investigate whether circulating HMGB1 levels is associated with disease activity in AAV.

Methods.-- Plasma samples from 74 patients with AAV in active stage and 46 patients with AAV in remission were collected. The plasma levels of HMGB1 were determined by ELISA. Associations between plasma levels of HMGB1 with clinical and pathological parameters were analyzed.

Results.-- Plasma levels of HMGB1 in active AAV patients were significantly higher than those in normal controls (6.11 (3.25-12.79) ng/ml vs. 1.12 (0.53-1.39) ng/ml, P < 0.001) and AAV patients in remission (6.11 (3.25-12.79) ng/ml vs. 3.15 (2.30-4.10) ng/ml, P < 0.001). Among the patients with active AAV, plasma levels of HMGB1 in PR3-ANCA positive patients were significantly higher than those in MPO-ANCA positive patients (16.36 (4.86-27.36) ng/ml vs. 5.96 (3.13-11.83) ng/ml, P = 0.042). Correlation analysis showed that plasma levels of HMGB1 correlated with initial Scr (r = 0.275, P = 0.018), eGFR (r = -0.277, P = 0.017), the Birmingham Vasculitis Activity Score (BVAS) (r = 0.308, P = 0.008) and C-reactive protein (CRP) (r = 0.309, P = 0.008). Among the patients with MPO-ANCA, those within the first quartile of plasma HMGB1 levels had significantly lower level of MPO-ANCA than those within the other three quartile.

Conclusion.-- Circulating HMGB1 level might reflect disease activity and renal involvement of ANCA-associated vasculitis.

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Pentraxin-3 and soluble tumor necrosis factor-like weak inducer of apoptosis (s-TWEAK) in anti-neutrophil antibody associated vasculitis (AAV)

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Introduction.-- The novel inflammatory mediators PTX3 and s-TWEAK have recently gained a lot of attention in the field of autoimmune disease, although little studied in AAV. PTX3 is a CRP like protein synthesized by vascular endothelial cells and macrophages amongst others. It is believed to more closely reflect local tissue inflammatory activity than CRP which is synthesized in the liver. TWEAK is a recently discovered member of the TNF superfamily with, through binding to its receptor Fn14, mediates different biological effects including exacerbation if the inflammatory response via induction of the NF-kB signaling pathway. The aim of this study was to measure s-TWEAK and PTX3 in active AAV and to correlate to disease activity and other clinical markers.

Methods.-- A total of 40 consecutive patients (20 men, 20 women, median age 58 years) with active AAV (33 new and seven with relapsing disease) were included in the study and followed longitudinally. Twenty-four patients were diagnosed with GPA, 15 with MPA and one with CSS. Disease activity was assessed using BVAS at baseline and at remission at 6 month. Levels of PTX3 and s-TWEAK were measured with an ELISA method together with CRP and creatinine. s-TWEAK was also measured in 20 healthy controls.

Results.-- PTX3 was significantly elevated at baseline compared to follow-up (4.66 ng/ml vs 1.75 ng/ml, P = 0.0003) and correlated to BVAS at baseline (rho 0.43, P = 0.006) but not to CRP or creatinine. Levels of s-TWEAK on the other hand remained unchanged throughout the study and compared to healthy controls.

Conclusion.-- PTX3 correlated well with disease activity independently of CRP and may be a biomarker worth exploring in AAV. s-TWEAK was not elevated in active AAV compared to remission or in healthy controls. These results do not exclude that TWEAK plays a role in the pathogenesis of AAV by tissue expression but do not support an important role for circulating s-TWEAK.

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P180

Epitope analysis of anti-myeloperoxidase antibodies in propylthiouracil-induced ANCA-associated vasculitis

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Introduction.-- Increasing evidences have suggested the linear epitopes of antineutrophil cytoplasmic antibody (ANCA) directed to myeloperoxidase (MPO) might provide clues to the pathogenesis of propylthiouracil (PTU)-induced ANCA-associated vasculitis (AAV). The current study mapped epitopes of MPO-ANCA in sera from patients with PTU-induced MPO-ANCA (with or without vasculitis) and primary AAV, aiming to analyzing certain epitopes associated with the development of PTU-induced AAV.

Methods.-- Six recombinant linear fragments, covering the whole length amino acid sequence of a single chain of MPO, were produced from E. coli. Sera from 17 patients with PTU-induced AAV, 34 patients with PTU-induced MPO-ANCA but without clinical evidence of vasculitis and 64 patients with primary AAV were collected at presentation. Twelve of the 17 patients with PTU-induced AAV also had sera at remission. The epitope specificities were detected by enzyme-linked immunosorbent assay using the recombinant fragments as solid phase ligands.

Results.-- Sera of PTU-induced AAV patients had a significantly higher reactivity to P fragment compared with primary AAV patients (52.9% vs. 14.1%, P < 0.001). Among the 12 PTU-induced AAV patients with sequential samples, the number of fragments recognized in remission was significantly less than that in initial onset (2.12 ± 1.90 vs. 0.42 ± 0.90, P < 0.001). Moreover, in patients with PTU-induced AAV sera in active stage had a significantly higher binding rate to P fragment than in remission (52.9% vs. 16.7%, P = 0.046).

Conclusion.-- Linear epitopes of MPO molecule might be associated more closely with the development of PTU-induced AAV. In particular, the P fragment might be an important epitope in PTU-induced AAV.

