Le NET, un espoir dans l'AVC ?

Michel PLOTKINE
EA 4475 Pharmacologie de la circulation cérébrale
Faculté des Sciences Pharmaceutiques
Université Paris Descartes.

Académie nationale de pharmacie
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Conflits d’intérêt

- Aucune collaboration industrielle depuis 2013

L’AVC , un problème majeur de santé publique.

- 80% des AVC sont ischémiques.
- Occlusion d’un vaisseau cérébral par un thrombus.
- Un seul médicament validé , un fibrinolytique, le rt PA
- Mais échec de la recanalisation dans 50% des cas !!

Objectif:

- Améliorer le taux de succès de la thrombolyse
- Identifier les responsables des échecs
- Rôle des PMN ?

Notion Basique: Chacun son Job !

PMN

Inflammation

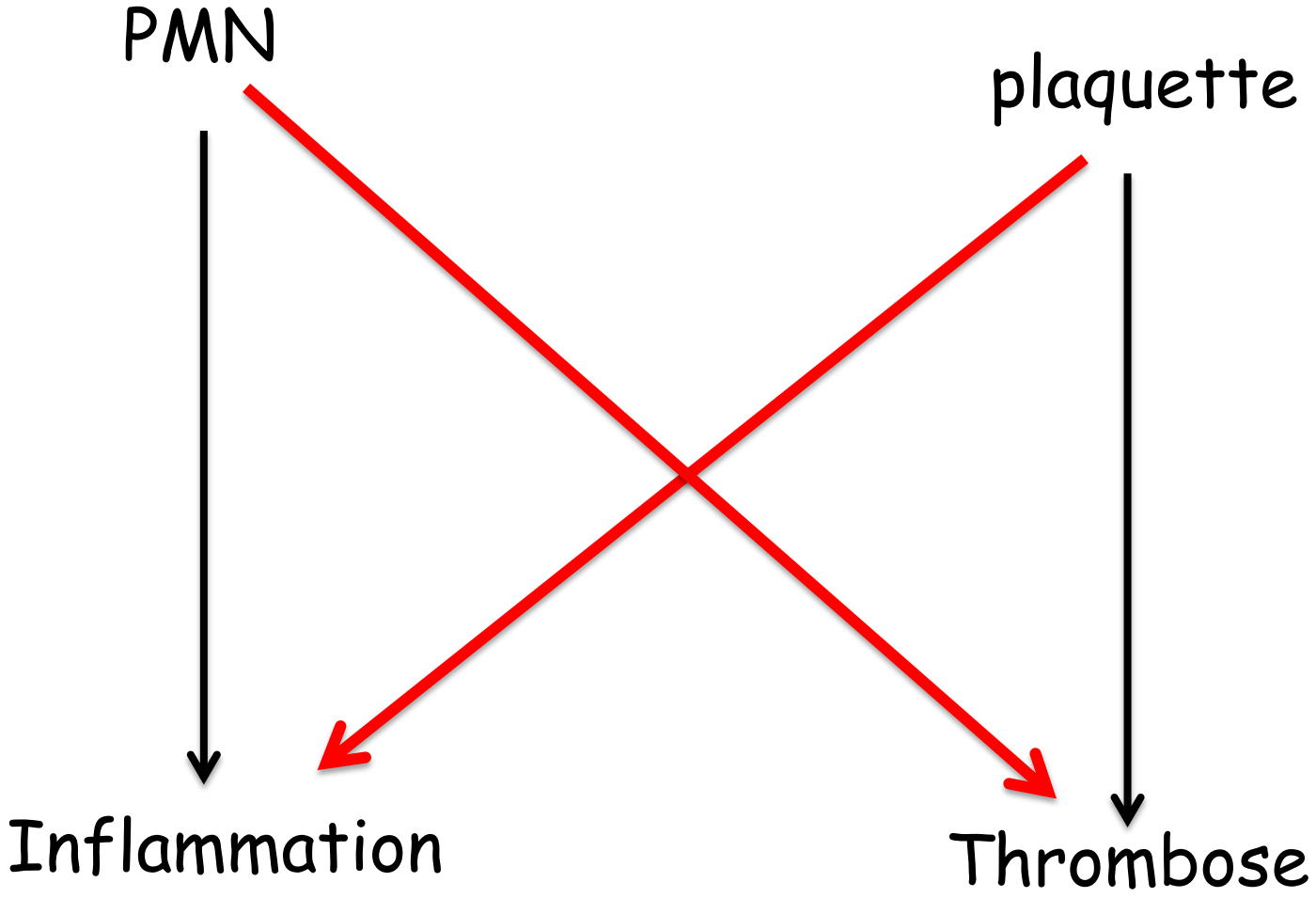
(Avant garde de l'immunité innée)

plaquette

Thrombose

(Avant garde de la coagulation)

Cross talk=collaboration



La thrombo-inflammation
ou immunothrombose !

Le PMN
La première ligne de défense contre les pathogènes !

Microbicide par plusieurs moyens:

1. Phagocytose (1909)

2. NETose (2004): le plan « B »

Quand la phagocytose est « débordée » ou quand la taille du pathogène ne permet plus sa mise en œuvre.

La NETose

Le PMN se sacrifie pour éradiquer le pathogène

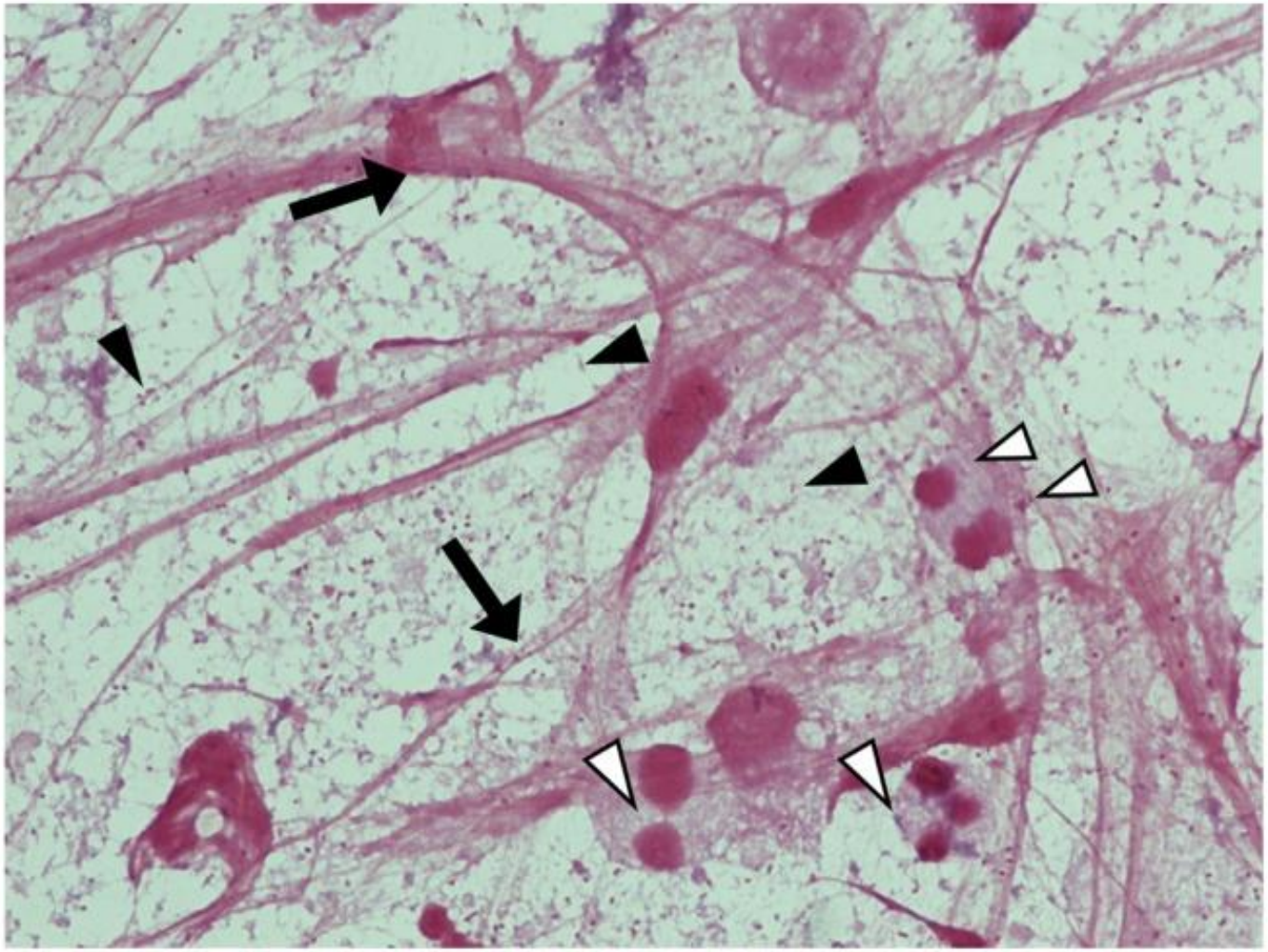
Mort des PMN différent de la nécrose et de l'apoptose.

