Immune interactions in chronic inflammation: old friends and new foes

Christoph J. Binder, MD, PhD

3rd International Danube Symposium

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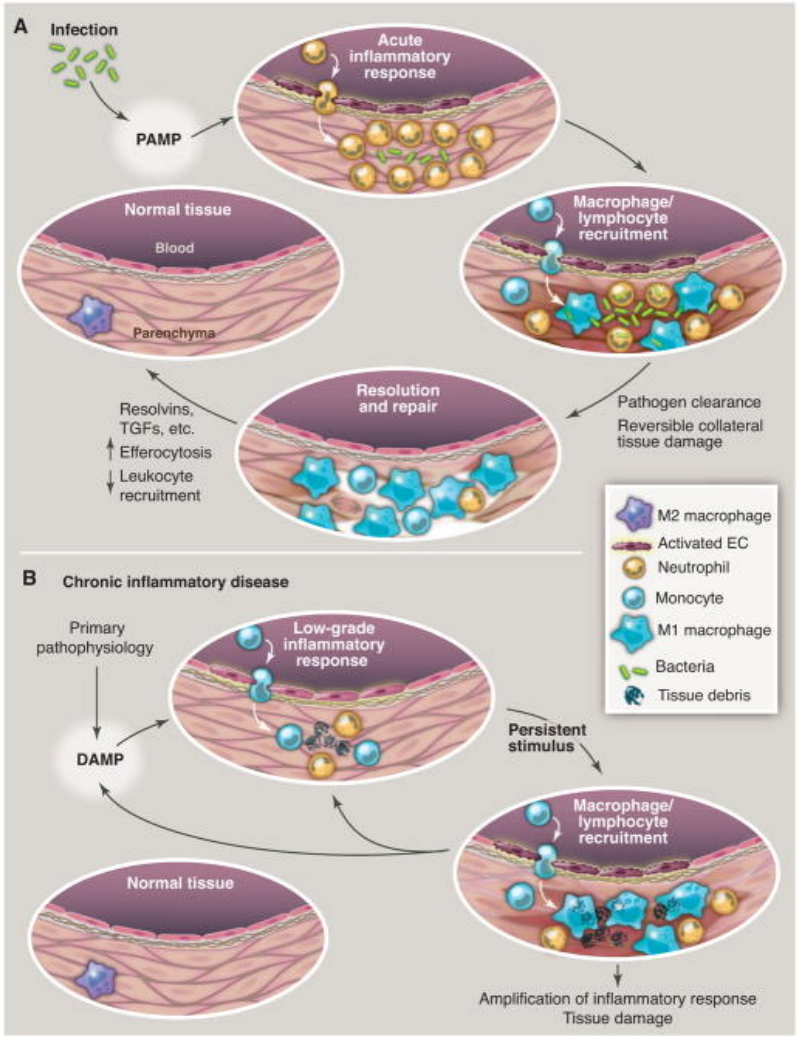
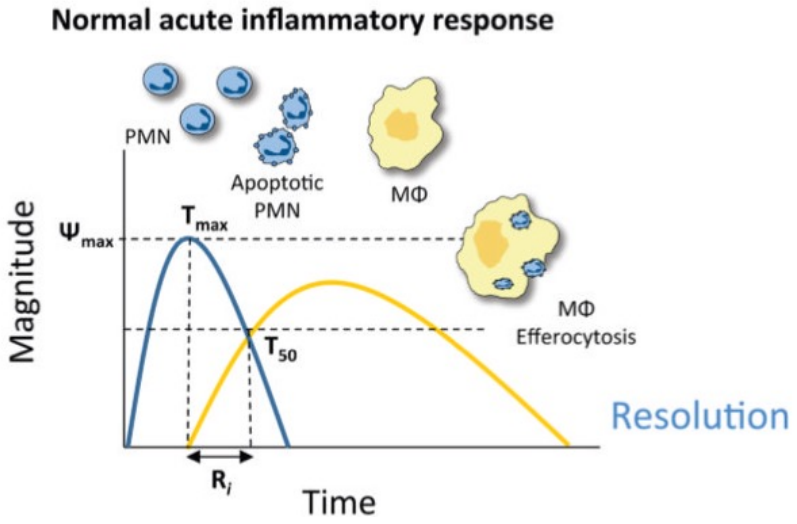
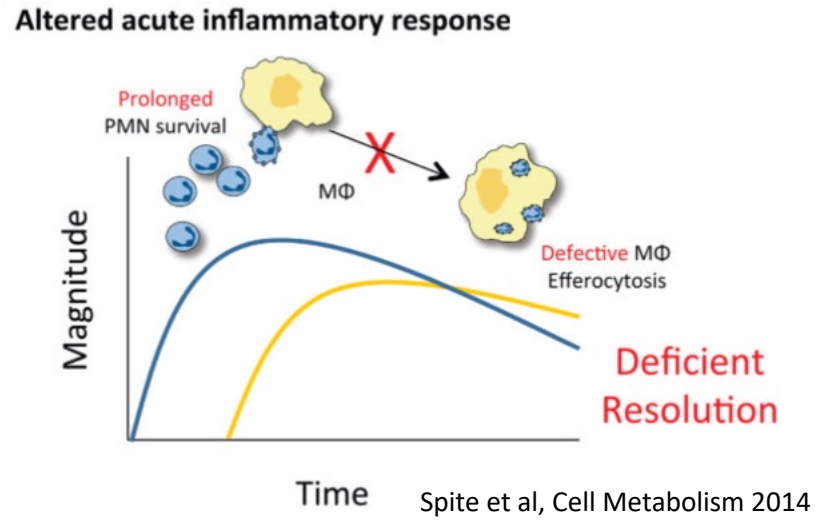
21. September 2023
Vienna

Impaired resolution triggers chronic inflammation



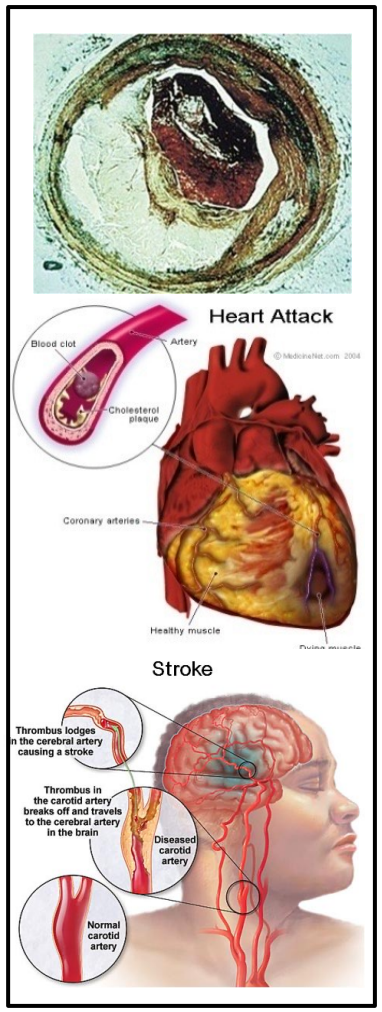
Tabas I & Glass CK, Science 2013

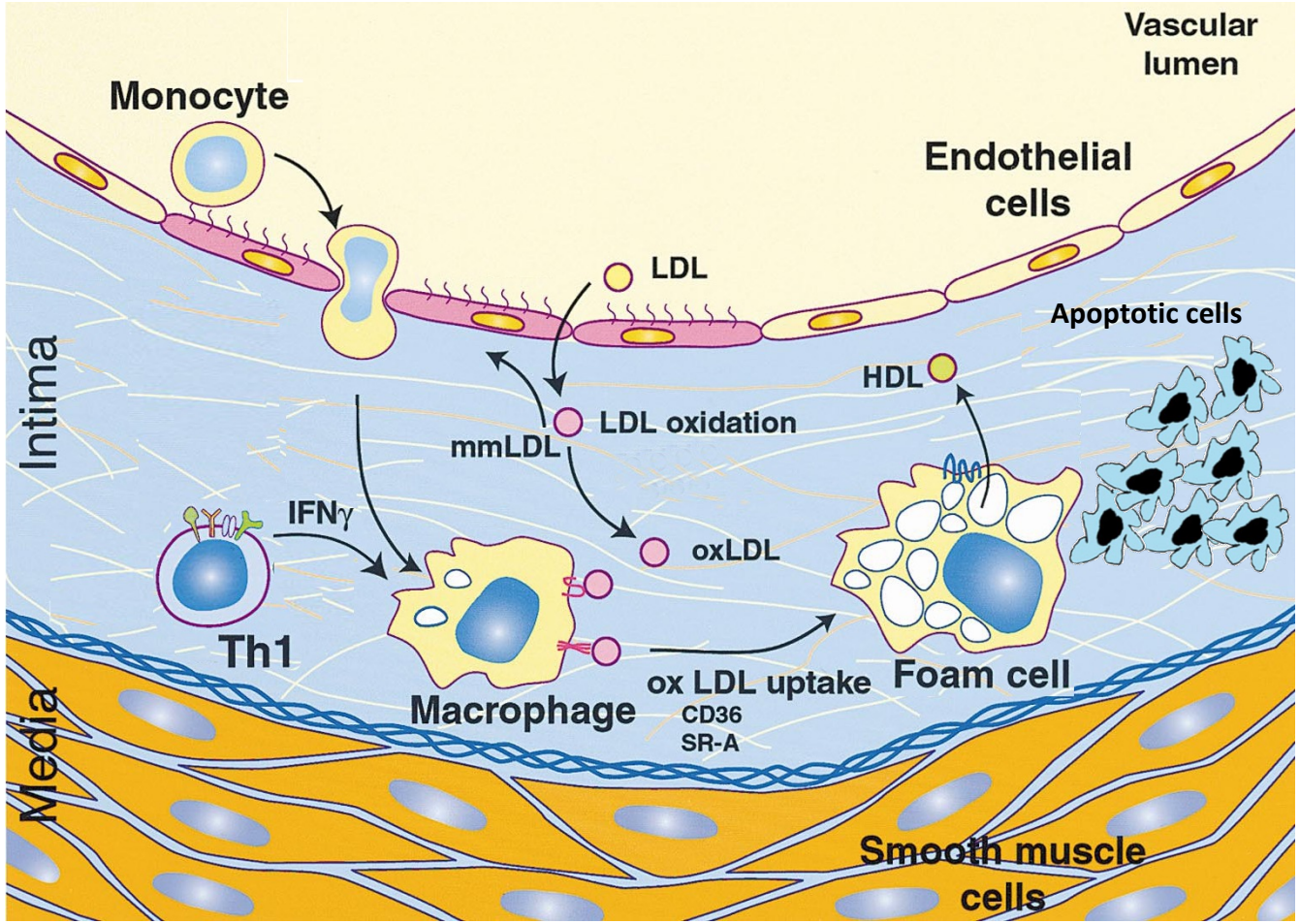




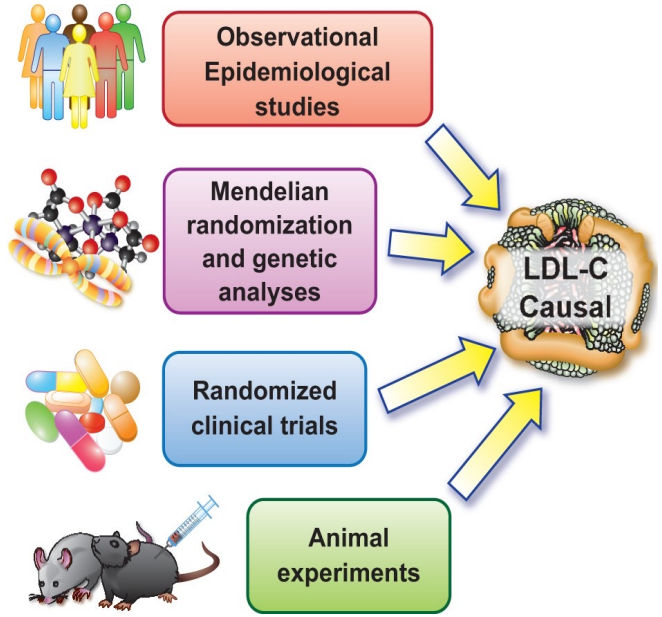
Spite et al, Cell Metabolism 2014

Atherosclerosis is a chronic inflammatory disease triggered by the accumulation of low-denisty lipoproteins (LDL)



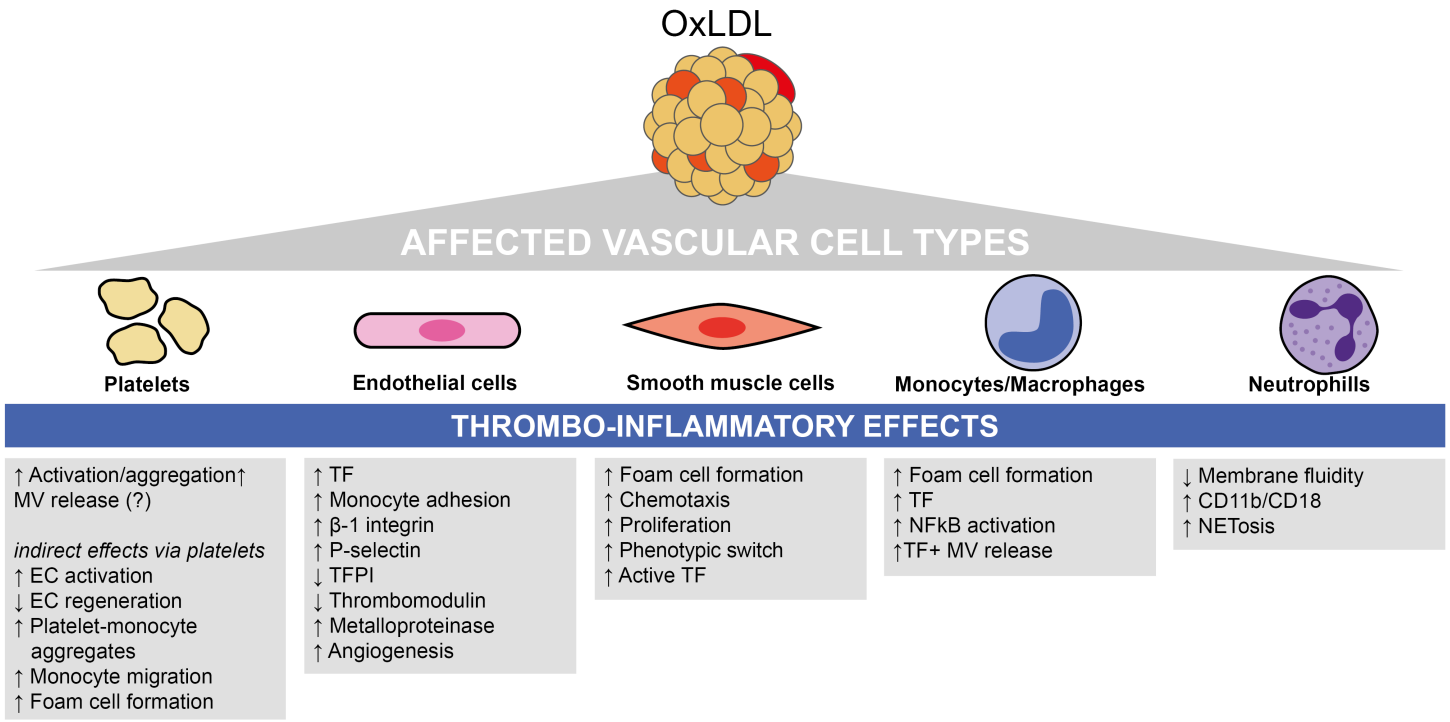


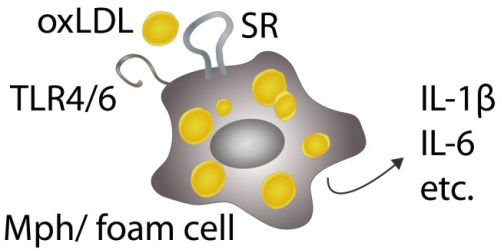
Modified from Glass & Witztum, Cell 2001



