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## **Vascular Inflammation: Blood Vessels under Attack** **November 13th, 2015 - Pasteur Institute Paris**

**(François Jacob auditorium, 125 places)**



**Session 1 – Immunology, inflammation and cardiovascular disease**  
**Ziad Mallat (University of Cambridge, Cambridge, United Kingdom):**  
***Innate and adaptive immune response in atherosclerosis***

**Session 2 – Pathogenesis and resolution of atherosclerosis**  
**Akos Heinemann (Graz University, Graz, Austria):**  
***Prostaglandins as regulators of leukocyte trafficking***

**Session 3 – Phagocytes in vascular inflammation (vasculitis)**  
**Barbara Walzog (Ludwig-Maximilians-Universität, Munich, Germany):**  
***Integrin signalling and neutrophil trafficking in innate immunity***

**Session 4 – Inflammation and vascular remodeling (aneurysm)**  
**Jean-Baptiste Michel (Paris, INSERM U1148, France):**  
***From atherothrombosis to adaptive immunity***  
***the example of aneurysm***



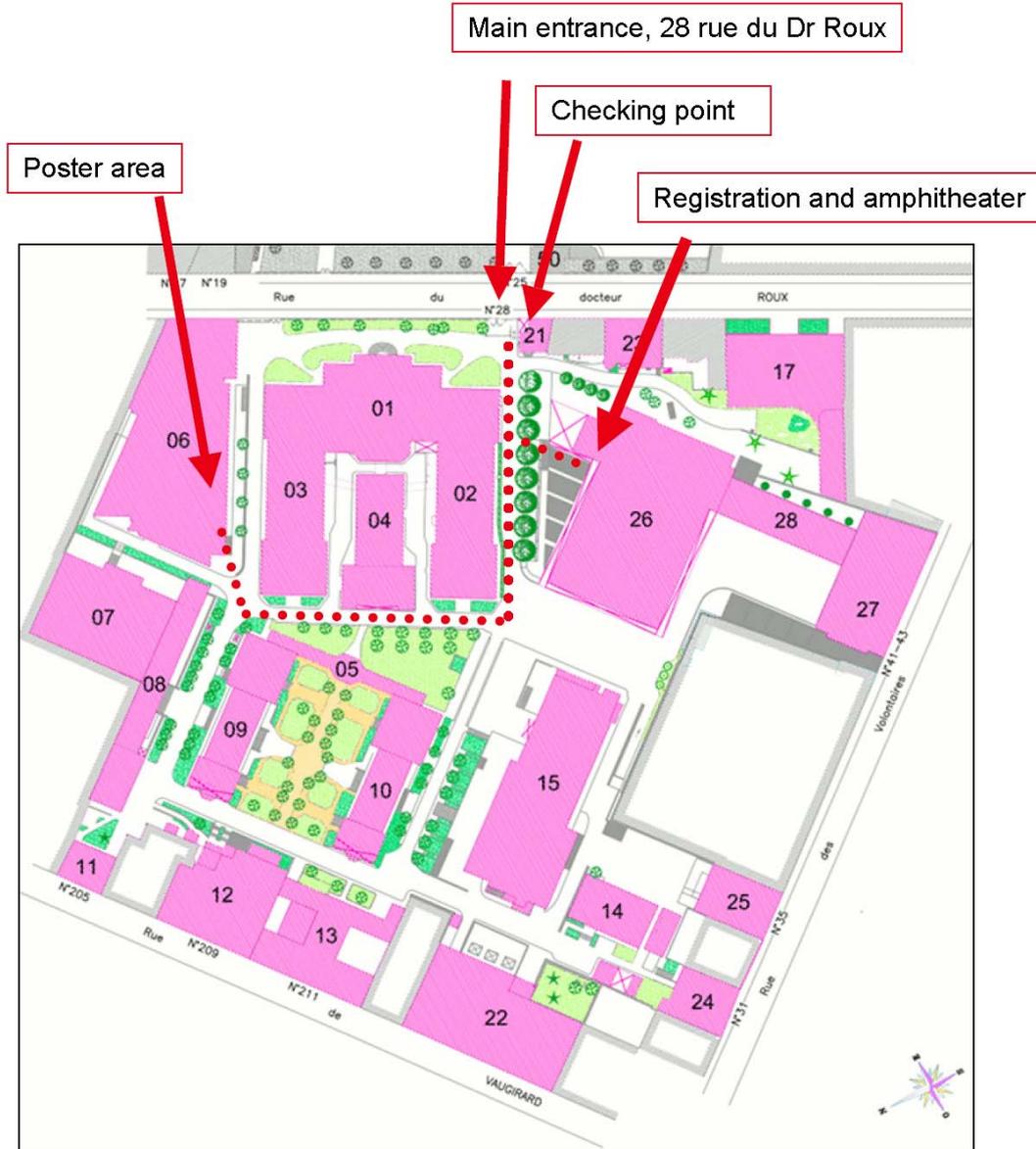
**Organizing committee: Xavier Norel, Jamel El-Benna, Véronique Witko-Sarsat,  
Florence Niedergang, Vincent Lagente, Emmanuel Letavernier, Michel Chignard**

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# MAP



# ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

November 13th, 2015 - Pasteur Institute, Amphitheatre François Jacob, Paris

Organizing committee: Xavier Norel, Jamel El-Benna, Véronique Witko-Sarsat, Florence Niedergang, Vincent Lagente, Emmanuel Letavernier, Michel Chignard

**8:30-9:00**     *Welcome of the participants*

**9:00-9:15**     **Message by Jamel El-Benna**

## **SESSION 1- Immunology, inflammation and cardiovascular disease**

*Chair: Barbara Walzog and Jamel El Benna*

**9:15-9:45**     *Innate and adaptive immune response in atherosclerosis*  
Ziad MALLAT (University of Cambridge, Cambridge, United Kingdom)

**9:45-10:00**     *The autoantigen proteinase 3 acts as a danger signal in autoimmune vasculitis*  
Véronique WITKO SARSAT\* (INSERM U1016, Institut Cochin, Paris, France)

**10:00-10:15**     *Microvascular endothelium alters the inflammatory profile of allogeneic CD4<sup>+</sup>-T lymphocytes after activation by HLA class II alloantibody binding*  
Nuala MOONEY\* (INSERM UMRs1160, Hôpital Saint-Louis, Paris, France)

**10:15-10:30**     *Human platelets differentially sense various Staphylococcus aureus exotoxins and adapt their subsequent immuno-modulatory molecule secretion profiles*  
Hind HAMZEH-COGNASSE\* (Faculté Médecine, GIMAP EA3064, St Priest-en-Jarez, France)

**10:30-11:15**     *Coffee break & Posters exhibition*

## **SESSION 2- Pathogenesis and resolution of atherosclerosis**

*Chair: Xavier Norel and Akos Heinemann*

**11:15-11:45**     *Prostaglandins as regulators of leukocyte trafficking*  
Akos HEINEMANN (Graz University, Graz, Austria)

**11:45-12:00**     *Inhibition of mPGES1 in human vessel, argument for cardiovascular safe alternative to Coxib*  
Gülsev ÖZEN\* (INSERM U1148, CHU X. Bichat, Paris, France)

**12:00-12:15**     *Heme oxygenase-1 –dependent anti-inflammatory effects of atorvastatin in zymosan-injected subcutaneous air pouch in C57BL/6 mice*  
Ghewa A. EL-ACHKAR\* (Faculty of Medicine, American University of Beirut, Lebanon)

