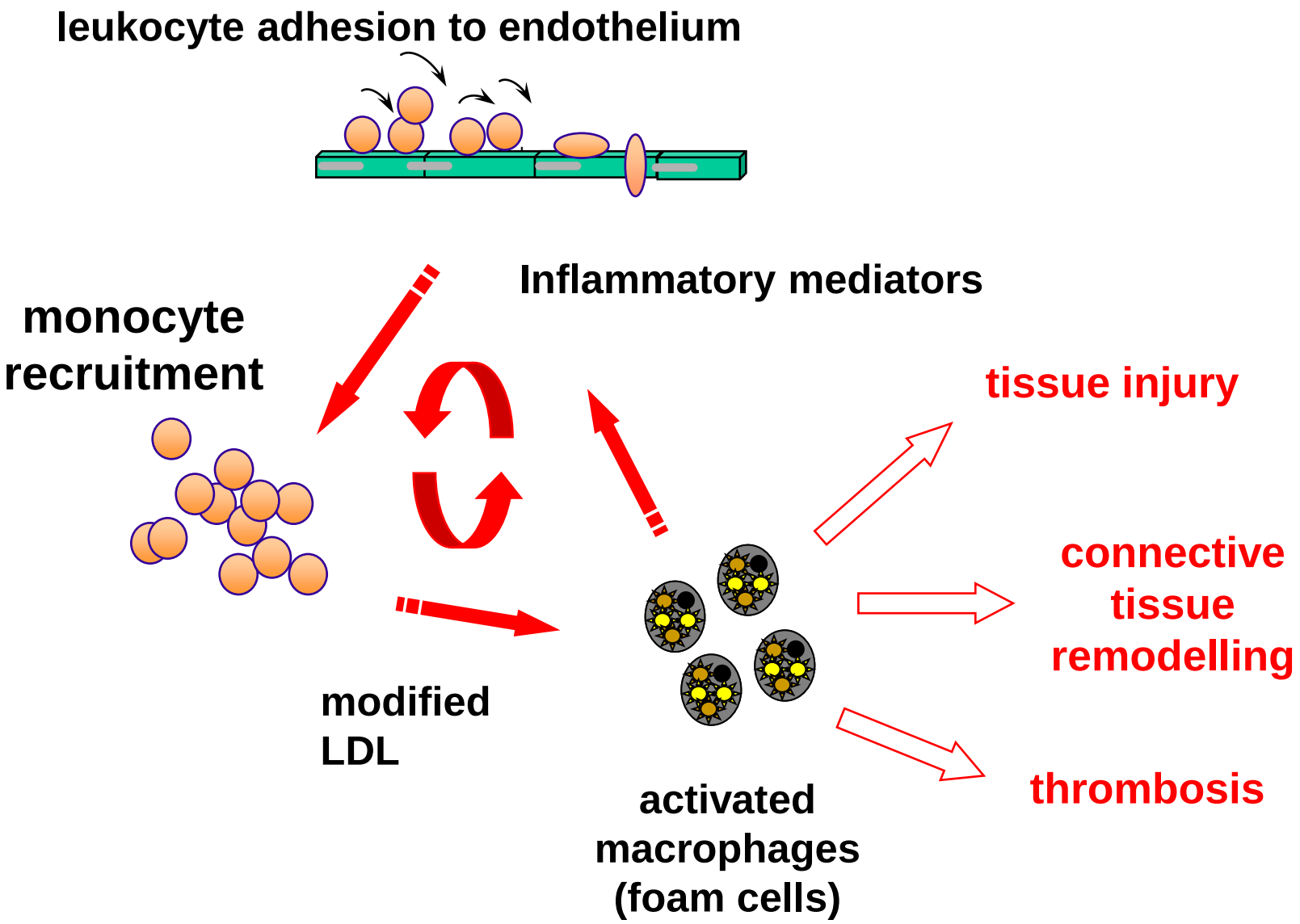
Inflammatory mediator release &/or cell death

Macrophage scavenger receptors

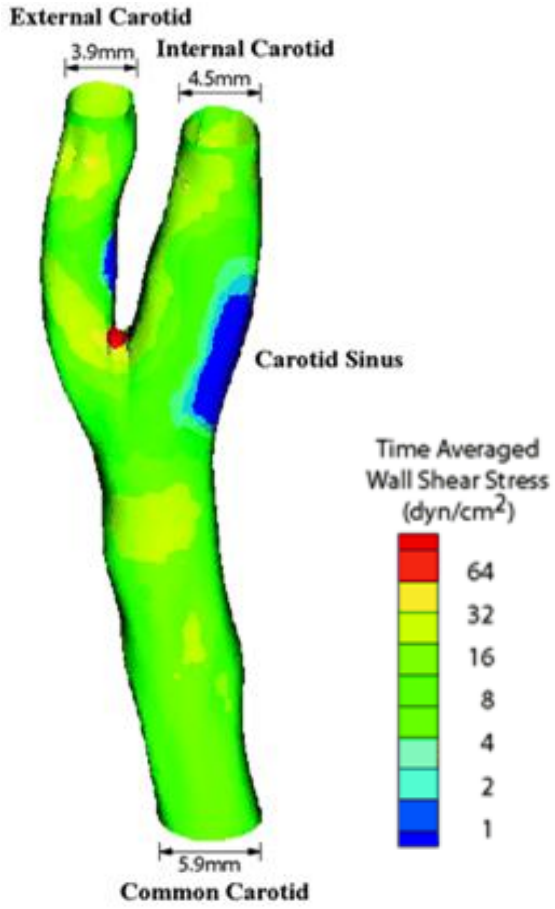


from Pluddemann et al (2007) Methods 43:207

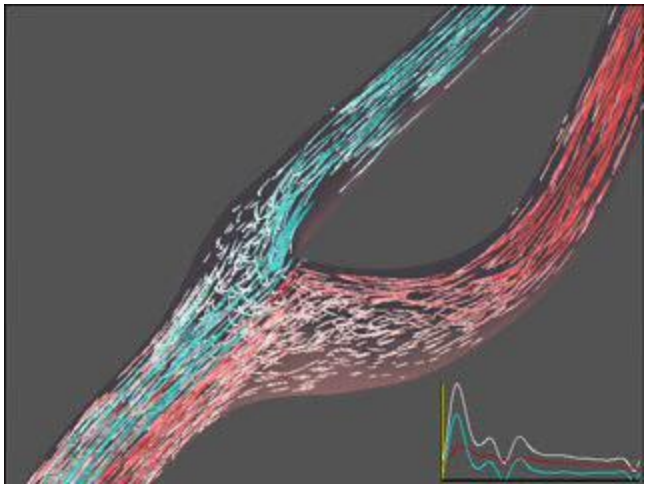
Inflammatory genesis of atherosclerosis



Branch points and curvatures are most susceptible to atherosclerosis



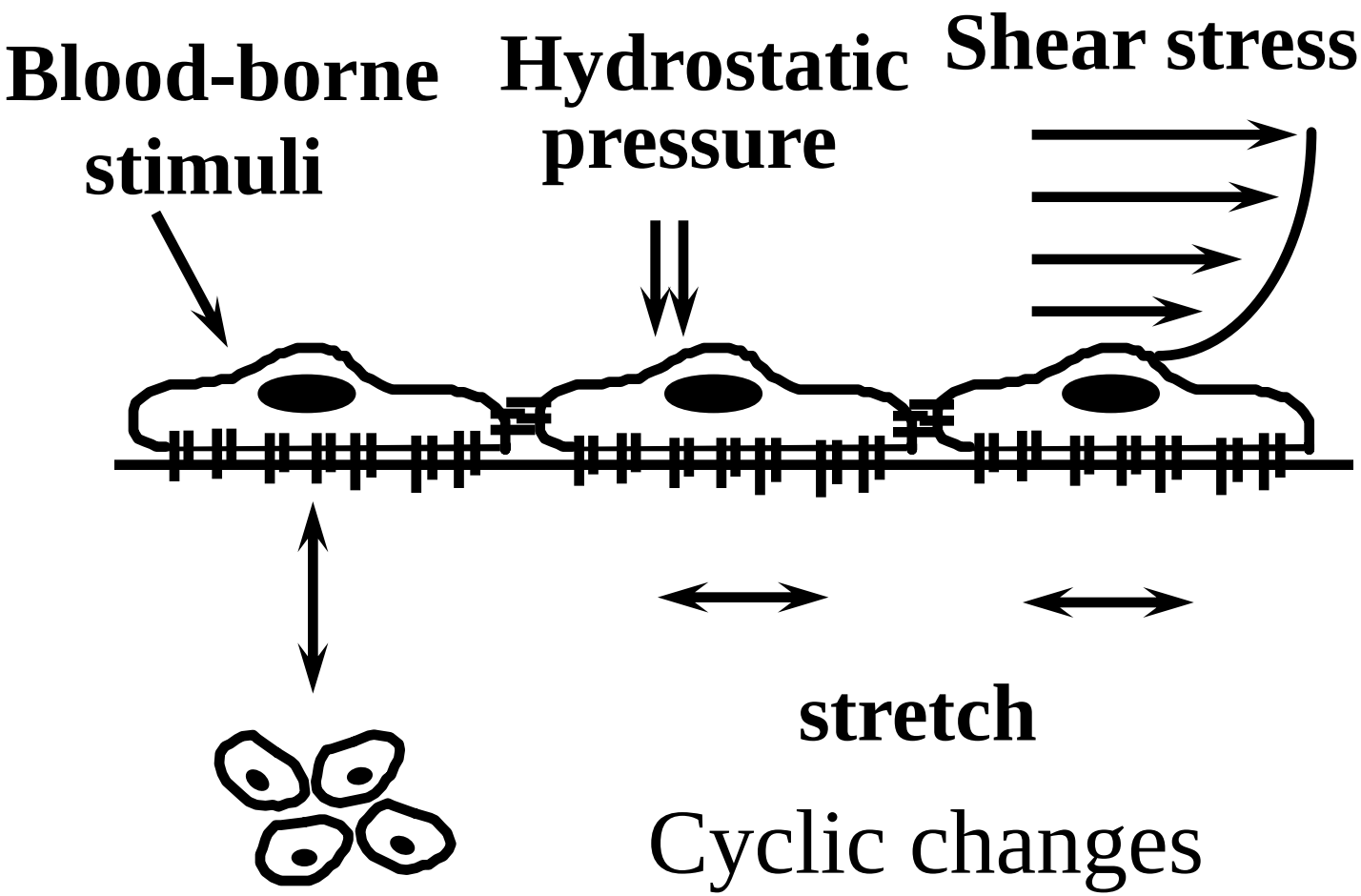
Dai et al. (2004) PNAS 101, 14871-14876



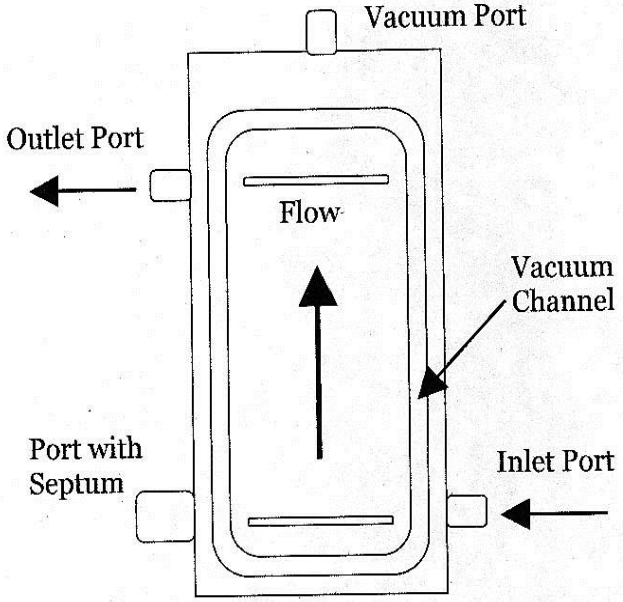
By Dr David Steinman
University of Western Ontario