Tokgözoğlu & Libby, Eur Heart J 2022

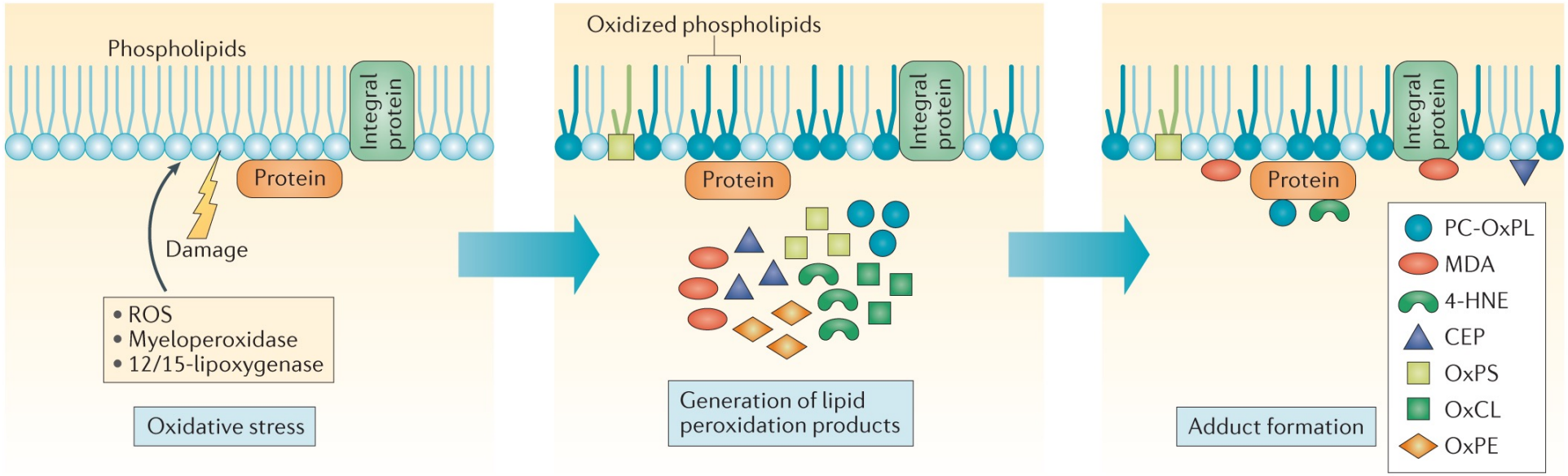
The proinflammatory actions of oxidized LDL





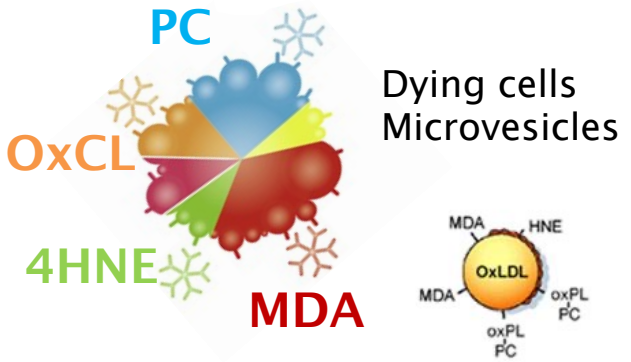
Obermayer et al, *JTH* 2018

Generation of Oxidation-specific Epitopes (OSE)



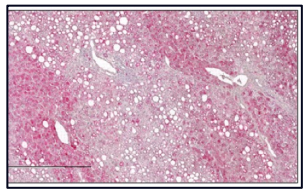
Binder et al., *Nature Rev. Immunol,* 2016

PC-OxPL….Phosphocholine of OxPL
MDA…….Malondialdehyde
4-HNE……..4-Hydroxynonenal
CEP…….Carboxyethylpyrrole
OxPS……..Oxidized Phosphatidylserine
OxCL……Oxidized Cardiolipin
OxPE……Oxidized Phosphatidylethanolamine



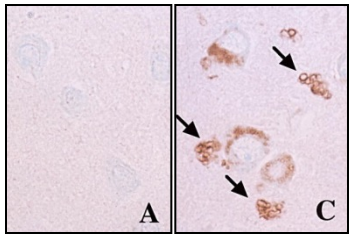
OSE are present in various pathological settings

Non-alcoholic steatohepatitis



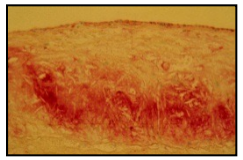
Sun et al. Cell Metabolism 2020

Alzheimer‘s disease



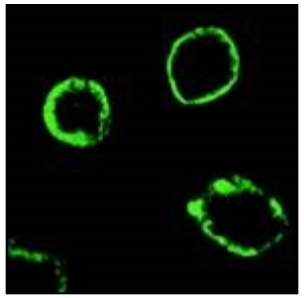
Dei et al. Acta Neuropath 2002

Atherosclerosis



Palinski et al. Arteriosclerosis 1996

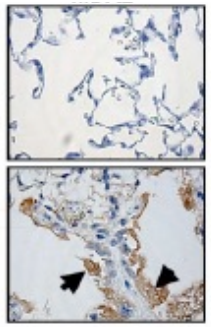
Apoptotic cells



Chang et al. PNAS 1999

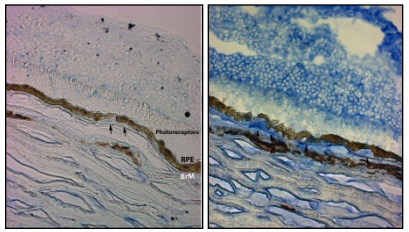
Oxidation-specific Epitopes

Acute lung injury



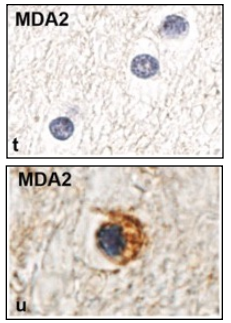
Imai et al. Cell 2008

Age-related Macular Degeneration



Weismann et al. Nature 2011

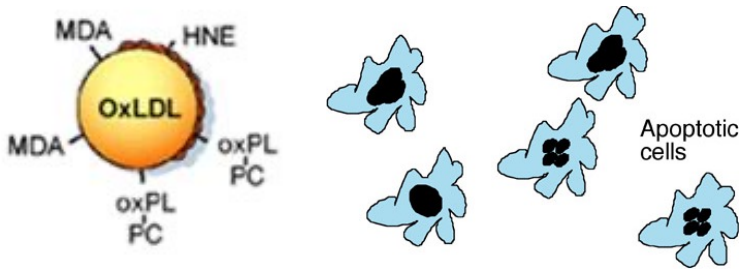
Multiple Sclerosis



Haider et al. Brain 2011

Oxidation-specific epitopes are targets of innate immunity

Accumulation of OxLDL, apoptotic cells, cell debris

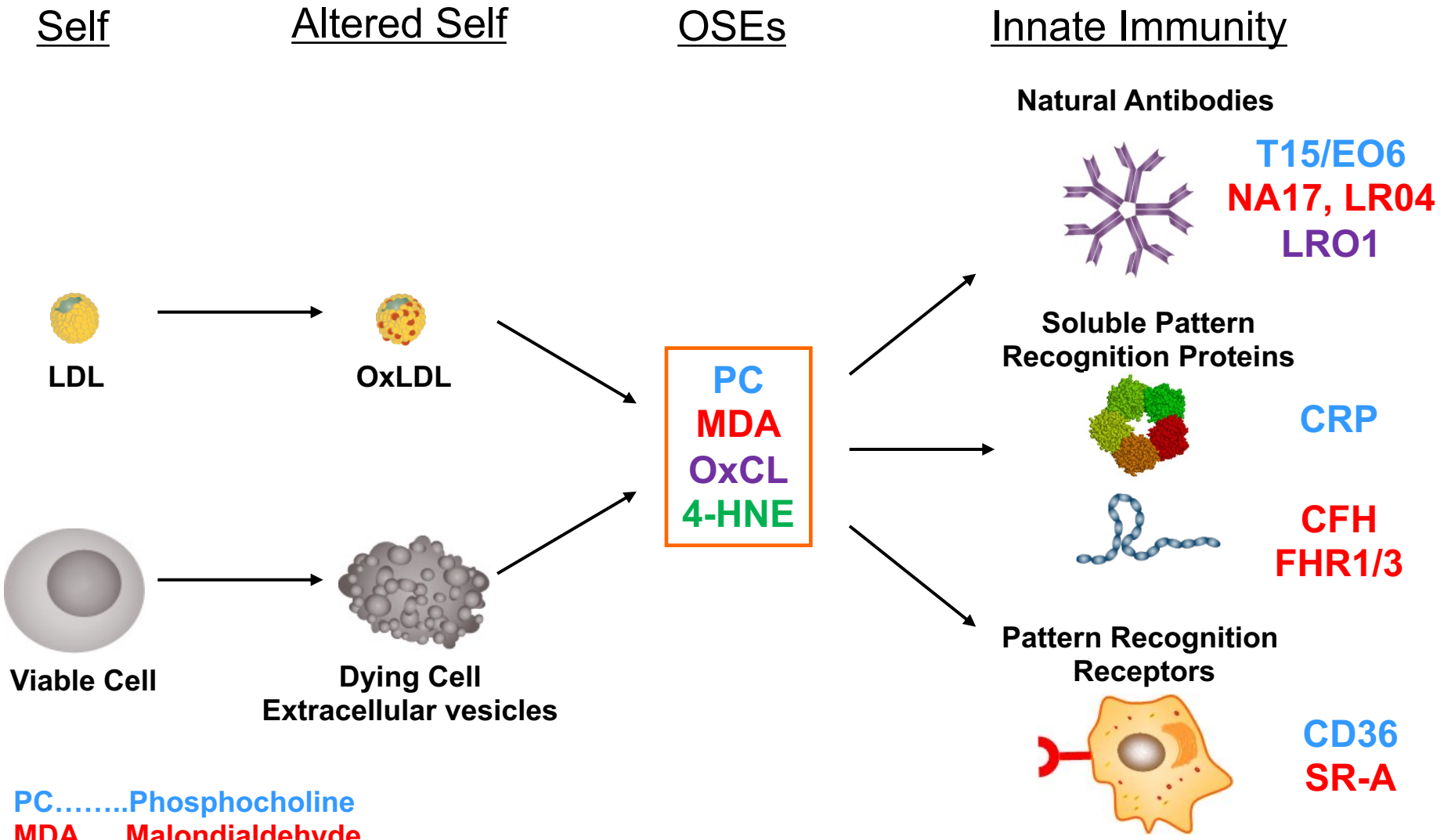


Contain lipid-peroxidation derived structures:

- Malondialdehyde (MDA/MAA)
- 4-Hydroxynonenal (4-HNE)
- Oxidized Phosphatidylcholine (OxPC)
- Oxidized Cardiolipin (OxCL)

Oxidation-specific epitopes are Danger Associated Molecular Patterns (DAMPs)

"Danger signals" recognized by Pattern Recognition Receptors (PRRs) of innate immunity (Scavenger Rec., TLRs, natural Abs, CRP, CFH)



PC……..Phosphocholine
MDA…..Malondialdehyde
OxCL….Oxidized Cardiolipin
4-HNE…4-Hydroxynonenal

Shaw et al., J Clin Invest 2000
Chang et al., PNAS 2002

Binder et al., Nature Med 2003
Chang et al., J Exp Med 2004

Boullier et al., J Lipid Res 2005
Tuominen et al., ATVB 2006

Chou et al., J Clin Invest 2009
Weismann et al., Nature 2011

Tsiantoulas et al., J Lipid Res 2015
Gruber et al., Cell Reports 2016

Busch, Hendrikx et al., Hepatology 2017
Alic, Papac-Milicevic et al., PNAS 2020

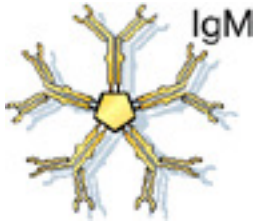
Natural IgM antibodies – old friends

Naturally occurring

Pre-existing antibodies

Primarily IgM

Secreted by B-1 cells



Germline encoded

Limited repertoire

Product of natural selection

Bind **microbial antigens**

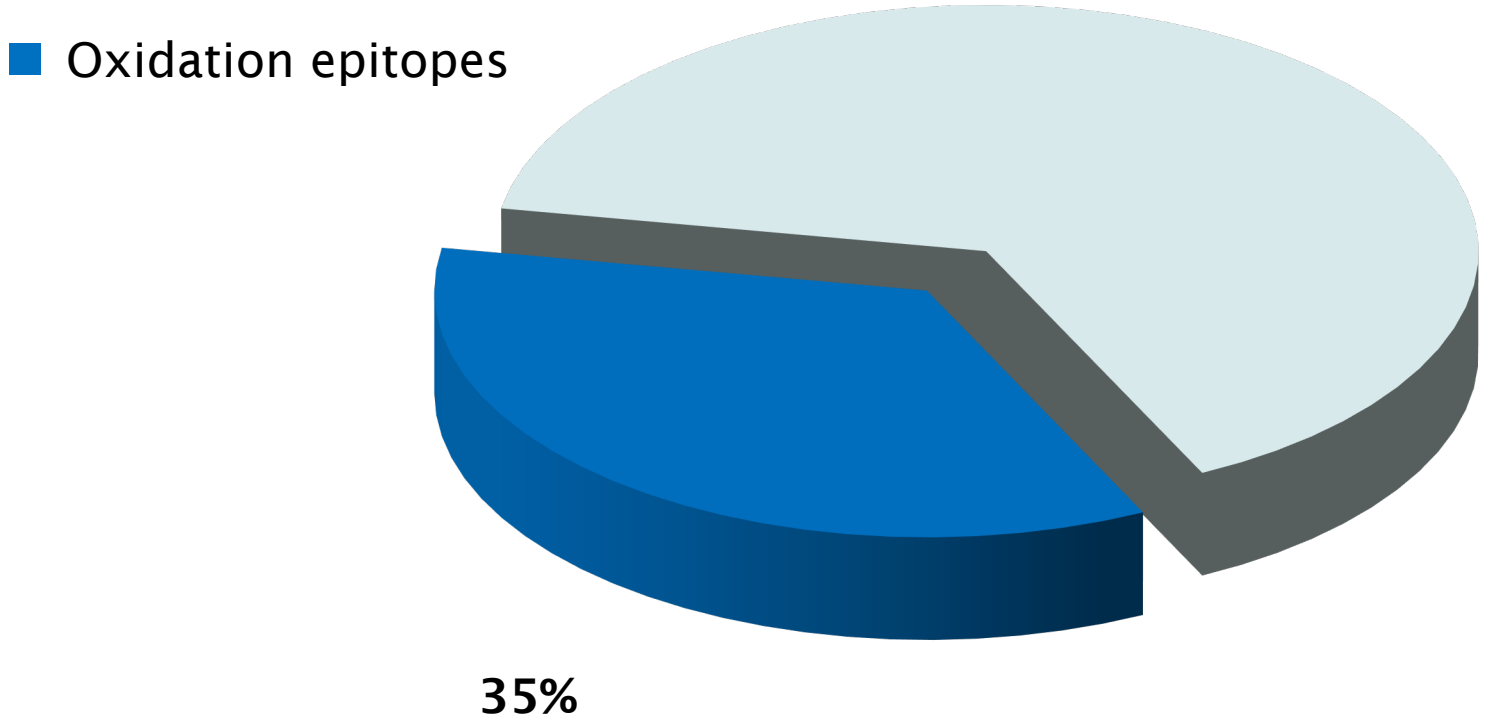
1. **First line defense**
   against bacterial & viral infections

self-antigens ("auto-reactive")

