ETTORE MAJORANA FOUNDATION AND CENTRE FOR SCIENTIFIC CULTURE
SCIENTIFIC AND TECHNOLOGICAL ADVANCEMENTS IN CARDIAC AND VASCULAR SURGERY

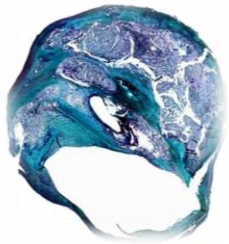
Clinical Institute of Medical and Chemical Laboratory Diagnostics

CIMCL

Medical University of Graz

KAGes

The vulnerable atherosclerotic plaque – improved management by cooperation between Radiology, Laboratory and Nano Medicine


Harald MANGGE
Medical University of Graz, Austria

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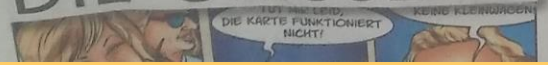


Atherosclerosis

Stable versus vulnerable AS plaque

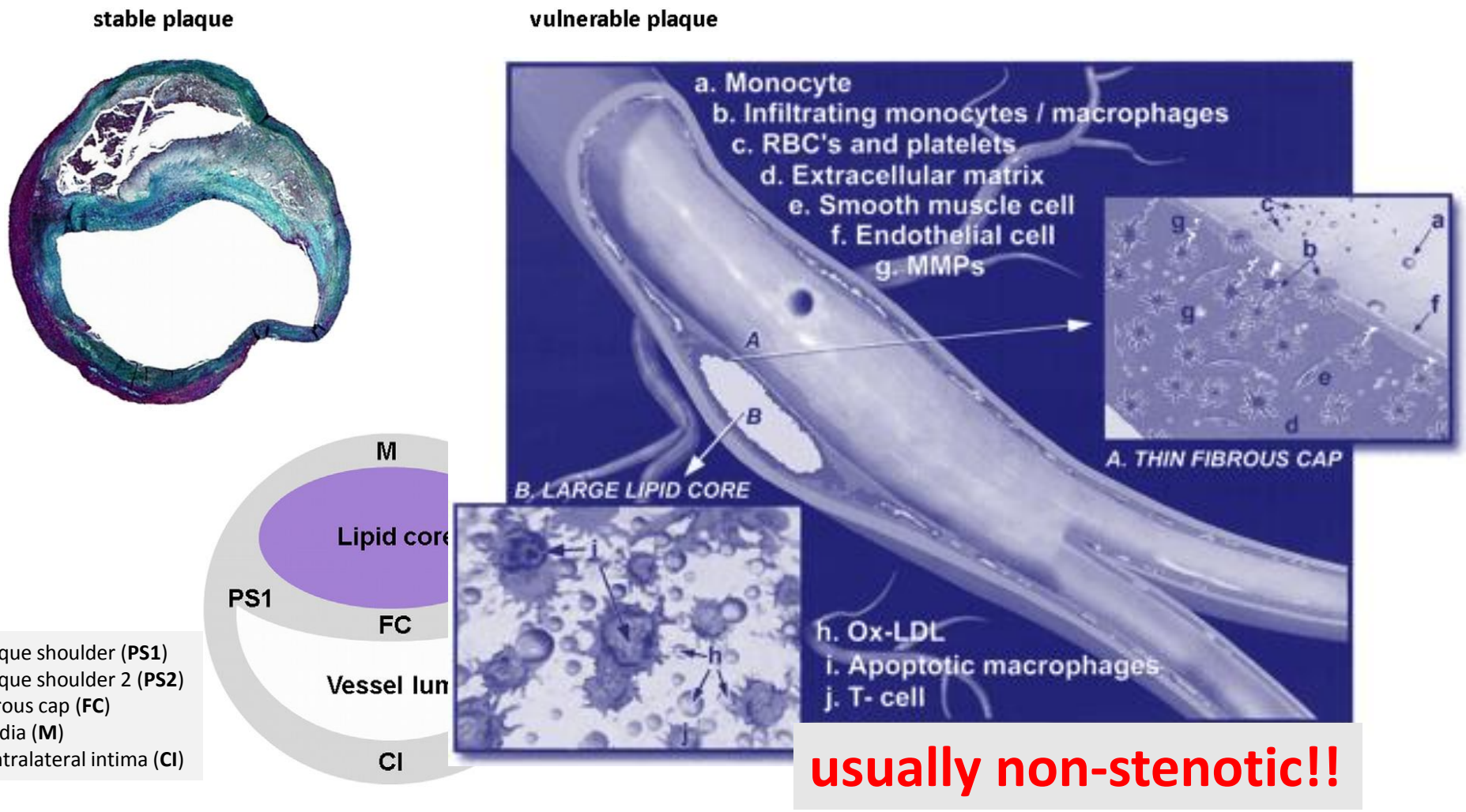
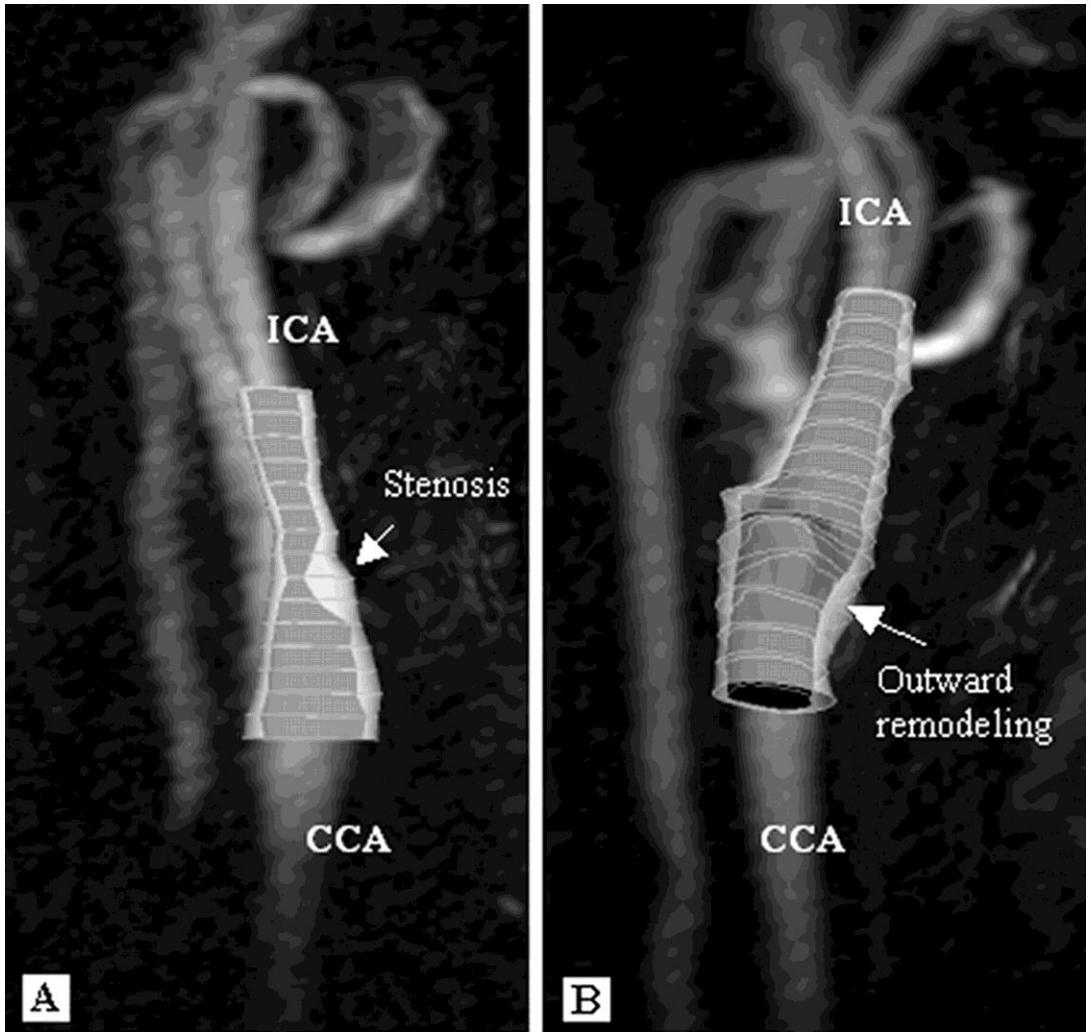


Figure from Dietel B, Garlichs C et al, Atherosclerosis 2013

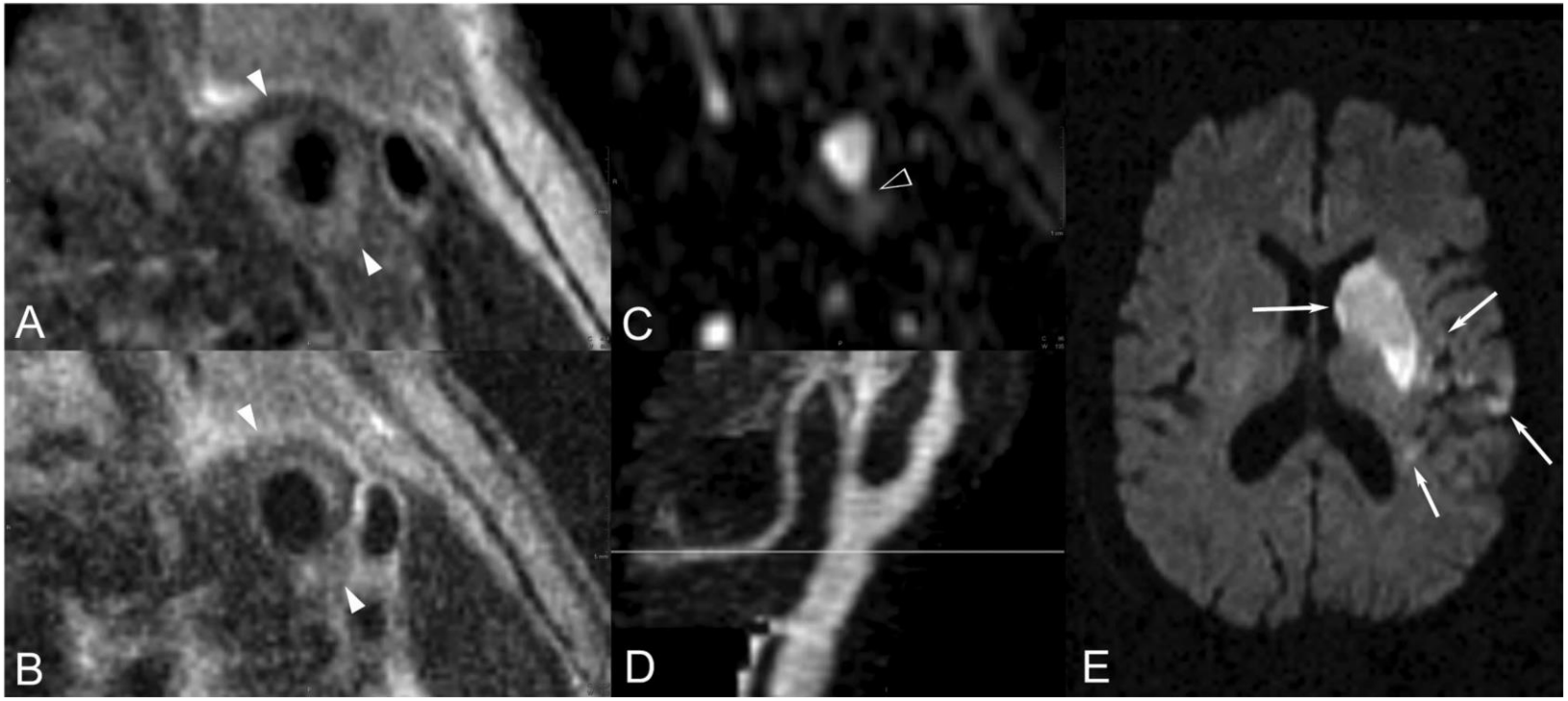


Non-stenotic AS lesions show dangerous outward remodeling

Immune-mediated inflammation plays an important role

Adame I M et al. Stroke. 2006;37:2162-2164

Nonstenotic ruptured, hemorrhagic plaque causes acute embolic stroke



Parmar J P et al. Circulation. 2010;122:2031-2038

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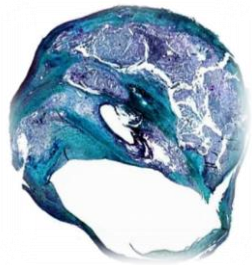
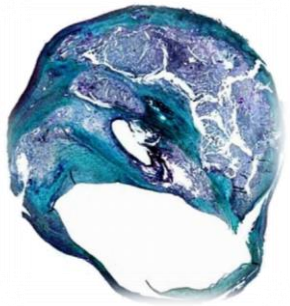
Atherosclerosis - *Vulnerability*

