

Les ANCA, à la Trap...

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d'allergologie**

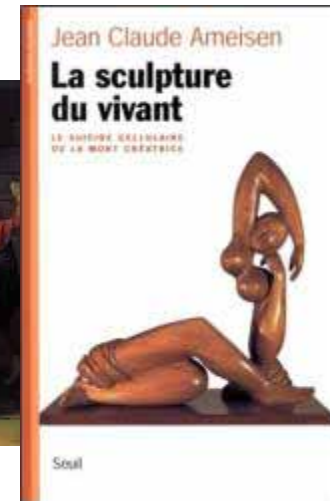


Cellule : 4 façons de mourir

Nécrose : dissémination façon puzzle !!!



Apoptose : suicide propre



Autophagie : zombi, ... et zarbi (survie au stress)



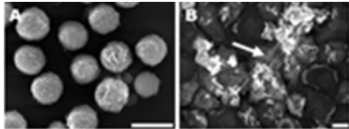
N Engl J Med, 2013, 368 (7) : 651-62

....et la NETose : suiciflingage des PNN



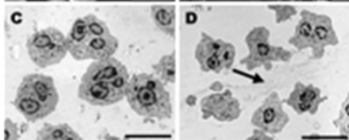
Brinkmann et al, Science, 2004 : 303 :1532-5 :
NET neutrophil extra-cellular traps

PNN
quiescent



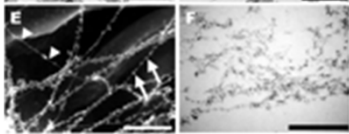
PNN
Activés : PMA

PNN
quiescent



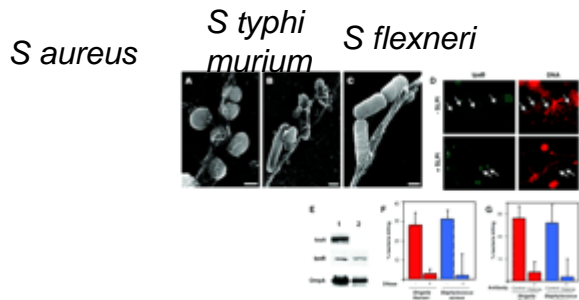
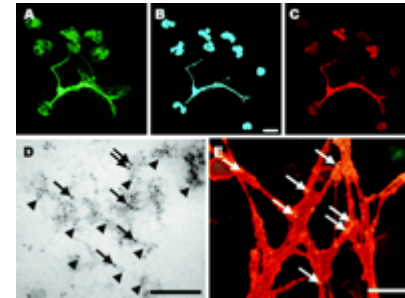
PNN
Activés : IL-8

SEM :
fibres

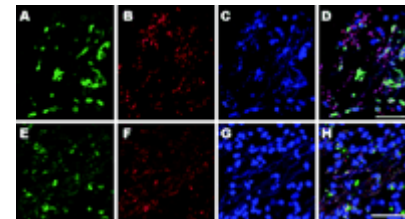


NET : pas de
membrane

élastase DNA nucléosome



➤ : élastase élastase
→ : histones



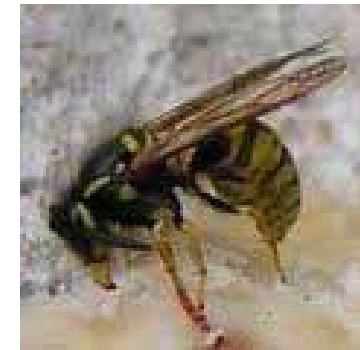
Shigellose
lapin

Appendicite
humaine

↓Bactéricidie par :
-DNase
-Anti-histone

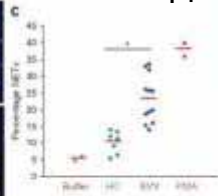
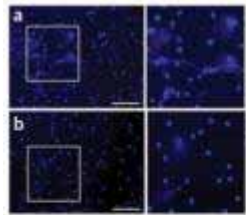
Brinkmann et al, Science, 2004 : 303 :1532-5 :
NET neutrophil extra-cellular traps

- PNN si infection :
 - kamikaze ou guêpe (dard)
 - Meurt en jetant ses filets de chromatine
 - Décorés de substances bactéricides en conformation native
 - NET :
 - burst dépendant
 - Inflammation dépendant ($\text{TNF}\alpha$, $\text{IFN}\gamma$)



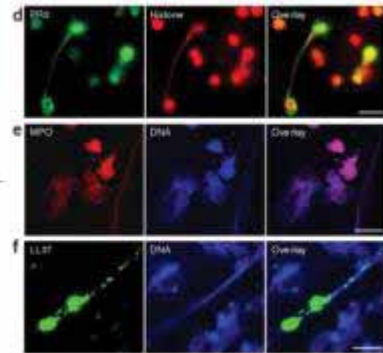
Kessenbrock et al , Nat Med 2009, 6(15) : 623-5
NET et AAV