- Risk factors are systemic but lesions are focal
- Low shear stress regions are susceptible
- Blood flow exerts shear stress on EC
- Endothelial cells detect shear stress

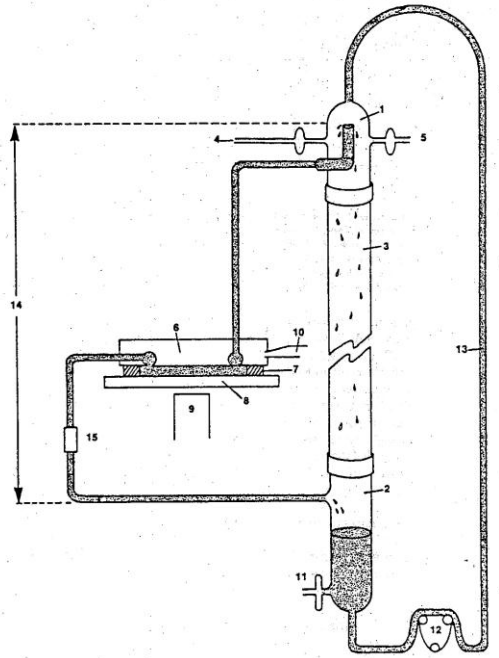
Effects of mechanical forces on endothelial function

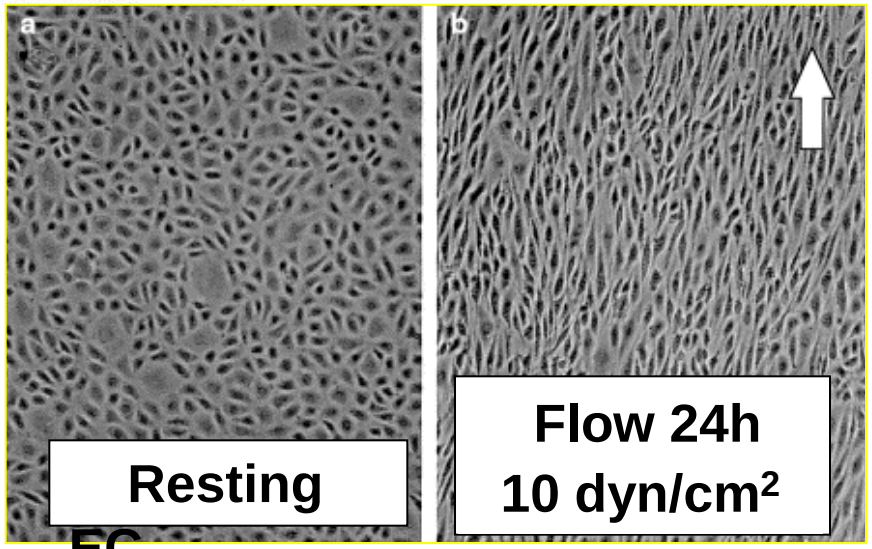


Use of parallel plate flow chamber for studying endothelial cells under flow



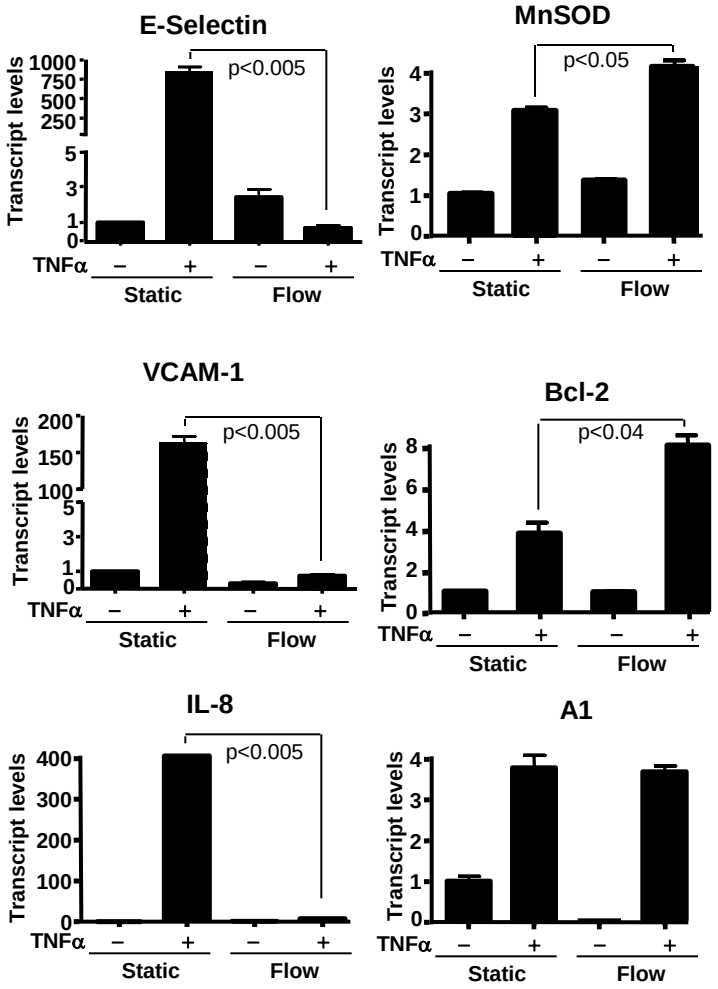
Flow Chamber





EC

Laminar flow suppresses proinflammatory gene expression but sustains cytoprotective responses in response to TNFα