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P181

IL-10 producing regulatory B-cells are diminished in ANCA-associated vasculitis

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Introduction.– B-cells have immuno-regulatory function acting as antigen-presenting cells. A separate subset of IL-10 producing B-cells (Bregs) has been identified in humans and mice regulating T-cell mediated immunity. In murine models, depletion of Bregs aggravates autoimmune responses. Furthermore, a defect of Bregs in human systemic lupus erythematosus has been reported. In the present study, we hypothesized that Bregs may be diminished or also defective in ANCA-associated vasculitis (AAV).

Methods.– Twelve healthy controls (HC) and 31 patients with AAV were enrolled. 22 AAV patients were in remission (AAV-r). 16 out of the AAV-r patients were untreated, 6 received immunosuppressive therapy at the time of sampling. 9 AAV patients presented with active disease (AAV-a) at the time of sampling. Bregs were defined as IL-10+ CD19+ B-cells. Bregs and T-cell subsets were determined via flow cytometry. Data is given as mean ± standard deviation.

Results.– Patients in remission and with active disease showed a diminished fraction of Bregs as compared to HC (CD19+ B-cells: %IL-10+: 8.9 ± 3.6% vs. 12.5 ± 3.2%, P = 0.01 and 6.7 ± 3.2% vs. 12.5 ± 3.2%, P = 0.001). IfN production by T-cells was negatively associated with Breg numbers (r = -0.69, P = 0.005) in untreated AAV-r. This association was not evident in active vasculitis or HC (r = -0.32, P = 0.68 and r = -0.10, P = 0.88).

Conclusion.– IL-10 producing B-cells are diminished in AAV. Furthermore, Bregs might regulate T-cell activation in quiescent AAV. This regulatory pathway may be lost in active AAV.

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**P182**

**Interleukin-21, B cell activating factor and unmethylated CpG oligodeoxynucleotides synergize in promoting anti-Proteinase 3 autoantibody production in vitro**

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Introduction.– Unmethylated CpG oligodeoxynucleotides (CpG-ODN), which resemble bacterial DNA, enhance ANCA production in vitro [1,2]. Recent studies have highlighted the role of IL-21 in plasma cell formation and antibody production by synergizing with B cell activating factor (BAFF). This study examined the possible contribution of CpG-ODN, IL-21, and BAFF in ANCA production.

Methods.– Peripheral blood mononuclear cells (PBMC) from 29 patients with PR3-AAV and 15 healthy controls (HC) were cultured in vitro for 12 days in the presence of BAFF and IL-21, with or without CpG-ODN. IgG production was measured by Phadia EliA and expressed in response units (RU). CpG-ODN effects on IL-21 receptor (IL-21R) expression on B cells, and influence of BAFF/IL-21/CpG-ODN treatments on B cell proliferation and plasma cell formation were analyzed by flow cytometry.

Results.– Stimulation with BAFF and IL-21 significantly increased IgG production in HC and patients and ANCA production in patient samples (RU median 1.36 (range 0.14–196.7) compared to 0.12 (0.00–0.14) in HC). ANCA production could be further augmented by addition of CpG-ODN (median 2.08 (0.14–239.4) versus 0.16 (0.10–0.28) in HC). CpG-ODN increased the percentage of IL-21R positive B cells from 57.5 ± 5.3% to 89.4 ± 1.3%. The combined treatment with BAFF, IL-21 and CpG-ODN markedly enhanced B cell proliferation compared to CpG-ODN or BAFF/IL-21 effect alone (CpG-ODN: 2.6 ± 1.2% vs BAFF/IL-21: 3.5 ± 1.1% vs BAFF/IL-21/CpG-ODN: 12.0 ± 5.0%). Furthermore, CpG-ODN induced plasma cell formation but this was not further enhanced by addition of BAFF/IL-21 (unstimulated: 0.9 ± 0.74% vs CpG-ODN: 34.3 ± 10.4% vs BAFF/IL-21/CpG-ODN: 29.8 ± 5.0%).

Conclusion.– IL-21, BAFF and CpG-ODN synergize in promoting IgG and PR3-ANCA production in vitro. This effect was associated with substantial B cell proliferation and CpG-ODN mediated plasma cell formation and IL-21R upregulation on B cells. Research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 261382.

References


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**P184**

**DNA from neutrophil extracellular traps is hypomethylated**

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Introduction.– Upon activation, neutrophils are capable of releasing neutrophil extracellular traps (NETs), in the form of decondensed DNA coated with microbial granule proteins, in order to trap and kill bacteria. NETs have been suggested as having a potential role in innate immunity due to their capability to stimulate pDCs. However, the mechanisms behind this immunostimulatory property of NETs remain elusive.

Methods.– The global methylation status of NET derived DNA was determined using a commercial methyl-Cytosine competition ELISA. We assessed the potential immunostimulatory properties of NET derived DNA by its ability to stimulate plasmacytoid dendritic cells, and IL9 reporter cells compared to normal genomic DNA.

Results.– NET DNA is significantly hypomethylated compared to genomic DNA from resting neutrophils and PBMCs. We found that NET DNA stimulated PBMCs to produce interferon-α and also IL9 reporter cells, but not after NET DNA had been treated with CpG methyltransferase. These results suggest that DNA hypomethylation during NETosis contributes NET DNA with the ability to stimulate IL9. We hypothesize that neutrophil DNA actively becomes hypomethylated during NETosis, and that this epigenetic modification may serve as a potential means to amplify the immune response during bacterial infection.

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