Pathological Mechanisms

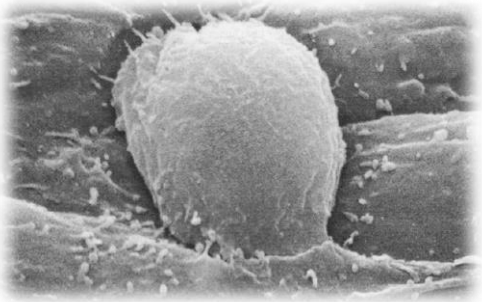


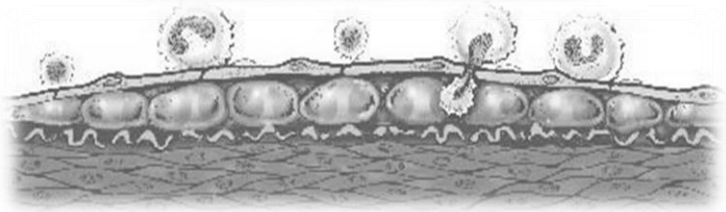
Atherosclerosis - *Vulnerability*



Inflammation

The monocyte/macrophage system





Atherosclerosis- *Vulnerability*

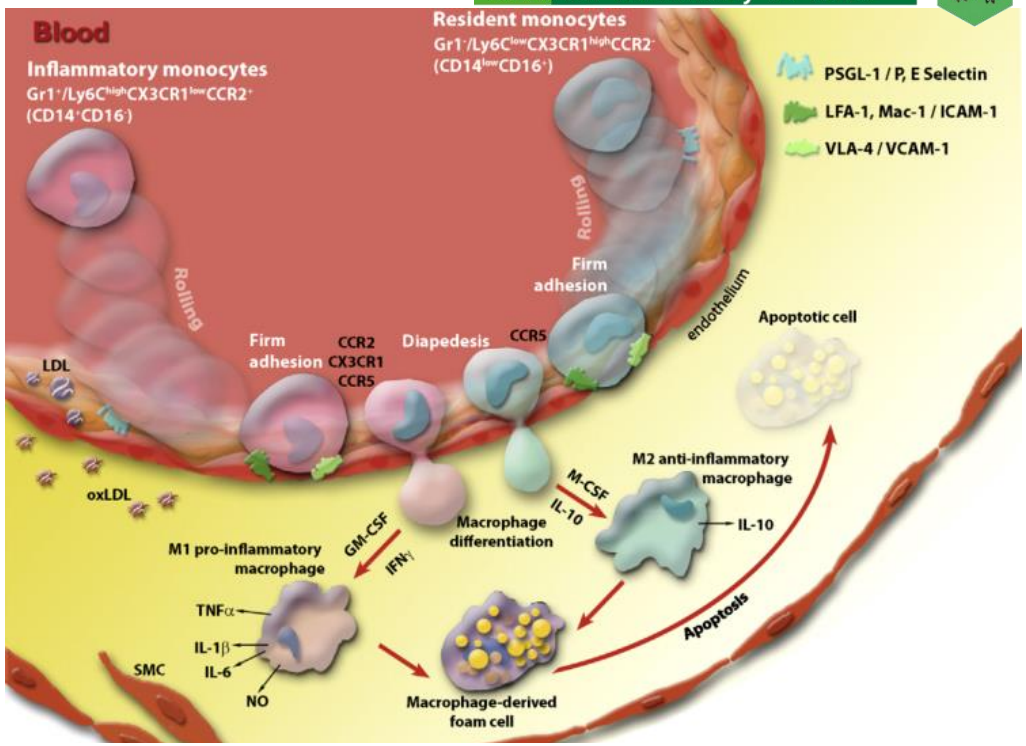
Central role of the macrophage

Intraplaque Macrophages

balance between „bad“ (M1) and „good“ (M2)

- Ly6C^{high} M1 macrophages produce proinflammatory TNF-a, IL-1, IL-6, NO
- Ly6C^{low} M2 macrophages secrete anti-inflammatory IL-10
- **After lipid up-loading**, **both** M1 and M2 macrophages turn into **foam cells**, which undergo apoptosis and thus create a necrotic center in later stages of the atherosclerotic process

Figures from
Madalina Fenyo et al Immunobiology (2013)
Aloke et al Journal of the American College of Cardiology (2012)

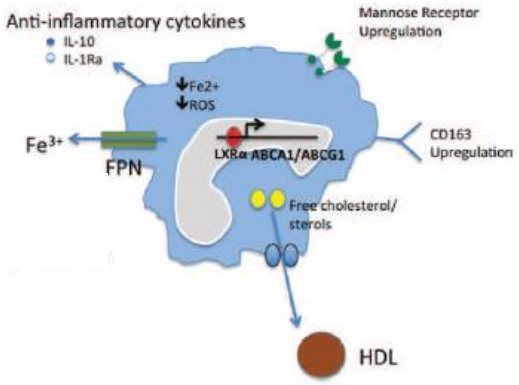


Induced by free hemoglobin/haptoglobin

M2 „subtype“

M(Hb) Macrophage

Stimulus:Hb:Hp
↑ anti-inflammatory Cytokines
↓ lipid uptake
↑ cholesterol efflux
Nonfoamy nature



Atherosclerosis- *Vulnerability*

Macrophage´s **janus face**

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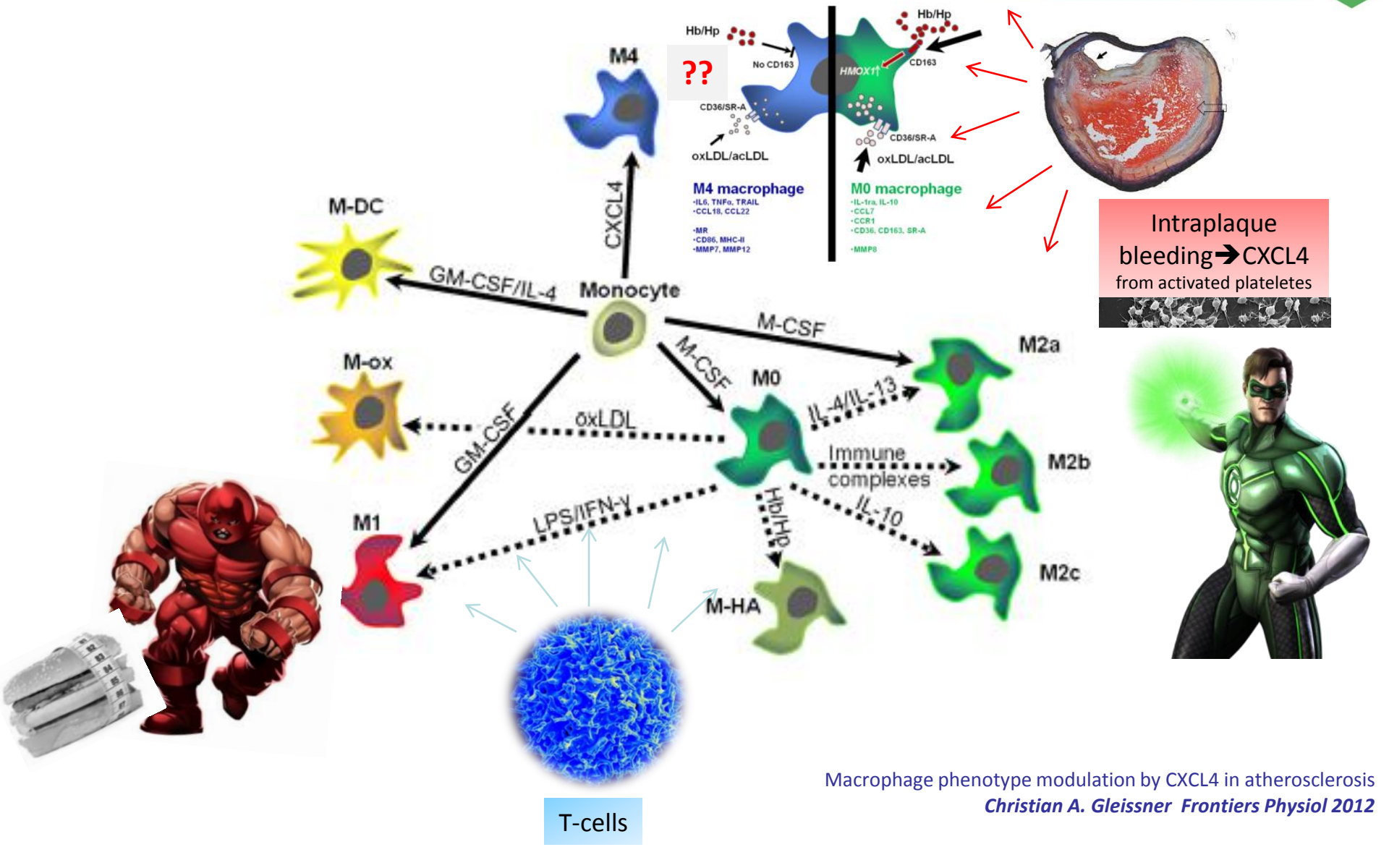
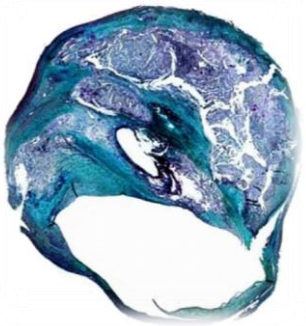


Macrophage phenotype modulation by CXCL4 in atherosclerosis

Christian A. Gleissner Frontiers Physiol 2012

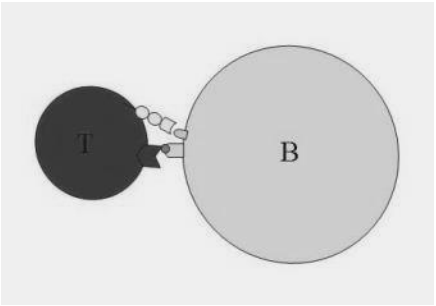
Atherosclerosis - *Vulnerability*

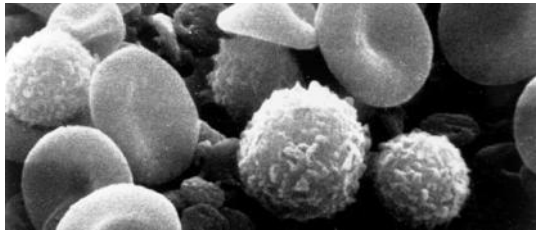


Inflammation

The adaptive immune response

T-helper cells, B-cells





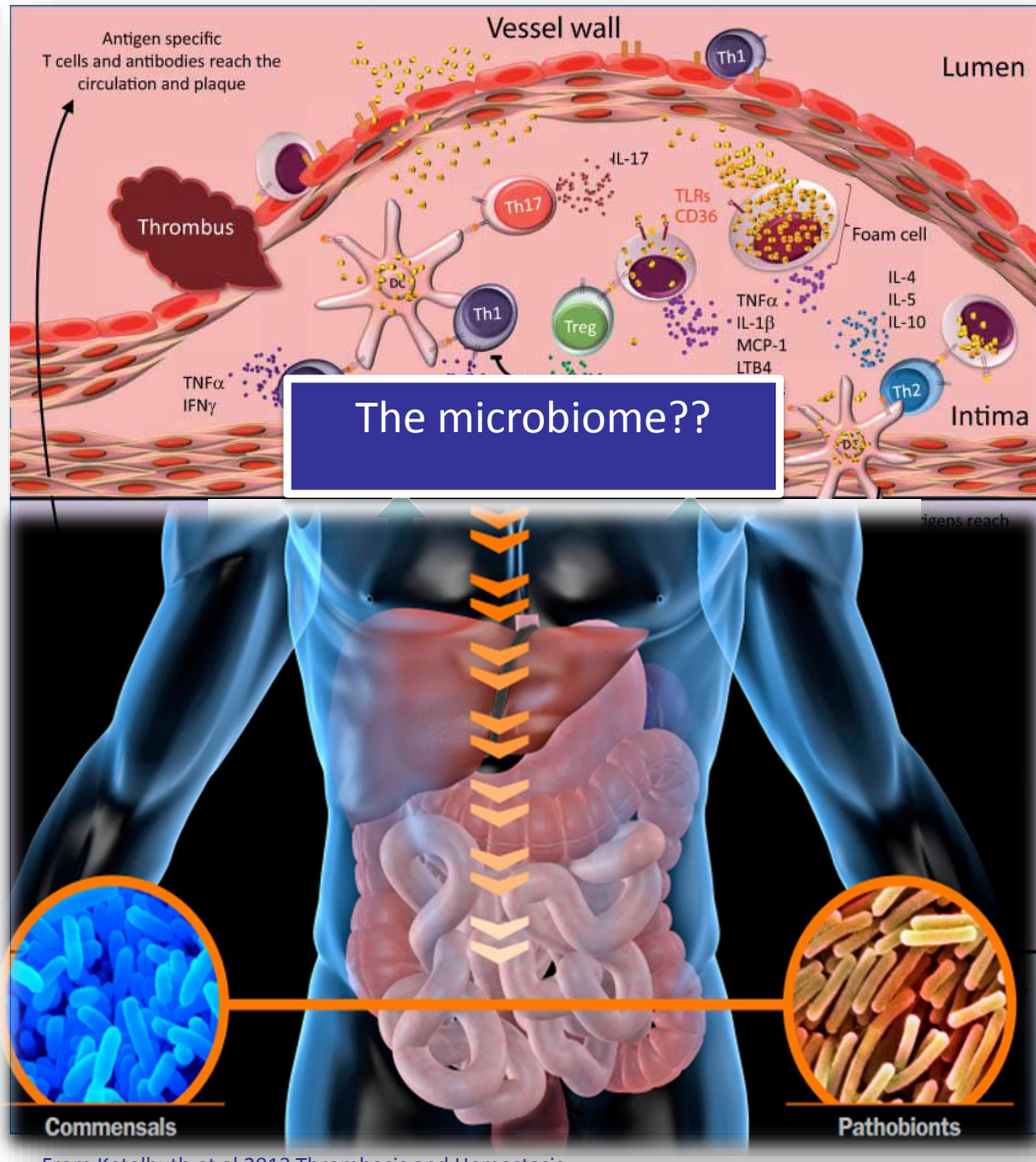
Atherosclerosis- *Vulnerability*

The adaptive immune response

TH1 Treg..

