Microparticules fibrinolytiques:

de la physiopathologie du sepsis à l'émergence d'un nouveau biomarqueur

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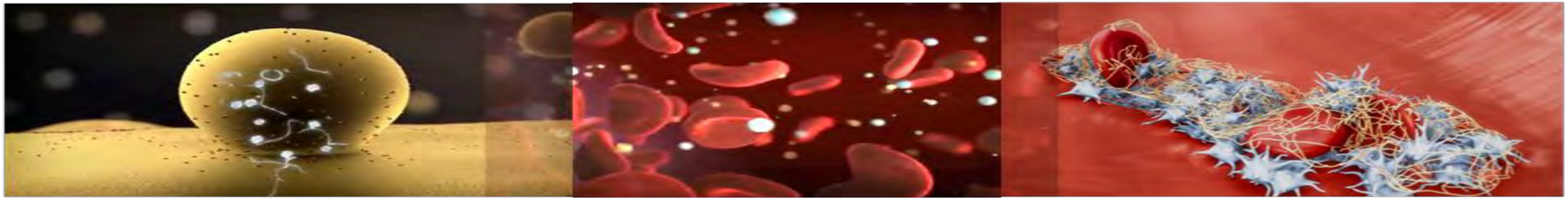
Doyen de la faculté de Pharmacie



Séance Académique – 26 février 2020 - 13h30 – amphithéâtre Boudier

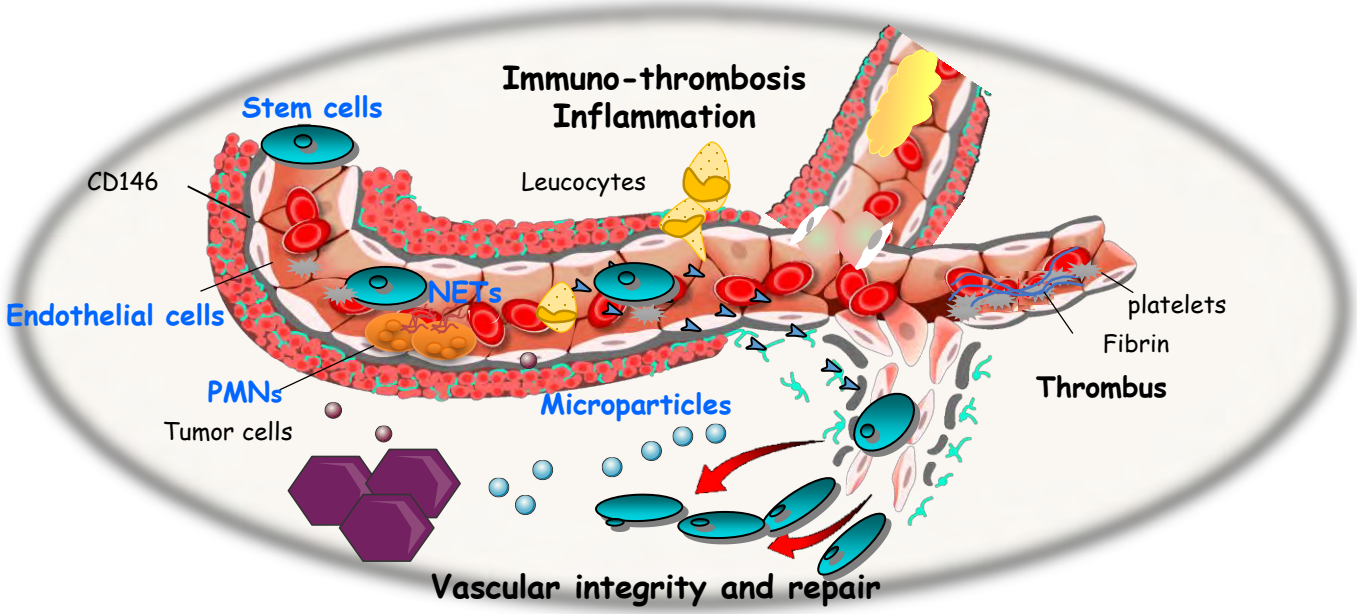
Scientific strategy

BASIC RESEARCH

Endothelial dynamic interactions with its environment

in immuno-thrombosis and vascular regeneration

New pathways



New targets

Vascular Monitoring Platform ↔ Non Invasive Biomarkers

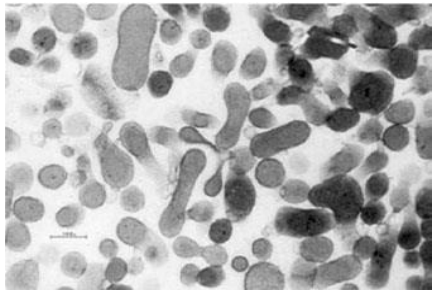
CERIMED ↔ Imaging

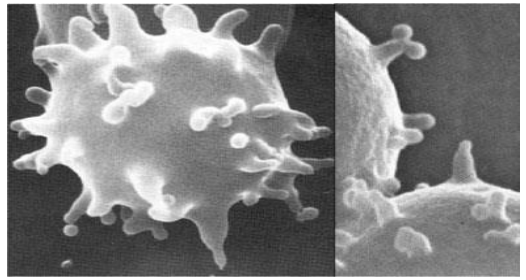
TRANSLATIONAL RESEARCH

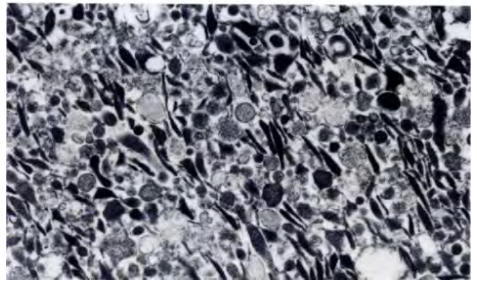
Innovative Biotherapies ↔ **Cell-based therapy unit INSERM _ CBT-1409**

Vascular personalized medicine

Microvesicles : concept evolution



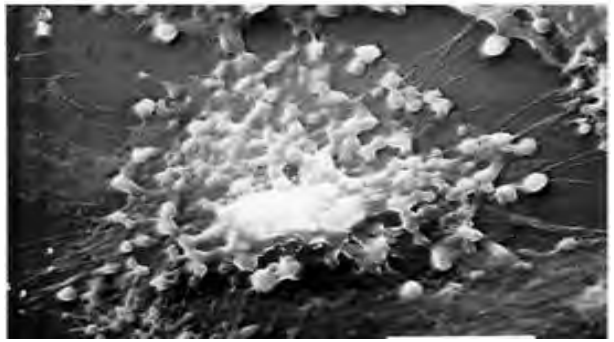




- Wolf's observation : « platelet dusts » (1967)
- Vectors of biological information
- Biomarkers
- Pharmacological targets
- Cell free therapy : « Vesiculotherapy »

B

E



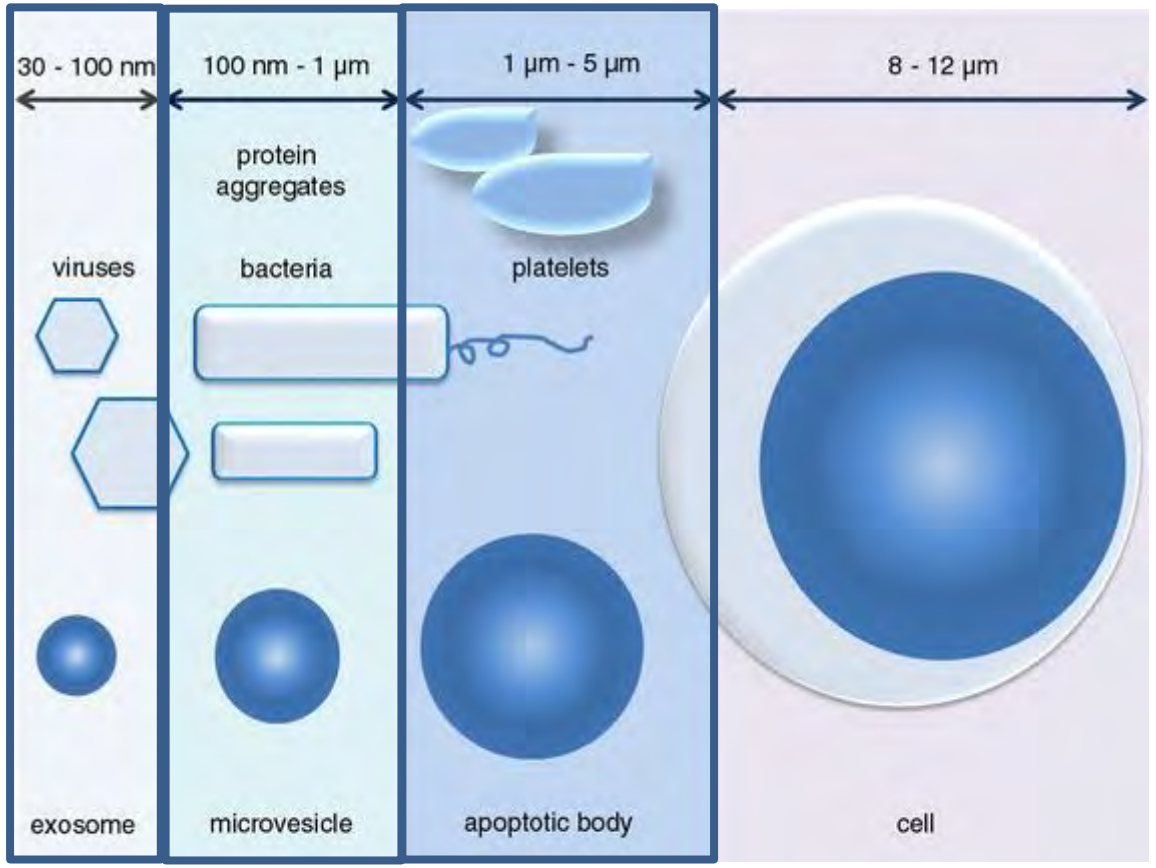
Characteristics of microvesicles

Extracellular vesicles

MISEV C Thery , JEV, 2018

« Small EV» « Large EV »

https://youtu.be/ogS_T_heSQY



György et al.Cell Mol Life Sci.2011

Diversity of Microvesicles

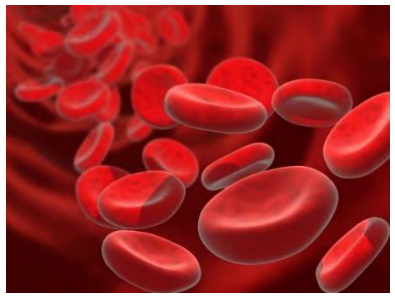
Diversity of biogenesis

Inducers

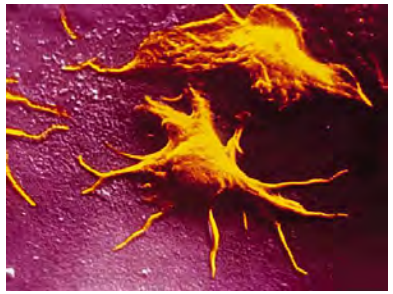
Agonistes plaquettaires (ADP, collagène)
Thrombine, PAI-1
Calcium ionophore
Complément
Anticorps
Hypoxie
Age
Flux sanguin
Apoptose
Molécules inflammatoires (LPS, cytokines)