**12:15-12:30**     *Influence of cholesterol on ROS production by NADPH oxidase, a cell free study*  
Rawand MASOUD\* (UMR 8000, Université Paris-Sud, Orsay, France)

**12:30-2:30**     *Lunch & Posters exhibition*

### SESSION 3- Phagocytes in vascular inflammation (vasculitis)

*Chair: Véronique Witko-Sarsat and Ziad Mallat*

**2:30-3:00** *Integrin signalling and neutrophil trafficking in innate immunity*

Barbara WALZOG (Ludwig-Maximilians-Universität, Munich, Germany)

**3:00-3:15** *F-actin-rich endothelial pores prevent vascular leakage during leukocyte diapedesis through local RhoA signaling in vivo*

Jaap D. VAN BUUL\* (University of Amsterdam, the Netherlands)

**3:15-3:30** *Proteinase 3 affects the production and function of microparticles: implications for inflammation and autoimmune vasculitis*

Katherine MARTIN\* (INSERM U1016, Institut Cochin, Paris, France)

**3:30-3:45** *Polymorphonuclear neutrophils, recruited by LTB4 into plaques, increased plaque proteolytic activity*

Marie-Anne MAWHIN\* (INSERM U1148, CHU X. Bichat, Paris, France)

**3:45-4:15** *Coffee break*

### SESSION 4- Inflammation and vascular remodeling (aneurysm)

*Chair: Emanuel Letavernier and Michel Chignard*

**4:15-4:45** *From atherothrombosis to adaptative immunity the example of aneurysm*

Jean-Baptiste MICHEL (INSERM U1148, CHU X. Bichat, Paris, France)

**4:45-5:00** *Endothelial hypoxia-inducible factor 2 $\alpha$  (HIF2 $\alpha$ ) mediates endothelial dysfunction and glomerular lesions in a hypertensive model*

Yosu LUQUE\* (INSERM UMRs1155, Hôpital Tenon, Paris, France)

**5:00-5:15** *Novel brain antioxidative activity in brain microvasculature*

Mikhail Y. GOLOVKO\* (University of North Dakota, Grand Forks, USA)

**5:15-5:30** *Endothelial to mesenchymal transition reflects the damage induced by microvascular inflammation in human renal grafts*

Alexandre HERTIG\* (INSERM UMRs1155, Hôpital Tenon, Paris, France)

\* indicates selected speaker from the abstracts



# **MEETING GREMI 2015**

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre Francois JACOB, Paris**

**ORAL PRESENTATIONS**

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

***The autoantigen proteinase 3 acts as a danger signal in autoimmune vasculitis***

**Véronique WITKO SARSAT**

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Granulomatosis with polyangiitis (GPA) is a necrotizing vasculitis associated with granulomatous inflammation and the presence of anti-neutrophil cytoplasmic antibodies (ANCA) directed against proteinase 3 (PR3). using a combination of in vitro and in vivo approach, we established that apoptotic cells expressing membrane PR3 altered the normal anti-inflammatory reprogramming of macrophages during efferocytosis and instead resulted in a pro-inflammatory response via the IL-1R1 and MyD88-dependent signaling pathway. This led to increased secretion of cytokines and chemokines, which can facilitate the recruitment of more PR3-expressing cells. The PR3-induced microenvironment favored interactions between naïve CD4+ T cells and pDCs in close proximity to macrophages and apoptotic neutrophils within granulomatous lesions of GPA patients. This was reproducible in a murine model with a skewed Th2/Th9 cell distribution with limited production of regulatory T cells and no Th17 cells. Addition of anti-PR3 ANCA induced Th17 cells, revealing a new mechanism of immune polarization specific to GPA. Accordingly, a Th9/Th2/Th17 skewed distribution of circulating CD4+ T cells was observed in GPA. Increased secretion of G-CSF, a cytokine known to stimulate myeloid cell production was also found. The ability of PR3 to disrupt immune silencing associated with clearance of apoptotic neutrophils constitutes a novel insight into the pathophysiology in GPA.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre Francois JACOB, Paris**

***Microvascular endothelium alters the inflammatory profile of allogeneic CD4<sup>+</sup>-T lymphocytes after activation by HLA class II alloantibody binding***

**J. Lion, C. Taflin, A. Cross, A. Haziot, D. Glotz, N. MOONEY**

Inserm, UMRs 1160, 75010 Paris, France ; LabEx Transplantex. AP-HP, Hôpital Saint-Louis, Service de Néphrologie et Transplantation, Laboratoire de Histocompatibilité, Paris, F-75010 France. Université Paris Diderot, Sorbonne Paris Cité, F-75013 France

Following renal transplantation, the microvascular endothelium is the first site of interaction between recipient peripheral blood mononuclear cells and the allogeneic donor graft. Alloreactivity between the recipient and the donor can lead to allograft damage resulting in transplant glomerulopathy characterized by duplication of the glomerular basement membrane targeting the renal endothelial capillary network.

Patients who have high levels of circulating alloantibodies directed against HLA class II antigens have an increased incidence of transplant glomerulopathy and this led to the suggestion that HLA class II antibody binding to the microvascular endothelium damages the allograft and results in capillary obliteration and interstitial fibrosis.

The allograft endothelium expresses HLA class II molecules in the steady-state and expression is highly increased under inflammatory conditions. We have demonstrated that endothelial cell expression of HLA class II allows activation and polarization of allogeneic CD4<sup>+</sup> T cells towards either pro-inflammatory (IL-17<sup>+</sup>) or functionally suppressive regulatory (FoxP3<sup>bright</sup>) subsets (Taflin et al PNAS 2011).

In view of the association between HLA class II alloantibodies and endothelial damage, we examined the outcome of HLA-DR antibody binding to endothelial cells on CD4<sup>+</sup>-T cell responses. HLA-DR antibody binding to endothelial cells activated phosphorylation of Erk, MEK and Akt. Pre-activation of endothelial cells with HLA-DR Ab or the F(ab'<sup>2</sup>) fraction before co-culture with allogeneic PBMC led to increased production of IL-6 by the endothelial cells and the use of selective Akt inhibitors revealed that IL-6 production was Akt-dependent. We next determined the outcome of HLA-DR antibody binding to endothelial cells on subsequent polarization of allogeneic CD4<sup>+</sup>-T. Pre-activation of endothelial cells with anti-HLA-DR antibody increased expansion of the Th17 subset and decreased amplification of the Treg population. The increased Th17 expansion was dependent on IL-6 produced by endothelial cells and these data were reproduced by activating endothelial cells with serum from an allosensitized patient containing relevant HLA-DR antibodies.