2. **"House keeping" functions**
   - Clearance of self-antigens or stress-induced neo-self

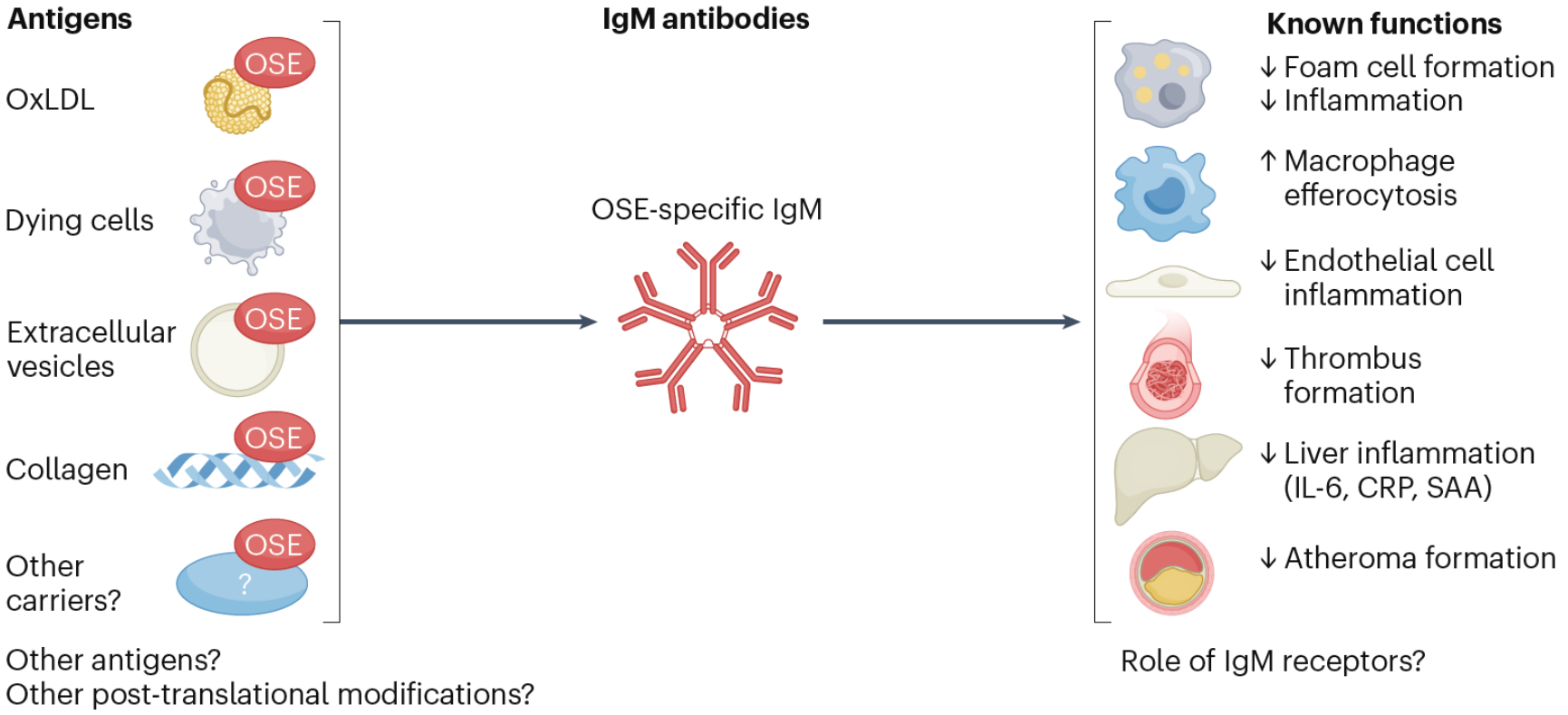
Binding properties of natural IgM antibodies

A **large part of** B-1 cell derived **natural IgM** binds to oxidation epitopes



Chou et al., *J Clin Invest* 2009

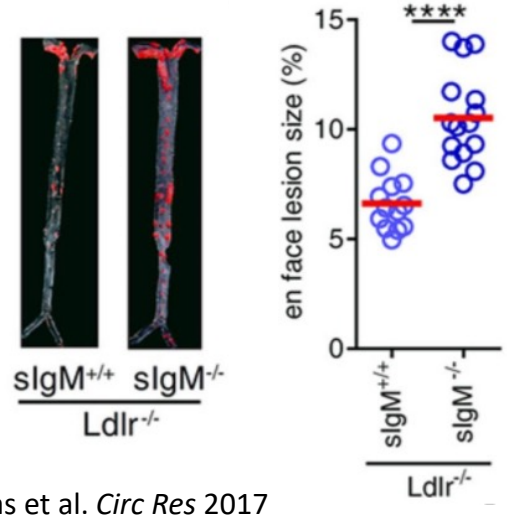
Functions of anti-OSE IgM depend on OSE-carrying antigens



Deroissart & Binder, *Nature Reviews Cardiology,* in press

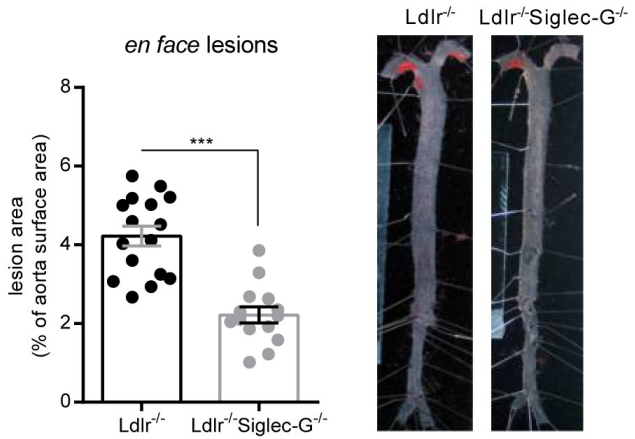
Natural IgM antibodies protect from atherosclerosis

Loss of secreted IgM

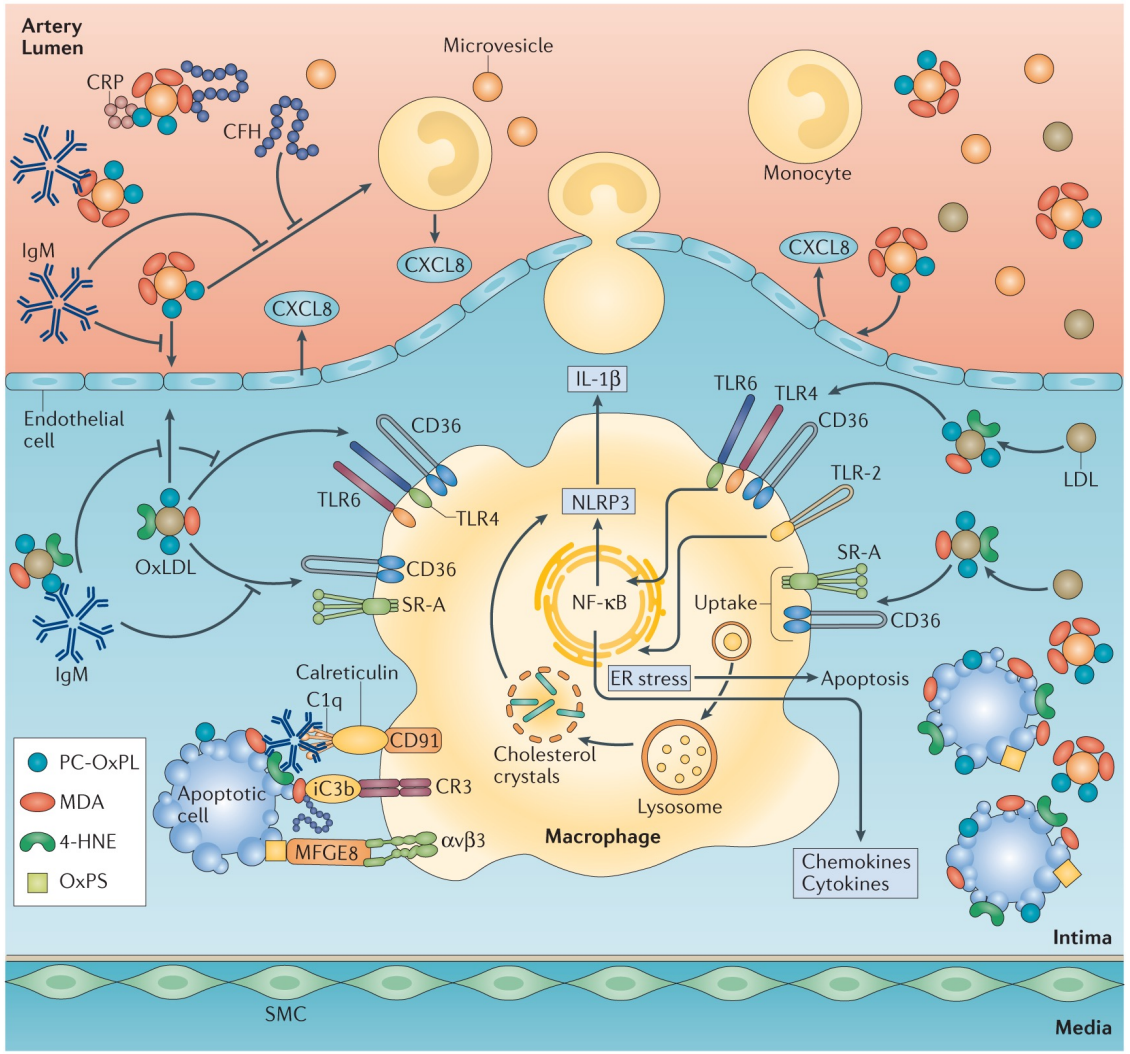


Tsiantoulas et al. *Circ Res* 2017

Increased natural IgM

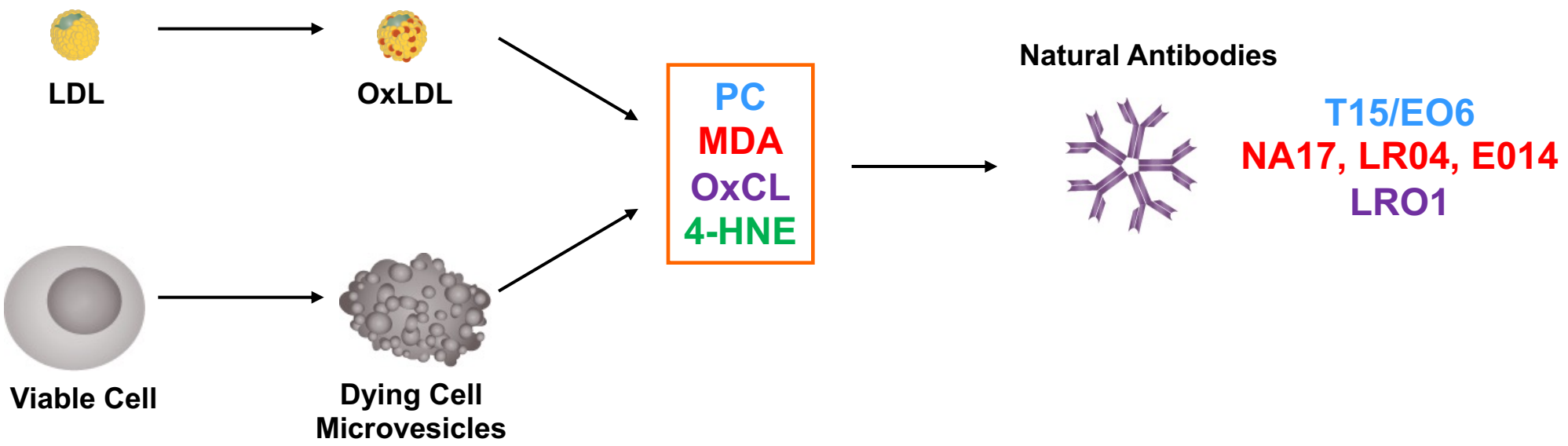


Gruber et al., *Cell Reports* 2016



Binder et al., *Nature Rev. Immunol* 2016

Self | Altered Self | OSEs | Innate Immunity



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Shaw et al., J Clin Invest 2000
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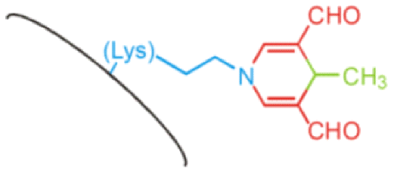
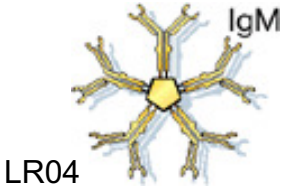
Tsiantoulas et al., J Lipid Res 2015
Gruber et al., Cell Reports 2016
Busch, Hendrikx et al., Hepatology 2017
Alic, Papac-Milicevic et al., PNAS 2020