Parental cells

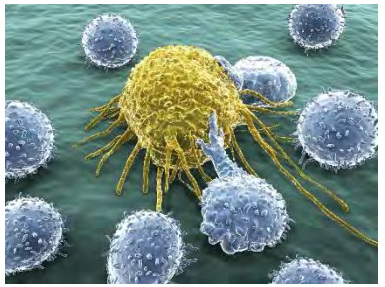
Globules rouges



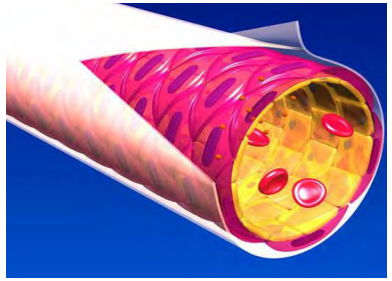
Plaquettes

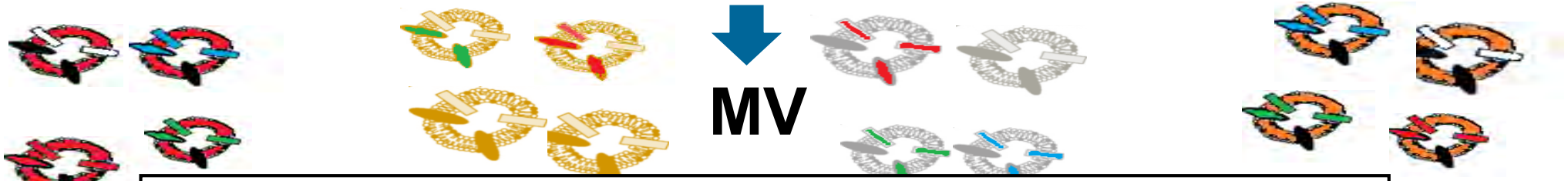


Leucocytes



Endothélium





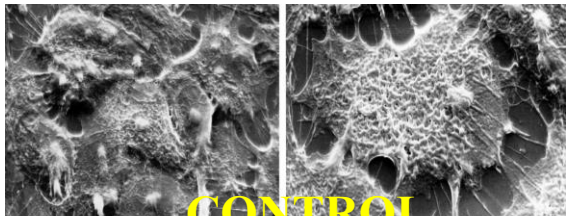
A Signature of cell responses to their environment

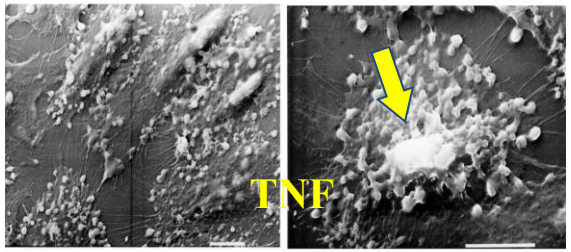
Diversity of functions

Diversity of structure and composition

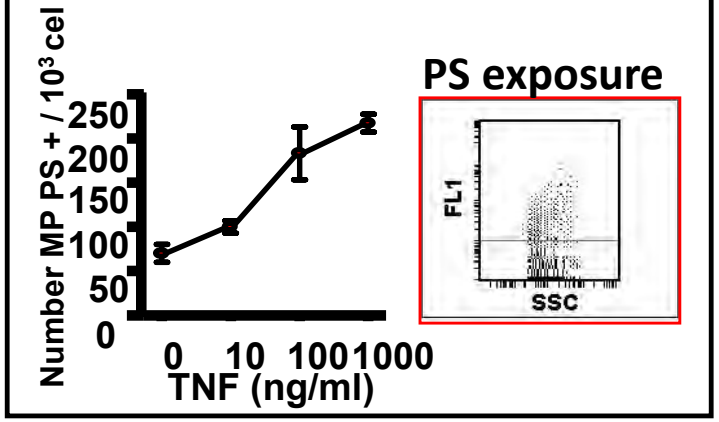
EMP generation in response to inflammatory stimulation

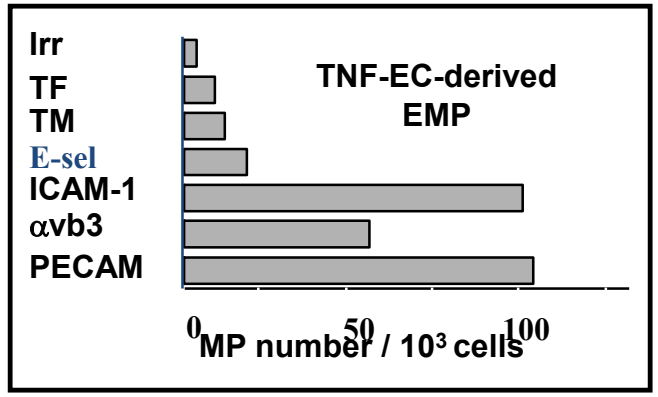
Combes et al, J Clin Invest, 1999



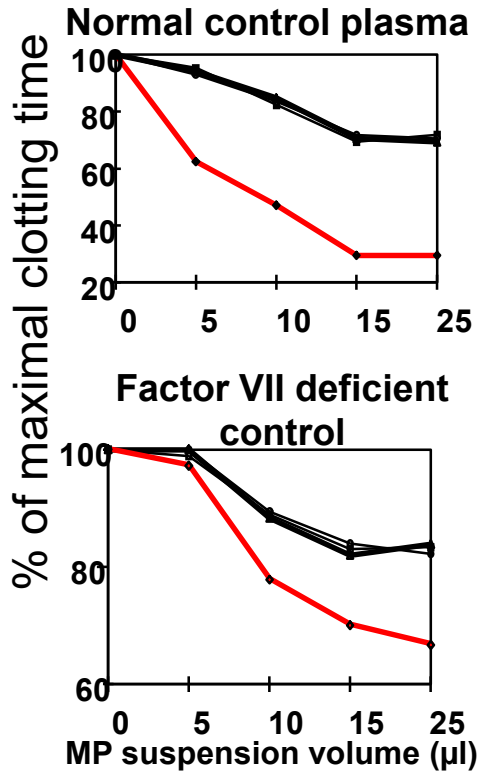


Heterogeneous diameter
(0.1 to 1 µm)





