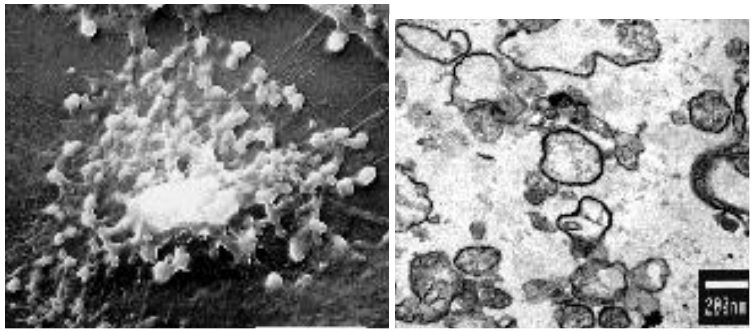
CBCS Summer School on cardiovascular Sciences

Nice June 15 th , 2011

The multiple faces of endothelial –derived microparticles From Basic Mechanisms to clinical applications



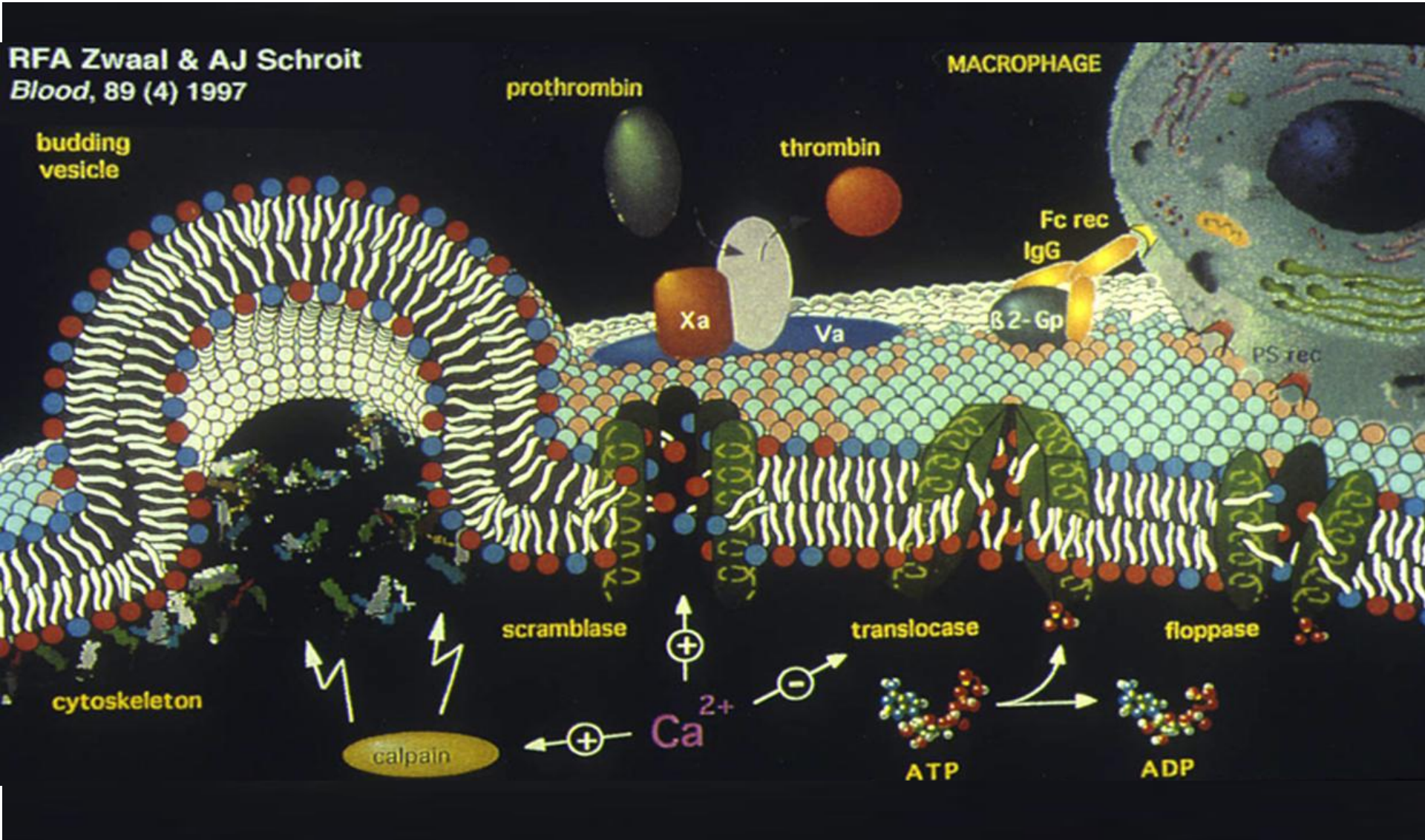
F Dignat-George

UMR 608 INSERM :Physiopathologie de l'endothélium

Université de la méditerranée

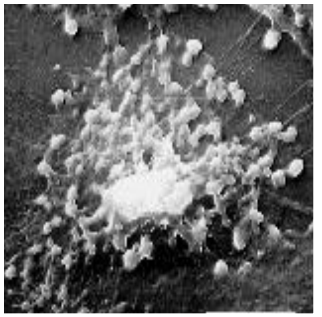
Marseille

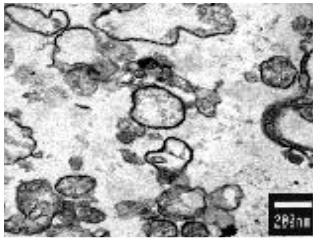
Membrane remodelling and microparticle formation

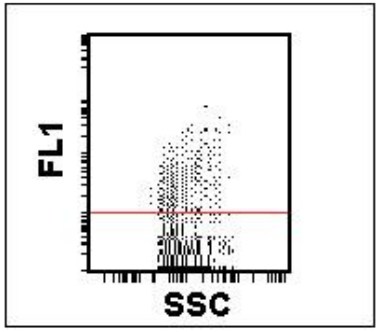


How to define microparticles?

- Vesicles resulting from blebbing of the cell membrane in response to cell activation or apoptosis
- Heterogeneous size (diameter: 0.1 to 1 µm).
- Expression of phosphatidylserine and antigens representative of their parent cells
- Biomarkers in vascular pathologies
- Conveyors of bioactive molecules

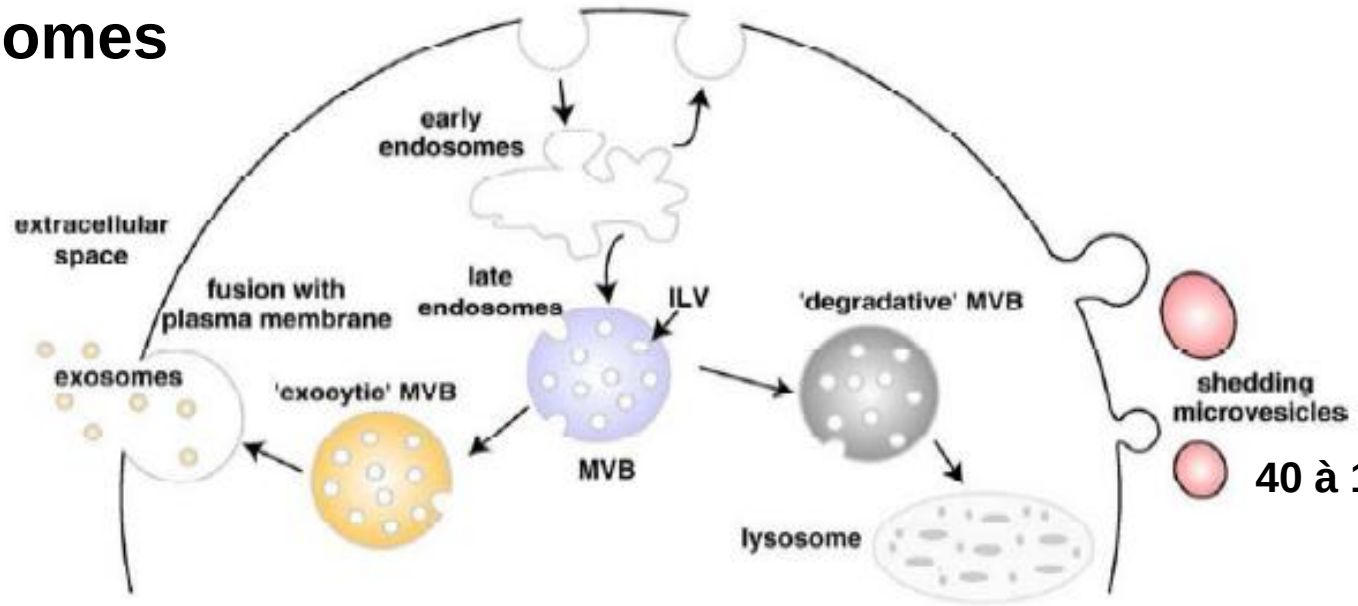






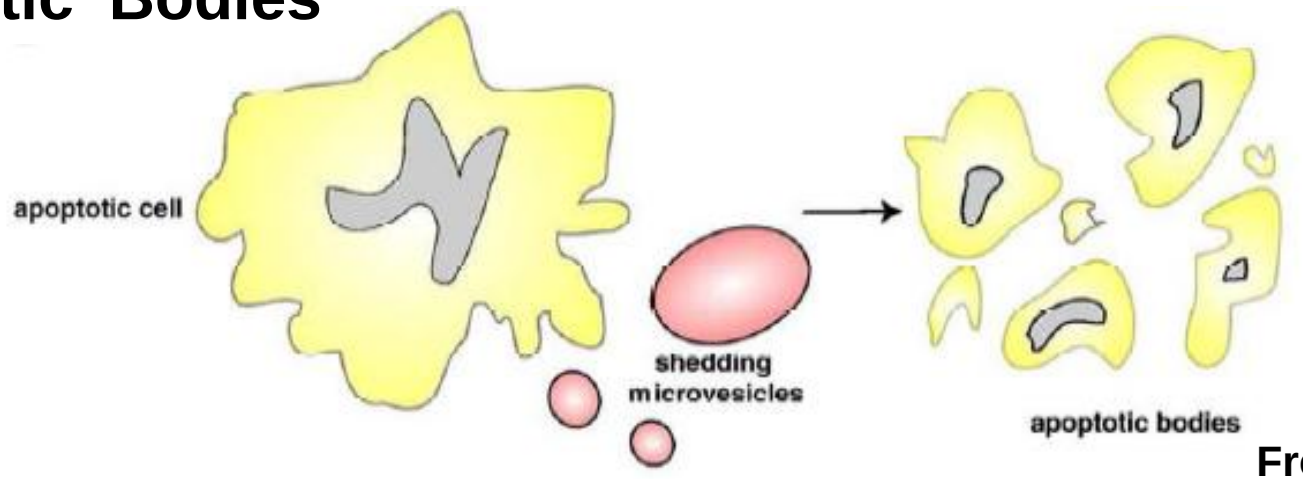
Microparticles and other vesicles

Exosomes



40 à 100 nm

Apoptotic Bodies



From 1 to 4 µm

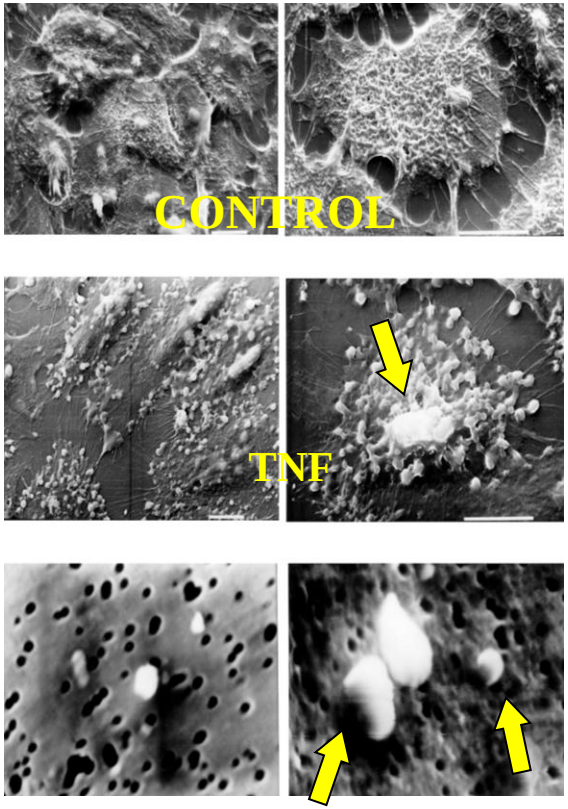
Mathivanan S. J. Proteomics 2010

Questions

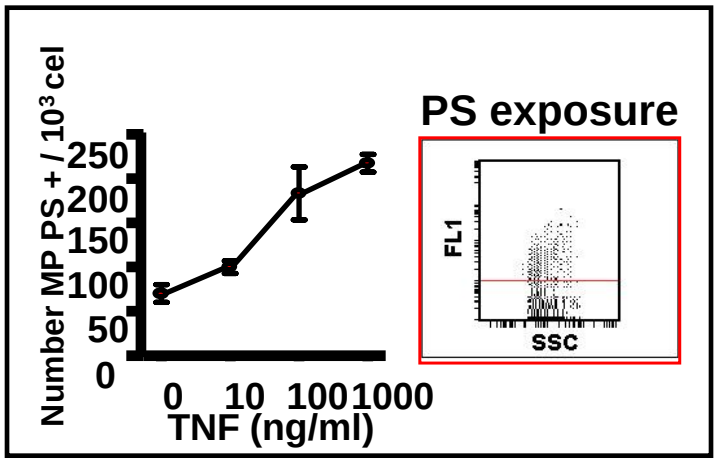
- **Multifaceted roles of Endothelial-derived Microparticles**
- **Mechanisms of formation**
- **Endothelial Microparticles in diseases**
- **Endothelial Microparticles : friends or foes?**

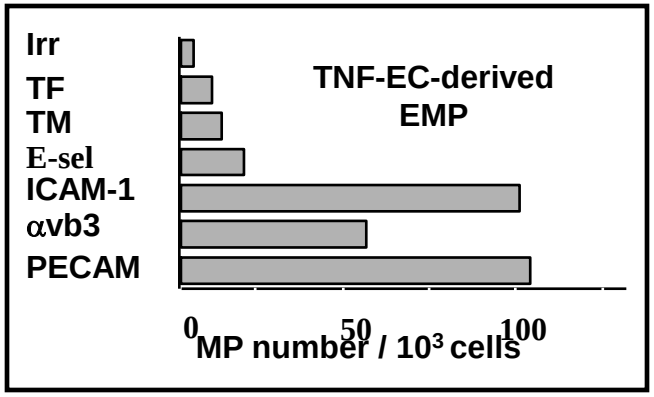
EMP generation in response to inflammatory stimulation

Combes et al, J Clin Invest, 1999

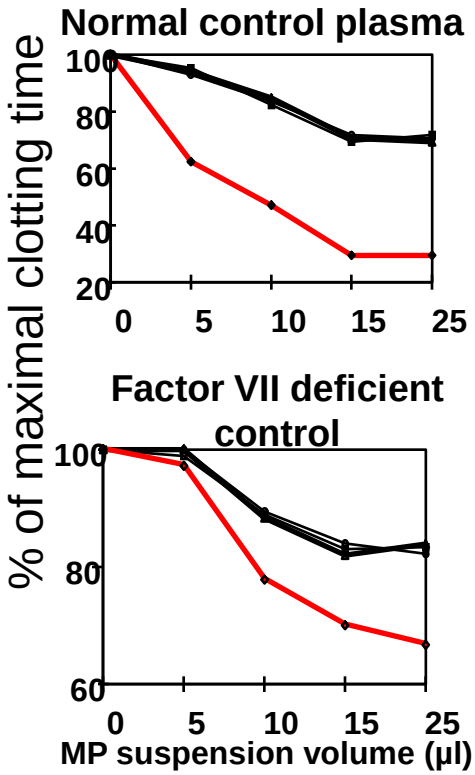


Heterogeneous diameter (0.1 to 1 µm)





Endothelial phenotypic features

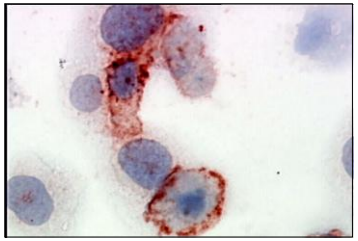


Pro-coagulant activity

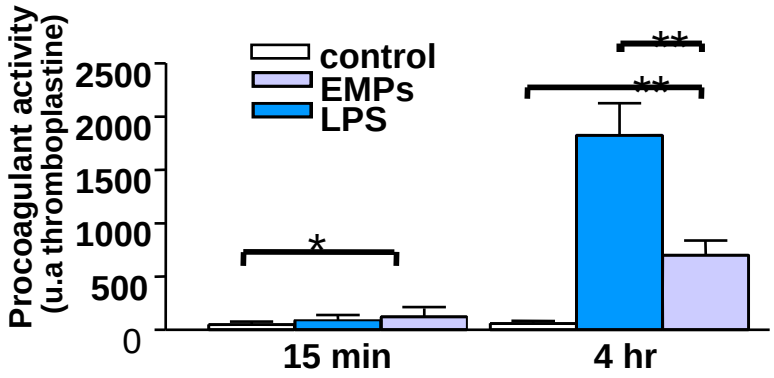
Interaction between EMP and monocytes participates in the amplification of procoagulant responses

Sabatier et al, Blood, 2002, 90:142

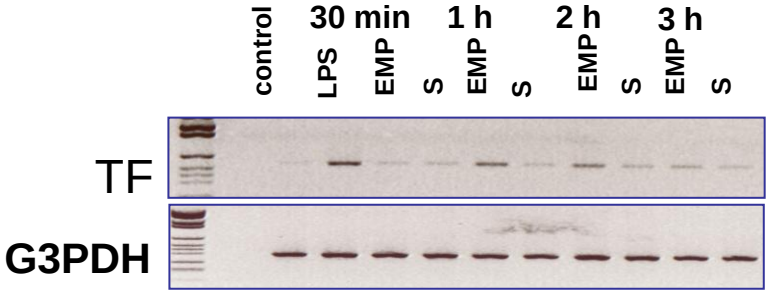
- ***EMP bind to THP-1 cells***



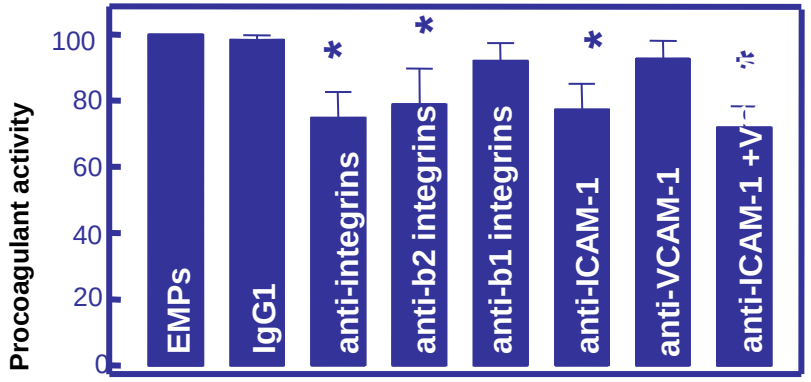
- ***EMP induce THP-1 procoagulant activity***