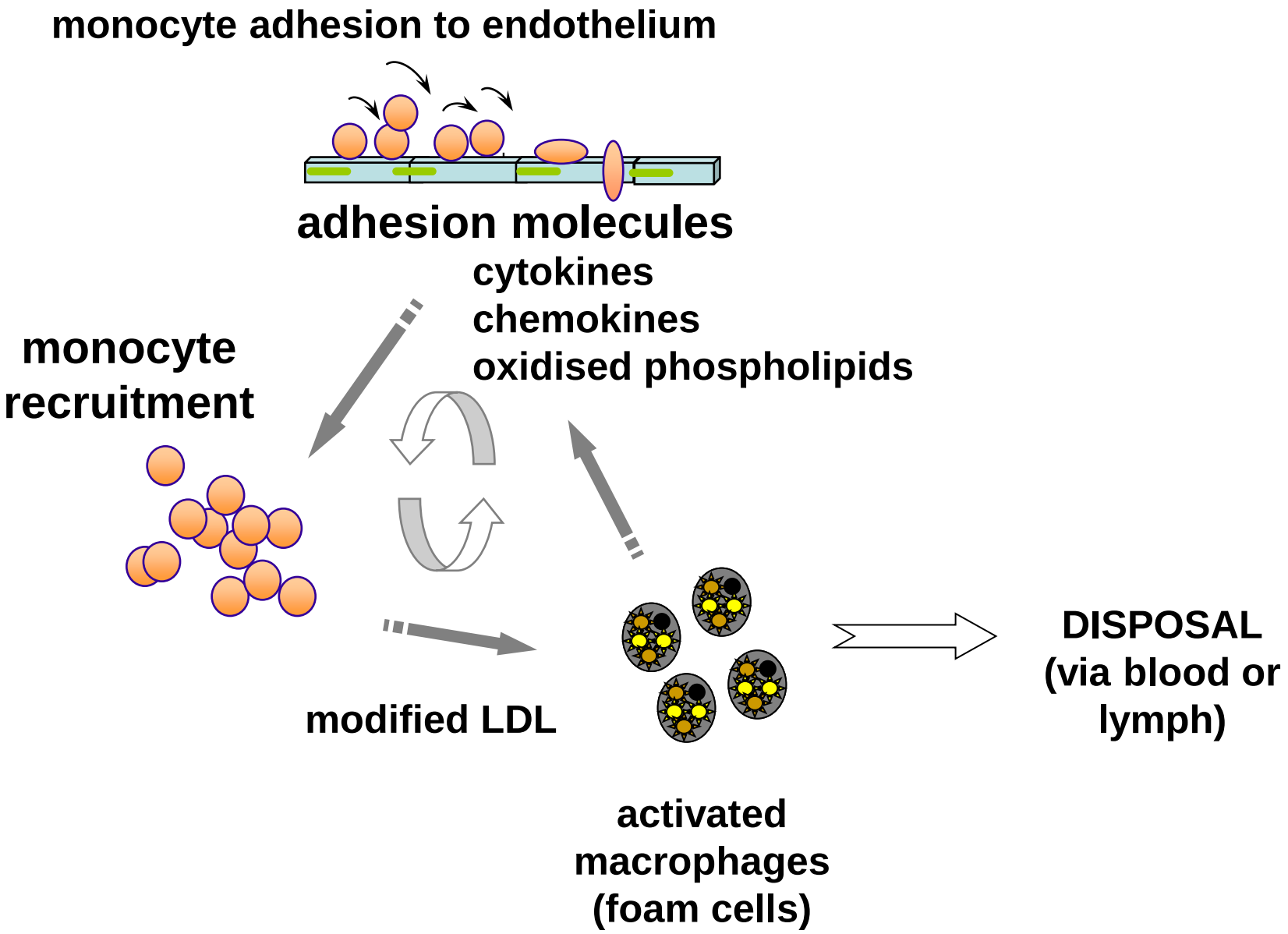


proinflammatory genes

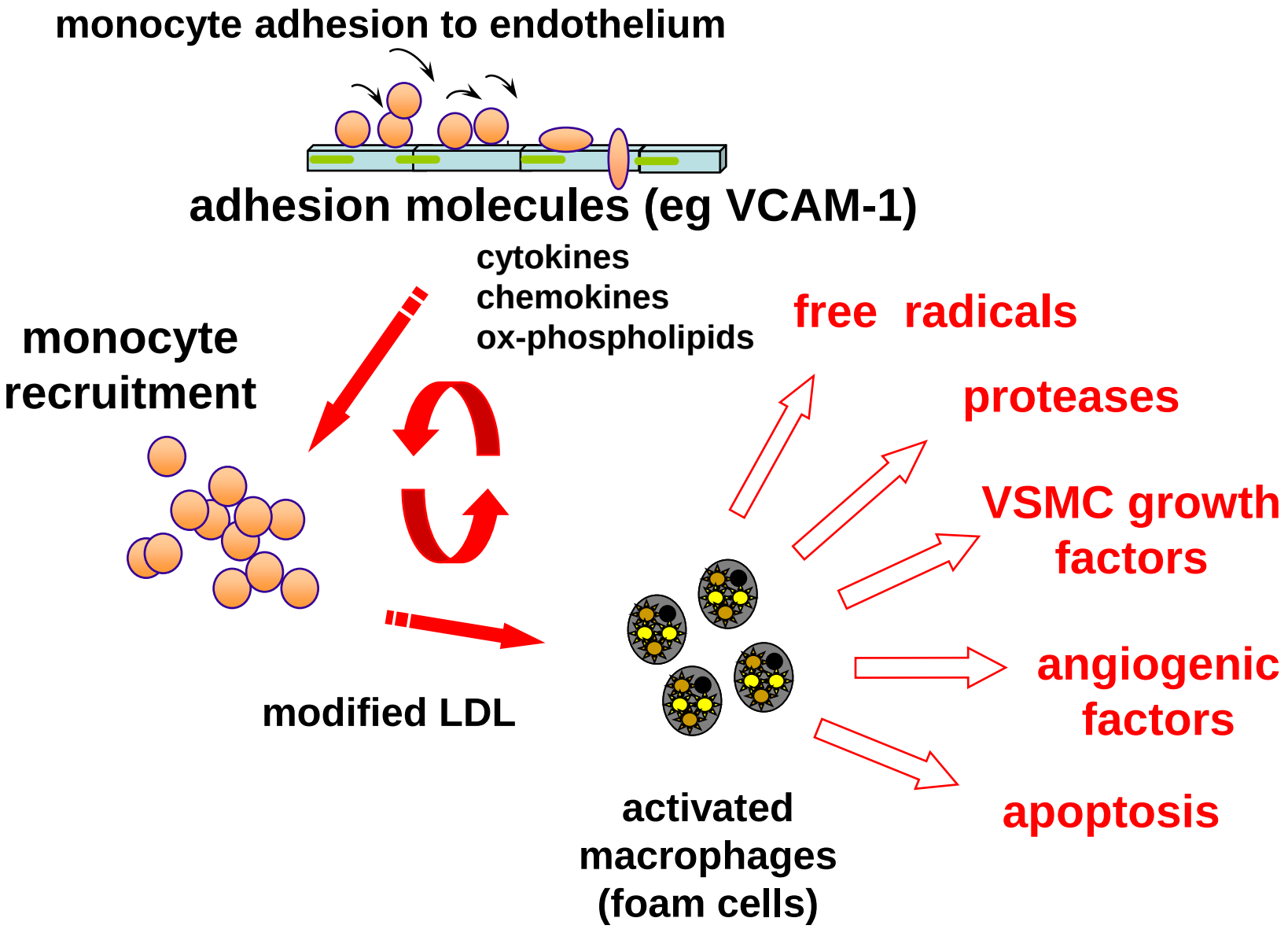
cytoprotective genes

Partridge et al (2007) FASEB J , 21:3553

Homeostatic debris disposal



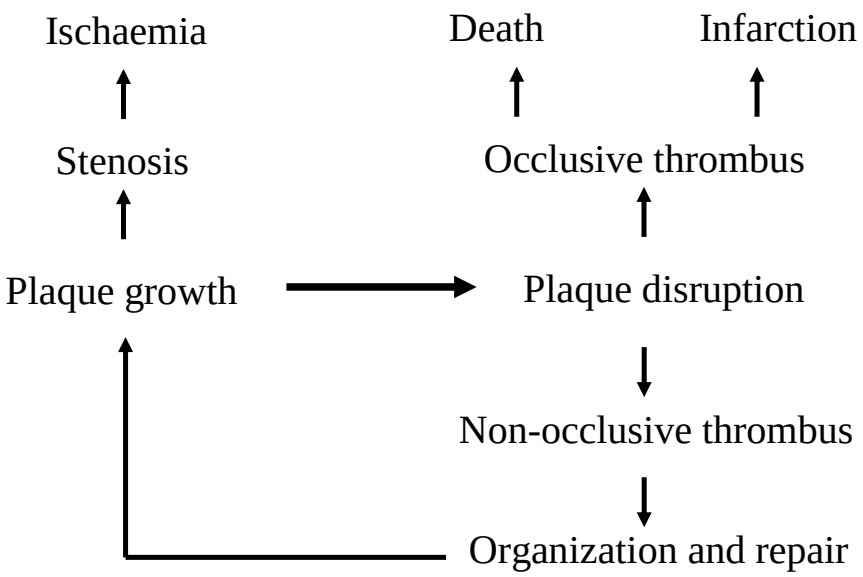
Inflammatory basis of atherosclerosis

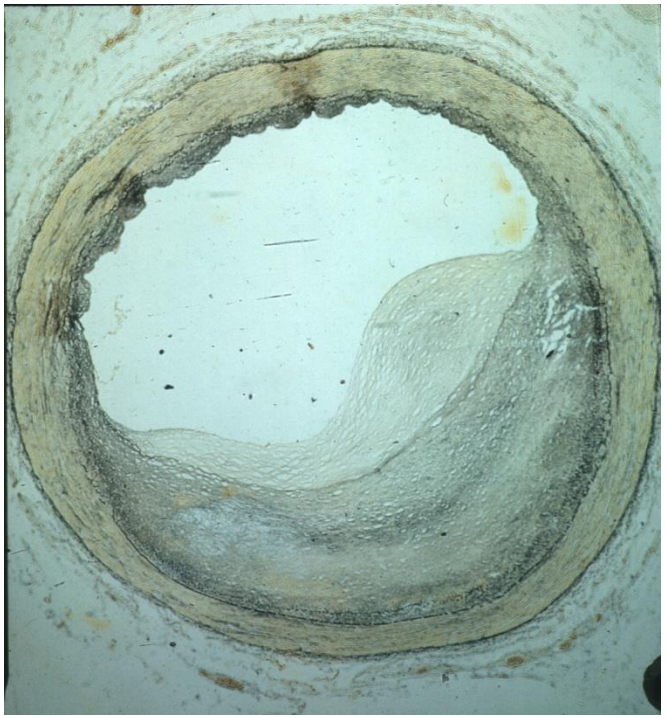


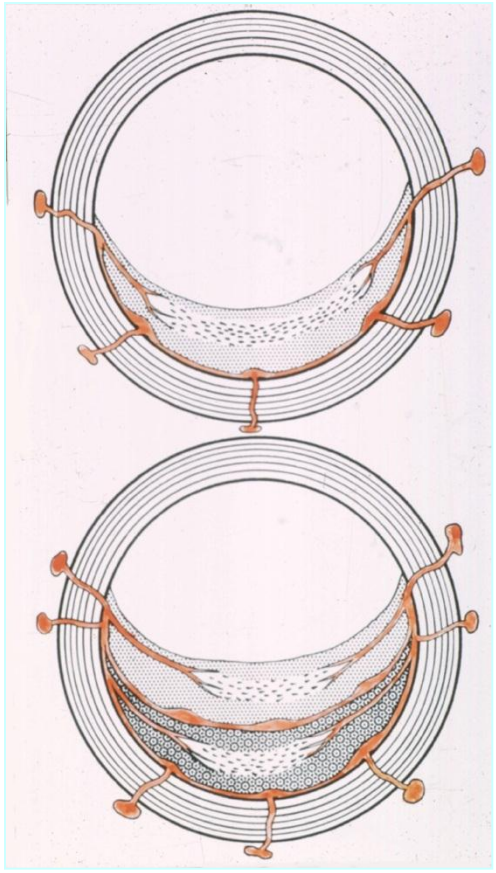
Macrophage uptake of LDL

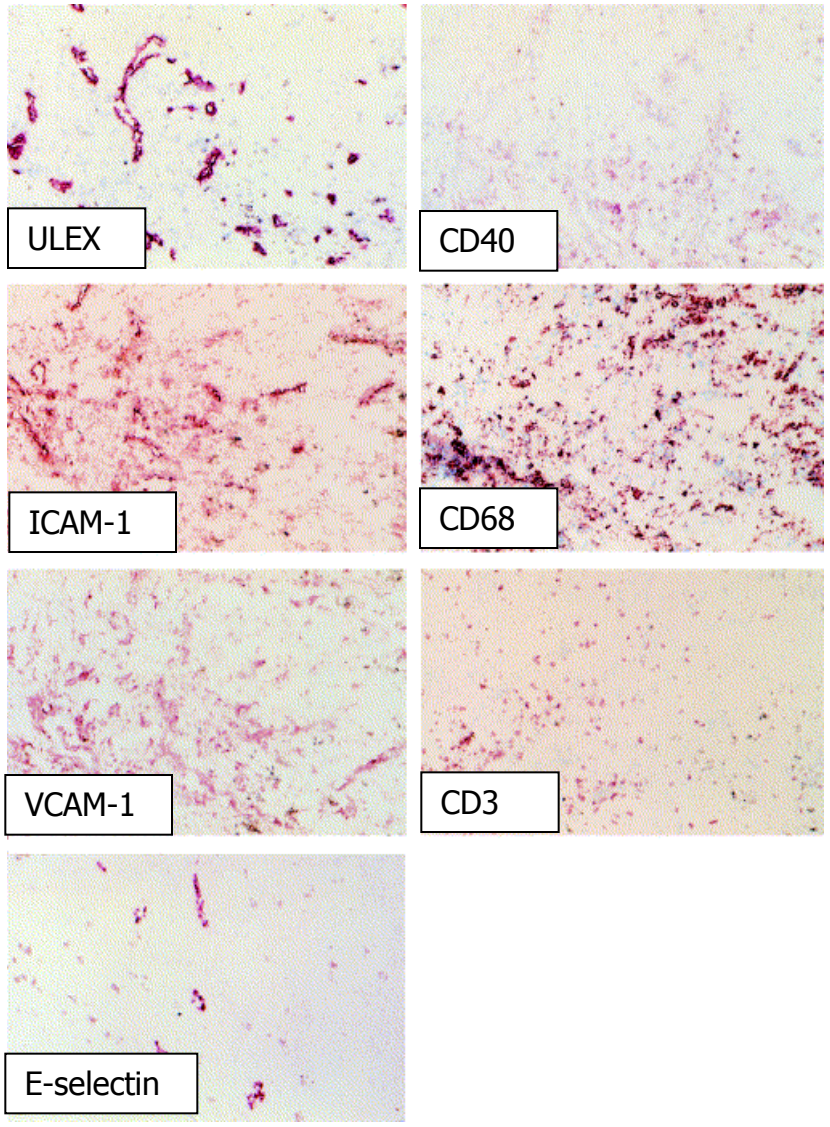
- Physiological uptake of LDL via LDL receptor controlled by receptor down-regulation
- Uptake of oxidised LDL by scavenger receptors is not regulated and results in “foam cell” formation.
- Cholesterol-laden macrophages die by apoptosis or necrosis and release proinflammatory cytokines and growth factors.