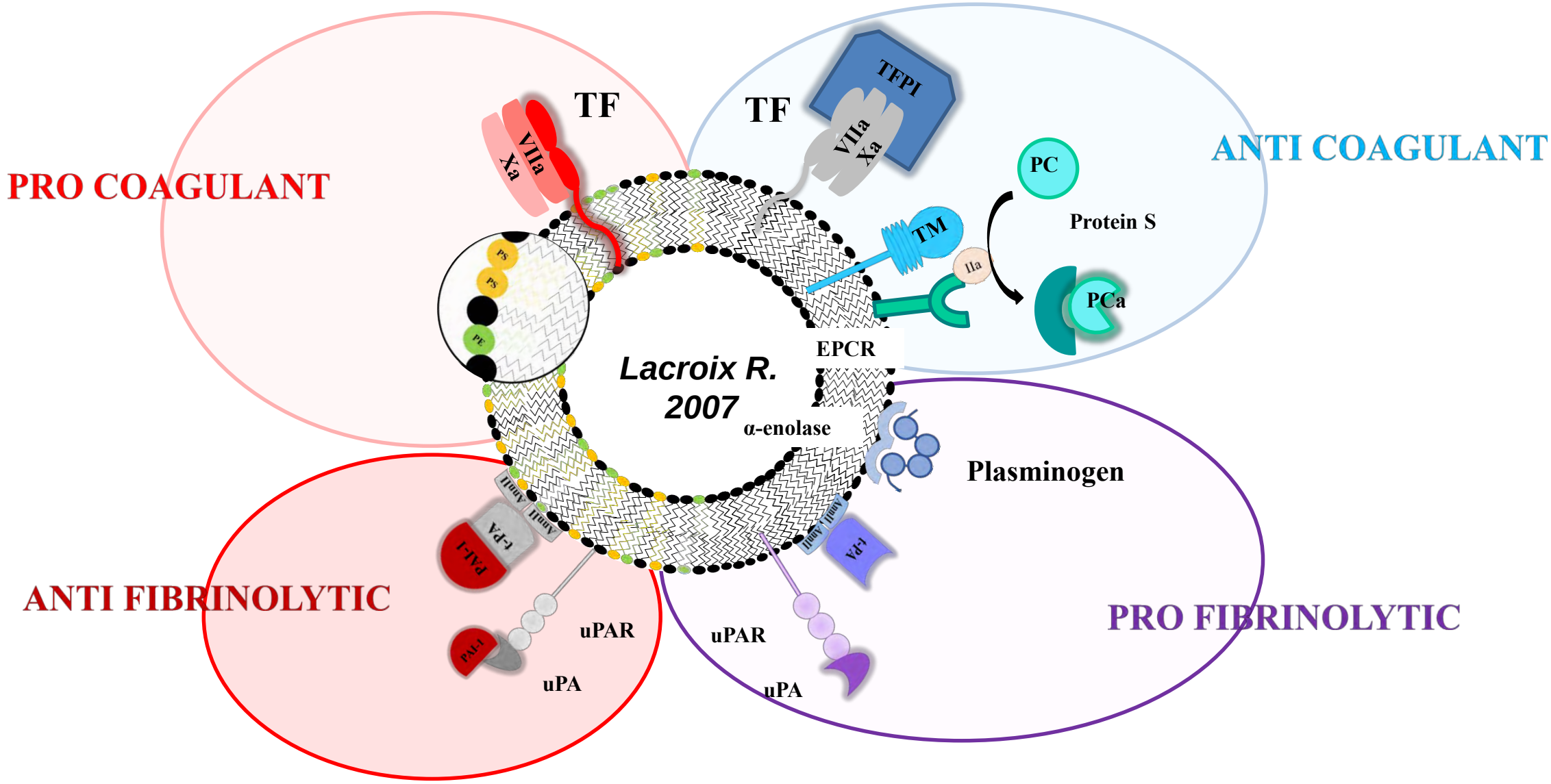
Endothelial phenotypic features



Pro-coagulant activity

EMP support procoagulant responses

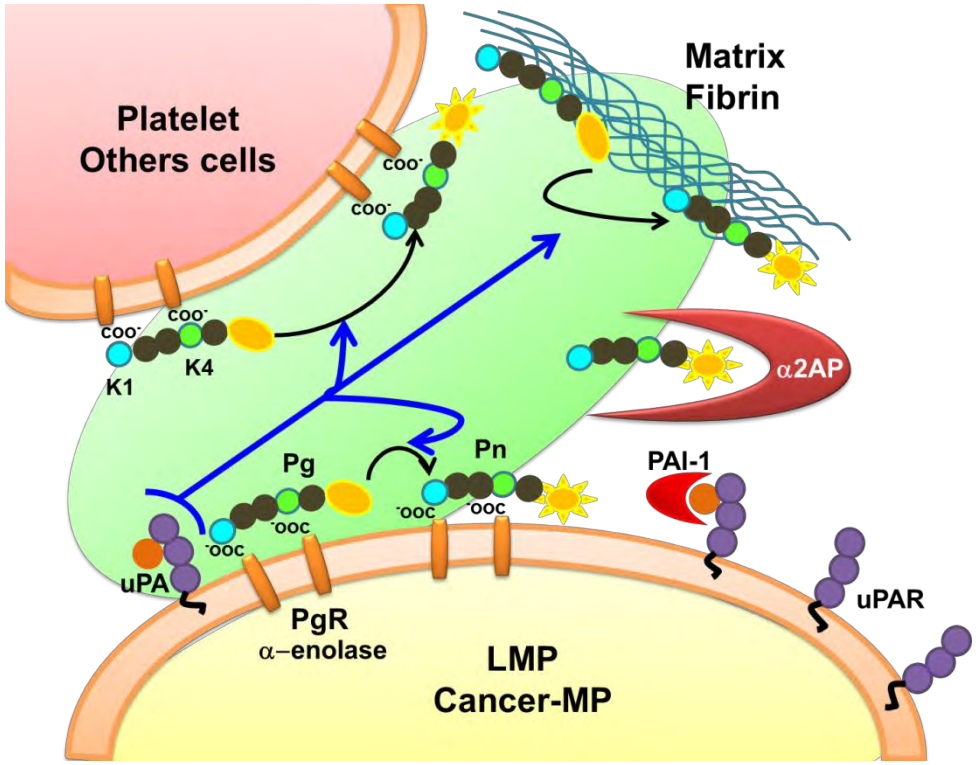
Revisited role in haemostasis



Lacroix et al. , Semin. Thromb. Haemost, 2019

Lacroix Blood 2007,

Take Home Messages



- MV are ambivalent catalytic surfaces supporting **fibrinolytic** enzymatic reactions
- MV PGC is dependent on **u-PA** and **t-PA** expression and is conveyed *in vivo* by **endothelial- leukocyte-** and **tumor-derived MVs**
- **MV** are not only **conveyors** but also **efficient biocaptors**
- MV-PGC is **detectable " *in vivo* "**

Pathophysiological role and Clinical pertinence in human diseases ?

Lacroix Blood 2007; Dejouvencel , Lacroix, Blood 2010; Lacroix et al: Haematologica 2012 ; Semin Thromb and Haemost 2013; JTH 2013

Septic shock

Physiopathology

Systemic response to an infection

+

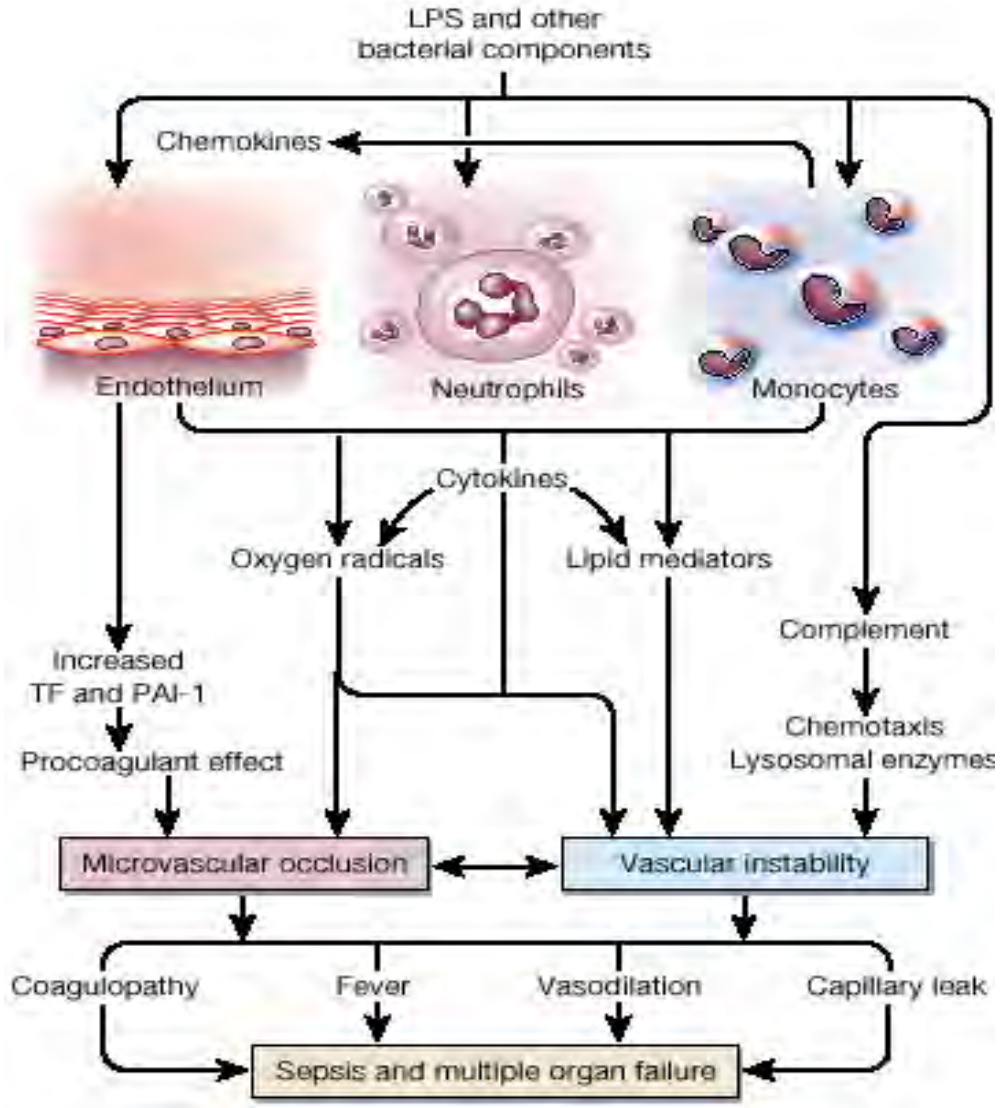
- Vascular inflammatory response
- Hemostasis imbalance with activation of the coagulation and abnormal fibrinolysis

(Disseminated Intravascular Coagulation)

Epidemiology

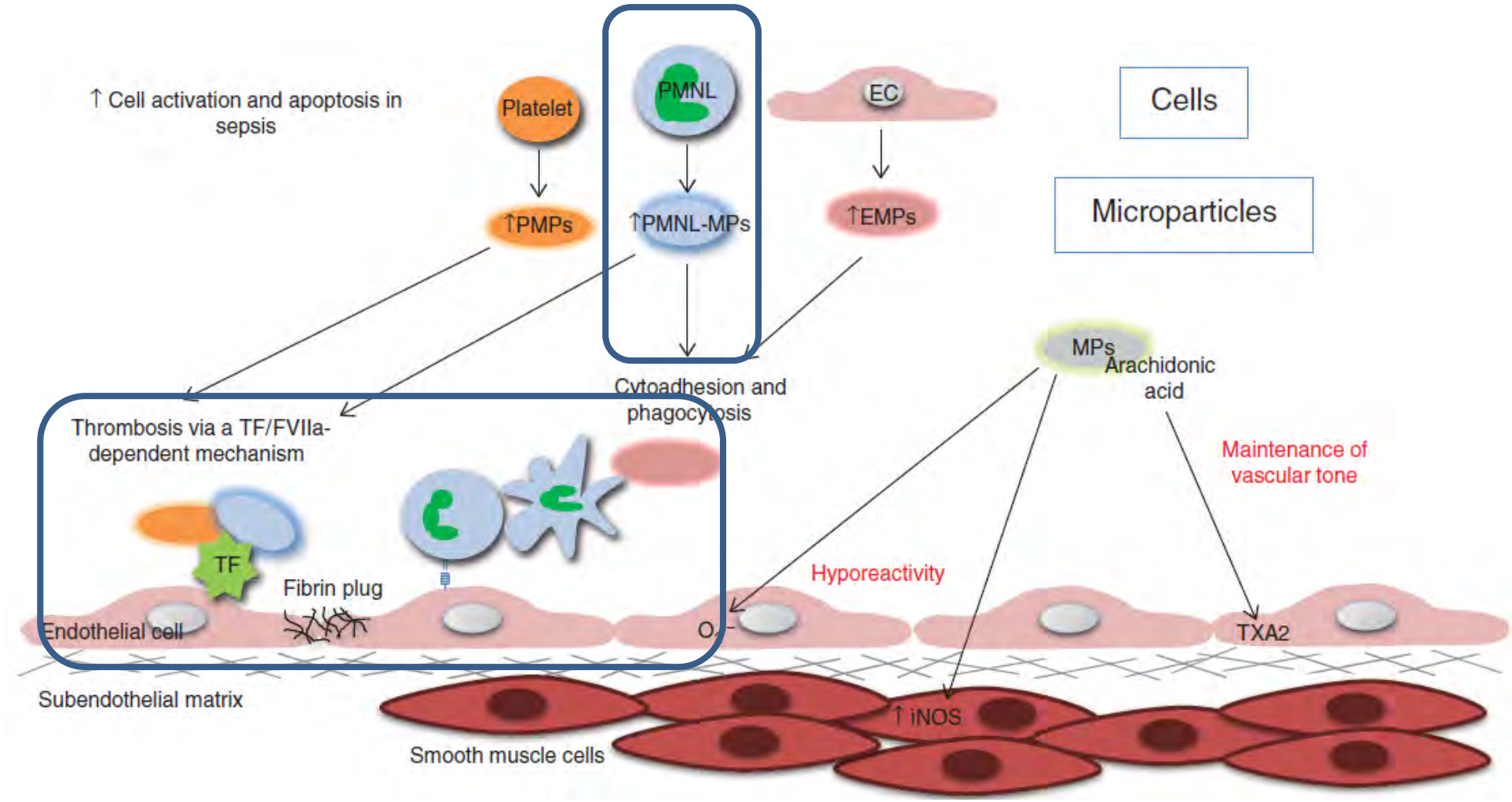
10 to 20% of patients in ICU

Mortality rate : 30 to 50%



Cohen, Nature: 2002 420:885

Microvesicles and sepsis



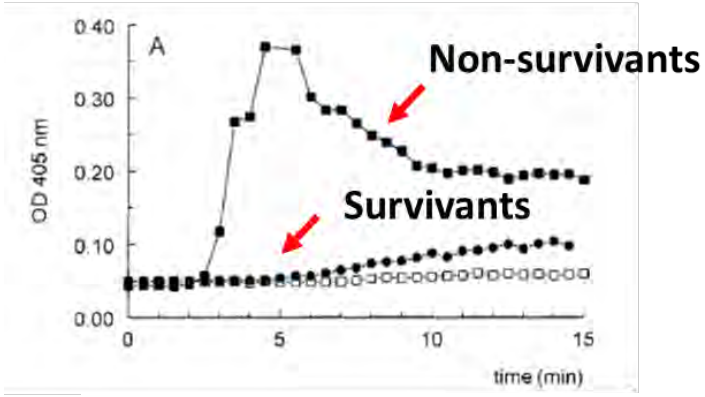
Coagulation **Inflammation** **Vascular dysfunction**

Reid et al. BJA 2012

Role of MVs in septic shock ?