- ***THP-1 TF mRNA is induced by EMP***



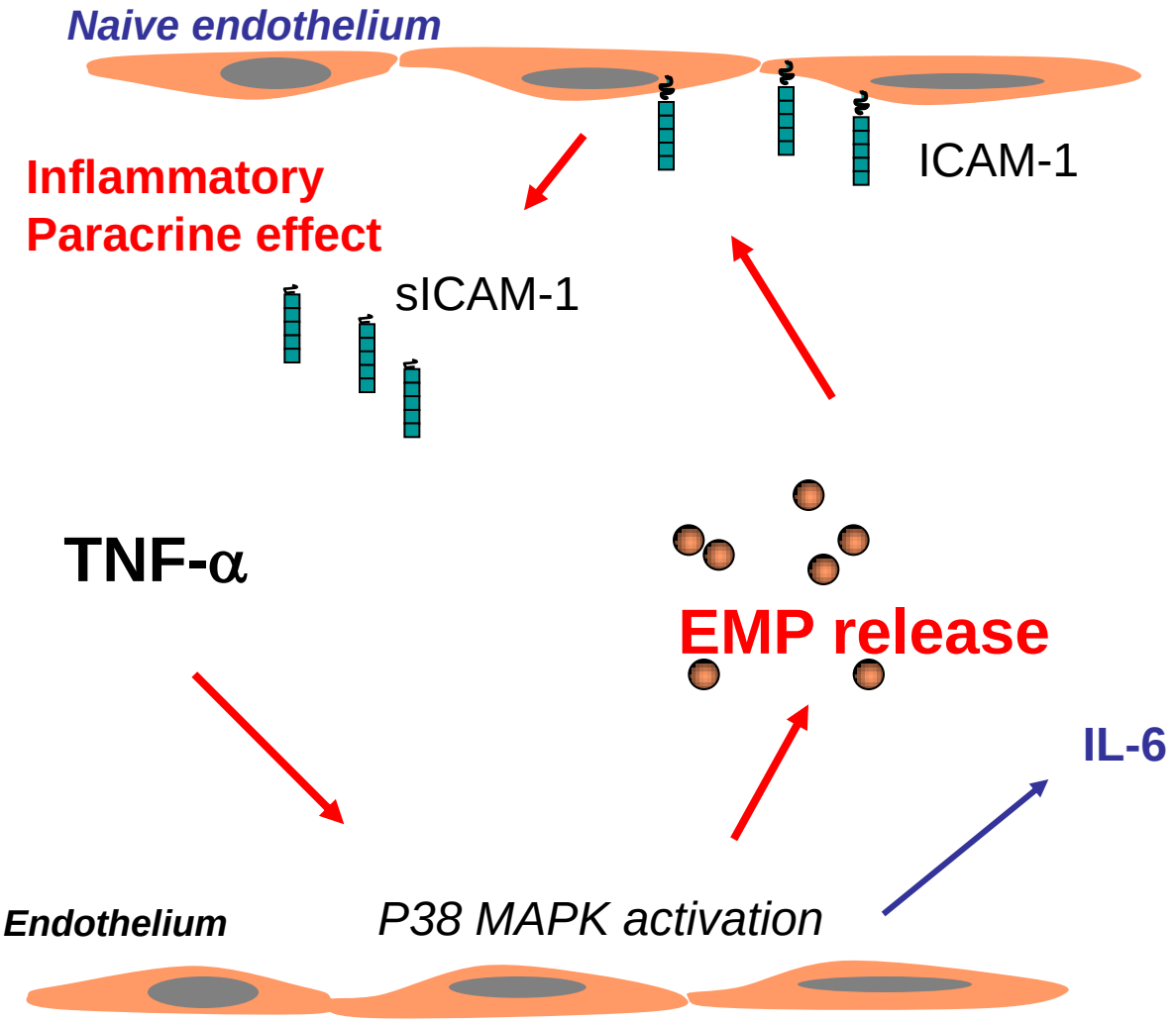
- ***Adhesion molecules are involved***

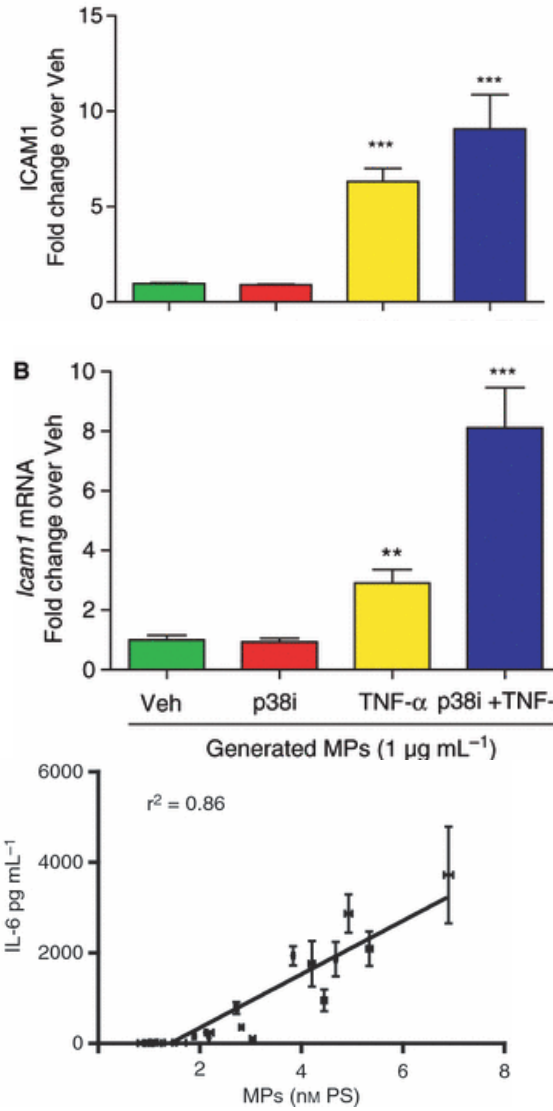


By coupling inflammation and coagulation, EMP/monocyte interaction is determinant for amplification of TF pathway

EMP elicit proinflammatory responses in naive endothelium

Curtis AM et al, J Thromb Haemost 2009

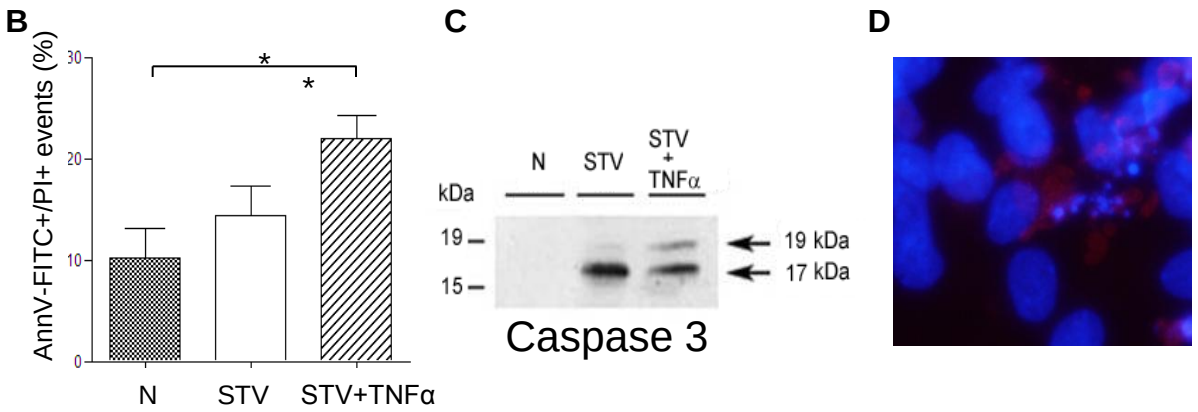




EMP : Cause and consequence of inflammation

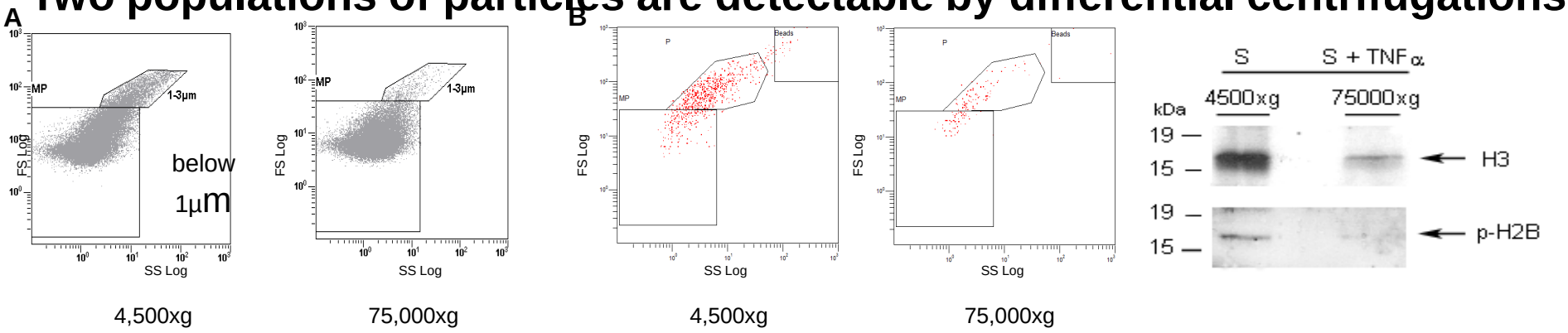
IL1 alpha , a major mediator of inflammation is conveyed by endothelial derived microparticles

(Y. Berda, G Kaplanski, submitted)



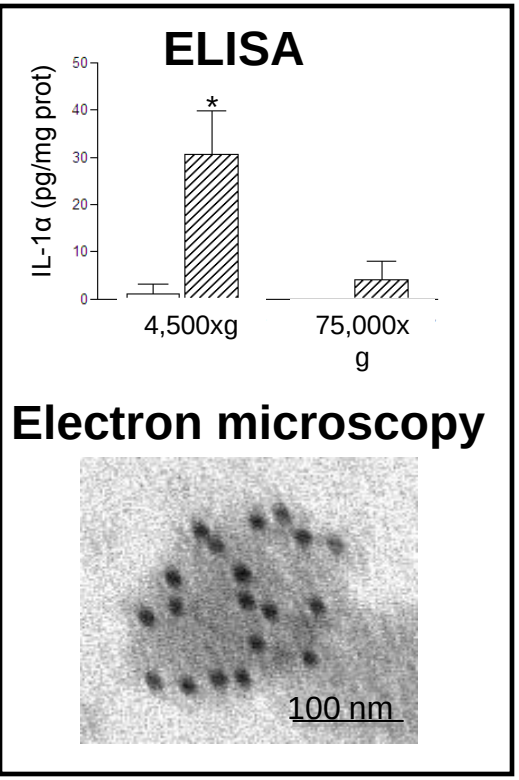
HUVECs submitted to serum starvation and TNFα stimulation release AnnV/PI+ particles expressing the cleaved form of Casp-3

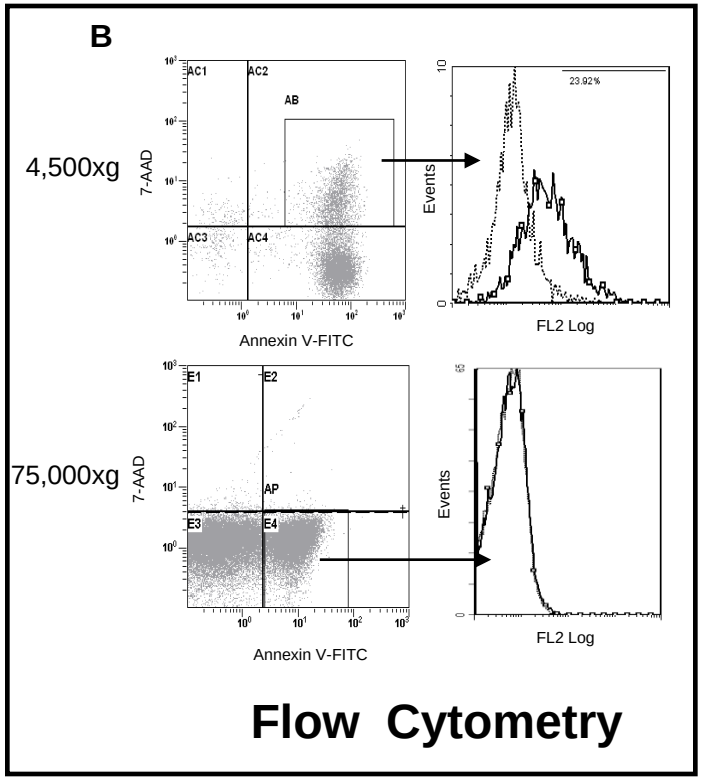
Two populations of particles are detectable by differential centrifugations

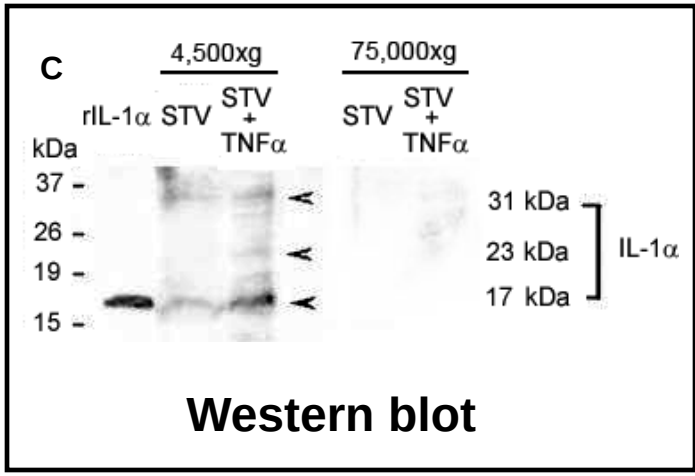


Endothelial –derived apoptotic bodies release active IL1 alpha

(Y. Berda , G. Kaplanski, submitted)







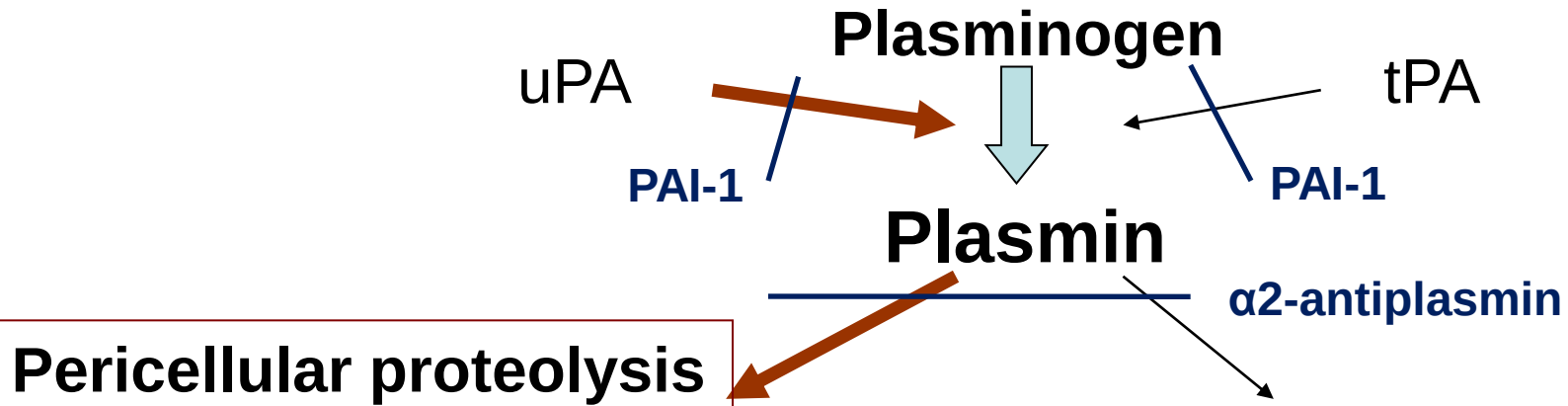
Apoptotic bodies conveying IL-1alpha :

-*in vitro*: promote MCP-1 and IL-8 production

-*in vivo:* promote peritoneal inflammation

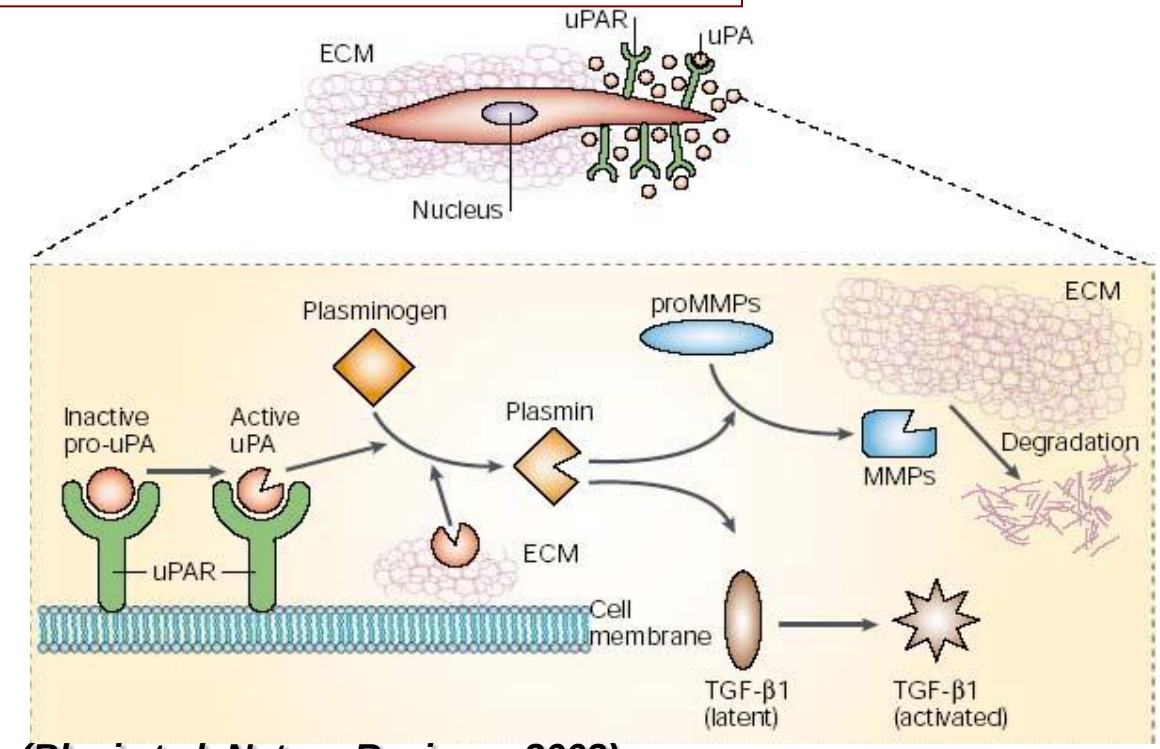
Danger-Associated Molecular Pattern (DAMP) playing a key role in « sterile » inflammation

Do MP support a link between hemostasis and angiogenesis by conveying plasmin ?



Pericellular proteolysis

Fibrinolysis



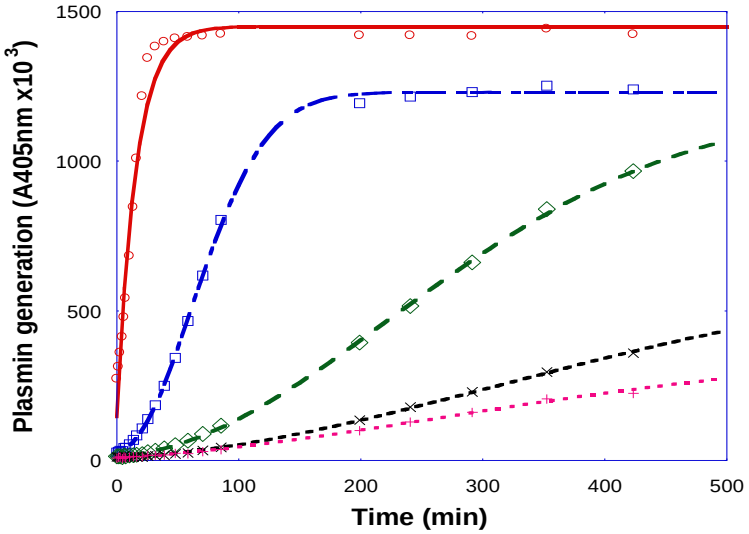
(Blasi et al, Nature Reviews, 2002)

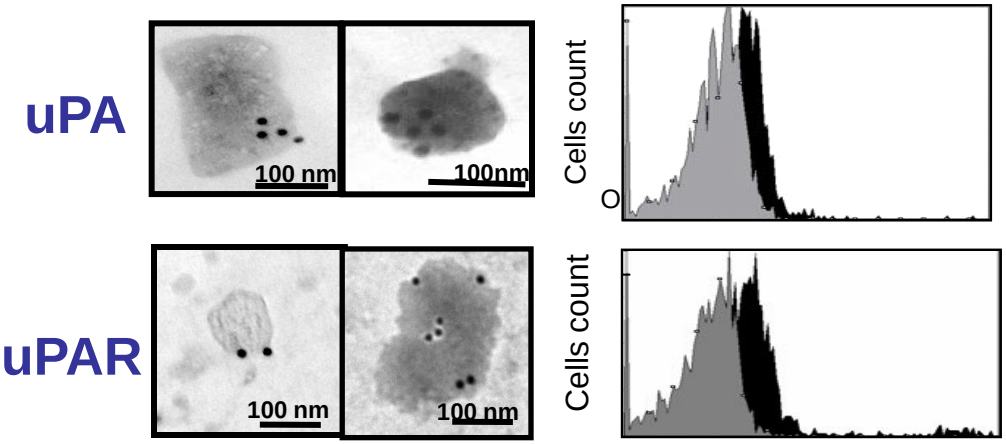
Tissue remodeling
Angiogenesis
Cancer spreading

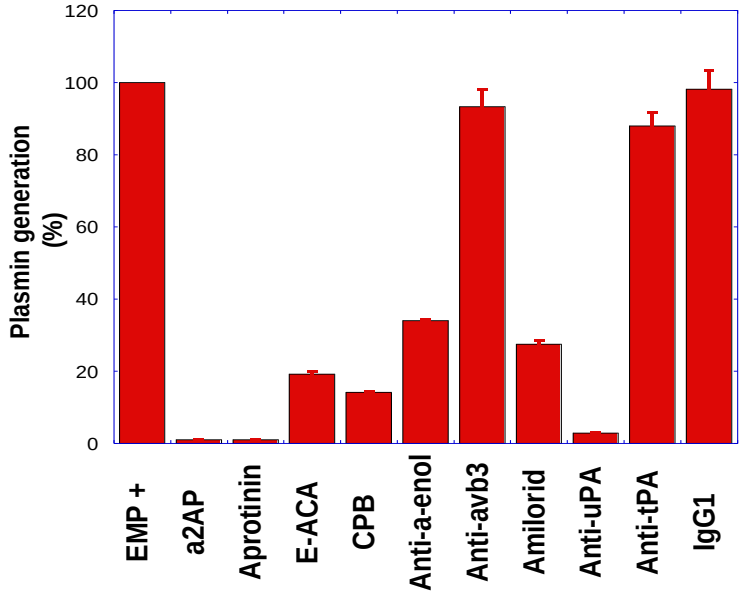
EMP behave as surface supporting Plasmin generation

