Expression profiling of *Helicobacter*-activated regulatory B cells

Sawsan Said, Guliz Tuba Barut and Ayca Sayi Yazgan
Istanbul Technical University, Turkey

ABSTRACT

The regulatory B and T cells have a pivotal role in balancing immune pathogenicity and protection. Recently, it has been shown that the regulatory T cells could reduce *H. pylori*-induced gastritis in mice, at the same time allows the bacterium to colonize the mucosa at higher densities. Moreover, it was reported that IL-10^{+} B cells were activated upon *Helicobacter* sonicate treatment through TLR2-MyD88 activation, which leads to differentiation of T regulatory-1 (Tr-1) cells from naïve T cells (1, 2). The interaction between Tr-1 and IL10^{+}B cells may prevent the gastric precancerous lesions and serve as a good immune modulator against *Helicobacter*. Recently, RNA profile of B10 cells was investigated by RNA-seq, and differentially expressed genes in B10^{+} and B10^{-}B cells were identified (3,4). CD9 was identified as a key surface marker for most mouse IL-10^{+} B cells, and PD-1 was differentially expressed in IL10^{+} / IL10^{-}B cells. In our study, we focus on understanding the expression profile of *Helicobacter* activated regulatory B cells by microarray analysis. Next, we aim to investigate the expression levels of the recently described genes that are expressed in B10 cells; CD9 and PDCD1(PD1) in our samples; unstimulated B cells, *H. felis* stimulated B cells, *H. felis* stimulated IL-10^{+} B cells and IL-10^{-} B cells. Based on our microarray and real-time PCR data, we found that *H.felis* stimulated IL-10 competent B cells differentially express both CD9 and PD-1 and PD-L1 compared to stimulated IL-10 negative B cells.

CONTACTS

Sawsan Said, Ph.D candidate
Istanbul Technical University, Turkey
Email: sawsansaid@gmail.com

Ayca Sayi Yazgan, PhD
Istanbul Technical University, Turkey
Email: sayi@itu.edu.tr

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INTRODUCTION

IL-10^{+} B cells are activated upon *Helicobacter* stimulation through TLR2-MyD88 pathway, activated B cells lead to differentiation of naïve T cells to T regulatory-1 (Tr-1) cells. The primary objectives of this study are to understand the expression profile of *Helicobacter felis* (*H.felis*) activated (Hact) regulatory B cells and to identify genes and transcription factors related to IL-10 production in (Hact) – IL-10^{+} Bregs.

METHODS AND MATERIALS

We obtained B cells from spleen of C57BL/6 mice. Then we stimulated B cells with *H.felis* sonicate for 16 hours. Afterwards, we isolated *H.felis* activated IL10 $^{+}$ / IL10 $^{-}$ B cells by magnetic isolation using Militenyi's kit. The expression profiling of *Helicobacter* activated regulatory B cells was performed by microarray analysis using Agilent Sure Print G3 Gene Expression Microarrays of Mouse (v2) 8x60K models. Next, we confirmed the differential expression of four genes,CD9, PD-1, Trip1 and NRP2, in IL-10 positive B cells by real-time PCR. Also, we investigated the expression levels of PD-1 and PDL-1 in *H.felis* activated B cells in RNA level by real-time PCR.

RESULTS

A. Microarray analysis was followed by bioinformatic analysis that used to plot heat map representation of 27, 122 genes, with highly variable expression among B cell groups, a plotted heat map of hierarchical clustering is based on distance similarity for probes and samples; unstimulated B cells, *H .felis* stimulated B cells, IL10 positive B cells and IL10 negative B. The microarray analysis shows 1615 up-regulated genes in IL10 + / IL10 – B cells & 794 down –regulated genes in IL10 + / IL10 – B cells,

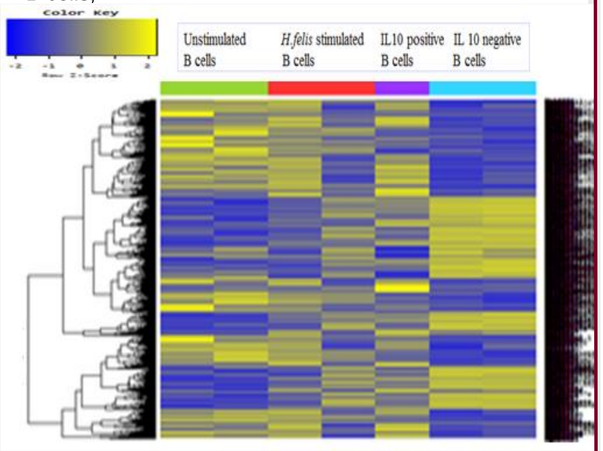

Figure 1. Cluster analysis-Hierarchical clustering heat map for all B cell groups. Cluster analysis includes two samples from each of the following group; unstimulated B cells, *H. felis* stimulated B cells, IL10 negative B cells and one sample of IL10 positive B cell

RESULTS

B. Real-time PCR data showed that CD9, PD-1, NRP2 , Trip1 and PD-L1 are highly expressed in IL10 positive B cells in comparison to IL10 negative B cells.