MDA epitopes are immunodominant oxidation-specific epitopes

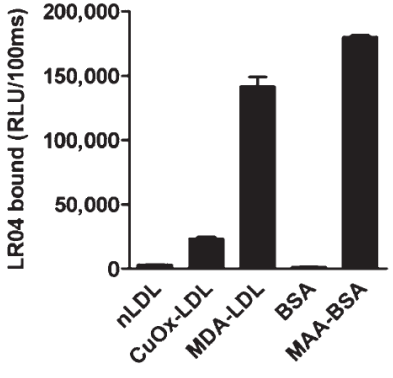
Malondialdehyde-acetaldehyde (MAA)-Lysine

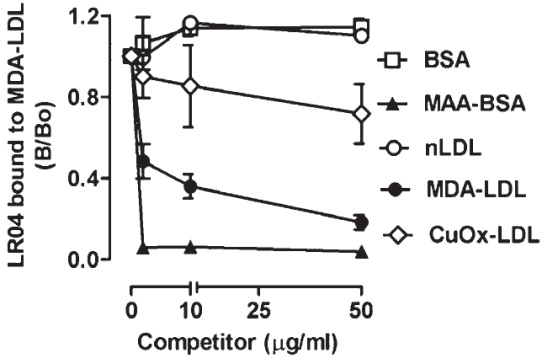


4-methyl-1,4-dihydro-3,5-pyridinedicarboxaldehyde (MDHDC)-epitope

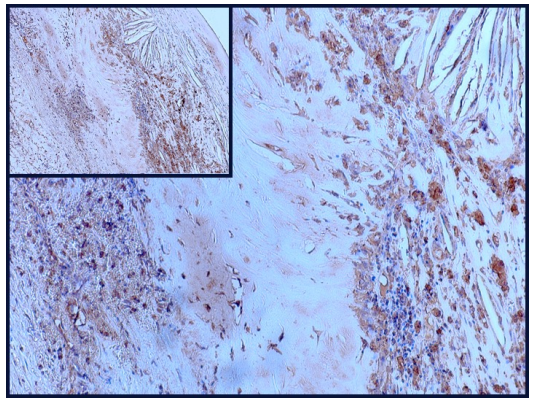


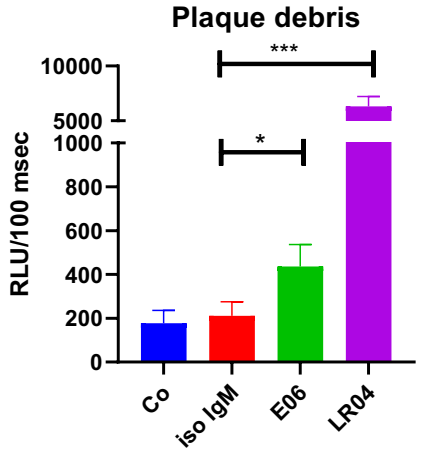
LR04 IgM has specificity for MDA-epitopes





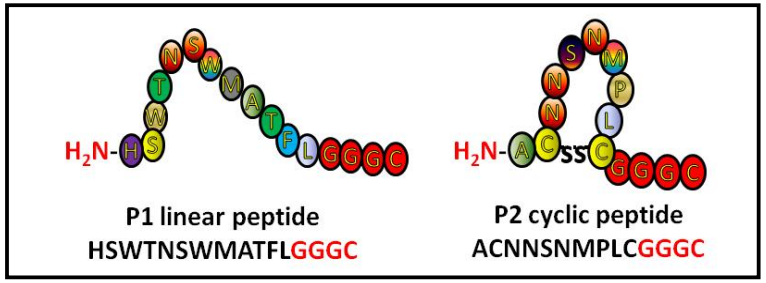
Binding of the MDA-specific IgM LR04 to coronary plaques and plaque debris



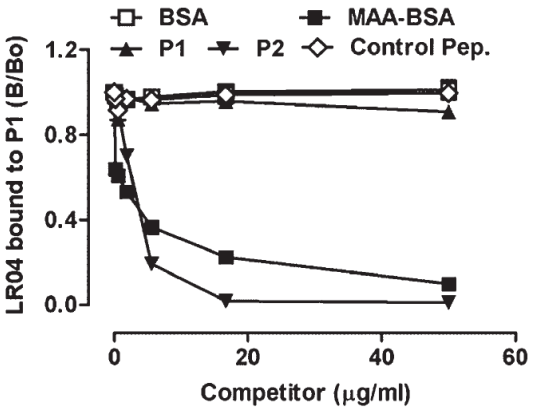


Taras Afonyushkin

Peptide mimotopes of MDA-epitopes



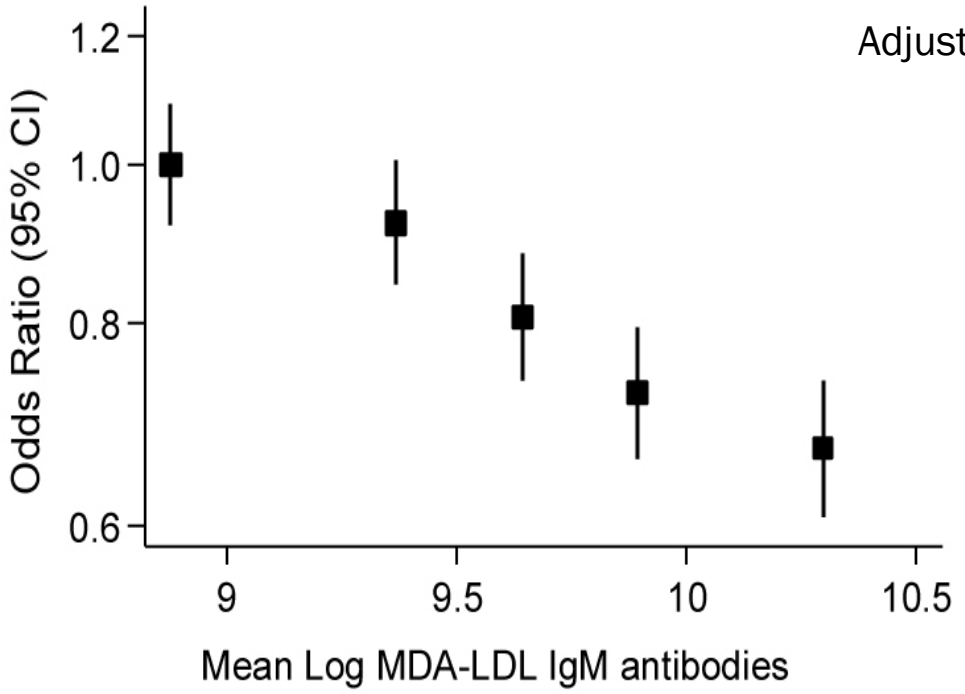
Immunocompetion assay



Amir et al., *J Lipid Res* 2012

Inverse association of anti-MDA-LDL IgM and myocardial infarction

Pakistani Risk of Myocardial Infarction Study PROMIS (4,559 cases and 4,617 controls)



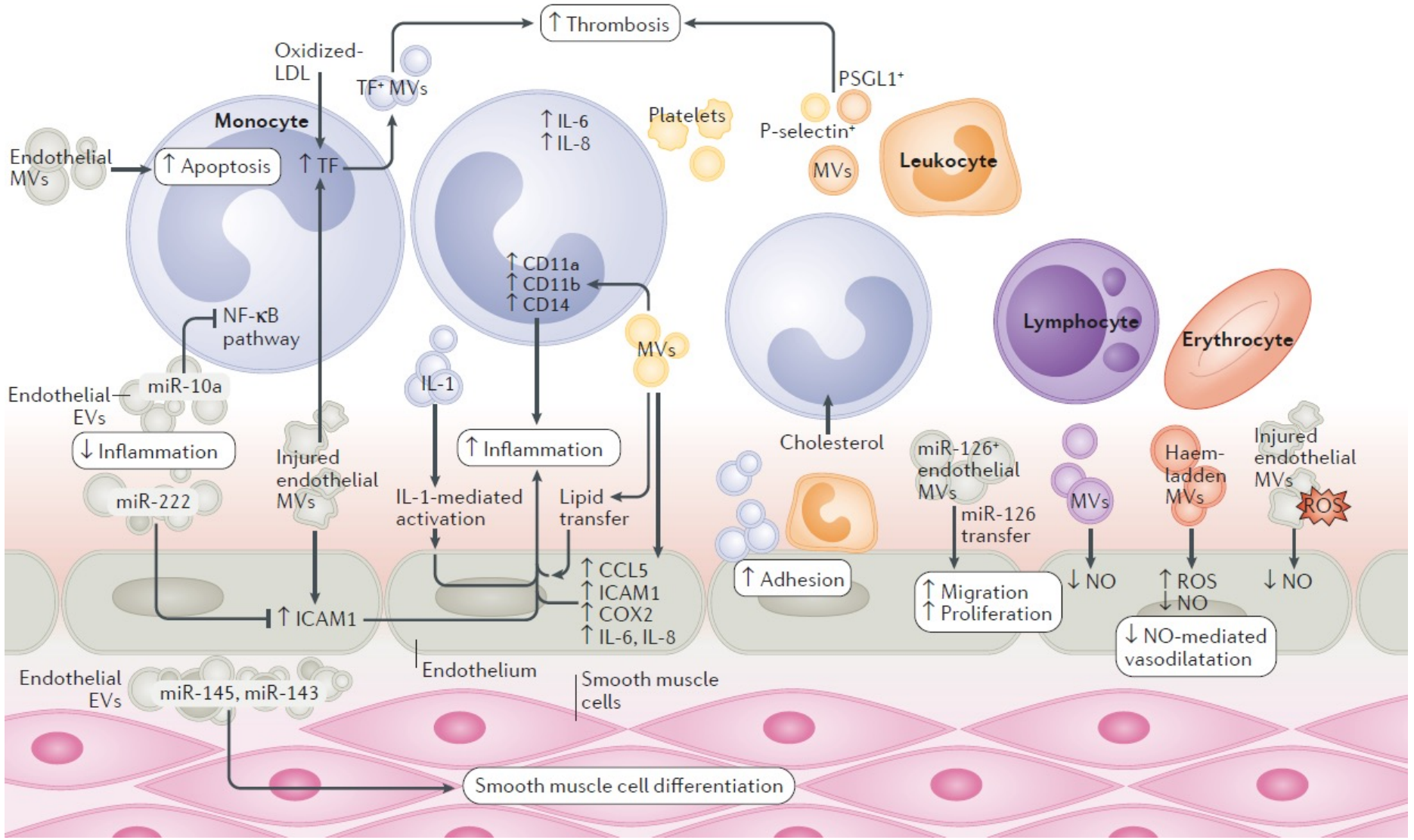
Adjusted for:

- Age
- Gender
- Tobacco
- Hypertension
- Diabetes
- LDL-C
- HDL-C
- TG

	IgM MDA-LDL	IgM MDA Mimotope P1
Across categories		
HR (95% CI) vs. reference		
Quintile 1	1.00 [Ref]	1.00 [Ref]
Quintile 2	0.90 (0.79-1.03)	0.82 (0.72-0.94)
Quintile 3	0.80 (0.70-0.92)	0.76 (0.67-0.87)
Quintile 4	0.75 (0.65-0.86)	0.73 (0.64-0.84)
Quintile 5	0.67 (0.58-0.77)	0.72 (0.62-0.82)
P value for trend	<0.001	<0.001
Per SD higher value		
HR (95% CI)	0.90 (0.87-0.94)	0.94 (0.90-0.98)
P value	<0.001	<0.001

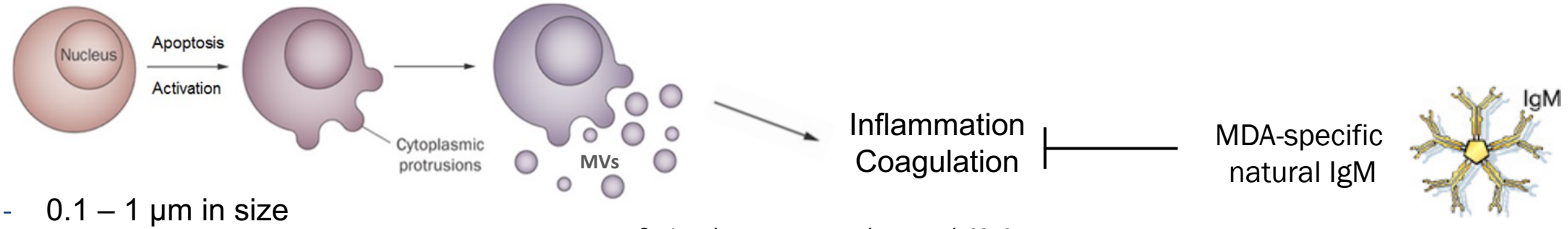
Taleb et al, *J Lipid Res* 2023

Extracellular vesicles in CVD – new foes



Boulanger et al., *Nature Rev Cardiol* 2017

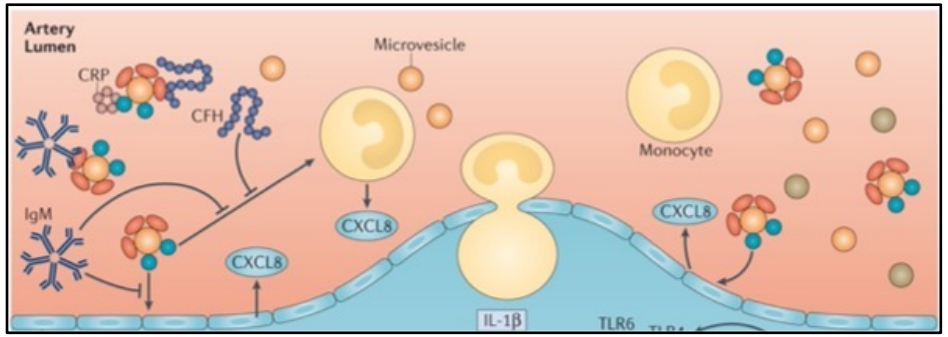
Microvesicles (MVs) are targets for anti-OSE IgM



- 0.1 – 1 µm in size
- Large extracellular vesicles
- Carry different mediators
- Circulate in plasma
- Increased levels in several diseases