Lacroix et al, Blood ,2007

EMP generate plasmin at their surface in a uPA-dependent manner





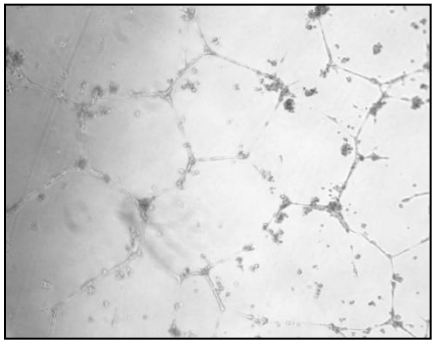


Plasmin generation by EMP : Potential impact in fibrinolysis and extravascular proteolysis

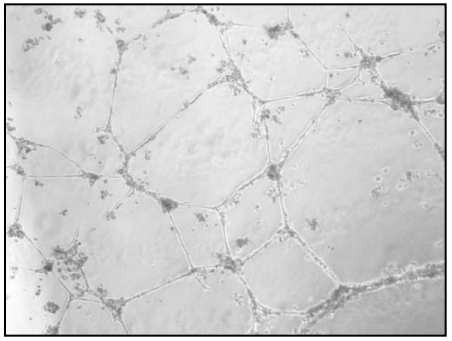
Does plasmin generation by EMP influence angiogenic properties of endothelial progenitors cells

Biphasic effect of EMP on vascular tube formation:

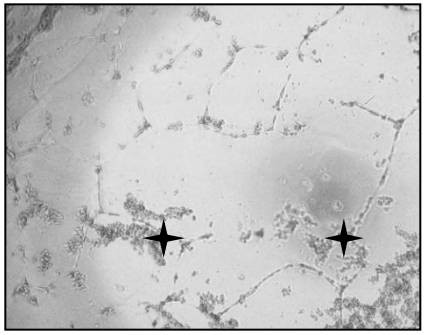
- low concentrations increase **-high concentrations have a reverse effect**



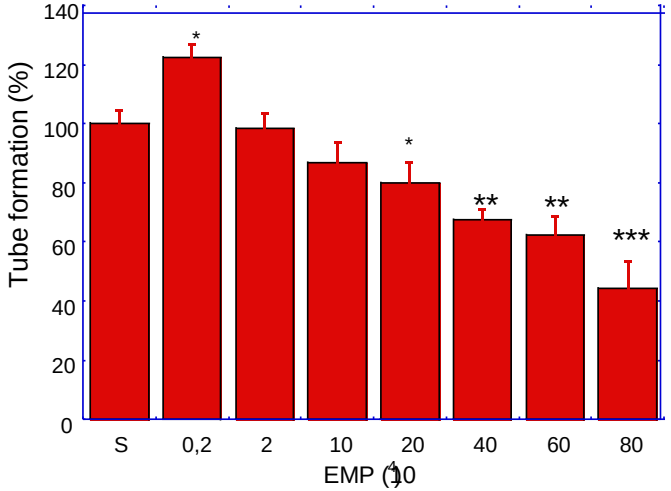
0 EMP



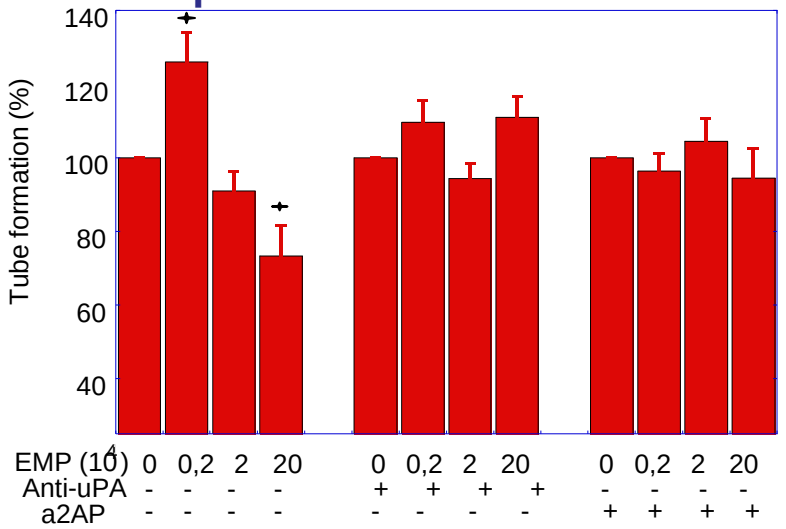
2.10^3 EMP



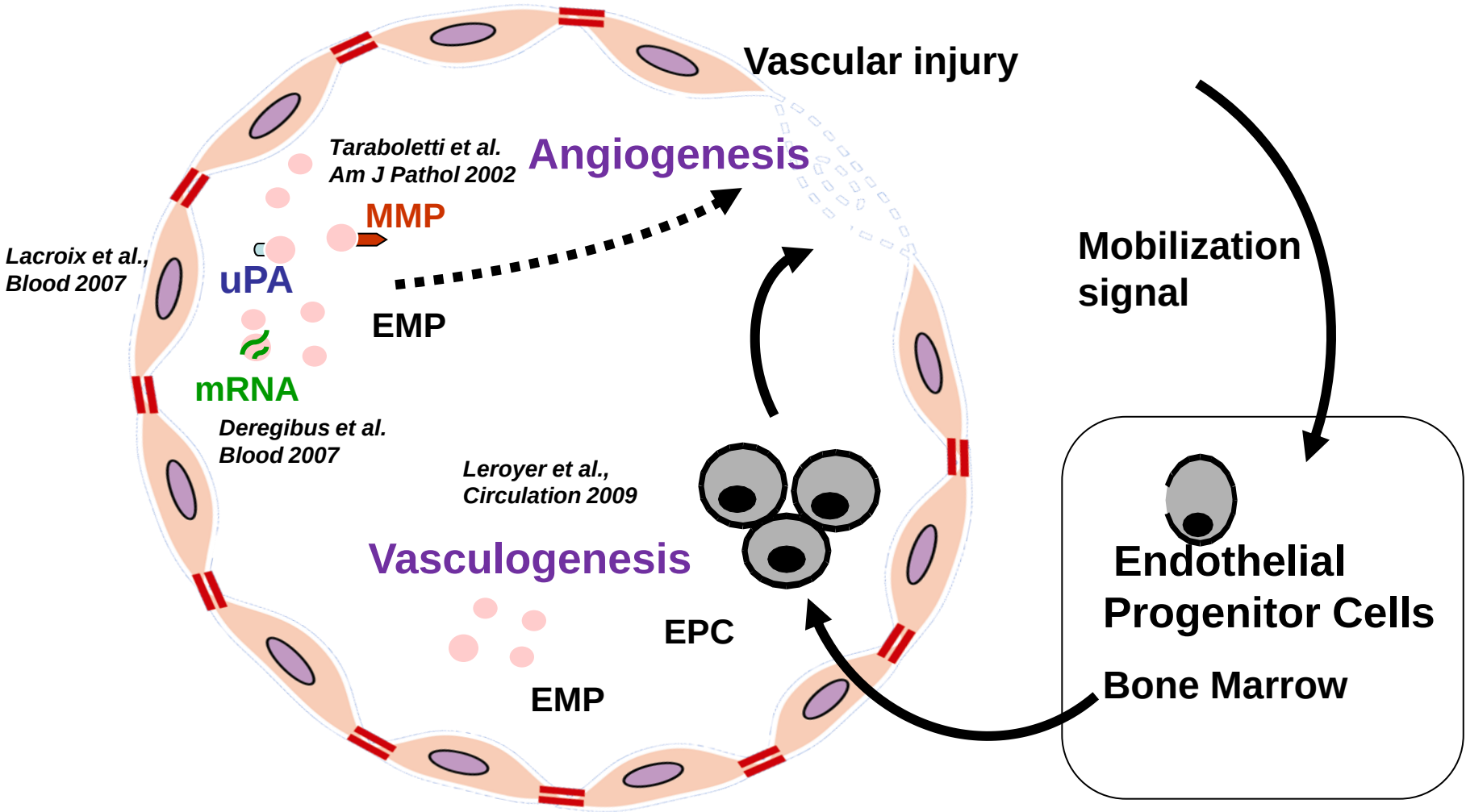
2.10^5 EMP



Inhibition of EMP effect by uPA or plasmin blockade



Endothelial microparticles
more than a signature of stress-injured and dying cells

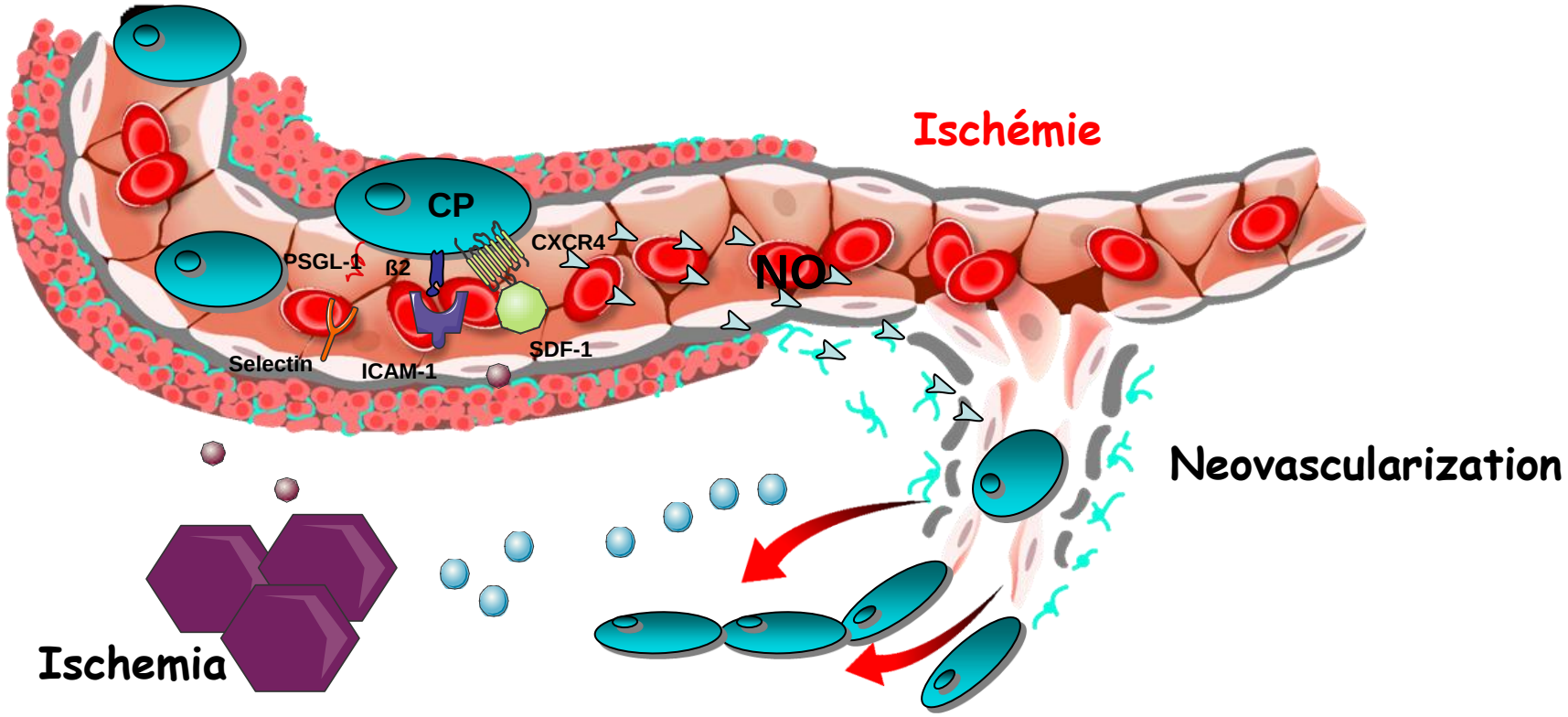


A survival message warning for the need of regenerative mechanisms and repair processes

Role of MP in Vasculogenesis

Leroyer et al., *Circulation* 2009

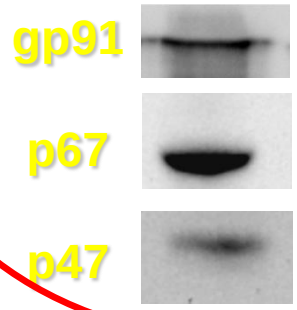
Hypothesis : MP produced *in situ* after ischemia could be the endogenous signal for post-ischemic neovascularization

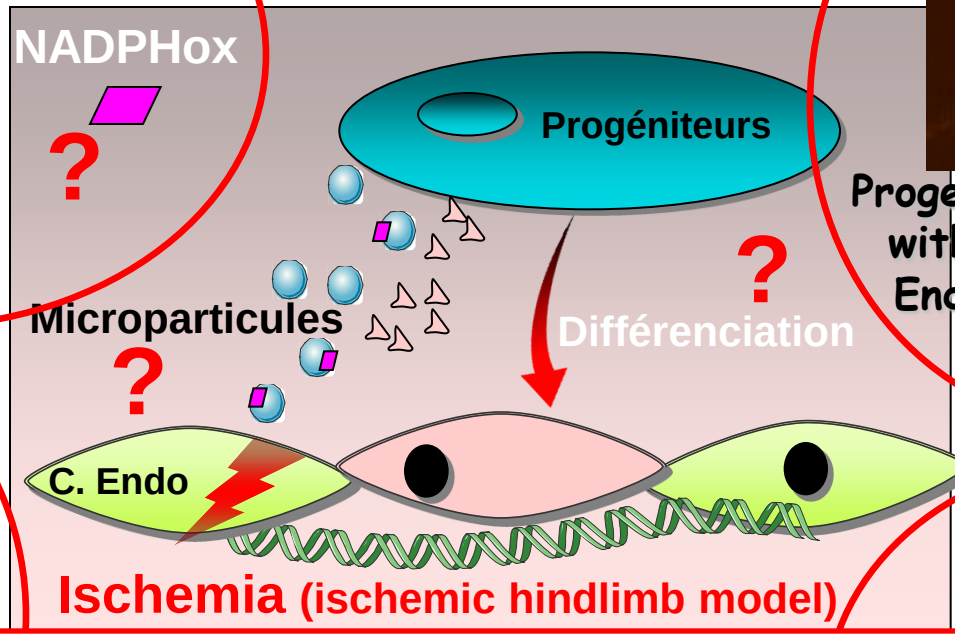


Role of MP in Vasculogenesis

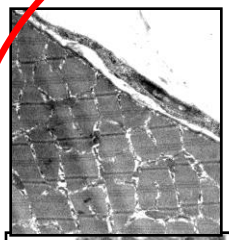
Leroyer et al., *Circulation* 2009

NADPHox subunits are expressed on ischemia induced EMPs

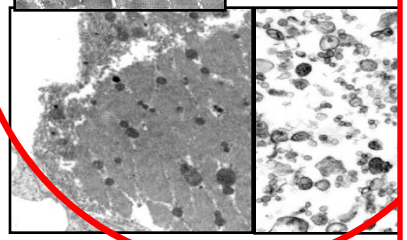




Progenitor cells incubated with MPs acquired an Endothelial phenotype



Ischemia produced EMP



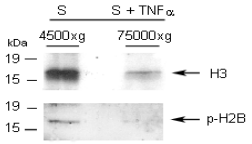
o injection
P primed PC
ovascularization

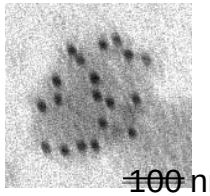
EMP produced during tissue ischemia stimulate progenitor cell differentiation and promote post-natal neovascularization in a ROS-dependent manner.

Endothelial microparticles :

A storage pool of bioactive vectors at the crossroad of thrombosis, inflammation and angiogenesis

Sabatier et al, J Cell Mol Med, 2009, Leroyer et al, Thromb Hamost, 2010

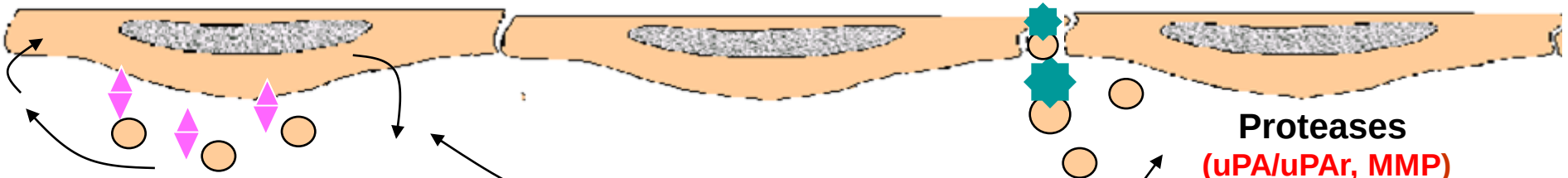




100 nm

IL-1alpha production,RANTES, ICAM1

Vascular remodeling – angiogenesis



Proteases

(uPA/uPAr, MMP)

