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). IFNγ 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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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 ELISA and PR3-ANCA production was quantified 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 ± 3.3% 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 microbicidal 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 TLR9 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 TLR9 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 TLR9.

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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