Optimisation of *in vitro* assays to assess cytokine production by human primary B-cells

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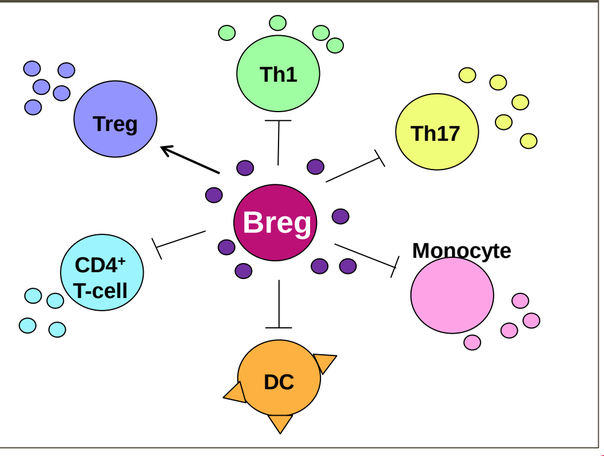
Background

- B-regulatory cells (Bregs) produce anti-inflammatory cytokine IL-10 to control and dampen immune cells (Lighaam et al., 2018).
- As shown in Figure 1, Bregs have many functions including:
  - Suppression of TNFα production by monocytes, downregulating Th1 and Th17 differentiation (thus reducing IFNγ and IL-17 production), and inhibiting $CD4^+$ proliferation (Flores-Borja et al., 2013; Iwata et al., 2011).
  - Impairing antigen presentation, and increasing differentiation of naïve $CD4^+$ T-cells into T-regulatory cells (Tregs) that secrete TGF-β and IL-10 (Bankó et al., 2017).
- Bregs have a protective role in inflammatory diseases, such as SLE and RA (Blair et al., 2010; Flores-Borja et al., 2013).

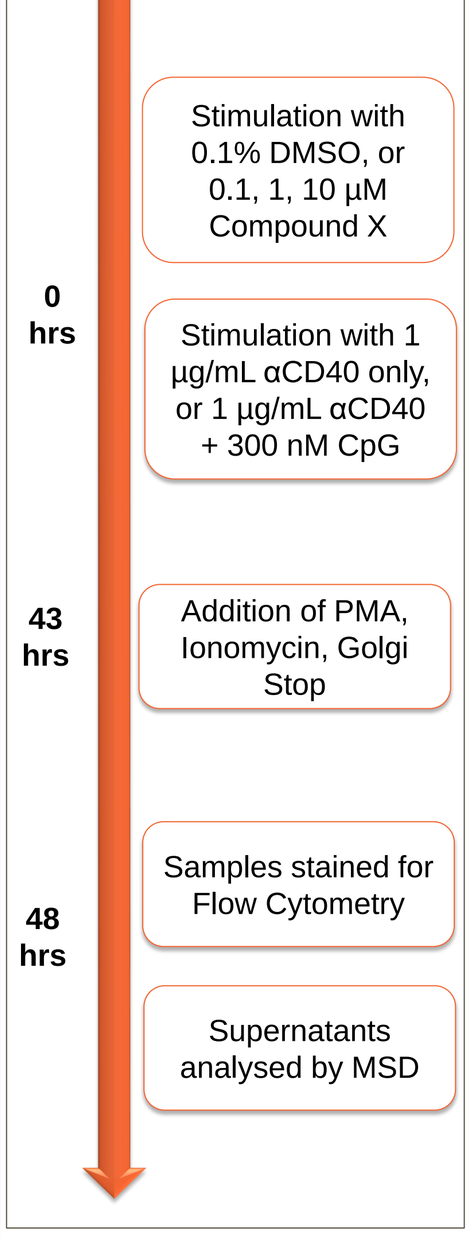
The aim was to investigate the phenotype of Bregs, based on CD27 and CD38 expression, and the effects of a potential Breg-enhancing drug (Compound X) on IL-10 secretion.