Renal transplant rejection is associated with increased intragraft pro-inflammatory Th17. These data reveal a novel mechanism of microvascular damage, activated by HLA-DR antibody binding to endothelial cells, and dependent on enhanced expansion of Th17 cells mediated by IL-6 and lessened amplification of Treg cells.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

***Human platelets differentially sense various Staphylococcus aureus exotoxins and adapt their subsequent immuno-modulatory molecule secretion profiles***

**Pauline Damien<sup>1</sup>, Adrien Chabert<sup>1</sup>, Fabrice Cognasse<sup>1,2</sup>, Cédric Badiou<sup>3</sup>, Fabrice Zeni<sup>4</sup>, Marie Ange Eyraud<sup>2</sup>, Charles Antoine Arthaud<sup>5</sup>, Bruno Pozzetto<sup>1,5</sup>, Olivier Dauwalder<sup>3,6</sup>, Francois Vandenesch<sup>3,6</sup>, Olivier Garraud<sup>1,7</sup>, Gérard Lina<sup>3,6</sup>, Hind HAMZEH-COGNASSE<sup>1</sup>**

<sup>1</sup>Université de Lyon, GIMAP-EA3064, F42023, Saint Etienne, France ; <sup>2</sup>EFS Auvergne-Loire, F42000, Saint-Etienne, France ; <sup>3</sup>CIRI, International Center for Infectiology Research, LabEx Ecofect, Université Lyon1, Inserm U1111, Ecole Normale Supérieure de Lyon, CNRS UMR5308, 69008 Lyon, France ; <sup>4</sup>Service de Réanimation Médicale, CHU de Saint-Etienne, 42270 Saint-Etienne, France ; <sup>5</sup>Laboratoire de Bactériologie-Virologie- Hygiène, Centre Hospitalo-Universitaire de Saint-Étienne, 42270 Saint-Etienne, France ; <sup>6</sup>Centre National de Référence des Staphylocoques, Hospices Civils de Lyon, 69500 Bron, France. <sup>7</sup>Institut National de la Transfusion Sanguine, 75739 Paris, France

### **Introduction**

Sepsis is accompanied by several biological clinical and biological features that evoke acute generalized inflammation. Platelets are involved in the pathophysiology of sepsis, as shown by the frequent occurrence of thrombocytopenia in this pathology, but the mechanisms of platelet depletion are not completely understood. Platelets react to bacterial components and secrete copious amounts of proinflammatory molecules. Staphylococcus aureus is one of the most frequent bacterial causes of sepsis in Europe. S. aureus and its components can bind to, and activate, platelets.

### **Methods**

To investigate the effects of S. aureus exotoxins on the capacity of platelets to release inflammatory cytokines/chemokines, platelets were exposed to staphylococcal exotoxins  $\alpha$ - and  $\gamma$ -hemolysin, Panton-Valentine leukocidin and toxic shock syndrome toxin-1. Platelet activation, aggregation and the release of soluble immunomodulatory factors, such as soluble (s)CD62P; soluble CD40-ligand (sCD40L); regulated on activation, normal T cell expressed and secreted (RANTES, CCL5) and stromal cell-derived factor-1alpha (SDF-1alpha) were assessed. Statistical significance of data was analyzed using a two-way ANOVA with repeated measures and a Bonferroni post-hoc test.

### **Results**

No phenotypic platelet activation or aggregation was observed upon toxin stimulation regardless of the toxin. Profiles of secreted immunomodulators appeared to be dependent on the staphylococcal exotoxin that was used for stimulation, with differential release of sCD62P, sCD40L and RANTES by platelets but not of SDF-1alpha. Platelet stimulation with combinations of exotoxins used at suboptimal concentrations resulted in variable release of sCD40L and RANTES compared with each exotoxin alone at the suboptimal concentration. In addition, some exotoxin combinations inhibited the release of RANTES by platelets in response to prolonged exposure.

### **Conclusions**

The results suggest that platelets differentiate between staphylococcal toxins and adapt their inflammatory response to perceived specific “danger” signals. This is of particular importance in the context the inflammatory phase of sepsis.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

***Inhibition of mPGES1 in human vessel, argument for cardiovascular safe alternative to Coxib***

**Gülsev ÖZEN<sup>(1,2)</sup>, Ingrid Gomez<sup>(3)</sup>, Armond Daci<sup>(1)</sup>, Lilia Boubaya<sup>(2)</sup>, Önder Teskin<sup>(4)</sup>,  
B. Sönmez Uydeş-Dogan<sup>(1)</sup>, Gökçe Topal<sup>(1)</sup> and Xavier Norel<sup>(2)</sup>**

<sup>1</sup>Istanbul University, Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkey; <sup>2</sup>INSERM U1148, CHU X. Bichat, 75018 Paris, France; <sup>3</sup>Cardiovascular Research Unit, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, S10 2JF, UK; <sup>4</sup>Gaziosmanpasa Avrupa Safak Hospital, Istanbul, Turkey

**Introduction :** Inhibition of prostaglandin (PG) synthesis by COX-2 (cyclooxygenase-2) inhibitors such as COXIBs is effective in reducing inflammation but their cardiovascular side effects limit their use. Microsomal prostaglandin E synthase-1 (mPGES-1) is a terminal PGE<sub>2</sub> synthase in the COX pathway. Recently mPGES-1 inhibitors have been suggested as a potential novel therapy, an alternative to COX-2 inhibitors. The aim of our study was to compare the effects of mPGES-1 inhibitor and COX-2 inhibitor on vascular tone under normal or inflammatory conditions in human internal mammary arteries (IMA) and saphenous veins (SV).

**Methods :** Using an organ bath system, a first concentration-response curve induced by norepinephrine (NE) was performed on isolated human vessels (IMA n=15; SV n=29): fresh or cultured (18h) in the presence or absence of inflammatory conditions (interleukin-1beta (IL-1beta) and lipopolysaccharide (LPS)). In addition, a second NE concentration-response curve was established after 30 min pre-incubation with mPGES1 inhibitor (Compound-3 (C3; Leclerc et al., 2013), 10 microM) or COX-2 inhibitor (DuP-697, 1 microM). PGE<sub>2</sub> and PGI<sub>2</sub> release from human vessels were measured in organ bath medium by ELISA.

**Results :** Under inflammatory conditions, the contractile responses induced by NE decreased in human SV or IMA. In these vascular preparations, incubation with C3 significantly attenuated the maximal contractions induced by NE (IMA -27±09%; SV -40±07%). These reductions were at least completely reversed after co-incubation with both C3 and IP (prostacyclin, PGI<sub>2</sub> receptor) antagonist Cay10441 (1 microM, 30 min). A similar effect was also detected in fresh vessels. In contrast, COX-2 inhibitor increased contractile response to NE in IMA (+69±19%) and did not modify SV tone under inflammatory condition. PGE<sub>2</sub> levels were significantly reduced after treatment with COX-2 or mPGES-1 inhibitors in both vessels. While PGI<sub>2</sub> levels were only reduced in presence of COX-2 inhibitor and increased by mPGES-1 inhibitor.

**Conclusion :** In contrary to COX-2 inhibitor, mPGES-1 inhibitor by increasing PGI<sub>2</sub> levels (increased PGH<sub>2</sub> availability for PGI<sub>2</sub> synthases) will reduce vascular tone reactivity and may prevent thrombosis. For these reasons, their use in the treatment of inflammatory diseases should be devoid of deleterious cardiovascular side effect.