- Intimal LDL oxidation
- Macrophages activation, foam cells, pro-inflammatory cytokines ↑, MMPs ↑
- Endothelial cells over-express adhesion molecules (VCAM-1, ICAM-1)
- **CD4⁺ T helper** of the **Th1 type** are activated by ApoB100 peptides, **IFNγ ↑, TNFα ↑**
- **Regulatory T cells (Treg)** down-regulate the process **TGFβ ↑, IL-10 ↑**
- Antigen loaded **Dendritic cells** (DCs) reach draining lymph nodes, spleen
- Naïve T cells develop into effector T cells re-enter the bloodstream, reach the AS lesion…

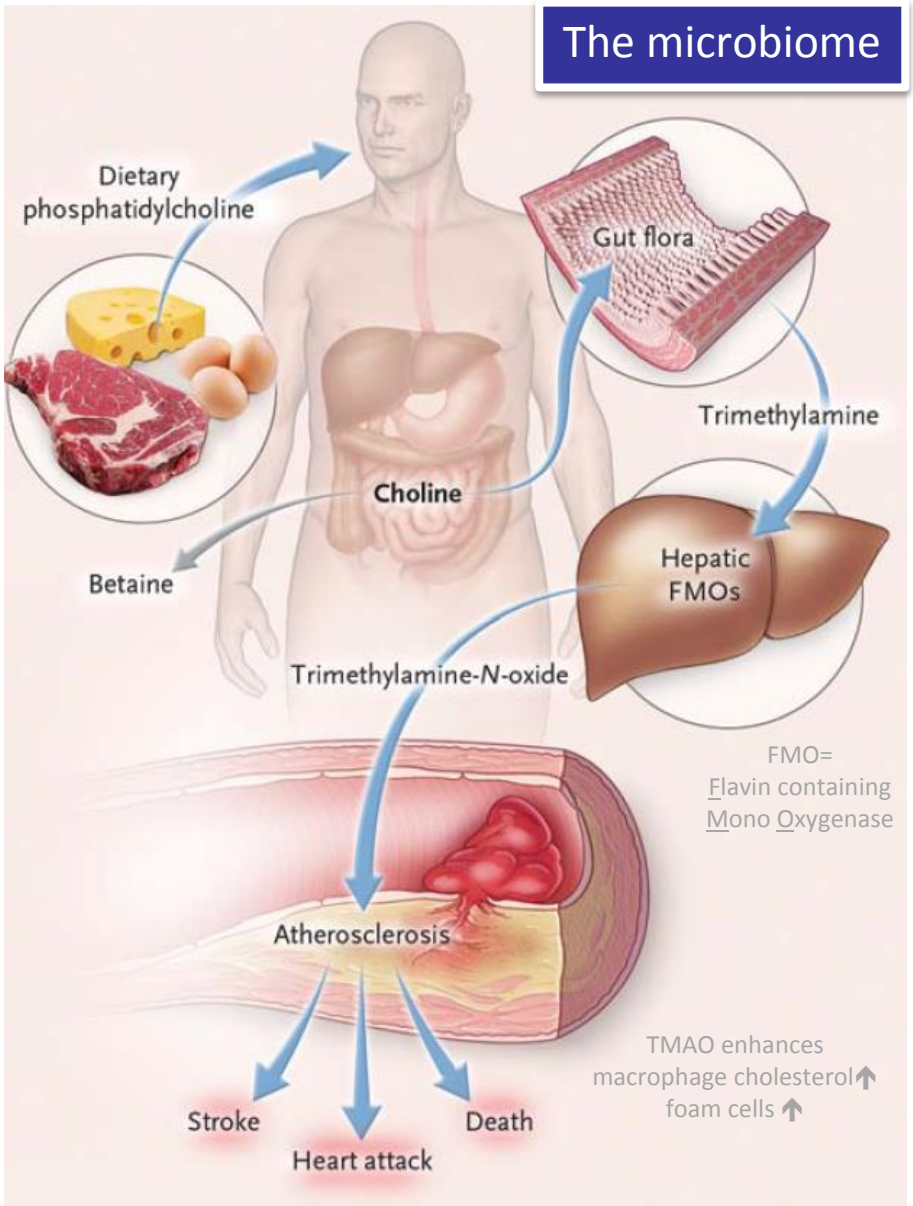
Critical amplification of the inflammatory response



From Ketelhuth et al 2013 Thrombosis and Hemostasis

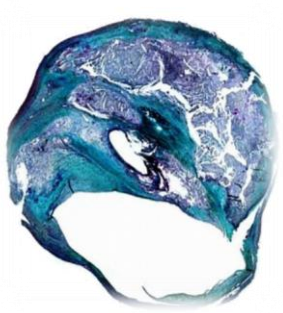
A new trigger of atherosclerosis

Trimetylamine (TMAO)



Tang et al NEJM 2013

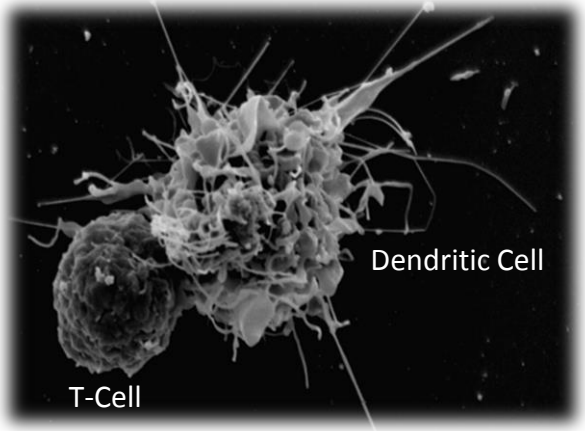
Atherosclerosis - *Vulnerability*



Inflammation

The innate immune response

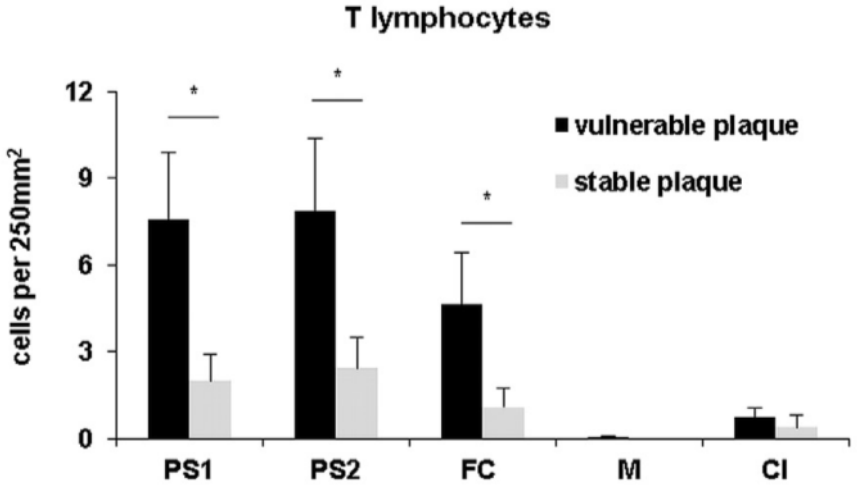
T-regulatory cells, Dendritic cells

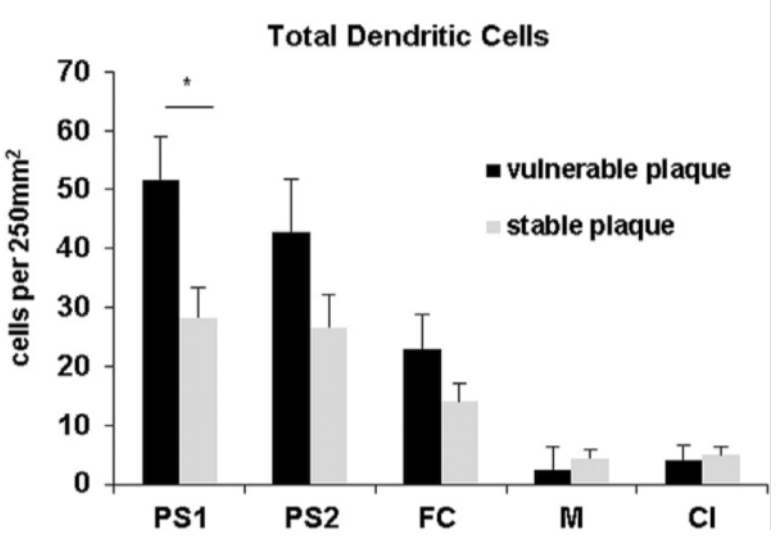


Atherosclerosis- *vulnerability*

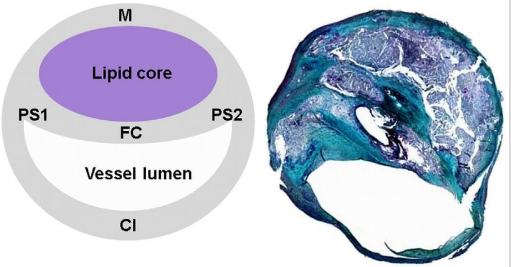
Innate immunity Tregs and DCs

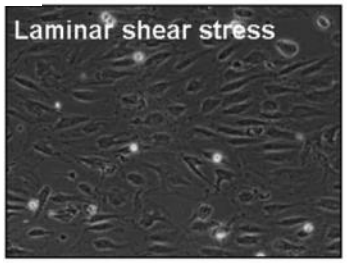
Regulatory T cells (Tregs) and **dendritic cells** (DCs) determine vulnerability

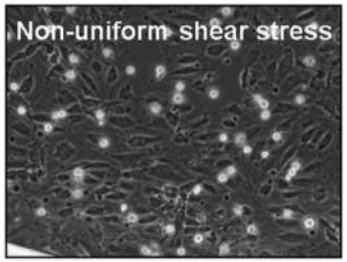




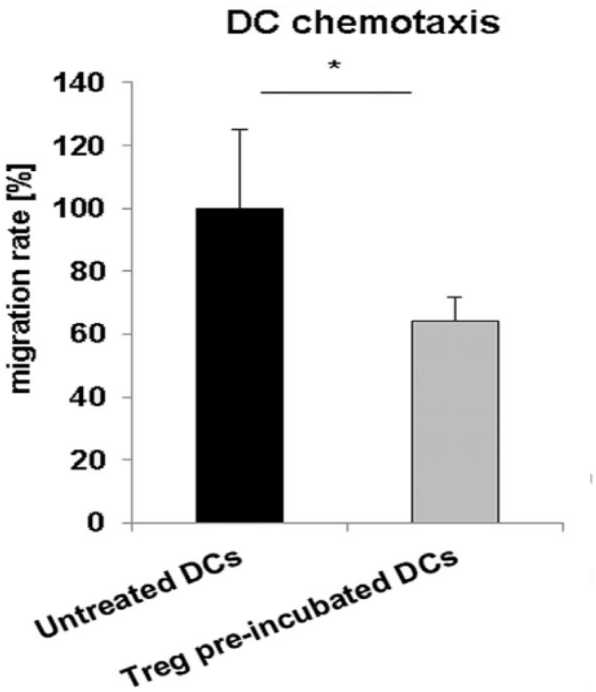
plaque shoulder (**PS1**)
plaque shoulder 2 (**PS2**)
fibrous cap (**FC**)
media (**M**)
contralateral intima (**CI**)





