NFκB: THE HUB OF INNATE IMMUNITY WITHIN THE VASCULAR VESSEL WALL

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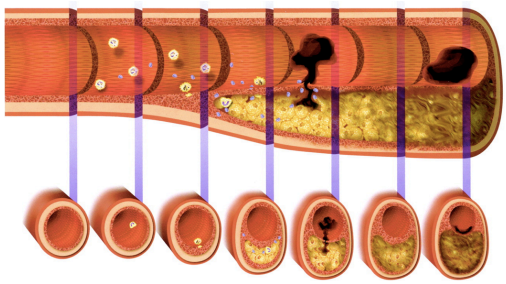
Learning outcomes

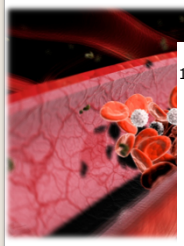
- To outline the fundamental mechanisms involved in the pathogenesis of atherosclerosis
- To illustrate the potential of targeting inflammatory signalling pathways in atherosclerosis, in particular the nuclear factor κB pathway

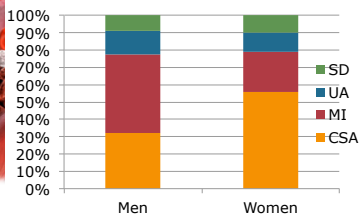
The paradox of atherosclerosis: a chronic process ....



Libby P Circulation 2001

… with predominant acute clinical manifestation



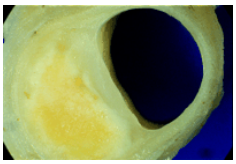


Framingham Heart Study Kannel WB Am J Card 1976; 329:1442

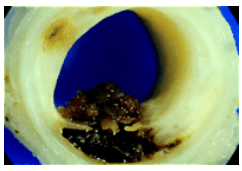
What is atherosclerosis?

The two facets of atherosclerosis

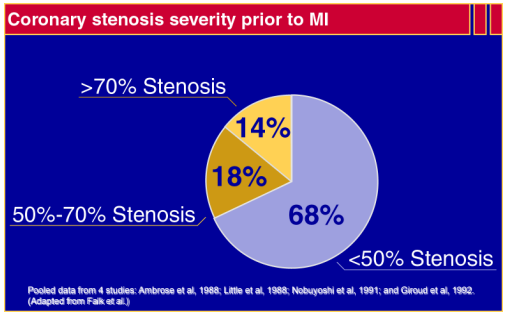
Stable



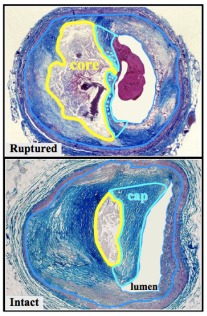
Unstable



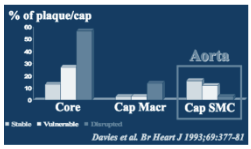
Myocardial Infarctions are caused by low-grade stenoses



The high risk atherosclerotic plaque



- ✓ Necrotic core >25% plaque area
- ✓ Fibrous cap < 65 μm
- ✓ Infiltration of inflammatory cells in the fibrous cap
- ✓ Loss of SMC in the fibrous cap



Site of Intimal Rupture or Erosion of Thrombosed Coronary Atherosclerotic Plaques Is Characterized by an Inflammatory Process Irrespective of the Dominant Plaque Morphology

Allard C. van der Wal, MD; Anton E. Becker, MD;
Chris M. van der Loos, PhD; Pranab K. Das, PhD