Beyer & Pisetsky, *Nature Rev Rheumatol,* 2010

Tsiantoulas et al., *J Lipid Res* 2015

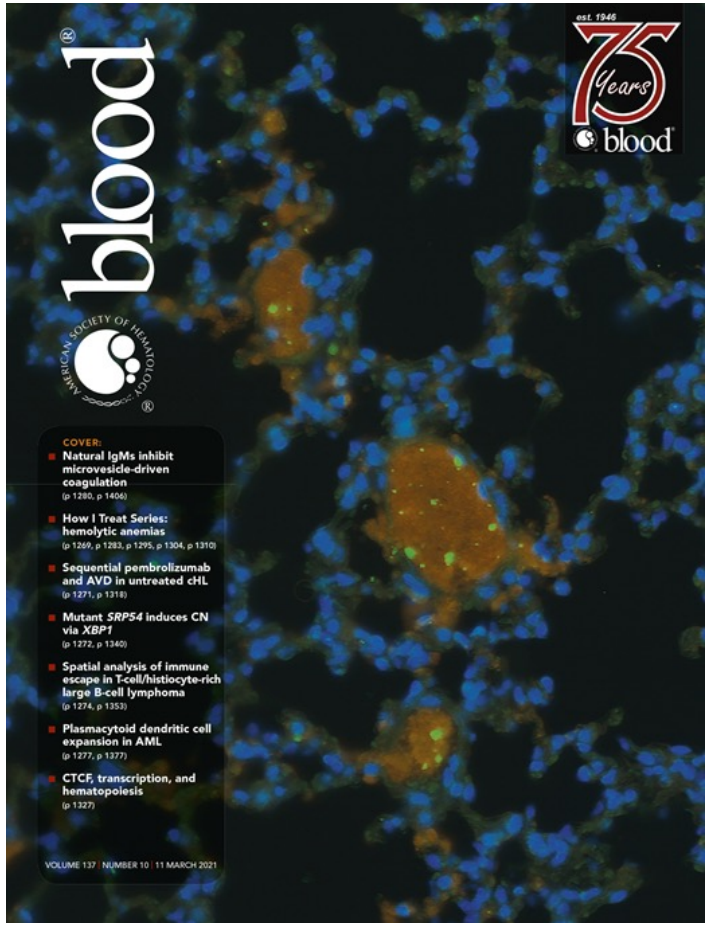


Binder et al., *Nature Rev Immunol, 2016*

THROMBOSIS AND HEMOSTASIS

Natural IgM antibodies inhibit microvesicle-driven coagulation and thrombosis

Georg Obermayer,[1,2,*] Taras Afonyushkin,[1,2,*] Laura Göderle,[1] Florian Puhm,[1,2] Waltraud Schrottmaier,[3] Soreen Taqi,[1] Michael Schwameis,[4,5] Cihan Ay,[6,7] Ingrid Pabinger,[6] Bernd Jilma,[4] Alice Assinger,[3] Nigel Mackman,[8] and Christoph J. Binder[1,2]



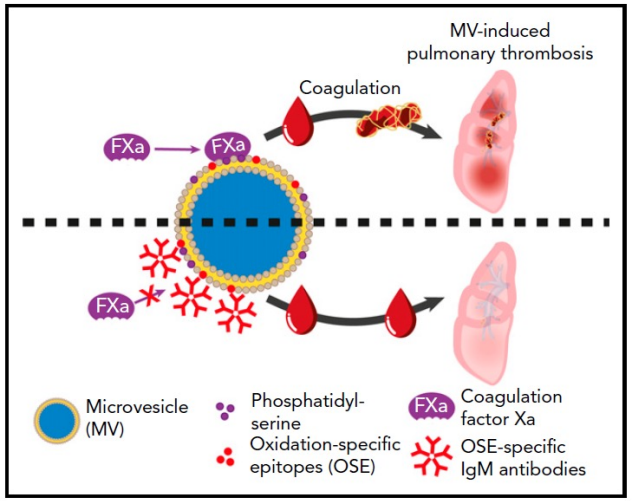
THROMBOSIS AND HEMOSTASIS

Comment on Obermayer et al, page 1406

Natural IgM antibodies help fend off thrombosis

Dorian O. Haskard | Imperial College London

It is tempting to view the immune system as a thrombosis driver,[1] but the article by Obermayer et al[2] in this issue of *Blood* provides a counterpoise through the discovery of an anticoagulant role for innate immunity. Their article linking circulating microvesicles with natural immunoglobulin M (IgM) antibodies is an intriguing story with high clinical significance.

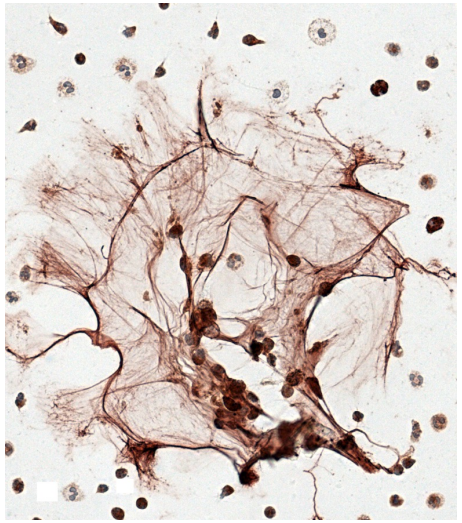


Neutrophil Extracellular Traps (NETs) trigger thrombosis

Clinical Track

Coronary Neutrophil Extracellular Trap Burden and Deoxyribonuclease Activity in ST-Elevation Acute Coronary Syndrome Are Predictors of ST-Segment Resolution and Infarct Size

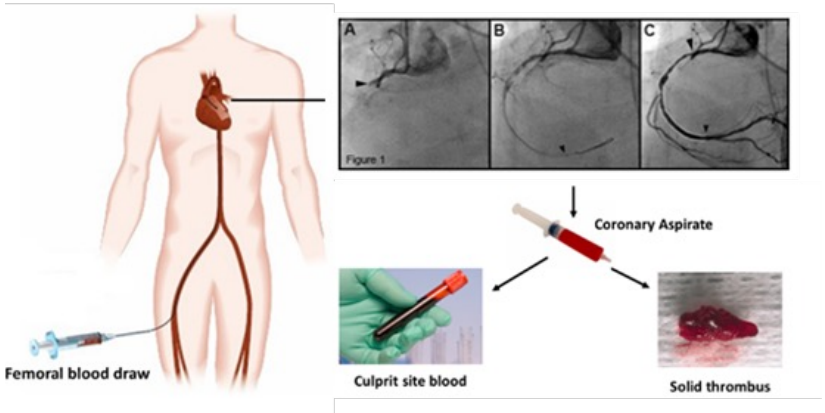
Andreas Mangold, Sherin Alias, Thomas Scherz, Thomas M. Hofbauer, Johannes Jakowitsch, Adelheid Panzenböck, Daniel Simon, Daniela Laimer, Christine Bangert, Andreas Kammerlander, Julia Mascherbauer, Max-Paul Winter, Klaus Distelmaier, Christopher Adlbrecht, Klaus T. Preissner, Irene M. Lang

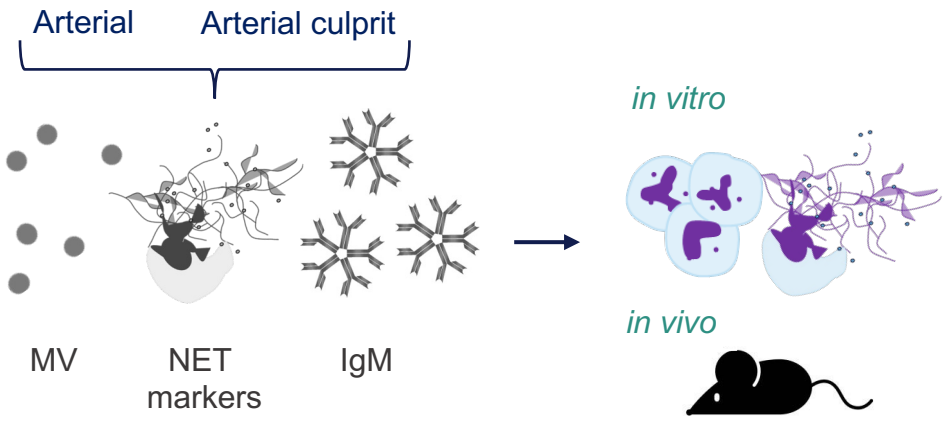


Mangold et al., *Circ Res 2020*

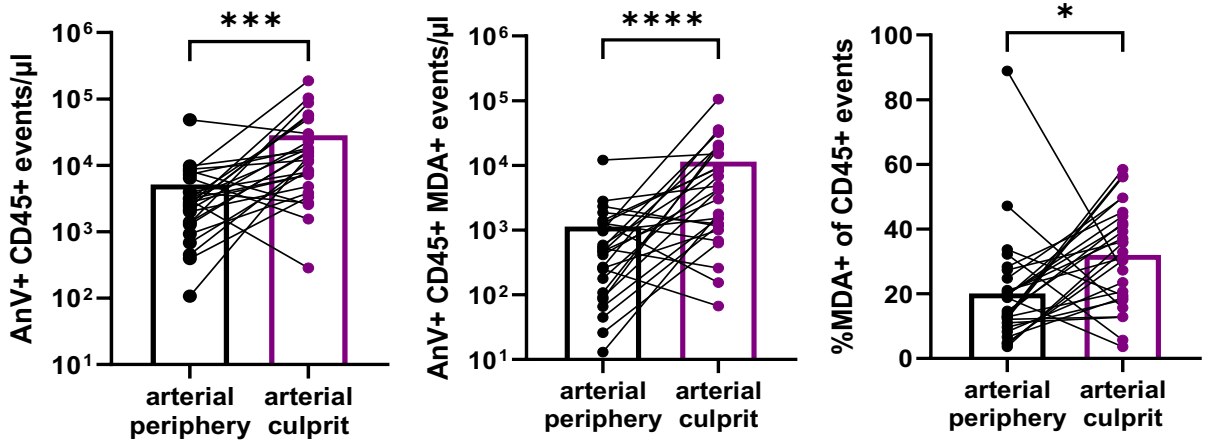
Concentration and frequency of leukocyte-derived MVs carrying MDA-epitopes are increased at the culprit site

STEMI patients (n=28)

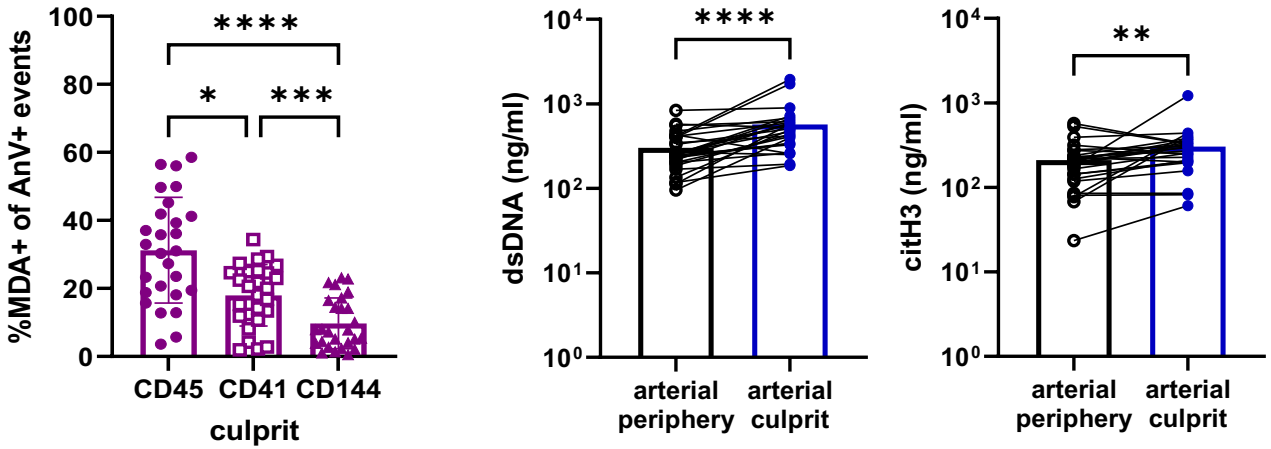




MDA+ leukocyte-derived MVs



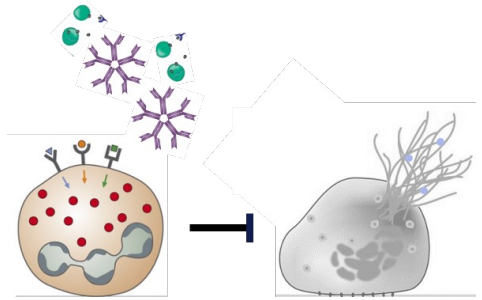
NETosis markers



Ondracek, Afonysuhkin et al., *in revision*

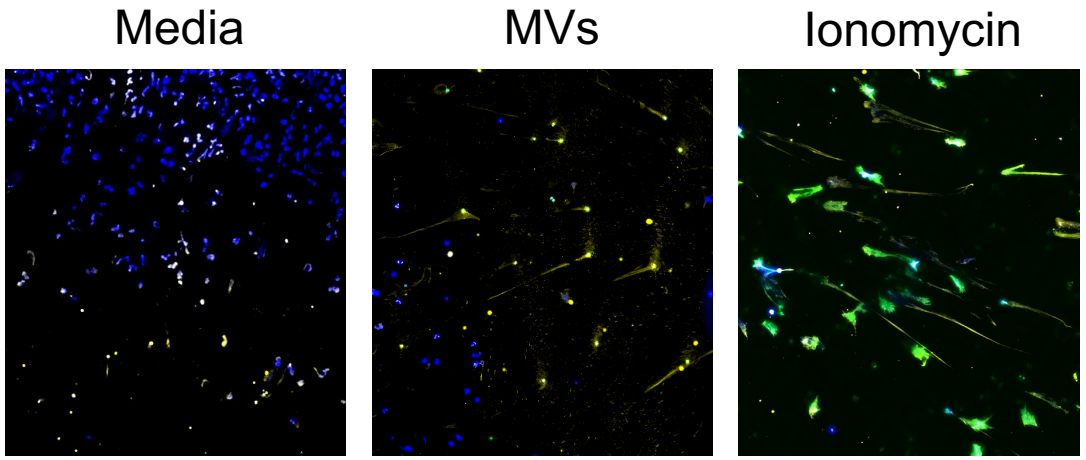
MDA-specific IgM inhibit NETosis induced by microvesicles (MVs)

Human neutrophils stimulated with MDA+ MVs from LPS-activated THP-1 cells in the presence of the MDA-specific IgM LR04 or an isotype control