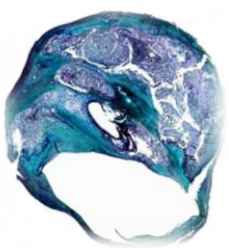


Dynamic adhesion of mature DCs to endothelial cell layer observed in bifurcation slides



From Dietel B, Garlichs C et al 2013 Atherosclerosis

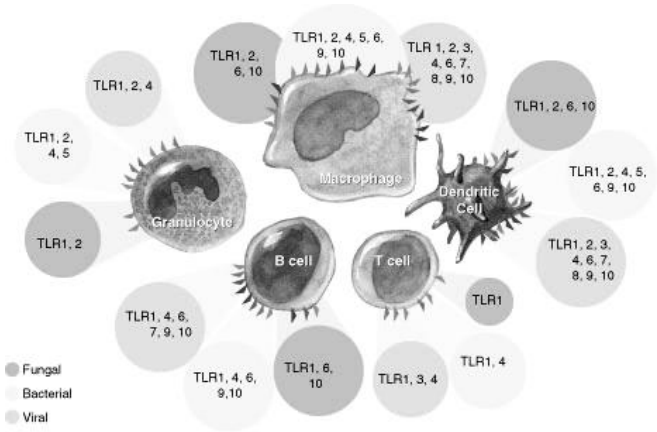
Atherosclerosis - *Vulnerability*



Inflammation

The innate immune response

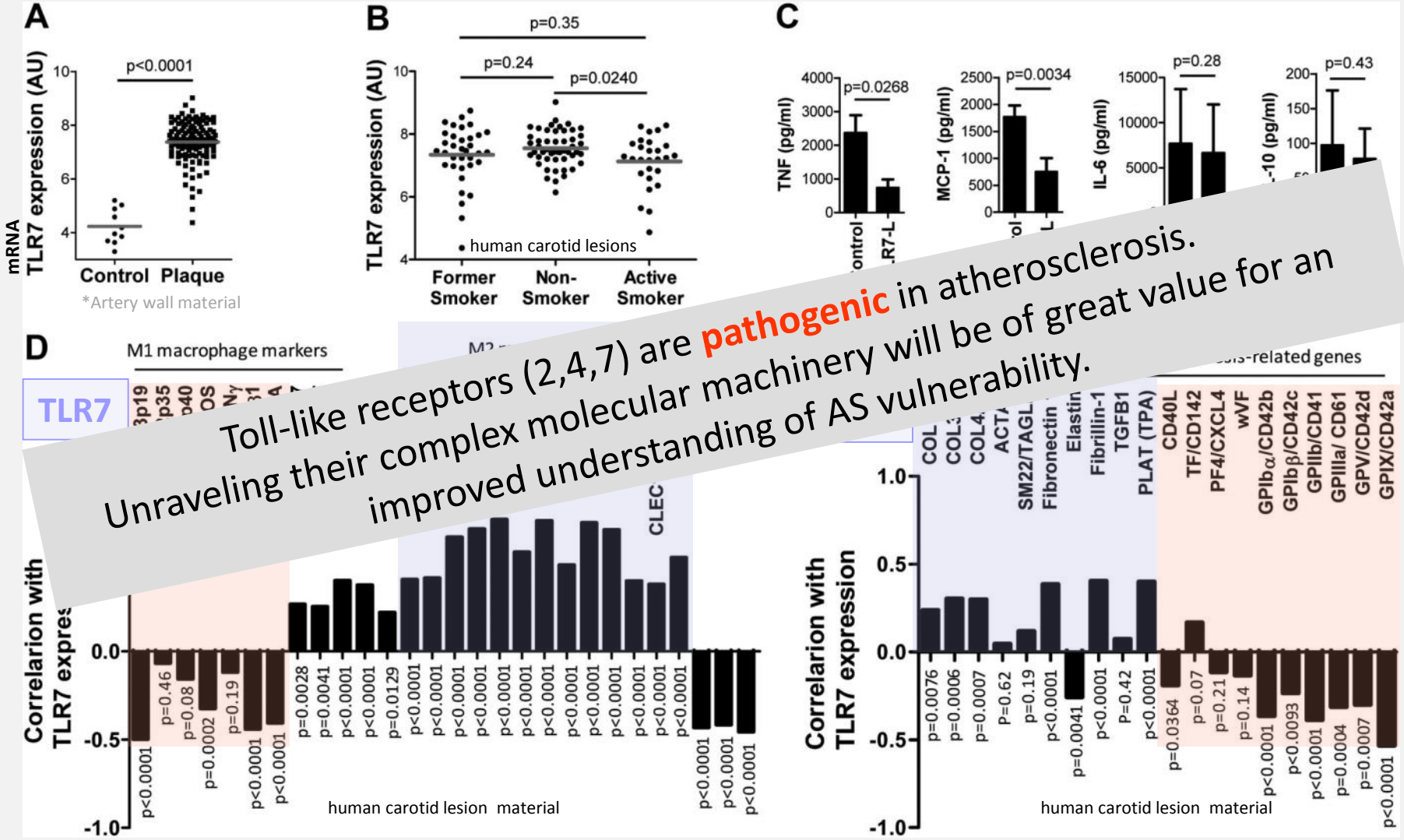
Toll like receptors



Atherosclerosis- *vulnerability*

Toll-Like Receptor 7 **protects** in human atherosclerosis

- TLR7 expression in human carotid plaques stimulates genes related to a more stable plaque phenotype



Toll-like receptors (2,4,7) are **pathogenic** in atherosclerosis.
Unraveling their complex molecular machinery will be of great value for an improved understanding of AS vulnerability.

*Specimens from patients undergoing carotid endarterectomy and specimens from healthy iliac arteries of organ donors

Salagianni et al Circulation 2013

Atherosclerosis - *Vulnerability*

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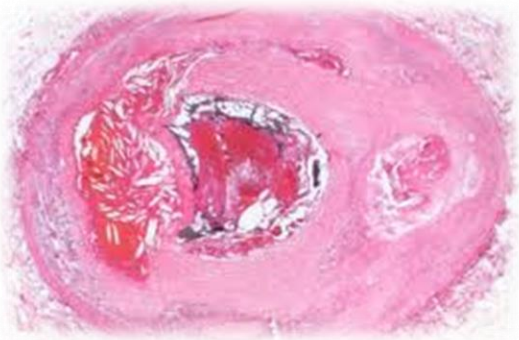
Intra-plaque hemorrhage



Takaya et al Circulation 2005

Atherosclerosis-*vulnerability*

The *balance* between *clotting* and *bleeding*

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Intraplaque hemorrhage

- found in culprit lesions of patients with unstable angina pectoris:

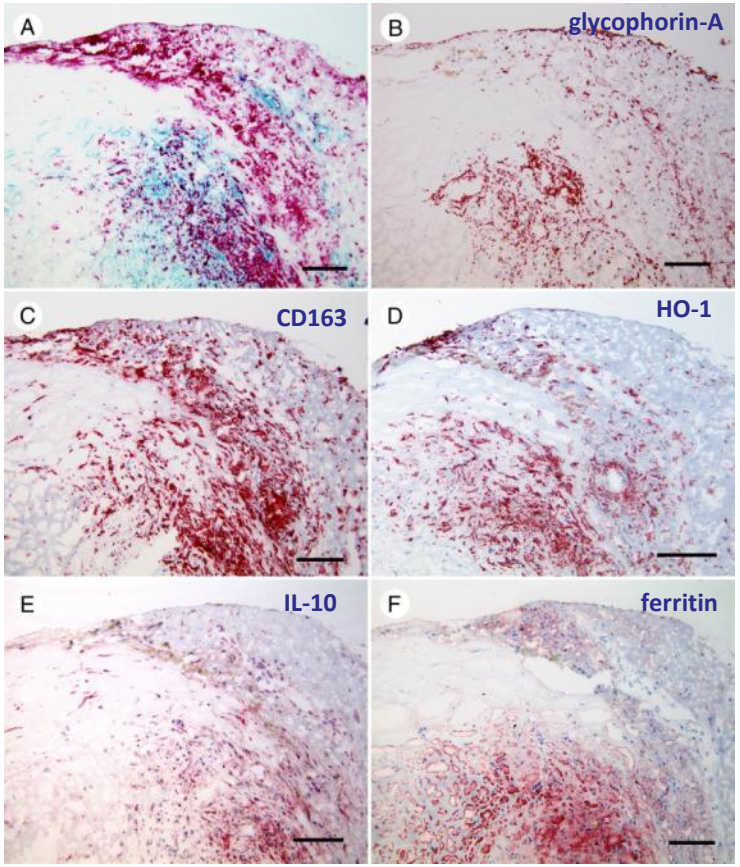
- **Hb/Hp scavenger receptor** (**CD163**)
- **IL-10**
- **hemoxygenase 1 (HO-1)**
- **Ferritin**
- **4-hydroxy-2-nonenal** (a major product of lipid peroxidation)*

Free hemoglobin induces **oxidative tissue damage** by hem iron, subsequent produced oxygen species are cleared by the macrophage Hb scavenger receptor (CD163),

Interleukin-10 and **ferritin** expression induced

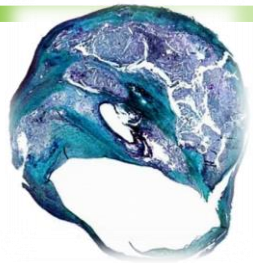
IL-10, **iron** content, **HO-1** activity - critical markers for vulnerability?
Potential targets for manipulation of events?

Atherectomy specimen from a **culprit lesion** in a patient with **Unstable Angina Pectoris (UAP)**



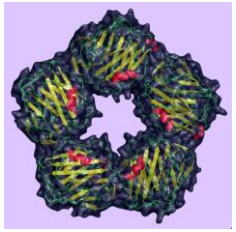
Double immunostaining for **smooth muscle cells (turquoise)** and **macrophages (red)**. B-F, Immunostaining for glycophorin-A (B), CD163 (C), HO-1 (D), IL-10 (E) and ferritin (F). Bar: A-F, 50 µm.
GlycophorinA =specific for erythrocytes

Yunoki et al Human Pathology (2013)
*Yunoki et al Eur Heart J (2009)
Cheng et al Circulation (2009)



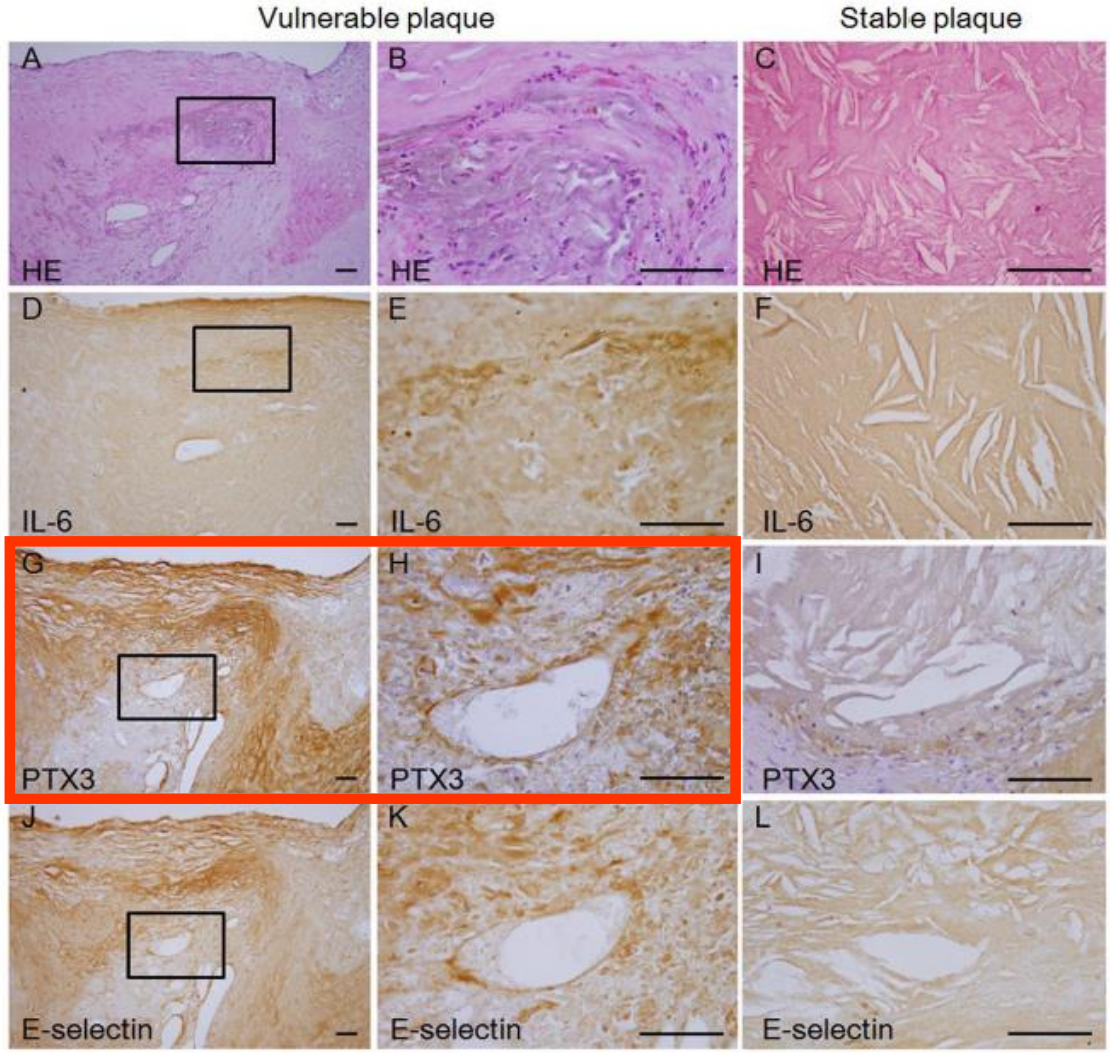
Atherosclerosis - *Vulnerability*

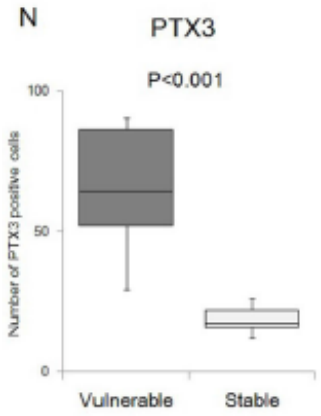
Selected potentially diagnostic Biomarkers involved in the former discussed mechanisms