Step-wise progression of atherosclerotic plaques



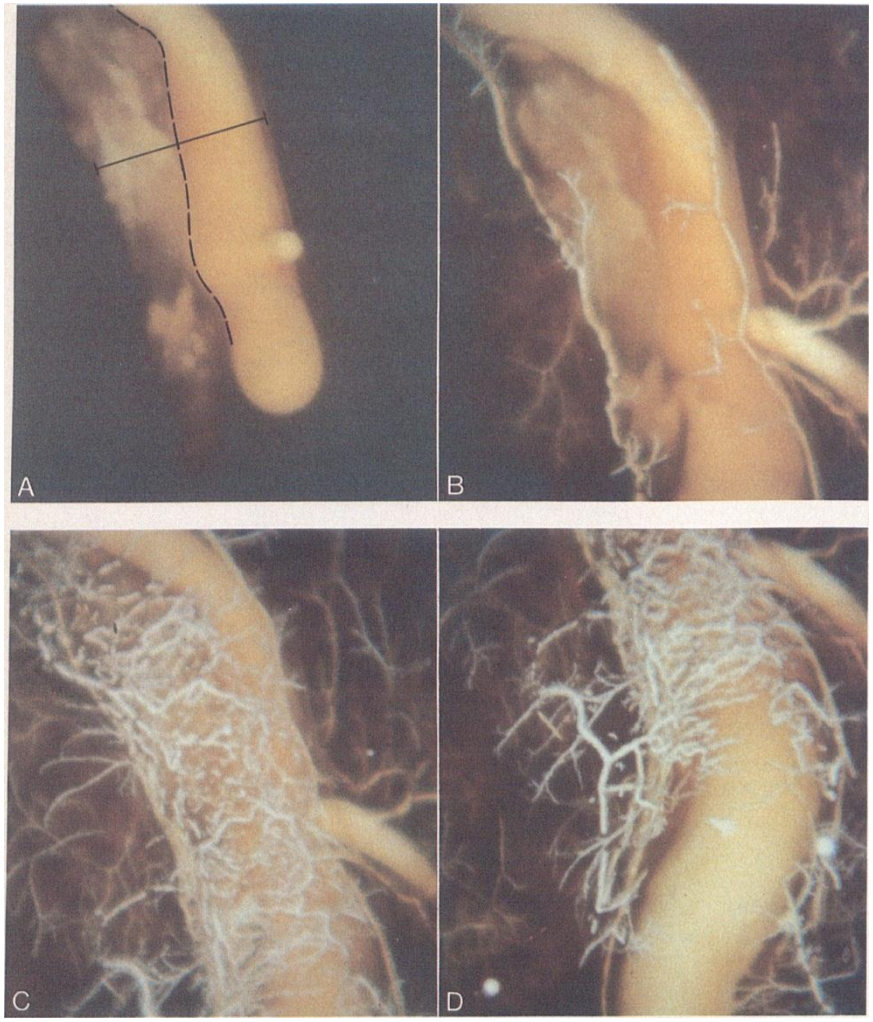






De Boer et al (1999) Cardiovasc Res 41:443.

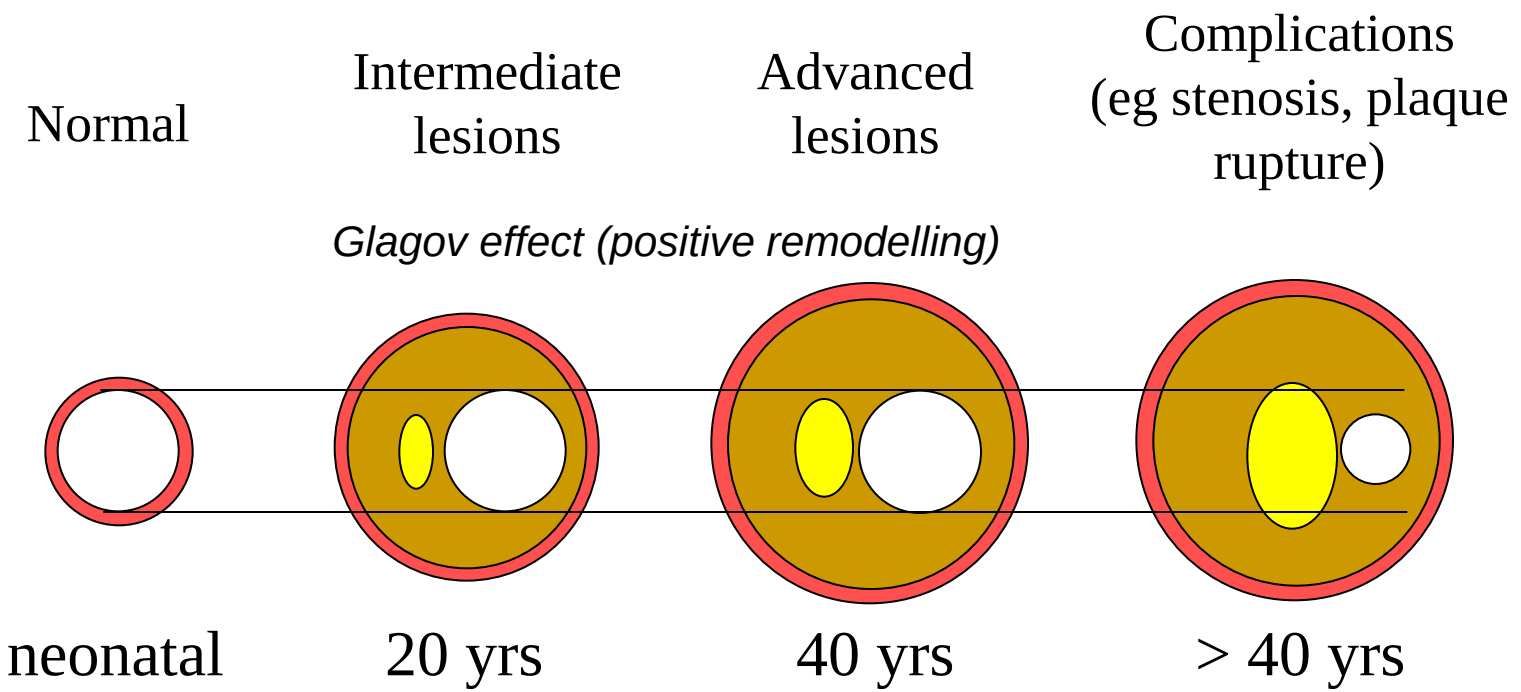
Intra-plaque haemorrhage and lesion progression



- poorly formed vessels with inadequate pericyte and basement membrane support
- density of microvessels correlates with density of activated macrophages
- intraplaque haemorrhage linked to acute clinical events – due to physical disruption
- extravasated erythrocytes provides a dual metabolic challenge –lipid from erythrocyte membranes and iron from heme

Barger AC et al. N Engl J Med 1984;310:175-177.

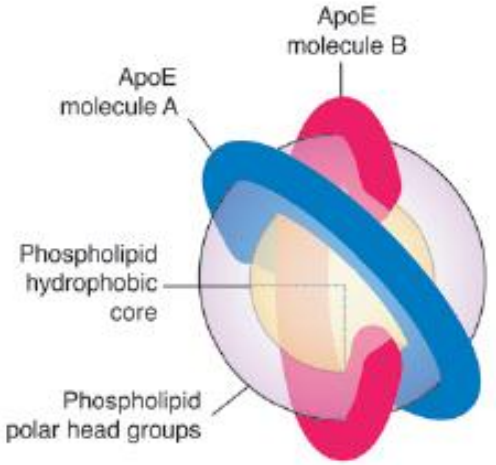
Natural history of atherosclerosis



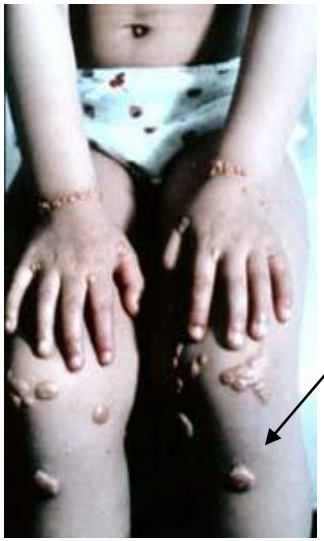
Seymour Glagov et al (1987) New Engl J Med 316:1371

Mouse models of atherosclerosis

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

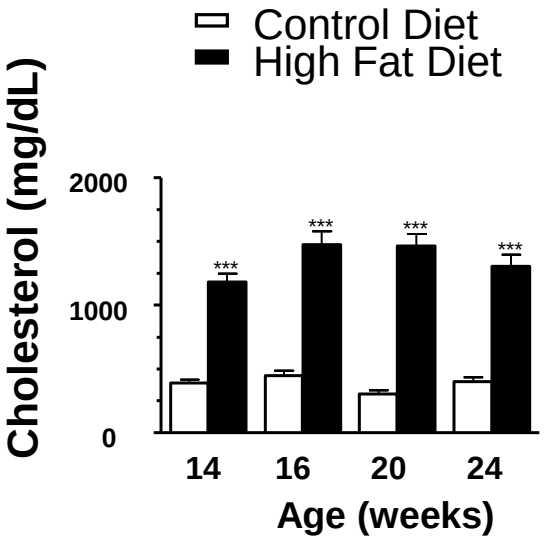


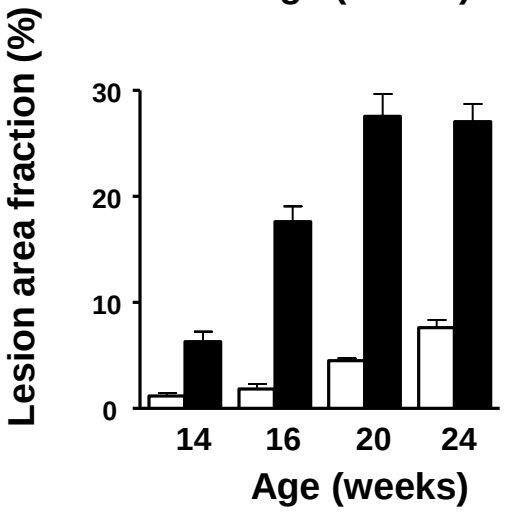
- **LDL receptor -/-**
  - Mutations in familial hypercholesterolaemia

