ANCA reactive B cells and neutrophils cross-talk in the pathogenesis of AAV: A model proposal

P.R. Hurtado, J. Nitschke, E. Hurtado-Perez, C.A. Peh
The Royal Adelaide Hospital, Adelaide, Australia

Introduction.— The current model of AAV pathogenesis is based on the role of circulating ANCA and its effect on primed neutrophils. However, published data of patients with AAV treated with Rituximab, which remove circulating B cells, shows that clinical remission correlates more to the decreasing number of circulating B cells than decrease in ANCA titre. Given that ANCA reactive B cells can be found in circulation in patients with AAV, we would like to hypothesize that these cells play a direct role in AAV pathogenesis. Here, we propose a model whereby activated neutrophils and ANCA-reactive B cells engage in intercellular cross-talk, which could potentially lead not only to neutrophil degradation and inflammation but also to the proliferation and differentiation of ANCA-reactive B cells. The model is based on the expression of complementary molecules on activated B cells and Neutrophils, such as Lymphtoxin A (Lta) and ICAM-1 (CD54) on B cells, and LTBR, LAF-1 and BAFF (CD268) molecules on neutrophils. Membrane expression of ANCA antigens on activated neutrophils or in NETs would act as an additional activation signal for B cell differentiation and ANCA production.

Methods.— PBMC from healthy individuals, as well as purified Neutrophils and B cells were cultures in the presence of TLR ligands for 24 and 48 hours. Phenotype studies of B cells and Neutrophils were carried out using directly labelled monoclonal antibodies and analyzed by flow cytometry while the gene expression was studies by RT-PCR.

Results.— Preliminary results show expression of Lta and CD54 on B cells and LTBR and LAF-1 on Neutrophils are modulated by TLR-ligands such as LPS, viral RNA and Cp6 oligonucleotides. Given the role of these molecules on cell adhesion and activation it is reasonable to speculate on the possibility of neutrophil-B cell and the resulting cell activation. If proven to be true, the model would potentially open new opportunities for disease monitoring and novel targets for therapeutic intervention of AAV.

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Coagulation activity in renal ANCA-positive vasculitis

A.E. Ekstrand1, R.L. Lassila1, I.J.K. Joutsi-Korhonen1, A.S. Salmela2
1. Helsinki university hospital, Helsinki, Finland
2. Vaasa central hospital, Vaasa, Finland

Introduction.— Incidence of thromboembolism in ANCA-associated vasculitis (AAV) is high (1–3). The profile of coagulation and fibrinolysis in AAV patients remains poorly characterized and we aimed to study it in association with disease activity in a prospective case control setting.

Methods.— Twenty-one AAV patients with renal disease (median eGFR 21 ml/min) were compared with controls: 20 patients having other mild chronic kidney disease (CKD) (group 2, eGFR 91) and 20 patients with moderate CKD (group 3, eGFR 44). Platelet count, anti-thrombin (AT), FVIII:C and von Willebrand factor ristocetin cofactor activities (VWF:RCo), VWF antigen (VWF:Ag), fibrinogen, prothrombin fragments (F1 + 2) and fibrin degradation product D-dimer were measured during the active and remission states of the disease and reported as median. Results.— The F1 + 2 was 2.6-fold and D-dimer 5-fold higher during the active AAV than in its remission (563 vs 212 pM and 3.0 vs 0.6 mg/L, P < 0.01 for both). During active AAV these values clearly exceeded also those of the control group 2 (F1 + 2 2164 pM, P < 0.001; D-dimer 0.2 mg/L, P < 0.013) and group 3 (F1 + 2 2244; D-dimer 0.3, P < 0.01 for both). Platelet counts and fibrinogen decreased during active AAV compared with the remission (294 vs 219 109/L, P < 0.011 and 6.4 vs 4.9 g/L, P = 0.022). Again, FVIII:C (22%), VWF:RCo (198%) and VWF:Ag (222%) were the highest among patients with active AAV, but remained elevated at remission. Interestingly, AT reached supranormal levels towards remission in AAV (101 vs 115%, P < 0.01). In AAV patients, two thromboembolic events occurred during the follow-up.

Conclusion.— Thrombin formation and especially fibrin turnover prevail during active AAV compared both with remission and other kidney

Tissue destruction in granulomatosis with polyangitis: Common histological pattern in mice and men

1. University medical, center Hamburg-Eppendorf, Hamburg, Germany
2. University hospital of Schleswig-Holstein, campus Kiel, Kiel, Germany
3. University hospital of Schleswig-Holstein, campus Lübeck, Lübeck, Germany

Introduction.— Tissue destruction related to Granulomatosis with Polyangiitis (Wegener’s)–(GPA) often affects the upper respiratory tract and is still poorly understood. Recent findings in a xenograft model of GPA in immunodeficient mice suggest that fibroblasts are key players in the destruction of human cartilage. In this work we compare morphological/cellular patterns of tissue destruction in GPA patients and GPA xenografts in immunodeficient mice.

Methods.— Nasal biopsies from GPA patients (n = 8) containing cartilage and/or bone fragments were evaluated for destruction by conventional histology. Cellular patterns, markers of differentiation and proliferation were characterized by immunohistochemistry (vimentin, CD68, TRAP, MMP1/3/13, CD31, IL-17RC). Xenografts of nasal mucosa from GPA patients with active endonasal disease (n = 10) and from sinusitis control patients were cotransplanted with healthy human cartilage in immunodeficient pfp/rag2−/− mice and were investigated in parallel.

Results.— Samples from GPA patients and xenografted GPA tissues displayed GPA-related cartilage/bone destruction. Anti-human vimentin and MMP1/3 staining showed fibroblasts to be the main mediators of tissue destruction in xenografts. Human tissues showed a more complex cellular pattern at sites of destruction including the expression of TRAP, IL-17-RC and CD31. This destruction is mediated by invading cells, not by necrosis or ischemia due to vasculitis. Nonetheless, the morphology of destruction was very similar in tissue samples from both sources.

Discussion.— Nasal cartilage/bone destruction in GPA is a cellular mediated process independent from the human circulation. Fibroblasts are dominant at sites of cartilage/bone destruction in GPA patients and GPA xenografts.

Conclusion.— New treatments focusing on fibroblast-mediated tissue destruction as MMP expression and cell migration offer new therapeutic options for tissue destruction in GPA refractory to standard treatment. Such therapeutic strategies can now be evaluated in our xenograft model.

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