Adaptive immune response

Pentraxin 3 - carotid plaque inflammation





Pentraxin 3 (PTX3)

increased

in carotid plaques

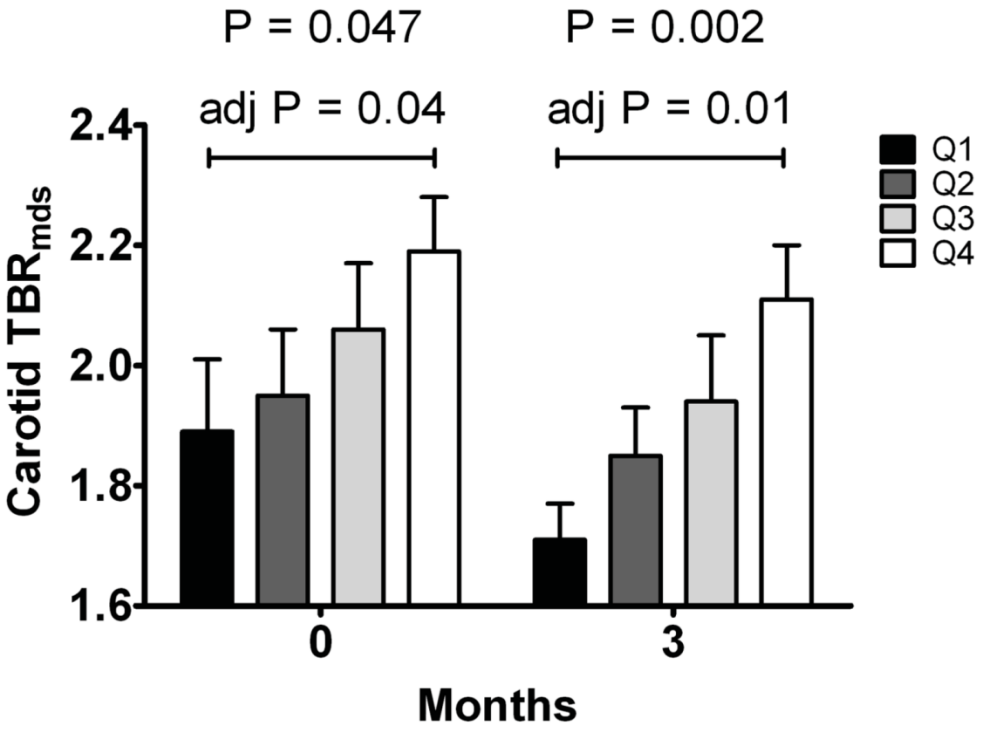
not expressed in the liver

AS plaque specific

PTX3 mainly expressed in DCs, ECs, SMCs, macrophages, fibroblasts

Monocyte/Macrophage

Myeloperoxidase - carotid plaque inflammation



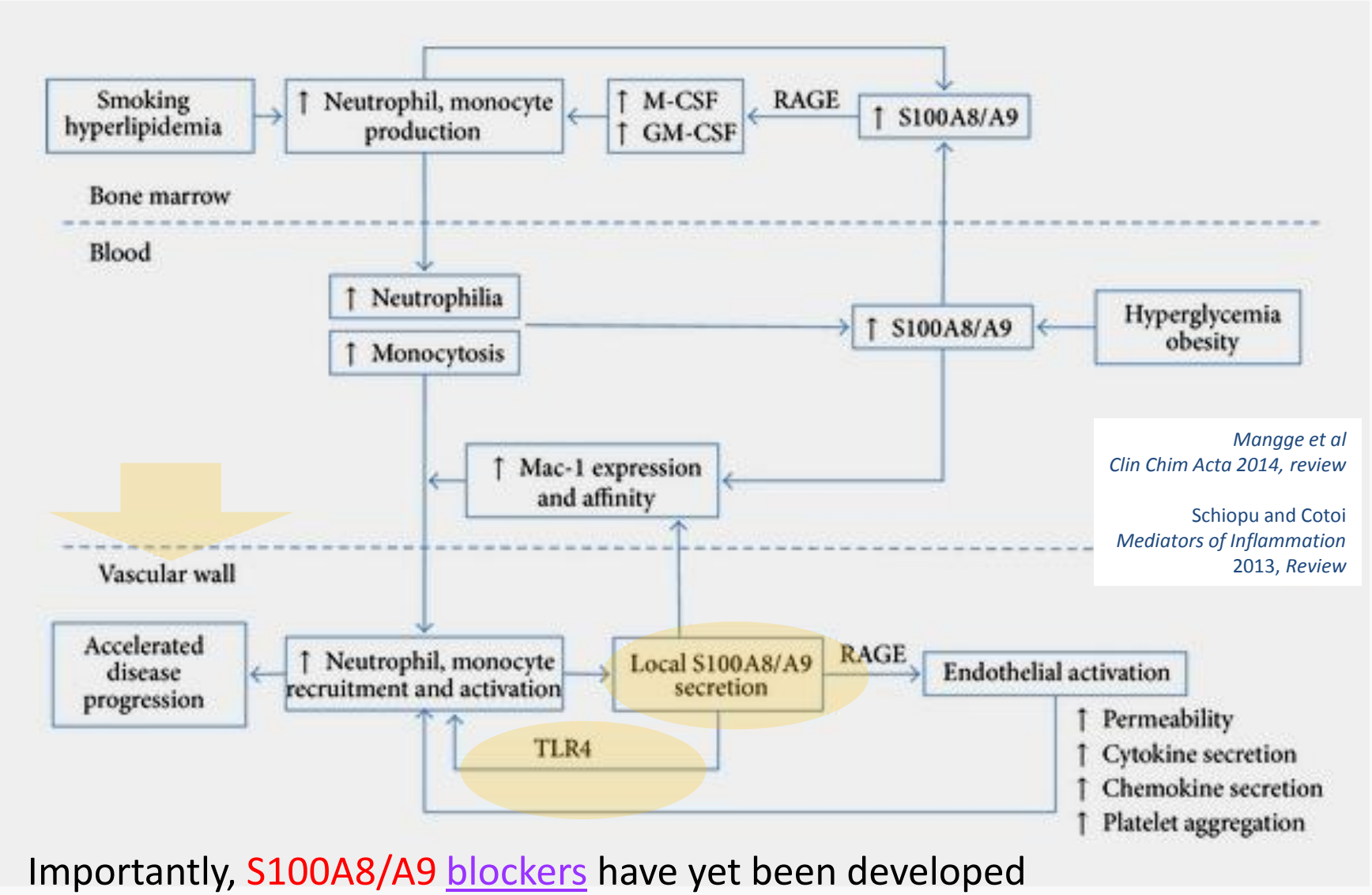
Plasma Myeloperoxidase
Quartiles

Raphaël Duivenvoorden et.al.
J A C C : CARDIOVASCULAR IMAGING
2013

Figure 1. Carotid TBR$_{mds}$ Values at Baseline and 3 Months' Follow-Up for Baseline MPO Quartiles

- Higher baseline myeloperoxidase (MPO) values were associated with higher baseline carotid target-to-background ratio of the most diseased segment (TBRmds) values
- Relation remained present at 3-month follow-up

Mangge et al
Clin Chim Acta 2014, review



Mangge et al
Clin Chim Acta 2014, review

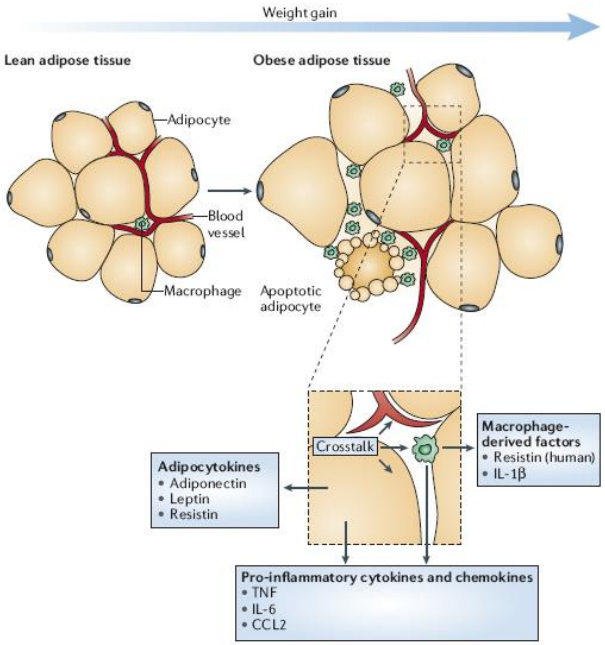
Schiopu and Cotoi
Mediators of Inflammation
2013, *Review*

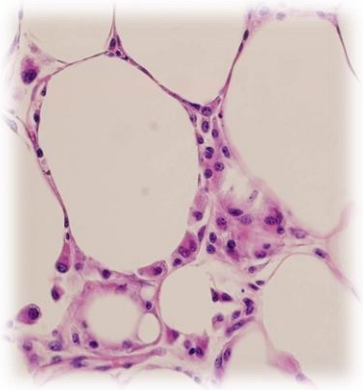
Importantly, S100A8/A9 blockers have yet been developed and are approved for clinical testing.

Atherosclerosis - *Vulnerability*

Selected potentially diagnostic Biomarkers

Adipokines

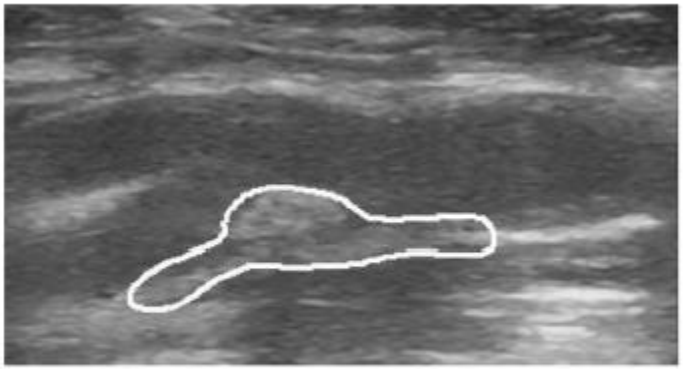




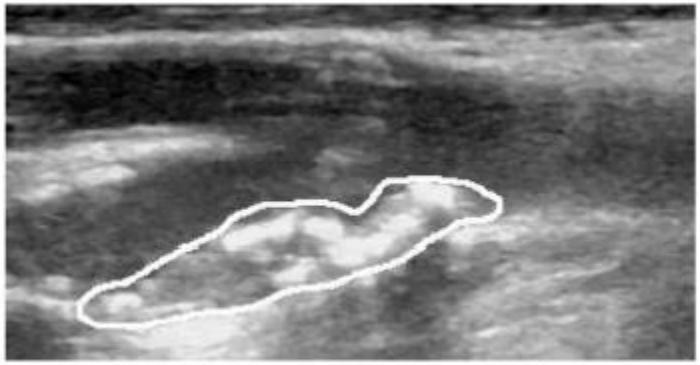
Inflammation related adipokines

Blood RBP4 and omentin-1

GSM = Grey Scale Median index



(a) stenosis: >70%, GSM: 20



(b) stenosis: >70%, GSM: 144

Differences in clinical and biochemical parameters in high-grade and low-grade carotid stenosis groups.