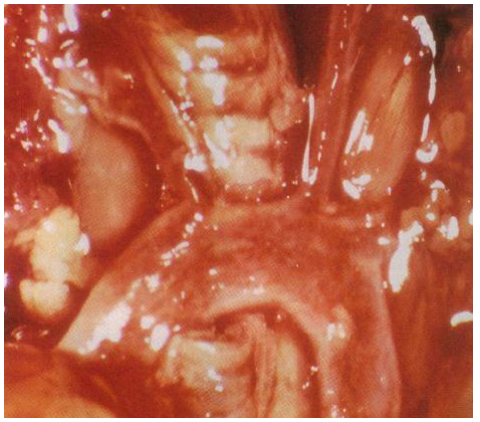


xanthoma

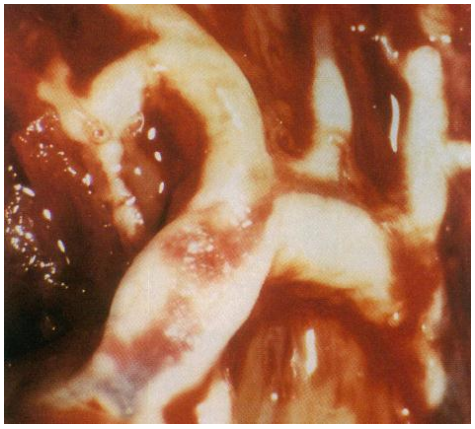
Atherosclerosis in *Ldlr*$^{-/-}$ mice





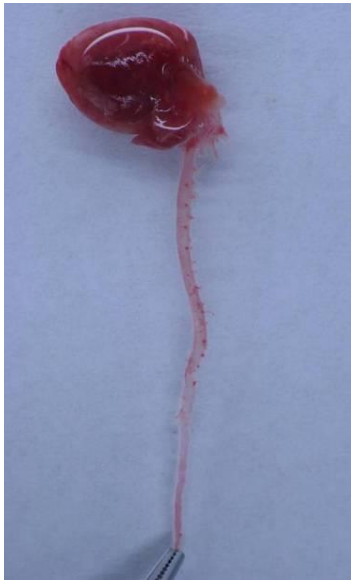


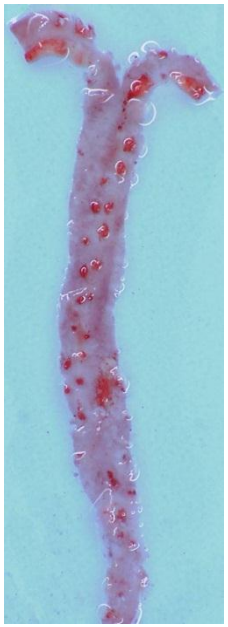
wild-type

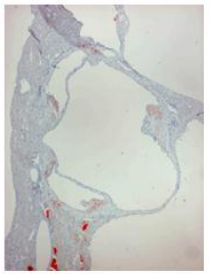
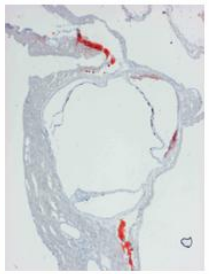
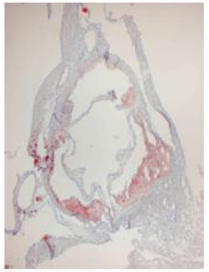
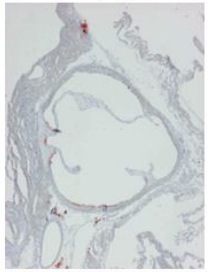
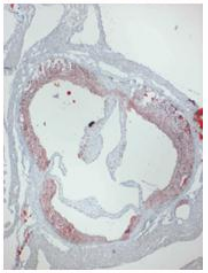
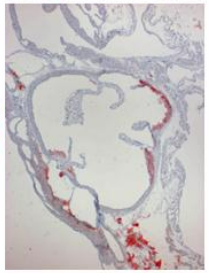
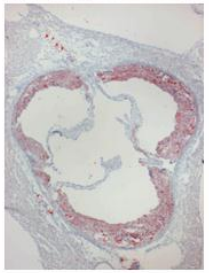
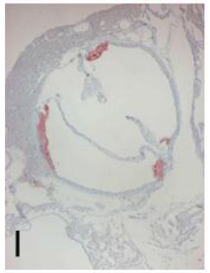


Ldlr-/- high fat 13 mo

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
TNFa
IL-4
IL-6
IL-12
IL-18
IFNg

Decelerators

IL1RA
TGFb
IL-10

Debris (eg modified lipoproteins, apoptotic cells)

Reversible fatty lesions

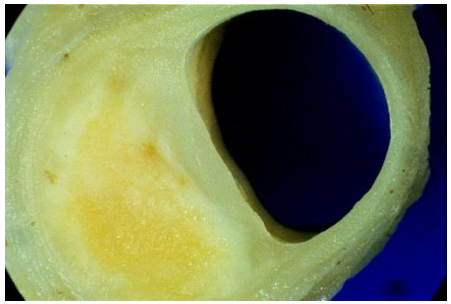
inflammatory drive + - *homeostatic innate immunity*

Irreversible remodelling

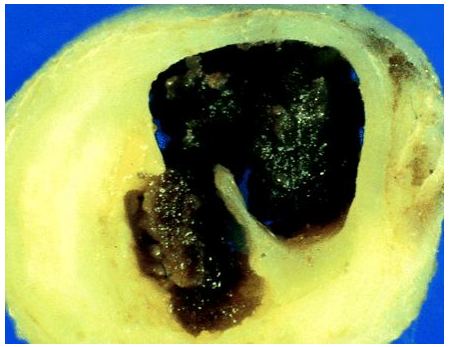
wound healing

environmental and genetic influences

inflammation

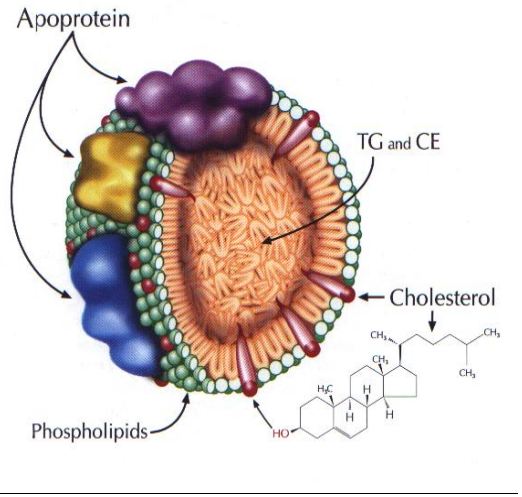


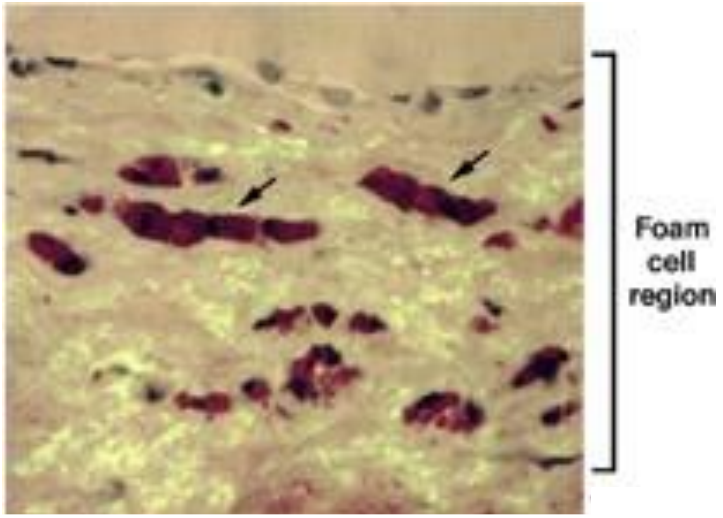
Stable plaques



Unstable plaques

Foam cells

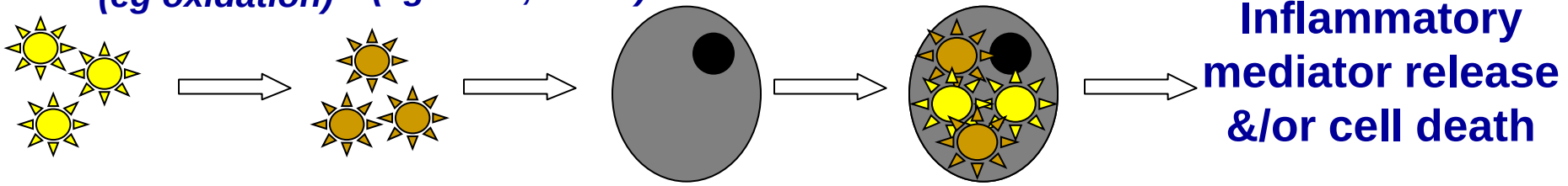




from Dr Howard K

lipoprotein modification (eg oxidation)

via scavenger receptors (eg SR-A, CD36)



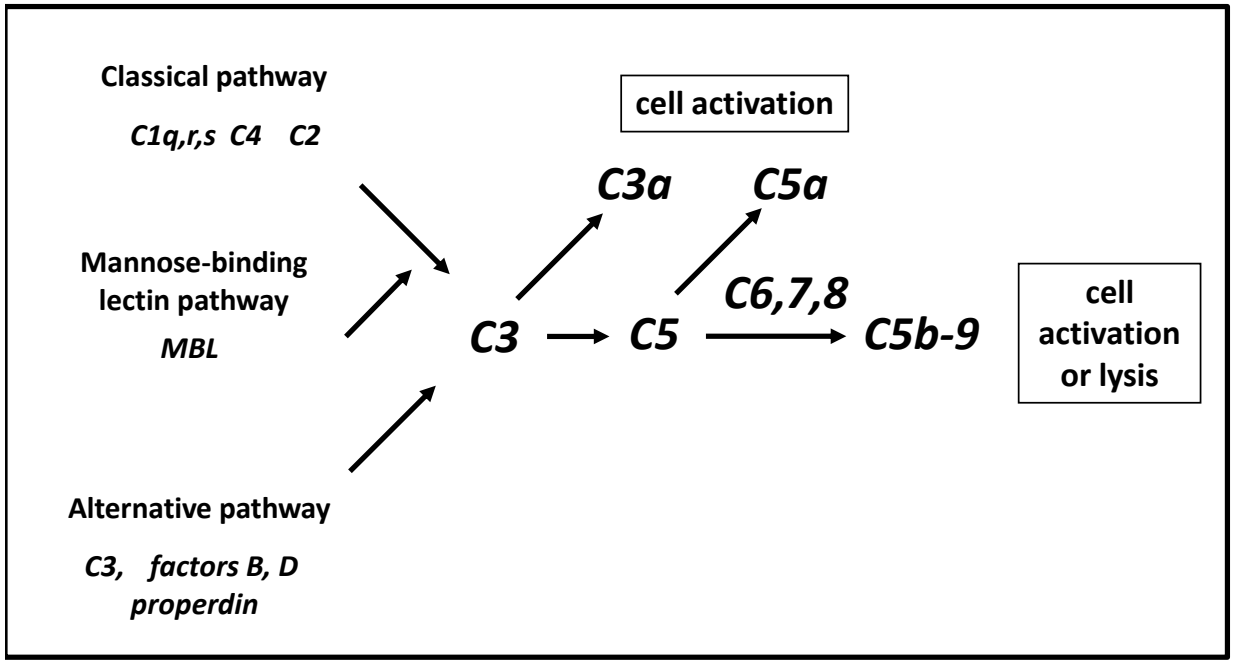
Macrophage

"foam cells"

