IL-4 acts through aryl hydrocarbon receptor to antagonize TLR7 induced double negative 2 B cells in lupus

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We recently showed that in systemic lupus erythematosus (SLE), IL-4R signaling is a powerful antagonist that can effectively suppress the development of activated naïve (aNAV) and CD11c⁺T-bet⁺ IgD⁻CD27⁻ double negative 2 (DN2) B cells promoted by both type I and type II IFNs. In the present study, we used the BXD2 mouse model of lupus to determine the mechanism of IL-4 in suppressing the development of DN2 B cells in vivo. Administration of IL-4 significantly inhibited the development of anti-Smith, anti-DNA, and anti-histone autoantibodies induced by TLR7 agonist R848 in BXD2 mice. This was associated with a decreased percentages of CD11c⁺T-bet⁺ IgD⁻ B cells. Feature-barcoding single cell RNA-sequencing analysis showed that IL-4 modulated B cell development at the transitional stage 2 (T2) and skewed naïve B cells to develop into the CD23⁺CD21⁻ follicular B cells. IL-4 induced the gene encoding interleukin-4-induced1 (IL4i1), an enzyme that metabolizes aromatic amino acids and this was associated with the upregulation of aryl hydrocarbon receptor (AhR) and downstream genes. Metabolomics analysis revealed IL-4 induced AhR agonistic metabolites in B cells including kynurenine (Kyn), indole-3-acetic acid, and indole-3-lactic acid. In the absence of IL-4, Kyn and a potent AhR agonist, formylindolo[3,2-b]carbazole (FICZ), significantly suppressed TLR7 plus IFNβ-induced DN2 B-cell development in vitro. Our results suggest that IL-4 acts through the IL4i1-AhR pathway to inhibit B-cell regulatory response to TLR7 and type I IFN. Identification of small molecular metabolites that act directly in B cells to induce homeostasis may lead to development of orally dosed metabolome modulating therapeutics efficacious in the treatment of SLE.