Differential TLR10 expression on B cell subsets

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Abstract

Toll-like receptors (TLRs) are important players in the innate immune system and are expressed by a variety of cells including B cells and dendritic cells. TLR10 is an orphan receptor that is expressed exclusively by B cells. To study TLR10 expression on different B cell subsets, we performed multi-color flow cytometric staining on normal human peripheral blood using a panel of antibodies against CD19, CD27, CD5, CD38, CD24 and TLR10. We found that CD27^{+} memory B cells show higher TLR10 expression compared to naïve CD27^{-} B cells. Further analysis revealed that CD38^{hi}CD24^{hi} B cells (which have been defined as regulatory B cells) exhibit intermediate TLR10 expression. Surprisingly, CD5^{+}CD27^{+}CD38^{lo} CD24^{lo} B cells showed the lowest TLR10 expression. These data suggest that TLR10 may be involved in different functions in different B cell subsets, which will be addressed in our ongoing work.

Background

TLRs play an important role in the innate immune system. They are type I transmembrane proteins composed of extracellular leucine-rich domains and an intracellular region consisting of a Toll-IL-1 receptor homology domain. TLR10, also known as CD290, can hetero-dimerize with TLR1 and 2. The ligand of TLR10 is still unknown. TLR10 is predominantly expressed on B cells and a higher expression has been reported on IgM^{+}IgD^{+}CD27^{+} memory B cells. Expression of TLR10 on regulatory and other B cell subsets has not been described.

**Figure 1.** TLR10 expression can be detected on peripheral blood B cells
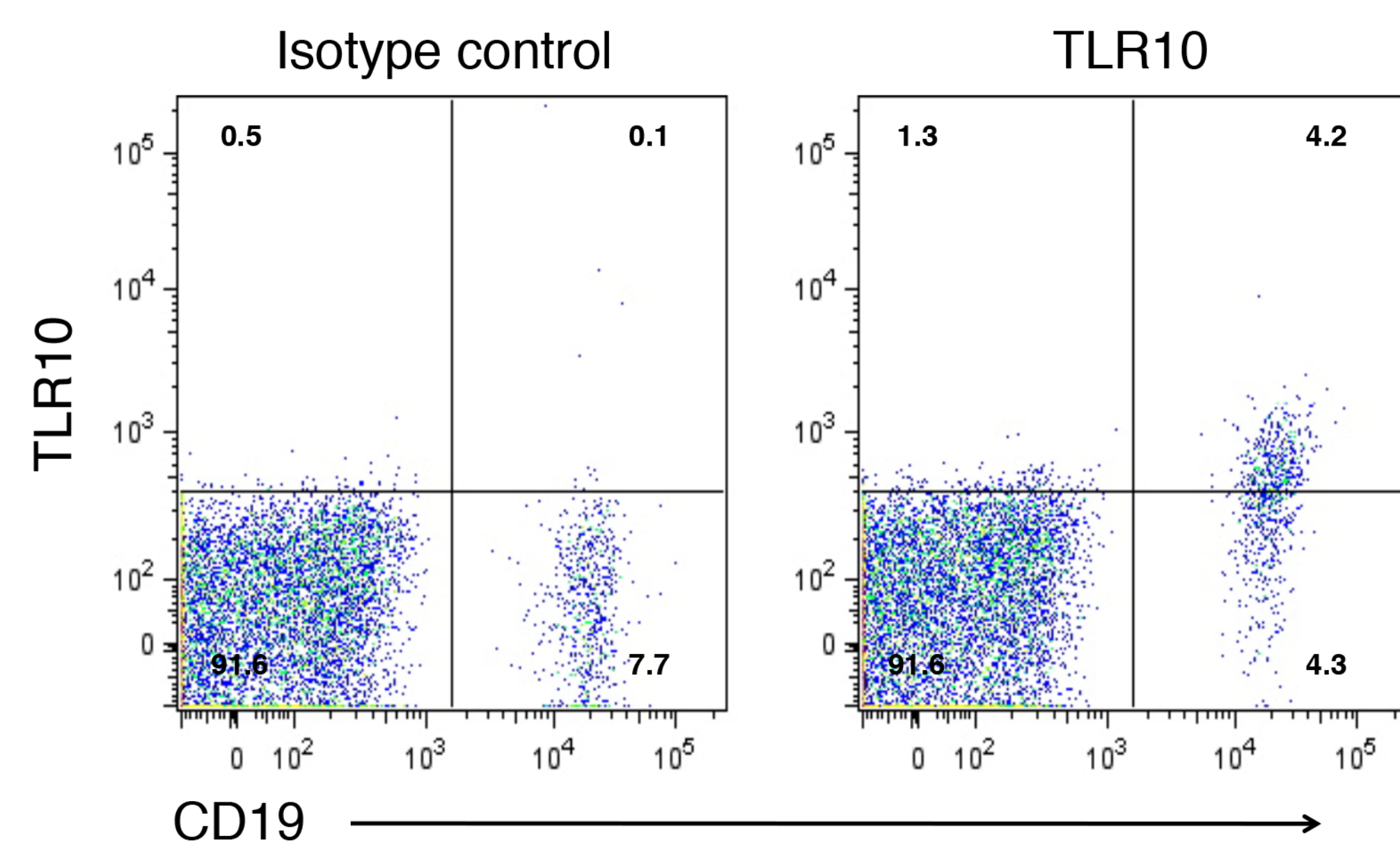
Human peripheral blood was stained with CD19 APC and TLR10 PE, followed by RBC lysis with BioLegend's RBC Lysis Buffer. Data from one representative donor is depicted (n=5).