Figure 1: Breg functions



Methods

Figure 2: Experimental Design

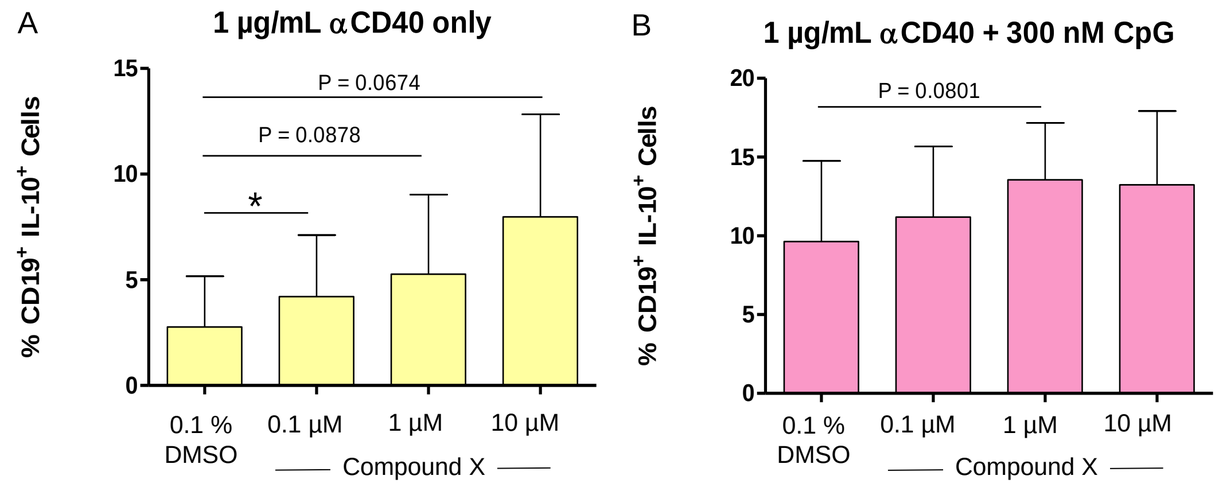


Results

The effects of Compound X and CpG on $CD19^+IL\text{-}10^+$ cell frequency and IL-10 secretion

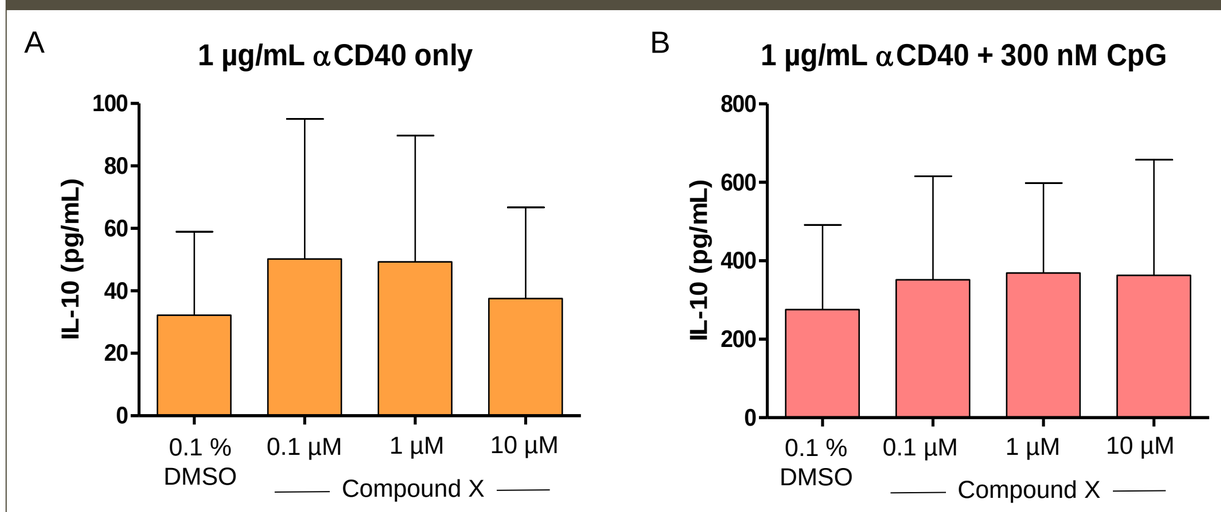
- Figure 3A also showed that Compound X increased the frequency of $CD19^+IL\text{-}10^+$ cells in a concentration-dependent manner.
- In Figure 3B, less effect was seen on the number of $CD19^+IL10^+$ cells by Compound X. This could be because:
  - CpG and Compound X interacted leading to inhibition.
  - Compound X was a competitive agonist at the Toll-like receptor 9 receptor.
  - IL-10 was already at saturated levels with CpG