Neutrophil Extracellular Traps Kill Bacteria

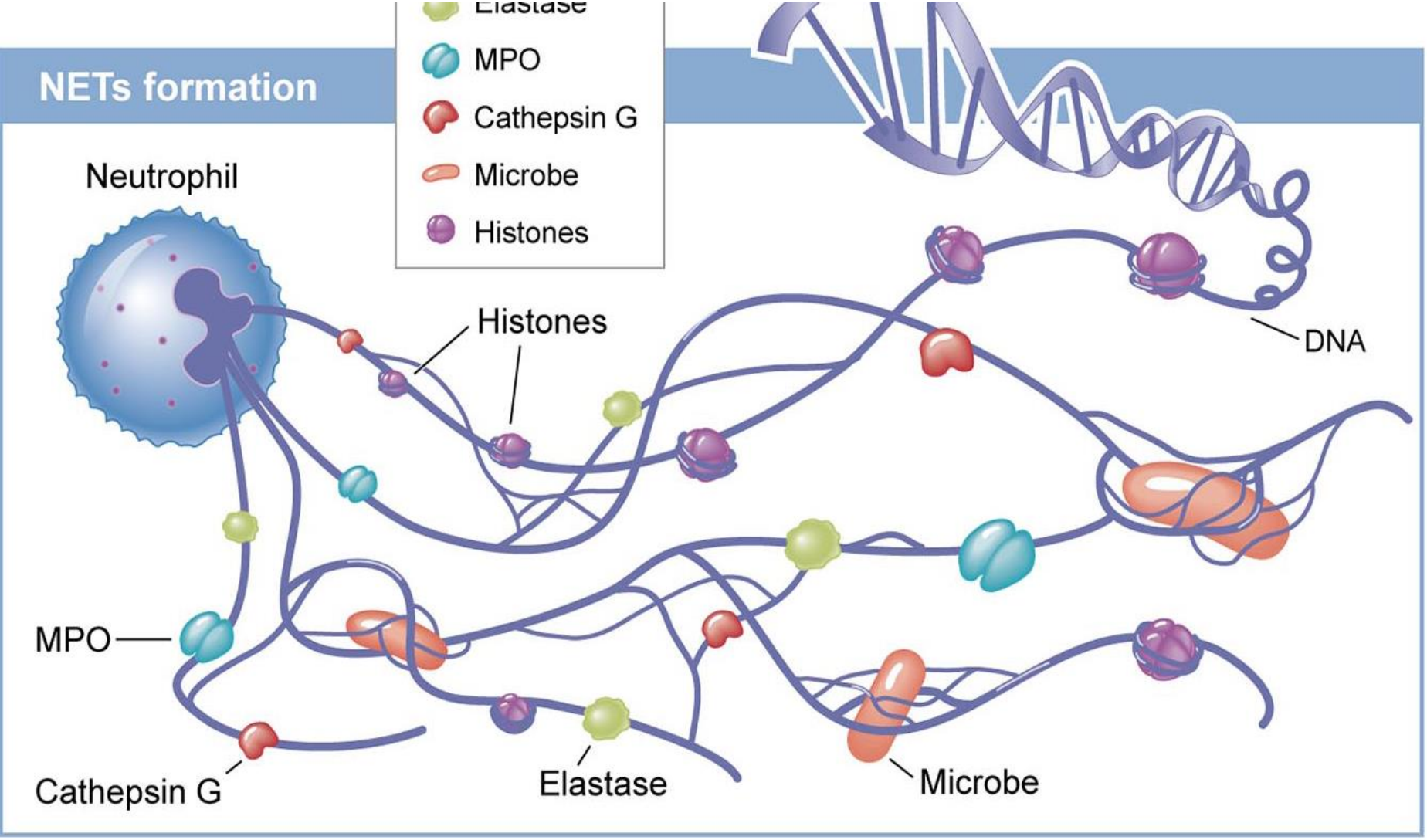
Volker Brinkmann,1 Ulrike Reichard,1,2 Christian Goosmann,1,2Beatrix Fauler,1 Yvonne Uhlemann,2 David S. Weiss,2 Yvette Weinrauch,3 Arturo Zychlinsky2*

Sciences, 2004, 303, 1532-5

Neutrophils release granule proteins and chromatin that together form **extracellular fibers** that bind Gram-positive and -negative bacteria. These neutrophil extracellular traps (NETs) degrade virulence factors and kill bacteria !



A large number of Gram-negative coccobacilli (black arrowheads), including a proportion phagocytosed by neutrophils (white arrowheads) and numerous fibrous NETs (arrows) are observed at higher magnification (1000×).


NETs formation
Elastase
MPO
Cathepsin G
Microbe
Histones
Neutrophil
Histones
DNA
MPO
Cathepsin G
Elastase
Microbe

Mécanismes de la NETose

- Rencontre avec un DAMP (LPS , HMGB1….).
- Activation de la NADPH oxydase (NOX)
- Stress oxydatif.
- Citrullination des histones par la PAD4.
- Décondensation du noyau.
- Expulsion du contenu dans le milieu extracellulaire.
- Formation du réseau piégeur ! (ou le trappeur au travail) .

- Déficit de NETose et risque infectieux en cas de défaut en NOX (granulomatose chronique familiale)
- Blocage par Inhibiteur de la NADPH oxydase (iodonium de diphenyléne)
- Blocage chez les NADPH-/-

PAD 4: Facteur clé de la NETose

- Transforme la fonction imine de l'arginine des histones en citrulline (citrullination des histones)
- Perte des charges + des histones.
- Décondensation de la chromatine

Outils:

- Existence de souris PAD4 -/-
- Existence d'inhibiteur de PAD 4

- Pas de NETose chez les PAD4-/-

PAD 4= peptidylarginine desiminase de type 4

Les armes des NETs

- Réseau de ADN décondensé.
- Décoré avec les histones et les nombreuses enzymes: protéases, élastase, myéloperoxydase…..

