SA-PO2206

De Novo Protein Synthesis of PR3 and MPO Autoantigens in Mature Neutrophils of Patients with ANCA Vasculitis Anshul K. Badhwar,[1] Elizabeth A. Alderman,[1] Akhil Muthigi,[1] Heng Ge,[1,2] Elisabeth Berg,[1] J. Charles Jennette,[1] Gloria A. Preston,[1] Ronald J. Falk.[1] *[1]UNC Kidney Center, University of North Carolina at Chapel Hill, NC; [2]Xian Jiaotong University, Xian City, Shaanxi Province, China.*

Background: Due to a defect in epigenetic silencing, circulating neutrophils from patients with ANCA disease express PR3 and MPO genes which are normally expressed only in bone marrow cells. We examine the processing of transcripts in mature neutrophils and whether increased transcription results in increased PR3/MPO protein.

Methods: A psoralen-biotin RNA probe complementary to sense PR-3 and MPO was used for hybridization on northern blot. Total RNA from 9 patients and 9 healthy donors was used to characterize the transcripts present. Quantitative RT-PCR of PR3 and MPO transcripts was used to confirm expression levels detected by northern blotting. Nascent protein synthesis was metabolically labeled with a methionine analog, selectively biotinylated, purified by magnetic streptavidin beads and detected by western blotting.

Results: Multiple isoforms of PR3 transcripts were observed by northern blotting of leukocyte RNA from patients with PR3-ANCA. Five of nine patients expressed at least one isoform of PR3 mRNA, and of the five, three patients expressed an alternatively spliced variant larger (approx. 100 to 400 additional nucleic acids) than currently annotated size. Unexpectedly, PR3 transcripts were also detected in three of nine healthy controls. Northern blotting was determined to be quantitative and correlated with levels of expression of both PR3 and MPO by standardized qRT-PCR assay. A novel polyadenylation site distal to the canonical site was associated with expression in circulating mature neutrophils and monocytes. Upregulation of PR3 and MPO transcripts was associated with de novo protein synthesis in four of four patients with MPO-ANCA. None of the 8 healthy donors tested produced significant levels of either protein.

Conclusions: The data indicate that neutrophils in the periphery produce both PR3 and MPO protein de novo and that the presence of previously unidentified isoforms of PR3 may lead to the production of altered forms of PR3 protein.

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SA-PO2207

Membrane Association of Proteinase 3, the Autoantigen in Granulomatosis with Polyangiitis (GPA), Expressed at the Membrane of Apoptotic Neutrophils, Is Essential for Impairing Their Phagocytosis by Macrophages Veronique Witko-Sarsat,[1] Arnaud Millet,[1] Magali Pederzoli-Ribeil,[1] Luc Mouthon.[2] *[1]Cochin Institute Immunology-Hematology Department, INSERM U1016- University René Descartes, Paris, France; [2]Internal Medicine Department, Cochin Hospital, Paris, France.*

Background: The removal of apoptotic neutrophils is a key event in the resolution of inflammation, its failure has been incriminated in chronic autoimmune diseases. We described that proteinase 3 (PR3) the autoantigen in granulomatosis with polyangiitis (GPA) was externalized during apoptosis and impaired the phagocytosis of apoptotic neutrophils by macrophages thus acting as a dont eat me signal (Kantari et al, Blood 2007). The aim of the study was to investigate whether PR3 membrane expression and/or its enzymatic activity was essential for this activity

Methods: Stable transfectant in RBL cells expressing a mutant of PR3 (PR34H4A) inable to insert into the plasma membrane was generated. The phagocytosis of apoptotic RBLPR34H4A by human monocyte-derived macrophages was studied in comparison with wild type RBLPR3. The enzymatic activity of apoptosis-induced membrane PR3 was studied for its ability to cleave extracellular matrix proteins such as fibronectin.

Results: The mutations of four hydrophobic (F180, F181, L228, F229) amino acids abrogated PR3 membrane anchorage and cells expressing this hydrophobic patch-deficient PR3 mutant (PR34H4A) did not inhibit macrophage phagocytosis thus confirming this importance of PR3 membrane association in this phenomena. We demonstrated that this "dont eat me" activity of membrane-associated PR3 was independent of its serine proteinase activity because 1) the enzymatically-dead mutant PR3S203A displayed the same activity and 2) that apoptosis-induced PR3 externalization did not result in an increased ability to cleave extracellular matrix proteins such as fibronectin.

Conclusions: Our conclusion is 1) that the molecular basis of PR3 "dont-eat-me signal" relies more on PR3 membrane anchorage but not on its enzymatic activity and ii) that PR3 "dont-eat-me" activity might potentiate the mechanisms of autoimmunity and be involved in the pathophysiology of ANCA-associated vasculitis.

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Endothelial Lineage Impairment and Increased PR3 Expression on Peripheral Cells of Endothelial Phenotype in Wegener´s Granulomatosis Susann Patschan,[1] Daniel Patschan,[1] Sabine Blaschke,[1] Gerhard A. Mueller.[1] *[1]Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany; [2]Göttingen; [3]Göttingen.*

Background: Wegener´s Granulomatosis (WG) is characterized by microvascular endothelial damage and by alterations of the endothelial progenitor cell (EPC) system. Interactions between anti-Proteinase 3 antibodies and their respective antigens (PR3) on neutro-phils are pathogenetically relevant in WG. Aim of this study was (I) to analyze total circulating EPCs and regenerative activity of blood-derived EPCs, and (II) to evaluate PR3 expression patterns on circulating myelomonocytic and endothelial cells in WG.