TNF α PNN :
a : ANCA IgG
b : IgG témoin



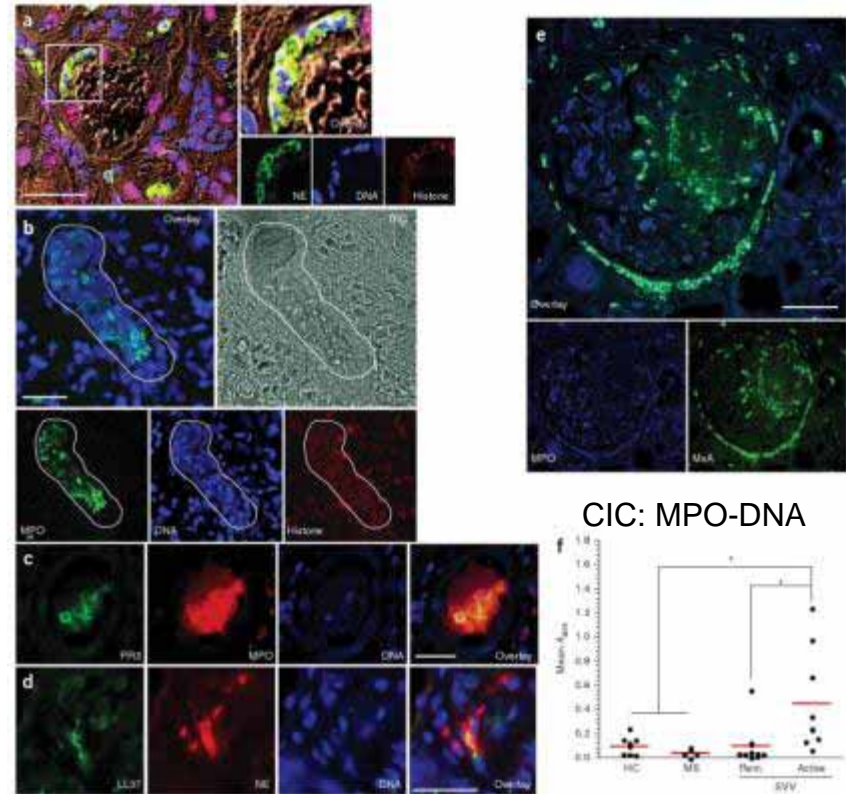
T+

PR3 Hist
MPO DNA Les 2
LL37 DNA



PBR AAV : colocalisation

LL37-DNA \Rightarrow + pDC \Rightarrow \uparrow IFN α \Rightarrow \uparrow MxA

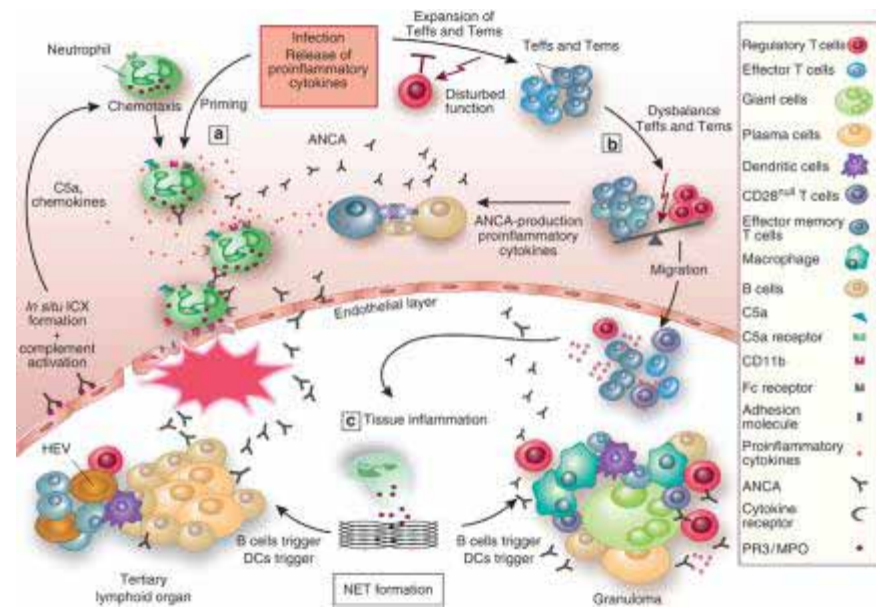


CIC: MPO-DNA

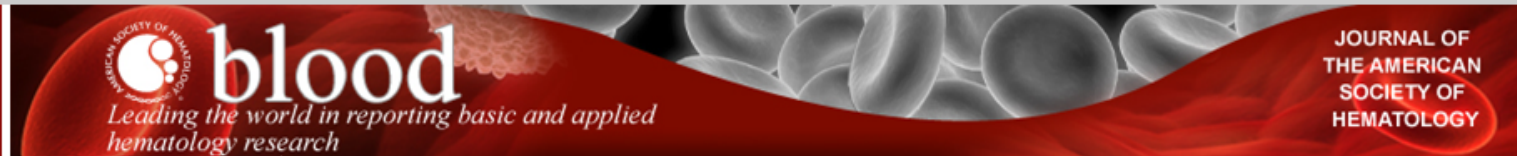
MxA : protéine A de résistance au myxovirus

Kessenbrock et al , Nat Med 2009, 6(15) : 623-5
NET et AAV

- AAV
 - *in vitro* et *in vivo* : ↑ NET
 - Avec PR3 et MPO
 - durant phase active
 - source de complexes chromatine/ANCA-Ag



Kidney Int 2010



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Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity

Sabina Sangaletti^{1,*}, Claudio Tripodo^{2,*}, Claudia Chiodoni¹, Carla Guarnotta², Barbara Cappetti¹, Patrizia Casalini³, Silvia Piconese¹, Mariella Parenza¹, Cristiana Guiducci⁴, Caterina Vitali¹, and Mario P. Colombo¹

Author Affiliations

Abstract

Antineutrophil cytoplasmic antibodies (ANCA) target proteins normally retained within neutrophils, indicating that cell death is involved in the autoimmunity process. Still, ANCA pathogenesis remains obscure. ANCA activate neutrophils inducing their respiratory burst and a peculiar form of cell death, named NETosis, characterized by formation of neutrophil extracellular traps (NETs), decondensed chromatin threads decorated with cytoplasmic proteins endorsed with antimicrobial activity. NETs have been consistently detected in ANCA-associated small-vessel vasculitis, and this association

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This Article

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Blood October 11, 2012
vol. 120 no. 15 3007-3018

Abstract *Free*

» Full Text *Free*

Full Text (PDF) *Free*

Supplemental Methods, Figures, Tables, and Video

All Versions of this Article:

blood-2012-03-416156v1
120/15/3007 *most recent*

Classifications

IMMUNOBIOLOGY
PHAGOCYTES,
GRANULOCYTES, AND
MYELOPOIESIS

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Objectif

- Est-ce que les NETs peuvent servir de transporteurs actifs pour transférer les auto-antigènes des PNN aux DC ?
 - ANCA dirigés contre épitopes conformationnels \Rightarrow nécessité molécules natives pour rupture de tolérance