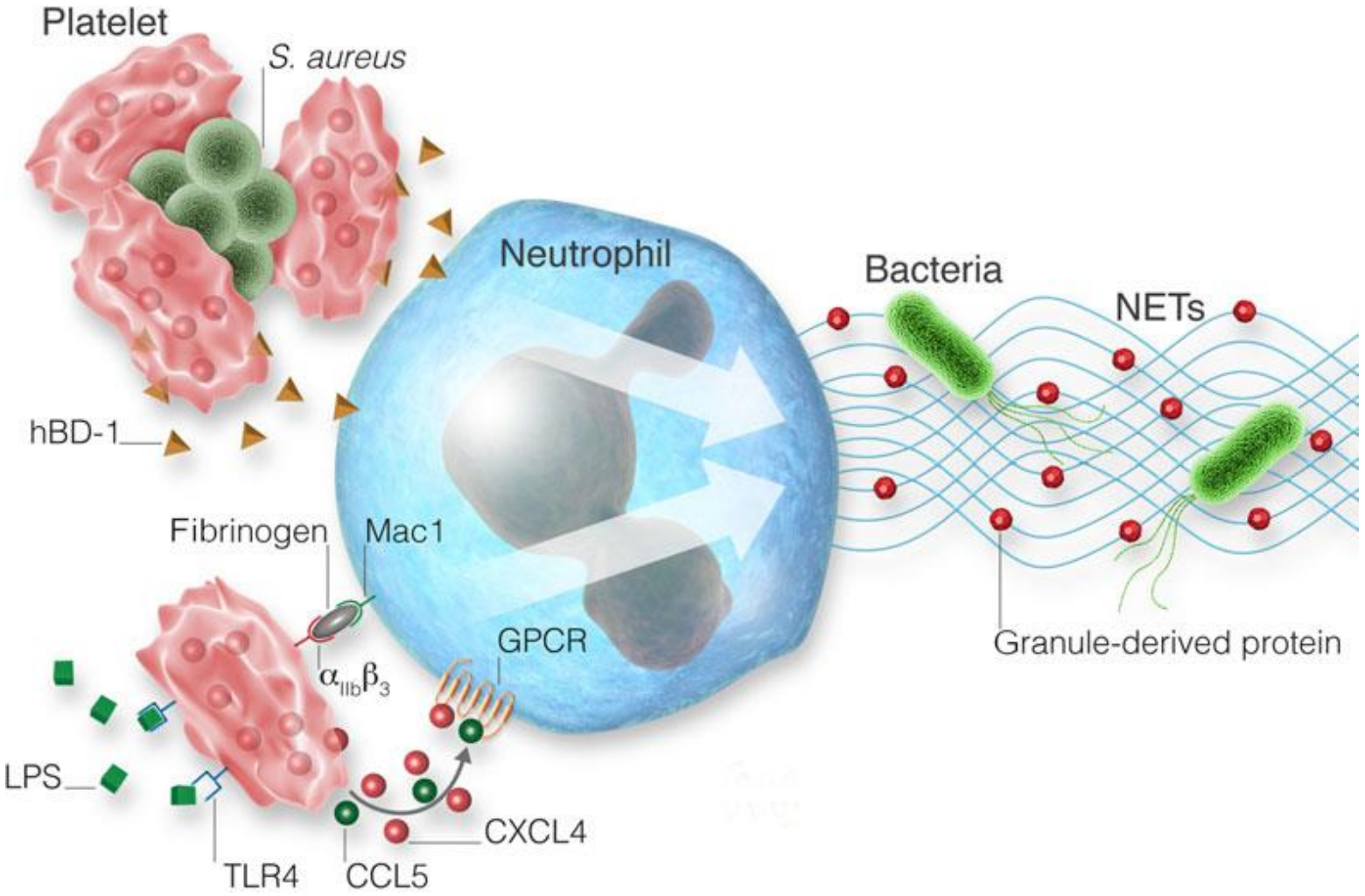
Un arsenal redoutable vis à vis des agents pathogènes !!!

Mais aussi pour l'hôte !

NET et coagulation

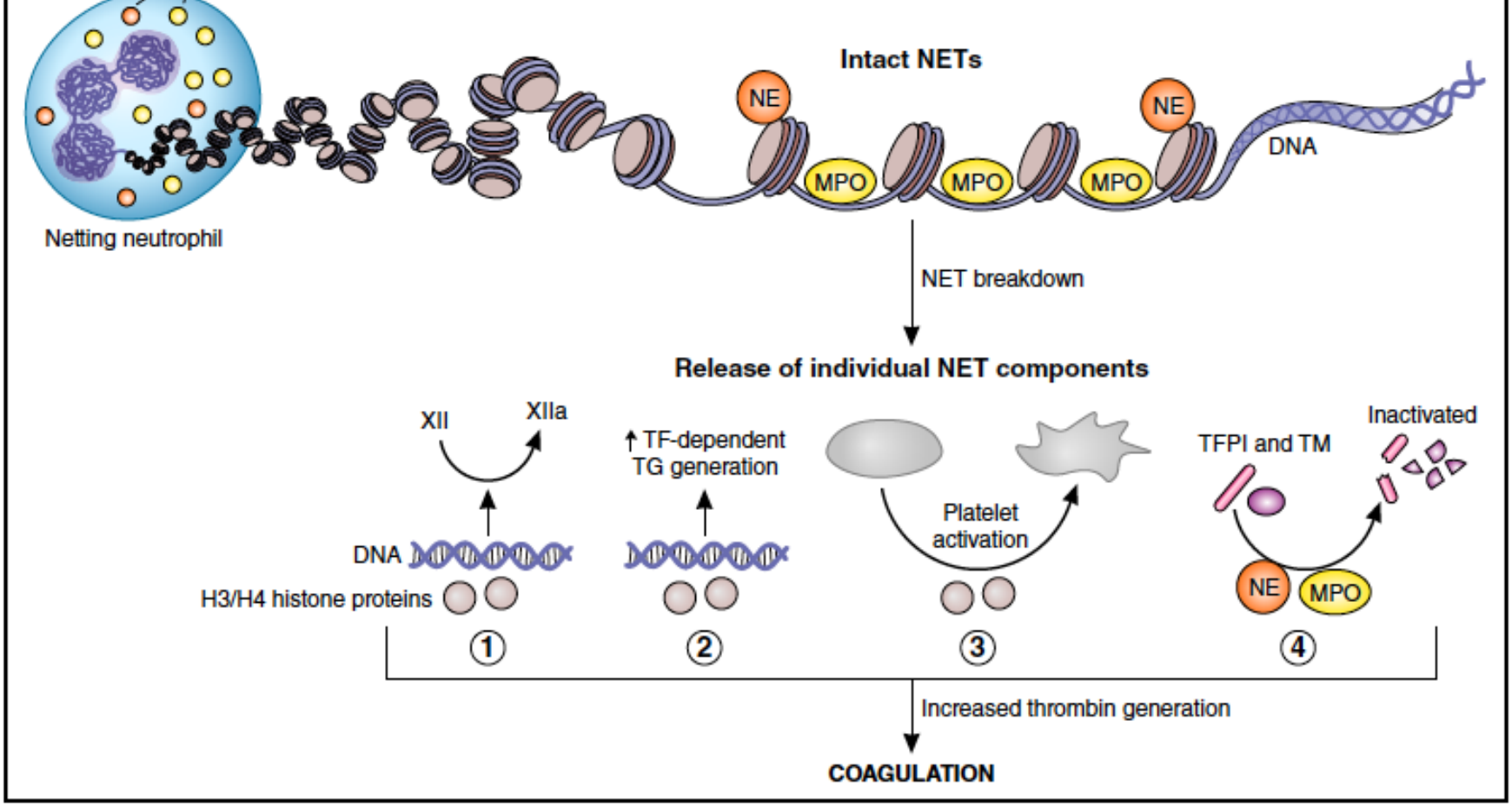
Les complexes PMN –plaquettes:

Une collaboration efficace mais……. dangereuse !



