A1 Calprotectin amplifies the inflammatory response
R. Pepper1, S. Hamour2, H.H. Wang2, T. Vogl1, T. Cook2, C. Pusey2
1. UCL Centre for Nephrology, London, United Kingdom
2. Imperial College London, London, United Kingdom
3. University of Muenster, Muenster, Germany

Introduction. Calprotectin (MRP8/14) is an endogenous TLR4 agonist, expressed in neutrophils, monocytes and infiltrating macrophages, promoting endothelial activation and transcription of proinflammatory cytokines. We have shown patients with active ANCA-associated vasculitis (AAV) have increased cell surface expression of calprotectin, with elevated cell surface and serum levels, and relapsers from the NORAM trial have higher early serum levels than non-relapsers. Calprotectin (+) macrophages are found in crescentic renal lesions but not sclerotic lesions, and calprotectin deficient mice (cal-/-) are protected in a nephrotoxic nephritis (NTN) animal model. We investigate the proinflammatory mechanisms of calprotectin on bone marrow derived macrophages (BMDMs), endothelial cells (EC) and mesangial cells (MC).

Methods. EC isolated from wild-type (WT) mice; BMDMs from WT, TLR4-/- and cal-/- mice; MC from WT and TLR4-/- mice- were stimulated with calprotectin. WT EC were co-cultured with WT BMDMs or cal-/- BMDMs. Cytokines measured in supernatants by ELISA. The phagocytosis ability of WT and cal-/- BMDMs were compared using opsonised beads. Serum calprotectin levels were measured in WT mice during NTN.

Results. The calprotectin induced increase in IL-8, TNF-α, MCP-1 in BMDMs was abrogated in TLR4-/- BMDMs (P < 0.001), but no differences seen in MC. Cal-/- vs WT BMDM stimulated with exogenous calprotectin demonstrate little pro-inflammatory activity and less TNF-α, IL-6, IL-8 (P < 0.005). The increase in IL-6, IL-8 and MCP-1 following co-culture of EC and WT BMDM was absent with cal-/- BMDM. Cal-/- BMDMs demonstrate decreased phagocytosis (P < 0.005). WT mice have increased serum calprotectin (correlates with thrombosis).

Conclusion. Calprotectin has inflammatory effects mediated by TLR4 on BMDMs and a TLR4 independent effect on MC possibly through mesangial RAGE receptors, known to bind calprotectin. Cal-/- BMDMs lack a pro-inflammatory effect, suggesting a role for calprotectin in amplifying endothelial and glomerular damage in AAV, and may be a potential therapeutic target.

http://dx.doi.org/10.1016/j.lpm.2013.02.003

A2 Proteinase 3 induces shape change in platelets through activation of the Rho/Rho-kinase signaling pathway
X. Peng, T. Kurz, C. Brommesson, M. Grenegård, M. Segelmark
Linköping University, Linköping, Sweden

Introduction. Interactions between neutrophils and platelets may be important in the pathogenesis of ANCA-associated vasculitis (AAV). Platelets have been shown to promote NETosis and AAV patients have an increased risk of thrombosis. Here, we explore the in vitro effect of proteinase 3 (PR3) on isolated human platelets.

Methods. Measurement of platelet shape change and aggregation with light transmission technique, light microscopy, immunofluorescence, measurement of protein phosphorylation by western blotting, measurement of cytosolic calcium.

Results. Low doses of PR3 (0.3 μg/ml–6 μg/ml) induced a rapid and dose-dependent shape change in platelets within seconds, but without inducing aggregation. Light microscopy showed that the platelets responded to PR3 by displaying a spherical morphology but no detectable formation of micro-aggregates. The shape change was dependent on the enzymatic activity as it could be inhibited by serum (alpha-1-antitrypsin) and PYDA (a specific low molecular weight inhibitor). The shape change was accompanied by a minor rise in intracellular calcium and by activation of the Rho/Rho-kinase pathway (measured by ROCK phosphorylation). Pre-incubation with the Rho-kinase inhibitor Y-27632, or the calcium chelator BAPTA/AM, antagonized the PR3-induced shape change and combining the agents abolished it. The addition of PR3 (0.6–3.0 μg/ml) dose-dependently reduced platelet aggregation induced by thrombin and the specific PAR1-agonist SFLLRN.

Conclusion. In vitro low concentrations of PR3-induced rapid activation of the Rho/Rho-kinase pathway resulting in a shape change and reduced ability to aggregation. Considering increased plasma concentrations as well as high surface expression of PR3 in AAV, these interactions may have relevance for the pathogenesis of thromboembolic events and vascular lesions in AAV.

http://dx.doi.org/10.1016/j.lpm.2013.02.004

A3 Identification of target antigens of anti-endothelial cell antibodies in patients with ANCA-associated systemic vasculitis: A proteomic approach
A. Régent1,2, H. Dib1,2, G. Bussone2,3, N. Tamas1,2, C. Federici1,2, C. Broussard1,2, L. Guilleuvin1, L. Mouthon1,3
1. Inserm U1016, Institut Cochin, CNRS UMR 8104, 8, rue Méchain, 75014 Paris, France
2. Université Paris-Descartes, 12, rue de l’École de Médecine, 75270 Paris, France
3. Pôle de Médecine Interne, Centre de Référence pour les vascularites nécrosantes et la sclérodermie systémique, Hôpital Cochin, Assistance Publique Hôpitaux de Paris, 27, rue du Faubourg Saint-Jacques, 75679 Paris Cedex 14, France