**Figure 2.** CD27^{+} memory B cells show higher TLR10 expression
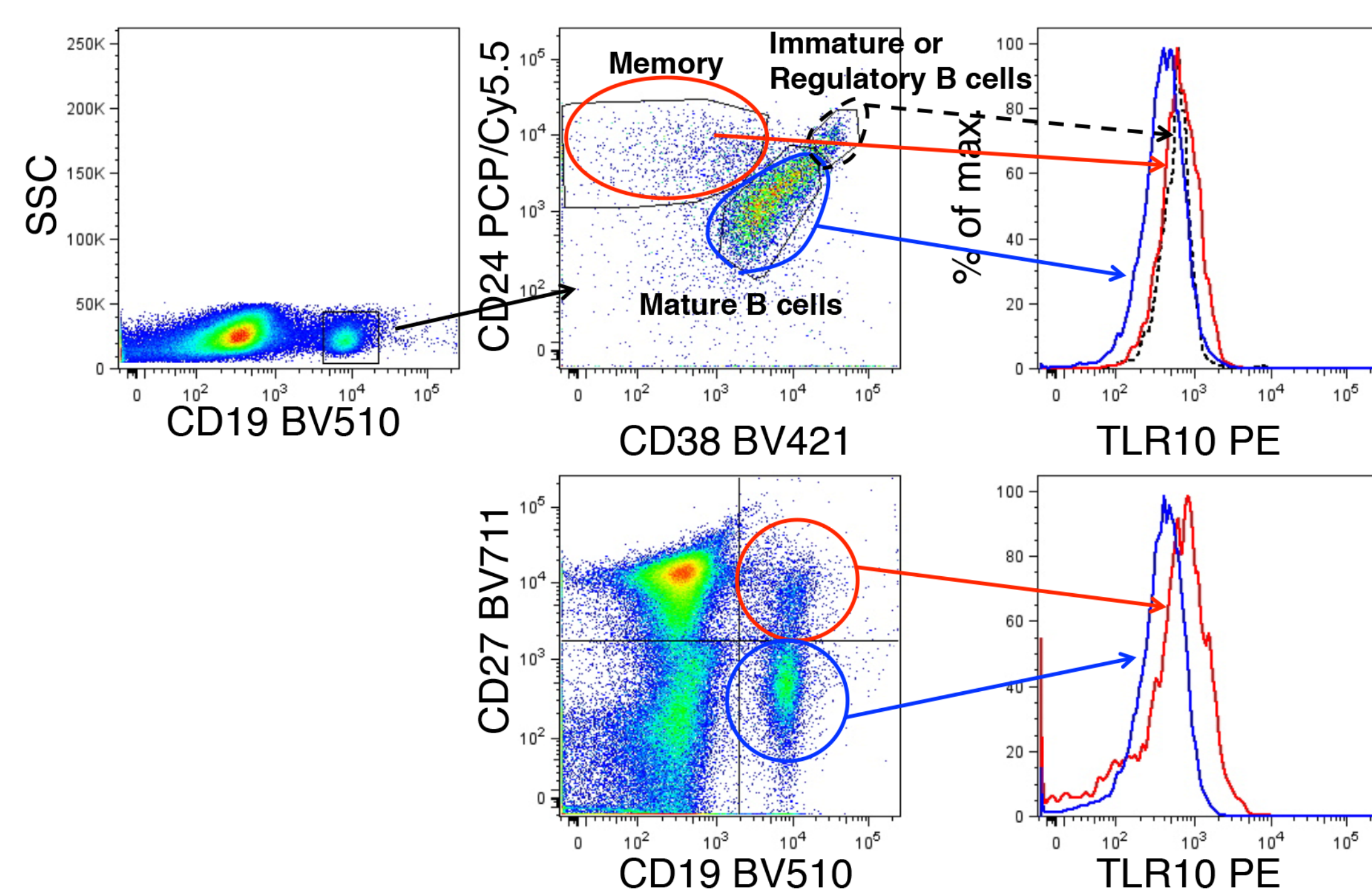
Human peripheral blood was stained with CD19 APC and TLR10 PE, followed by RBC lysis with BioLegend's RBC Lysis Buffer. Data from one representative donor is depicted (n=5).