Mechanisms of platelet-mediated NET formation

Platelets in leucocyte recruitment and function
Jan Rossaint1,2 and Alexander Zarbock1,2*
Cardiovasc Res, 2015



Activated neutrophils release NETs, which are scaffolds of decondensated DNA and histones. Nouboussie et al report that individual NET components such as free DNA and histone proteins promote thrombin generation (TG) through the following 3 pathways: (1) activation of the contact pathway via factor XII, (2) amplification of tissue factor (TF)–dependent TG, (3) and activation of platelets via histones H3 and H4. (4) In addition, natural anticoagulants such as tissue factor pathway inhibitor (TFPI) and thrombomodulin (TM) can be inactivated by myeloperoxidase (MPO) and serine proteases (ie, neutrophil elastase [NE]). Professional illustration by Patrick Lane, ScEYEnce Studios.

in: C Schulz et al. Blood 2017

NETs

- Activent la coagulation
- Lèsent les Cellules endothéliales

2 bonnes raisons d'être thrombogène !!!

NETs et thrombose artérielle

- Démontré dans ischémie myocardique expérimentale
- Mise en évidence dans IM en clinique

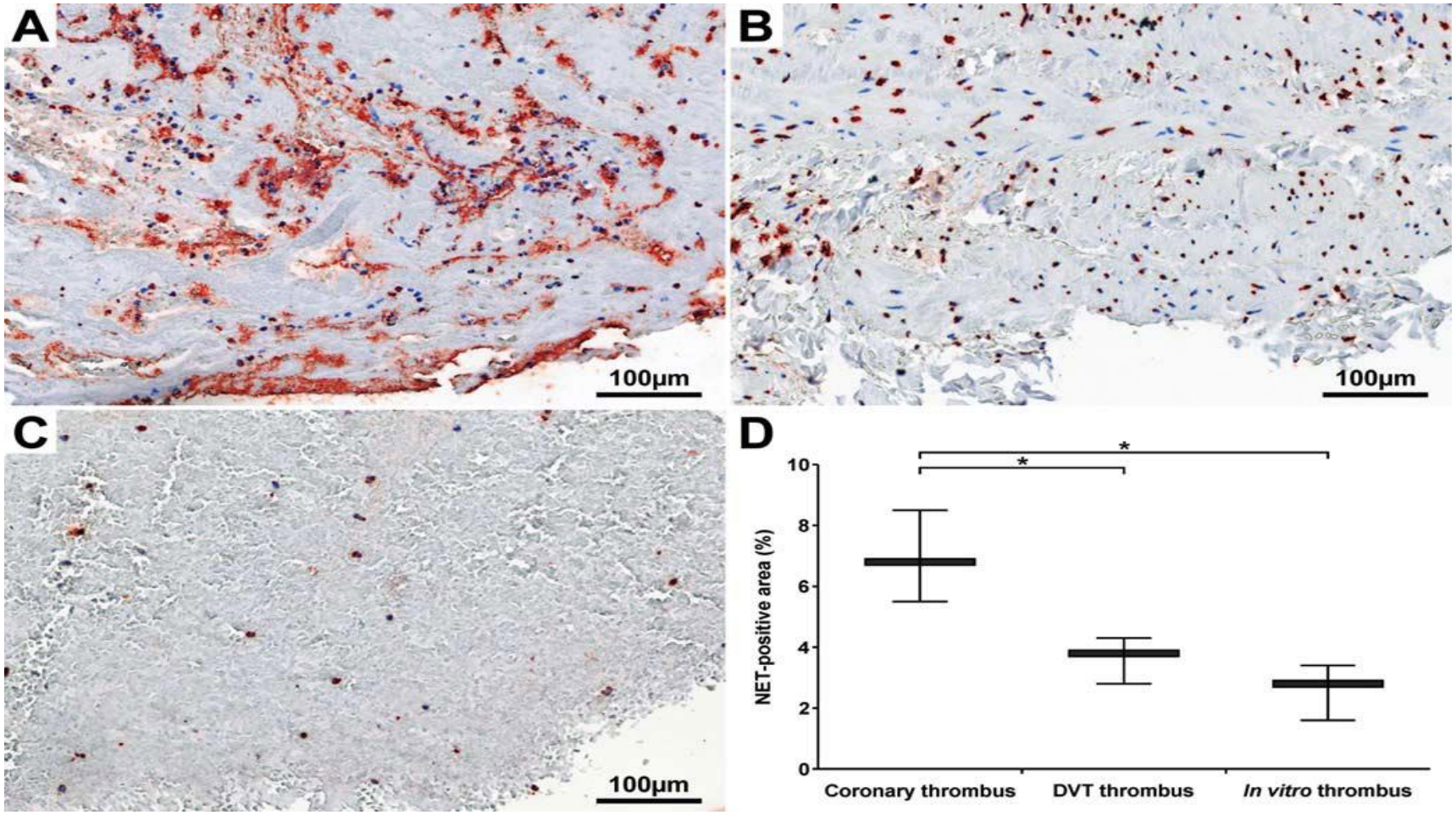


Figure 4: Quantification of NETs in vascular thrombi. NET staining was performed with an anti-DNAhistone antibody and is visible in red, cell nuclei are blue. A coronary thrombus representative of 30 consecutive coronary particulate aspirates is shown (panel A). A deep vein thrombus (DVT) representative of 7 subsequent samples is shown (panel B), and an in vitro whole blood clot representative of clotted whole blood samples from 4 healthy donors is shown (panel C). Data are expressed as "NET-positive area" in percent of total thrombus area (Panel D, each *$p<0.05$).(Hoffmann et al. 2015)

NETs et fibrinolyse

Stabilisation de la fibrine par l'association ADN –histone !!

- Augmentation de l'épaisseur des fibres
- Augmentation de la rigidité des thrombus
- Résistance à la fibrinolyse
- DNase potentialise l'effet du rt PA