# MEETING GREMI 2015

## *VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK*

November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris

*Heme oxygenase-1 –dependent anti-inflammatory effects of atorvastatin in zymosan-injected subcutaneous air pouch in C57BL/6 mice*

**Ghewa A. EL-ACHKAR**<sup>1,2</sup>, May F. Mrad<sup>1</sup>, Eva Hamade<sup>3</sup>, Ayad Jaffa<sup>1</sup>, Roberto Motterlini<sup>2</sup>, Aïda Habib<sup>1,4</sup>

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Statins possess many beneficial pleiotropic properties as they exert anti-inflammatory, antioxidant, anti-proliferative and anti-thrombotic effects. Statins also regulate the expression and activity of heme oxygenase-1 (HO-1), an inducible redox-sensitive cytoprotective protein responsible for the degradation of heme. This study was conducted to investigate the effect of atorvastatin on inflammation in C57BL/6 mice using the subcutaneous air pouch model and analyze the mechanism involved. Inflammation was induced by injection of 1 % zymosan in the pouch. Atorvastatin (5 mg/kg, i.p.) and/or the inhibitor of HO-1 tin protoporphyrin IX (12 mg/kg, i.p.) were administered daily for 9 days before zymosan injection and on the same day of zymosan injection. The number of cells in the exudates and the gene expression of inflammatory markers were evaluated. We found that compared to zymosan-treated mice, atorvastatin strongly reduced the cell influx by 61% ( $p \leq 0.001$ ), the levels of prostaglandin E2 by 32% ( $p < 0.05$ ) and interleukin-6 by 61% ( $p < 0.05$ ). Similarly atorvastatin markedly reduced the gene expression of inflammatory markers (interleukin 6, interleukin 1 alpha, pro-interleukin 1 beta / nlrp3 inflammasome, tumor necrosis factor alpha, chemokine (C-C motif) ligand 3 and chemokine (C-C motif) ligand 4) as well as the protein expression of cyclooxygenase-2 by 64% ( $p < 0.001$ ) and inducible nitric oxide synthase by 75% ( $p < 0.001$ ). Co-treatment of mice with atorvastatin and tin protoporphyrin IX fully prevented the effect of statin on cell influx and tumor necrosis factor alpha and partially reduced the levels of inflammatory markers suggesting a contribution of HO-1 in statin-mediated regulation of inflammation. Our results identify HO-1 as one of the targets of statins in modulating the inflammatory response in vivo.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

### ***Influence of cholesterol on ROS production by NADPH oxidase. A cell free study***

**R. MASOUD, T. Bizouarn, C. Houee Levin**

Laboratoire de chimie physique, UMR 8000, université Paris Sud 91405 Orsay France

NADPH oxidase, a multi-subunit enzyme complex catalyzes an intense production of superoxide ions ( $O_2^{\bullet-}$ ), precursor of reactive oxygen species (ROS) [1]. Deregulation of NADPH oxidase activity is associated with several pathologies including inflammatory and cardiovascular diseases [2]. It was shown that the membrane part of NADPH oxidase is present in lipid rafts and the lipid composition is determinant for the activation of membrane proteins [3]. Our aim was to investigate the influence of cholesterol on the activation process of NADPH oxidase in a cell-free system. Our results clearly show that cholesterol is not efficient activators of NADPH oxidase like arachidonic acid [4], however it trigger a basal low superoxide production. A higher concentration of cholesterol, if present during the assembly process of the enzyme, has an inhibitory role on the production of  $O_2^{\bullet-}$ . Added cholesterol acts on both cytosolic and membrane components of NADPH oxidase, leading to imperfect assembly. Our results support the idea that lipid environment is determinant for ROS regulation and that cholesterol could interact with NADPH oxidase constraining its capacity to generate  $O_2^{\bullet-}$ . Therefore, cholesterol plays a role in the regulation of NADPH oxidase which might further contribute to other diseases.

1. Bedard, K. & Krause, K. H. (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology, *Physiological reviews*. 87, 245-313.
2. Rodino-Janeiro, B. K., Paradela-Dobarro, B., Castineiras-Landeira, M. I., Raposeiras-Roubin, S., Gonzalez-Juanatey, J. R. & Alvarez, E. (2013) Current status of NADPH oxidase research in cardiovascular pharmacology, *Vascular health and risk management*. 9, 401-28.
3. Vilhardt, F. & van Deurs, B. (2004) The phagocyte NADPH oxidase depends on cholesterol-enriched membrane microdomains for assembly, *The EMBO journal*. 23, 739-48.
4. Souabni, H., Thoma, V., Bizouarn, T., Chatgililoglu, C., Sifaka-Kapadai, A., Baciou, L., Ferreri, C., Houee-Levin, C. & Ostuni, M. A. (2012) trans Arachidonic acid isomers inhibit NADPH-oxidase activity by direct interaction with enzyme components, *Biochimica et biophysica acta*. 1818, 2314-24.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre Francois JACOB, Paris**

***F-actin-rich endothelial pores prevent vascular leakage during leukocyte diapedesis through local RhoA signaling in vivo***

**Niels Heemskerk, Lilian Schimmel, Chantal Oort, Taofei Yin, Bin Ma, Kobus van Unen, Bettina Pitter, Stephan Huvencers, Joachim Goedhart, Yi Wu, Eloi Montanez, Abigail Woodfin,  
Jaap D. VAN BUUL**

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In response to inflammation, leukocytes exit the vasculature through transient openings in the endothelium without causing plasma leakage. Here, we investigated the mechanism by which endothelial cells simultaneously prevent vascular leakage and allow leukocytes to cross the endothelial lining. We show that the endothelial small GTPase RhoA is required to maintain a tight EC barrier during leukocyte diapedesis. Depletion in vitro or inhibition of endothelial RhoA in vivo results in increased vascular leakage of FITC-dextran, but did not alter neutrophil adhesion or transmigration. By examining local RhoA activation using a novel RhoA biosensor, we found that RhoA was transiently activated around transmigrating neutrophils. At this stage, endothelial cells assemble F-actin structures around endothelial pores, which are inhibited by RhoA depletion. Furthermore, Myosin-II was locally activated at endothelial pores, in vivo. Our work shows that endothelial pore confinement prevents vascular leakage during leukocyte extravasation which is driven by a basolateral actomyosin-based structure that requires activation of RhoA.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

***Proteinase 3 Affects the Production and Function of Microparticles: Implications for Inflammation and Autoimmune Vasculitis***

**Katherine MARTIN<sup>1,2</sup>, Magali Pederzoli-Ribeil<sup>1,2</sup>, Min Yin<sup>3</sup>, Philippe Frachet<sup>4</sup>,  
Philippe Saas<sup>5</sup>, Chantal Boulanger<sup>3</sup>, Véronique Witko-Sarsat<sup>1,2</sup>**

<sup>1</sup>INSERM U1016, Paris, France ; <sup>2</sup>CNRS-UMR 8104, Paris, France ; <sup>3</sup>Hôpital Européen Georges Pompidou, Paris, France ;

<sup>4</sup>Joseph Fourier University Grenoble, Grenoble, France ; <sup>5</sup>Inserm UMR1098, Besançon, France

### **Background**

Microparticles (MP) are generated from the plasma membrane following activation or apoptosis and can modulate inflammation, coagulation and vascular function. They contain high levels of phosphatidylserine (PS) on their surface and possess a range of molecules from the parent cell. MP levels in patients with active vasculitis are significantly higher than healthy controls. Proteinase 3 (PR3), the autoantigen in Granulomatosis with polyangiitis, is expressed at the plasma membrane under basal conditions and also during neutrophil apoptosis. Membrane PR3 on apoptotic neutrophils can interfere with their clearance by macrophages and promote a pro-inflammatory phenotype. Importantly, PR3 is co-externalized with PS during apoptosis and mutations within the hydrophobic patch region of PR3 prevents this association.

