Abstracts: Oral presentations

A1  
Calprotectin amplifies the inflammatory response  
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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 elevated cell surface and serum levels, and relapsers from the NORA 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/-/- BMDM (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.

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A2  
Proteinase 3 induces shape change in platelets through activation of the Rho/Rho-kinase signaling pathway  
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Introduction.– Interactions between neutrophils and platelets may be important in the pathogenesis of ANCA-associated vasculitis (AAV). Platelets have been shown to promote NET-osis 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 transmittance 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.

Discussion.– In vitro low concentrations of PR3 induced rapid activation of the Rho/Rho-kinase pathway resulting in a shape change and reduced ability to aggregate. 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.

Conclusion.– PR3 can directly affect platelet morphology and function.  
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A3  
Identification of target antigens of anti-endothelial cell antibodies in patients with ANCA-associated systemic vasculitis: A proteomic approach  
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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.