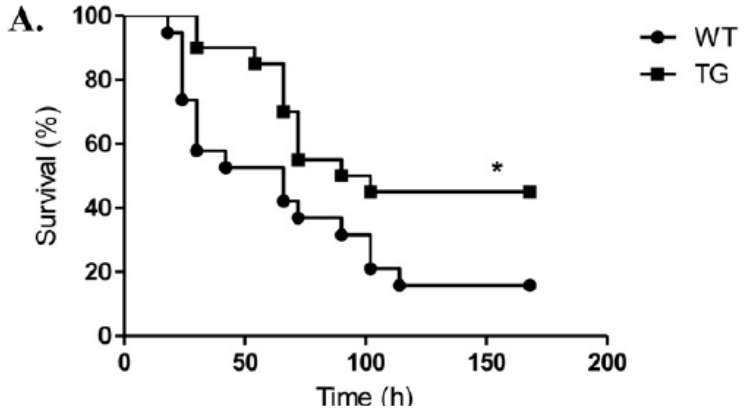
Deleterious role

Thrombin generation



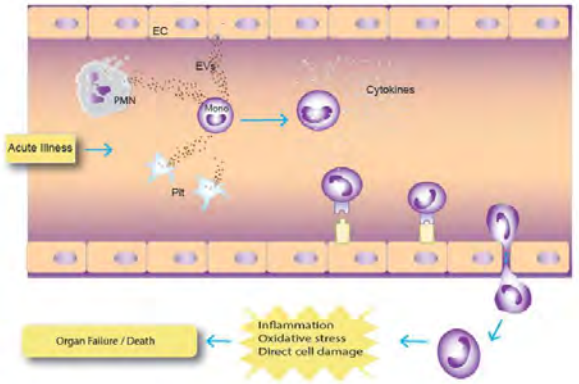
Nieuwland, Blood, 2000

Calpastatin effect



Zafrani, AJRCCM, 2012

Monocyte activation

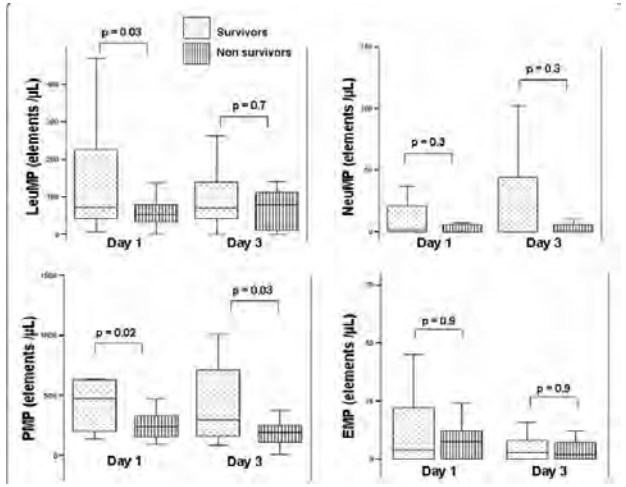


Danesh, Front Immunol, 2018

Role of MVs in septic shock ?

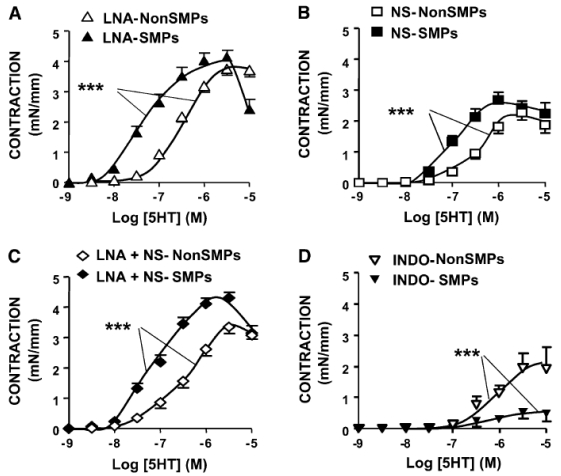
Protective role

PNN-MVs associated with survival



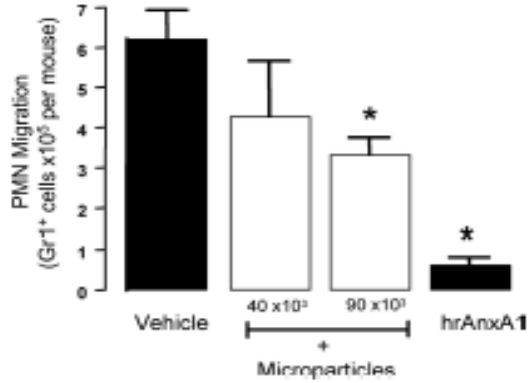
Guervilly et al. Critical Care 2011

Vascular hyporeactivity prevention



Mostefai, AJCTM 2008

Anti-inflammatory properties (AnA1, TGFb)



Dalli, Blood, 2008
Gasser, Blood, 2004

Role in septic shock ?

Hypothesis

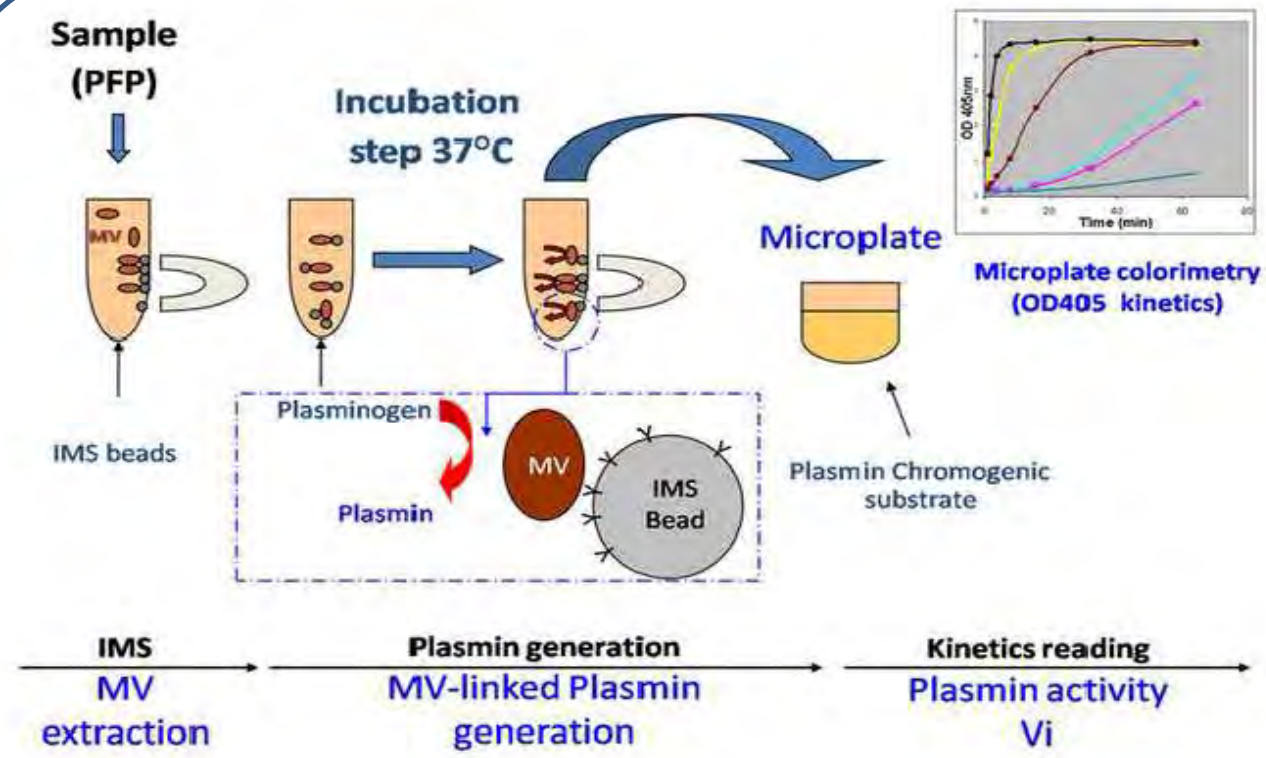
Increased plasmin generation capacity confers to MVs a protective role in sepsis

Objectives

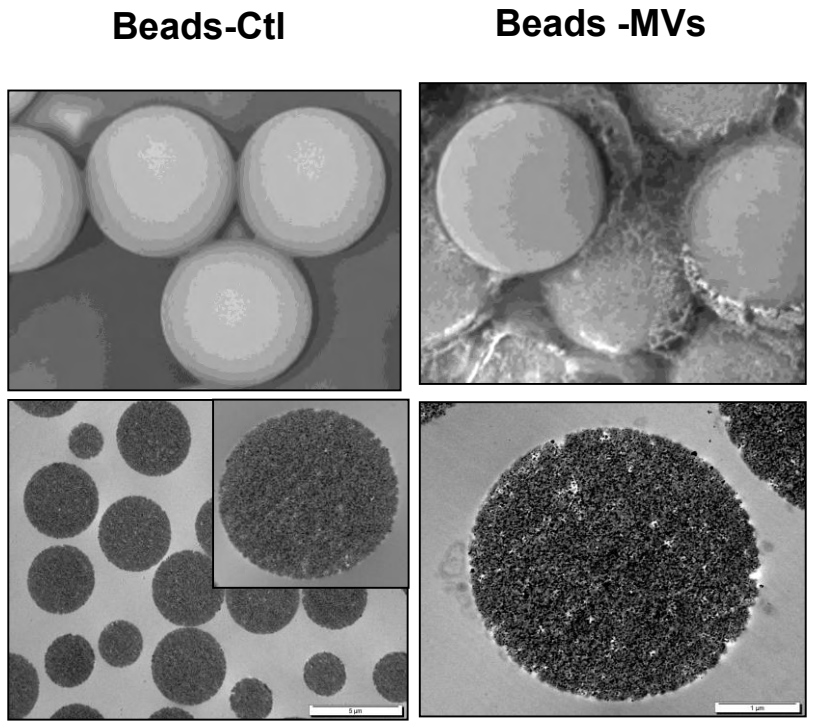
- develop an innovative assay to reproducibly measure MP-PGC in clinical samples
- validate this hypothesis in a pilot clinical study