Méthodes

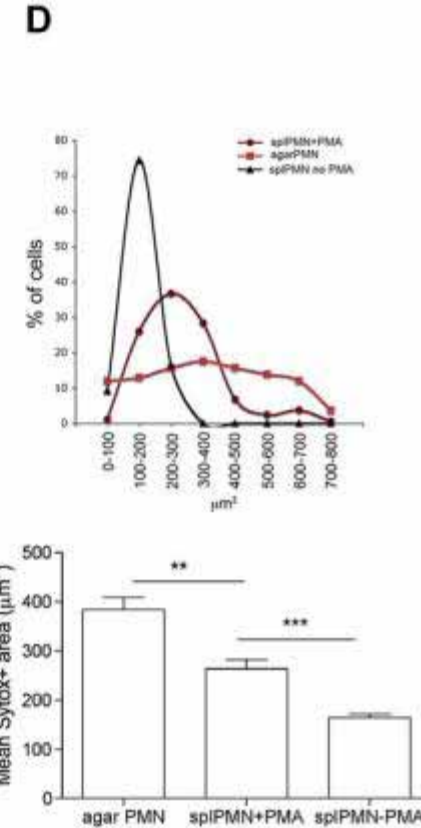
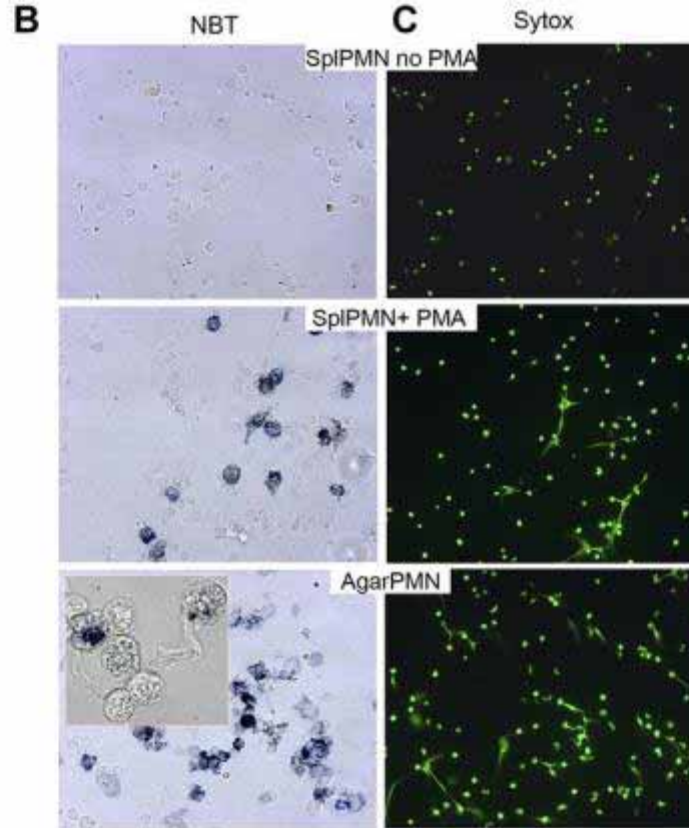
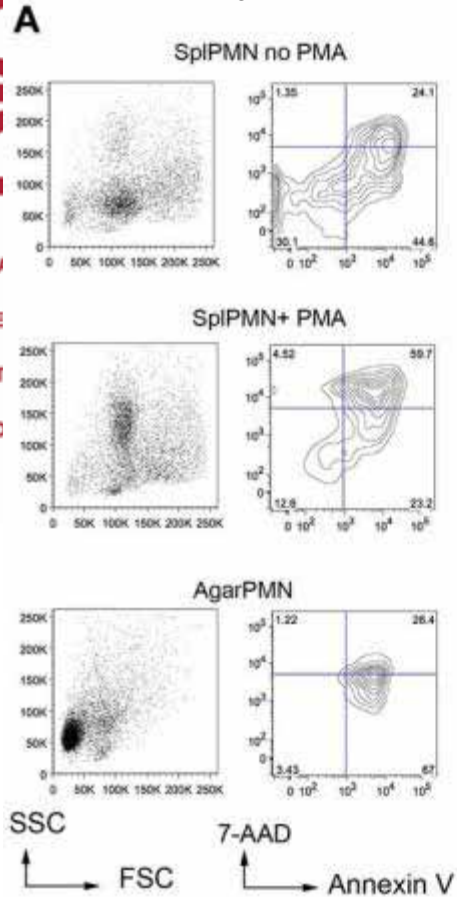
- Détection des NET :
 - ❶ PNN sur Lames de verres poly-D-Lys
 - ❷ Marqueur du DNA :
 - colorant (SYTOX green : que DNA extra-cellulaire)
 - ou Ac anti-Histone
 - ❸ fixation (PFA 2%)
 - ❹ Ac anti-MPO, - PR3, - élastase
- Induction :
 - NETose :
 - PMA (phorbol 12-myristate 13 acétate)
 - Apoptose :
 - Ac anti-Fas/CD95
 - Nécrose :
 - 4 cycles congélation/décongélation

Méthodes

- Co-cultures :
 - mDC + PNN
 - Marquage anti-MPO conjugué (AlexaFlour-488)
 - Lecture confocale
- Immunisation souris :
 - mDC
 - PNN
 - DC + PNN
 - DC + PNN + DNase
 - Mesure :
 - Anti-MPO
 - Anti-PR3
 - histologie

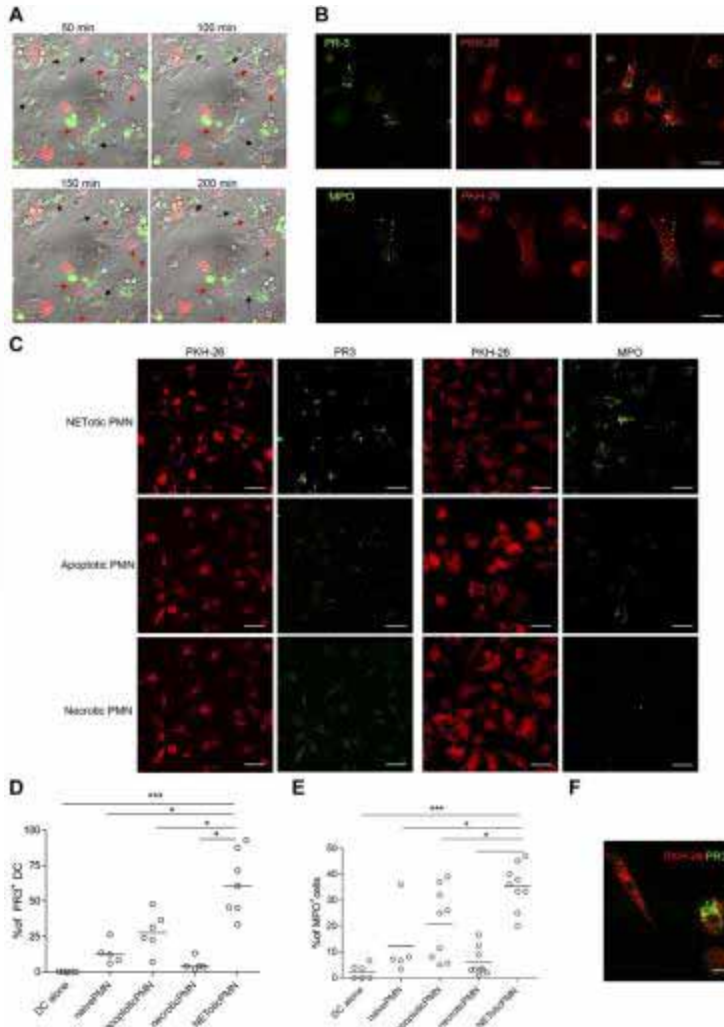
Inflammatory, agar-elicited neutrophils spontaneously die of NETosis ex vivo.

- V+AAD+ : apoptotic PMN
- V+AAD- : early apoptotic PMN
- V-AAD+ : necrotic PMN
- V-AAD- : live PMN