Figure 3: Compound X increases IL-10 expression in $CD19^+$ cells



Mean ± SD, n = 3, *P < 0.05

Figure 4: CpG increased the amount of IL-10 secretion



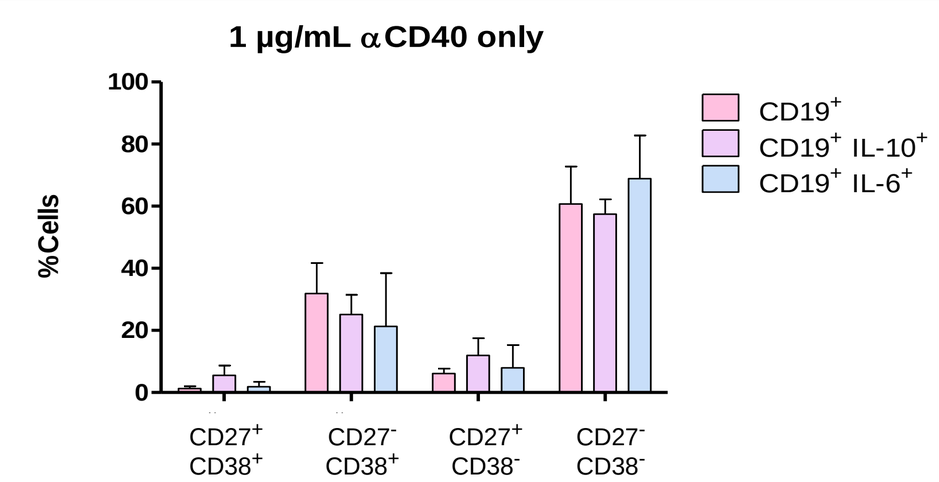
Mean ± SD, n = 3

- Figures 4A and 4B showed that higher concentrations of Compound X did not greatly affect the amount of IL-10 released into the supernatants.
- This could suggest that Golgi Stop was effective at inhibiting the release of IL-10.

Phenotyping Bregs using CD27 and CD38 as extracellular markers

- $CD19^+IL\text{-}10^+$ cells were predominantly $CD27^-CD38^-$ under αCD40 only stimulation conditions (Figure 5). A similar trend was observed for αCD40 and CpG.
- For all $CD19^+$ cells, there was 17.7-51.6% $CD27^-CD38^+$ expression. Hence, this implies that most of the B-cells were naïve-like, and there were some activated B-cells.
- However, different stimulation conditions may affect the phenotype of B-cells.

Figure 5: $CD19^+IL\text{-}10^+$ cells were predominantly $CD27^-CD38^-$



Mean ± SD, n = 3

Conclusions

- αCD40 and CpG induced more $CD19^+IL10^+$ cells than αCD40 only.
- Compound X increased the number of $CD19^+IL\text{-}10^+$ cells under αCD40 only stimulation conditions.
- αCD40 and CpG induced more IL-10 secretion than αCD40 only.
- Compound X did not have a statistically significant effect on IL-10 secretion by B-cells.
- $CD19^+IL\text{-}10^+$ B-cells were primarily $CD27^-CD38^-$ naïve-like cells.

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