Introduction.— High mobility group box 1 (HMGB1) is a nuclear protein that acts as an alarm when released by necrotic or activated cells. High serum HMGB1 levels have been associated with active nephritis as well as with granulomatous manifestations in patients with antineutrophil cytoplasmatic antibody (ANCA)-associated vasculitis (AAV). This study aims to evaluate HMGB1 levels, CD4+ T-cells and effector memory T-cells (CD4+ TEM) in urine from patients with AAV and active nephritis.

Methods.— Twenty-four AAV patients with active nephritis and 10 healthy controls (HC) were evaluated. Diagnosis of GPA or microscopic polyangiitis (MPA) was based on the European Medicines Agency algorithm whereas patients with isolated renal involvement, ANCA positivity and/or biopsy-proven pauci-immune necrotizing glomerulonephritis were classified as renal limited vasculitis (RLV). Urinary levels of HMGB1 were measured by Western blot while CD4+ T-cells and CD4+ TEM cells (CD4+CD45+RO+CCR7−) in urine were analyzed by flow cytometry.

Results.— Median urinary intensity of HMGB1 was higher in AAV patients than in HC (5.15 (IQR 3.52–9.25) vs. 2.58 (1.88–4.24); P = 0.006) whereas no difference was found among different AAV subsets regarding median urinary intensity of HMGB1 (GPA: 4.1 (2.6–16.4) vs. MPA: 5.1 (4.4–5.6) vs. RLV 5.7 (1.4–13.6); P = 0.951) and HMGB1/creatinine ratio (GPA: 0.93 (0.29–3.91) vs. MPA: 1.29 (0.90–1.89) vs. RLV 0.54 (0.14–1.28); P = 0.186). Urine levels of HMGB1 were positively correlated with CD4+ T-cell counts in urine (rho: 0.407; P = 0.034) whereas there was a tendency for a positive correlation with CD4+ TEM cells and CD4+ TEM cells (CD4+CD45+RO+CCR7−) in urine. No correlation could be found between urine levels of HMGB1 and 24 hour proteinuria (rho: 0.065; P = 0.390) or creatinine clearance (rho: −0.049; P = 0.832).

Conclusion.— Urinary HMGB1 levels are increased in AAV patients with active nephritis when compared to HC and are associated with CD4+ T-cells in urine. These findings suggest that urine HMGB1 is a biomarker for renal activity in AAV.

Further readings

http://dx.doi.org/10.1016/j.lpm.2013.02.263

P193

HMGB1 in ANCA-associated vasculitis: A longitudinal study
A.W. Silva De Souza1, J. Westra1, J. Bijzet1, P.C. Limburg1, M. Bijl2, C.A. Stegeman2, C.G.M. Kallenberg2
1. University medical center Groningen, Groningen, Netherlands
2. Martini hospital, Groningen, Netherlands

Introduction.— Extra-cellular high mobility group box 1 (HMGB1) acts as an alarm and has been shown to be a biomarker of disease activity in systemic lupus erythematosus (SLE). This study aims to assess anti-HMGB1 antibodies and HMGB1 levels as biomarkers for disease activity and predictors of relapsing disease in ANCA-associated vasculitis (AAV).

Methods.— AAV patients with active disease and healthy controls (HC) were evaluated for anti-HMGB1 antibodies by an in house ELISA while serum HMGB1 levels were measured longitudinally in AAV patients at presentation, during remission, prior to and at relapses by a commercial ELISA kit.

Results.— HMGB1 levels were similar between AAV patients at presentation (n = 52) and HC (n = 35) (2.64 ± 1.80 ng/ml vs. 2.39 ± 1.09 ng/ml; P = 0.422). AAV patients with renal involvement had lower HMGB1 levels than patients without renal involvement at presentation (2.35 ± 1.48 ng/ml vs. 3.52 ± 2.41 ng/ml; P = 0.042). A negative correlation was observed between HMGB1 levels and 24-hour proteinuria (P = −0.361, P = 0.028). Forty-nine AAV patients were evaluated for HMGB1 levels during follow-up and no differences were observed between relapsing and non-relapsing patients (P = 0.350). No significant increase in HMGB1 levels was observed prior to a relapse comparing to the remission period and changes in HMGB1 levels were not associated with an increased risk for relapse in AAV. Positivity for anti-HMGB1 antibodies was low in patients with active AAV (3 out of 24 patients).

Conclusion.— Serum HMGB1 levels at presentation are lower in patients with renal involvement. Relapses are not preceded or accompanied by significant rises in HMGB1 levels and changes in HMGB1 levels are not related to ensuing relapses. Anti-HMGB1 antibodies are present in only a few patients in AAV. In contrast to SLE, HMGB1 does not seem to be a useful biomarker for disease activity and relapses in AAV.

Further readings

http://dx.doi.org/10.1016/j.lpm.2013.02.264

P194

Functionally effect regulatory B cells in patients with active ANCA vasculitis
UNC, Chapel Hill, USA

Introduction.— Immunopathogenesis of ANCA disease is B cell dependent; yet, how B cell subsets contribute is unknown. CD19+CD24hiCD38hi Bregs play a role in immunological tolerance by suppression of TH1 cells via IL-10 secretion. We hypothesize that a decrease in functional, IL-10 producing B cells is a component of B cell dysregulation that accompanies active ANCA disease.

Methods.— We examined 140 ANCA patients samples and 19 healthy controls by flow cytometry. To determine the competency of B cell subsets to produce IL-10 in patients with ANCA disease, PBMCs were stimulated with CD40 ligand and CpG DNA and processed for intracellular staining of IL-10.

Results.— Patients with ANCA disease had similar percentages of Bregs as healthy individuals: 14% vs 10%; p = 0.2. In sub-group analysis, active MPO ANCA patients had significantly less Bregs than those in remission (14.3 vs 7; P = 0.01). In contrast, regardless of Breg percentage, B cells from patients with active disease produced significantly less IL-10 than patients in remission (15%); P = 0.0096). Patients in remission are similar to healthy individuals with regard to IL-10 producing B cells (26%) and 26% (P = 0.9), respectively.

Discussion.— ANCA patients and healthy individuals had similar percentages of Bregs; yet, active ANCA patient B cells produced less IL-10. These data suggest that Bregs are present in patients with active ANCA disease but functionally impaired.

http://dx.doi.org/10.1016/j.lpm.2013.02.265