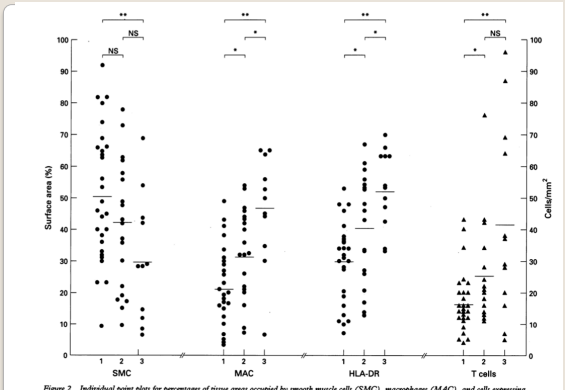
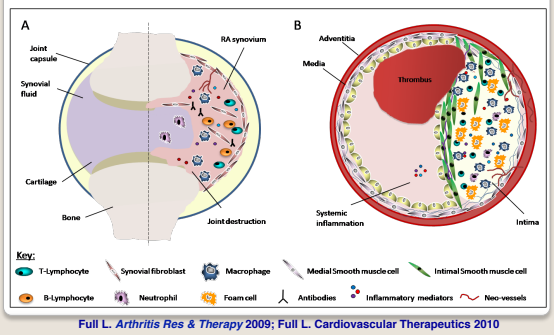


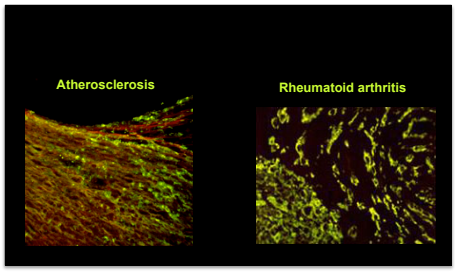
Figure 2 Individual point plots for percentages of tissue areas occupied by smooth muscle cells (SMC), macrophages (MAC), and cells expressing HLA-DR (HLA-DR) and the number of T lymphocytes per mm² for each atherectomy specimen in the three patient groups. Note the considerable overlap between the three groups. Bars represent mean values. *P < 0·05; **P < 0·005.

Similarities between atherosclerosis & rheumatoid arthritis



Full L. *Arthritis Res & Therapy* 2009; Full L. Cardiovascular Therapeutics 2010

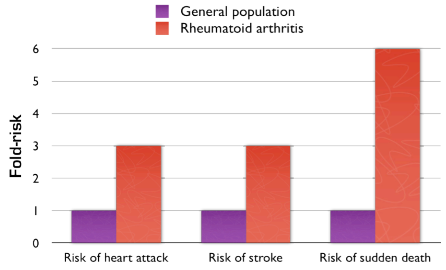
Upregulation of HLA-DR expression in atherosclerosis & rheumatoid arthritis



L. Jonasson, J. Holm, O. Skalli, G. Gabbiani and G.K. Hansson, J Clin Invest 76 (1985)

Feldman & Maini *Immunological Reviews* 2008 (Images kindly provided by Dr Tom Kennedy)

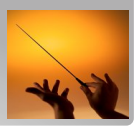
CVD is the major fatal complication of RA



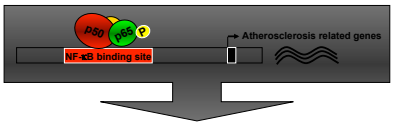
RA as bad risk as diabetes

Nuclear factor κB (NFκB)

- Transcription factor involved in inflammatory gene expression
- "Director" of the inflammatory response

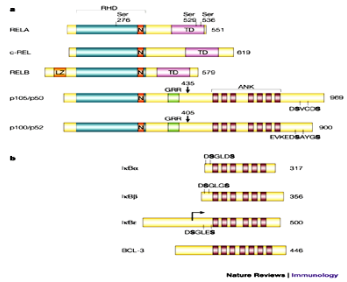


NFκB regulates the main biological events in the atherosclerotic plaque

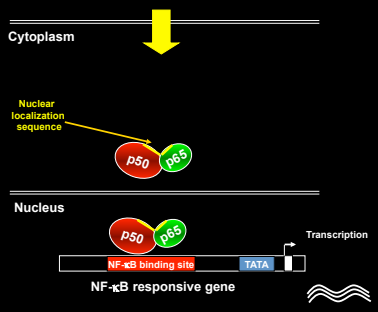


Recruitment of mononuclear cells	MCP-1, RANTES, MGSA/*gro*, E-selectin, ICAM-1, VCAM-1, MAdCAM-1
Inflammation & Immunity	IκBα, IL-1, IL-2, IL-6, IL-8, TNFα, G-CSF, M-CSF, GM-CSF, MCP-1, RANTES, MGSA/*gro*, MHC-I, MHC-II, CD80, CD86
Apoptosis & Proliferation	XIAP, c-IAP1, c-IAP2, Cyclin D
Thrombosis & vessel homeostasis	iNOS, COX-2, tissue factor, PAI-1, MnSOD, MMP-2, MMP-9, PTX3, RAGE