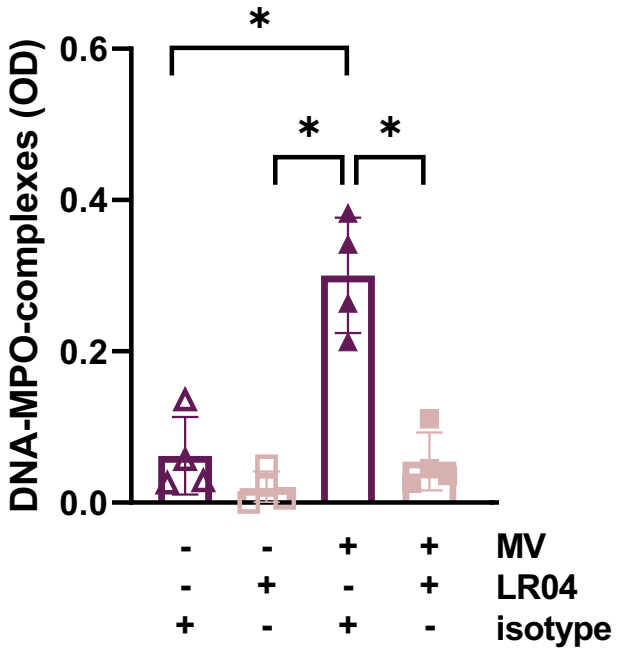


Stimulation with MVs

Release of NETs, DNA, Histones, MPO, other granular proteins



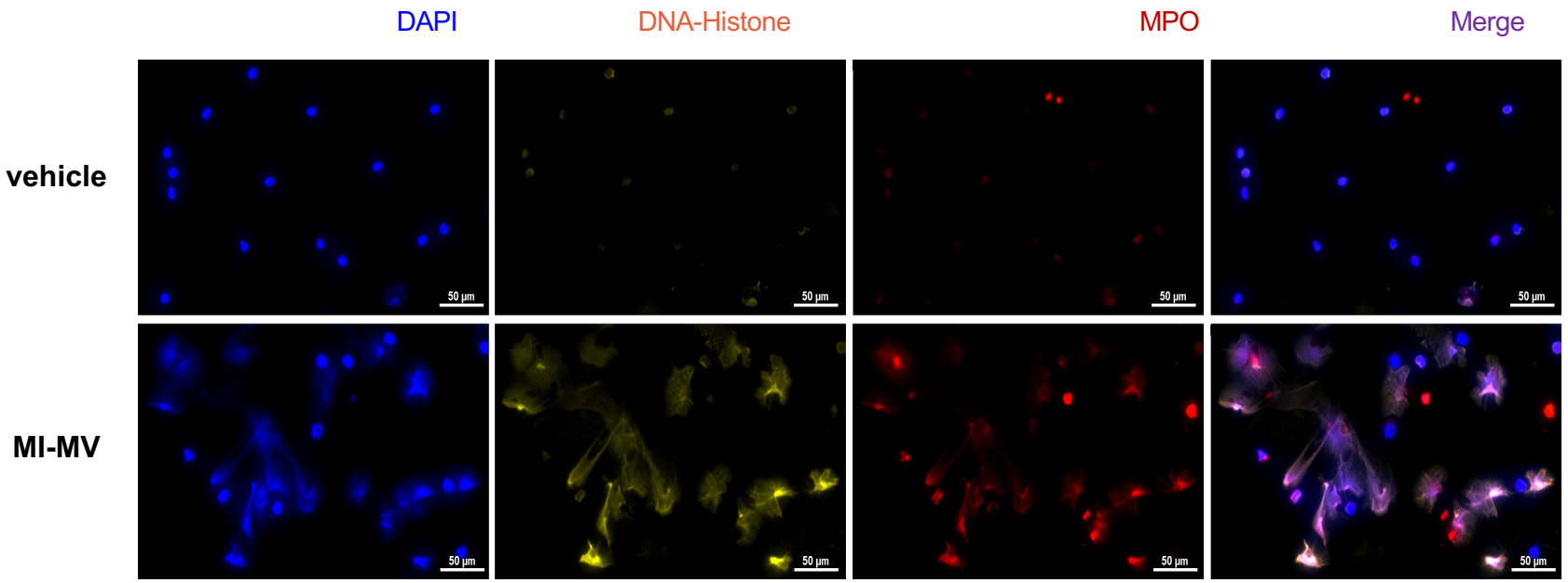
Blue, DAPI
Yellow, DNA-Histone
Green, citH3

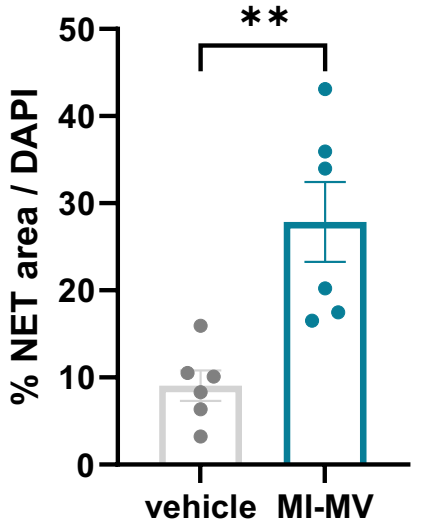


Ondracek, Afonysuhkin et al., *in revision*

Circulating MVs from MI patients trigger NETosis

HL60 neutrophils stimulated with MVs from STEMI patients (MI MV) for 3 hours

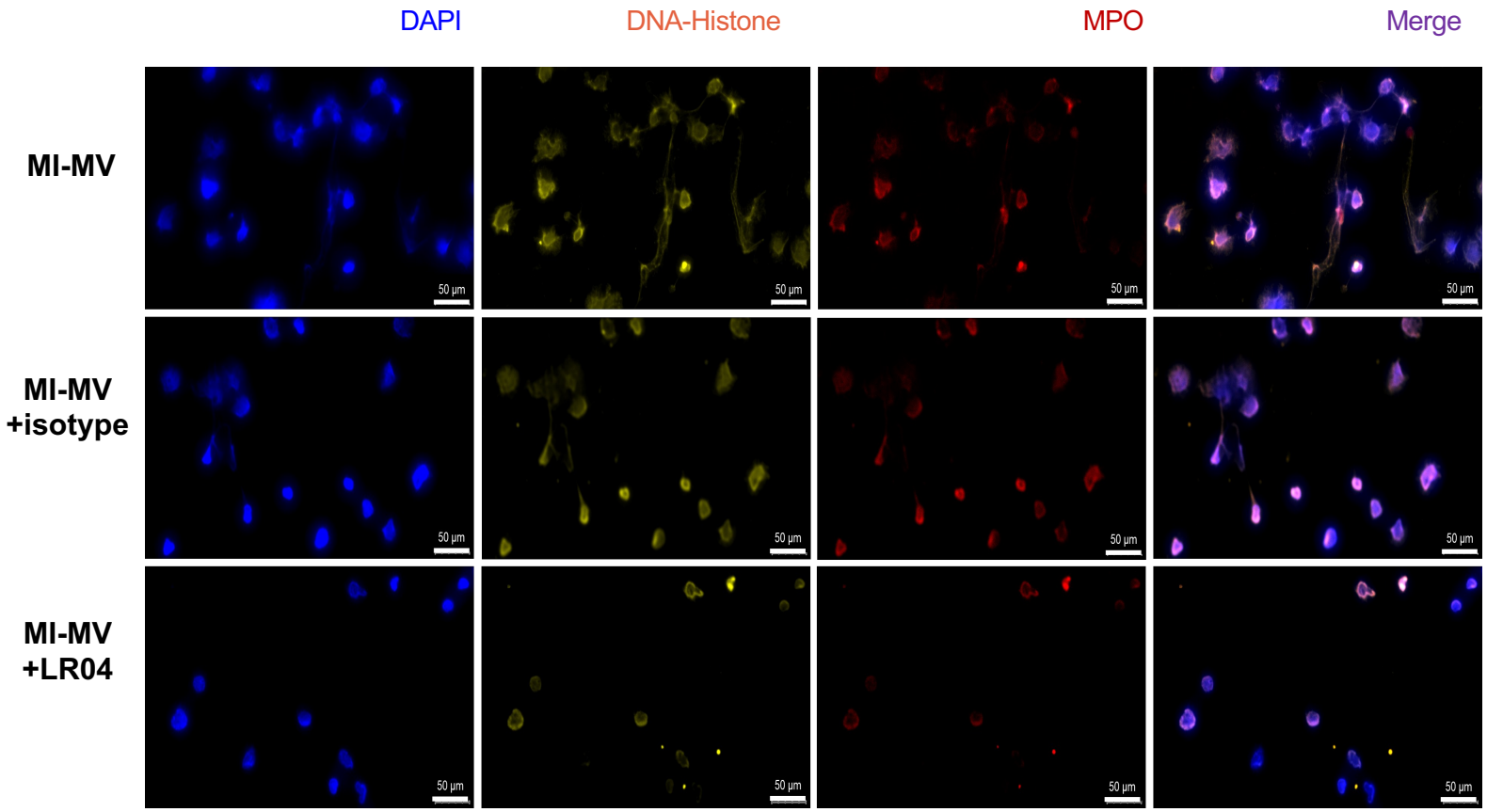


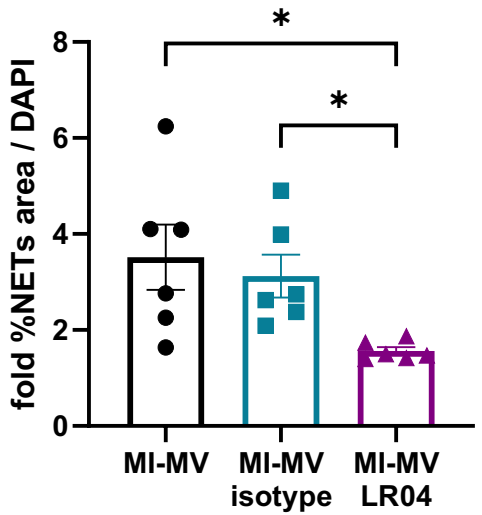


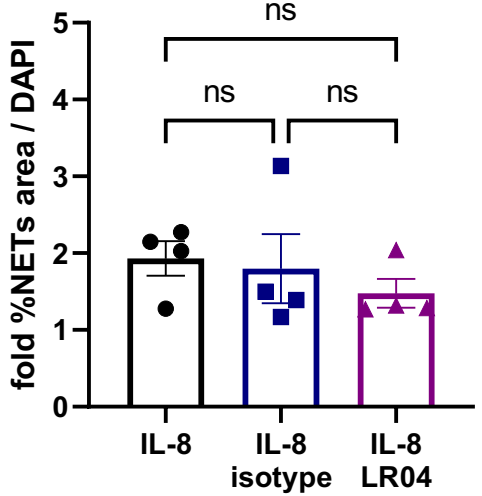
Ondracek, Afonysuhkin et al., *in revision*

MDA-specific IgM inhibit NETosis induced by MVs from MI patients

HL60 neutrophils stimulated with MI MVs for 3 hours in the presence of the MDA-specifc IgM LR04 or an isotype control

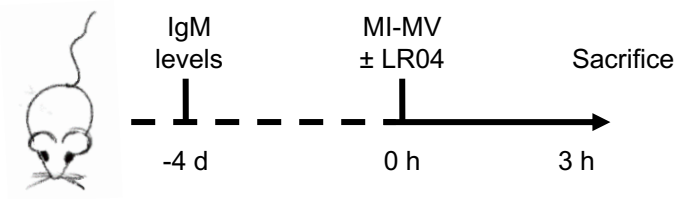


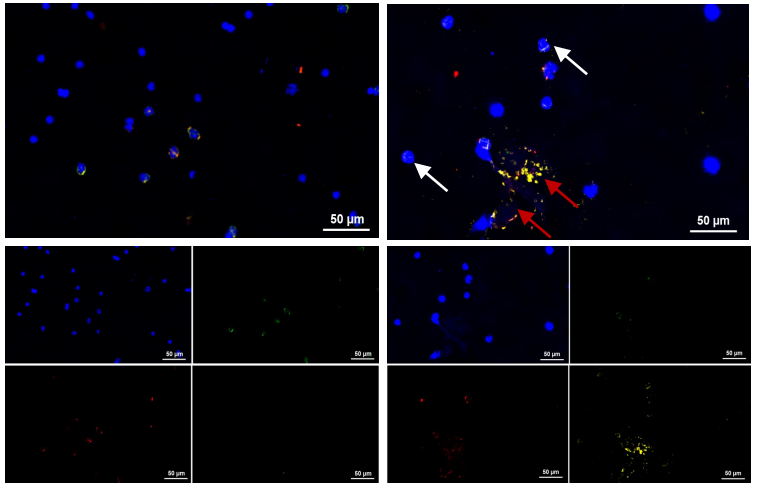




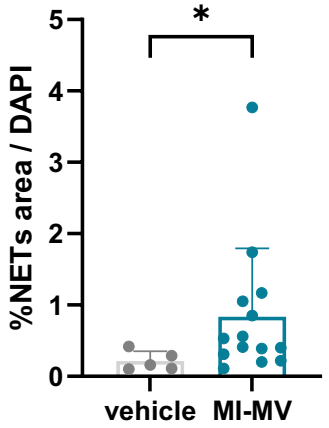
Ondracek, Afonysuhkin et al., *in revision*

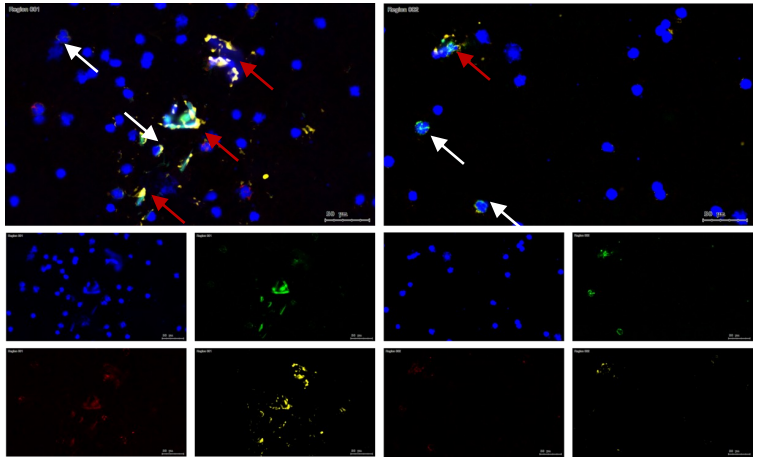
MDA-specific natural IgM reduce NET formation induced by circulating MVs from MI patients in vivo