A new bioassay to measure MV- derived plasmin generation capacity

Cointe et al ,JEV 2018



Licenced patent STAGO: PCT/FR08/00767



Sensitivity, specificity , reproducibility

MV-PGC assay: validation in septic shock

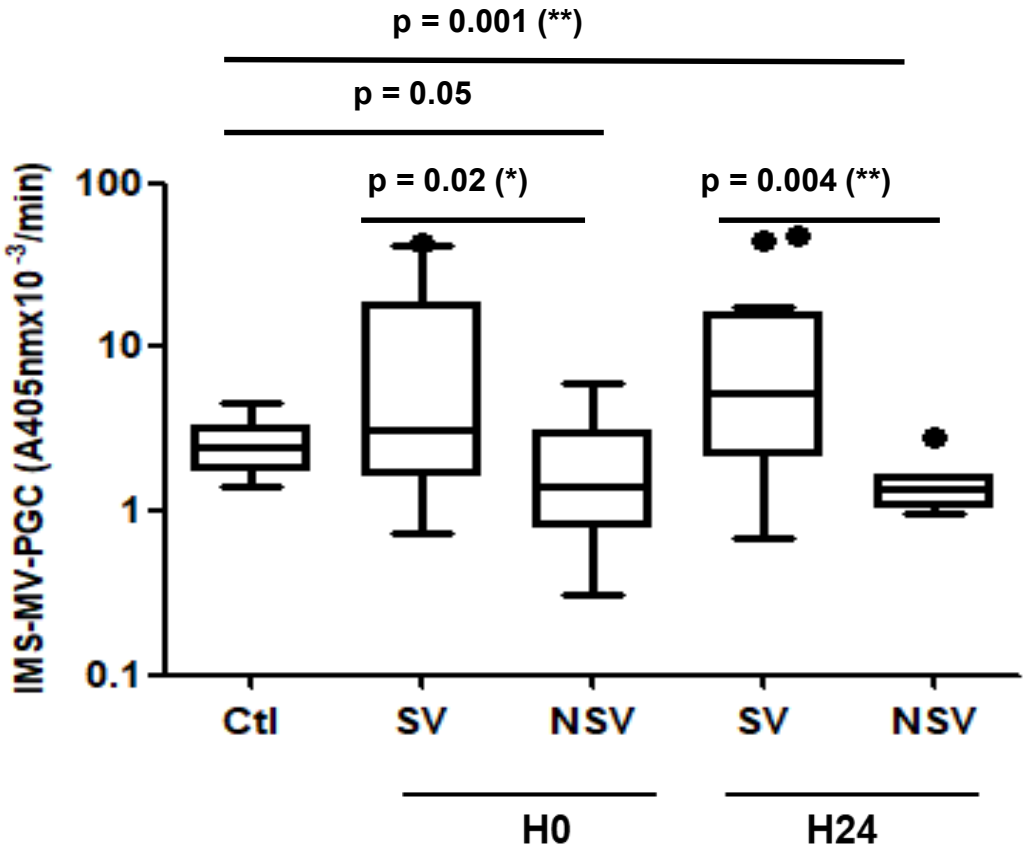
Cointe et al ,JEV 2018

Pilot study : 32 patients with septic shock

MV dependent plasmin generation analyzed on 2 samples (H0-H24) within the first 24h after diagnosis.

Mortality rate : 37% (20 S, 12 D)

	Total	Survived	Died	p-value
Age (SD)	63 (20)	57 (21)	72 (12)	p>0.03
IGS II (SD)	62 (22)	53 (16)	79 (21)	p = 0.003
Comorbidities	No difference			
Treatments	No difference			
Sepsis origin	No difference			
Germ type	No difference			



High levels of MV-PGC are associated with a better survival in septic shock

Role in septic shock ?

Hypothesis

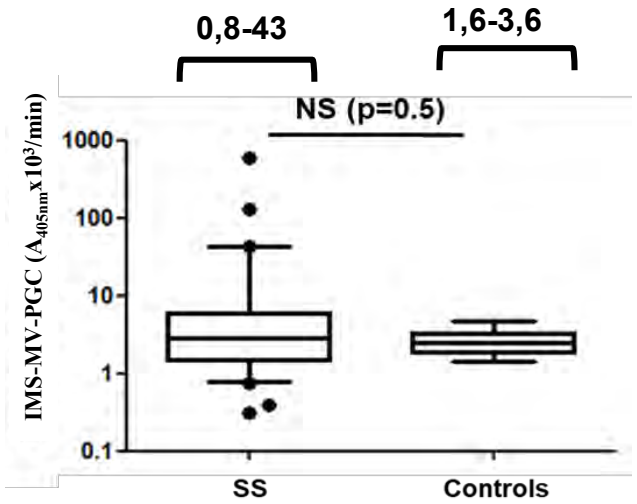
Increased plasmin generation capacity confers to MVs a protective role in sepsis

Objectives

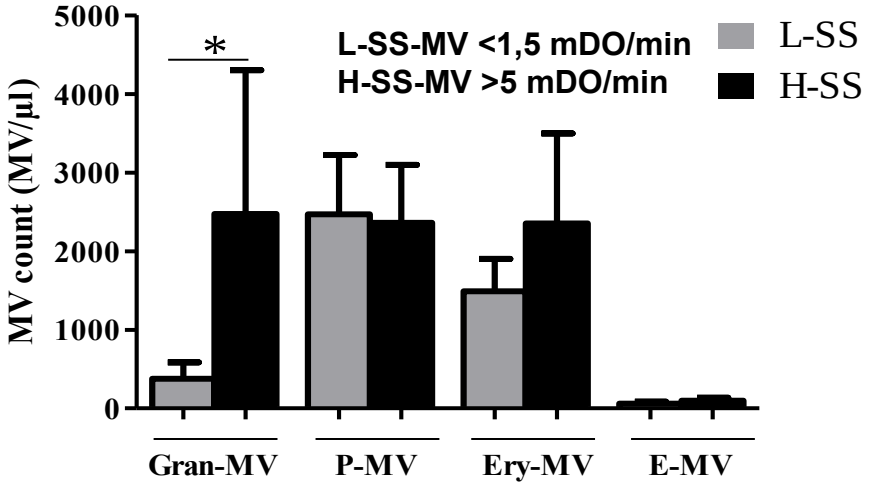
- Decipher the cellular and molecular basis of heterogeneous profile of MV-PGC
- Evaluate whether PGC confers to MV the capacity to lyse a thrombus in vivo

Heterogeneous profile of MV-PGC among SS patients MVs

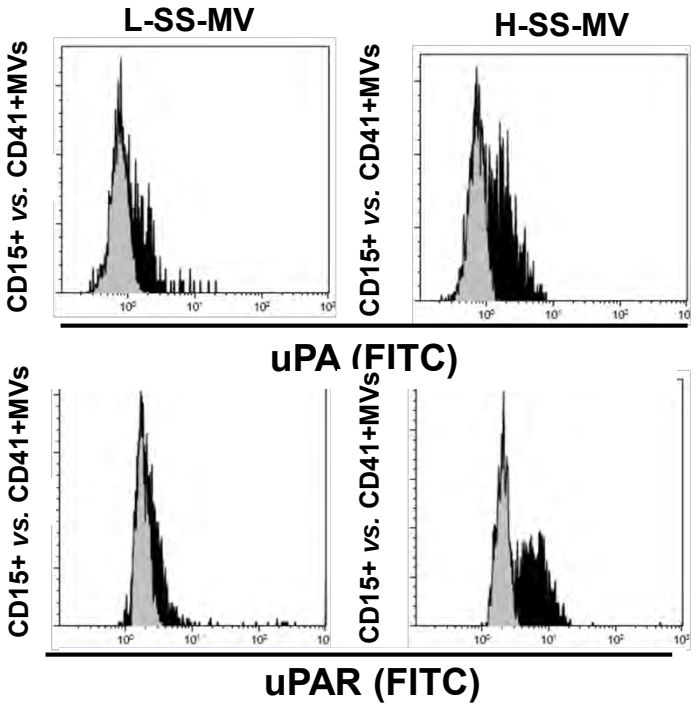
MV Plasmin Generation Capacity



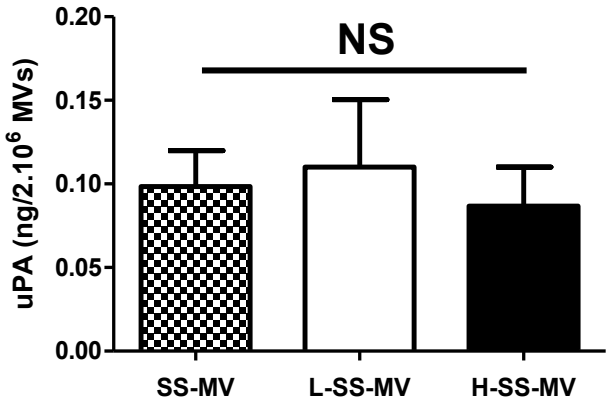
Flow cytometry



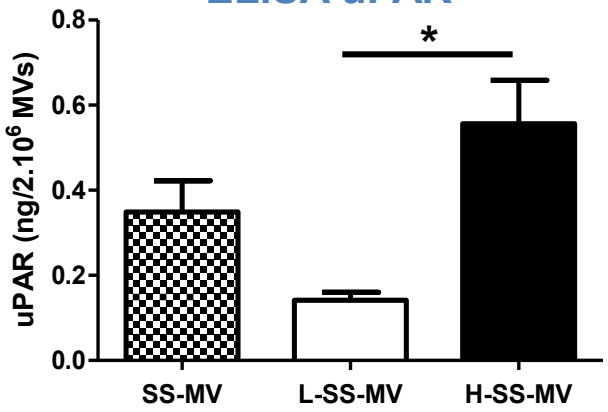
Flow cytometry



ELISA uPA



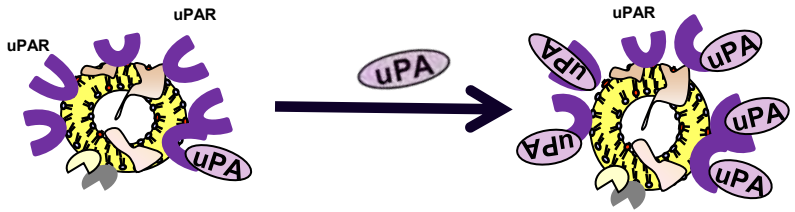
ELISA uPAR



MV-PGC heterogeneous profile is driven by the amount of Gran –MV but also by the surface expression of uPA/uPAR expression on Gran-MVs

Lytic capacity of MVs

LPS model



LPS-MV
"L-SS-MV"

uPA-LPS-MV
"H-SS-MV"

Thrombus lysis

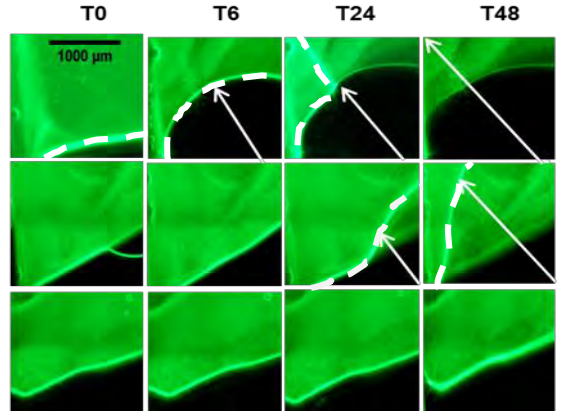
Thrombus in suspension

→ Release fluorescence
Adaptated from Boulaftali et al.