NFκB/Rel and IκB families

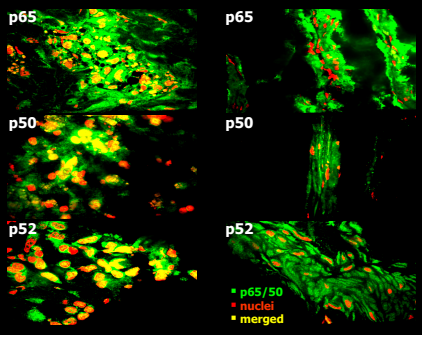


Model of NFκB activation



Activated NFκB in carotid plaques

Carotid endarterectomies ; Immunoconfocal images (60x)



NFκB and acute coronary syndromes

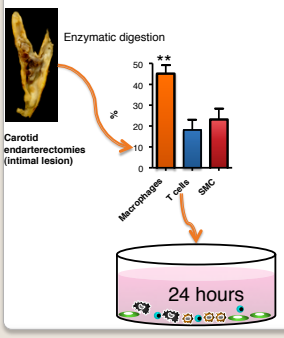
- Nuclear translocation of p65 is higher in unstable coronary atherectomies (Wilson SH, *Atherosclerosis* 2002)
- NFκB activation
  - ✓in PBMC from peripheral circulation (Ritchie M, *Circulation* 1998)
  - ✓in myocardial biopsies (Valen G, *Cardiovasc Res* 2000)

KO	Lesion size
CCL2/CCR2	▼ (64-79%)
CCL5/CCR5	▼ (44-53%)
MyD88	▼ (59-62%)
TLR-2	▼ (55%)
TLR-4	▼ (54%)
TNFa	▼ (50-83%)
IL-1/IL-1R	▼ (40-67%)
IL-12	▼ (52-68%)
IL-18	▼ (25-34%)
IFNg/IFNgR	▼ (59-75%)

Murine atherosclerosis is an inflammatory disease

Kleeman R Cardiovascular Res 2008

Functional studies in human atherosclerosis



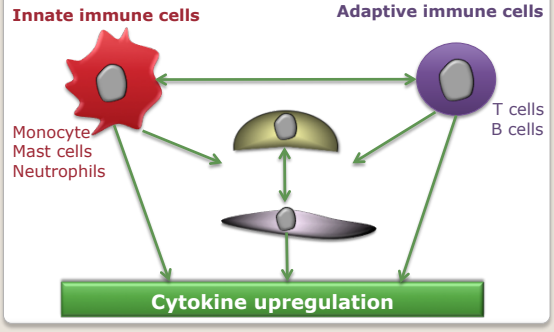
	Cytokine	% Detected
75-100 %	TNFα	96
	TNFβ	1
	IL1α	68
	IL1β	87
50-75	IL2	3
	IL4	58
	IL5	1
	IL6	100
	IL10	94
	IL12 (p40)	62
	IL12 (p70)	7
	IL15	4
	IL17	3
	IFNα2	56
25-50	IFNγ	14
	M-CSF	94
	GM-CSF	96
	IL11	0
	IL29	0
0-25	CCL2/MCP-1	100
	CX3CL1/FRACTALKINE	89
	CXCL10/IP-10	90
	CCL5/RANTES	96
	CXCL9/MIG	76
	CXCL7	100
	CXCL6	13
	CXCL11/ITAC	9
	CCL14a	100
	CCL19/ELC/MIP3b	0
	CCL20/LARC/MIP3a	67

Monaco C PNAS U S A 2004;101:5634-9

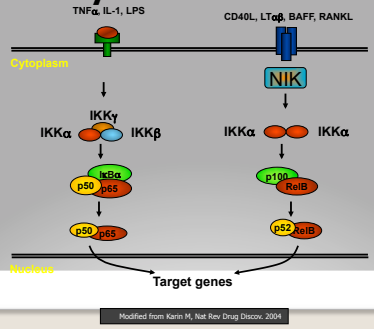
N=67

Q: What drives cytokine production in human disease?