### **Objective**

Given that PS is a major component of MP, we sought to determine whether PR3 affects MP production or function. Methods: RBL cells transfected with a control plasmid, wild type PR3 or PR3 mutated within the hydrophobic patch were stimulated with calcium ionophore or gliotoxin, MP collected with ultracentrifugation and flow cytometry used to determine the number of MP. The activity of these MP was measured by assessing their capacities to modulate NADPH-oxidase in human neutrophils. Functional experiments were conducted in the presence of ANCA to determine if they had a modulatory role.

### **Results**

RBL cells expressing PR3 produced significantly less MP during activation and apoptosis. This reduction was dependant on its ability to associate with the membrane, as mutating the hydrophobic patch restored production. Functionally, MP generated from PR3 expressing cells after calcium ionophore activation induced a significantly larger respiratory burst in neutrophils compare to control MP. Interestingly, while MP generated during apoptosis from control or mutant PR3 cells inhibited the respiratory response, those isolated from PR3 expressing cells exhibited an increased respiratory burst compared to both control and PR3-4H4A expressing cells.

### **Conclusion**

We clearly show that while PR3 expressing cells produce less MP, MP from these cells were more potent at potentiating inflammation.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

***Polymorphonuclear neutrophils, recruited by LTB4 into plaques, increased plaque proteolytic activity***

**MAWHIN Marie-Anne, Fabre Jean-Etienne**

LVTS, Institut National de la santé et de la Recherche Médicale U1148, Hôpital Bichat, Paris, France

### **Aim**

Atherosclerosis is recognised as being based upon immune responses. The role of polymorphonuclear neutrophils (PMNs) in atherosclerosis is just beginning to emerge. PMNs have been evidenced in plaques, but no studies have yet shown how they are recruited into plaques and whether they are activated inside plaques. We aim to assess whether PMNs can be chemo-attracted into plaques and whether they can increase the proteolytic activity of the plaque.

### **Methods**

We quantified the production of LTB4 by EIA in atherosclerotic plaques of ApoE<sup>-/-</sup> mice subjected to a chow diet, a high fat diet, or treated with LPS. We injected labelled neutrophils in order to compare their capacity to invade plaques in mice with an impaired production of LTB4 (ApoE<sup>-/-</sup>-5-Lo<sup>-/-</sup>) to control mice (ApoE<sup>-/-</sup>-5-Lo<sup>+/+</sup>). To study the effect of a large number of PMNs on plaques, we deposited isolated PMNs on a carotid artery adventitia of 60 week-old ApoE<sup>-/-</sup> mice and examined their entry in the plaque by FLIM. The proteolytic activity of plaques after PMN delivery was assessed using in situ zymography.

### **Results**

Mice that had been treated with LPS showed a significant production of LTB4. Following LPS injections to enhance LTB4 production by plaques, PMNs entry was significantly decreased in plaques of ApoE<sup>-/-</sup>-5-Lo<sup>-/-</sup> mice compared to ApoE<sup>-/-</sup>-5-Lo<sup>+/+</sup> mice. PMNs entered plaques after peri-adventitial delivery. Once inside the plaque, PMNs significantly increased proteolytic activity for collagen type I and type IV (respectively 2.8- and 2.5-fold increase, p<0.001). Both activities were inhibited by the broad MMP inhibitor, 1-10 phenanthroline.

### **Conclusion**

Plaques, that had been stimulated with LPS, produced LTB4, which contributed to the recruitment of PMNs. Once they had invaded the plaque, PMNs can release the content of their granules, leading to an increase in proteolytic activity. This study suggests that PMNs might contribute to plaque vulnerability.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

***Endothelial hypoxia-inducible factor 2 $\alpha$  (HIF2 $\alpha$ ;) mediates endothelial dysfunction and glomerular lesions in a hypertensive model***

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Endothelial dysfunction has a central role in chronic kidney disease pathophysiology. Endothelial HIF2 $\alpha$  has shown a protective role in a kidney ischemia-reperfusion model in mice associated with a decreased leukocyte recruitment.

Our study evaluates the role of endothelial HIF2 $\alpha$  during angiotensin 2 induced hypertension. For that purpose we infused angiotensin 2 for 42 days to wild type and endothelial HIF2 $\alpha$  knock-out mice (Cdh5.cre Epas1lox/lox).

After chronic angiotensin 2 infusion Cdh5.cre Epas1lox/lox developed increased glomerulosclerosis and albuminuria than controls. Histological analysis showed severe podocyte lesions including foot process fusion. We didn't observe a significant difference in inflammatory cell infiltrates (CD3, macrophages) and a decreased capillary density. Hemodynamic study using Doppler ultrasonography showed an endothelial dysfunction in KO mice. That was confirmed by impaired glomerular endothelial NOS activation in Cdh5.cre Epas1lox/lox mice.

In conclusion, endothelial HIF2 $\alpha$  mediates glomerular injury in an hypertensive model through impaired endothelial NOS response.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre Francois JACOB, Paris**

### ***Novel brain antioxidative activity in brain microvasculature***

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Antioxidative activity in the brain protects against a variety of neurodegenerative disorders in which oxidative stress play a role such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis. One antioxidative activity is related to the ability to hydrolyze oxidized polyunsaturated fatty acids (PUFA) from esters, thus defining the role of esterases as antioxidants. Oxygen exchange is indicative of esterase activity. Therefore, we looked at the exchange of oxygen on prostaglandin E2 (PGE2), an arachidonic acid oxidation product that is formed in situ on PUFA esters via nonenzymatic oxidative pathways. We determined the esterase activity in different rat brain compartments both in vivo and in vitro by measuring  $^{18}\text{O}$  exchange on the carboxyl group of deuterium labeled PGE2 using LC-MS. While there was oxygen exchange in all of the compartments, the choroid plexus showed the highest exchange rate of  $^{18}\text{O}$ . We also treated a primary neuronal culture with increasing concentrations of  $\text{H}_2\text{O}_2$  up to 500  $\mu\text{M}$  in the presence and absence of an esterase inhibitor, and measured LDH release as an indicator of cytotoxicity. The inhibitor caused a dramatic, up to 5 fold, increase in  $\text{H}_2\text{O}_2$  cytotoxicity with increased  $\text{H}_2\text{O}_2$  concentration. The inhibitor alone did not display cytotoxic effect. Using pharmacological inhibition, we demonstrated that this esterase activity is distinct from known esterases that have antioxidant properties such as paraoxonase or platelet activating factor acetyl hydrolase. These results indicate that there is high esterase activity in the brain and this activity may be neuroprotective against oxidative damage.

# MEETING GREMI 2015

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François JACOB, Paris**

***Endothelial to Mesenchymal transition reflects the damage induced by microvascular inflammation in human renal grafts***

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**Introduction:** Antibody-mediated rejection (ABMR) is defined by the occurrence of microvascular inflammation in the graft, and is a leading cause of allograft loss. Sensitive and reliable markers of antibody-endothelium interaction during ABMR are not currently available for routine use, though.

**Methods:** Using immunohistochemistry, we retrospectively studied the diagnostic value of fascin-1, a marker of endothelial-to-mesenchymal transition (EndMT), for ABMR in 53 renal transplant biopsies, including 20 with ABMR, 24 with cell-mediated rejection, and 9 normal grafts. We validated our results in a second independent set of 74 unselected biopsies, and in an animal model of acute ABMR. In vitro analysis were undertaken to determine whether anti-HLA allo-antibodies were sufficient to induce EndMT in endothelial cells.

