REGULATION OF EXPRESSION AND FUNCTION OF NEGATIVE IMMUNOMODULATORY RECEPTORS IN B-CELLS: IMPLICATIONS FOR THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Background and Objectives Fine tuning of B-cell activation and differentiation depends on convergent signals from the B-cell receptor (BCR), costimulatory/coinhibitory membrane receptors, and Toll-like receptors. We sought to examine the expression and function of the coinhibitory receptors programmed death-1, PD-1, programmed death-ligand-1, PD-L1, and B and T lymphocyte attenuator (BTLA) in B-cells from healthy donors, and from patients with systemic lupus erythematosus (SLE), the prototype of systemic autoimmune disease characterised by activated B-cells and production of high-titre autoantibodies in long-lived plasma cells.

Materials and Methods Peripheral blood CD19+ B cells were purified from healthy donors (n = 11) and active SLE patients (n = 15; SLE disease activity index 8.3 ± 2.7 [mean ± SEM]). PD-1, PD-L1, and BTLA were examined by flow cytometry in naïve (CD19+CD27+), memory/transitional (CD19+CD27-), and plasma B-cells (CD19+CD27+) at baseline and following stimulation. Activation, differentiation, and proliferation (CFSE dilution) of B-cells were examined in the presence or absence of the BTLA ligand, HVEM. Western blot was used to assess the phosphorylation of intracellular kinases.

Results In healthy donors, the coinhibitory receptors PD-1 and PD-L1 were significantly upregulated on circulating plasma cells compared to transitional/memory and naïve B-cells (PD-1: 36 ± 7%, 14 ± 3%, 2.0 ± 0.5%; PD-L1: 94 ± 2%, 83 ± 5%, 62 ± 8%, respectively, p < 0.001). BTLA was expressed by 93–100% of B-cells, and mean fluorescence intensity was significantly higher in plasma cells (334 ± 146 versus 127 ± 12 in naïve B-cells, p = 0.048). BCR activation enhanced the expression of all three receptors in normal B-cells; addition of CpG-ODN (TLR-9 ligand) further induced PD-1 and PD-L1 but not BTLA-expression, whereas addition of the cytokines IL-4, IL-10, or IL-21 reduced PD-1 and BTLA levels. In vitro crosslinking of BTLA resulted in reduction of BCR-induced phosphorylation of ERK, CD80/CD86 and BAFF-receptor expression, as well as in inhibition of cell proliferation (divided cells: 5.3 ± 0.4% versus 17.7 ± 0.1% in anti-IgM-stimulated cells). In comparative analysis, SLE patients exhibited significantly higher PD-1 expression on plasma-cells compared to healthy donors (65 ± 8% versus 36 ± 7%, p = 0.002), whereas there was no difference in PD-L1 or BTLA. Preliminary studies suggest distinct roles for PD-1 and BTLA in regulation of activation and maturation of B-cells in healthy controls and in the context of lupus.

Conclusions The coinhibitory receptors PD-1, PD-L1 and BTLA demonstrate differential expression among B-cell subsets and they are induced upon stimulation with important implications for the regulation of B-cell activation, proliferation and differentiation. aberrancies in the expression and function of coinhibitory receptors in SLE plasma B-cells could contribute to enhanced autoantibody-forming capacity and disease pathogenesis.