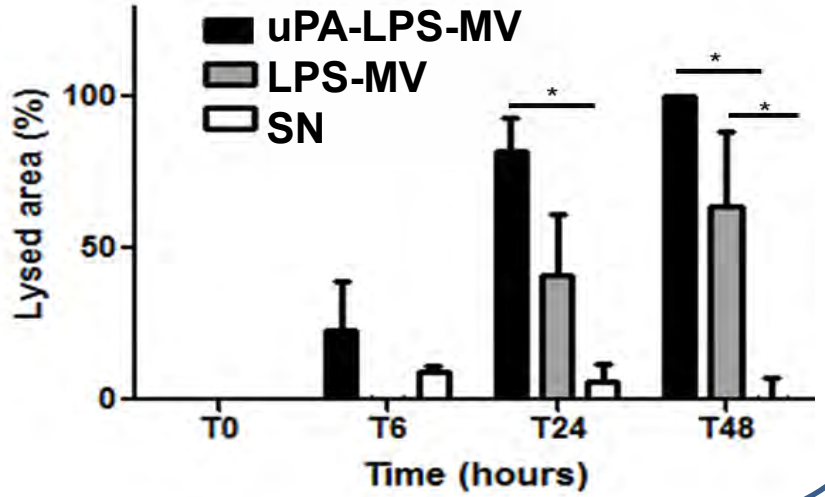
Thrombus in microchamber

→ Retraction of fluorescence front lysis
Adaptated from Collet et al.

Microscopic front lysis retraction assay



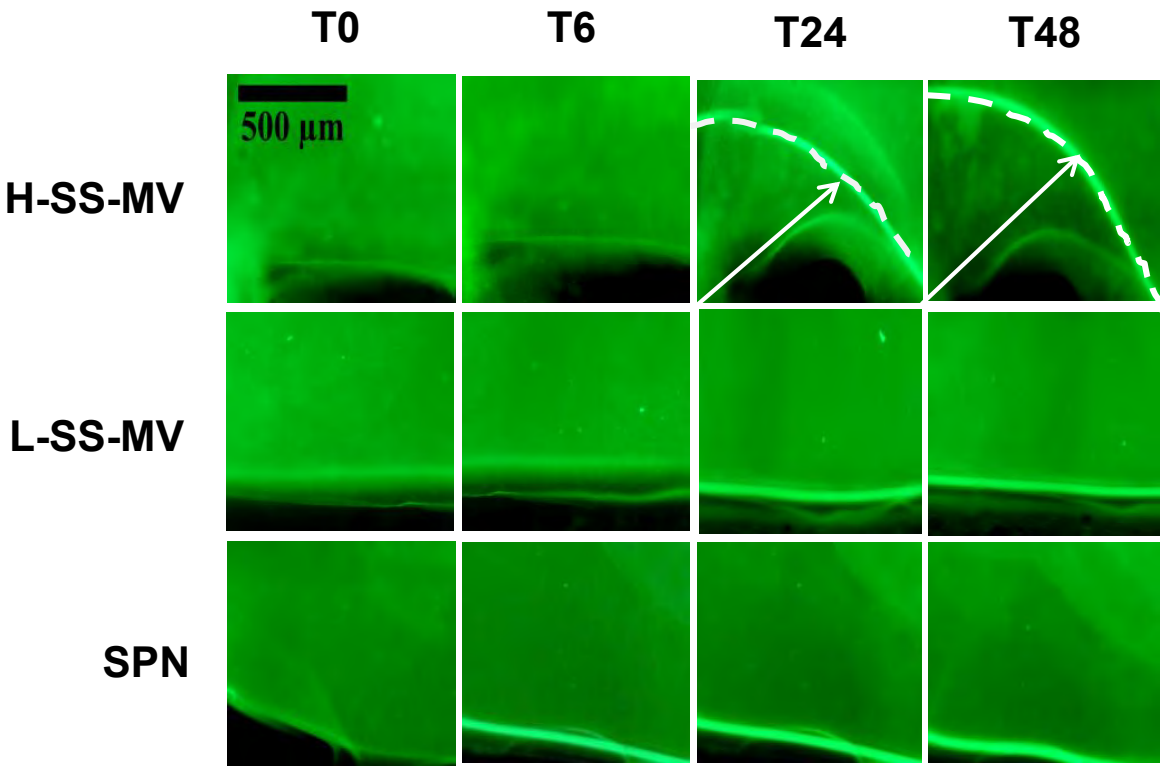
Lysed area



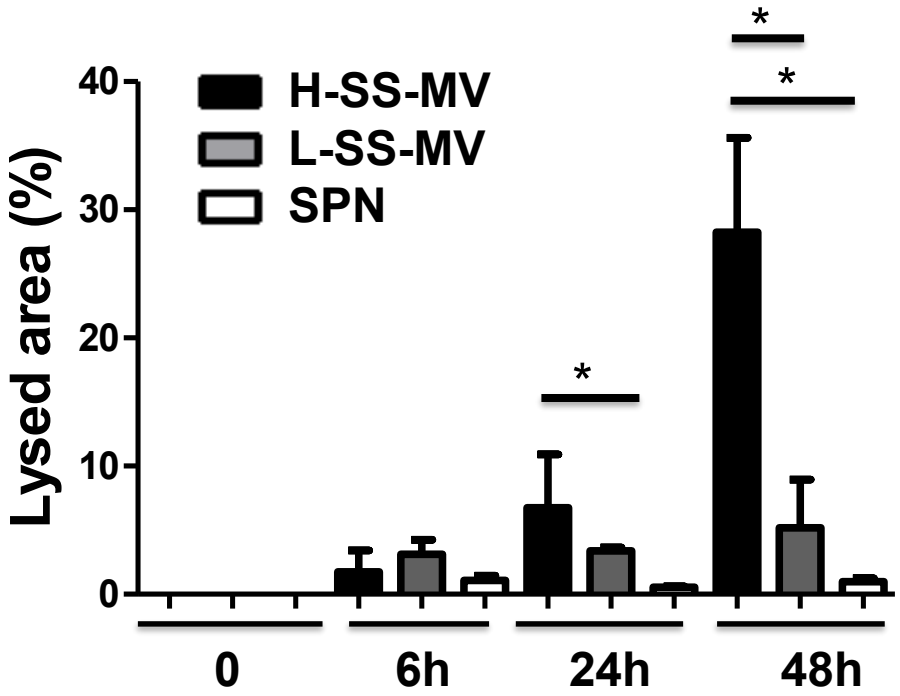
High PGC level confers to MV the capacity to lyse a thrombus

PGC confers to SS MVs the capacity to lyse a thrombus *in vitro*

Microscopic front lysis retraction assay

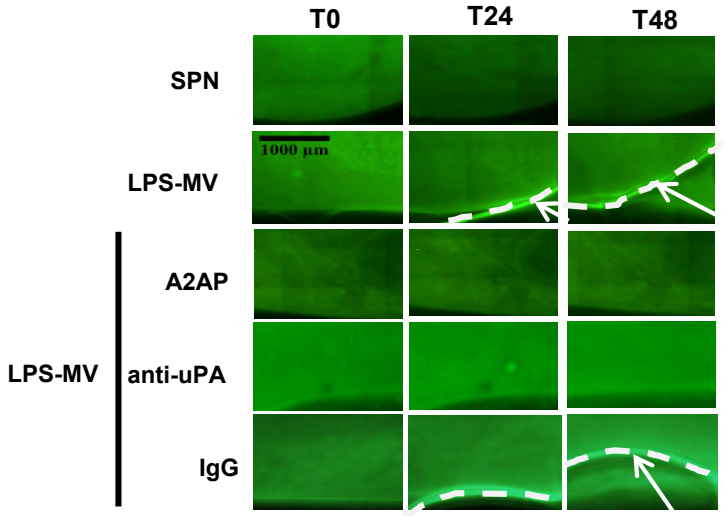


Lysed surface

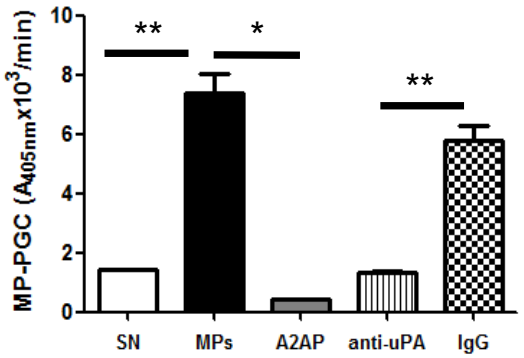


Lysis rate is driven by the PGC level of SS-MVs

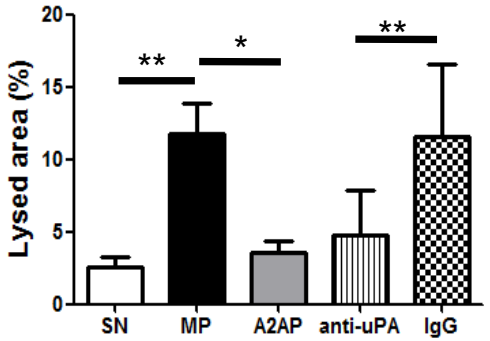
Molecular mechanisms of MVs thrombolytic effect