**Figure 3.** Highest TLR10 expression is detected on a subset of CD5^{+} memory B cells
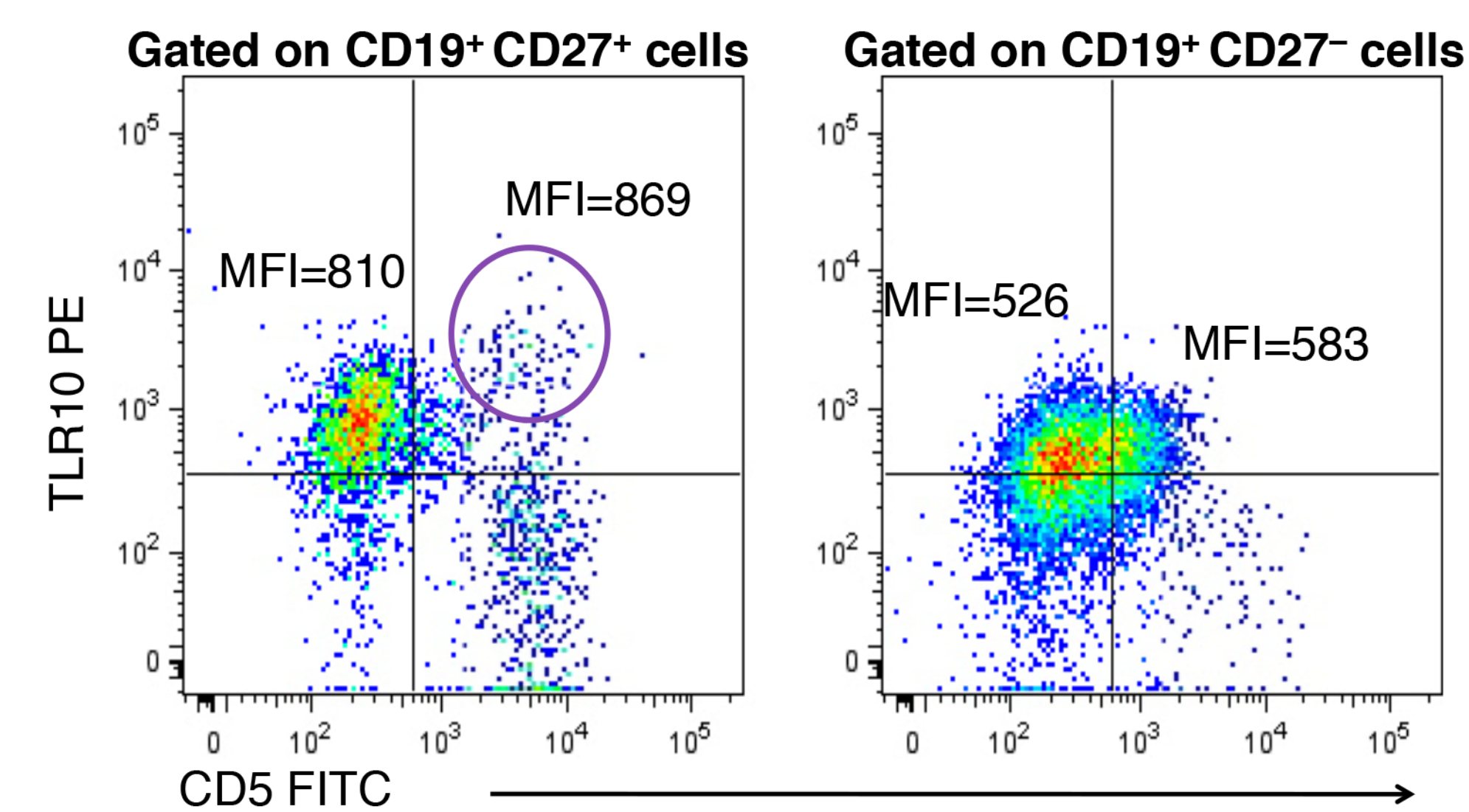
Amongst the memory B cells, highest TLR10 expression was observed on a subset of CD5^{+}CD27^{+} B cells (left panel). One representative donor is depicted. Dot plots are gated on CD19^{+}CD27^{+} cells.

