**Low dose IL-2 enhances the generation of IL-10-producing immunoregulatory B cells**

**Introduction:** Regulatory B cells (Bregs) have been shown to ameliorate disease severity in murine models of lupus nephritis, multiple sclerosis, inflammatory bowel disease, allergy and transplant rejection, principally via the cytokine interleukin-10 (IL-10), but whether Bregs can be induced in humans by therapeutic manipulation is currently unknown.

**Methods:** We undertook a transcriptomic analysis of IL-10-producing B cells in humans to delineate cytokine receptor expression, followed by *in vitro* stimulation of murine and human peripheral blood and splenic B cells with IL-2 to assess the impact on cytokine production. B cells were also co-cultured with CD4 T cells to measure their immunoregulatory capacity. Finally we tested the *in vivo* relevance of our observations by assessing IL-10-producing B cells in mice and n= 6 patients treated with low dose IL-2 (Proleukin).

**Results:** IL-10-producing B cells expressed transcripts of all components of the IL-2 receptor (CD25, CD122 and CD132) and CD25 was significantly higher on IL10+ versus IL10- B cells. We found that surface CD25 was upregulated on a subset of mouse and human splenic B cells following stimulation with toll-like receptor (TLR) agonists and CD40L, rendering these cells receptive to IL-2. The addition of IL-2 to these activated B cells significantly augmented IL-10 production, whilst pro-inflammatory cytokines such as IL-6 and tumour necrosis factor alpha (TNF-α) were unchanged, resulting in a skewing of B cells towards a regulatory phenotype. Consequently, co-culture of IL-2-treated B cells with activated CD4 cells led to a reduction in T cell production of TNF-α. *In vivo*, in mice and patients treated with low dose IL-2, we observed a significant increase in IL-10-producing B cells.

**Conclusions:** Together, our data suggest that low dose IL-2 may be a useful strategy to promote the generation of regulatory B cells *in vivo*, with therapeutic implications for autoimmune disease.