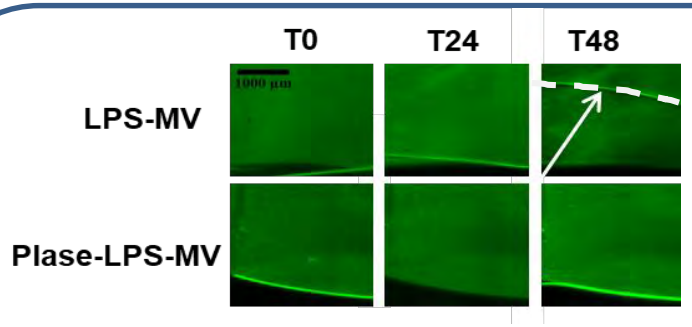
Plasmin generation



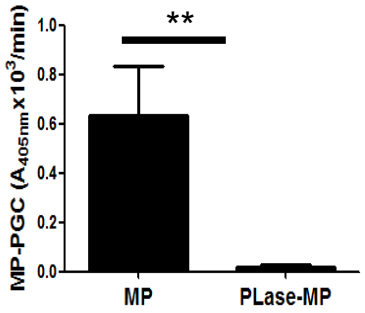
Lysed surface



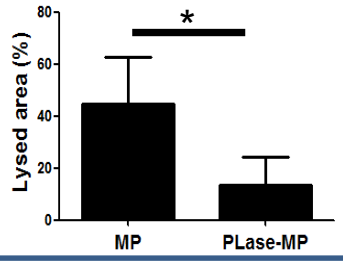
➔ Thrombus lysis is dependent on plasmin generated by its activator uPA



Plasmin generation



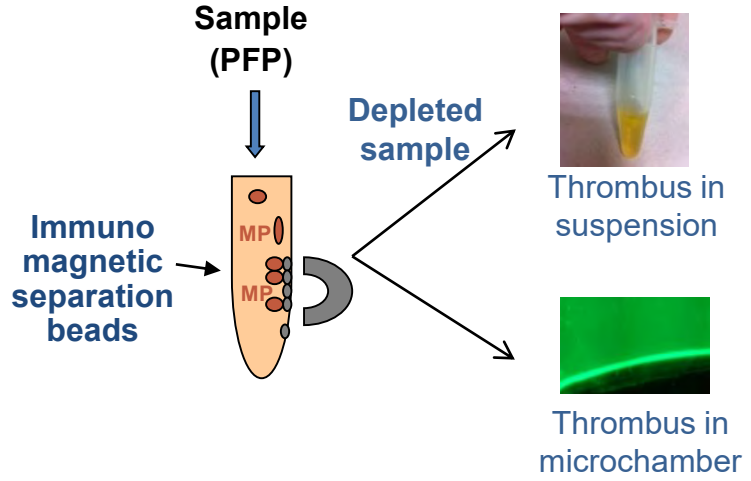
Lysed surface



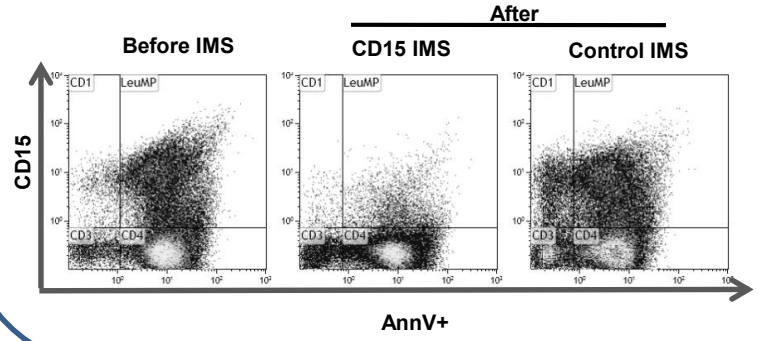
➔ Thrombolytic effect is uPAR-dependent

➔ In the LPS model, the MV thrombolytic effect is driven by PGC level and uPAR expression

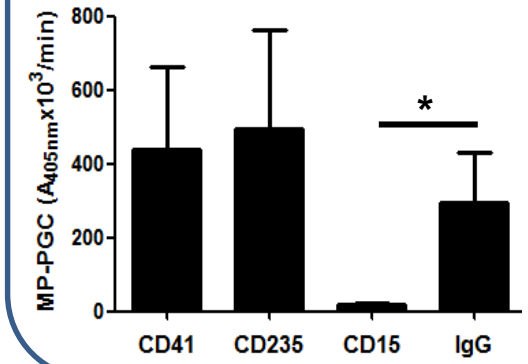
Cellular mechanism of MVs thrombolytic effect