↓

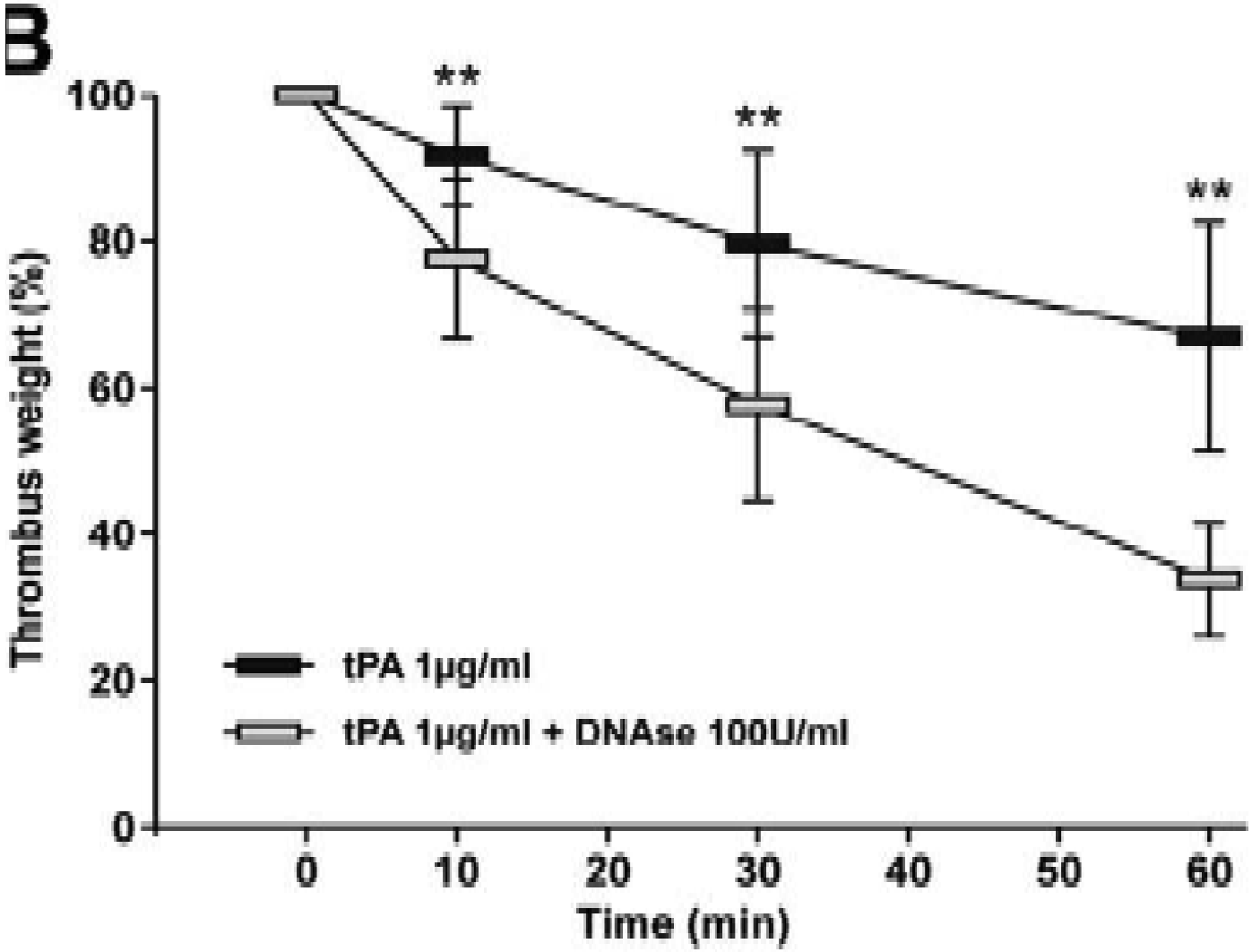
Le thrombolytique du futur

Association fibrinolytique + « NETolytique » ?

- Mechanical stability and fibrinolytic resistance of clots containing fibrin, DNA, et Histones. Longstall et coll. JBC 2013;288:6946-56.

- DNA, histones and NETs exert anti-fibrinolytic effects in a plasma environment. Varju et coll. Thromb haemost 2015; 113.6 E pub.

La DNase accélère in vitro la lyse d'un thrombus coronarien humain induite par la rtPA. (Hoffmann et al , 2015)