Sangaletti S et al. Blood 2012;120:3007-3018

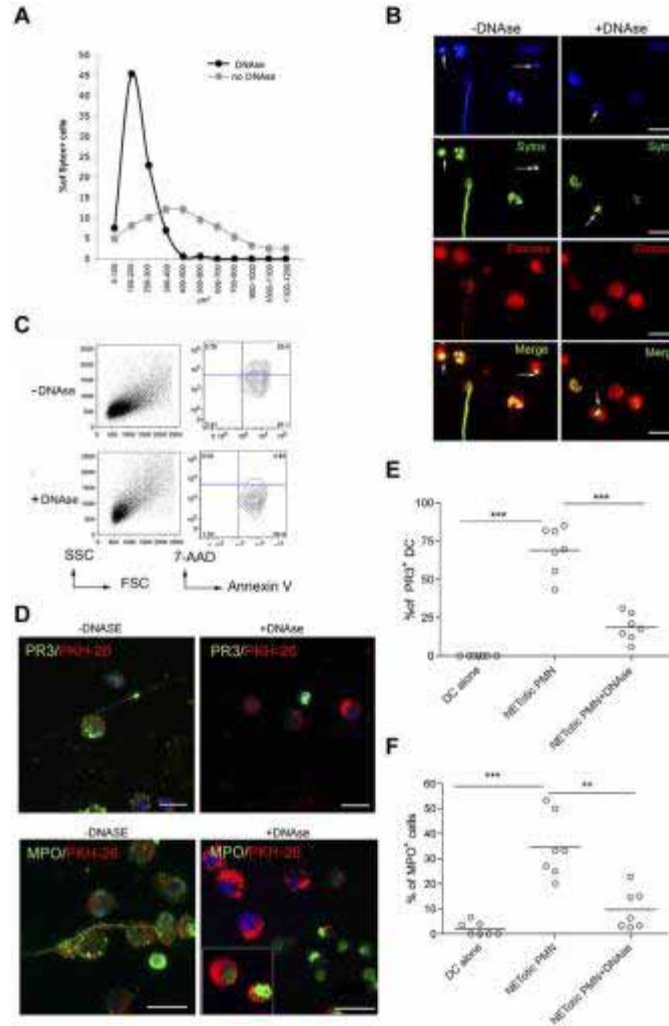
s interact with NETotic PMNs and are loaded with proteins from NET. (A) NETs persistently interact with mDCs.



A : interaction mDC/PMN nétotiques
B : chargement en ANCA Ag des mDC
C : NET > apoptose, et nécrose = 0
D et E : quantification
F : Pr3 dans corps apoptotiques

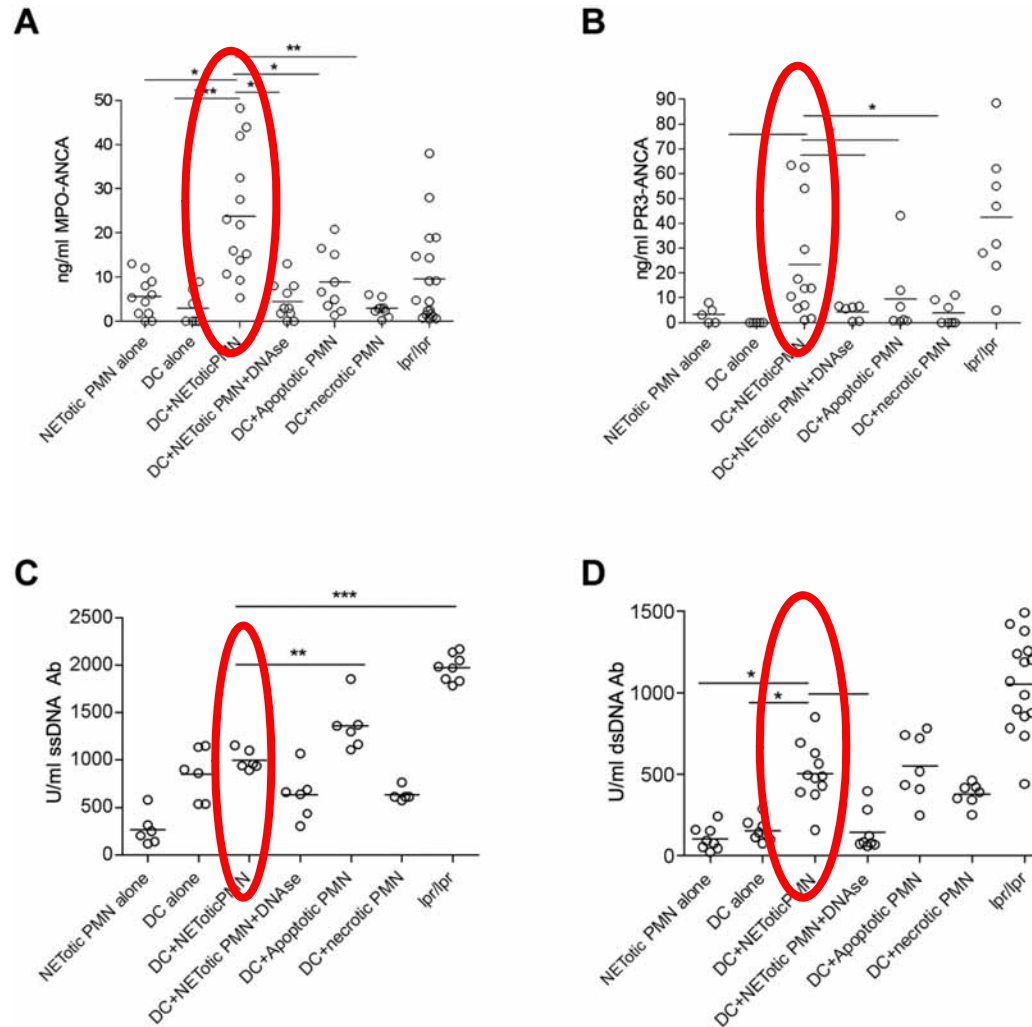
Sangaletti S et al. Blood 2012;120:3007-3018

reatment of mDC-PMN coculture abrogates DC uploading with neutrophil proteins.



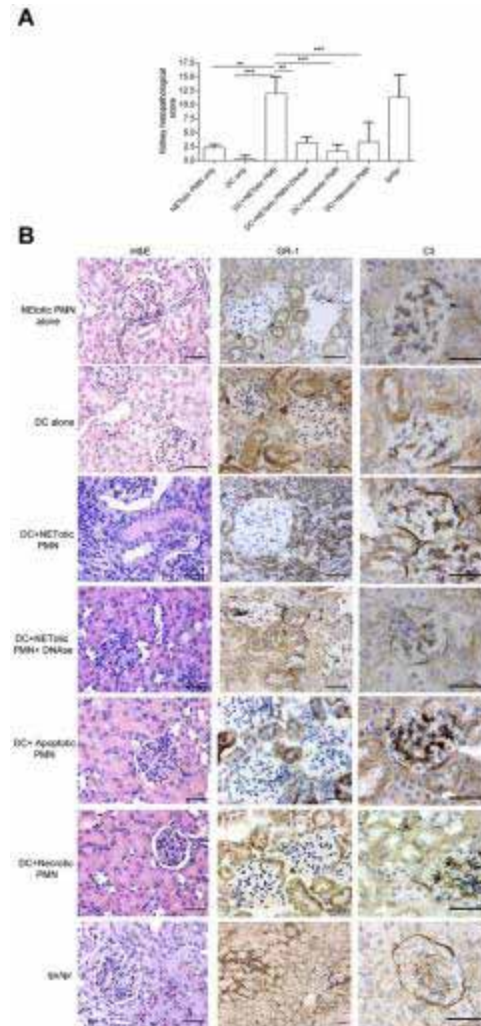
Sangaletti S et al. Blood 2012;120:3007-3018

In vivo injection of NET-loaded mDCs induces autoantibody production.