Inflammatory mediator release &/or cell death

Role of complement in atherosclerosis

Apoptotic cells
Cholesterol crystals
Denatured LDL
Microparticles
Immune-complexes
Endotoxin

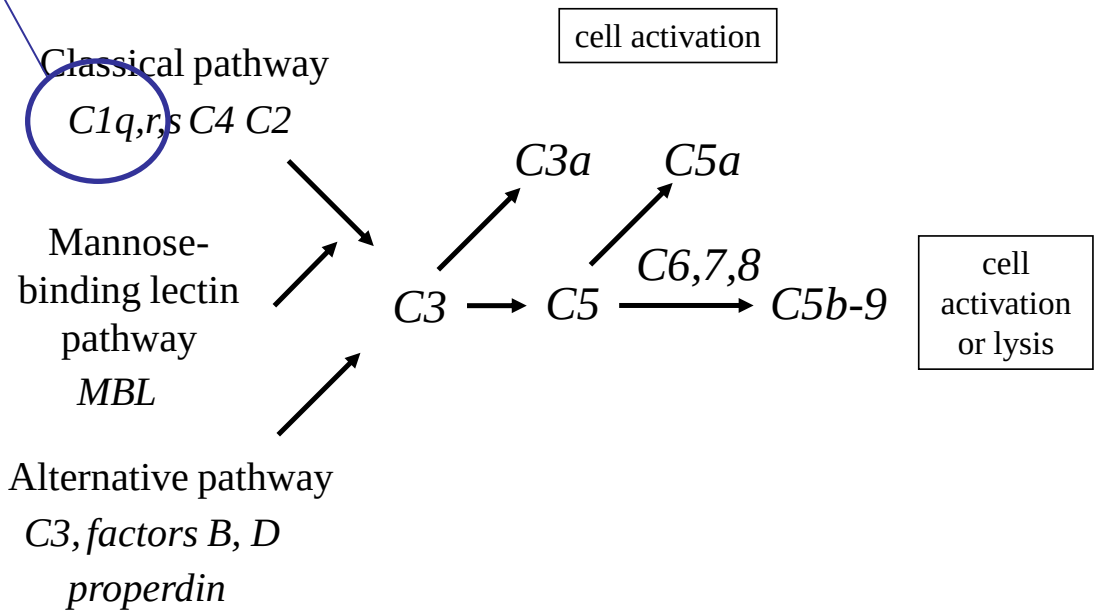


Immunohistological evidence for complement activation in human atherosclerosis

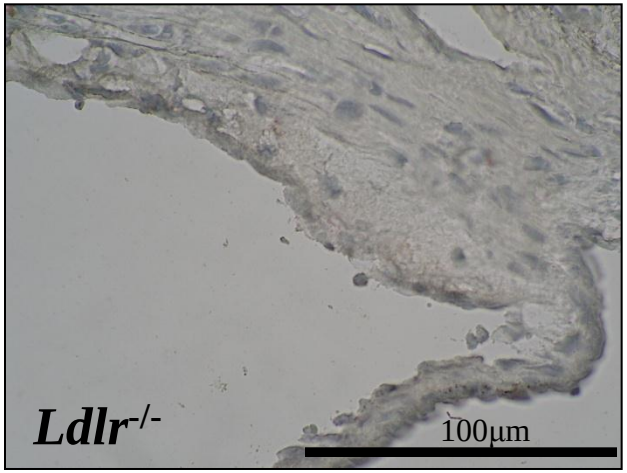
C6 deficient rabbits on a high fat diet are protected (*Geertinger & Sørensen 1977; Schmiedt et al 1998*)

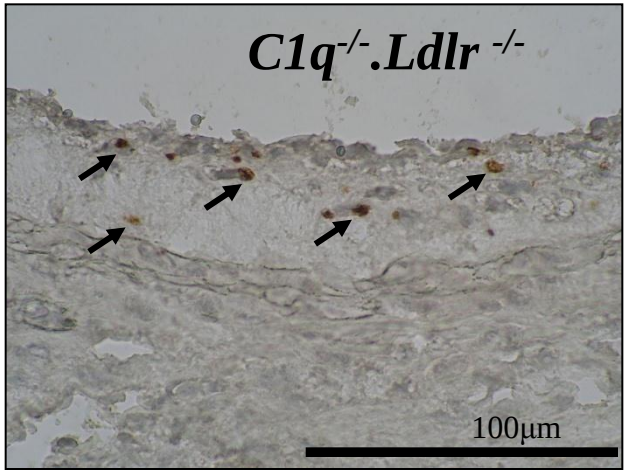
$C3^{-/-}$ mice show impaired progression to complex lesions *(Buono et al, 2002)*

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

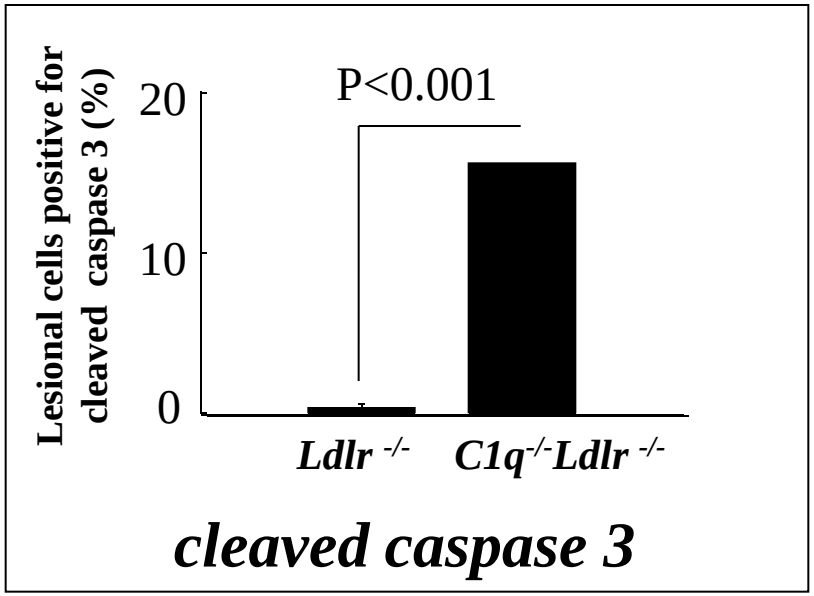


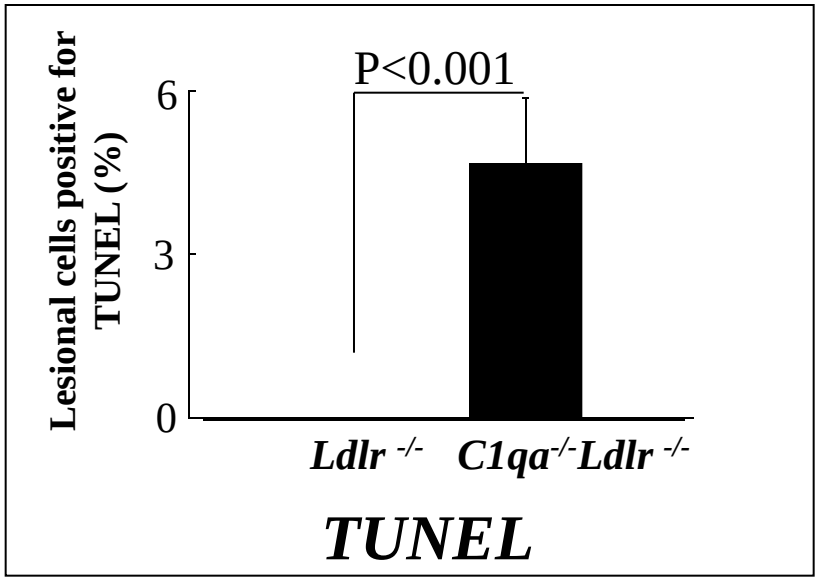
C1qa$^{-/-}$*Ldlr*$^{-/-}$ mice show impaired apoptotic cell clearance





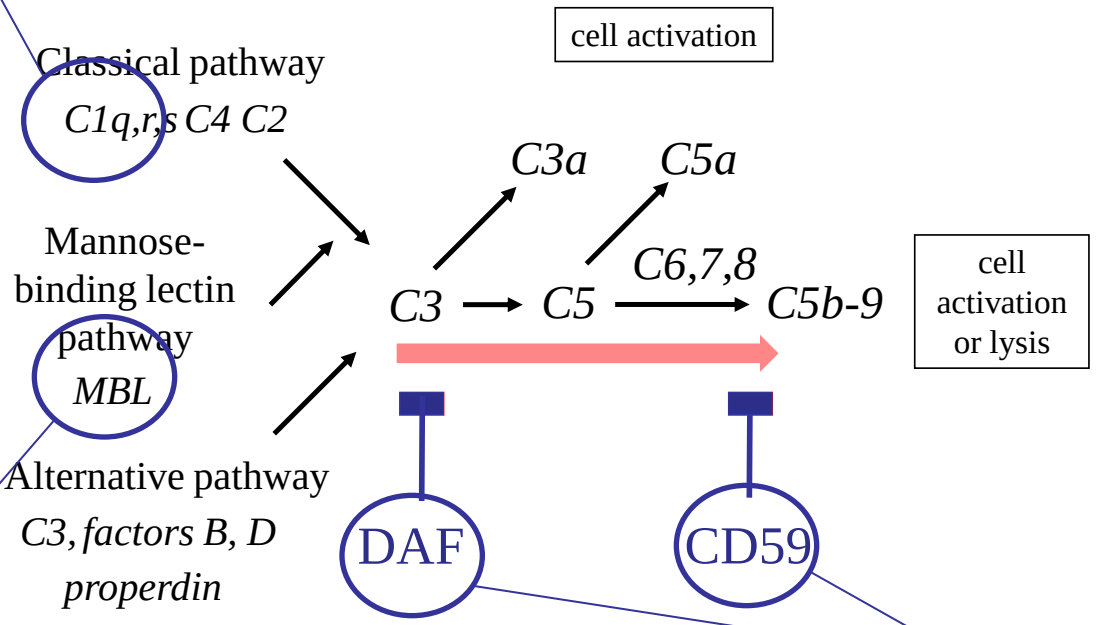
cleaved caspase 3





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*)