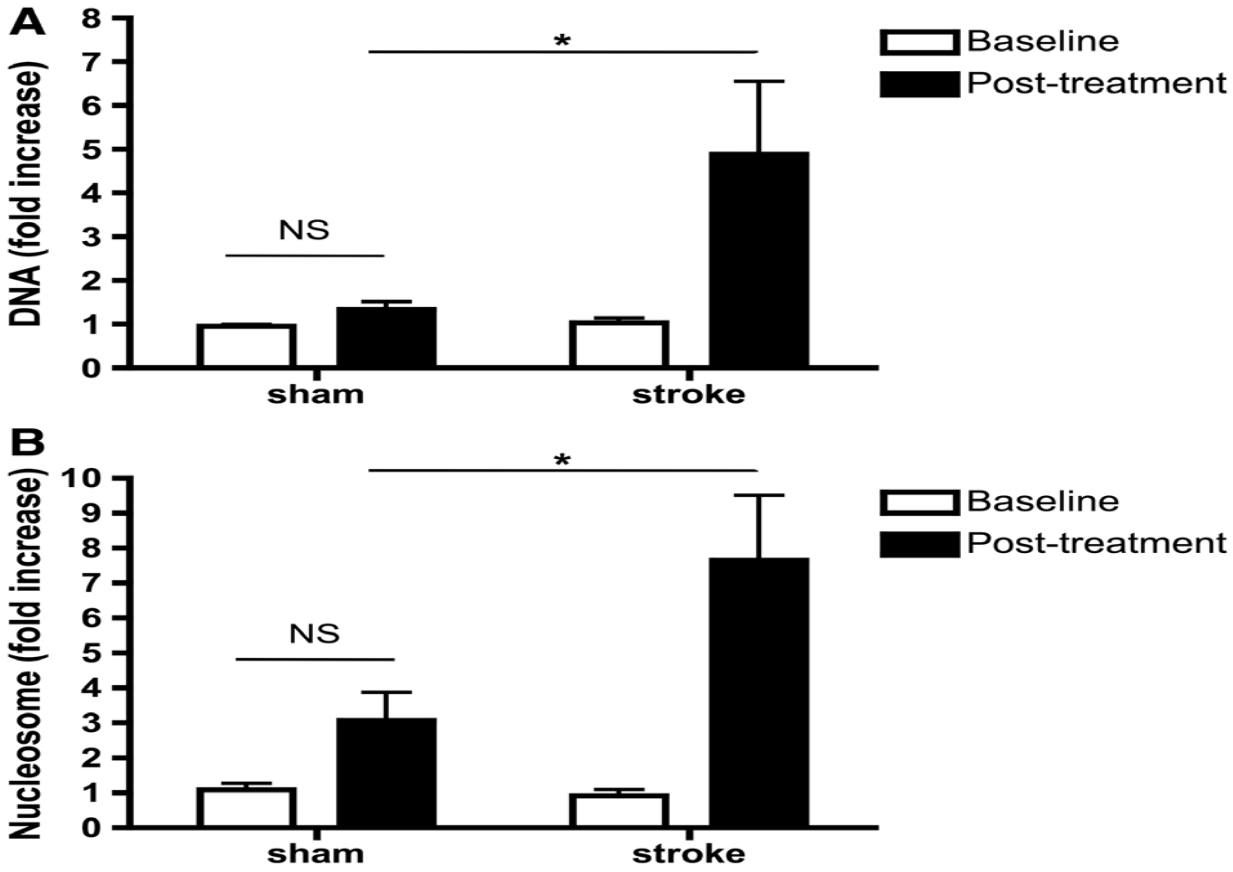


NETs et Ischémie cérébrale

Extracellular chromatin is an important mediator of ischemic stroke in mice

De Meyer et coll. ATVB 2012;32:1884-1891

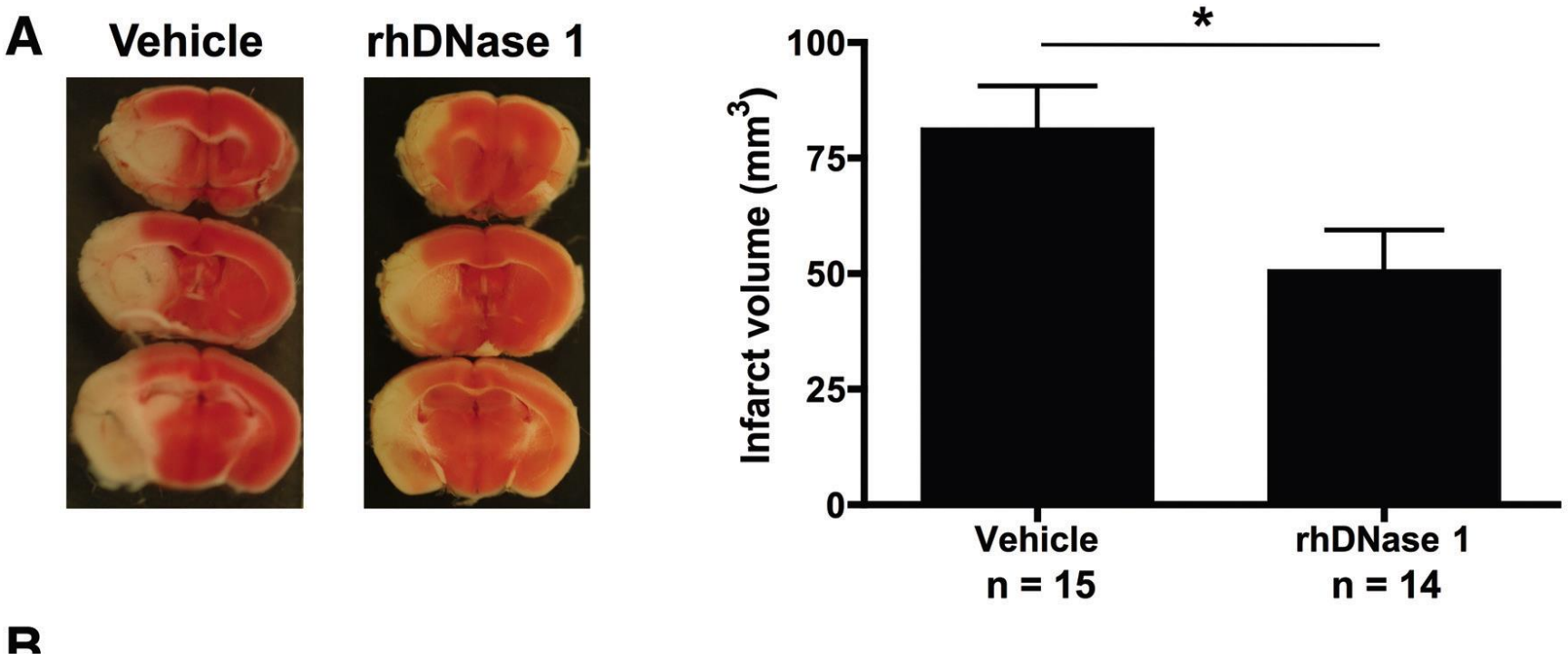
Extracellular Chromatin Is generated after Ischemic Stroke in Mice



DNA and nucleosomes are increased after 2 hours occlusion and 22h reperfusion in MCAO in mice. (« Post treatment »= after 22h reperfusion.

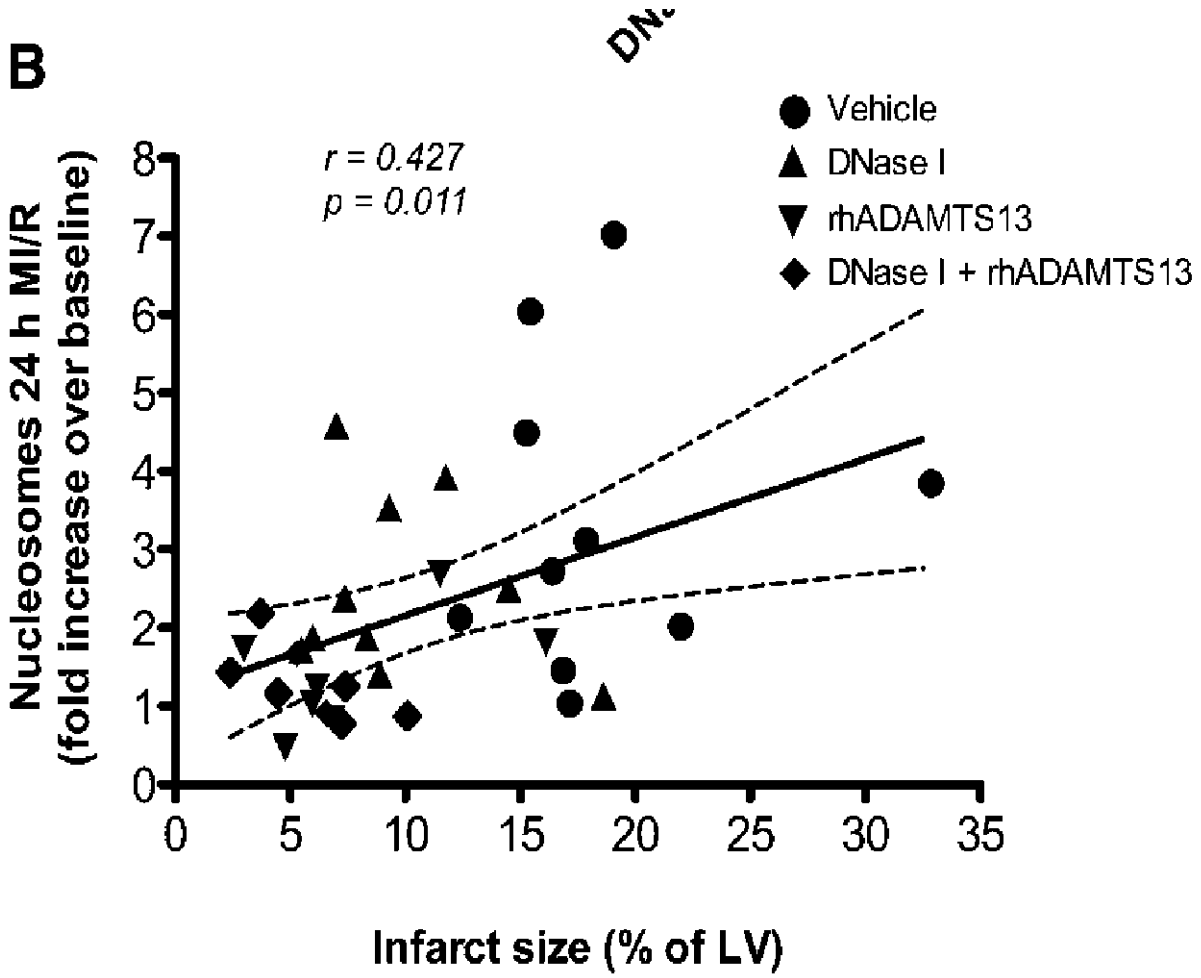
De Meyer et coll. ATVB. 2012, 32,1884-91.

Extracellular Chromatin Is an important mediator of Ischemic Stroke in Mice



Recombinant human DNase 1 (rhDNase1) improves outcome after ischemic stroke.Wild-type mice treated with rhDNase 1 or vehicle
De Meyer et coll. ATVB. 2012, 32,1884-91.