Both innate and adaptive immunity play a role in atherosclerosis

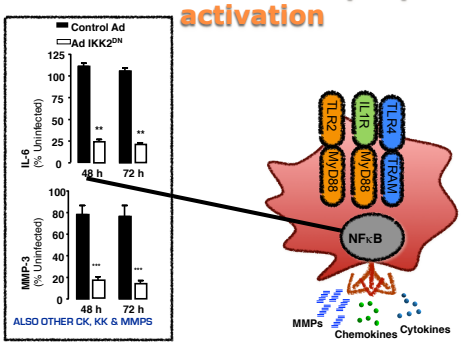


Classical and alternative pathway of NFκB activation



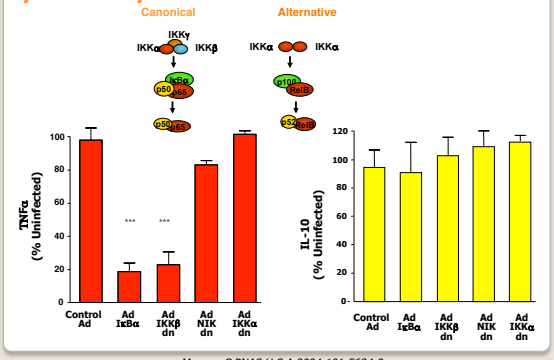
Modified from Karin M, Nat Rev Drug Discov. 2004

NFκB drives human plaque activation



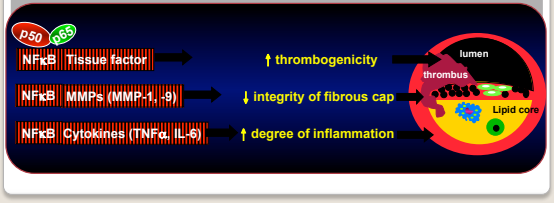
Monaco C., Circulation 2009; 120:2462

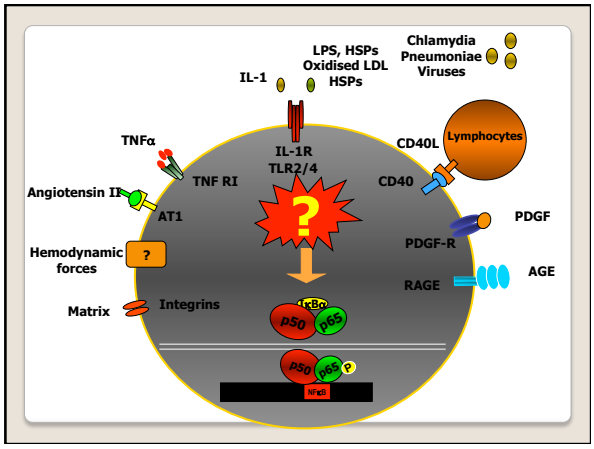
Selective inhibition of pro-inflammatory cytokines by NFκB blockade



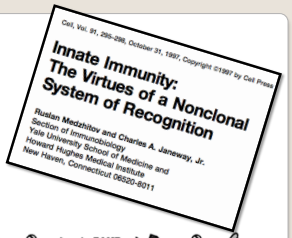
Monaco C PNAS U S A 2004;101:5634-9

NFκB regulates the thrombotic potential of the atherosclerotic plaque





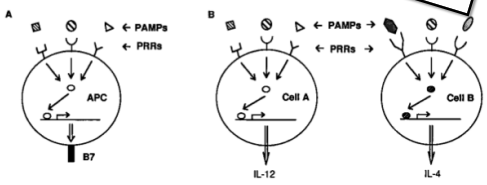
Innate immunity: the "dirty little secret" of immunology



Cell, Vol. 91, 295–298, October 31, 1997, Copyright ©1997 by Cell Press

Innate Immunity: The Virtues of a Nonclonal System of Recognition

Ruslan Medzhitov and Charles A. Janeway, Jr.
Section of Immunobiology
Yale University School of Medicine and
Howard Hughes Medical Institute
New Haven, Connecticut 06520-8011