Taraboletti et al. Am J Pathol 2002

Lacroix et al, Blood, 2007

Jouvencel , Lacroix R et al, Blood 2010

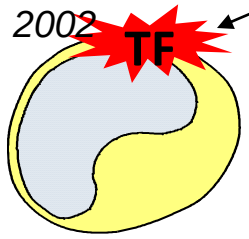
Inflammation

Rautou et al, Circ research, 2011

, Mause, ATVB 2005, Curtis et al, 2009

Coagulation

Sabatier et al, Blood, 2002



TNF

LPS

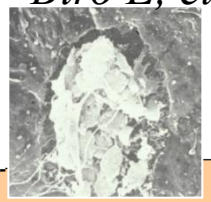
Monocytes

EMP

Combes et al, J.Clin Invest 1999

Thrombus formation

Biro E, et al. JTH, 2003

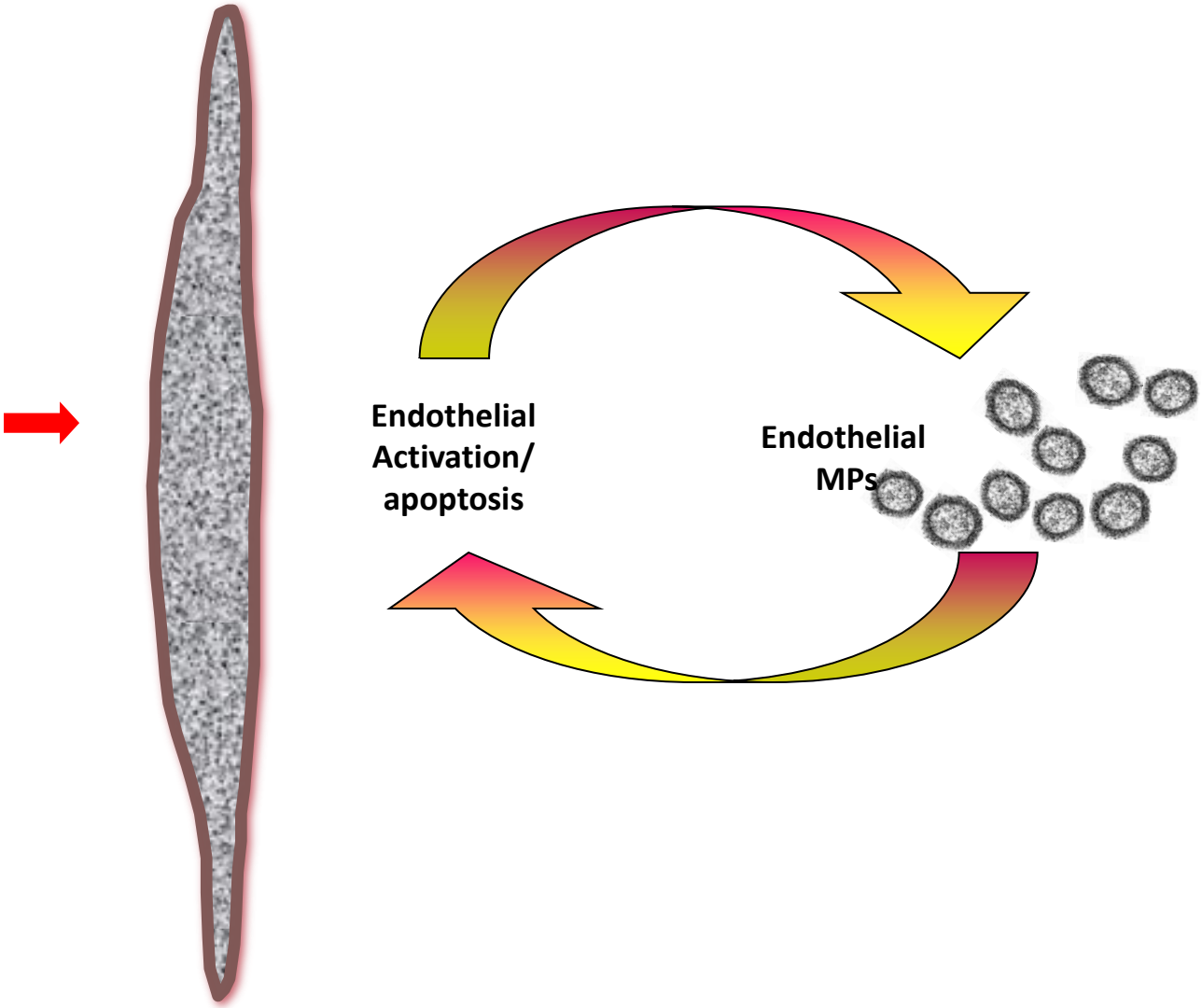


Questions

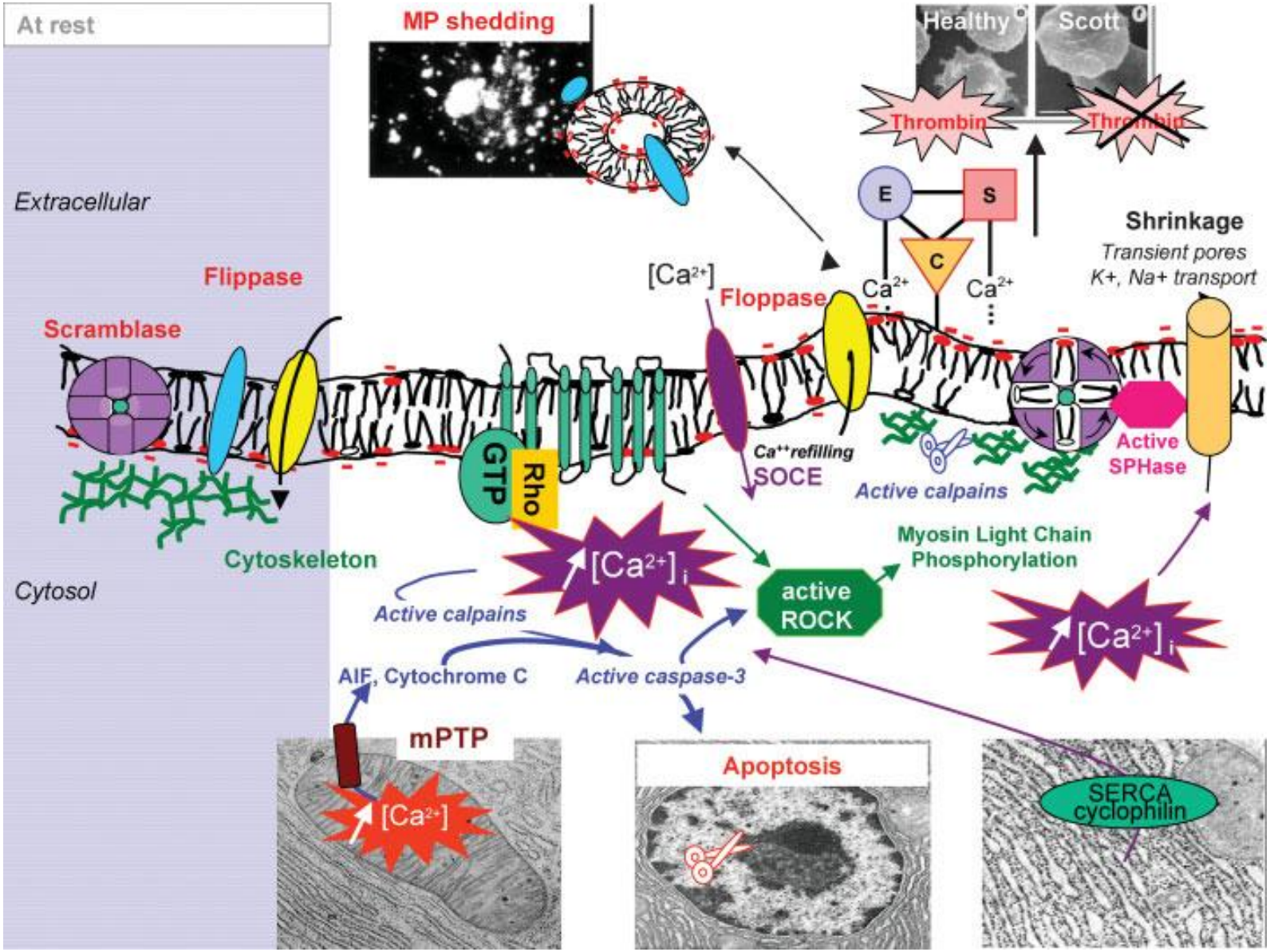
- **Multifaceted roles of Endothelial-derived Microparticles**
- **Mechanisms of formation**
- **Endothelial Microparticles in diseases**
- **Endothelial Microparticles : friends or foes?**

Inducers of vesiculation

TNFα
LPS
Camptothecin
CRP
PAI
Thrombin
Uremic toxins
Ox-LDL
Cytokines
ROS
Apoptosis inducers
Low shear stress?
……



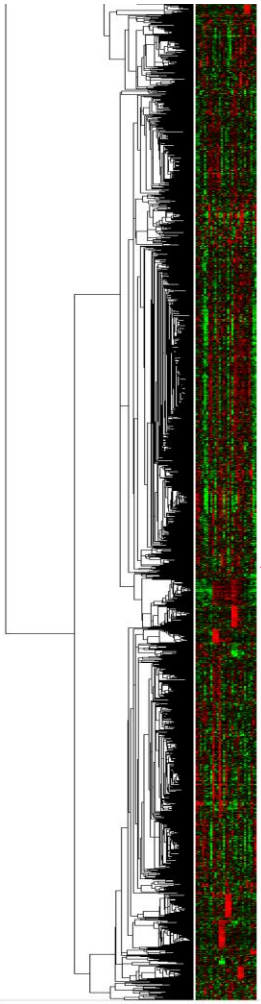
Mechanisms of formation



Morel O. Seminar in thromb and haemost 2010

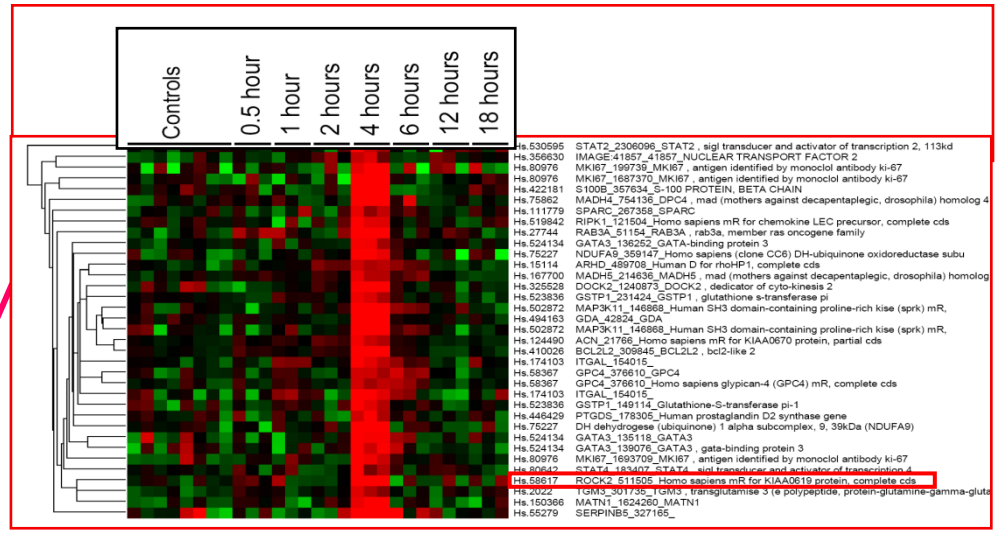
Thrombin activates pathways supporting EMPgeneration

Sapet et al, Blood, 2006

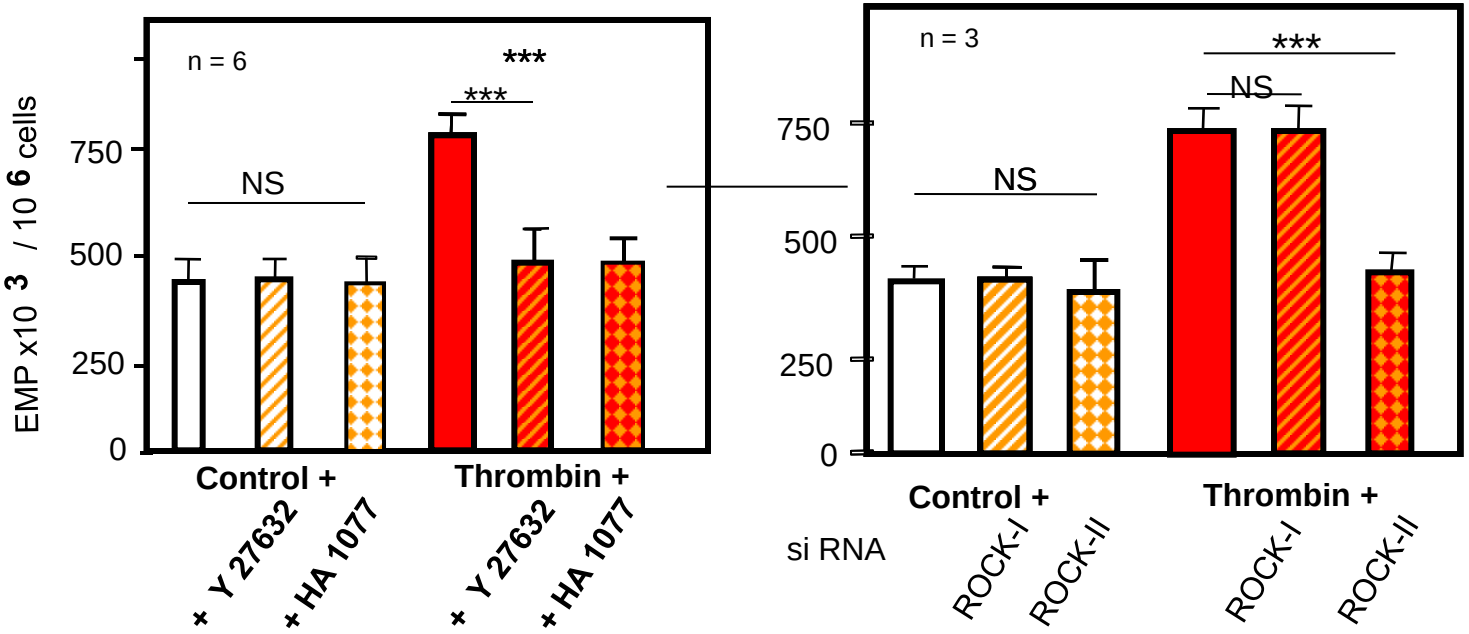


Up: 159 genes

Down: 37 genes



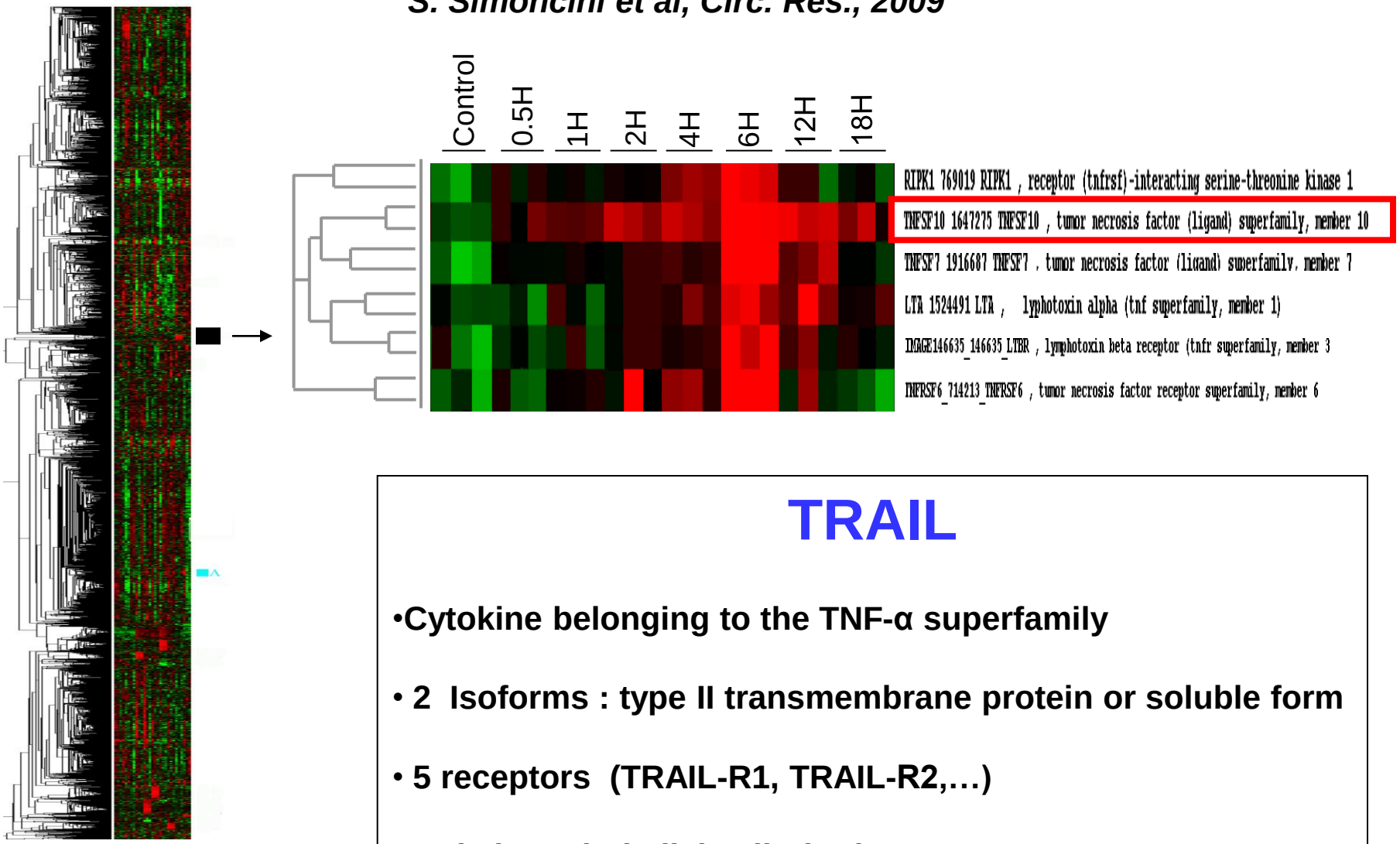
Cytoskeleton organization ROCK-II



ROCK-II is involved in EMP generation induced by thrombin

Thrombin induces the expression of TRAIL/Apo2L In endothelial cells

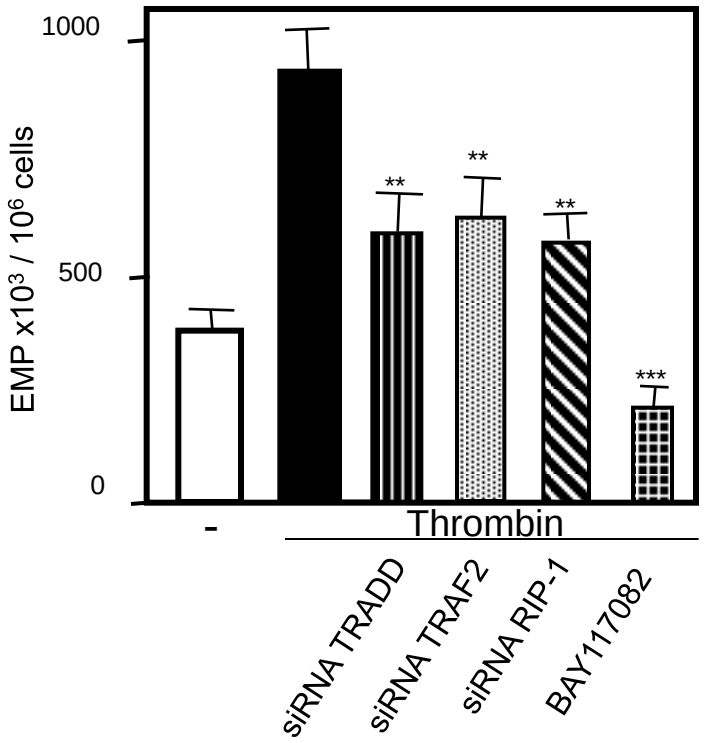
S. Simoncini et al, Circ. Res., 2009

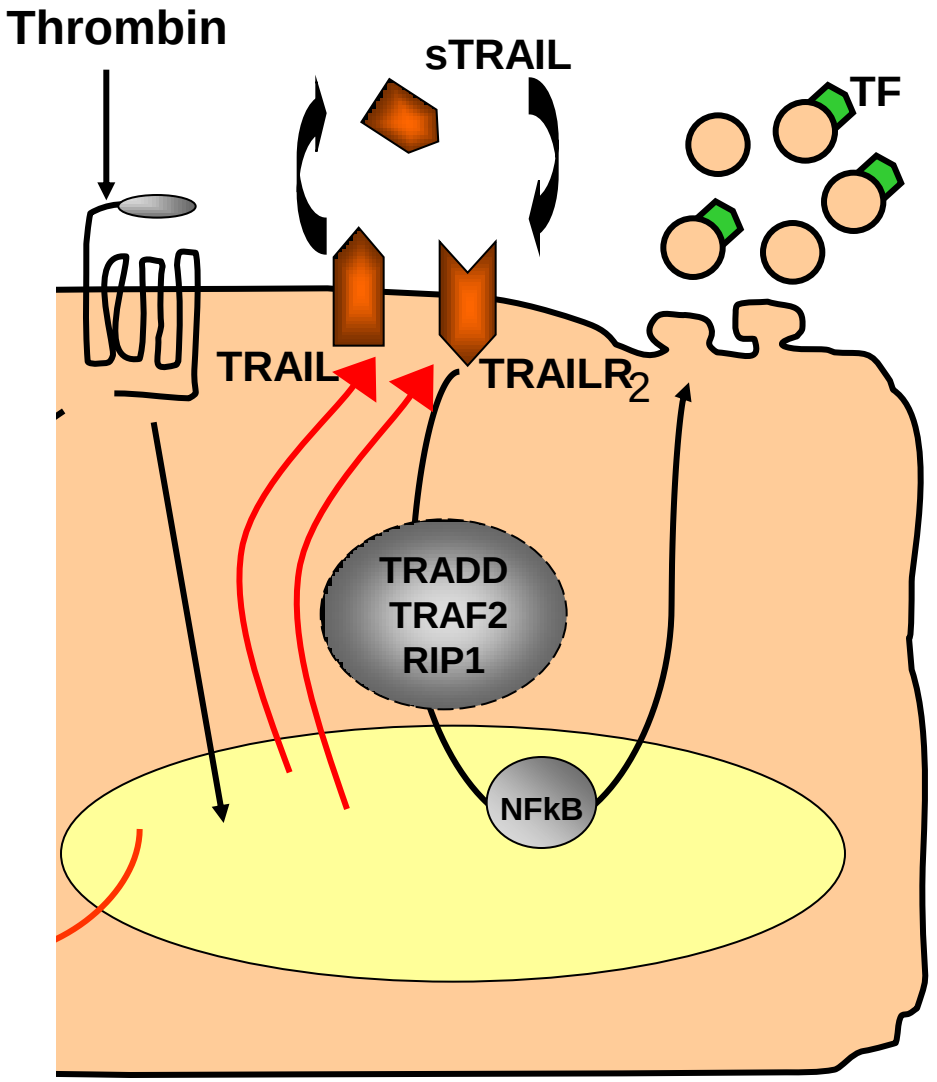


TRAIL

- **Cytokine belonging to the TNF-α superfamily**
- **2 Isoforms : type II transmembrane protein or soluble form**
- **5 receptors (TRAIL-R1, TRAIL-R2,…)**
- **Role in endothelial cells (EC) ?**

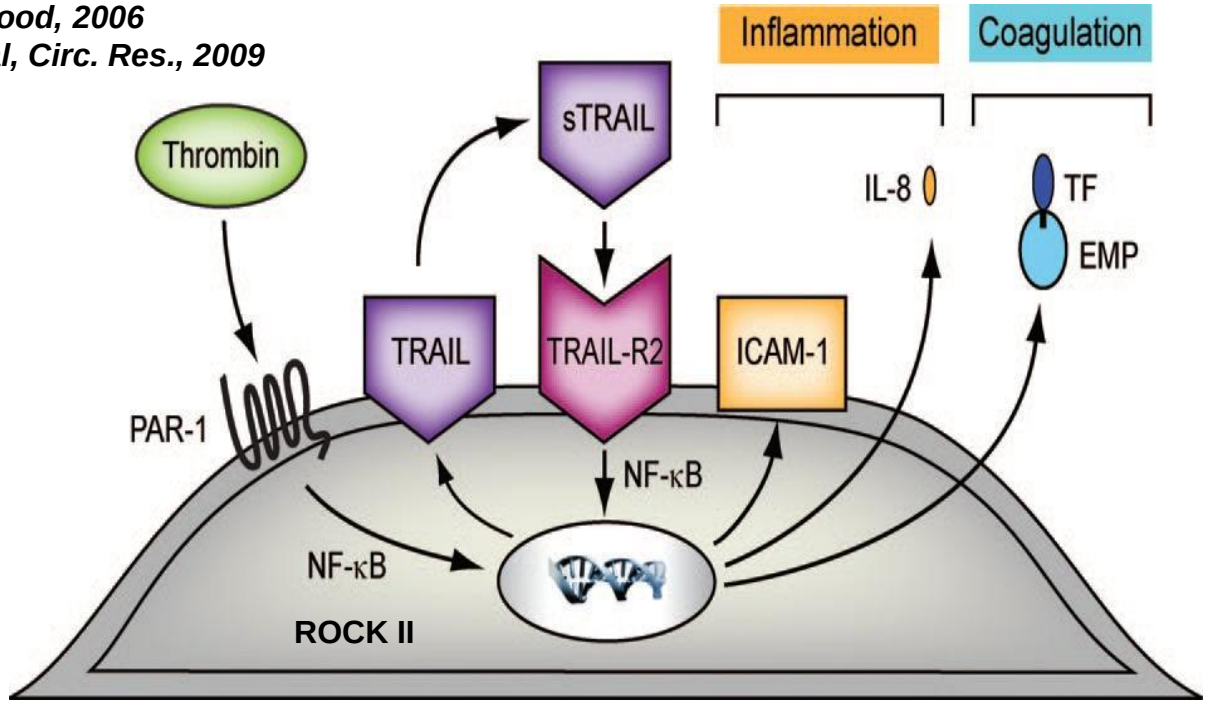
Thrombin induces the release of EMP bearing TF by an amplification loop involving TRAIL/ TRAIL R2