Variables	High-grade carotid stenosis group ($N = 225$)	Low-grade carotid stenosis group ($N = 75$)	p
RBP-4 (mg/L)	51.44 ± 16.23	38.39 ± 8.85	<0.001
Omentin-1 (ng/ml)	490.41 ± 172	603.20 ± 202.43	<0.001
TPA (mm^2)	0.93 ± 0.37	0.50 ± 0.24	<0.001
GSM score	44.36 ± 15.40	63.63 ± 22.75	<0.001

Variables	Established carotid atherosclerosis ($N = 300$)	Control group ($N = 73$)	p
RBP4 (mg/L)	48.18 ± 14.12	25.74 ± 10.72	<0.001
Omentin-1 (ng/ml)	518.61 ± 191.10	815.3 ± 185.32	<0.001

Independent associations of total plaque area with variables using linear standard multiple regression analysis ($R^2 = 0.201$, $p = 0.005$).

Variables	Total plaque area		
	beta	p	95% CI
RBP4	0.242	0.021	0.193–0.312

CI, confidence intervals; hsCRP, high-sensitivity C-reactive protein.

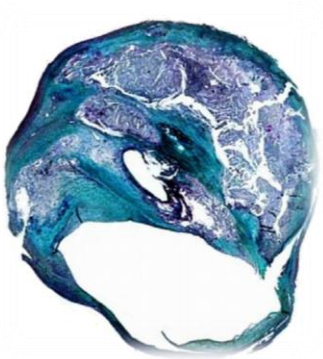
Retinol binding protein 4 ↑

Omentin-1 ↓

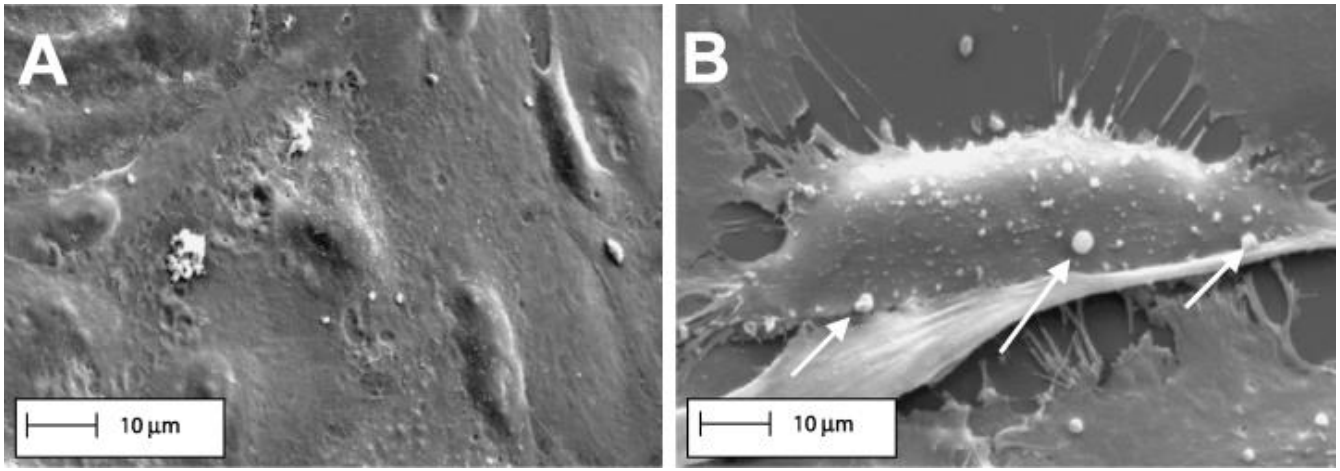
with AS severity and echolucency

Kadoglu et al
Atherosclerosis 2014, PMID: 24956535

Atherosclerosis - *Vulnerability*

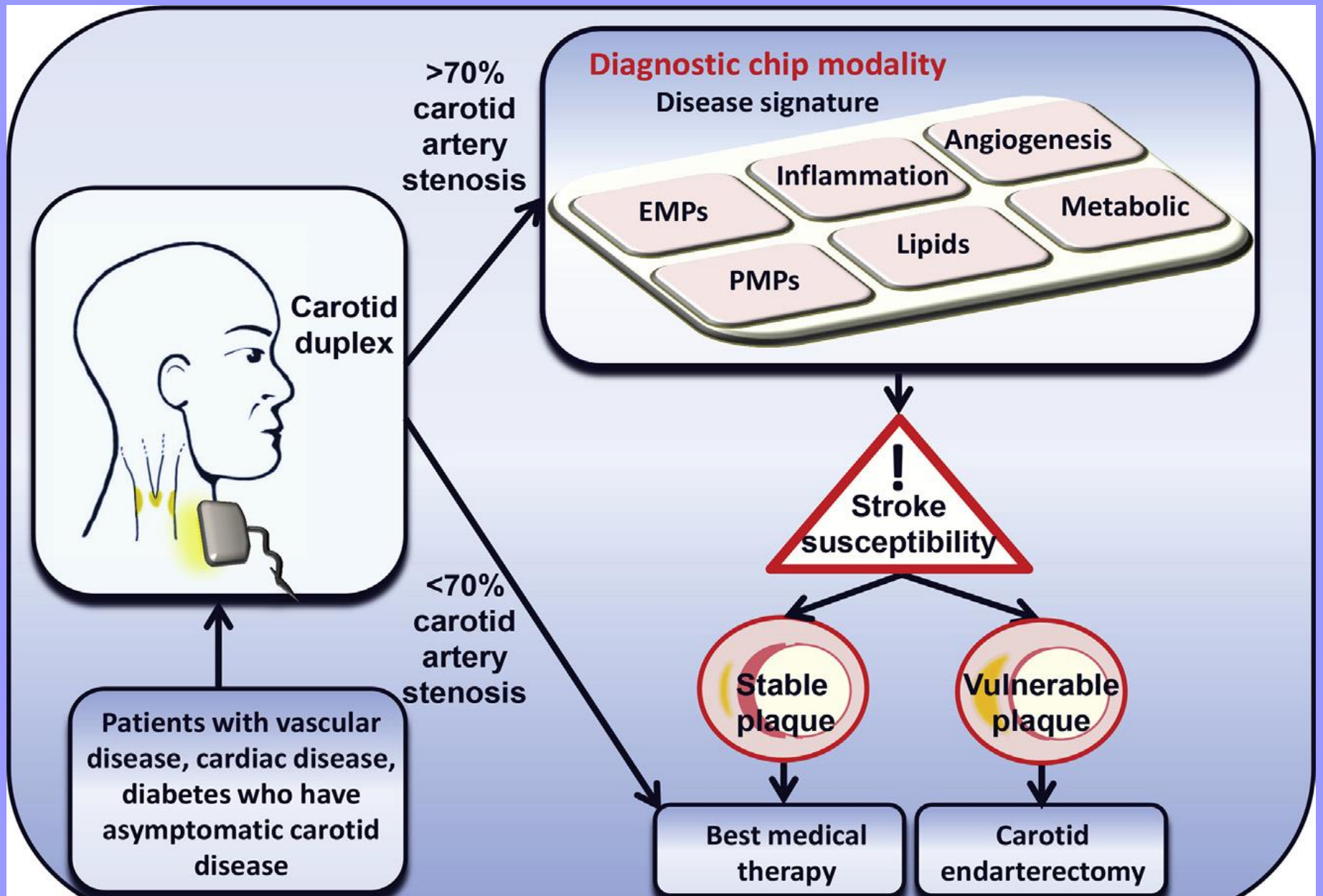


Blood celluar microparticles as conveyors of information for the whole process



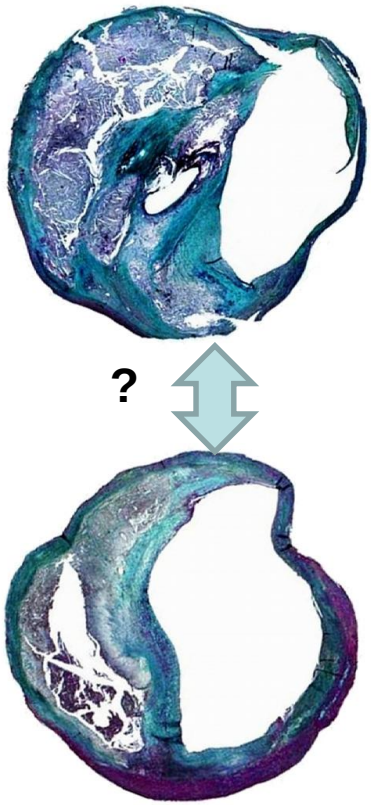
Diamant et al EuJClinInves, 2004
Umbilical vein endothelial cells (A) and the formation of microparticles after stimulation of the cells with interleukin-1α (B).

A holistic approach for improved prediagnosis



Conclusion - how to "catch" the vulnerable phase

Blood and/or *intra-plaque* biomarkers?



Candidates

- Proinflammatory: plasma S100A8/A9, plasma myeloperoxidase, plasma RBP4, *plaque* pentraxin 3, *plaque* iron content
- Anti-inflammatory: plasma omentin-1, plasma interleukin 10

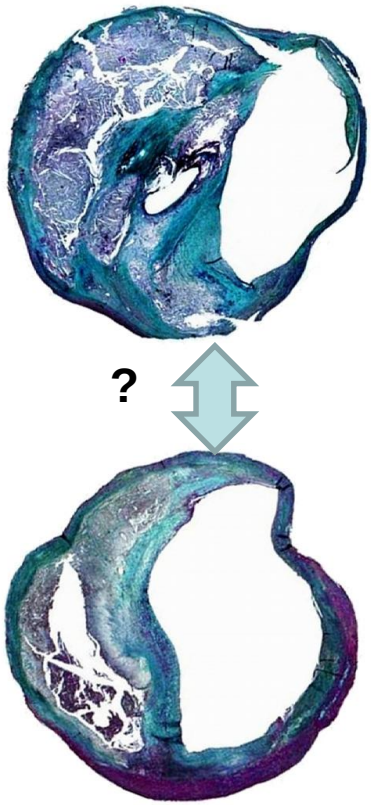
Main problem - "patient" stratification

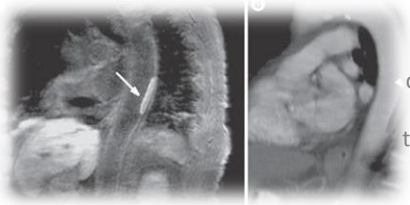
Screen in asymptomatic individuals. Who? At which time? Influence of Co-morbidities.

Elevated only in the acute phase – **too late…**

Conclusion - how to “catch” the vulnerable phase

"Intelligent” contrast agents?





In vivo MRI of human thrombi using a fibrin-targeting peptide conjugated to gadolinium-tetraazacyclododecane tetraacetic acid (EP-2104R) Shaw, S. Y. (2009) *Nat. Rev. Cardiol.*

Problems, hurdles

- **To find** a target–ligand combination **specific** for the vulnerable plaque.
- The specific binding should be reversible, pharmacokinetics favorable, and should generate a robust contrast change.
- **To find** the right animal model, and the development validation pipeline to human application.

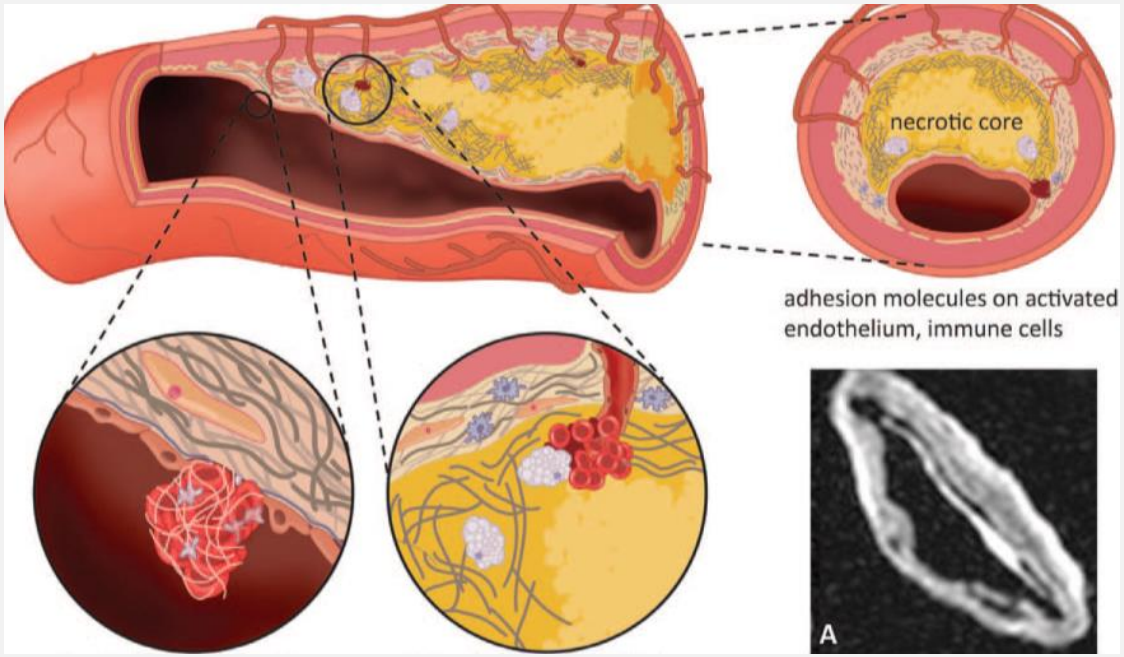
Main problem - "patient" stratification

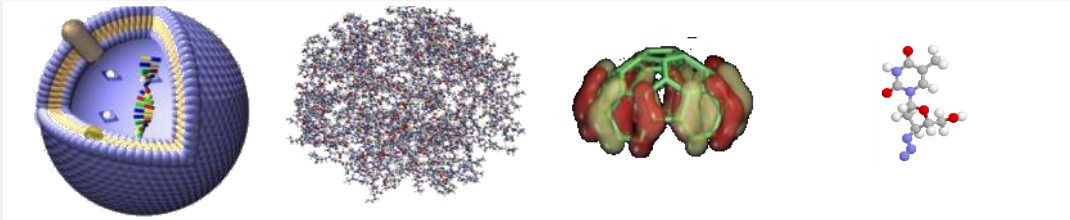
Screen in asymptomatic individuals. Who? At which time?