Innate immunity vs. adaptive immunity

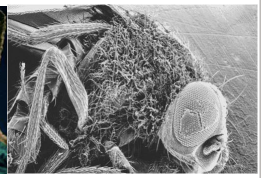
Table I. *Innate and adaptive immunity*

Property	Innate Immune System	Adaptive Immune System
Receptors	Fixed in genome; rearrangement not necessary	Encoded in gene segments; rearrangement necessary
Distribution	Nonclonal; all cells of a class identical	Clonal; all cells of a class distinct
Recognition	Conserved molecular patterns (LPS, LTA, mannans, glycans)	Details of molecular structure (proteins, peptides, carbohydrates)
Self–non-self discrimination	Perfect: selected over evolutionary time	Imperfect: selected in individual somatic cells
Action time	Immediate activation of effectors	Delayed activation of effectors
Response	Costimulatory molecules; cytokines (IL-1β, IL-6); chemokines (IL-8)	Clonal expansion or anergy; IL-2; effector cytokines (IL-4, IFN-γ)

Presidential Address to The American Association of Immunologists : The Road Less Traveled by: The Role of Innate Immunity in the Adaptive Immune Response

Charles A. Janeway, Jr.

J Immunol, 1998;161;539-544

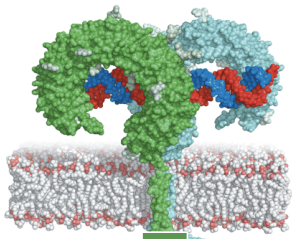



"*Toll*"= great, amazing, weird in German

Fungal infections in absence of fruit fly "*Toll*"

HUMAN *Toll*-like receptors

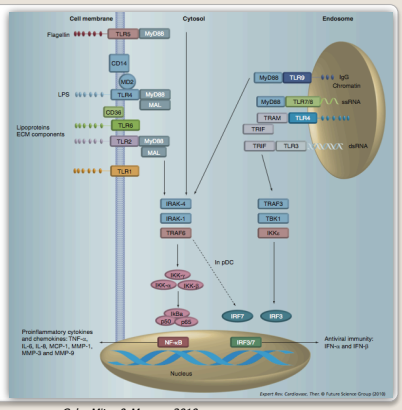
Self vs. non self



Danger vs. non dangerous signals

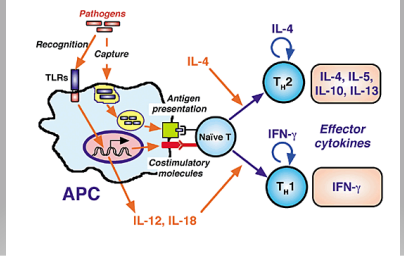
Immune response

Toll-like Receptor signalling



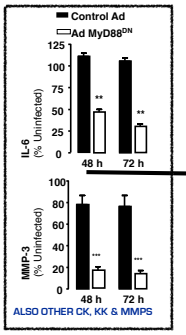
Cole, Mitra & Monaco 2010

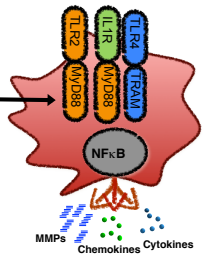
Toll-like receptors: critical proteins linking innate and acquired immunity