Figure 4. TLR10 expression is down-regulated following activation with LPS and ligation of CD40
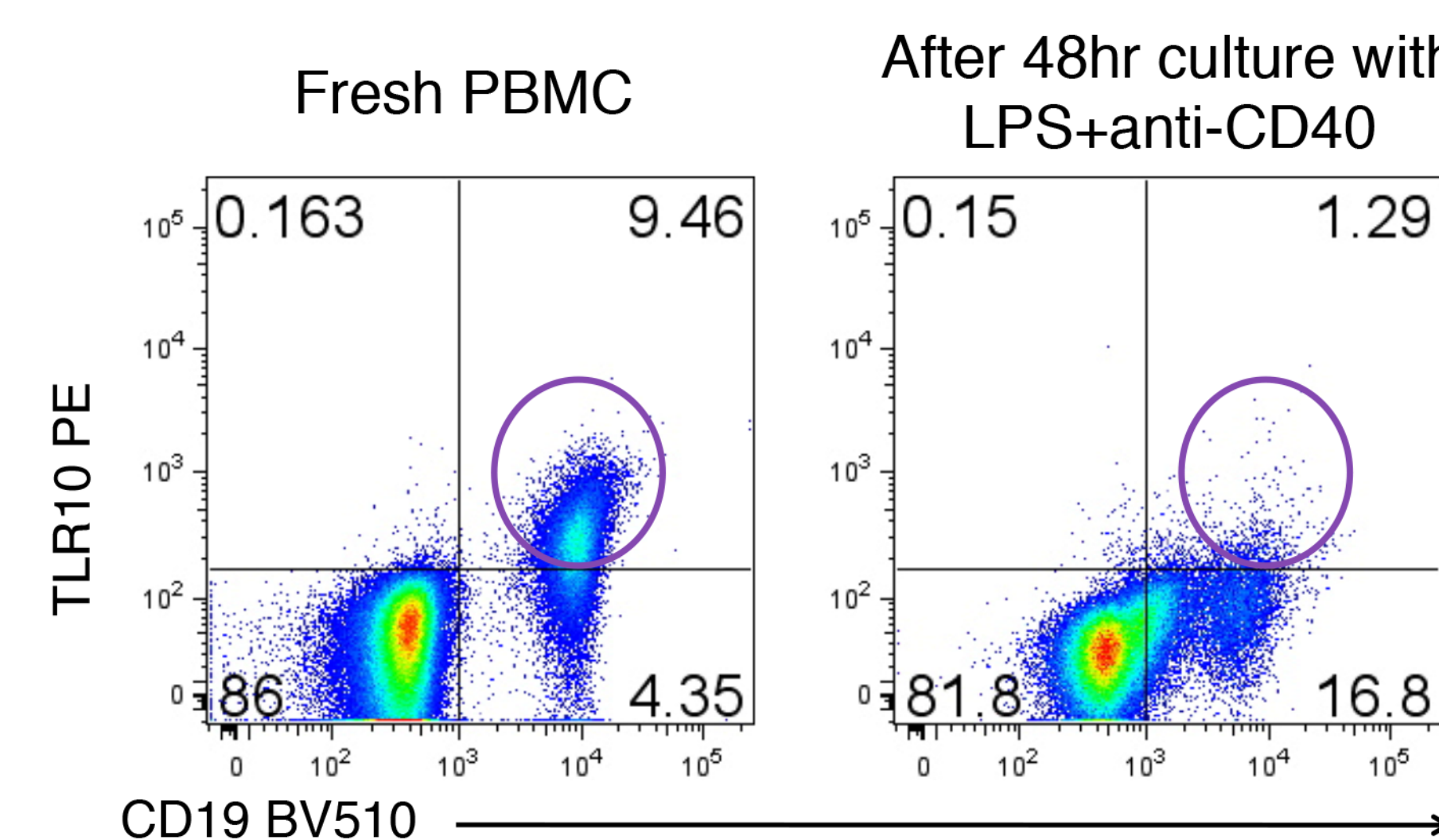
Peripheral blood mononuclear cells were stained with CD19 APC and TLR10. Data from one representative donor is depicted (n=6). Note significant reduction in TLR10 expression following activation with LPS and/or anti-human CD40 (clone HB14). Dot plots are gated on lymphocyte population.