Introduction. Anti-endothelial cell antibodies (AECA) are frequently detected in anti-neutrophil cytoplasm antibodies (ANCA)-associated systemic vasculitis (AAV) and are considered to play pathological roles but their antigenic specificities are still unknown. We used a proteomic approach combining two-dimensional electrophoresis and immunoblotting to identify the target antigens of AECA in patients with ANCA-associated vasculitis.

Methods. Sera from 30 ANCA-associated vasculitis patients [12 with Granulomatosis with polyangiitis (GPA), nine with microscopic polyangiitis (MPA), nine with Churg-Strauss syndrome (CSS)], tested in pools of three sera, were compared to a sera pool from 12 healthy controls (HC). Serum IgG reactivity was analyzed by use of a 2-D electrophoresis and immunoblotting technique with normal human umbilical vein endothelial cell (HUVEC) antigens.
Results.— Serum IgG in the HC sera pool recognized 85 protein spots and serum IgG from patients with AAV recognized $134 \pm 65$ different protein spots. We focused on protein recognized by at least 3/4 pools of patients with GPA and 2/3 pools of patients with MPA and CSS and not by HC and identified 20, seven and eight proteins, respectively. In addition, among the 330 spots recognized by at least one pool of patients with AAV, ten different spots were recognized by at least 6/10 pools. Among identified proteins, IgG reactivity was detected against alpha-enolase, lamin A/C and protein disulfide-isomerase A3. Interestingly, Ingenuity Pathway Analysis revealed that most of these antigens interact with TGF-β and signalling pathways such as JNK and MAPK.

Conclusion.— AECA detected in patients with AAV recognize cellular targets playing key roles in cell biology. Target antigens interact with protein and complexes known to play a crucial role in AAV pathophysiology.

http://dx.doi.org/10.1016/j.lpm.2013.02.005

A5 Orthotopic heart transplantation in eosinophilic granulomatosis with polyangiitis

M. Groh1, G. Masciocco2, E. Kirchner3, A. Kristen4, G. Bortman5, S. Vannous6, M. Rosenberg7, A. D'armini8, C. Pellegrini9, A. Brucato9, G. Gregorini10, M. Frigerio11, J.J. Scali12, L. Calabrese3, L. Guillevin1

1. Department of Internal Medicine, Paris, France
2. Department of Cardiology and heart Transplantation, Milan, Italy
3. Department of Rheumatologic and Immunologic Disease, Cleveland, USA
4. Department of Cardiology, Angiology, and Respiratory Medicine, Heidelberg, Germany
5. Department of Cardiovascular surgery, Buenos Aires, Argentina
6. Department of Cardiovascular and thoracic surgery, Paris, France
7. Department of Internal Medicine III (Cardiology and Angiology), Kiel, Germany
8. Department of cardiothoracic surgery, Pavia, Italy
9. Department of Rheumatology, Internal Medicine and Emergency Medicine, Milan, Italy
10. Division of Nephrology, Brescia, Italy
11. Department of cardiology and heart Transplantation, Brescia, Italy
12. Department of Rheumatology, Autoimmune and Metabolic Bone Diseases, Buenos Aires, Argentina

Introduction.— Heart involvement is the leading cause of death in eosinophilic granulomatosis with polyangiitis (EGPA) [1]. Transplant outcome after orthotopic heart transplantation (OHT) due to EGPA-related cardiomyopathy has been reported in a single patient [2].

Patients.— We conducted an international retrospective study of patients who went through OHT for EGPA-related cardiomyopathy between October 2000 and December 2009. A complete PubMed review was also performed.

Results.— Nine patients were identified. All had negative ANCA serology and acute congestive heart failure due to severe eosinophilic myocarditis (mean troponin level $14.6 \pm 20.5 \mu g/L$ and LVEF value $24 \pm 6\%$). EGPA and EGPA-related cardiomyopathy diagnoses were synchronous in five patients and in the other four patients were separated by 12 to 21 months.

Six patients’ explanted hearts (67%) showed histologic evidence of EGPA. Four patients (44%) died of sudden death after OHT, with survival time ranging from three to 60 months. Follow-up for the five surviving patients (56%) is 55 to 102 months. EGPA relapse after OHT occurred in six patients (67%), within a period of two to 48 months.

Discussion.— This is the largest case series of patients who have gone through OHT for EGPA-related cardiomyopathy. It is essential to identify cardiac involvement early during the course of EGPA, for prompt treatment with corticosteroids and cyclophosphamide may allow recovery of the cardiac function [3,4]. In refractory patients, OHT can be performed with respect to the International Society of Heart and Lung Transplantation (ISHLT) guidelines [5], but it is of poor-prognosis. After OHT, recent data suggests that tacrolimus (TAC) and mycophenolate