La taille de la nécrose est correllée aux nucléosomes



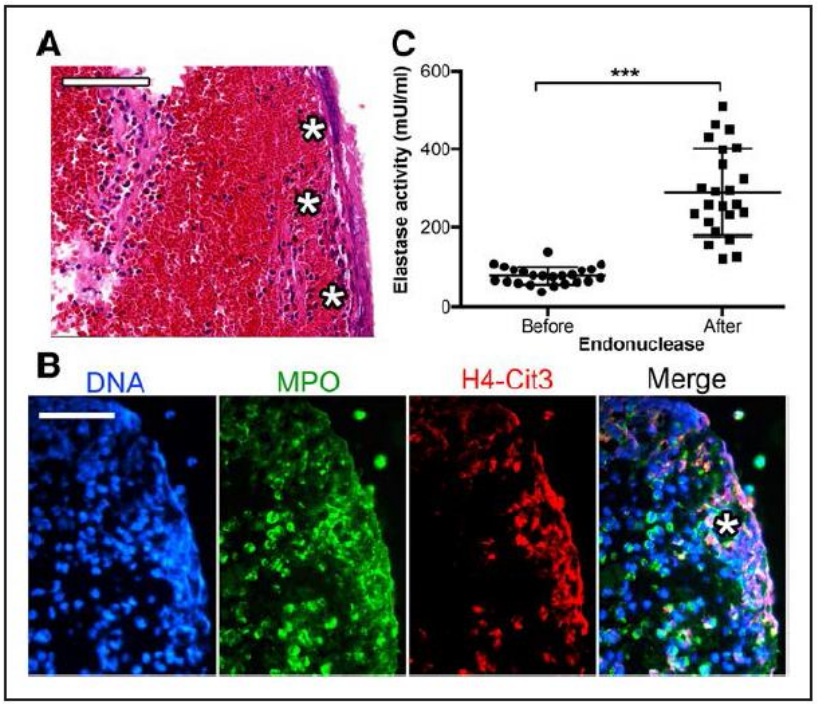


Figure 1. Neutrophil extracellular traps (NETs) are constitutively present in acute ischemic stroke thrombi. The presence of neutrophils and NETs in acute ischemic stroke (AIS) thrombi was investigated by immunohistological analysis. **A**, Representative image of a thrombus stained by hematoxylin/eosin showing the abundance of polymorphonuclear cells and extracellular nucleic acid (*) predominantly located in the thrombus outer layer. Scale bar=50 μm. **B**, Representative images of a thrombus stained for DNA (DAPI; Sigma-Aldrich), and with antibodies against myeloperoxidase (rabbit antihuman MPO antibody, Dako) and citrullinated histone H4 (rabbit antihuman Histone H4 citrulline 3 [H4-Cit3] antibody, Millipore). Note the presence of NETs at the thrombus periphery. Scale bar=50 μm. **C**, The presence of NETs in AIS thrombi was investigated by measurement of DNA-associated neutrophil elastase activity. The dot plot shows the elastase activity measured in supernatants of thrombi before and after endonuclease treatment (n=23; $P<0.0001$).

Ducroux et coll, stroke 2018.

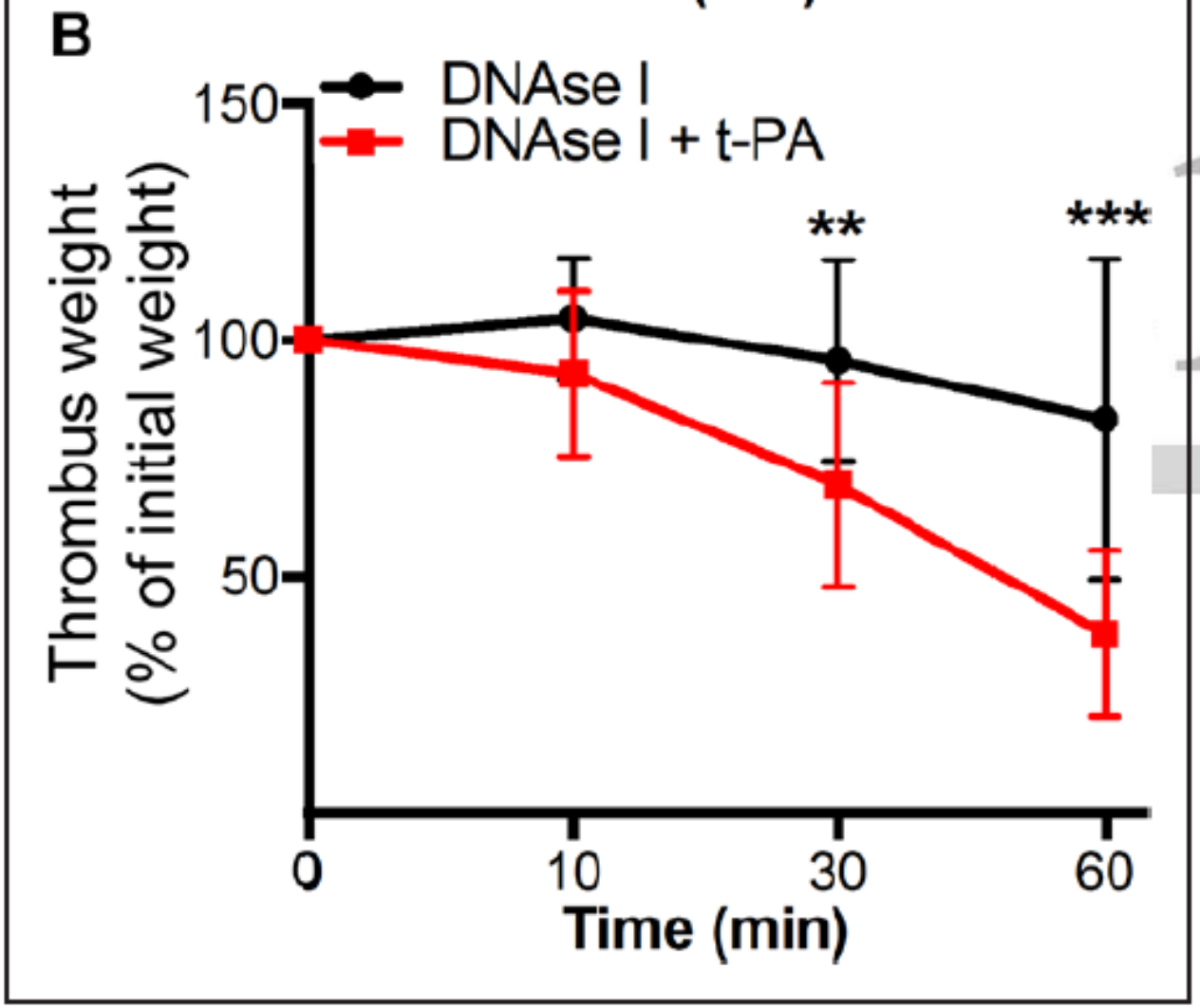
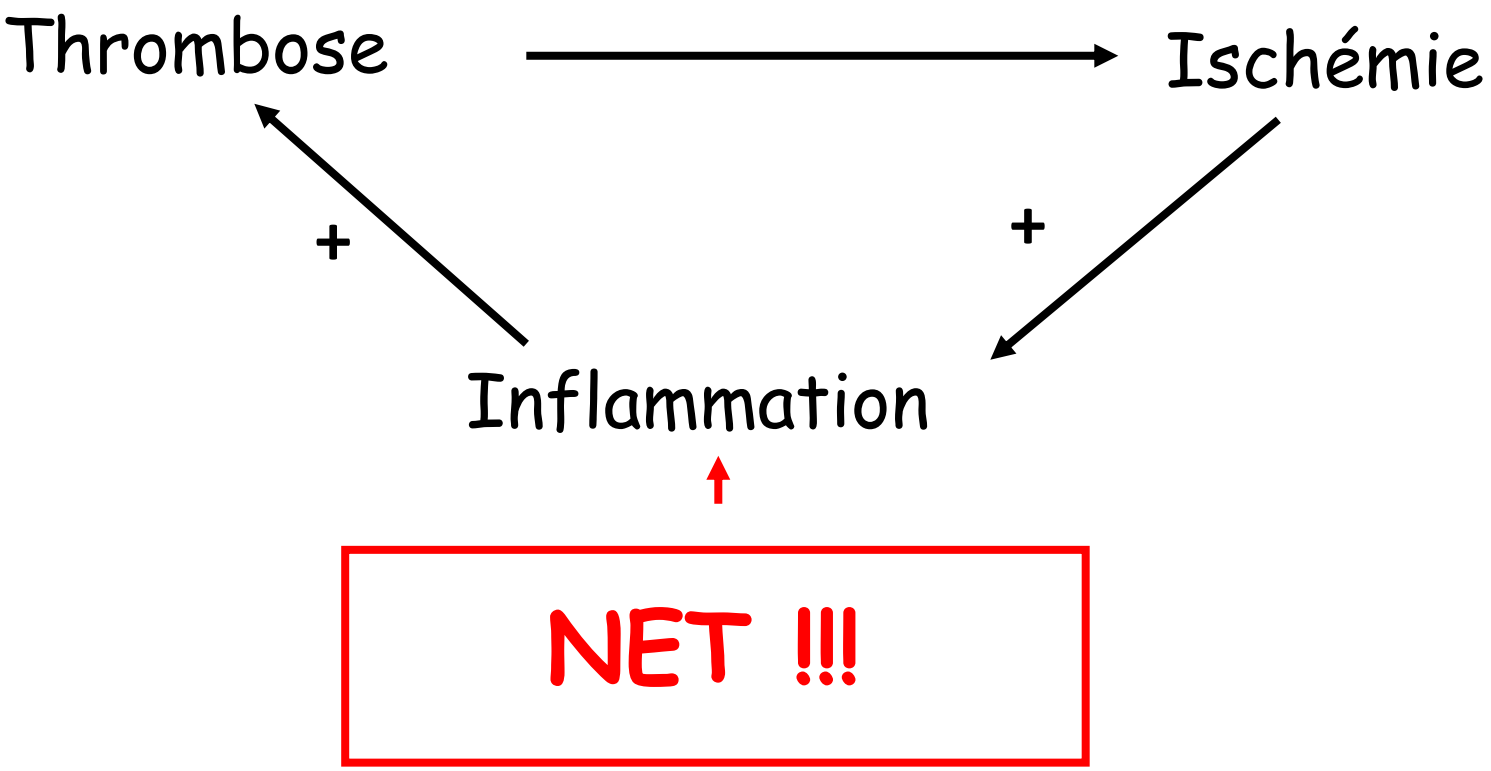