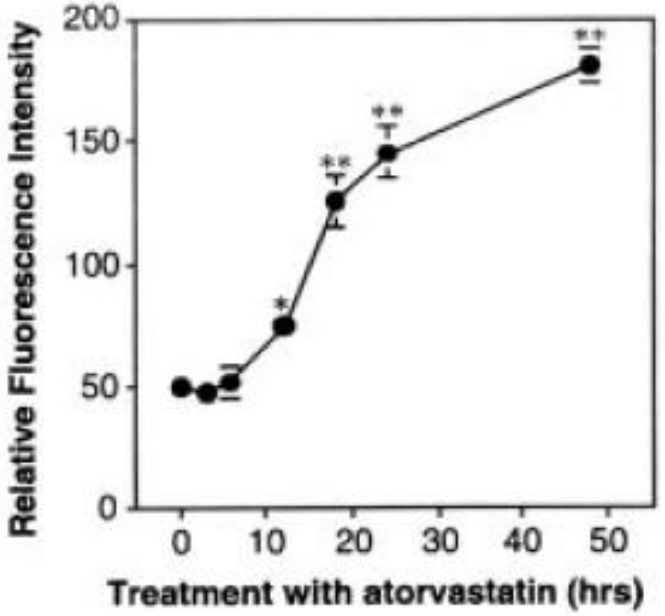
Accelerated atherosclerosis in mice with MBL-A and MBL-C deficient bone marrow (*Matthijsen et al 2009 Circulation*)

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

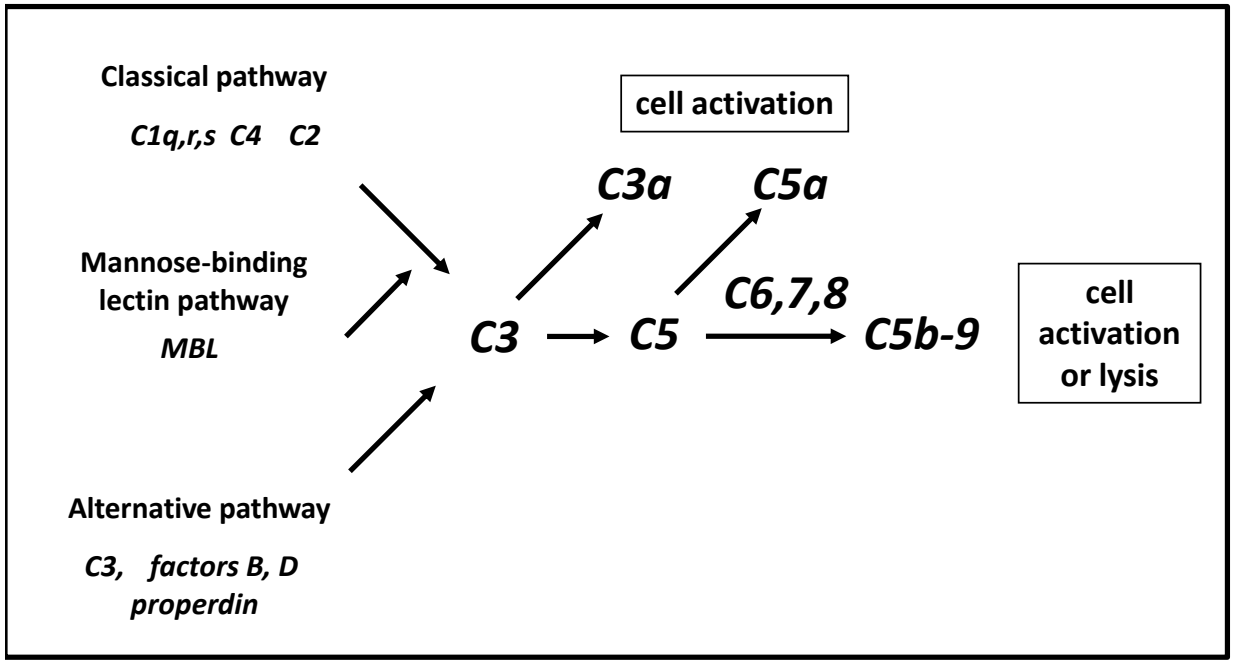


CD59 is upregulated by hypoxia and shear-stress

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

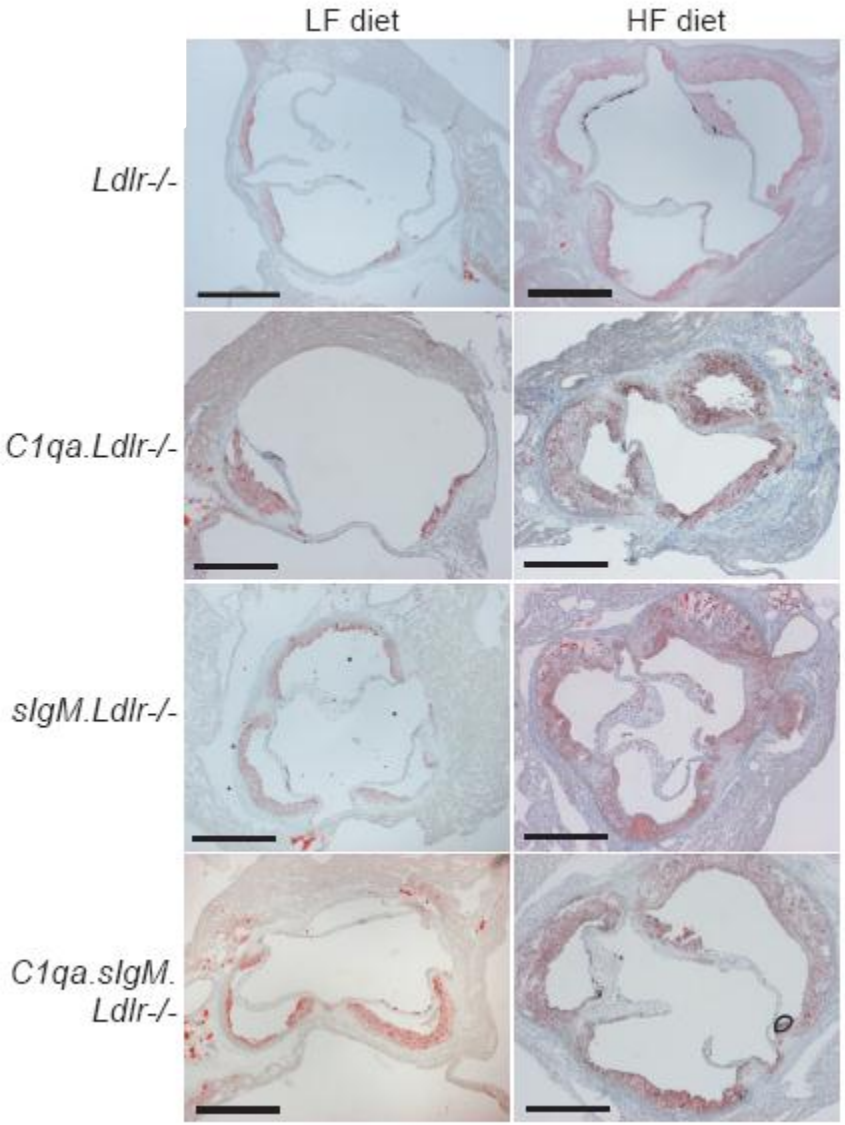
Role of complement in atherosclerosis

Apoptotic cells
Cholesterol crystals
Denatured LDL
Microparticles
Immune-complexes
Endotoxin

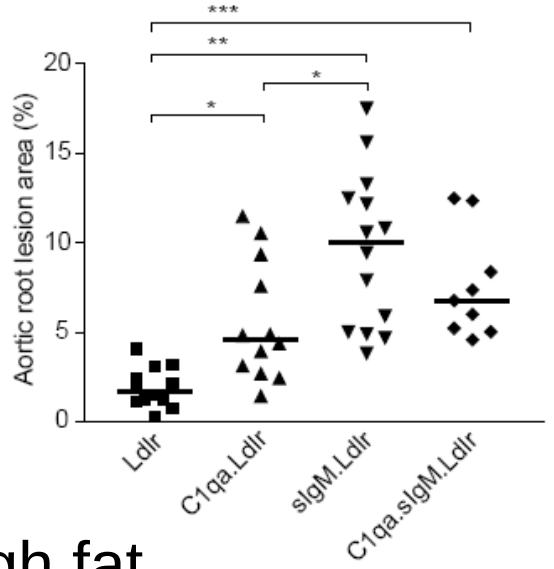


What is the role of IgM natural antibodies in the system?

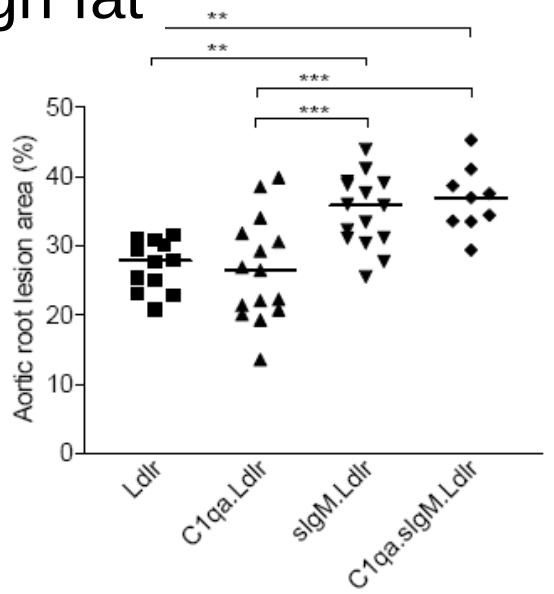
IgM deficiency accelerates atherosclerosis