Akira S, Nature Immunology 2001

MyD88 drives human plaque activation

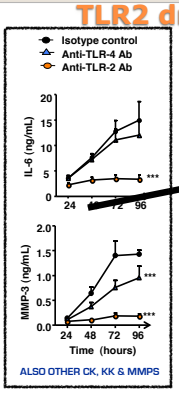


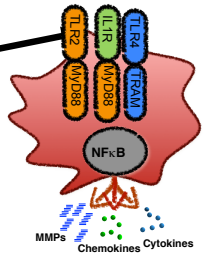


Monaco C., Circulation 2009; 120:2462

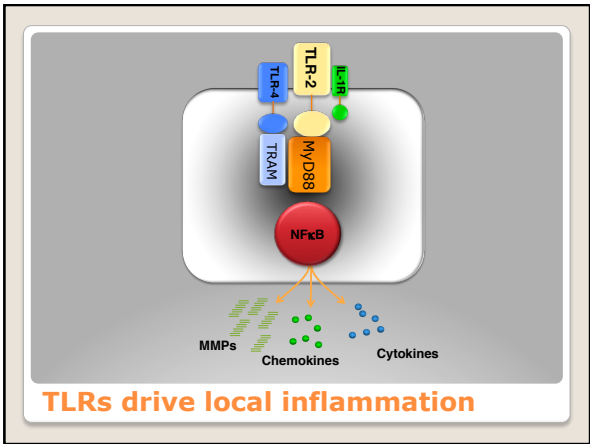
TLR2 drives human plaque activation

IN MURINE MODELS GENETIC DELETION OF TLR2, TLR4 AND IL1R HAVE EQUIVALENT EFFECT





Monaco C., Circulation 2009; 120:2462



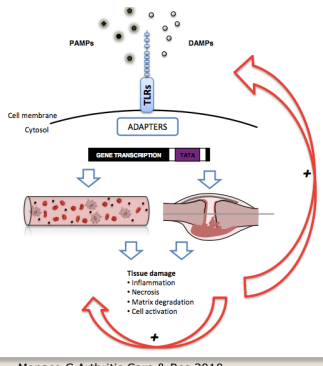
TLRs drive local inflammation

Table 2. TLRs and endogenous activators

Receptor	Ligand	Source
TLR1	β-defensin 3	Released from activated/necrotic cells
TLR2	HSP60, 70, Gp96, HMGB1, HMGB1-nucleosome complexes, β-defensin 3, surfactant proteins A and D, eosinophil derived neurotoxin, antiphospholipid antibodies, serum amyloid A	Released from activated/necrotic cells
	Biglycan, versican	Induced upon tissue damage
	Hyaluronic acid fragments	Degradation of tissue
TLR3	mRNA	Released from activated/necrotic cells
TLR4	HMGB1, surfactant proteins A and D, β-defensin 2, HSP60, 70, 72, 22, Gp96, S100A8, S100A9, neutrophil elastase, antiphospholipid antibodies, lactoferrin, serum amyloid A, oxidised LDL, saturated fatty acids	Released from activated/necrotic cells
	Biglycan, fibronectin EDA, fibrinogen, tenascin-C	Induced upon tissue damage
	Heparin sulphate fragments, hyaluronic acid fragments	Degradation of tissue
TLR5	Unknown	Unknown
TLR6	Unknown	Unknown
TLR7/8	Antiphospholipid antibodies, ssRNA	Released from activated/necrotic cells
TLR9	IgG-chromatin complexes, mitochondrial DNA	Released from activated/necrotic cells
TLR10	Unknown	Unknown
TLR11	Unknown	Unknown

Monaco C Arthritis Care & Res 2010

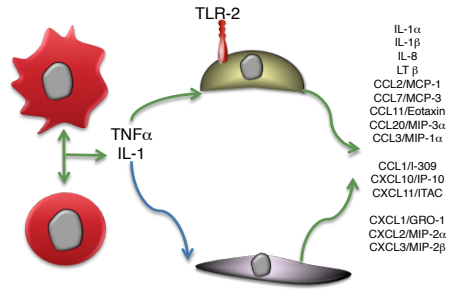
Endogenous TLR agonists in inflammatory diseases



Monaco C Arthritis Care & Res 2010

Q: How does the cytokine pattern affects resident vascular cells?

Cytokine cascade in atherosclerosis



- ✓ Cytokine production in human atheroma is quantitatively different between stable and unstable plaques
- ✓Innate immunity and in particular TLR-2 plays a predominant role in cytokine and MMP production in human atherosclerosis
- ✓ Exposure to cytokines changes gene expression and behavior of resident vascular cells

Conclusions

Jennifer Cole
Leena Viiri
Nagore Astola
Amanda Cross
Mike Goddard
Mika Falck-Hansen
Anusha Seneviratne
Louise Full
Joseph Shalhoub
Ilona Kruzynska-Ziaja

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Cytokine biology of atherosclerosis