**Results:** Endothelial cells of the peritubular capillaries in human and rodent grafts with ABMR expressed strongly fascin, whereas those from normal renal grafts did not. In human recipients, the level of expression of this EndMT marker was significantly associated with current ABMR criteria including capillaritis, glomerulitis, peritubular capillary C4d deposition, and donor-specific antibodies. These markers allowed us to predict subsequent occurrence of disease. EndMT markers were more specific than capillaritis for the diagnosis and prognosis of ABMR and predicted late (up to 4 years after biopsy) renal graft dysfunction and proteinuria. In the second independent set of 74 renal graft biopsies, the EndMT markers for the diagnosis of ABMR had a sensitivity of 100% and a specificity of 85%. In vitro, allo-antibodies were not sufficient to induce EndMT.

**Conclusion:** The expression of fascin, an actin-bundling protein typical of mesenchymal cells, by endothelial cells, is associated with microvascular inflammation and as such is sensitive and reliable diagnostic tool for ABMR, that predicts late loss of allograft function.

**MEETING GREMI 2014**

***VASCULAR INFLAMMATION:  
BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Paris**

**POSTERS**

# MEETING GREMI 2014

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Paris**

### **P1**

#### **The role of CLEC9a in atherosclerosis development**

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Necrotic core formation during the development of atherosclerosis is associated with a chronic inflammatory response and promotes accelerated plaque development and instability. We hypothesized

that sensing of necrotic cells by the C-type lectin receptors CLEC9A plays a determinant role in the inflammatory response of atherosclerosis.

Reconstitution of lethally-irradiated Ldlr<sup>-/-</sup> with bone marrow from CLEC9A<sup>-/-</sup> mice significantly reduced atherosclerotic lesion size in aortic root (-45%, p=0,0059) after 5 weeks of FAT diet and (-40%, p=0,0017) after 7 weeks of FAT diet, as compared to mice transplanted with wild-type bone marrow-derived cells. However, no effect of CLEC9A was observed after 13 weeks of FAT diet (p=0,4996),

suggesting early effect of CLEC9A on atherosclerosis development.

The same phenotype was observed in 20-week-old Apoe<sup>-/-</sup>CLEC9A<sup>-/-</sup> compared to Apoe<sup>-/-</sup> mice put on chow diet (-50%, p=0,0022).

Interestingly, an increase of IL-10 expression (+60%, p=0,0093) was observed in spleens of mice deficient for CLEC9A. Furthermore, the beneficial effect observed in CLEC9A<sup>-/-</sup> was abolished in CLEC9A<sup>-/-</sup>IL-10<sup>-/-</sup> compared to IL-10<sup>-/-</sup> (p=0,4452). Altogether, we showed that sensing of necrotic cores by CLEC9A promoted atherosclerosis development and this effect was mediated through a decrease of IL-10 production.

# MEETING GREMI 2014

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Paris**

### **P2**

**Involvement of IL-1 and Oncostatin M in acanthosis and microvascular stenosis in Hypertensive Leg Ulcer**

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Hypertensive leg ulcer (HLU) is an inflammatory disease characterized by intense pain, alteration of vascularisation and skin necrosis. The optimal treatment relies on surgical removal of necrotic tissues covered by a split-skin graft. We studied histomorphology of the lesions and investigated the involvement of inflammatory cells and cytokines to further define physiopathology of HLU. We report epidermis acanthosis and a preferential occlusion of the precapillary arterioles with infiltration of neutrophils, macrophages and T lymphocytes in the dermis. OSM, IL-1beta and IL-6 were overexpressed in the ulcer, whereas the Th17 derived cytokines were not. In vitro, the addition of IL-1beta and OSM promoted acanthosis and destructuration of reconstructed epidermis. Exogenous IL-1beta and OSM synergistically induced epidermal acanthosis in mice. These data show that OSM and IL-1beta are not only a biological characteristic signature of HLU, but these cytokines reflect a specific inflammatory state, directly involved in the pathogenesis. We suggest that anti-cytokines biotherapies could be an alternative strategy to surgery to treat HLU.

# MEETING GREMI 2014

## *VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK*

November 13th, 2015 - Pasteur Institute, Paris

### P3

#### **Different modulation of neutrophil activities by platelets**

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**Background :** During inflammatory reactions, neutrophils are one of the first cells of the immune system recruited at the reaction site. All steps of neutrophil activation - mobilization, infiltration, effector functions- need to be finely tuned to ensure optimal effectiveness while limiting collateral tissue damage. In various models of inflammation, platelets have been shown to promote neutrophil recruitment, to modulate neutrophil histotoxic activities, and to repair vascular damage associated with neutrophil trafficking. Depending on the experimental model, platelets were shown to either stimulate or inhibit neutrophil histotoxic activities. However, the basic mechanisms governing this regulation are still poorly understood. Here, we investigated the determinants of the platelet action towards neutrophil histotoxic activities.

**Methods :** In a first set of experiments, isolated human neutrophils were incubated with washed platelets. These experiments were performed in the absence or presence of TNF-alpha and with or without culture insert to prevent contact between the two cell types. In some experiments collagen or thrombin was added to activate platelets. In a second time, the same experiments were performed in the presence of endothelial cells (EC) cultured on cell culture inserts. Neutrophil activation markers such as elastase, myeloperoxidase activity and ROS production were measured in each experiment.

In vivo, immune-complex (IC) mediated inflammation was elicited by i.v injection of BSA combined with either i.v or i.p injection of anti-BSA IgG in wild-type or thrombocytopenic mice.

**Results :** In vitro, platelets co-incubated with control or TNF-alpha-stimulated neutrophils reduced neutrophil elastase and myeloperoxidase activity. This inhibition was not modified by the presence of platelet agonists and was abolished when the contact between platelets and neutrophils was prevented. In the presence of EC cultured, the same inhibitory effects were observed except when neutrophils were pre-incubated with EC prior to platelet addition.

In vivo, in a model of IC-mediated inflammation, we observed that platelets exerted opposite effects towards neutrophil histotoxic activities depending on whether the reaction was induced systemically in the bloodstream or locally, in the peritoneal cavity.

**Conclusion :** Together, our preliminary results suggest that platelets inhibit the histotoxic activities of unstimulated and stimulated neutrophils through contact-dependent mechanisms unless neutrophils are engaged and primed by interactions with EC. Thus, our results designate neutrophil/EC interactions as a crucial determinant of the stimulatory effect of platelets on neutrophil histotoxic activities.

# MEETING GREMI 2014

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Paris**

### **P4**

**CD31 engagement modulates Neutrophils-Endothelial cells interactions under physiological flow**

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Studies using genetic deletion or molecules able to block CD31 engagement have indirectly suggested that CD31 is involved in neutrophil trans-endothelial migration. Nevertheless, as an ITIM-bearing receptor, CD31 functions go beyond its adhesive properties and indeed nothing is known about the dynamic of CD31 expression and the role of its engagement in neutrophils rolling and adhesion onto activated endothelial cells.

By using flow cytometry and fluorescence microscopy, we found most of CD31 extracellular portion is rapidly lost, leaving a membrane-proximal extracellular fragment expressed by activated neutrophils. We found that the presence of a synthetic peptide, able to engage the lingering CD31 extracellular fragment, sustains in a dose-dependent manner the phosphorylation of the intracellular CD31 ITIM 713 motif, SHP2 activation and CD31 clusters upholding.