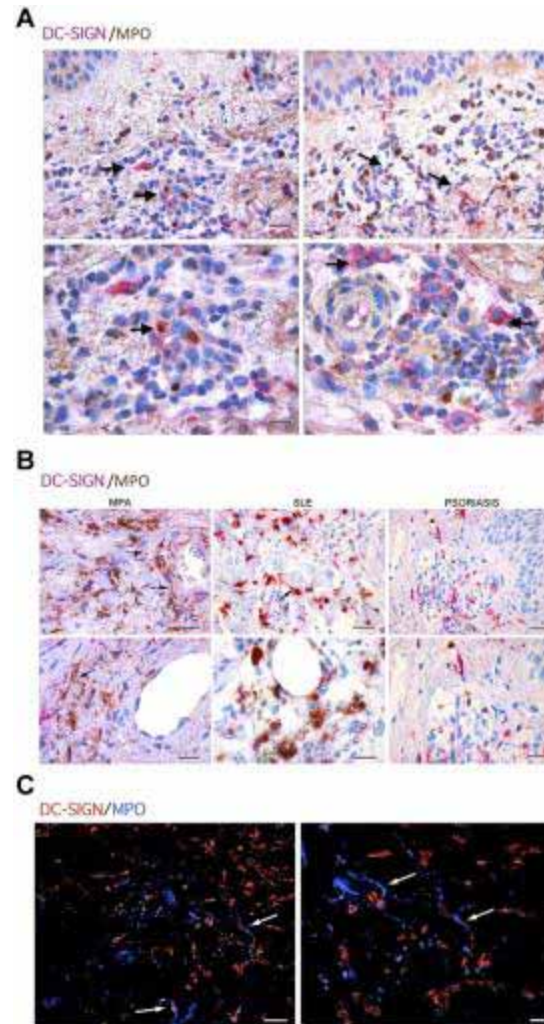
Sangaletti S et al. Blood 2012;120:3007-3018

NET-loaded mDCs injected in mice induce renal damage.



Sangaletti S et al. Blood 2012;120:3007-3018

eutrophil interaction in skin biopsies from patients with MPO-ANCA-associated microscopic polyangiitis.



Sangaletti S et al. Blood 2012;120:3007-3018

conclusion

- Transfert ANCA Ag :
 - PNN nérotiques \Rightarrow mDC
- mDC chargées \Rightarrow \uparrow ANCA et lésions
 - \neq
 - ANCA apoptotiques : non pathogènes
 - ANCA nérotiques : pathogènes

Abnormal Conformation and Impaired Degradation of Propylthiouracil-Induced Neutrophil Extracellular Traps

Implications of Disordered Neutrophil Extracellular Traps in a Rat Model of Myeloperoxidase Antineutrophil Cytoplasmic Antibody–Associated Vasculitis

Daigo Nakazawa,¹ Utano Tomaru,¹ Akira Suzuki,² Sakiko Masuda,¹ Risa Hasegawa,¹
Toshiaki Kobayashi,¹ Saori Nishio,¹ Masanori Kasahara,¹ and Akihiro Ishizu¹

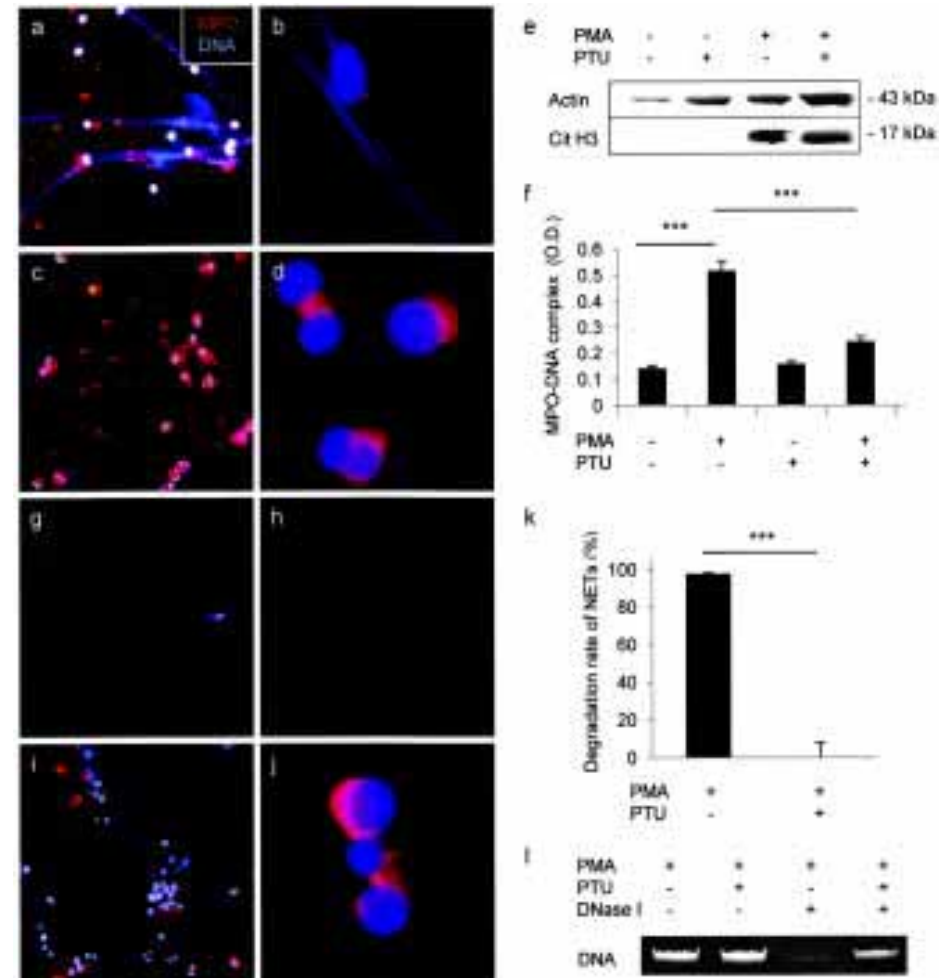
Objective. Neutrophil extracellular traps (NETs) are composed of DNA and antimicrobial proteins, including myeloperoxidase (MPO). Recent studies have demonstrated that impaired regulation of NETs could trigger an autoimmune response. Propylthiouracil (PTU), an antithyroid drug, is associated with a risk of MPO antineutrophil cytoplasmic antibody (ANCA) production and MPO ANCA–associated vasculitis (MPO AAV). This study was undertaken to clarify the mechanism of MPO ANCA production, using the PTU-induced model of MPO AAV.

which had been induced by PMA with PTU using rat neutrophils, produced MPO ANCA and developed pulmonary capillaritis. When rats were given oral PTU with intraperitoneal injection of PMA, pauci-immune glomerulonephritis and pulmonary capillaritis occurred with MPO ANCA production in the serum.

