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

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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 Lyphoptokin 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 CpG 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.

http://dx.doi.org/10.1016/j.lpm.2013.02.256

Coagulation activity in renal ANCA-positive vasculitis

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Introduction.—Incidence of thrombembolism 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, antithrombin (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 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 dimer 5-fold higher during the active AAV than in its remission (563 vs 212 P < 0.01 for both). During active AAV these values clearly exceeded also those of the control group 2 (F1 + 2 1 + 2 M, P < 0.01 for both). Platelet counts and fibrinogen increased during active AAV compared with the remission (294 vs 219 P < 0.011 and 6.4 vs 4.9 g/l, P < 0.022). Again, FVIII:C (228%), VWF:RCo (198%) and VWF:Ag (222%) were the highest among patients with active AAV, but remained elevated at remission. Interestingly, AT reached supernormal 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

P187

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

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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, IL17-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.

http://dx.doi.org/10.1016/j.lpm.2013.02.257

P185
Glycosylation and sialylation of anti-proteinase 3 antibodies in granulomatosis with polyangiitis is associated with increased inflammatory cytokines

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Introduction. – Interaction between immunoglobulin G (IgG) and its receptors is determined by glycosylation of Asn297 on the antibody’s Fc portion. Previous in vitro studies have demonstrated that T cell receptors is determined by glycosylation of Asn297 on the antibody’s Fc portion. Previous in vitro studies have demonstrated that T cell

Methods. – Serum was collected from 38 healthy controls (HC) and 49 patients with GPA and anti-proteinase 3 (PR3) antibodies. IgG was purified from serum using Protein A-Sepharose beads. Anti-PR3 specific IgG (aPR3) was isolated using a PR3 specific ELISA plate. Glycosylation of IgG was determined by mass spectrometry. Clinical data was collected prospectively on 10 GPA patients (GPAc) and serum cytokines were measured by multiplex assay. All patients gave informed consent.

Results. – aPR3 was only isolated from patients with GPA and was predominantly IgG1. Agalactosylated total IgG1 was more common in GPA than HC (52% vs 29%; P < 0.001) and agalactosylation was even more common in aPR3 specific IgG1 (59%; P = 0.006). Galactosylation (Gn) and sialylation (Sn) were lowest in aPR3, low in GPA IgG1 and highest in HC IgG1. Although reduced Gn and Sn were associated with GPA within the 10 GPAc patients there were positive correlations between cytokine concentrations, aPR3 (but not IgG1) Gn and time to remission (table I). There were less significant correlations between aPR3 Sn and cytokines. There were no correlations between Gn or Sn of GPA IgG1 and aPR3. There was no correlation between Gn or Sn and Birmingham Vasculitis Activity Score.

Discussion. – Our data suggests that although reduced Gn and Sn is associated with GPA compared to HC the proinflammatory cytokine environment in patients drives increased Gn of autoantibodies and is associated with an increased time to remission indicating that T cell activity may be important in determining autoantibody glycosylation and time to remission.

http://dx.doi.org/10.1016/j.lpm.2013.02.258

P189 LAMP-2, a potential novel receptor on human monocytes derived dendritic cells (MDDC)

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Introduction. – Pauci-immune-focal-necrotizing-glomerulonephritis (FNGN) is an inflammatory disease associated with autoantibodies (ANCA). Lysosomal-membrane-protein 2 (LAMP-2) is a recently defined target of circulating autoantibodies in addition to the more commonly known targets [1]. LAMP-2 is a type-2 membrane-protein that is used as lysosome and late endosomal marker and with essential roles in chaperone mediated autophagy and antigen presentation [2]. LAMP-2 is also present on the cell-surface although its role was never studied. Here, we focus on the role of LAMP-2 as a specific endocytic receptor acting on freshly isolated monocytes and iDCs with possible implication in antigen presentation.

Methods. – Monocytes Derived Dendritic Cell and activation CD14+ monocytes are isolated from PBMCs using MACS, culture along 7 days with GM-CSF and IL-4. The activation status is checked using CD80, CD83 MHCI by FACS. Uptake experiment DCs are co-cultured with anti LAMP-2 antibody and LPS stimulated DCs. Uptake was absent in MDDC from an individual with genetic LAMP-2 deficiency. As expected, LAMP-2 is a inflammatory disease associated with autoantibodies (ANCA). Lysosomal-membrane-protein 2 (LAMP-2) is a recently defined target of circulating autoantibodies in addition to the more commonly known targets [1]. LAMP-2 is a type-2 membrane-protein that is used as lysosome and late endosomal marker and with essential roles in chaperone mediated autophagy and antigen presentation [2]. LAMP-2 is also present on the cell-surface although its role was never studied. Here, we focus on the role of LAMP-2 as a specific endocytic receptor acting on freshly isolated monocytes and iDCs with possible implication in antigen presentation.

Discussion. – Circulating autoantibody directed against LAMP-2 cause direct activation of DCs and increase dextran uptake, therefore It is probable that this antibody are responsible for breaking of the tolerance leading to ANCA production. Biological implication implies the possibility that H4B4 mimic other immunogenic molecules.

http://dx.doi.org/10.1016/j.lpm.2013.02.259

Table I

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<td>% aPR3 Gn</td>
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