Nanotechnology for Atherosclerosis?

1. NPs have a ***high*** *surface to volume ratio* well suited for coating with targeting molecules

2. the ***size (10-~500 nm)*** fits well for targeting the "party"





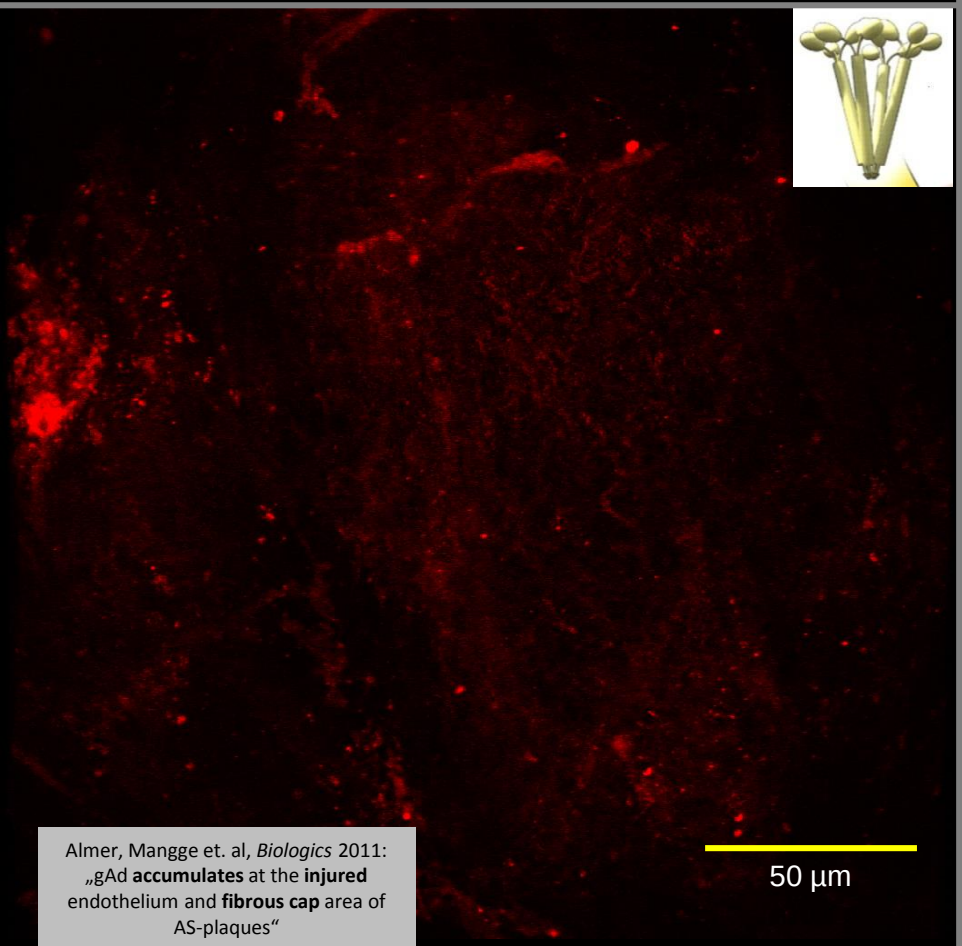
80-200 nm	~ 50 nm	~ 10 nm	1-5 nm
Liposomes	Dendrimers	Buckyballs	Drugs

Yunoki et al Human Pathology (2013)

Results, confocal laser scan microscopy (CLSM)

Full-length versus globular Adiponectin (***ex vivo*** data)

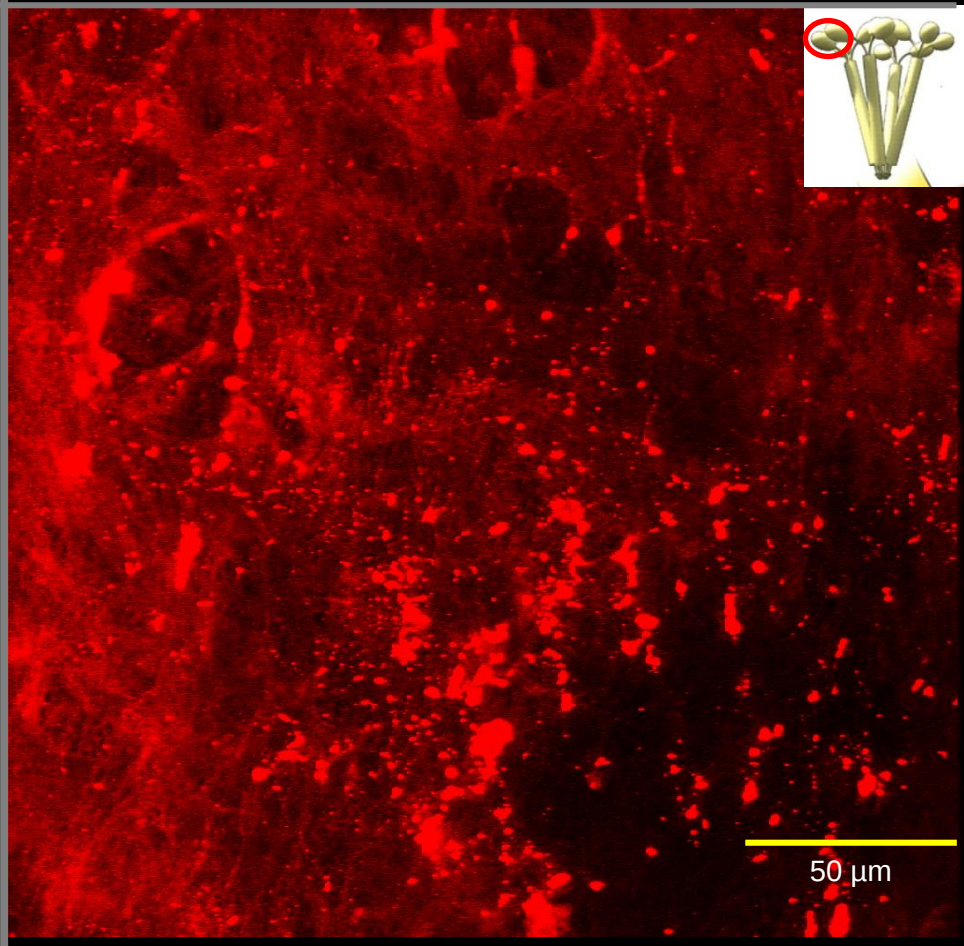
Plaque stained with **full-length** Ad^{ATTO655}



Almer, Mangge et. al, *Biologics* 2011: „gAd **accumulates** at the **injured** endothelium and **fibrous cap** area of AS-plaques“

30 µg OligoAd-ATTO655/ml Krebs-Henseleit Buffer + 1% BSA
120 min at 37°C on the shaker

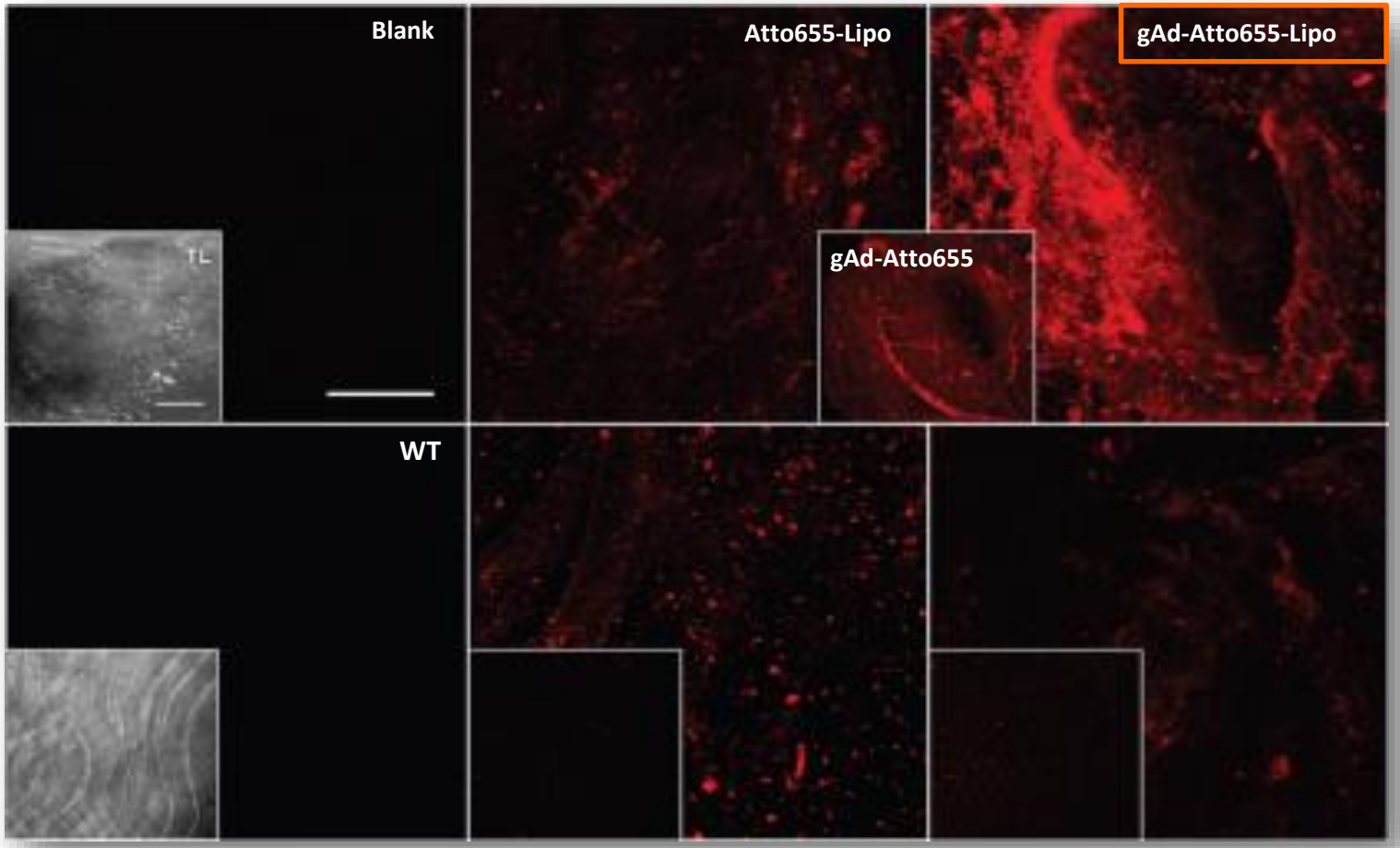
Plaque stained with **globular**Ad^{ATTO655}

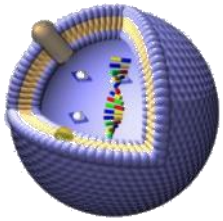


20 µg gAd-ATTO655/ml Krebs-Henseleit Buffer + 1% BSA
120 min at 37°C on the shaker

Adiponectin coated "stealth liposomes"

gAd and gAd-Lipos accumulate at atherosclerotic plaques surface *ex vivo*





Plaque

1-2 h incubation (37°C)

20 µg/mL gAd-Atto655

Atto-655 = red

Scale bars = 50 µm

Aorta

Almer et al. International Journal of Nanomedicine 2011:6 1279-1290

“Conclusion”