Conclusion. Our findings indicate that abnormal conformation and impaired degradation of NETs induced by PTU are involved in the pathogenesis of PTU-induced MPO ANCA production and MPO AAV. These findings suggest that disordered NETs can be

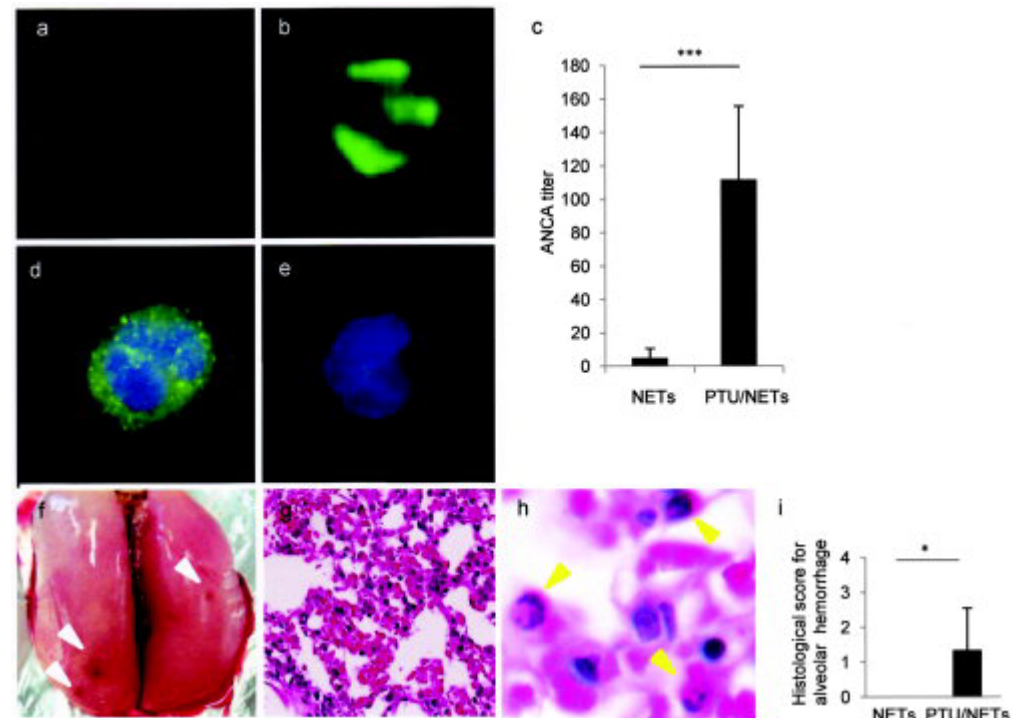
Objectif : influence de PTU sur formation des NETs

- PTU :
 - 30 % MPO-ANCA, parfois AAV
 - Dans PNN : MPO transforme PTU
- Induction NET par PMA :
 - PTU :
 - $\Rightarrow \downarrow$ NET et résistance à DNase
 - a : PMA
 - b : PMA + PTU
 - c : PMA + DNase
 - d : PMA + PTU + DNase
 - $\Rightarrow \downarrow$ complexes DNA-MPO



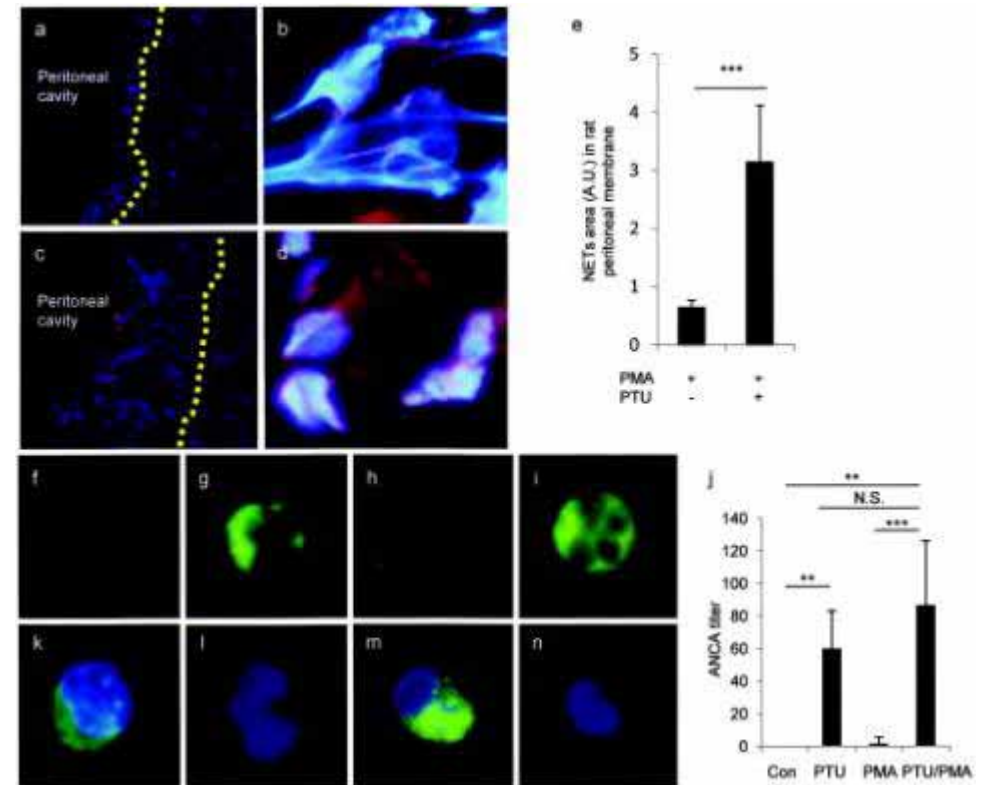
rats WKY immunisés par NETs

- Immunisation :
 - NETs PMA :
 - a : ANCA IFI
 - Nets PMA+PTU
 - b : pANCA IFI
 - d et e : MPO-ANCA
 - d : NRS
 - e : anti-MPO
 - HIA (f, g, h et i) : 4 rats/6
 - avec PNN