DAPI, citH3, MPO, Ly6G





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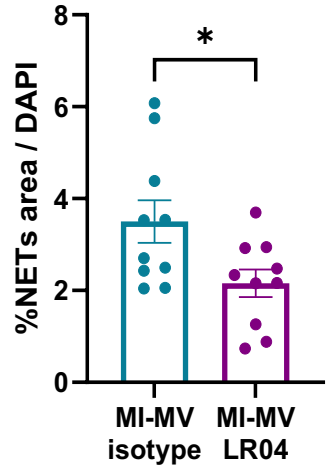
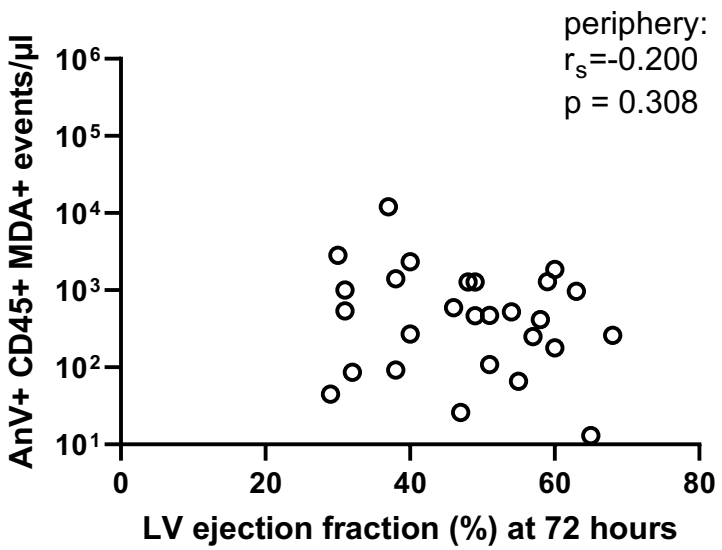


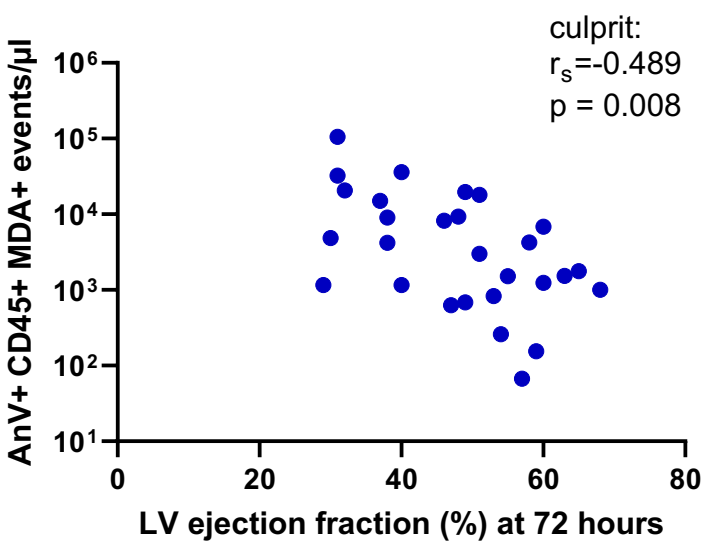
Ondracek, Afonysuhkin et al., *in revision*

Ratio between NETogenic MDA+ MVs and NETosis-inhibiting MDA-specific IgM predict heart function after MI

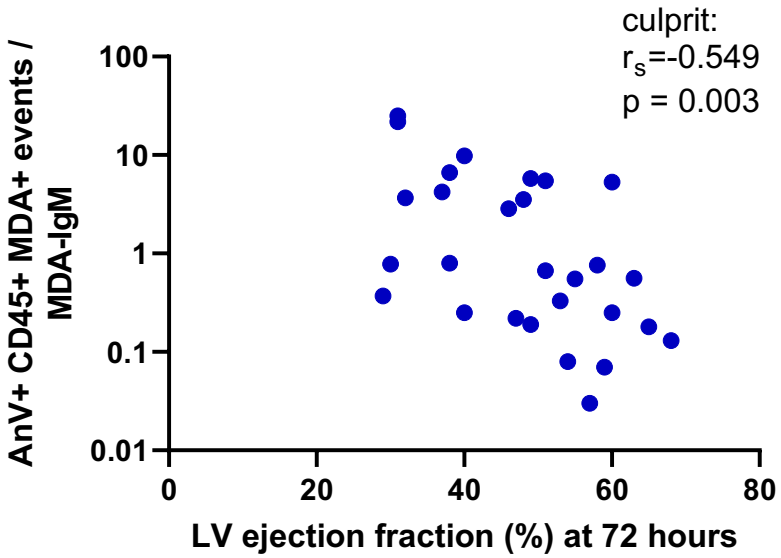
MDA+ MVs in arterial periphery



MDA+ MVs arterial culprit



MDA+ MVs/MDA IgM ratio

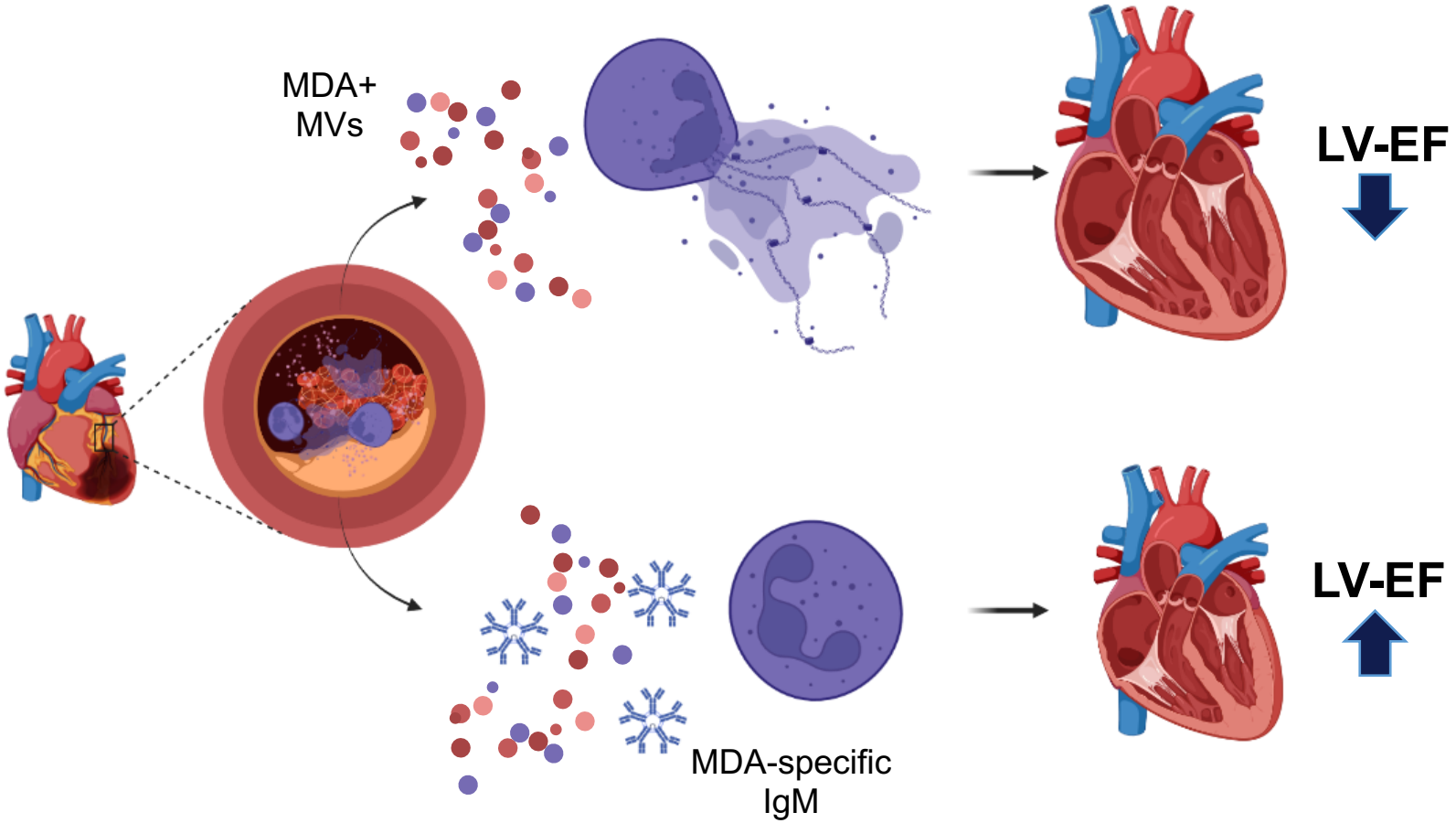


- arterial periphery
- arterial culprit

cMRI-derived LV-EF after 72 hours

Ondracek, Afonysuhkin et al., *in revision*

Natural IgM reduce MI-associated damage by inteferring with MV-induced NETosis



Summary

- Oxidation-specific epitopes (OSE) are lipid peroxidation-derived structures that are present on oxidized LDL, dying cells and a subset of microvesicles.
- OSE are universal marks of many chronic inflammatory lesions and conditions.
- OSE represent DAMPs that are targets for housekeeping functions of innate immune immunity and can act as pro-inflammatory danger signals.
- Low levels of natural IgM antibodies with specificity for OSE are associated CV and thrombotic risk.
- MDA+ microvesicles are increased at the culprit arterial site of MI patients and MDA-specific IgM protect from microvesicle-induced NETosis and thrombosis.

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Joseph L. Witztum
Sam Tsimikas

FWF Der Wissenschaftsfonds.

Leducq Foundation

WWTF VIENNA SCIENCE AND TECHNOLOGY FUND

FFG Forschung wirkt.

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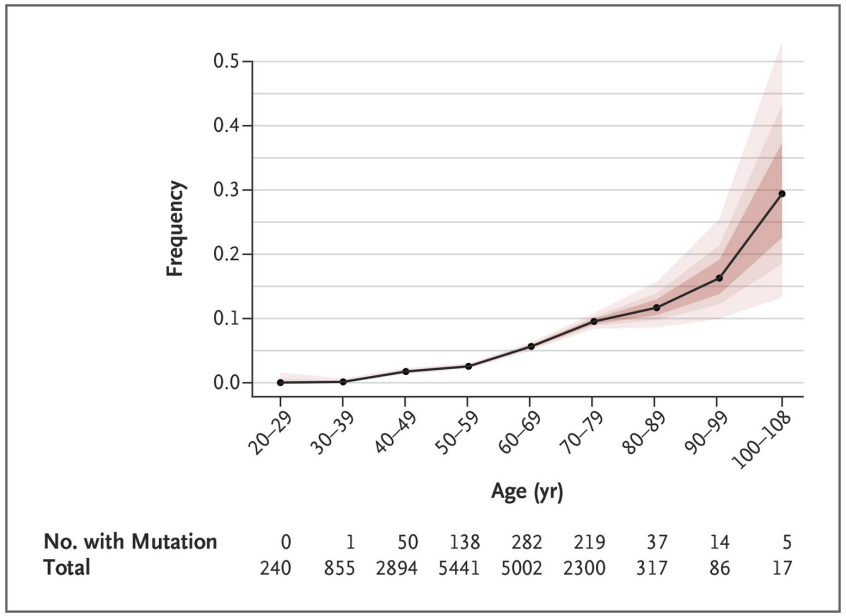
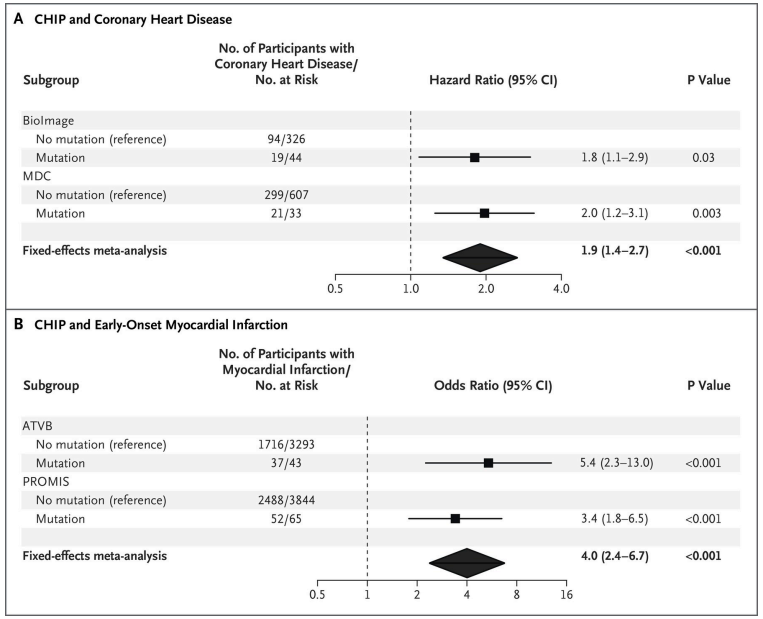
Clonal Hematopoesis of Indeterminate Potential (CHIP)

- **Common age-related condition**
- **Somatic mutations** leading to **clonal expansion** of **haematopoietic stem cells**
- Mutations in the transcriptional regulators *DNMT3A*, *TET2*, *JAK2*, and *ASXL1*
- CHIP is associated with **increased risk** of **haematological malignancies** and all cause mortality, but also **40% increased risk** of **CVD**



Jaiswal et al., New Engl J Med 2014



Jaiswal et al., New Engl J Med 2017

Clonal Hematopoiesis of Indeterminate Potential (CHIP)