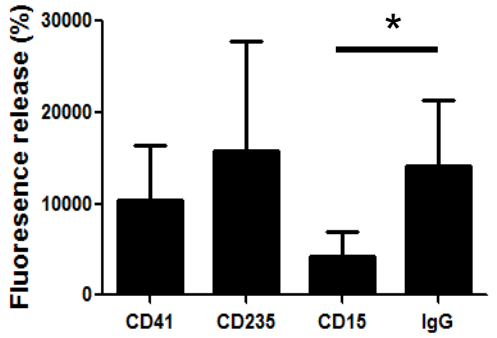
Depletion efficiency



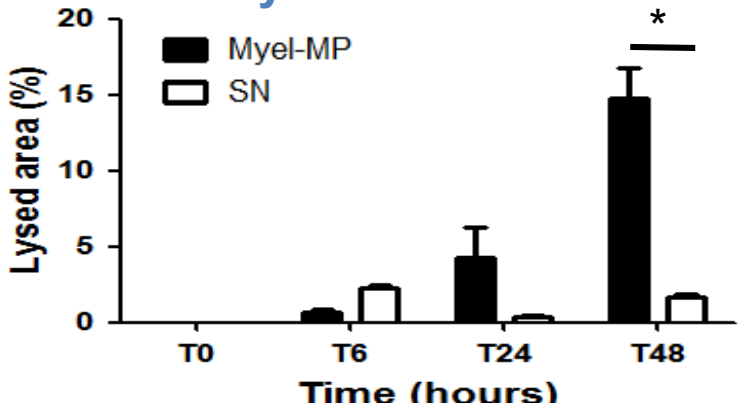
Plasmin generation



Fluorescence release



Lysed surface



➔ **MVs derived from granulocytes support thrombus lysis**

Infusion of uPA Boosted Gran-MVs improves mice survival

J0 : PLC

Injection 10^7 uPA-Gran-MVs « H-SS-MVs » Gran-MVs « L-SS-MVs » SPN

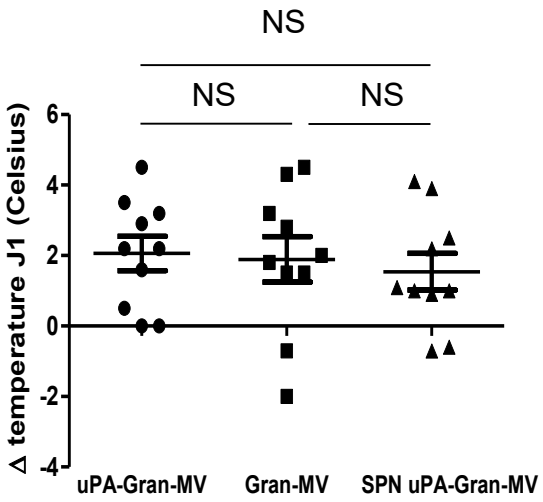
J5 : Death

J1 J2 J3 J4

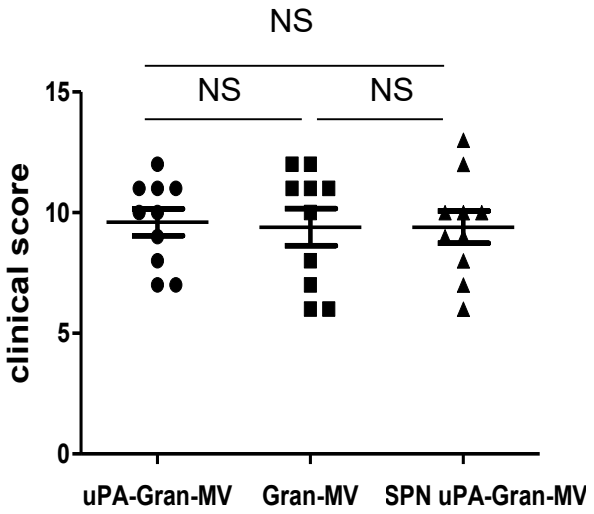
Histology

Monitoring

Clinical parameters

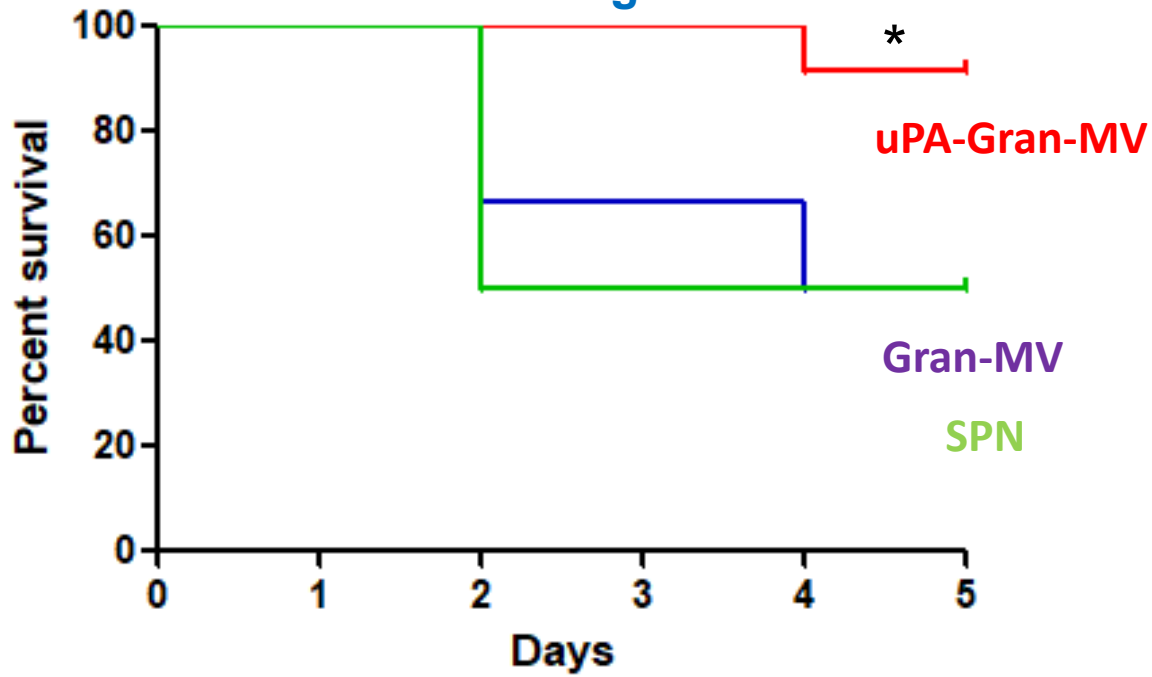


Temperature



Clinical score

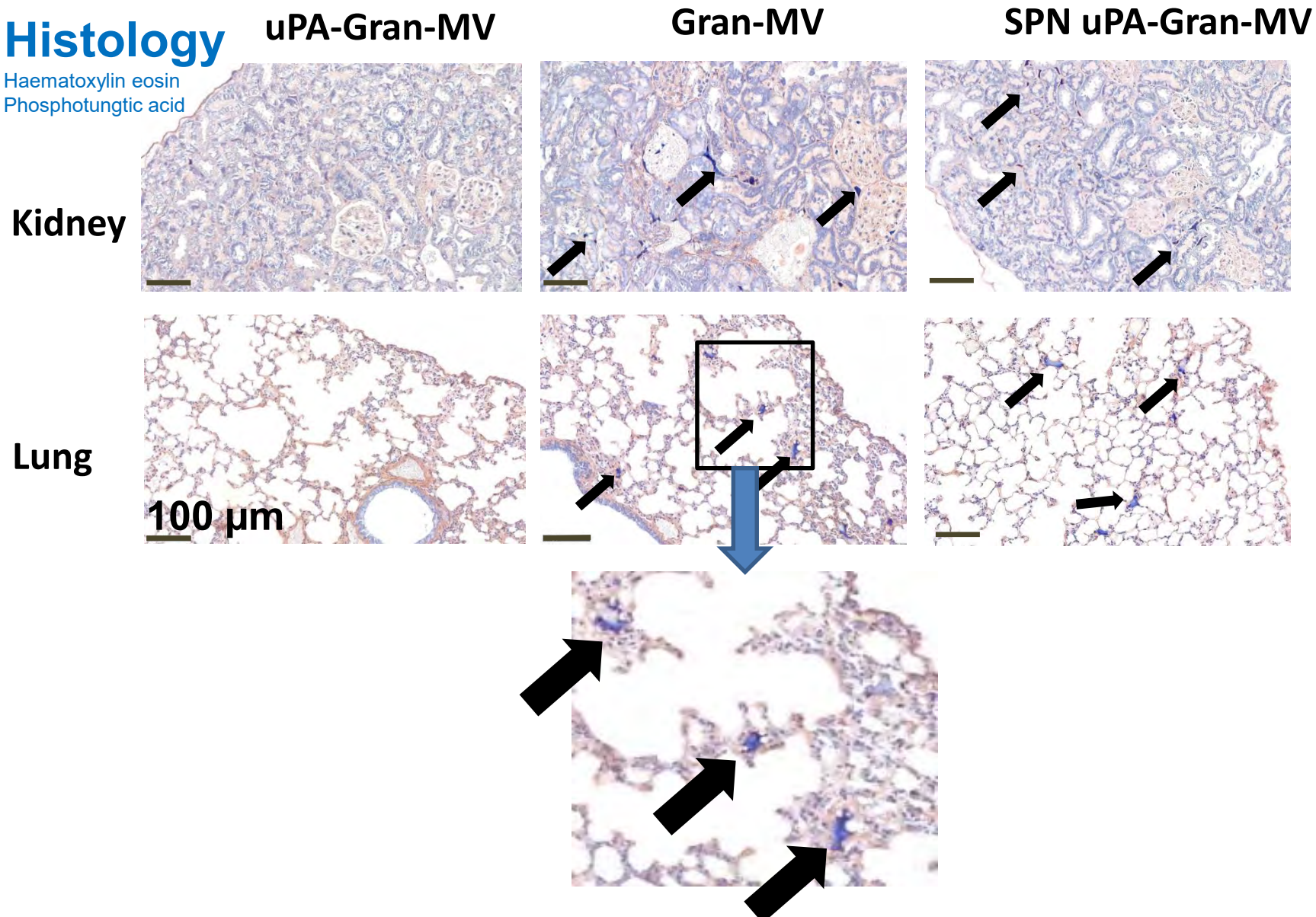
Pourcentage survival



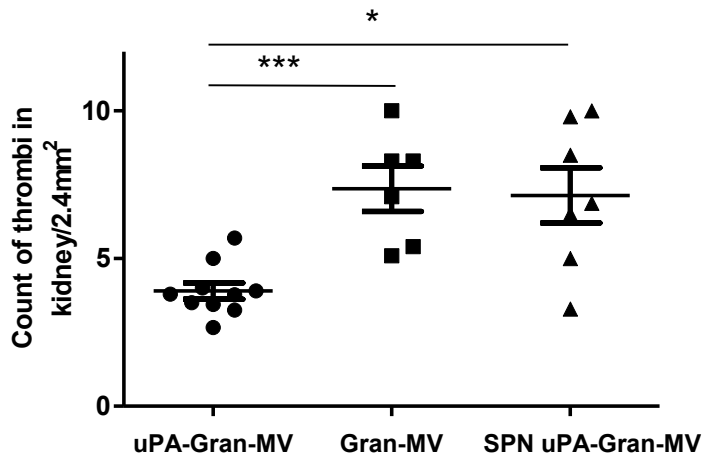
Infusion of uPA- boosted Gran-MVs reduces thrombus formation

Histology

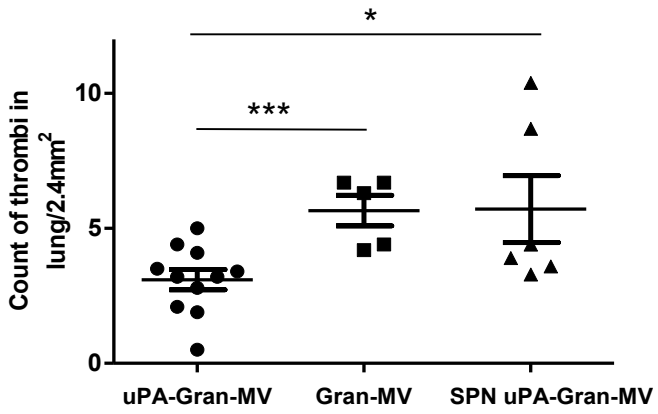
Haematoxylin eosin
Phosphotungtic acid



Number of thrombi in kidney



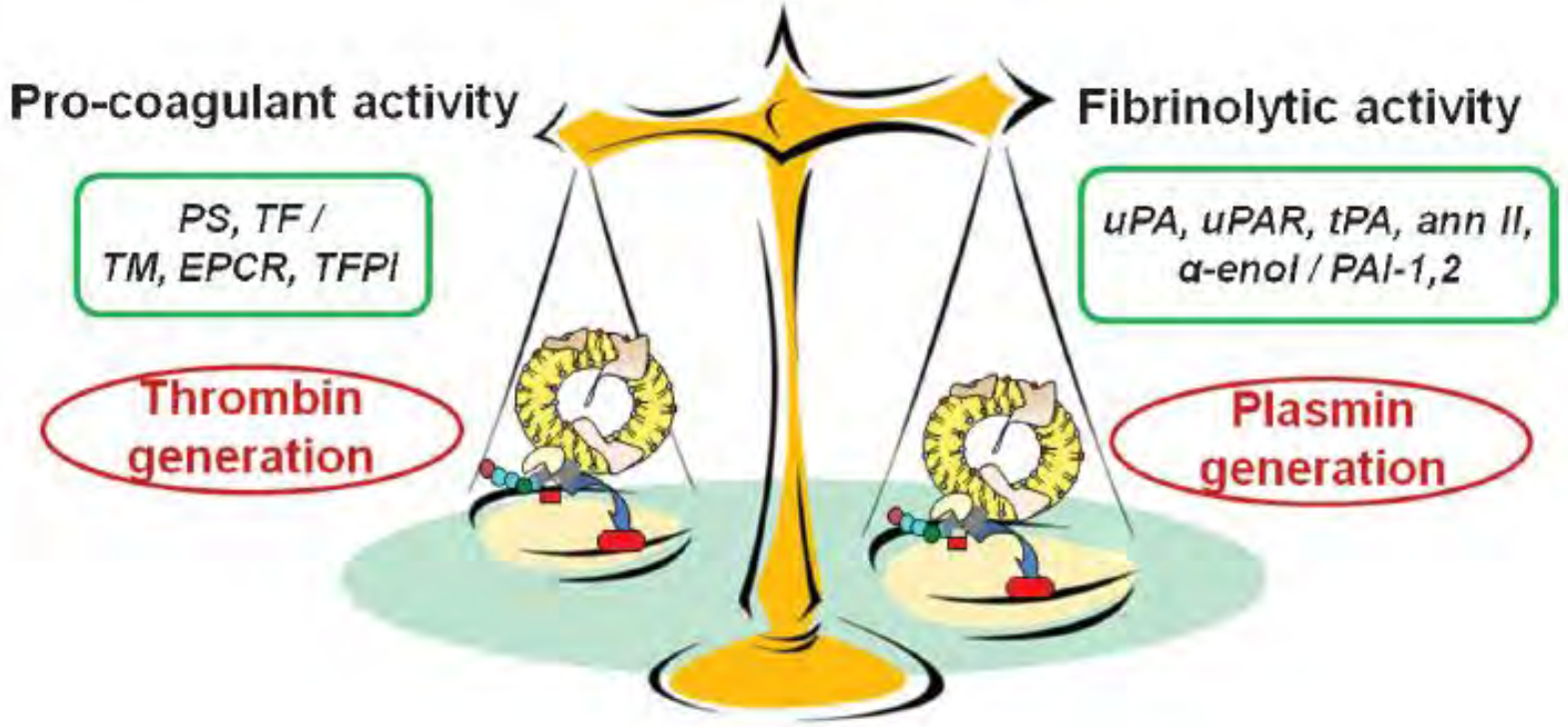
Number of thrombi in lung



Conclusion

- MV from septic shock patients display a heterogeneous PGC profile
- MV-PGC heterogeneous profile is driven by u-PA/ u-PAR system conveyed by granulocyte-derived MV
- Thrombus lysis rate is driven by the PGC levels of SS MV in vitro
- Gran MV with high PGC levels reduce clot formation, improve mice survival, confering a protective effect of Gran MV in a mice model of sepsis

MV : integrative micro-system tuning the haemostatic balance



« MV-dependent plasmin generation capacity»

- a more comprehensive view on their beneficious and deleterious role in septic shock
- revisit MV definition as a potential prognosis biomarker
- pave the way for novel therapeutic strategies targeting fibrinolysis imbalance

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