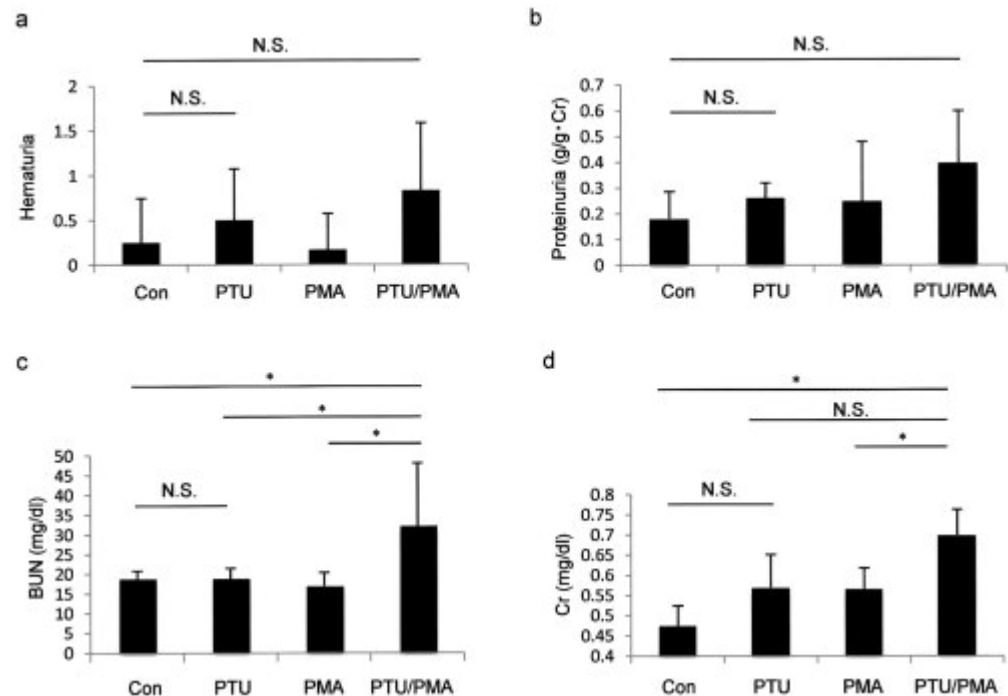
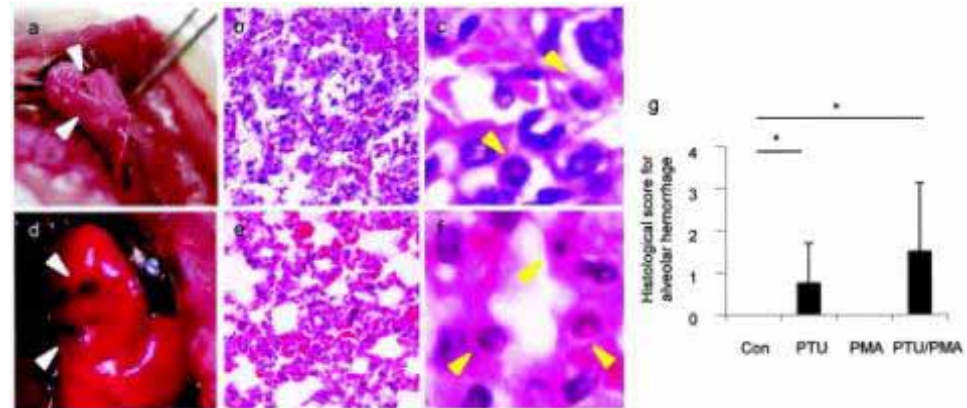
Modèle de MPO AAV chez rat WKY

- 4 groupes :
 - 1 : Contrôle (f)
 - 2 : PTU oral (g)
 - 3 : PMA IP (h)
 - 4 : PTU oral + PMA IP (i)
- ⇒ ↓ dégradation des NETs des PNN péritonéaux du groupe 4
- ⇒ ANCA groupe 4



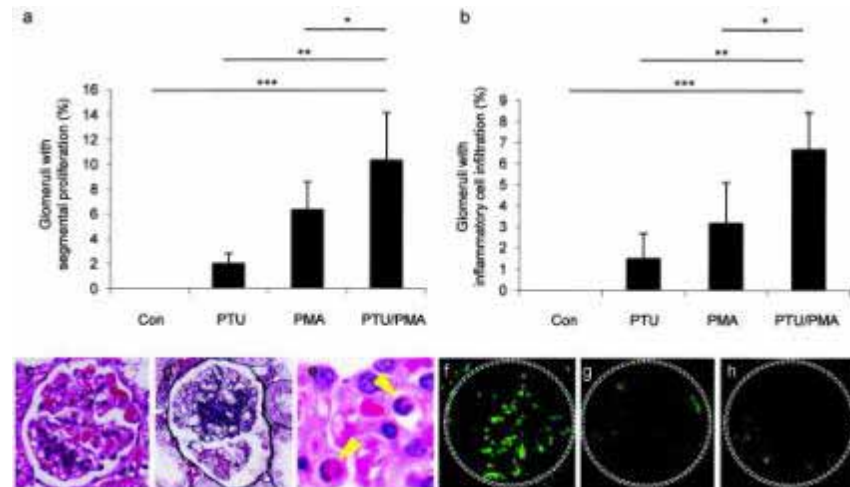
Modèle de MPO AAV chez rat WKY

- 4 groupes :
 - 1 : Contrôle
 - 2 : PTU oral
 - 3 : PMA IP
 - 4 : PTU oral + PMA IP
- ⇨ HIA :
 - 1 : 0/4
 - 2 : 2/4
 - 3 : 0/6
 - 4 : 6/6
- Fonction rénale : pas de différence significative
 - Tendence : ↑ groupe 4



PBR

- 4 groupes :
 - 1 : Contrôle
 - 2 : PTU oral
 - 3 : PMA IP
 - 4 : PTU oral + PMA IP
- ⇨ : ↑ significative
 - 4 : croissants
 - 4 : infiltrats inflammatoires