Thrombin induced EMP : Autocrine loop amplifying the interface coagulation / inflammation

C.Sapet et al, Blood, 2006
S.Simoncini et al, Circ. Res., 2009



EMP generation induced by thrombin :

:

Early pathway involving NF kB activation and ROCK-II isoform

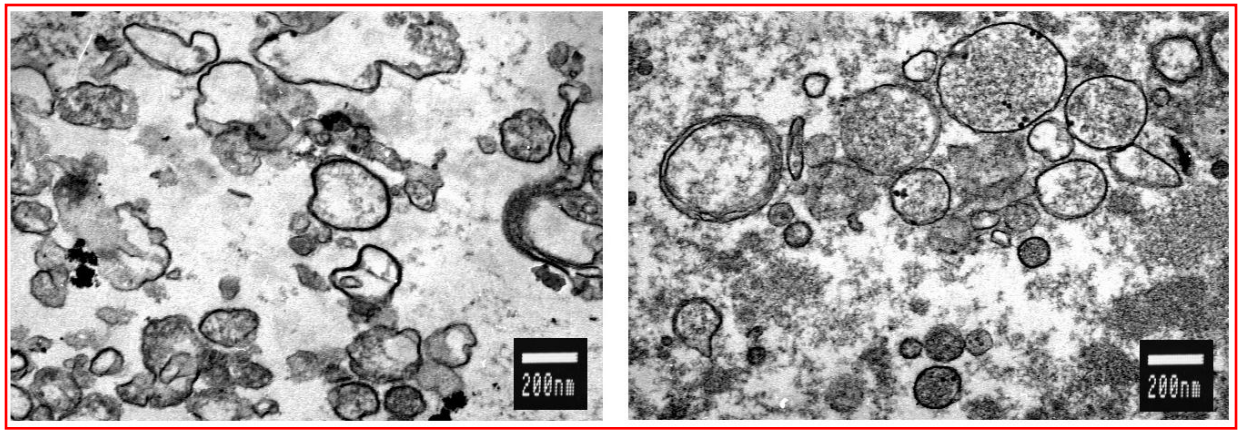
Late pathway involving indirect NF kB activation by newly synthesized TRAIL/TRAIL R

Pharmacological control of endothelial vesiculation : a potential novel therapeutic strategy for the treatment of inflammatory and thrombotic disorders

Questions

- **Multifaceted roles of Endothelial-derived Microparticles**
- **Mechanisms of formation**
- **Endothelial Microparticles in diseases**
- **Endothelial Microparticles : friends or foes?**

Microparticles in human diseases



MP from TNF-stimulated HUVEC *MP from human plasma*

International Society on Thrombosis and Haemostasis

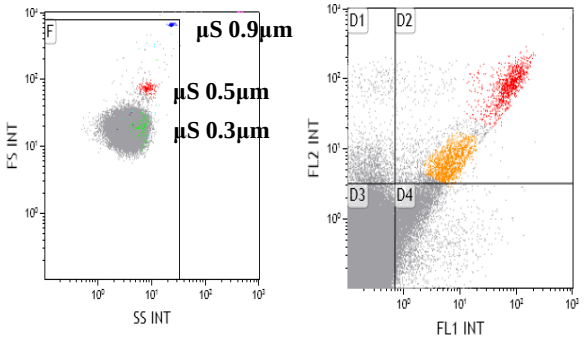
Scientific and Standardization Committee (SSC)
in vascular biology
F. Dignat-George

http://www.isth2010.com/

Standardization of Pre-analytical and analytical steps

A critical challenge ……

How to mesure microparticules ?

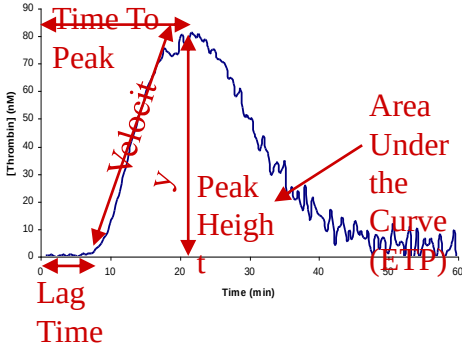


Flow cytometry

VE cadherin, Endoglin, E selectin,CD146

Numeration

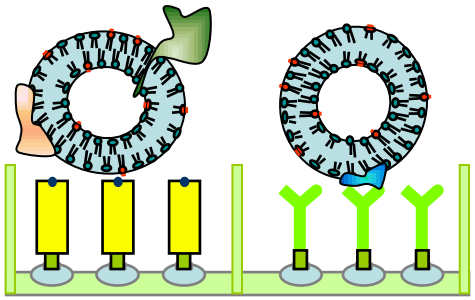
Fluorescent Calibrated beads (*Patent : n° 9908505)*
(Robert et al, J.Thromb. Haemost. 2008)
(Lacroix et al; J Thromb Haemost,2009)



Functional assays

PS /Prothrombinase activity
TF/ Thrombin generation
Procoagulant potential

tPA: uPA / plasmin activity
Patent : n° 07/04060
Proteolytic activity



Captured assays (JM Freyssinet / F Toti)

Endothelial antigen/prothrombinase activity
Procoagulant potential / Cellular origin

EMP significance in vascular diseases

Sabatier et al, J Cell Mol Med, 2009

Biomarkers of endothelial activation/injury

- **Correlation with disease activity and with endothelial dysfunction**

Vasculitis *(Brogan et al, Arth Rheum, 2004)*
Coronary artery disease *(Werner N et al, 2006)*
End stage renal disease *(Amabile N et al, 2006)*
Sickle cell anemia *(Shet AS, Blood, 2003)*

Hypertension *(Chironi et al , Hypert 2009)*
Diabetes *(Sabatier et al, Diabetes 2002 Chahed et al, Diabetes 2009)*
Met syndrome *(Helal, Nutrition and Metabolism, 2010)*
Renal failure *(Jourde et al, J. Throm. Haemost., 2009 Faure et al, J Throm. Haemost., 2006)*

- **Pronostic significance, vascular risk**

Stroke *(Simak et al 2006)*
Type 2 diabetes mellitus *(Koga et al, 2005,Morel 2007)*
Patients at risk for CHD *(Nozaki et al, 2009)*

Lupus anticoagulant *(Combes , J. Clin. Invest. 2001)*
AntiphospholipidSyndrome *(Dignat-George , J. Throm. H. 2003*
Bed rest *Sabatier , Am J Physiol, 2010)*
Kydney transplantation *(Al Massarani Am. J. Transpl. 2008)*

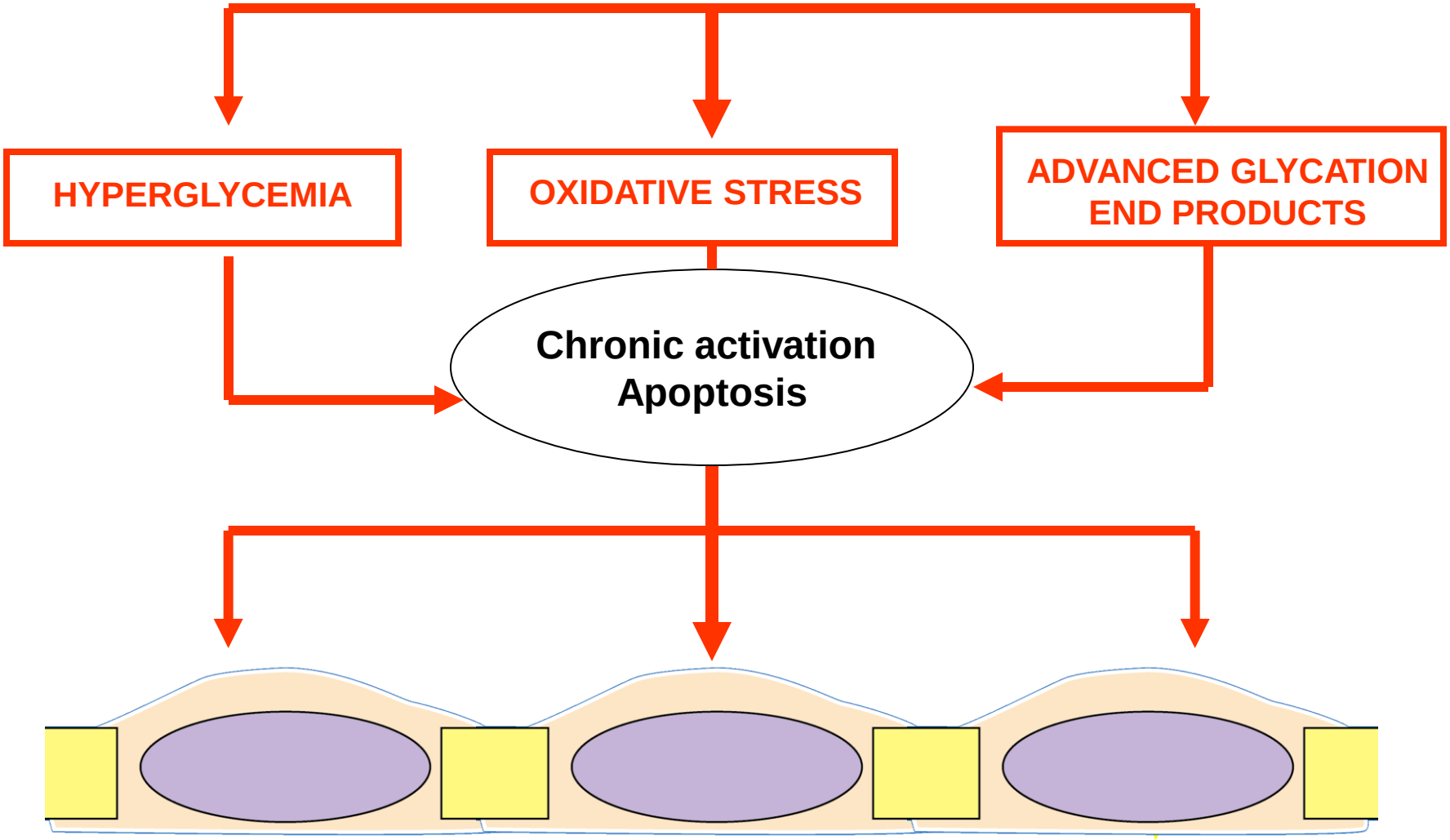
- **Response to Treatment monitoring**

Calcium inhibitors *(Nomura et al, 2007)*
Pioglitazone *(Esposito et al, ATVB 2006)*
Eicosapentanoic *acid* *(Morel et al, Thromb Haemost, 2004*
Transplantation *(Massarani Am. J. Transpl. 2009)*
Insulinothérapie *(Darmont et al, Thromb. Research 2010)*

Pathogenic vectors

Bioactive vectors disseminating procoagulant ,proinflammatory and proteolytic activites in the blood

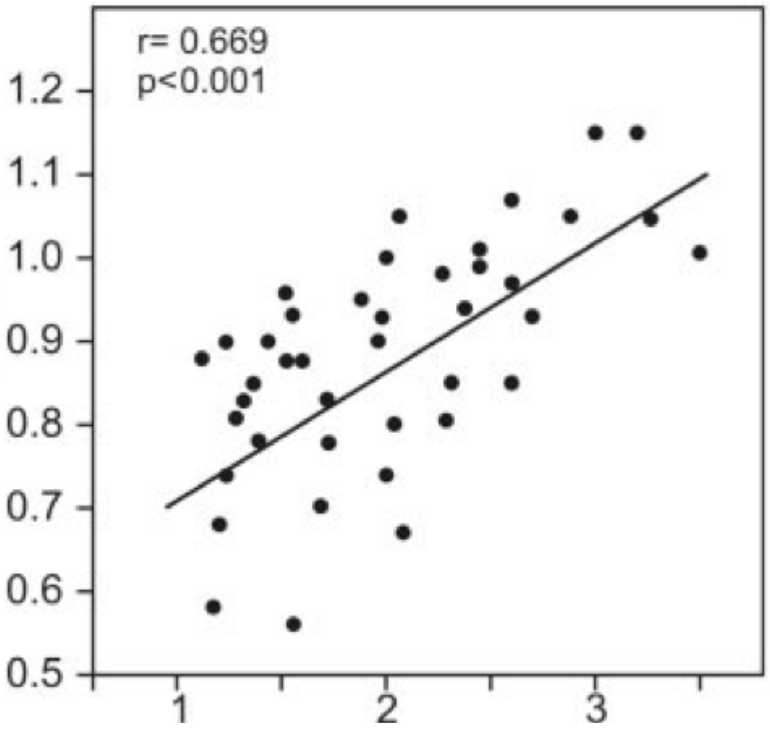
Diabetes



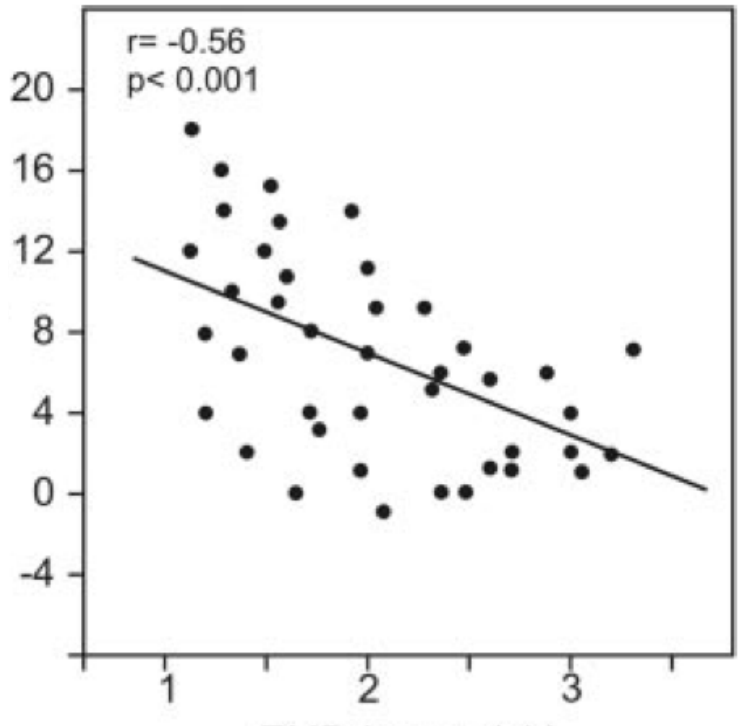
Prothrombotic and proinflammatory status through MP release?

Endothelial microparticles and endothelial dysfonction In obese women

Waist-to-hip ratio



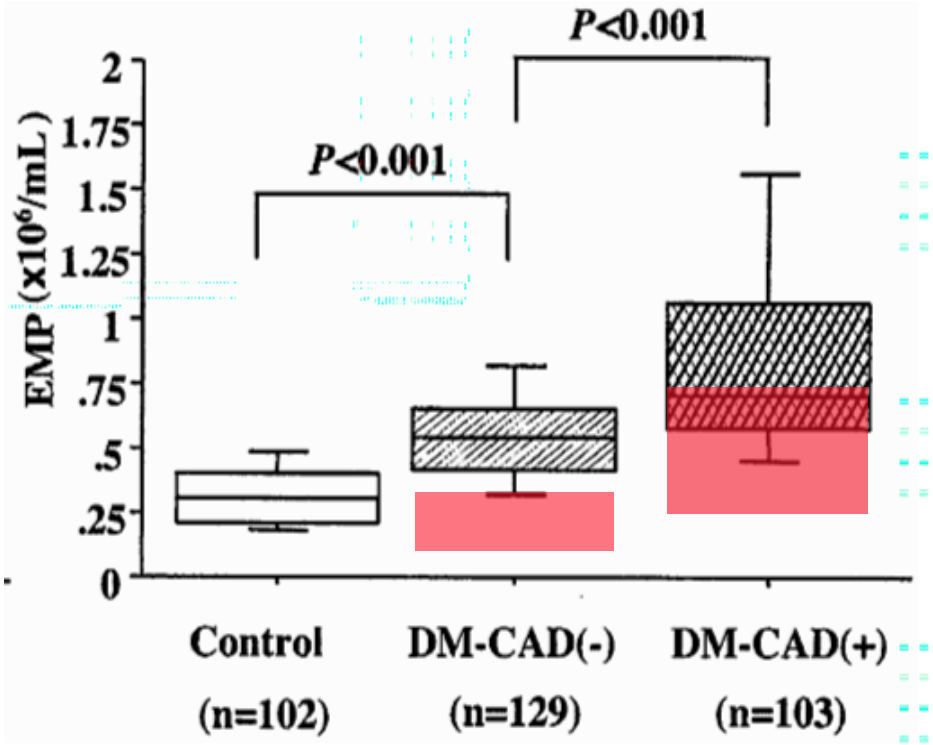
Flow-mediated dilatation

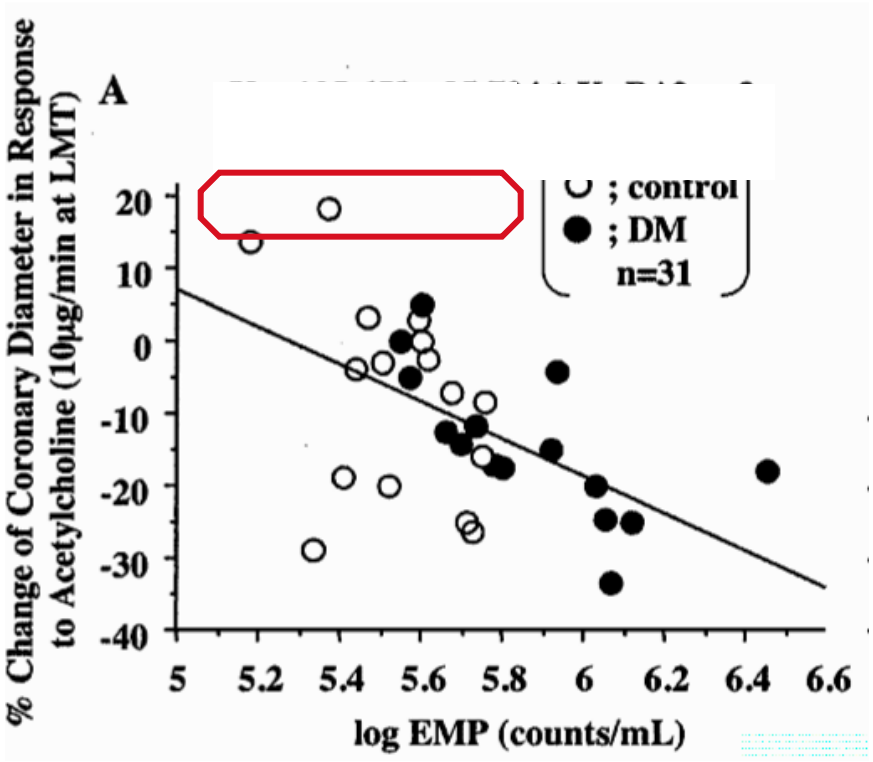


CD31+ CD42- MPs (count / microL)

Esposito *et al*., J. Clin. Endocrin. Metabo, 2006

Endothelial microparticles, vascular risk and Endothelial function in diabetic patients with C.A.D





Koga et al, JACC 2005, 45; 1622-30

MPE levels are predicitve of CAD in diabetes

- Multivariate analysis of risk factors for CAD in patients with diabetes:

– **EMP (high):**	**p=0.0002**
– **ICAM (high)**	**p= 0.3**
– **HDL (low)**	**p=0.02**
– **LDL (high)**	**p=0.04**
– **DM duration (>12yrs)**	**p=0.02**
– **Hypertension**	**p=0.03**

Koga et al, JACC 2005, 45; 1622-30

Pharmacological control of MP and diabetes prevention

- **Ca 2+ channel inhibitor (Nifedipine)** : Reduction of EMP and PMP shedding in hypertensive patients with type 2 diabetes

  (Nomura et al, J Human Hypertension, 2005)

- **Intensive insuline therapy :**decrease of EMP and PMP levels and procoagulant activity in Poorly controlled type 2 diabetic patients *(Darmont et al, Thomb Research , 2010)*

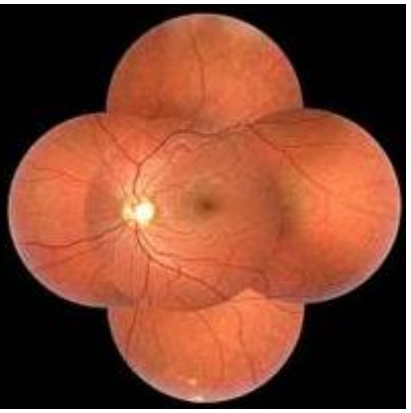
- **Antioxydant (Vitamine C)** : Protective effect of on endothelial damage reflected by decreased EMP levels in diabetic patients with MI . *(Morel et al, J. Thromb Haemost, 2003)*