Methods: Blood samples from WG patients were analyzed for total and for Flk-1+ myolomono-cytic cells. Healthy donors served as controls. For evaluating the proliferative activity of EPCs, a colony forming unit assay (CFU) was performed. PR3 expression by the cells was quantified by cytometric analysis. Serum Angiopoietin 1 and serum TNF-α were measured by ELISA.

Results: A total of 21 healthy donors (12 female, 9 male [40.3 ±9.2 years]) and 31 WG patients (13 female, 18 male [59.2 ±15.3 years]) were included into the study. The total percentages of EPCs were not different between the two groups. WG patients displayed lower proliferate activity of EPCs. In addition PR3 expression was significantly higher in the total as well as in the Flk-1+ (sub)population of myelomonocytic cells in WG. Finally, WG patients showed lower mean serum levels of Angiopoietin 1 and higher mean serum levels of TNF-α as compared to controls, the serum levels of both cytokines did not linearily correlate with either clinical activity or the total number of circulating EPCs or the numbers of colonies formed (EPC regeneration).

Conclusions: In addition to reduced EPC regeneration and decreased serum levels of Angiopoietin 1, both indicating impairement of the endothelial system, patients with WG show sig-nificantly increased expression of PR3 in the total and in the Flk-1+ myelomonocytic cell population. These data imply, that PR3 could be involved in the pathogenesis of microvascular endothelial damage in patients with WG.

SA-PO2209

C4d in Thrombotic Microangiopathy: Cause or Consequence? Jamie S. Chua, Hans J. Baelde, Ingeborg M. Bajema, Jan A. Bruijn, Danielle Cohen. *Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Background: Complement activation, whether caused by excessive activation or inadequate regulation, is known to play a major role in thrombotic microangiopathy (TMA). We previously showed that glomerular C4d deposition is associated to development of TMA in patients with lupus nephritis and antiphospholipid syndrome (APS). The aim of this study was to investigate whether C4d is also present in other forms of TMA and whether this marker for classical complement activation could identify patients with an antibody- or immune complex mediated TMA.

Methods: We investigated the presence of C4d and MBL depositions on 47 renal biopsies or autopsies with histologically proven TMA. Patients were divided into 2 groups: A first group with TMA in association with auto- or alloimmune disease (including SLE, APS, renal transplantation and stemcell transplantation) (n=27) and a second group of patients with clinically confirmed Hemolytic Uremic Syndrome (HUS) (n=20). Deposition patterns of C4d and MBL were scored blindly and semi-quantitatively in glomeruli, peritubular capillaries (PTC), arterioles and arterial branches.

Results: In general, C4d deposition was found in 94% of TMA cases, independent of the underlying clinical setting. Glomerular C4d deposition was present in 85% of allo- and autoimmune cases and in 80% of HUS cases (P=0,456). Arteriolar C4d deposition was found in 48% of allo- and autoimmune cases, in 60% of HUS cases (P=0,421) and was mainly observed in vessels obstructed by microthrombi. Diffuse C4d depositions in PTCs were only present in two cases of *de novo* TMA in renal allografts. Co-localization of C4d with MBL never occurred.

Conclusions: C4d is found in virtually all TMA cases, independent of the underlying clinical condition. Since C4d and MBL do not co-localize, C4d seems to represent classical pathway activation. However, rather than reflecting the cause, the classical pathway may be the consequence of severe endothelial damage or vascular remodeling in TMA. In addition, these data suggest that C5-inhibition (Eculizumab) could benefit the full spectrum of TMA patients, which is in line with recent successful results of C5 inhibitors in Shiga-toxin associated HUS.

SA-PO2210

Membranoproliferative Glomerulonephritis: Identification of New Diseases Associated Complement Genes Qian Chen,[1] Christoph Licht,[2] Gunter B. Wolf,[3] Christine Skerka,[1] Peter F. Zipfel.[1] *[1]Infection Biology, Leibniz Institue for Natural Product Researach and Infection Biology, Jena, Germany; [2]Hospital for Sick Children, Toronto, Canada; [3]Internal Medicine, University Hospital, Jena, Germany.*

Background: Membranoproliferative glomerulonephritis (MPGN) is a rare kidney disease characterized by hematuria, proteinuria and complement deposit formation, particularly at the glomerular basement membrane of the kidney. In order to define the mechanisms of this severe renal disease, we set up a European MPGN registry. Factor H-, CFHR1-, and Factor B-sequence variations, as well as copy number variations in the Factor H-CFHR gene cluster were assayed for 34 MPGN patients, as well as 67 healthy individuals. Two patients had a three nucleotide deletion causing absence of Lysine 224 in SCR4 Factor H. For the Factor B gene two major allelic variants, i.e. c.95G>A; R32Q- and c.672C>T; Y224Y appeared with higher frequencies in the patients vs controls (0.106 vs. 0.076 and 0.025 vs 0.008, respectively). The CFHR gene cluster shows different CFHR1 haploptypes as well as copy number variations. In the patient group homozygous deletion of a chromosomal segment which includes the CFHR1-CFHR3 genes was more frequent among patients as compared to the control group (Delta CFHR1 17.7%; n=6) vs the control group (Delta CFHR1 3.3%; n=2). The overall frequency of this allelic deletion was similar in both groups, which is explained by a higher frequency of the heterozygous alleles in the control group, suggesting that one copy of CFHR1 has a protective effect. Additional copy number variations were identified in MPGN patients. One patient had three allelic copies of the CFHR3/CFHR1 segment and two related patients showed a novel heterozygous deletion