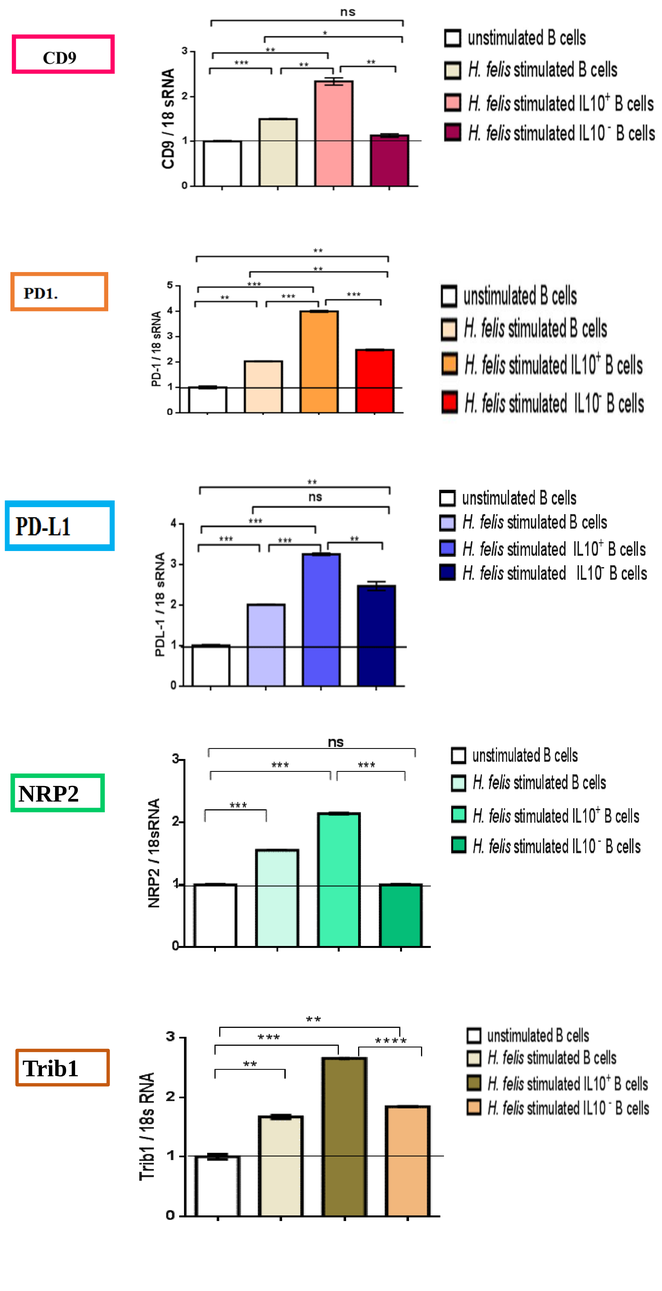

Figure 2. The average of the fold change of CD9, PD-1, PDL-1, NRP2 and Trip1 expression in three independent B cell experiments. B cell groups; unstimulated B cells, *H. felis* stimulated B cells , *H. felis* stimulated IL10 $^{+}$ and *H. felis* stimulated IL10 $^{-}$Bcells

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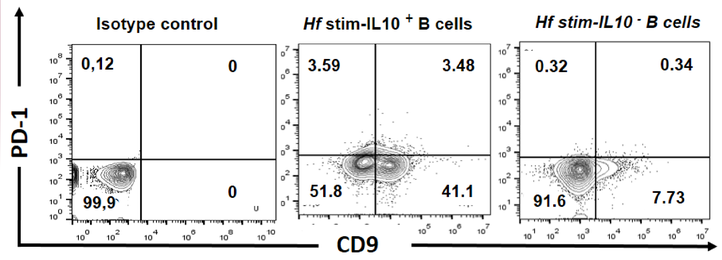

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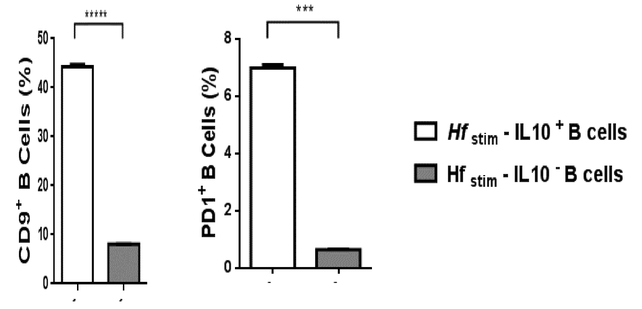

Figure 3. Flow cytometry staining showing a representative expression of PD-1 and CD9 in *H. felis* $_{stim}$ – IL10^{+} / IL10^{-} B cells. D, Bar graphs represent frequency of PD-1 and CD9 expression in *H. felis* $_{stim}$ – IL10^{+} / IL10^{-} B cells

Conclusion / Discussion

Our microarray analysis revealed 1615 up-regulated genes and 794 down –regulated genes in IL10 $^{+}$B cells compared to / IL10 $^{-}$B cells. We confirmed differentially expression of CD9, PD1, and PDL-1, Trip-1 and NRP2 by real-time PCR. CD9 and PD1 were previously shown to be highly expressed in IL-10 positive B10 cells. Also, we detected that PD-1 and CD9 are highly expressed in *H.felis* stimulated IL10 $^{+}$ B cells compared to IL10 $^{-}$ B cells in protein level.

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