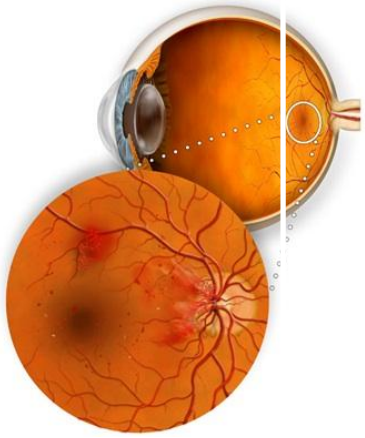
Part of the beneficial effect could be linked to decreased pathogenicity of procoagulant MP

Diabetic Retinopathy

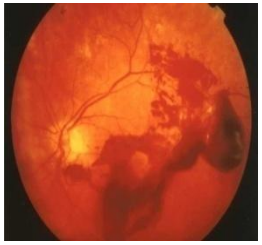
Leading cause of vision impairment and blindness

Healthy Subject





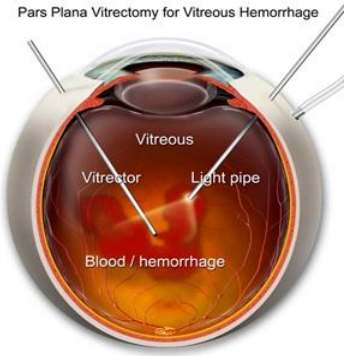
Proliferative Diabetic Retinopathy



- *Alteration of retinal vasculature*
- *Increased local activation and apoptosis of retinal, neural and endothelial cells*
- *Pathological angiogenesis in the vitreous cavity*

Therapeutic strategies

Vitrectomy

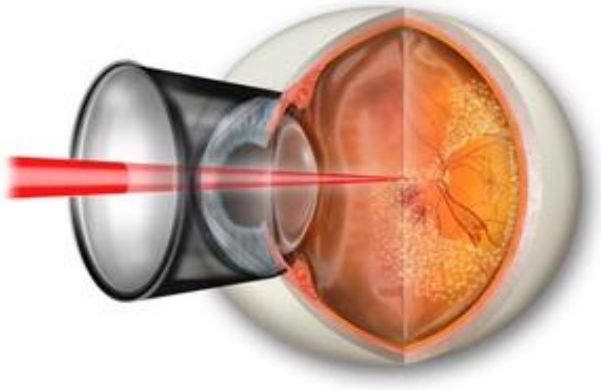


+ Photocoagulation

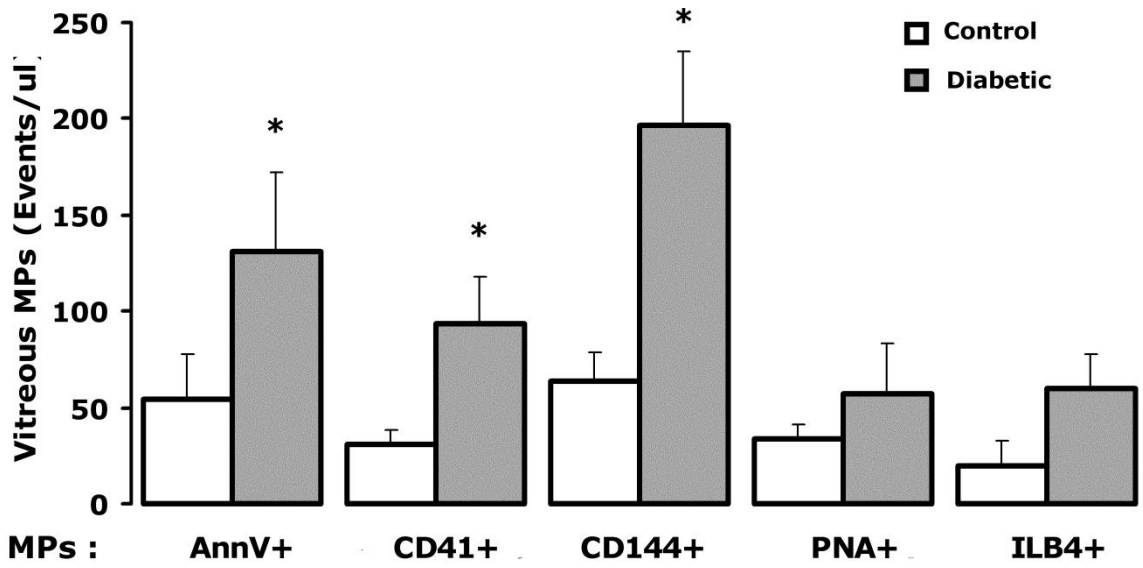
Or

Anti VEGF antibody

Bevacizumab



Characterization of vitreous MP

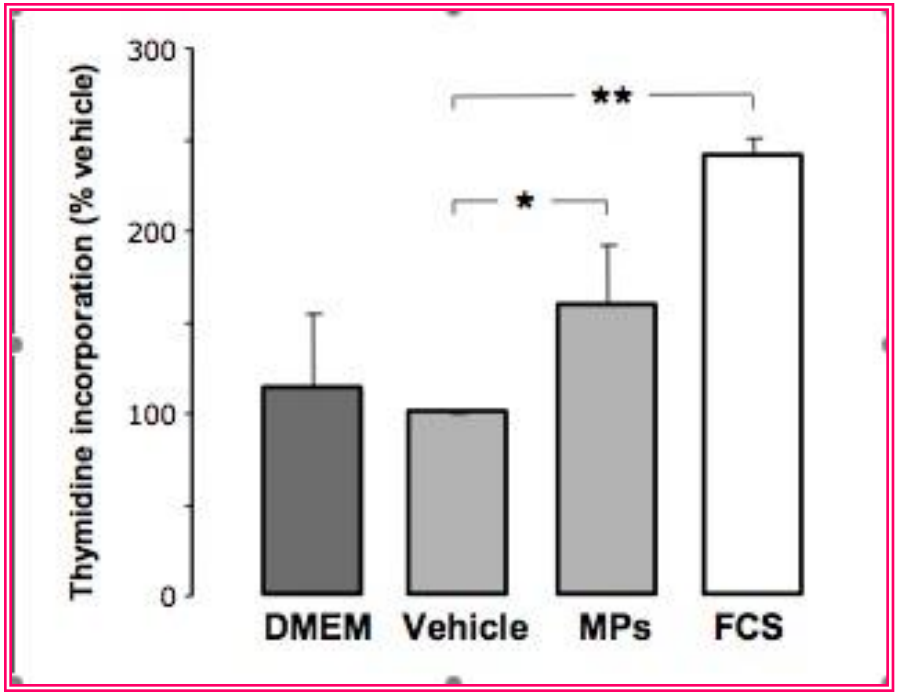


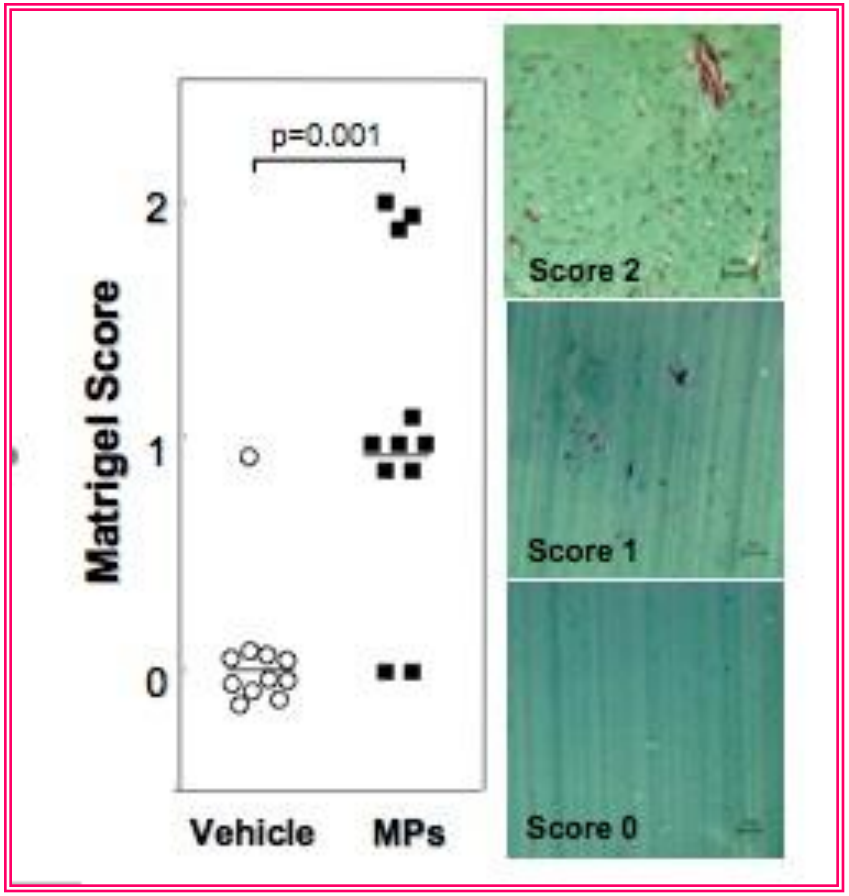
Majority of vitreous MP is of endothelial origin (CD144+)

PNA (peanut agglutinin) and ILB4 (isolectinB4) are retina markers

Chahed, Leroyer et al., *Diabetes* 2009

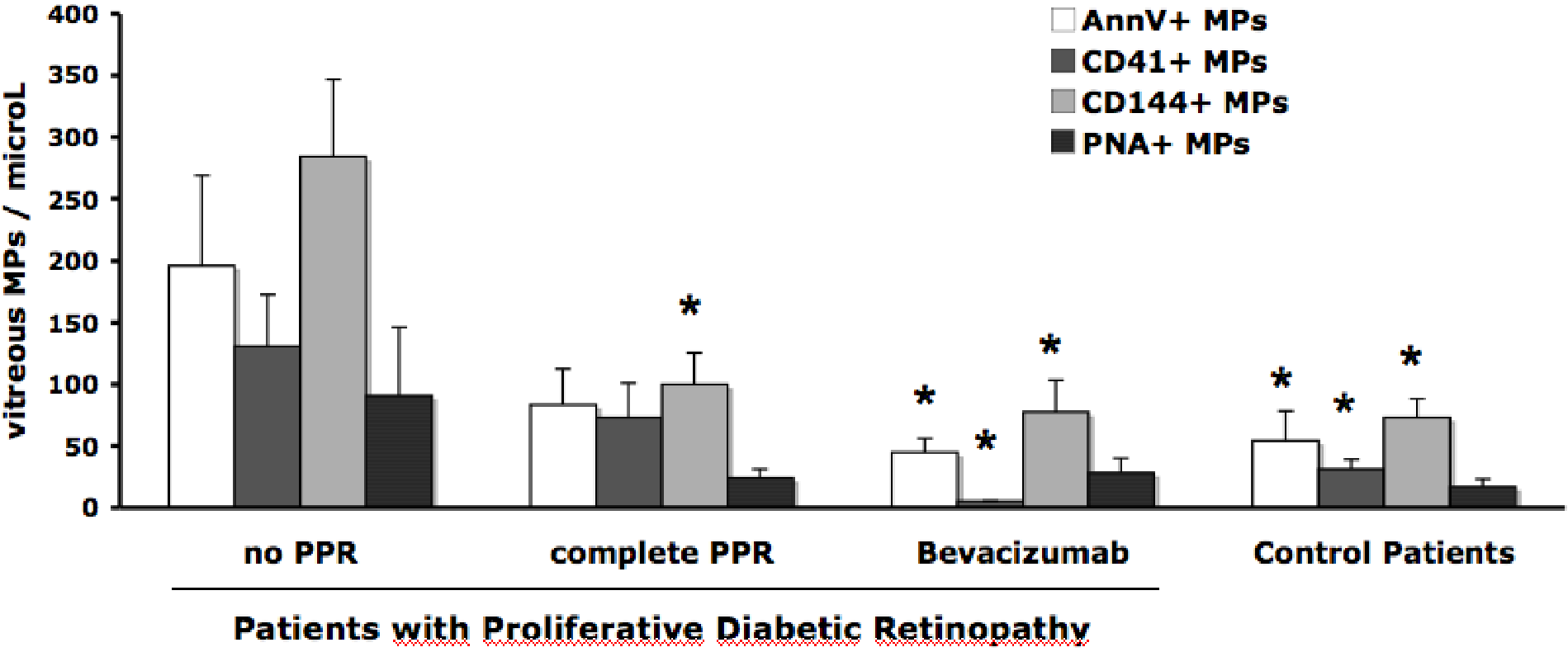
Vitreous MP induce endothelial cell proliferation and neovessel formation





Chahed, Leroyer et al., *Diabetes* 2009

Effect of therapies on vitreous MP levels in PDR



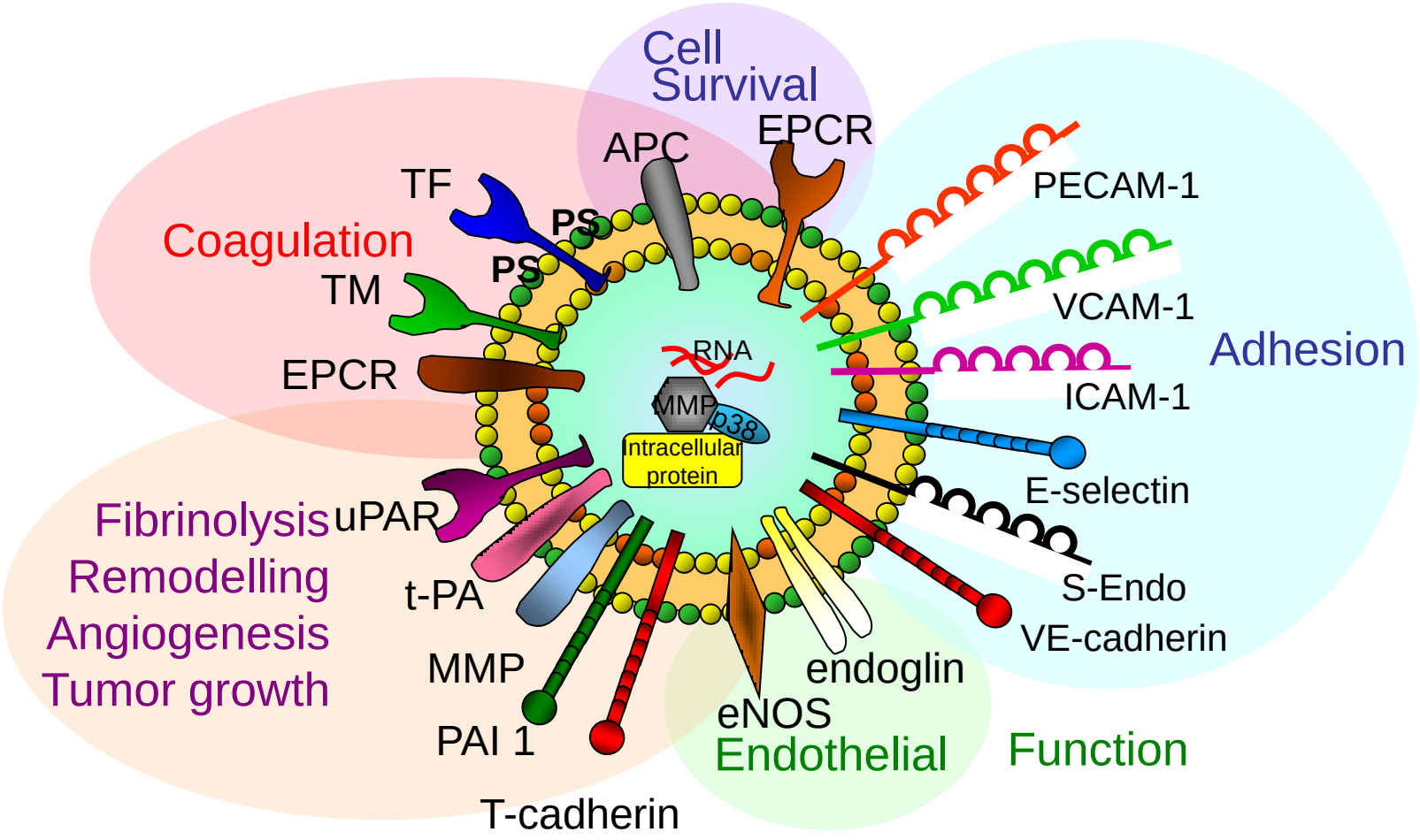
Chahed, Leroyer et al., *Diabetes* 2009

Questions

- **Multifaceted roles of Endothelial-derived Microparticles**
- **Mechanisms of formation**
- **Endothelial Microparticles in diseases**
- **Endothelial Microparticles : friends or foes?**

Multifaceted Endothelial microparticles

F Dignat -George and C Boulanger , ATVB, 2010

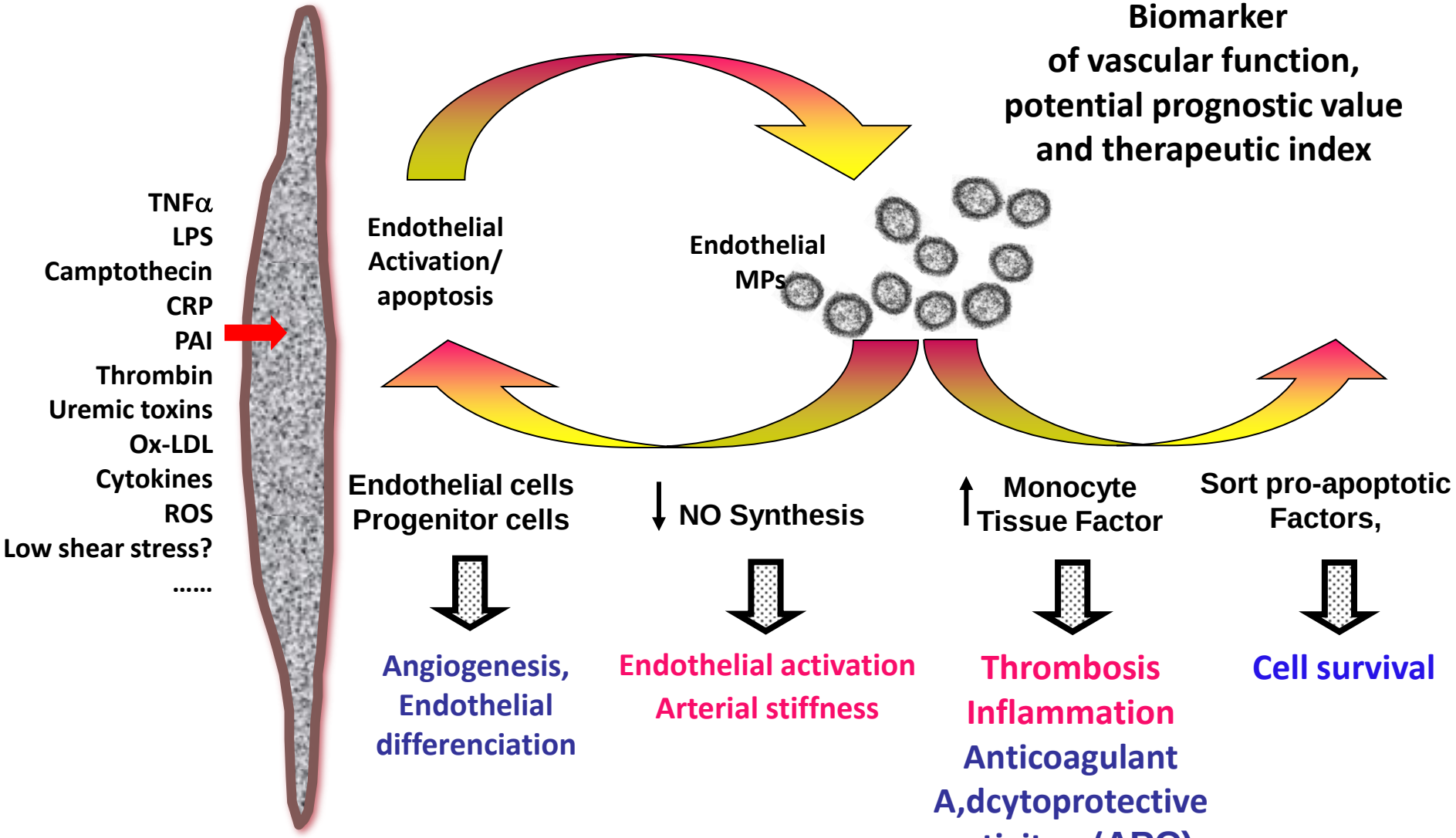


MP are conveyors of cell information
with major role in inflammation, thrombosis and angiogenesis

***But are they* friends or foes?**

The many faces of endothelial-derived microparticles

F Dignat- George and C Boulanger , ATVB, 2010



Questions and Challenges for the Future

MP are conveyors of cell information

with major role in inflammation, thrombosis and angiogenesis

- Use animal models to understand the pathophysiological role of MP
- Extend our knowledges on the molecular mechanisms controlling cell vesiculation
- Manipulate MP generation using pharmacological approaches

Future in vivo and in vitro studies will delineate when and how EMPs plays Dr Jekill or Mister Hyde

Clinical applications : New horizons in vascular disorders

- Key players in vascular complications associated with a disease state
- Promising markers to identify patients with vascular risk
- Novel targets for therapeutic strategies based on the pharmacological modulation of MP number and/or procoagulant activity?