We therefore evaluated the effect of such CD31 agonist peptide on neutrophil adhesion onto activated endothelial cells. Male, 8-week old C57BL/6 mice received an i.v. injection of rhodamine and were subjected to vital fluorescence video microscopy after local application of ionomycin (1µM) onto a segment of mesenteric venule. While mice receiving vehicle showed strong leukocyte recruitment in term of rolling and firm adhesion after 3 minutes on the ionomycin-treated venule, the administration of the CD31 agonist (2,5mg/kg) before surgery resulted in a durable 60% inhibition of leukocytes adhesion onto the activated endothelium (n=4/group). These observations were supported by separated in vitro experiments using a flow system in which purified human neutrophils were let stream with a post-capillary flow rate (5 dynes/cm<sup>2</sup>) on human coronary artery endothelial cell (HCAEC) that were previously cultured until confluence under continuous flow on cellix VENAFLUOR® chambers and activated overnight with 10ng/ml of TNFalpha. As compared to control cells, which exhibited the classical rolling-adhesion cascade, neutrophils pre-treated with the homotypic peptide (100µM) virtually did not arrest onto the activated HCAEC.

We conclude that CD31 signalling can consistently modulate neutrophils interactions with activated endothelial cells and may be crucial to control inappropriate neutrophil recruitment in different vascular inflammatory conditions.

# MEETING GREMI 2014

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

November 13th, 2015 - Pasteur Institute, Paris

### **P5**

#### **Role of connexin 43 in renal ischemia/reperfusion**

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**Background** : We have previously demonstrated that the gap junction protein connexin 43 (Cx43) is upregulated in chronic renal disease in humans and rodents. In this study we investigated the role of Cx43 in renal ischemia/reperfusion (I/R), which plays major roles in AKI and renal transplant graft failure.

**Methods**: I/R injury was induced in mice by arterial clamping for 25 min after contralateral nephrectomy, and reperfusion of varying duration (3-72 h). Kidney tissues were assessed for histological evaluation, immunofluorescence, RT-PCR and Western blotting. In separate studies, we administered oligonucleotide antisense (AS) for Cx43, and examined its effects on I/R.

**Results** : Serum creatinine (sCr) was markedly impaired in I/R mice at 24 h ( $104.8 \pm 18.8$  mM vs. C  $6.7 \pm 4.6$  mM,  $p < 0.05$ ). Regarding the histological damage, the inner cortex was the primary site of histological lesions at 24 h (histological scores  $3.63 \pm 0.12$  vs. C  $0.06 \pm 0.02$ ,  $p < 0.05$ ), but at 72 h the tubular necrosis was mostly repaired and the main site of lesions shifted to the medulla ( $4.13 \pm 0.07$  vs. C  $0.08 \pm 0.05$ ,  $p < 0.05$ ). Massive neutrophil infiltration occurred in the inner cortex at 24 h (GR1 staining  $2.89 \pm 0.53$  % vs. C  $0.008 \pm 0.003$  %,  $p < 0.05$ ), but rapidly decreased after 48 h ( $0.45 \pm 0.10$  %,  $p < 0.05$  vs. 24h). In contrast, significant macrophage infiltration was seen in the inner cortex and the medulla at 48 h (CD3 staining  $0.28 \pm 0.06$  %,  $0.73 \pm 0.14$  % vs. C  $0.03 \pm 0.01$ ,  $0.02 \pm 0.01$ ,  $p < 0.05$ ), and increased in the latter at 72 h ( $1.77 \pm 0.91$ ,  $p < 0.01$  vs. 48h). Cx43 was de novo expressed in the proximal tubules of the inner cortex compared to controls after 24h. Administration of Cx43AS blunted neutrophil infiltration (GR1 staining,  $0.66 \pm 0.28$  for AS vs. C  $1.87 \pm 0.24$ ,  $p < 0.05$ ) leading in decreased sCR levels and lower histological damage as well at 24 h.

**Conclusions** : Cx43 is upregulated in renal I/R, and its inhibition improved renal structure and function, indicating a key role of this protein in AKI.

# MEETING GREMI 2014

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

November 13th, 2015 - Pasteur Institute, Paris

### **P6**

#### **SERPINE2 expression and function in neutrophils**

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Inflammation and coagulation play pivotal roles in the pathogenesis of vascular disease ranging from arterial disease to arthritis and severe septic infections. Increasing evidence points to extensive cross-talk between these two systems, whereby inflammation leads to activation of coagulation, and coagulation further augments inflammation. Thrombin, the end product of the coagulation cascade, is the main driver of this cross-talk. Thrombin activation and activity are tightly regulated by proteins belonging to the serpin superfamily (serine protease inhibitor). Recently, protease nexin-1 (PN-1, encoded by SerpinE2) has emerged as the most potent endogenous inhibitor of thrombin. PN-1 can regulate all stages of the host response to trauma, including clotting / coagulation, inflammation, cell migration and proliferation, and tissue remodelling. PN-1 is an inhibitory serpin barely detectable in plasma but expressed by vascular cells, platelets and inflammatory cells. However the potential role of PN1 in regulating inflammatory processes is not known. PN-1 has been detected in monocytes/macrophages, but no data are available concerning its expression and potential function in neutrophils.

We have demonstrated by immunoblot and flow cytometry the presence of PN-1 in human and murine neutrophils. PN-1 is secreted after neutrophil activation by LPS and is detected on neutrophil surface. The measurements of myeloperoxidase activity and of the generation of reactive oxygen from neutrophils isolated from bone marrow of PN-1-KO and WT mice showed that PN-1-deficiency leads to a significant decrease of neutrophil activities. Neutrophil vascular recruitment induced by topical application of LTB4 on mesenteric veins was analysed by intravital microscopy. The neutrophil recruitment was much less important in PN-1-KO mice compared with WT. There is also a tendency to a lower recruitment of neutrophils in PN-1-KO mice compared with WT mice after intraperitoneal administration of LPS.

Altogether, our data suggest that PN-1 can positively regulate inflammatory responses of neutrophils.

# MEETING GREMI 2014

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Paris**

### **P7**

#### **Heme induces complement activation on endothelial cells via TLR-4/P-selectin axis**

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Heme is a damage associated molecular pattern, derived from ruptured erythrocytes during hemolysis, able to stimulate endothelial cells (EC) and to induce complement activation. The objective of this work was to identify the mechanisms responsible for heme-induced complement deposition on EC.

The C3 deposition and the levels of complement regulators MCP and DAF as well as C3b-binding platforms P-selectin and properdin on the cell surface were measured by FACS. The interaction of P-selectin with C3b and C3(H<sub>2</sub>O) was detected by SPR. The C3 deposition in vivo in mice kidneys after heme injection was measured by immunohistochemistry and immunofluorescence.

Exposure of EC to heme resulted in decrease of MCP and DAF expression and up-regulation of P-selectin but no properdin binding. Addition of purified physiological heme scavenger hemopexin completely prevented complement activation and the modifications of the surface expression of MCP, DAF and P-selectin. To distinguish the contribution of the decrease of the regulators versus expression of the C3-binding platform molecule, blocking of MCP and DAF or silencing of MCP was performed. Minimal or no increase in C3 deposition was detected, ruling out the contribution of the regulators. Blocking of P-selectin resulted in 50% reduction of C3 deposition. P-selectin bound C3b and also C3(H<sub>2</sub>O), thus partially explaining the complement activation on EC surface.