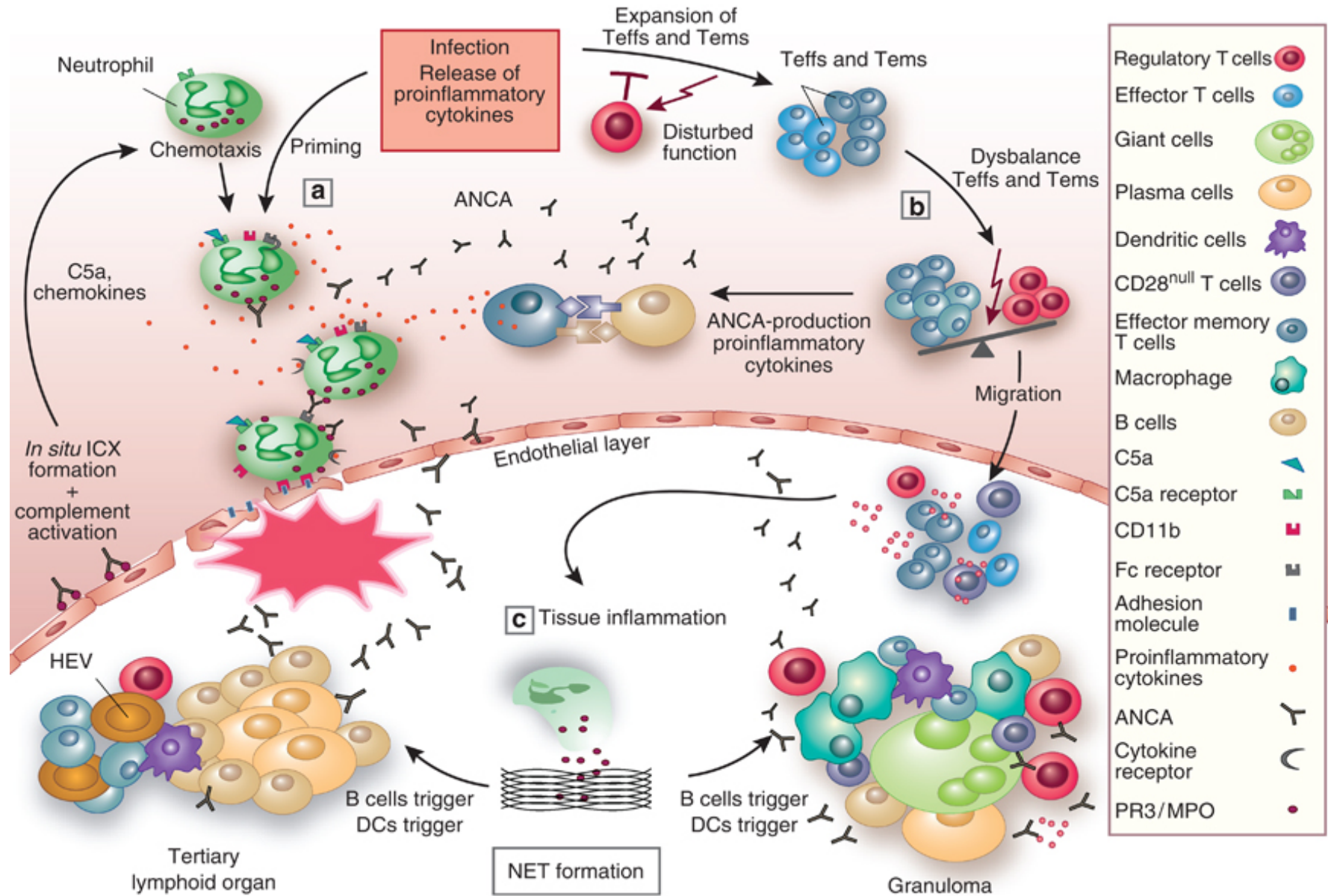


discussion

- PTU :
 - bloque TPO (iodination de T4)
 - Homologie TPO/LPO/MPO
- \Rightarrow : \uparrow $\frac{1}{2}$ vie des NETS
 - en modifiant réponse à DNase
 - \uparrow MPO-ANCA et HIA
- Mécanisme général rupture tolérance :
 - Complexe LL37-DNA \Rightarrow pDC et psoriasis

questions

- MPO-ANCA :
 - Capable de transfert?
- MPO : cible de PTU?
 - Altération des NETs : par inhibition de activité MPO?
- complexes MPO-PTU : \Rightarrow MPO-ANCA ?
- Autres AAV iatrogènes : implication NETose?



11th Dresden Symposium on Autoantibodies

September 1-4, 2013

Infection, Tumors and Autoimmunity



Chairman:

Karsten Conrad (Dresden, Germany)

Co-Chairman:

Edward K. Chen (Gaithersburg, USA)

Sunday, 01.09.2013

12:00 - 22:00	Registration
14:00 - 14:30	Welcome & Introduction
14:30 - 16:00	Pathogenesis of autoimmune diseases – Novel aspects A new synthesis for antibody-mediated immunity Arturo Casadevall (New York; USA) Neutrophil extracellular traps – Role in infection and autoimmunity Arturo Zychlinsky (Berlin, Germany)
16:00 - 16:30	Coffee break
16:30 - 18:40	Autoimmunity in infections and tumors The ASIA syndrome: Adjuvants in infections, silicone and vaccines; autoimmunity and cancer <i>Yehuda Shoenfeld (Tel Hashomer, Israel)</i> Review Cancer and Autoimmunity <i>N.N.</i>
19:00 - 22:00	Welcome Reception

Monday, 02.09.2013

09:00 - 10:30	<p>NETosis, infection and autoimmunity</p> <p>NETs: the missing link between cell death and systemic autoimmune diseases? <i>Erika Darrah, Felipe Andrade (Baltimore, USA)</i></p> <p>NETs and induction of ANCA <i>Elena Csernok (Lübeck, Germany)</i></p>
10:30 - 11:00	Coffee break
11:00 - 12:30	<p>Autoimmunity in infections and tumors</p> <p>Vitamin D in infection, autoimmunity and cancer <i>Yehuda Shoenfeld (Tel Hashomer, Israel)</i></p> <p>Target of autoantibody in HCV patients during interferon/ribavirin therapy <i>Ed Chan (Gainesville, USA)</i></p> <p>Novel vaccine against autoimmunity - harnessing parasitic infection for autoimmunity and cancer <i>Miri Blank (Tel Hashomer, Israel)</i></p>
12:30 - 14:00	<p>Lunch break</p> <p>Industrial Exhibition and Poster Viewing</p> <p>EASI Lunch Meeting (EASI members only)</p>
14:00 - 15:30	<p>Clinical relevance of tumor associated autoantibodies</p> <p>Autoantibody signatures: progress and perspectives for early cancer detection <i>Jerome Solassol (Montpellier, France)</i></p>



12:20 am - 12:30 am **A10** - High genetic diversity in nasal *Staphylococcus aureus* isolates from granulomatosis with polyangiitis (GPA) patients.
M.M. Van Timmeren, C. Glasner, T. Stoberneck, T.F. Omansen, E.C. Raangs, J.W. Rossen, J.P. Arends, G.A. Kampinga, W.J.B. Van Wamel, A. Rutgers, C.A. Stegeman, C.G.M. Kallenberg, P. Heeringa, J.M. Van Dijk (Groningen, Rotterdam, The Netherlands)

12:45 am - 2:15 pm **ROCHE SATELLITE SYMPOSIUM:
RITUXIMAB IN VASCULITIS**
Amphitheatre Farabeuf
Moderator: Kirsten de Groot (Offenbach, Germany)

S1 - Rituximab for ANCA-Associated Vasculitis: the Experience in the United States.
Jeremy M. Clain, Ulrich Specks, (Rochester, USA)

S2 - Rituximab for ANCA-associated vasculitis: the UK experience.
David Jayne (Cambridge, UK)

S3 - Rituximab for ANCA-associated vasculitides: the French experience.
Loïc Guillevin (Paris, France)

Conclusions : *Kirsten de Groot (Offenbach, Germany)*

2:30 pm - 4:30 pm **PLENARY SESSION 2: INNATE AND ADAPTATIVE IMMUNITY IN THE RESOLUTION OF INFLAMMATION**
Amphitheatre Farabeuf
Moderators: Coen Stegeman (Groningen, The Netherlands), Sylvain Perruche (Besançon, France)

2:30 pm - 3:00 pm **L13** - Apoptosis, apoptotic cell clearance and resolution of inflammation.
Adriano G. Rossi (Edinburgh, UK)

3:00 pm - 3:30 pm **L14** - Immunomodulatory properties of apoptotic cells.
Sylvain Perruche (Besançon, France)

3:30 pm - 3:45 pm **A11** - Neutrophils in ANCA vasculitis - old and apathetic.
T. Hellmark, Å. Pettersson, D. Selga, A. Bengtsson, Å. Johansson (Adelaide, Australia)

3:45 pm - 4:00 pm **A12** - Phagocytosis of apoptotic cells expressing PR3 impaired macrophage anti-inflammatory reprogramming.
A. Millet, J. Mocek, M. Pederzoli-Ribeil, F. Cormier, V. Baud, V. Witko-Sarsat (Paris, France)

4:00 pm - 4:15 pm **A13** - ANCA disease patients have defective Treg function exacerbated by expansion of a suppression-resistant effector population.
M. Free, D. Bunch, J.A. Mcgregor, C. Poulton, B. Jones, E. Berg, S. Hogan, Y. Hu, G. Preston, C. Jennette, R. Falk, M. Su (Chapel Hill, USA)

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