Conclusion. – In conclusion, this data suggests that when LAMP-2 is on the membrane acts as a specific receptor for internalization of extracellular molecules. After internalization, the ligand can be found into the MIMC causing as well the maturation of DCs up-regulating CD80/83.

References

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P190
Autoantibodies for several antigens in neutrophil cytoplasm other than PR3 and MPO also promote release of NETs from neutrophils
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Introduction. – Neutrophil extracellular traps (NETs) were first described as web-like structures that trap and neutralize microbes at sites of infection. NETs are comprised of chromatin components and neutrophil cytoplasmic proteins, and it has been reported that NETs are involved in autoimmunity such as SLE or ANCA-associated vasculitis (AAV). It has also been reported that autoantibodies themselves for important cytoplasmic autoantigens of neutrophils such as MPO and PR3 induce NETs production. However, the role of other neutrophil cytoplasmic antigens for the production of NETs is unclear. We investigated how autoantibodies for these antigens are involved in NETs productions.

Methods. – Human peripheral blood neutrophils were obtained from healthy donors. Neutrophils were cultured with PMA (control) or PMA plus several kinds of anti-neutrophil cytoplasmic antigens for 3 hours. Cells were stained with Hoechst 3,3342, Sytox Green and anti-MPO antibody. The percentage of NETs producing cells and the quantitation of nuclear decondensation were analyzed using Image J software. We distinguished between cells that released NETs fiber and cells that just died of NETosis.

Results. – Anti-MPO antibody strongly promoted both NETosis induction and release of NETs fiber. Although anti-PR3 antibody promoted NETosis induction, it did not increase the release of NETs fiber. Antibody for cathepsin G, found in the azurophil granule, also promoted NETosis induction only. There were some other antibodies, such as anti-lactoferrin and anti-neutrophil elastase, that promoted both NETosis and release of NETs fiber.

Discussion. – It has been thought that ANCA-es have an important role in the pathogenesis of AAV partly because ANCA promotes NETs production by neutrophils at sites of vasculitis. We showed that not only anti-MPO and anti-PR3 but also antibodies for other neutrophil cytoplasmic antigens promote NETs production. The mechanism these antibodies induce NETosis and what they do in the pathogenesis of AAV are to be elucidated.

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P191
Antibodies to LAMP-2 alter lysosome function and attenuate chaperone-mediated autophagy in human macrophages
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Introduction. – Lysosome-associated membrane protein-2 (LAMP-2) is membrane protein that traffics from the cell surface to lysosomes. It maintains lysosomal integrity and is rate limiting for chaperone-mediated autophagy (CMA). Anti-LAMP-2 antibodies are frequent in patients presenting with ANCA-associated vasculitis (AAV) [1,2]; whether they affect LAMP-2 function is unknown. Here, we determine whether antibodies to LAMP-2 compromise lysosome function and affect cell survival of human macrophages.

Methods. – Human THP-1 cells and monocyte derived macrophages (MDM) incubated alone or together with monoclonal antibodies to LAMP-2 (H4B4) or isotype control (CD4). IgG uptake was measured by confocal microscopy and Western blotting (WB); and viability and apoptosis by trypan blue and caspase-3. Lysosomes were purified (lysosome kit) and their proteins quantified by WB and fluorescence. CMA was assessed by lysosomal acquisition of HSc70 and import of cytoplasmic proteins.

Results. – H4B4 was selectively taken up by THP-1 cells and significantly increased apoptosis and cell death after 24 hours. It accumulated in lysosomes and significantly reduced LAMP-2 but not LAMP-1: CD4 did not. Lysosomal HSc70 was also reduced and correlated with LAMP-2 – implying a reduction in basal CMA. H4B4 abrogated of the stress-induced CMA response as shown by measuring the CMA substrate GAPDH and the reduced import of cytosolic proteins. H4B4 also increased lysosomal pro-cathepsin D but not mature cathepsin D, suggesting lysosomal integrity was also affected. H4B4 had similar on MDM.

Discussion. – H4B4 is specifically taken up by macrophages and traffics to lysosomes where it reduces membrane LAMP-2 and disrupts function. It markedly inhibits basal CMA and abrogates its stress-induced augmentation. There are clear implications for macrophage function is obvious not least because CMA is critical for antigen presentation to T cells.

Conclusion. – H4B4-triggered CMA dysfunction provides the relevant molecular mechanism for cell death by antibodies to LAMP-2.

References

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P192
Urinary HMGB1 levels are associated with CD4+ T-cells in urine in patients with ANCA-associated vasculitis and active nephritis
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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 in urine (rho: 0.336; P = 0.068). 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 finding suggest that urine HMGB1 is a biomarker for renal activity in AAV.

Further readings

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P193
HMGB1 in ANCA-associated vasculitis: A longitudinal study
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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

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P194
Functionally effect regulatory B cells in patients with active ANCA vasculitis
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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 flowcytometry. 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.

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