Candidate heme receptors, resulting in P-selectin expression, were searched by a high-throughput phosphorylation analysis. Activation of inflammatory TLR-4 signaling pathway was detected. Blockade of TLR-4 by TAK-242 prevented the P-selectin expression (without effect on MCP and DAF) and reduced ~50% the complement deposition on heme-exposed EC.

The capacity of heme to induce complement activation was validated in vivo, after heme injection in C57Bl/6 mice. C3 deposition was detected in kidney glomeruli, tubules and blood vessels.

The results demonstrate for the first time a link between TLR-4 activation by heme and complement deposition on EC. P-selectin plays a key role in this process, serving as a platform for C3. The ability of hemopexin and TAK-242 to reduce complement deposition on EC highlights their therapeutic potential in hemolytic conditions, where complement activation may be a link between hemolysis and vascular lesions.

# MEETING GREMI 2014

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Paris**

### **P8**

#### **The clinical utility of Myeloperoxidase testing**

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Myeloperoxidase (MPO) is the most abundant constituent of the azurophilic granules of polymorphonuclear leucocytes (neutrophils, monocytes and some of tissue macrophages). When secreted into the extracellular space, MPO appears to be responsible for converting LDL cholesterol to its oxidised form, which then attaches itself to the endothelial plaques of coronary arteries. MPO may play a direct role in the pathogenesis of cardiovascular disease (CVD). It is involved in :

- 1) lipid peroxidation, converting LDL to an atherogenic form and HDL to a dysfunctional form ;
- 2) destabilization and rupture of atherosclerotic plaques ; and
- 3) vasoconstriction and endothelial dysfunction.

Thus, MPO has been studied as a biomarker of CVD.

Elevated plasma levels of MPO have been linked to increased risk for :

- 1) CAD in low-risk populations
- 2) major adverse cardiovascular events (MACE) in patients with angiographically confirmed CAD
- 3) death or nonfatal myocardial infarction (MI) in patients with ST-segment elevation MI (STEMI)
- 4) cardiovascular complications of diabetes.

The determination of MPO could have an important role in the identification of patients who are at high risk of CVD and CAD particularly those aged 45 or more, where the mortality from CVD is markedly increased.

Salivary diagnostics holds great promise as an effective modality for the early diagnosis of cardiovascular diseases. Salivary markers include C-reactive protein (CRP), myoglobin (MYO), creatinine kinase myocardial band (CK-MB), cardiac troponins (cTn), and myeloperoxidase, which, when used in combination with an ECG, shows a positive correlation with myocardial infarct patients as compared to healthy controls.

Many advantages of saliva as a clinical tool over serum and tissues are noninvasive collection of sample, smaller sample aliquots, good cooperation with patients, cost effectiveness, easy storage and transportation, greater sensitivity, and correlation with levels in blood.

# MEETING GREMI 2014

## *VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK*

November 13th, 2015 - Pasteur Institute, Paris

### P9

**Poly([R,S]-3,3-dimethylmalic acid) nanoparticles as a controlled-release delivery system for an antithrombotic drug**

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Advancement in polymer science and engineering led to new polymers for controlled delivery of therapeutic drugs<sup>1</sup>. Poly([R,S]-3,3-dimethylmalic acid) (PDMMLA) and its hydrophobic derivatives can be considered as a family of promising polymers. PDMMLA is polyester synthesized for temporary therapeutic applications. The encapsulation of a bioactive coumarinic derivative is one of these applications, for this we tried to encapsulate warfarin as a model drug to optimize the controlled release settings.

Experimental : A series of amphiphilic statistical copolyesters with different acid/hexyl proportion 70/30, 50/50 and 30/70 (mol/mol) belonging to the poly([R,S]-3,3-dimethylmalic acid) (PDMMLA) family were synthesized<sup>2,3</sup>. Statistical polymer which contains 30% of acid and 70% of hexyl was chosen to investigate encapsulation and transporting efficiency of warfarin by nanoprecipitation method. Polymer nanoparticles were prepared by a nanoprecipitation solvent evaporation method, and characterized by Transmission Electronic Microscopy (TEM) for nanoparticles morphology and nanosizer for size and charge studies. Controlled release of active principle was followed by inverse phase HPLC. TEM analysis evidenced spherical nanoparticles (Figure 1), which present an average size in physiological medium of about 80 nm (Figure 2) with an only slight evolution of the size distribution over the study (28 days).

# MEETING GREMI 2014

## VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK

November 13th, 2015 - Pasteur Institute, Paris

### P10

#### Modulation of Inflammasome gene expression in arterial wall cells

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Cytokines play an important role in the regulation of the inflammatory process in atherosclerosis. Endothelial, smooth muscle cells and monocyte-derived macrophages bring into play cell surface and intracellular receptors in order to sense pathogens, cell damage molecules, and/or circulating oxidized proteins conditioning the expression of cytokines and the progression of the disease. Among these receptors, Nod-like receptors (NLRs) take part in the formation of multiprotein complexes, called inflammasomes, which associate an NLR, an adaptor protein called ASC (apoptosis-associated speck-like protein containing a CARD), and the pro-inflammatory protein caspase-1. The activation of inflammasomes by different stimuli including cholesterol crystals triggers the proteolytic cleavage of pro-caspase-1 into active caspase-1, which, in turn, converts the inactive precursors of IL1b and IL18 into their mature forms. Both cytokines play a crucial role in systemic inflammation as they act on various target organs and induce pro-inflammatory gene expression thereby amplifying the inflammatory response. Inflammasomes have taken a central stage in numerous inflammatory diseases and their implication in atherosclerosis is emerging

The aim of our study was to establish the pattern of inflammasome gene/protein expression in cells of the arterial wall under basal conditions and after activation with a proinflammatory stimuli (oxidized LDL, LPS, IFNg).

**Methods :** Mononuclear cells were isolated from human buffy coats using Ficoll gradient. Monocytes were selected by adherence and differentiated to macrophages in the presence of human serum. Human, aortic endothelial cells, or smooth muscle cells were commercially available. Cells were treated with 100µg/ml oxidized LDL, LPS or IFNg for different time periods. Total RNA was isolated from treated cells and mRNA expression was studied using RT-QPCR for inflammasome genes. Cell lysates and cell supernatants were also collected in order to study inflammasome protein expression and cytokine secretion.

**Results :** During in vitro differentiation of human monocytes to macrophages, the mRNA and protein expression of NLRP3 and pyrin is down-regulated. Proinflammatory stimuli like IFNg or LPS differentially regulate NLRP3 and pyrin gene expression where as ASC and caspase 1 are not modulated. Proinflammatory cytokine expression is strongly upregulated when cells are exposed to LPS or IFNg but this induction is not correlating with NLRP3 caspase 1, or pyrin regulation. Secretion of cytokines and mainly IL1b or IL18 requires an additional to LPS or IFNg stimuli. The effect of oxidized LDL on inflammasome gene expression is dependent on the cell type.

**Conclusion :** Our results (in progress), suggest that inflammasome genes are differentially expressed and regulated during atherosclerosis, underlying a cell specific role of each gene/protein in the inflammatory process.

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# **NOTES ON MEETING GREMI 2015**

## ***VASCULAR INFLAMMATION: BLOOD VESSELS UNDER ATTACK***

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BLOOD VESSELS UNDER ATTACK***

**November 13th, 2015 - Pasteur Institute, Amphitheatre François Jacob, Paris**

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