The challenge remains

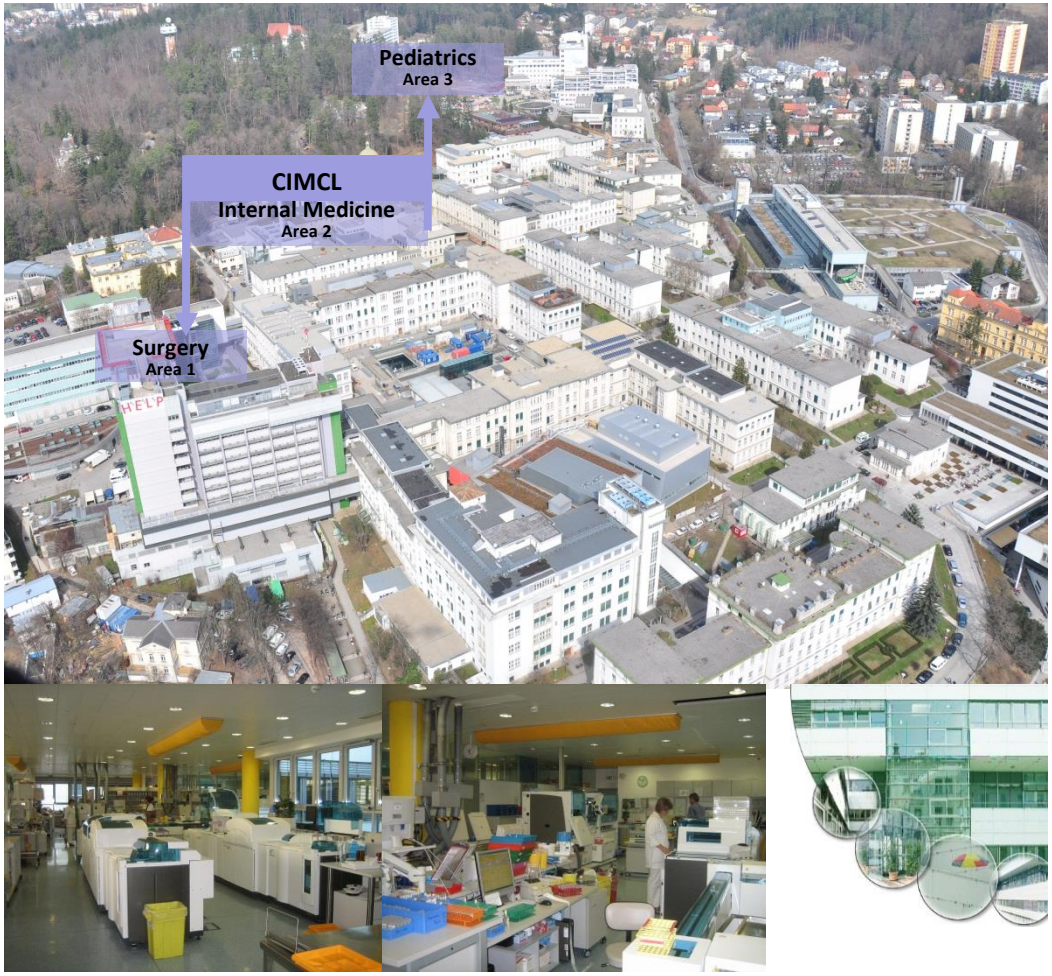
1.How can we diagnose a vulnerable AS lesion at the “**right time”** in a still asymptomatic person/patient?

2.How to treat this person effectively, **without side** effects? Any complication in a yet healthy person is inacceptable!

3.The cooperation between **Nanotechnology, Laboratory Medicine** and **Radiology** may help in this context by the development of NP constructs between stealth liposomes and targeting biomarkers.

Thanks for your attention!

Laboratory Medicine



- Clinical Institute for Medical and Chemical Laboratory Diagnostics, CIMCL

Total clinical laboratory diagnostics for the tertiary universitary hospital center of styria, R&D, teaching

~**7** Mill Analysis/year

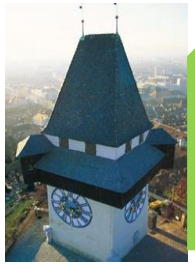
127 coworkers

KAGES: 4 MDs, 4 PhDs, **87** Technicians , 18 other staff

MUG: 4 MDs, 3 PhDs, 7 Technicians

Cardiovascular Research KIMCL Graz

Two Lead Projects



STYJOBS / EDECTA

STYrian Juvenile OBesity Study / Early Detection of Atherosclerosis

Medical University, Karl Franzens-University, Technical University of Graz

Paracelsus Medical University Salzburg, Medical University of Innsbruck

Daniel Weghuber, Barbara Eder, Katharina Paulmichl, Dietmar Fuchs

Maryland School of Medicine, Baltimore

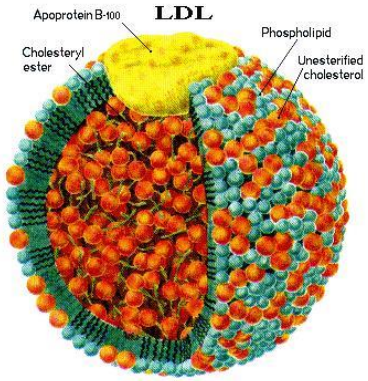
Gloria Reeves,Teodor T. Postolache

EU-Project NanoAthero

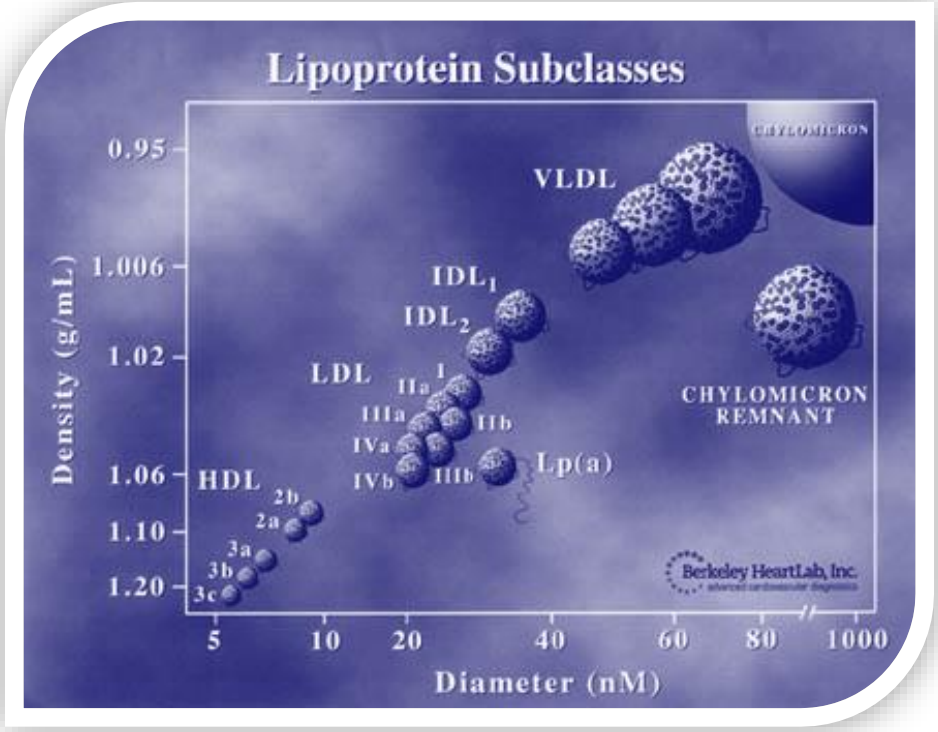
16 Partners from 10 Countries

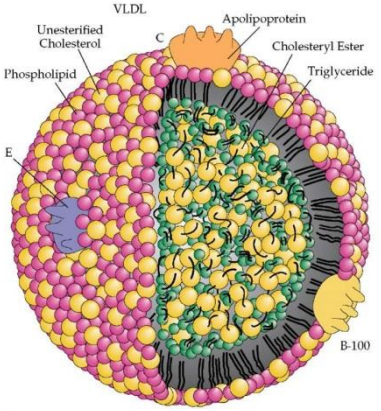
Austria, France, Denmark, Germany, Great Britain, Hungary, Italy, Israel, Netherlands, Swiss

Atherogenic Lipoproteins



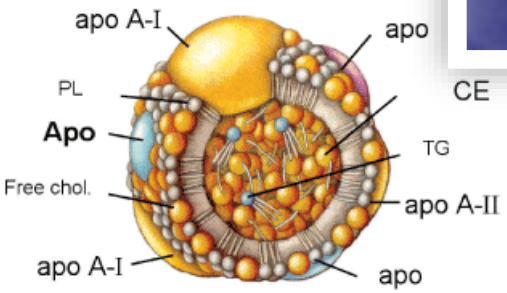
Small dense LDL



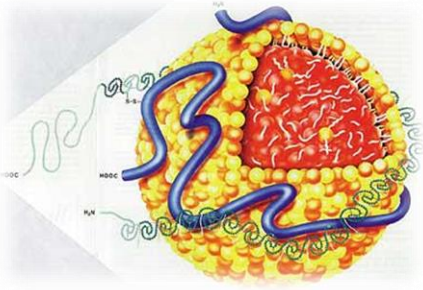


Triglyceride-rich remnants

Dysfunctional HDL

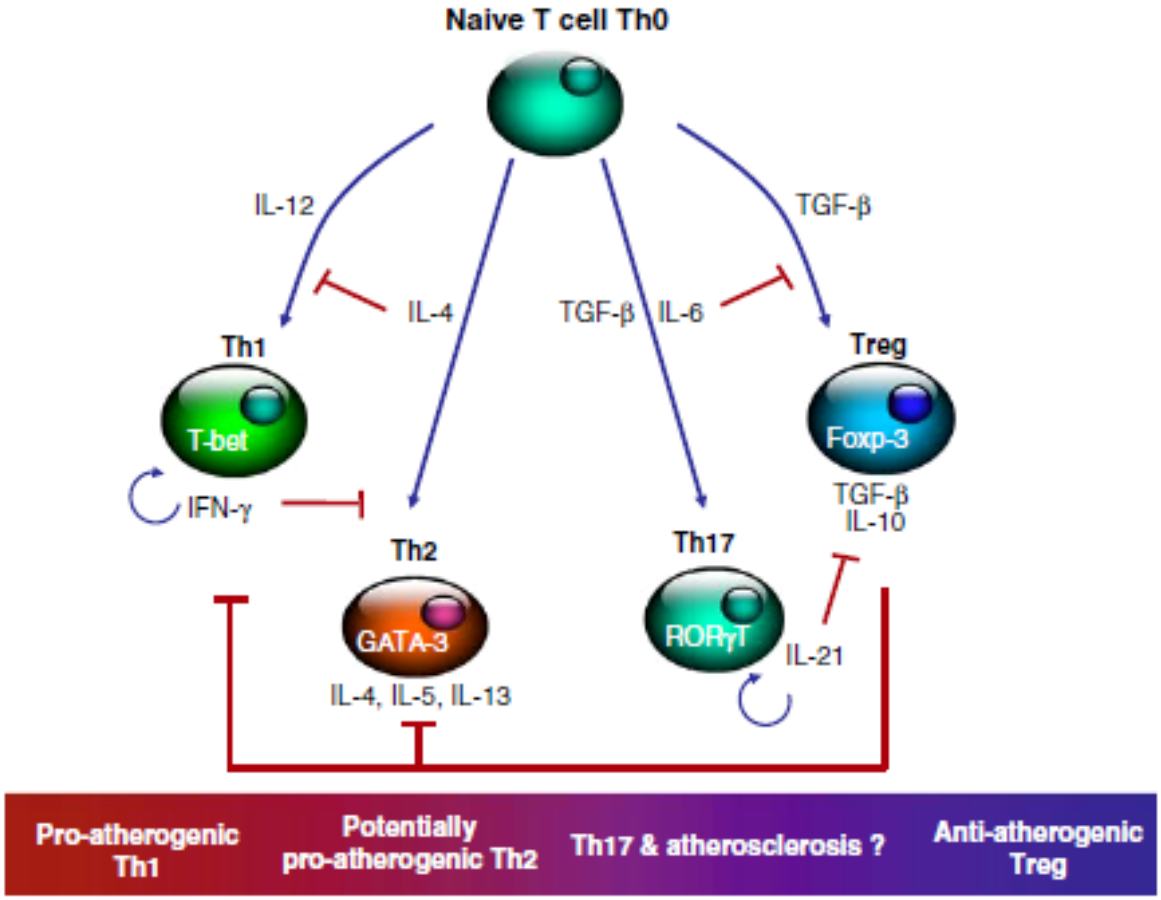


Lipoprotein (a)



Atherosclerosis- *vulnerability*

Control of inflammation - "bad" and "good" T-cells



Hafid Ait-Oufella, Tedgui et al Semin Immunopathol 2009
Mangge et al Clin Chim Acta rev1 2014, Curr Med Chem 2010