**A4**

**Proteinase 3 (PR3) is a phosphatidylserine-binding protein that can bind microparticles: Relevance in the context of granulomatosis with polyangiitis (GPA)**


**Introduction.** Proteinase 3 (PR3), the autoantigen in GPA is found in azurophilic granules of PMN and is expressed at the plasma membrane. Under PMN activation, PR3 is secreted in the extracellular environment and plays its proinflammatory functions. Unlike its homologs, PR3 is co-externalized with phosphatidylserine (PS) at an early stage of neutrophil apoptosis. This affinity of PR3 for PS could be relevant in the context of microparticles that are released from different cell types, including endothelial cells. Microparticles, emitted from apoptotic or activated cells, are membrane vesicles exposing PS on their surface, which are related to disease activity in GPA and which are reported to induce endothelial cell proinflammatory phenotype.

**Objective.** The aim of our study was to investigate whether PR3 has an affinity for PS and whether it could bind microparticles-derived from endothelial cells to mediate biological activities.

**Methods and results.** Surface plasmon resonance experiments and phospholipids-coated membranes provided evidence that PR3 was a PS binding protein and that this interaction depended on its hydrophobic patch. We next demonstrated that exogenous soluble PR3 was able to bind to apoptotic RBL cells expressing PS at their surface. This binding was significantly decreased after knocking down the phospholipid-scramblase 1 gene that was responsible for PS externalization in these cells. We next demonstrated that exogenous PR3 was able to bind endothelial-derived microparticles and not platelets-derived ones, suggesting a cell specificity.

**Conclusion.** We propose that PS constitutes a receptor for soluble PR3, secreted from activated neutrophils. We hypothesized that PR3 expressed on microparticles might mediate pro-inflammatory functions and/or immunomodulatory activities. Further studies will be required to evaluate whether targeting PR3/PS interaction might be relevant in the context of GPA, to dampen PR3 pro-inflammatory deleterious roles.

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**A5**

**Orthotopic heart transplantation in eosinophilic granulomatosis with polyangiitis**


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**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 ± 20.5 μg/L and LVEF value 24 ± 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
mofetil (MMF) are superior to micro-emulsion cyclosporine A and azathioprine respectively [6,7]. However, data regarding the use of TAC and MMF in EGPA is scarce. Conclusion.– EGPA should not be a limitation to OHT, which can be performed with respect to the ISHLT guidelines. There is no optimal immunosuppressive strategy. Arrhythmia is a burden. Further data is needed.

References


A6

Cluster analysis to explore subclassification of eosinophilic granulomatosis with polyangiitis (Churg-Strauss)


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Introduction.– Results from descriptive studies of eosinophilic granulomatosis with polyangiitis (EGPA) suggest distinct clinical subclasses that may be determined by anti-neutrophil cytoplasmic antibody (ANCA) status. We used hierarchical cluster analysis to explore whether EGPA could be subclassified.

Methods.– We used standardized retrospective data for a cohort with clinical diagnoses of EGPA followed in four tertiary referral centers. Hierarchical cluster analysis involved the Ward method with 12 input variables assessed at diagnosis: constitutional symptoms; mucocutaneous, ophthalmologic, ear, nose and throat, cardiovascular, gastrointestinal, renal, and central nervous system involvement; peripheral neuropathy; non-fixed lung infiltrates; and ANCA positivity. The resulting clusters were described by their most prominent summary characteristics. The distribution of clinical variables was analyzed by ANCA status with chi-square test.

Results.– The dataset included 262 EGPA cases diagnosed between 1984 and 2012. ANCs were detected in 30.9% of cases. Cluster analysis revealed three clusters of 39 (cluster 1), 92 (cluster 2) and 131 subjects (cluster 3). Cluster 1 was characterized by renal involvement (84.6%) and high ANCA positivity (92.3%), cluster 2 by virtually absent renal involvement (3.3%) and ANCA positivity (4.3%) and cluster 3 by an intermediate phenotype with renal involvement (13%), ANCA positivity (31.3%) and frequent cardiovascular involvement (59.5% vs. 17.9% and 35.9% for clusters 1 and 2, respectively) and gastrointestinal involvement (42% vs. 15.4% and 12%, respectively). ANCA positivity was associated with renal disease (P < 0.0001), peripheral neuropathy (P = 0.005) and constitutional symptoms (P = 0.02).

Conclusion.– Cluster analysis of EGPA, although reinforcing the link between ANCA positivity and renal involvement in the disease, does not suggest that it is composed of clearly separated and mutually exclusive subclasses.

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S7

Spleen tyrosine kinase (SYK) inhibition in experimental autoimmune glomerulonephritis (EAG)

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Introduction.– SYK has a critical role in immunoreceptor signalling. SYK inhibition with Fostamatinib (FOS) prevents immune-mediated injury in several animal models. The effect of SYK inhibition on autoantibody production, however, is not well defined. We aimed to address this question in EAG, an autoantibody dependent model of crescentic glomerulonephritis (CGN).

Methods.– In EAG, rats immunized with rat GBM antigen (c3) at day 0 develop autoantibodies to c3 and CGN by day 18, and have lung haemorrhage (LH) at day 36. In study 1, animals (n = 8/group) received either FOS 40 mg/kg or vehicle (VEH) by twice daily gavage from day 0–18, in order to examine the effects of SYK inhibition on induction of autoimmunity. In study 2, animals received FOS 40 mg/kg or VEH from day 18–36, to study the effects of treatment on established disease. In both studies, animals were monitored until Day 36. Cytokine production by nephritic glomeruli was examined ex vivo. SYK expression in rat and human tissue was assessed by immunohistochemistry (IHC).

Results.– Results of studies 1 and 2 are summarised in figure 1. There was a 58% reduction in the number of c3-specific B cells in FOS treated rats (P < 0.01), as demonstrated by ELISpot assay.