Figure 5. Following *in vitro* activation, IL-10-expressing cells are detected predominantly in TLR10^{lo} cells
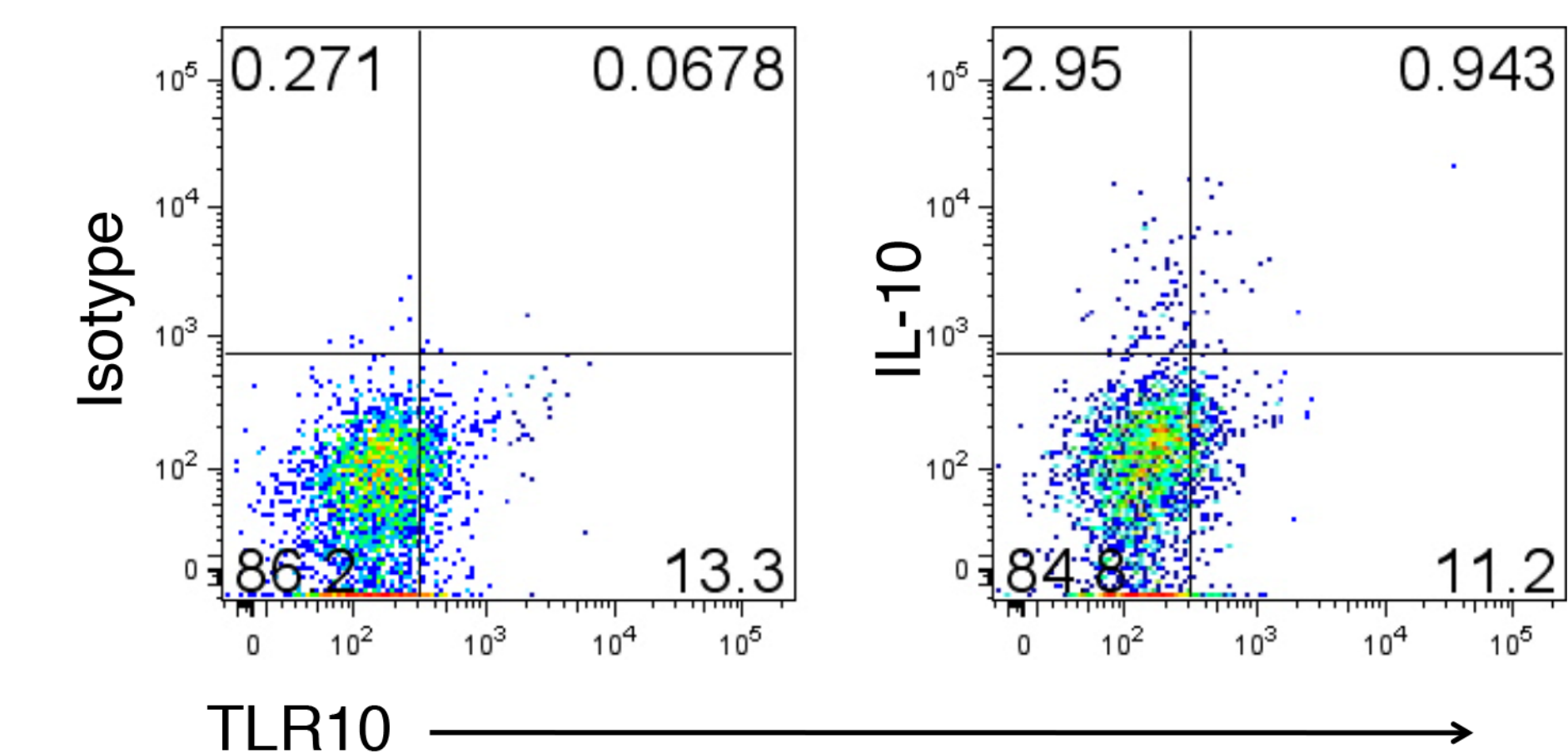
Peripheral blood mononuclear cells were stimulated with LPS and anti-human CD40 (clone HB14) for 48hrs. PMA (50ng/ml) and ionomycin (1µg/ml) were added during the last 5 hours of the stimulation. The cells were harvested and first stained with BioLegend's Zombie Yellow™ Fixable live/dead dye and then stained with CD27 FITC, TLR10 PE, CD24 PE/Cy7, IgD APC/Cy7, CD38 BV421™, CD19 BV510™ and IgM PerCP/Cy5.5. The cells were then fixed and permeabilized using BioLegend's 10X Permeabilization buffer. Cells were acquired on BD LSRFortessa™. Dot plots are gated on CD19^{+} cells.

Results

1. About 50% of peripheral blood B cells express TLR10.

2. Highest TLR10 expression is detected on a subset of CD5^{+} memory B cells.

3. CD38^{hi}CD24^{hi} regulatory B cells exhibit intermediate TLR10 expression compared to memory and naïve B cells.

4. Stimulation with LPS and anti-human CD40 significantly reduces TLR10 expression.

5. IL-10 expressing B cells are detected in the TLR10^{lo} subset, but these results are inconclusive, as TLR10 is down-regulated following *in vitro* activation.

Conclusions

1. Future studies using sorted TLR10-expressing B cell populations will aid in defining the precise role of TLR10 in IL-10 production, as TLR10 is down-regulated following *in vitro* activation with LPS and anti-human CD40.

2. Additional studies using siRNA will help identify the precise role of TLR10 in cytokine production and understand its role in disease.

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