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J M Freyssinet
F Toti

INSERM U919
Caen

E. Angles Cano

INSERM U 970

C Boulanger

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Y Berda
N Jourde
S Niock

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Diagnostical Stago .

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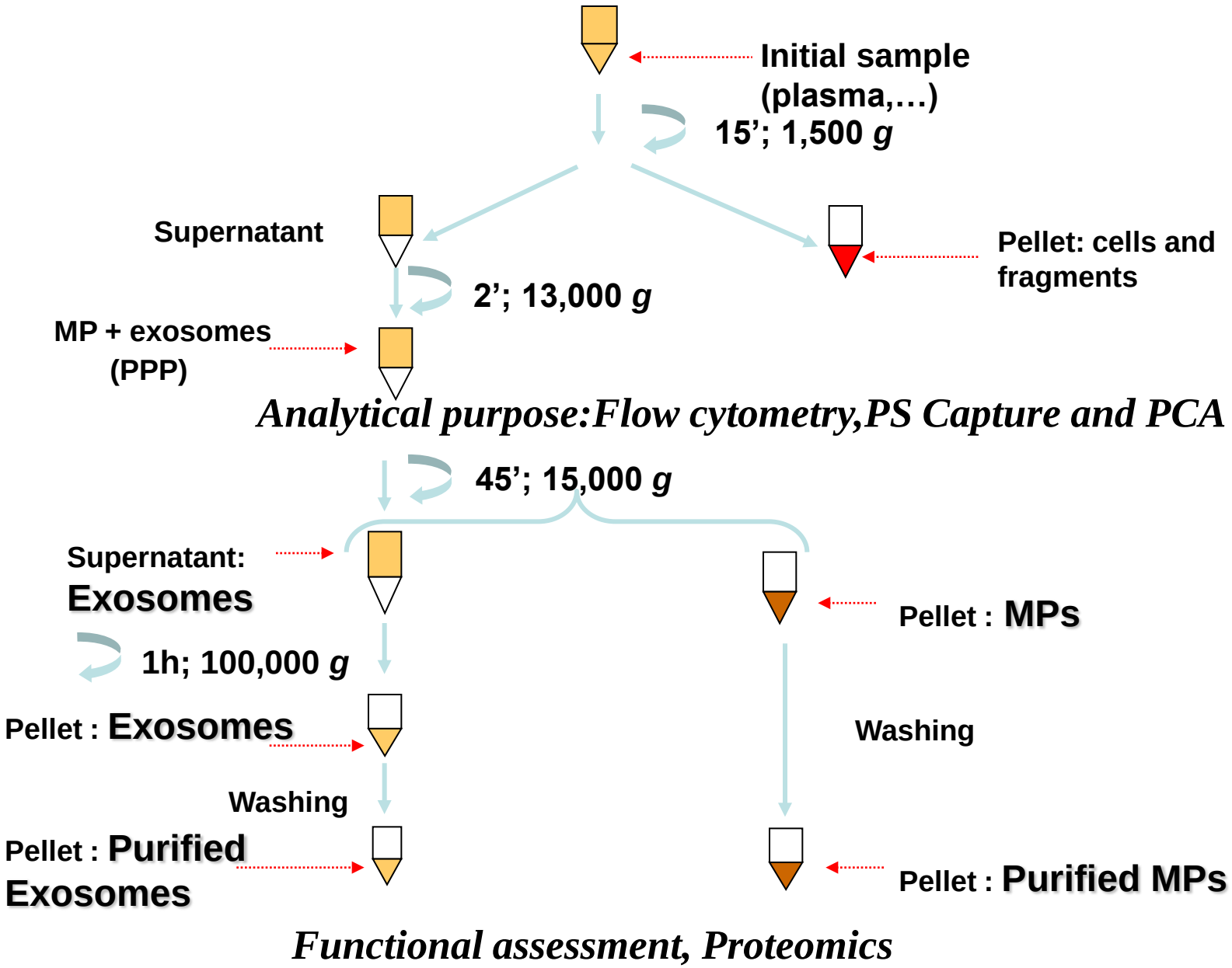
Harvard Medical School, Boston

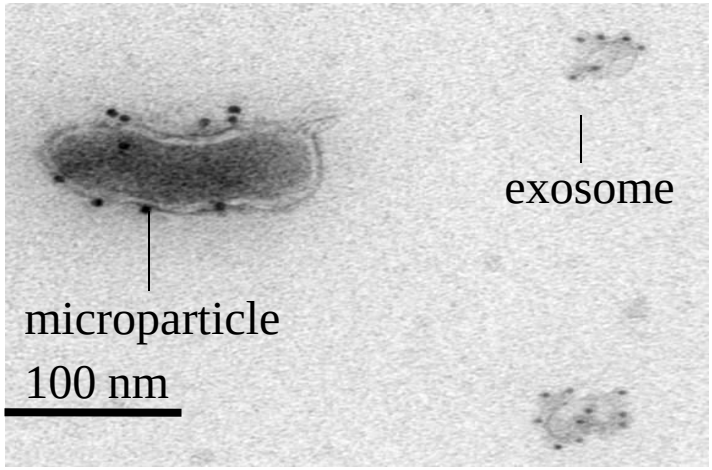
V. GUREWICH

Chapel Hill , North Carolina
N Key , N Mackman

Separation of microparticles from exosomes



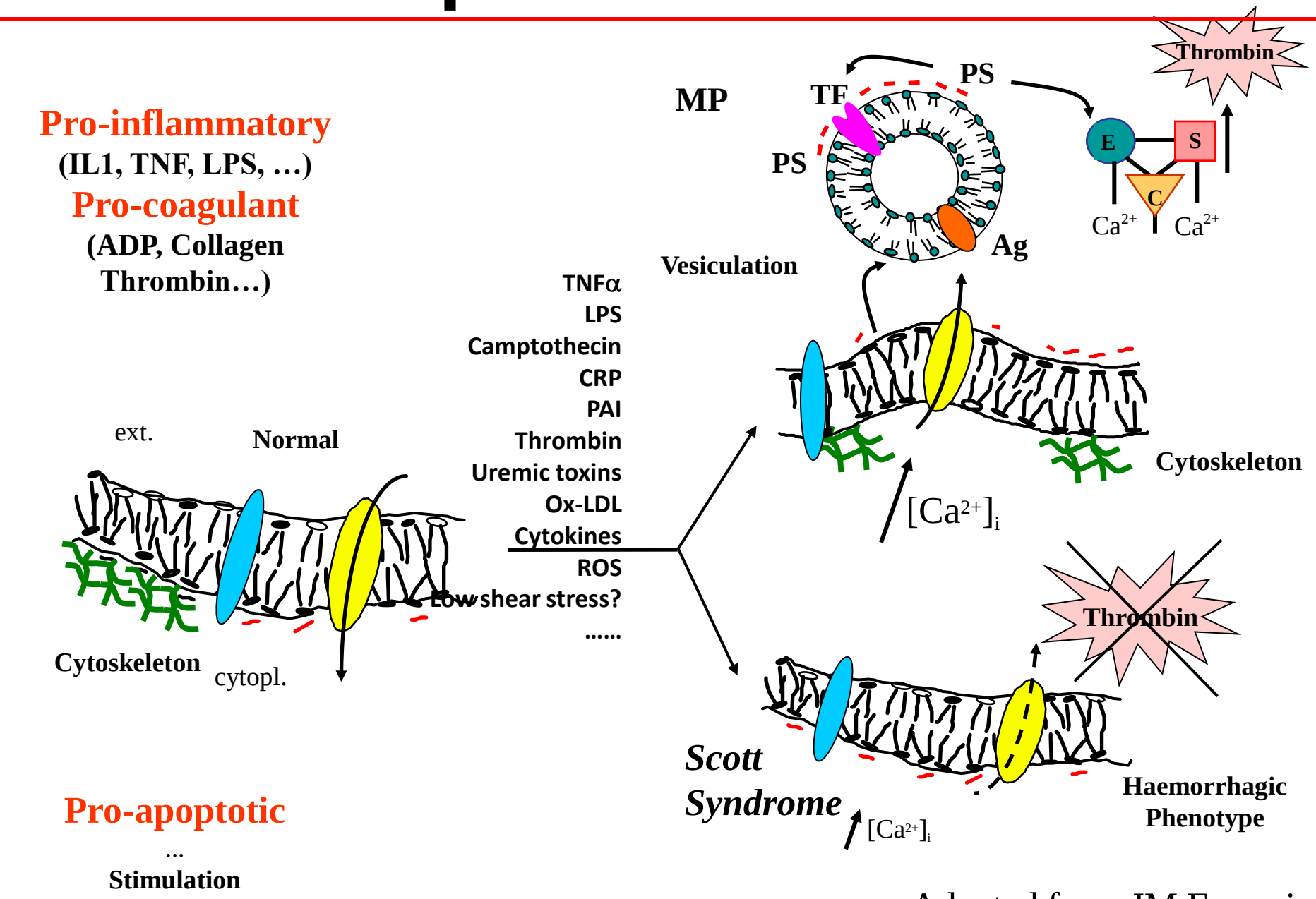
Microparticles *versus* Exosomes



Heijnen in Michelson (ed) *Platelets* (Academic Press), 2002

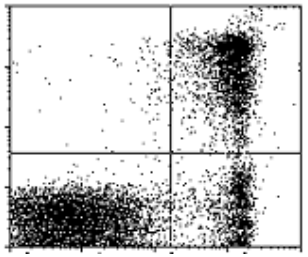
	Microparticles	Exosomes
Formation	Spontaneous blebbing	Exocytosis (endosomes, multivesicular bodies)
Size	0.1 to 1 µm	0.03 to 0.1 µm
Markers	Representative of cells	Tetraspan +++ (CD9, CD63, CD81), MHC, Lactadherin
PS expression	+	-
Procoagulant	+	-
Obtention	High speed centrifugation	Ultracentrifugation

Microparticle formation



Adapted from JM Freyssinet

The pre-analytical step is critical with numerous pitfalls



<u>Step 1:</u> Blood collection and sample processing

- Way of collection.
- Needle
-Anticoagulant…
-Impact of post-prandial hyper TG…

<u>Step 2:</u> Transportation

-Temperatures
- delay??…

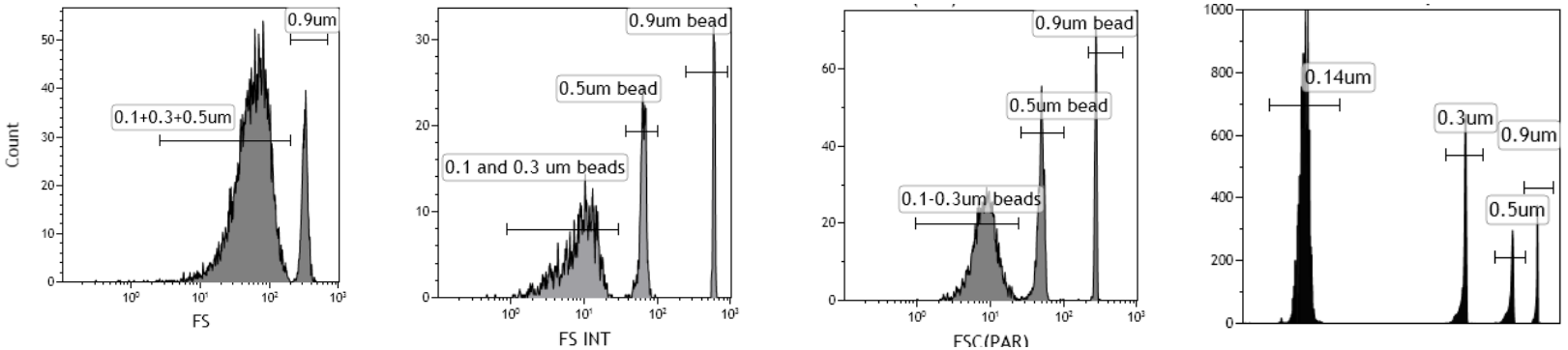
<u>Step 3:</u> MP obtention and Sample storage:

-Whole Blood /PFP
-Centrifugation speed
- Freezing

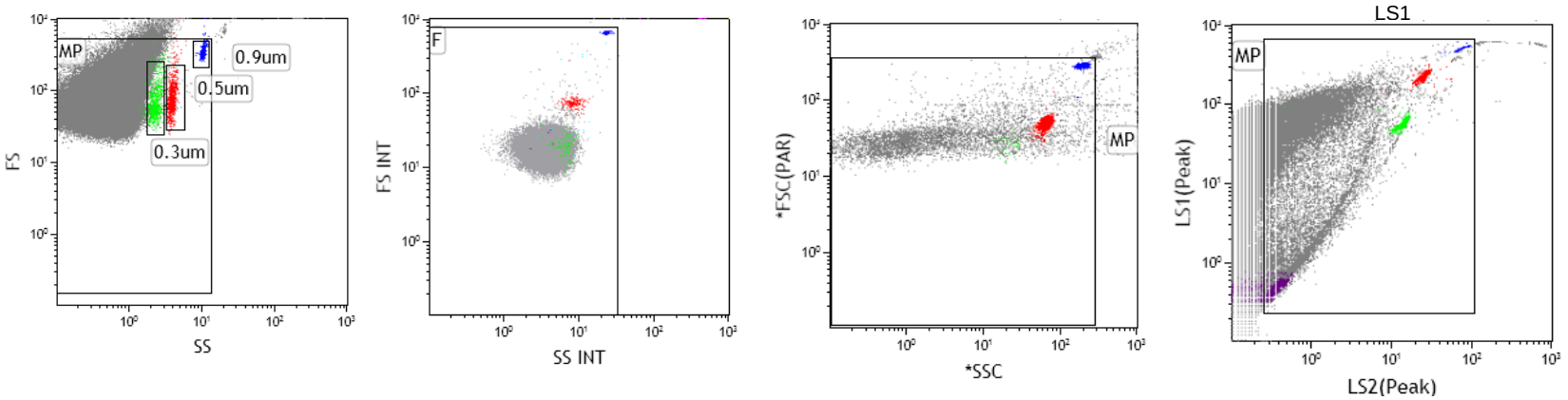
1.NEW GENERATION FLOW CYTOMETERS

FC500 | Gallios | Influx | Apogee A50

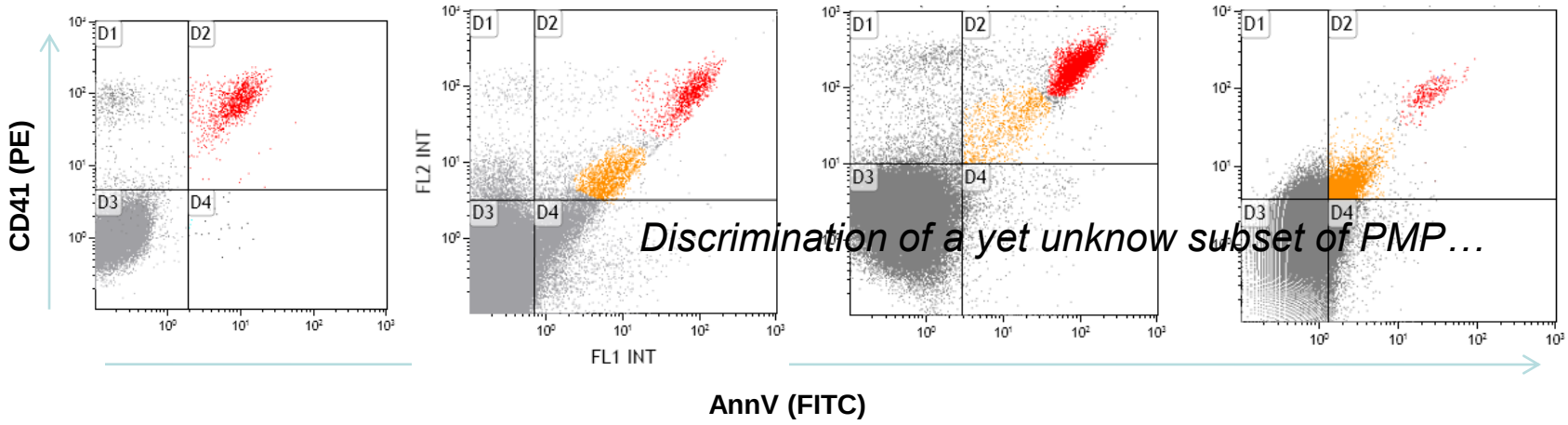
A Beads resolution



B Background Noise



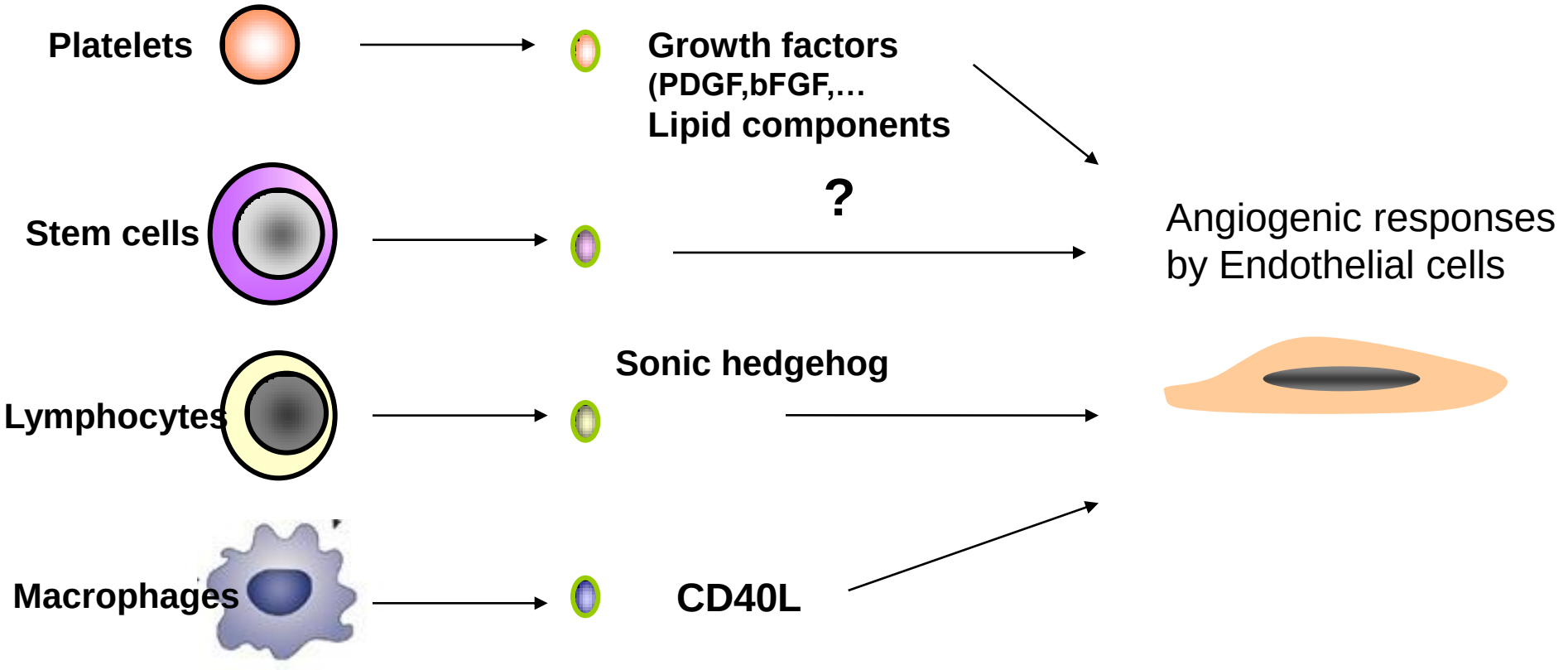
C PMP



Discrimination of a yet unknow subset of PMP…

On the Trail of Microparticles

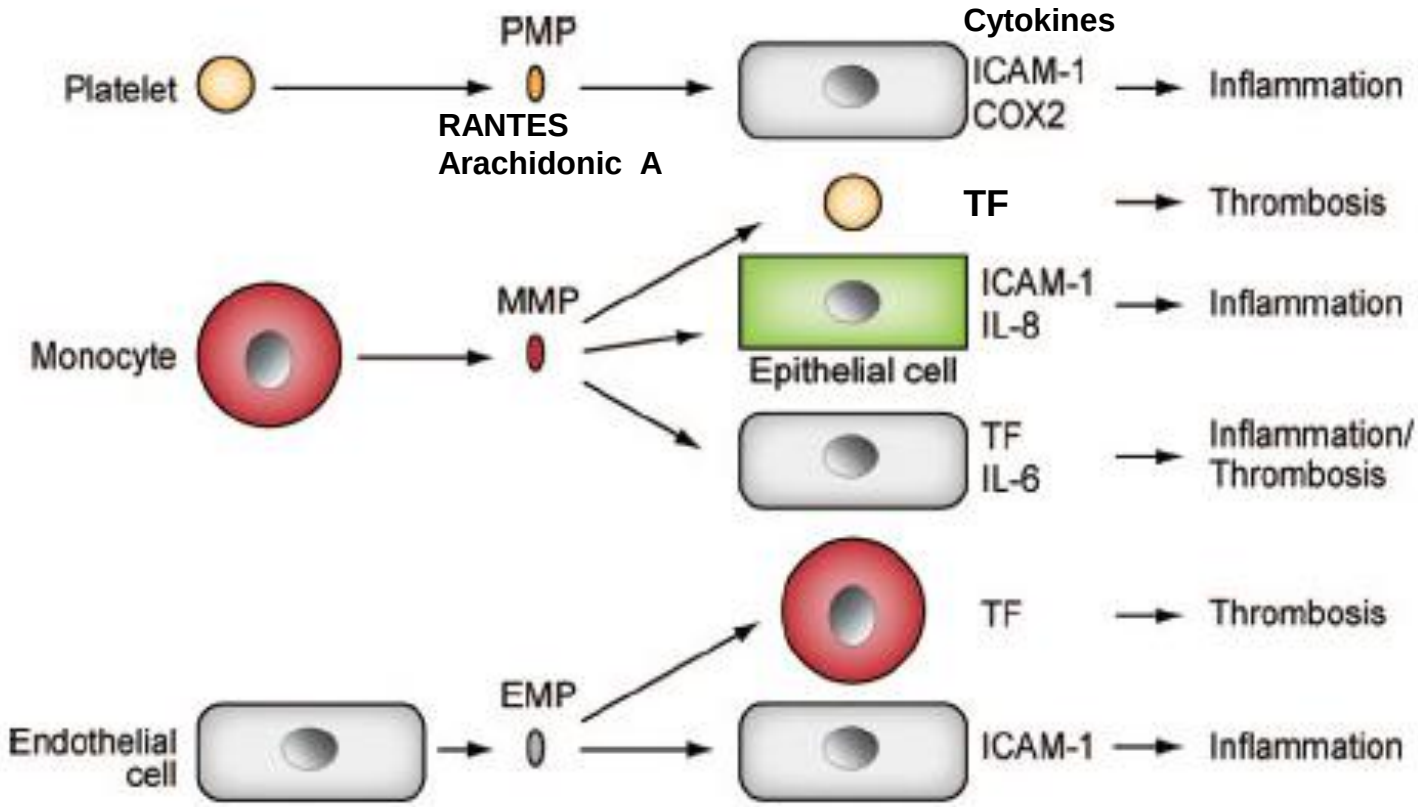
Benameur T, Pharmacol Rep 2009



A link between thrombosis, inflammation and angiogenesis

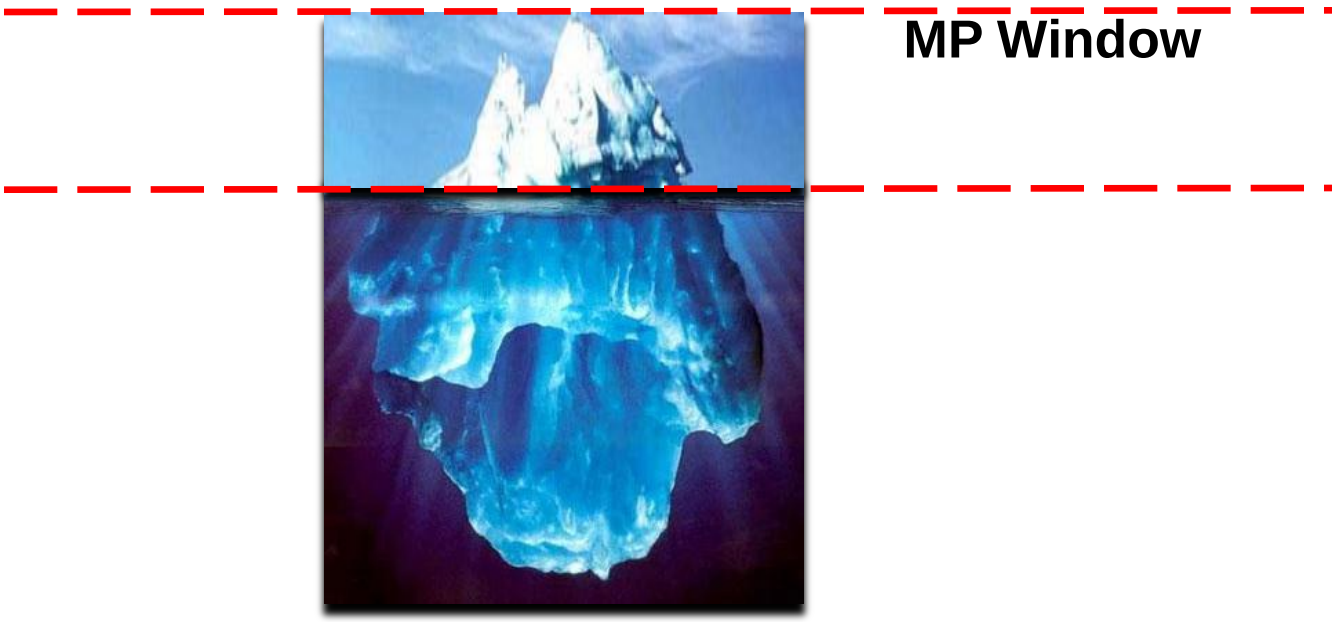
On the Trail of Microparticles

Nigel Mackman, Circ. Res. 2009



at the crossroad between thrombosis and inflammation

SIZE LIMITATION

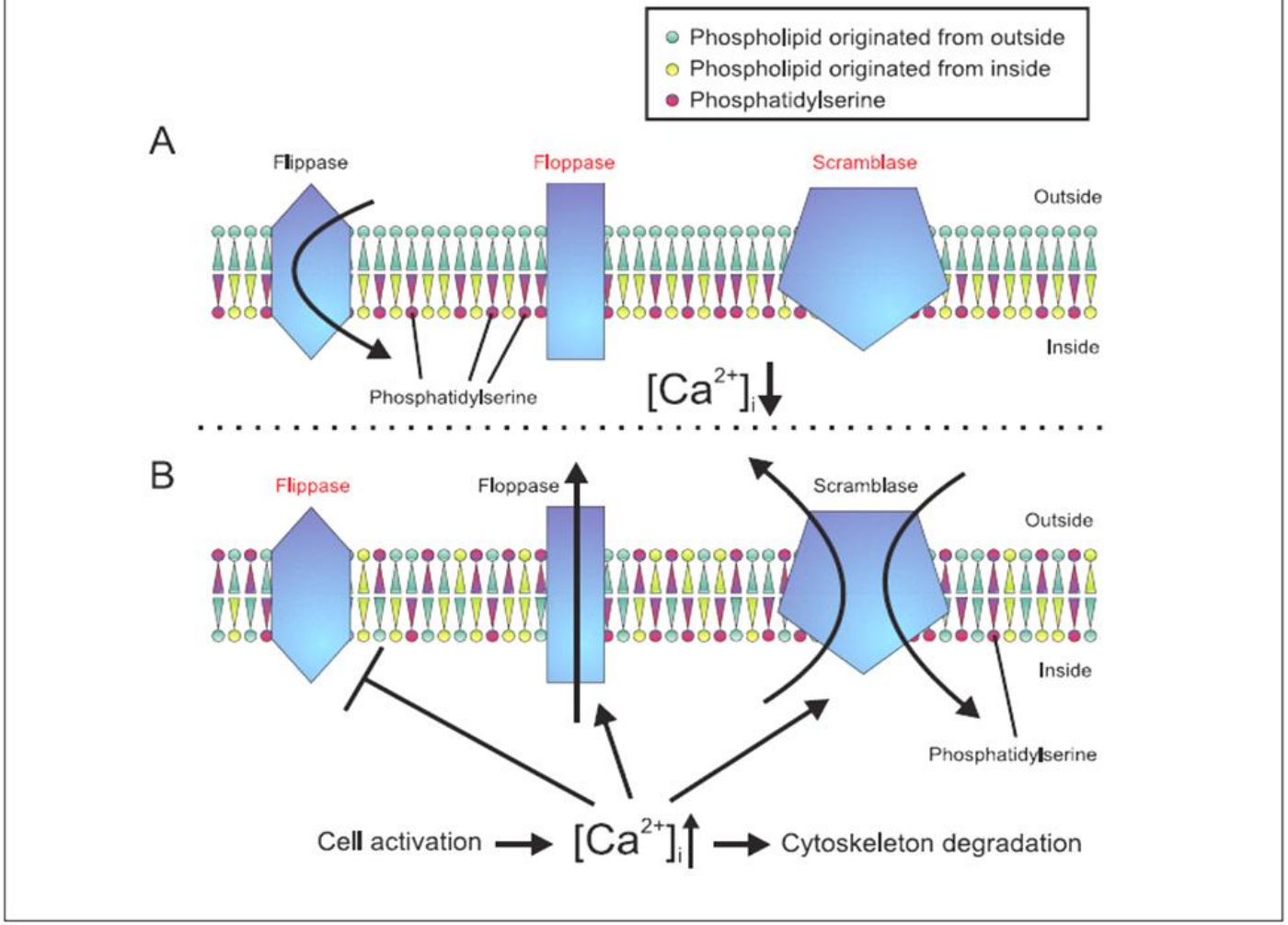


✓ **The limit flow cytometry :Previous generation of flow cytometers only detect the visible part of the iceberg**:

✓ II- **The Challenge : detect all MP, whatever the size**

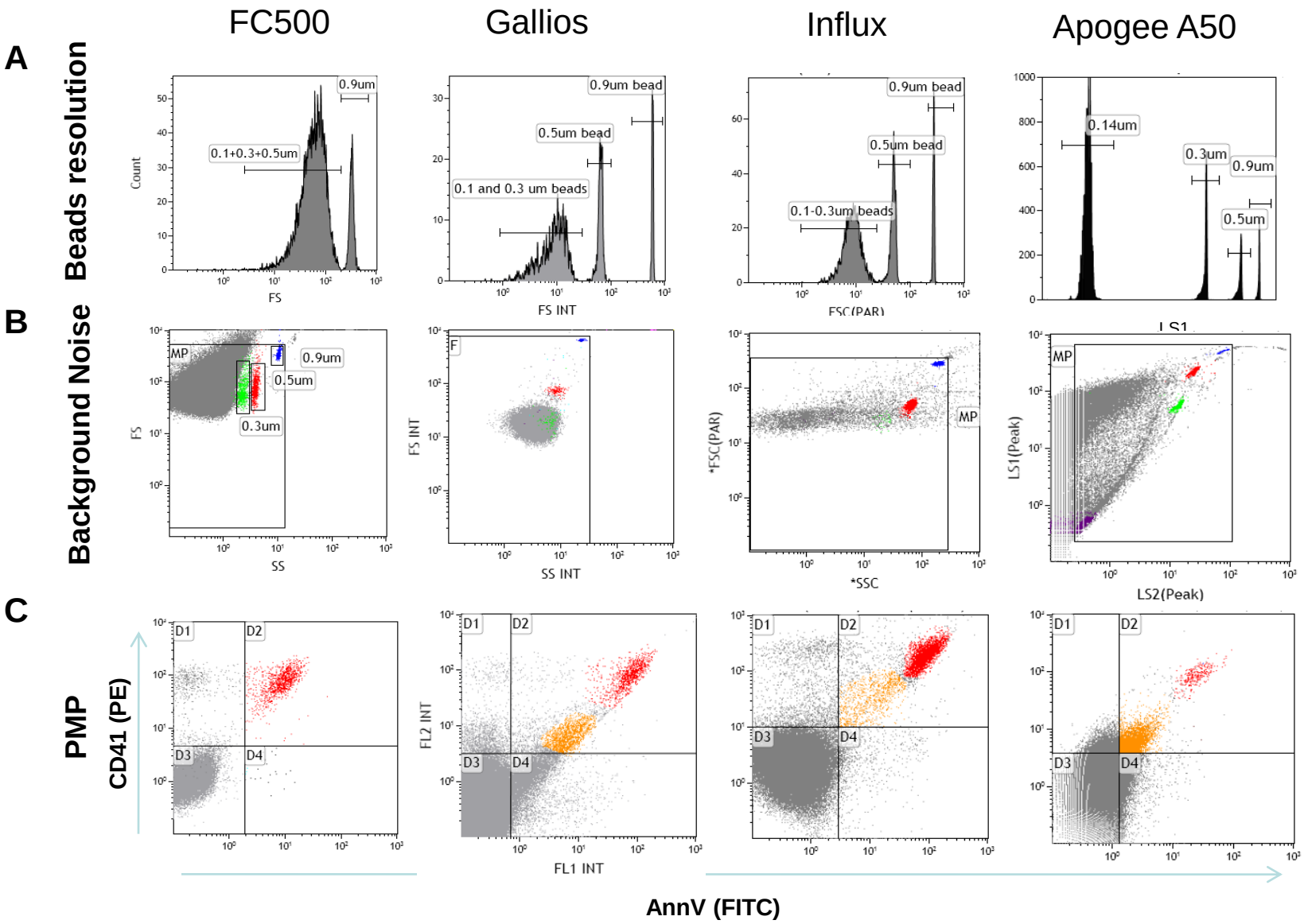
How to overcome the limits of Flow cytometry ?

Remaniement des phospholipides membranaires

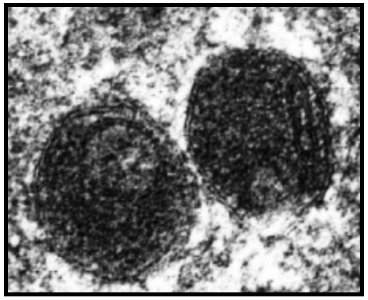


Nouvelle génération de cytomètre en flux

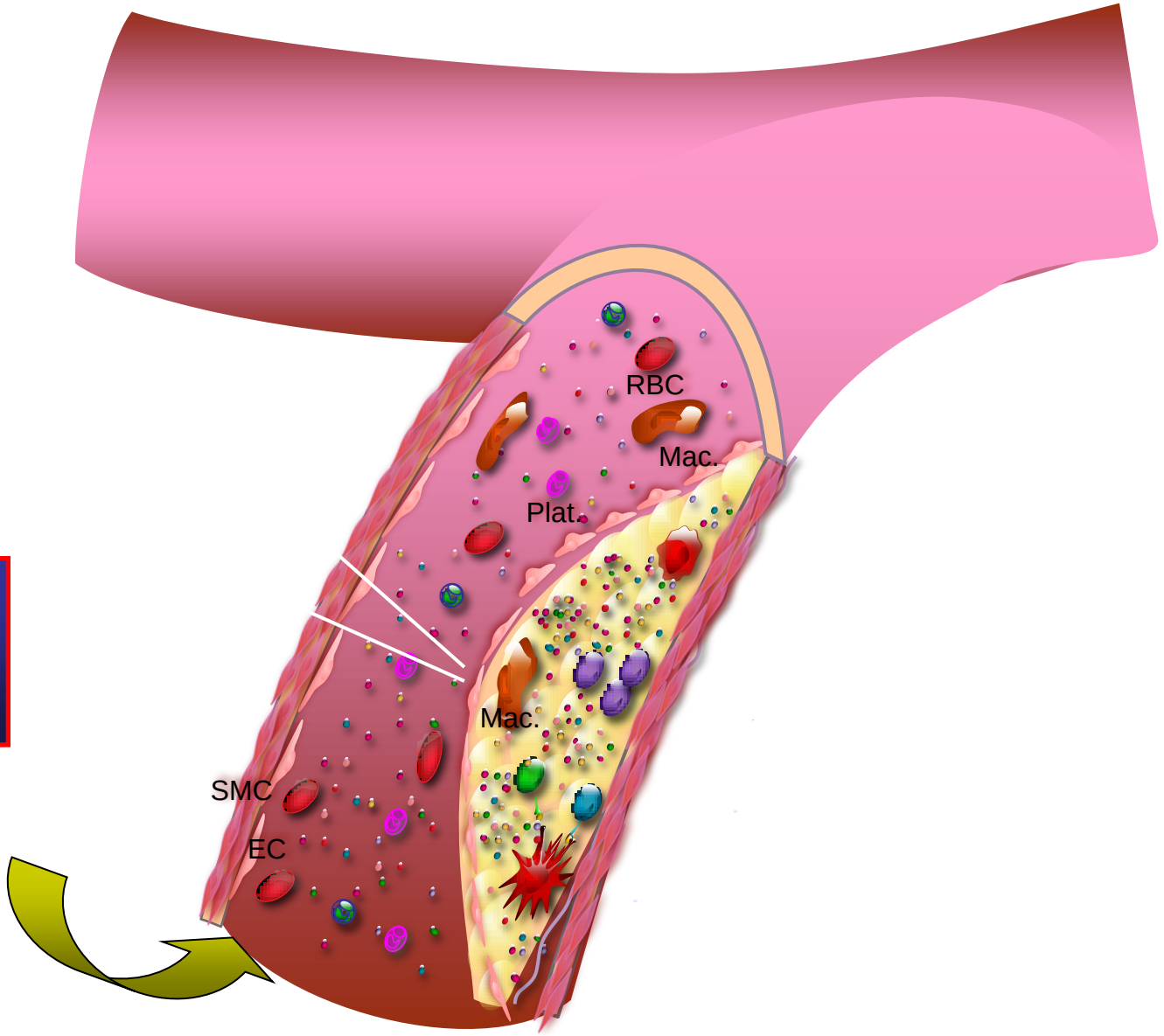
Lacroix R. et al. Seminar in Thromb. and Haemost. 2010



The multiple faces of endothelial derived microparticles

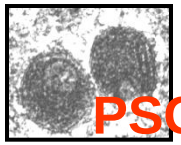


COAGULATION
INFLAMMATION
ANGIOGENESIS



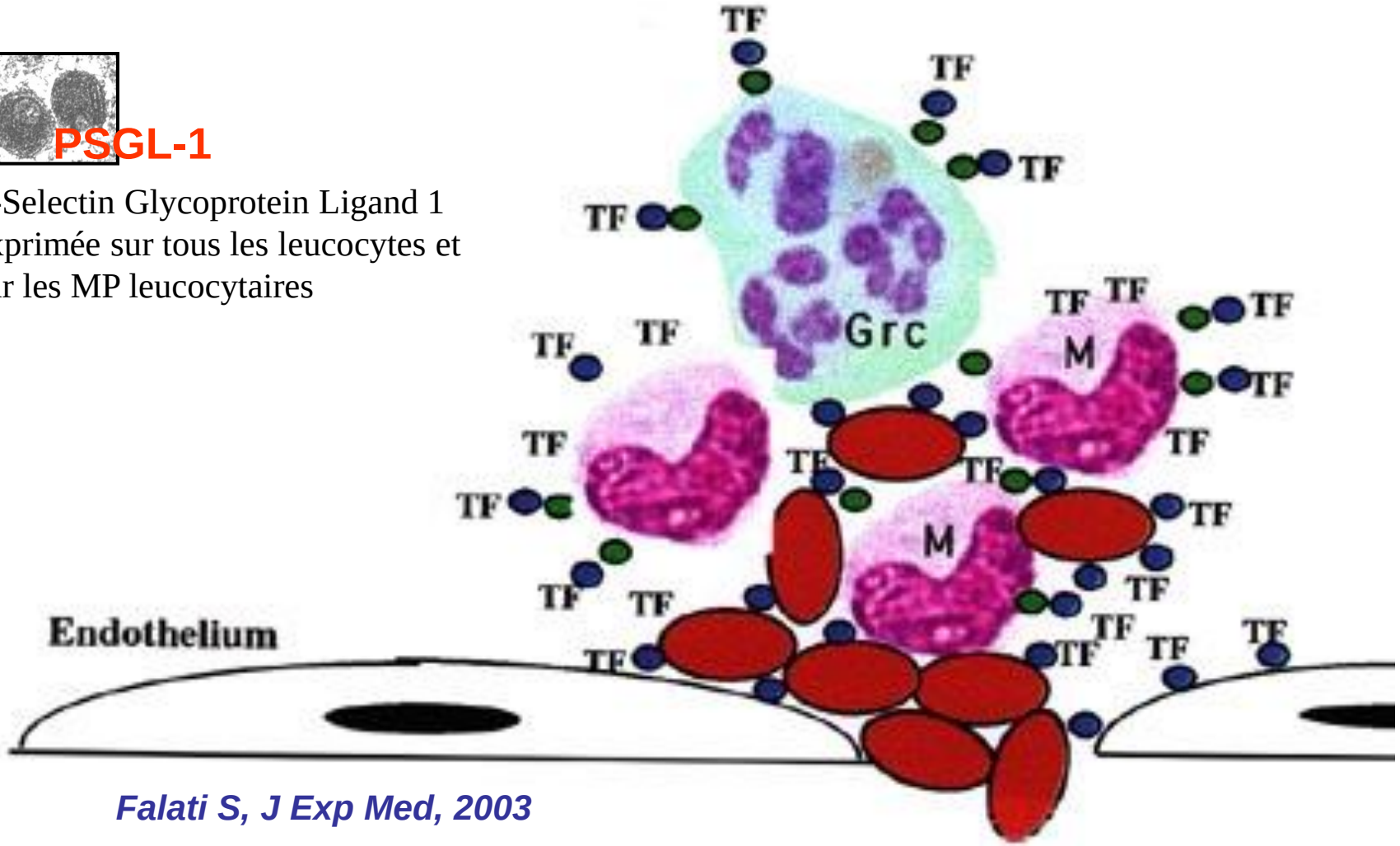
Rôle dans la coagulation et dans la formation du thrombus

Les MP sont capturées par les plaquettes du thrombus via l'interaction de la **PSGL-1** exprimée par les MP et la P-sélectine plaquettaire. La concentration de FT augmente au niveau du thrombus, ce qui accélère la formation de fibrine.



PSGL-1

P-Selectin Glycoprotein Ligand 1 exprimée sur tous les leucocytes et sur les MP leucocytaires



Falati S, J Exp Med, 2003