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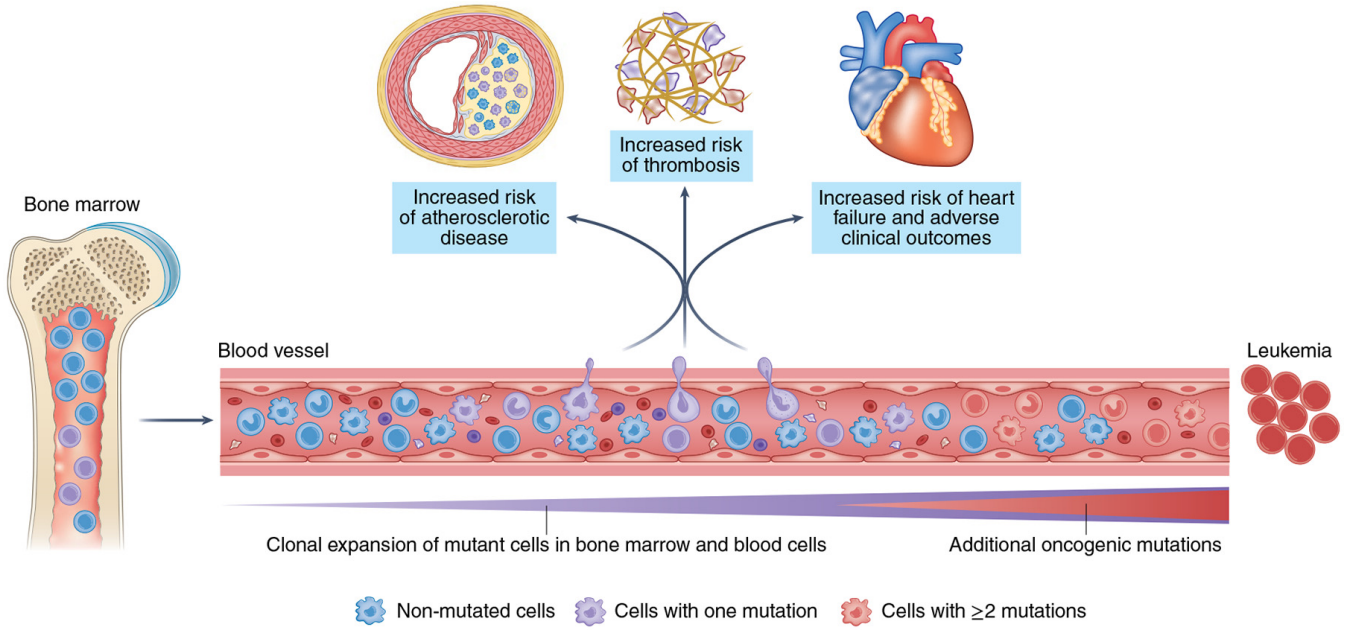
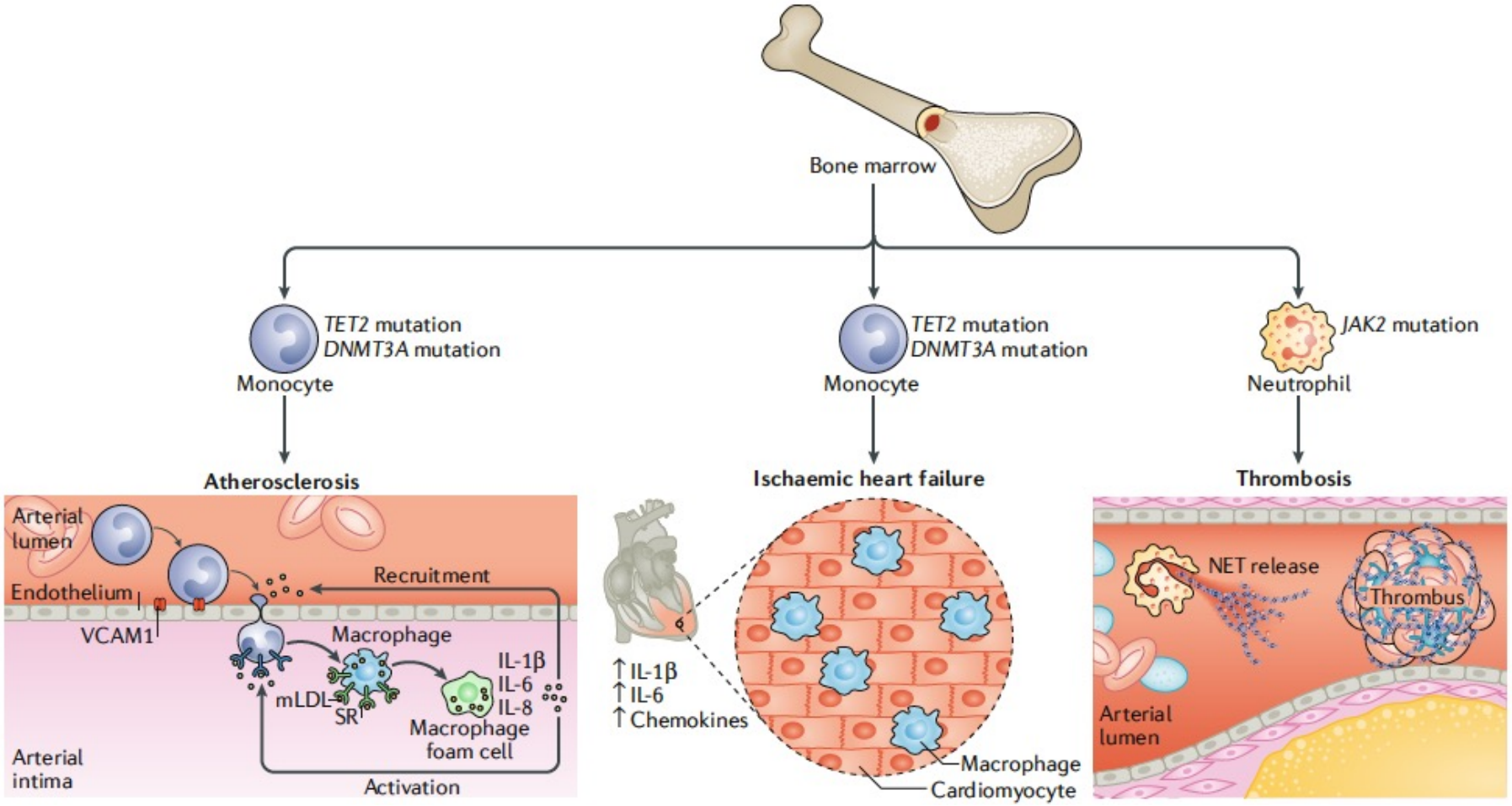


Tall & Fuster, Nature Cardiovasc Res 2022

Mechanisms mediating increased cardiovascular risk of CHIP



Jaiswal & Libby, Nature Reviews Cardiol 2019

Inflammation and Carotid Artery Risk for Atherosclerosis Study (ICARAS)

- Single-center study of 1268 patients with asymptomatic carotid atherosclerosis
- Average age: 69 years
- Prospectively followed for >12 years
- Main outcome data: Progression of atherosclerosis and CV mortality

Circulation
Volume 111, Issue 17, 3 May 2005; Pages 2203-2209
https://doi.org/10.1161/01.CIR.0000163569.97918.C0

VASCULAR MEDICINE

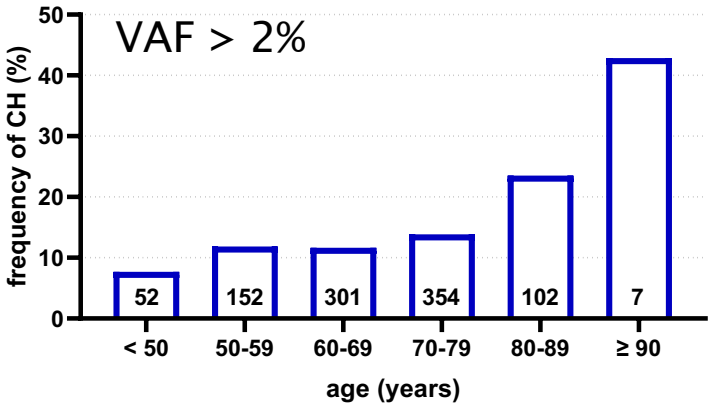
Inflammation and Carotid Artery—Risk for Atherosclerosis Study (ICARAS)

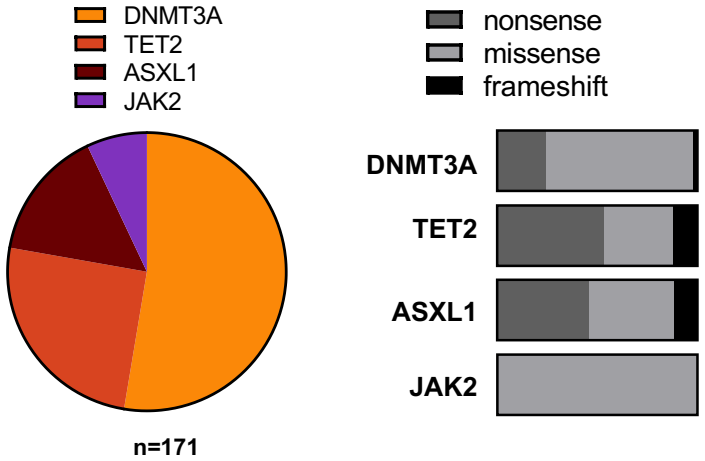
Martin Schillinger, MD, Markus Exner, MD, Wolfgang Mlekusch, MD, Schila Sabeti, MD, Jasmin Amighi, MD, Robert Nikowitsch, Ewald Timmel, Bernhard Kickinger, Christoph Minar, Matthias Pones, Wolfgang Lalouschek, MD, Helmut Rumpold, MD, Gerald Maurer, MD, Oswald Wagner, MD, and Erich Minar, MD

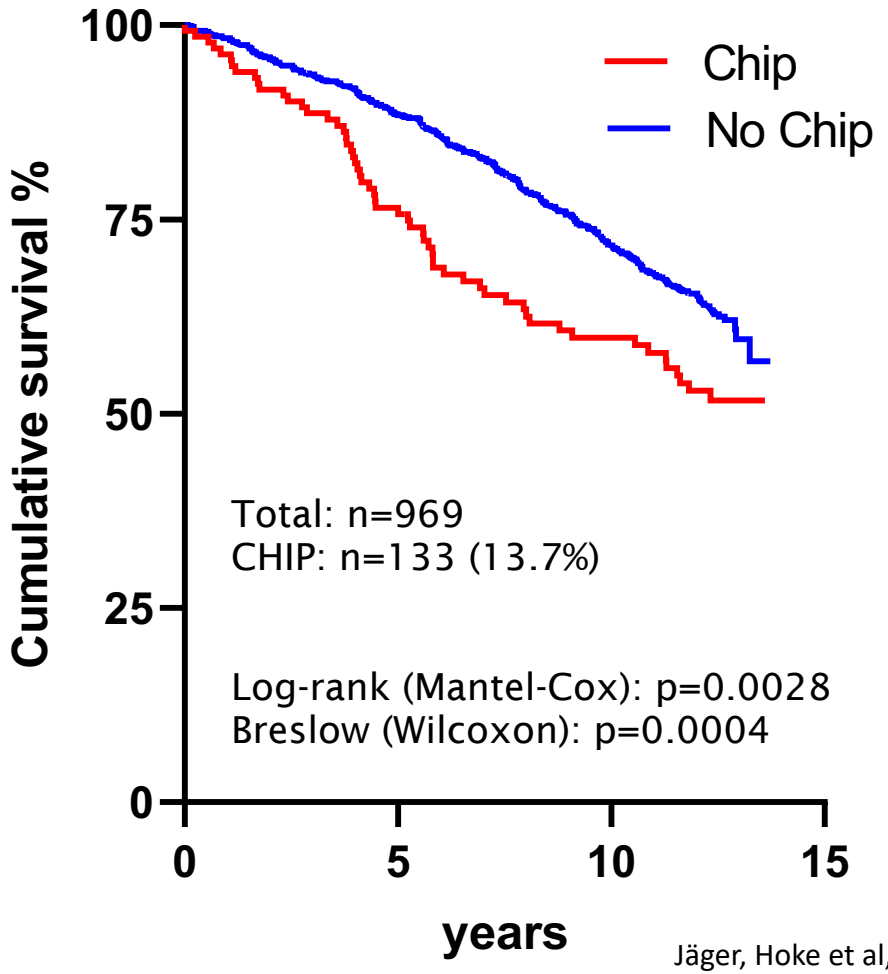
Demographic Data and Clinical Characteristics			
Age (years)	69 (60-76)	HbA1c (%)	5.9 (5.6-6.5)
Males/females	793 (63%) / 475 (37%)	Family history of atherosclerosis	688 (54%)
Body mass index (kg/m²)	26.1 (24.0-28.7)	History of MI	303 (24%)
Smoking status	1-10 cigarettes/d (131/10%) 11-20 cigs/d (107/8%) >20 cigs/d (116/9%)	History of stroke	199 (16%)
Arterial hypertension	861 (68%)	Serum creatinine (mg/dL)	1.05 (0.93-1.22)
Hyperlipidemia	830 (66%)	hs-CRP (mg/dL)	0.29 (0.13-0.63)
Total cholesterol (mg/dL)	205 (175-238)	Serum amyloid A (mg/dL)	6.3 (<3.8-10.9)
LDL cholesterol (mg/dL)	118 (93-148)	Statin treatment	726 (57%)
HDL cholesterol (mg/dL)	49 (41-60)	Diabetes	88 (7%)

Schillinger et. al, Circulation 2005

CHIP increases cardiovascular risk in ICARAS

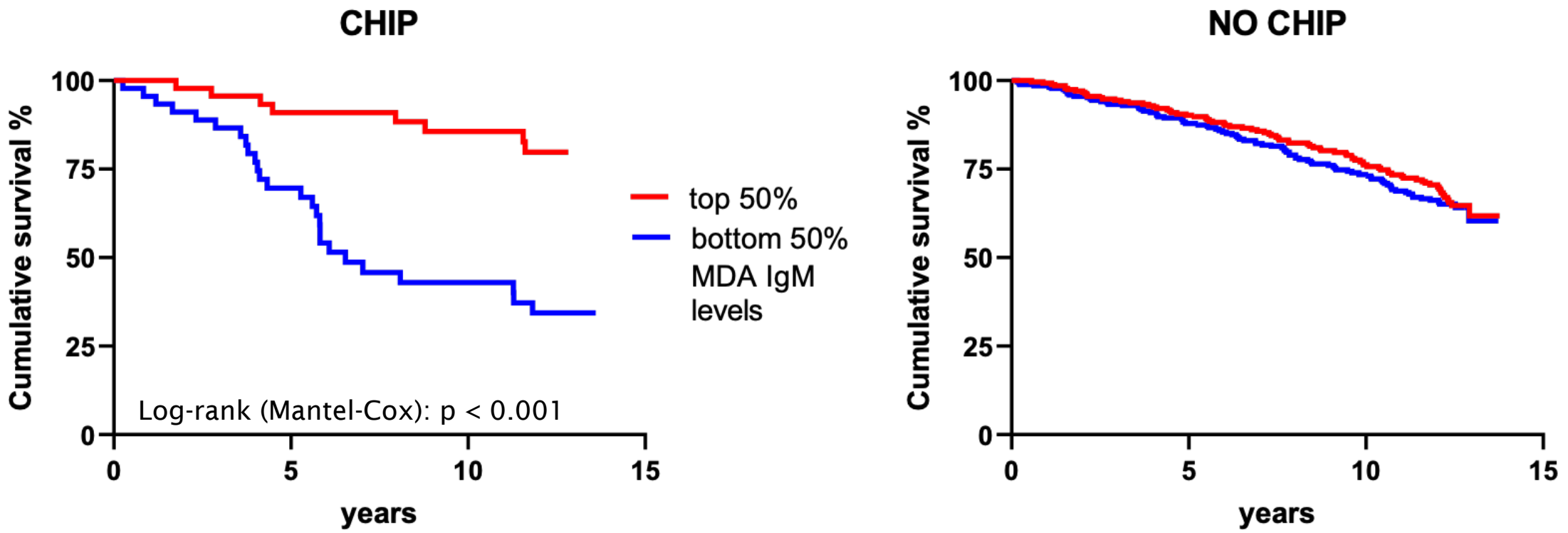






Jäger, Hoke et al, unpublished

High MDA-specific IgM levels reduce the increased risk associated with CHIP



Adjusted for: Age, Gender, Smoking, hsCRP, LDL-C, Past MI, Past stroke (p=0.021)

N = 639: 91 with CHIP (8 JAK2, 20 ASXL1, 50 DNMT3A, 28 TET2) and 548 without CHIP

Afonyushkin, Jäger, et al, unpublished