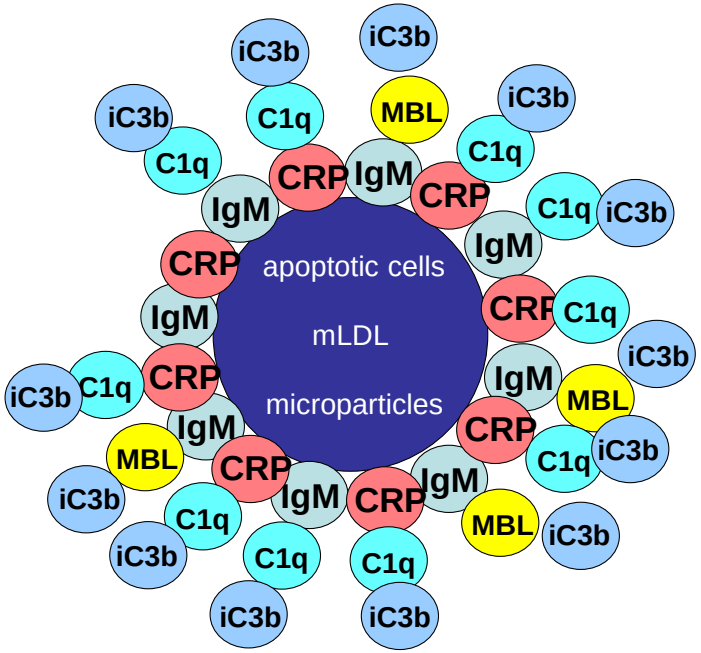
Low fat



High fat



Lewis et al 2009 Circulation 120:417



Homeostatic clearance

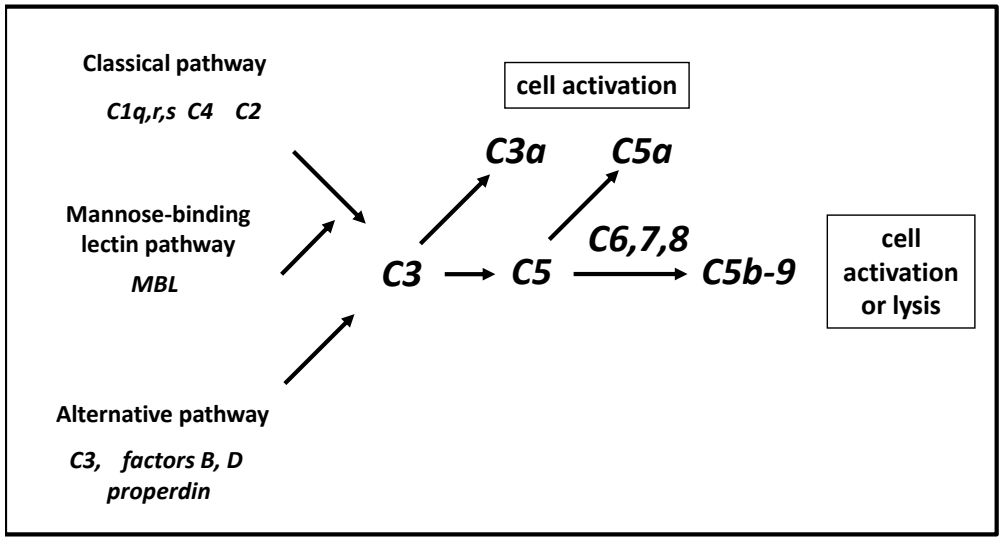
Complement, antibodies and atherosclerosis

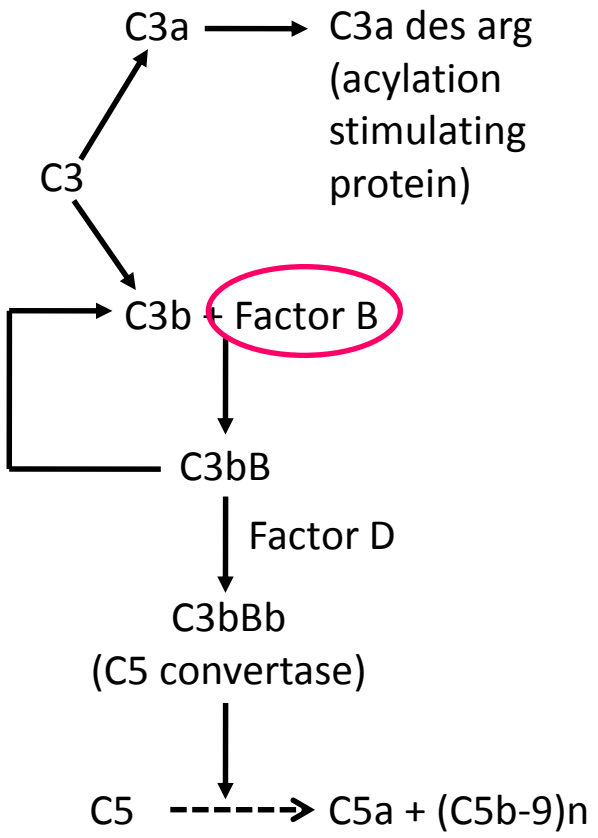
- Deficiency in serum IgM, C1q or MBL accelerates atherosclerosis in mice, indicating protective functions.

- The complement regulators DAF and CD59 shield the arterial wall from the potential proinflammatory effects of proximal pathway activation.

- What is the role of the Alternative Pathway?

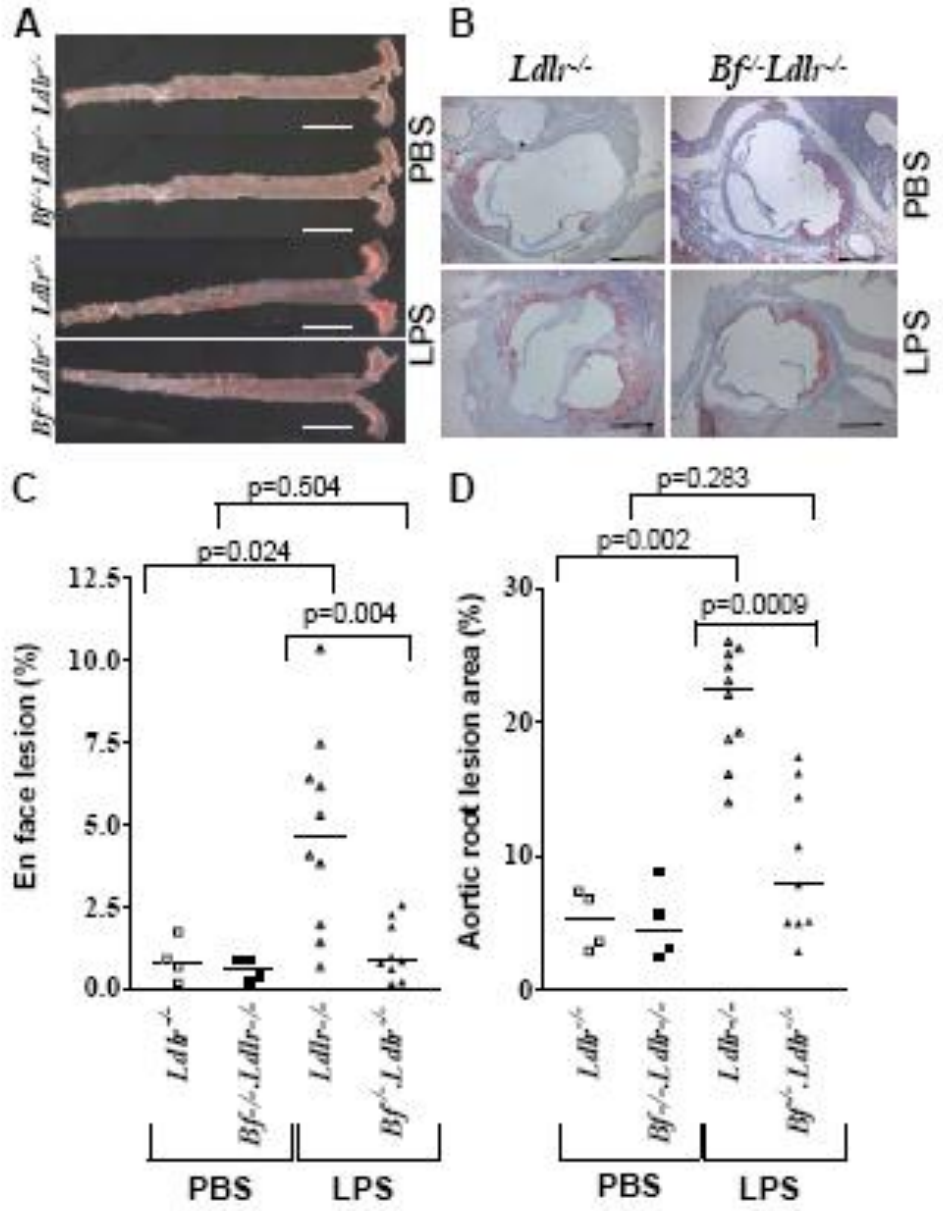
Alternative pathway



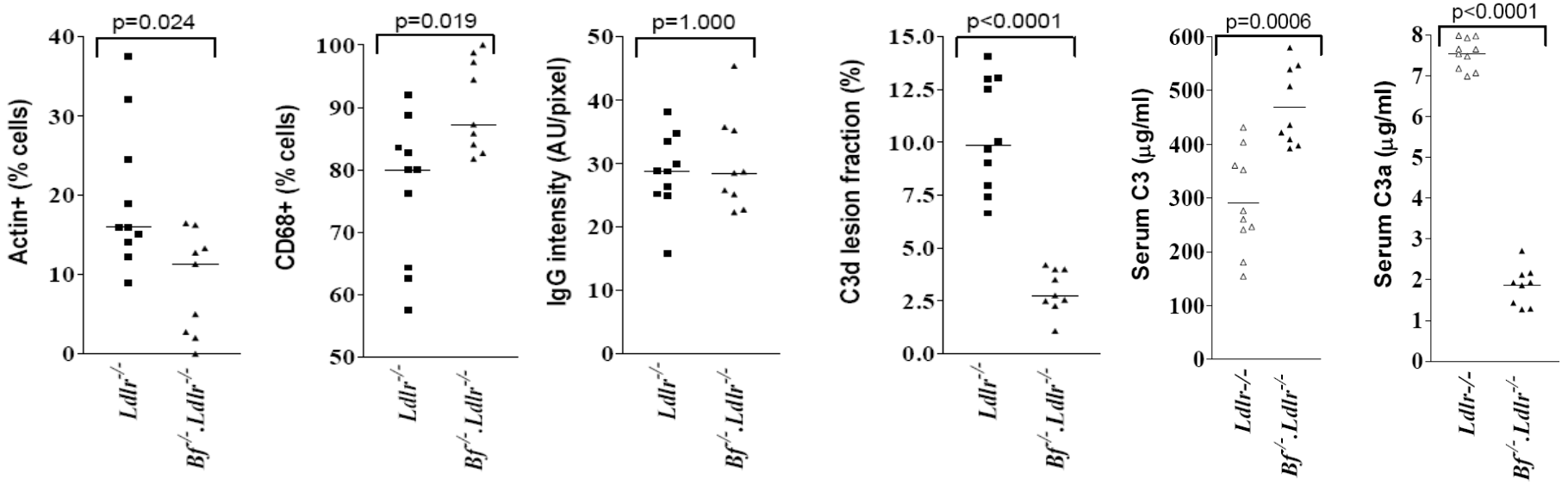


Hypothesis Activation of the alternative pathway stimulates proinflammatory downstream complement activation by over-riding complement inhibitors and is pro-atherogenic

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 (2012) Circulation 122:1948

Complement, antibodies and atherosclerosis

- These data add to the developing paradigm of the bifunctional role of the complement system 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.

Summary

- Atherosclerosis can be viewed as a dynamic chronic autoinflammatory disease of arteries

- The innate immune system regulates 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 immune mechanisms

- The interplay between proinflammatory and wound healing pathways governs plaque development

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Myles Lewis
Talat Malik
Sheng Yun

British Heart Foundation

wellcome trust