Figure 3. DNAse 1 potentiates tPA-induced thrombolysis ex vivo. Acute ischemic stroke thrombi recovered by endovascular therapy were incubated with tPA and DNAse 1, and their lysis was followed by measurement of thrombus wet-weight evolution over time. Mean baseline weight of thrombi was 14, 6±8, and 4 mg.

Coagulation et ischémie reperfusion



Question:

- Les NETs sont-ils une « druggable target » dans la thromboinflammation de l'AVC ?

Les pistes:

- Le stress oxydatif
- La PAD4
- La DNase

Les anti NETs, un classe du futur ?

Pathologies impliquant les NEts

- Infections
- Sepsis
- Maladies autoimmunes (présence d'anticorps contre des histones citrulinées)
  - Lupus
  - Polyarthrite rhumatoïde
  - Diabète type 1
- Thrombose (veineuse et artérielle)
- Trauma cranien
- Drépanocytose
- Ischémie reperfusion (SNC, myocarde , foie, périphérique…)
- VILI (ventilatory induced lung injury)
- Tumeurs (rôle prométastatique et prothrombogène)
- Maladie de Behcet
- Atherosclérose … Etc…..

- Un marché potentiel considérable !!!!

MERCI

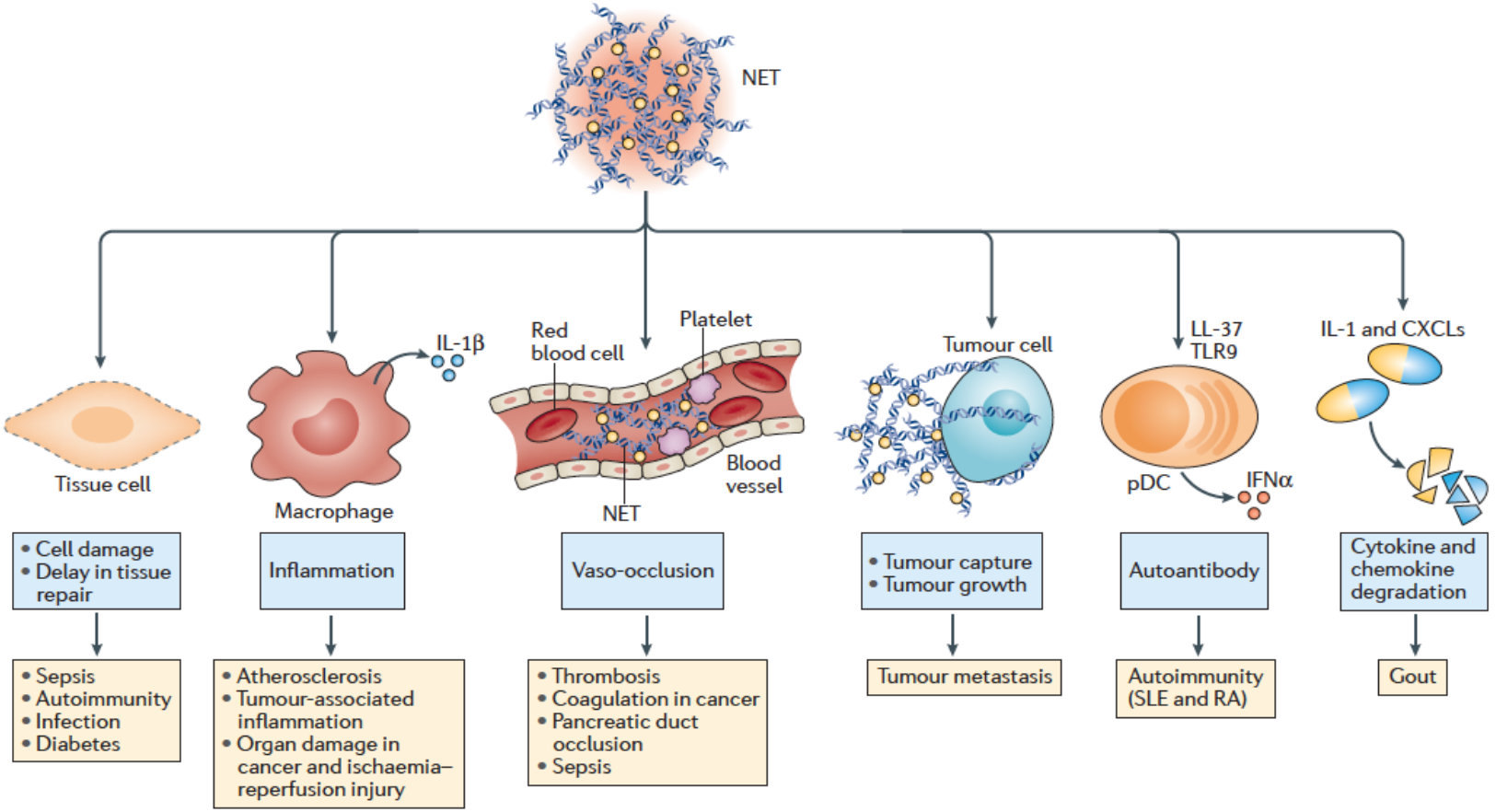


Figure 4 | **Mechanisms of NET-mediated pathology.** Neutrophil extracellular traps (NETs) cause pathology in a number of conditions through several mechanisms. Direct cell damage is implicated in infection, sepsis, autoimmunity and diabetes. By licensing macrophages for inflammation, NETs drive atherosclerosis. The increased propensity for NETosis promotes inflammation and organ damage in cancer and ischaemia–reperfusion injury. NET formation in the circulation promotes coagulation, vascular occlusion and thrombosis. NETs in capillaries can also capture and, potentially through other mechanisms, promote tumour metastasis. Finally, although NETs can promote inflammation, an accumulation of NETs promotes the resolution of inflammation through the degradation of cytokines and chemokines. CXCLs, CXC-chemokine ligands; IFNα, interferon-α; pDC, plasmacytoid dendritic cell; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TLR9, Toll-like receptor 9.

Papayannopoulos Nature rev Immunol , 2018