BREG FUNCTION OF HUMAN MARGINAL ZONE B CELLS MAY BE MODULATED BY BAFF-EXPRESSING DENDRITIC CELLS

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Basic Sciences

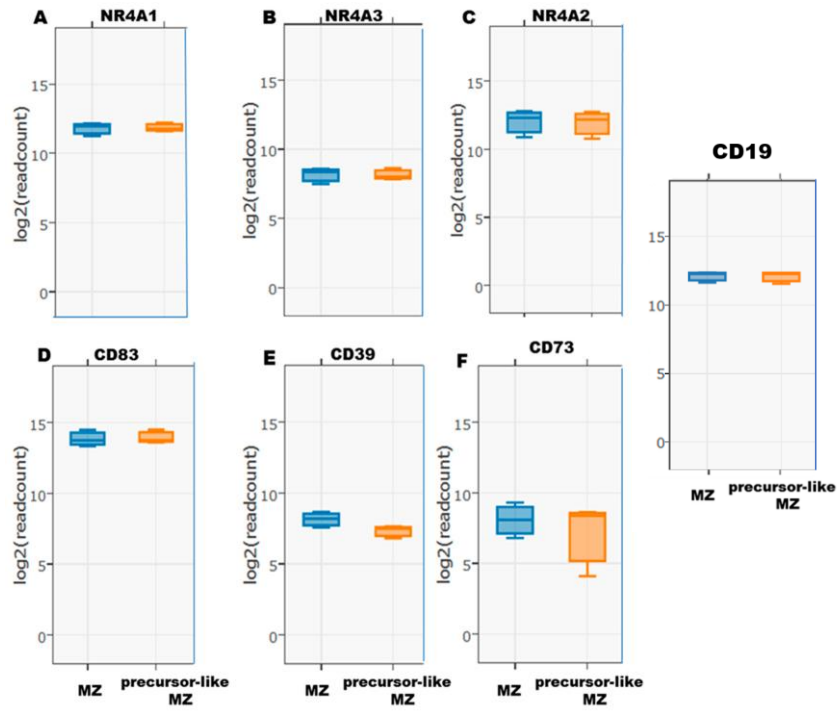
Introduction

Marginal zone B cells (MZ) and Precursor-like MZ (MZp).

- « Gatekeepers of innate immunity »; (Zouali, M. 2011). They are localised in the secondary lymphoid organs, at the marginal zone and can recirculate in humans.
- They are capable of T-independent responses via TLR receptors; Also capable of antigen-capture, trafficking and antigen-presentation.
- Human MZp are $CD19^{+}$, IgM^{+}, $CD1c^{+}$, $CD27^{+}$, $CD21^{low}$ and $CD10^{+}$, while conventional MZ are $CD21^{hi}$ and $CD10^{-}$. Similar populations have been associated with a regulatory potential (Breg).
- MZp possess powerful Breg potential due to the expression of several regulator markers *ex-vivo* (Doyon-Laliberté et al. 2019), such as:
  - The nuclear factors **NR4A1, NR4A2 and NR4A3** (essential for FOXP3 expression in Tregs).
  - CD39 and CD73 , responsible for the adenosine pathway.
  - **CD83**, which was shown to be important to their Breg function (Doyon-Laliberté et al. 2019).

Dendritic cells (DC) and BAFF

- DCs are the « orchestra chief » of the immune system, able to cross the bridge between innate and adaptive immunity. They are involved in the T-independent responses (such as MZ T-independent activation).
- They are a major producer of B Lymphocyte Stimulator/Activation Factor (BLyS/**BAFF**).
- BAFF is a key survival and differentiation factor for B cells, and is important for the selection of the MZ B cells pool. It can be found in a membrane-bound or a soluble form. It's produced by DCs, T cells, neutrophils, and other cell populations.



Doyon-laliberté et al. Antibodies (2019)

Rational and Hypothesis

- In HIV infected patients, blood frequency and IL-10 production of MZp are increased (Fontaine, J. et al. *Blood* 2011; Chagnon-Choquet, J. et al. *PLoSONE* 2014).
- In the same patients, serum BAFF is increased, and BAFF is overexpressed by DC and Monocyte-derived Dendritic Cells (Mo-DC). This increase is related to viral and non-viral factors such as Nef and the microbial translocation (Fontaine, J. et al. 2011, Chagnon-Choquet et al. *JID* 2015).
- Preliminary data from our lab has shown that BAFF is important for the shaping of Breg responses of MZp.
- Given the importance of BAFF to the shaping of the MZ pool and our preliminary data concerning BAFF, we sought to investigate how DCs could affect MZp's Breg function. More precisely, we decided to analyse the interplay between $BAFF^+$ DCs and MZp B cells as to regulation of their regulatory markers in **non-infected** tonsillar donors.

Methodology

- Measure of serum BAFF of $NR4A3^{-/-}$ mice (These mice are incapable of generating Mo-DC (Boulet, S. et al. *PNAS* 2019) by ELISA.
- Measure of total tonsillar BAFF surface levels and BAFF levels of DC, Mo-DC and $CD3^+$ T cells from 4 different donors by flow cytometry.
- Coculture of total tonsillar DCs of a single donor (KAG-017) and autologous B cells (1:10 ratio) for 24 hours in presence of absence of an Anti-BAFF blocking antibody. PMA/ionomycin was used as a positive control.
- Measure of frequencies of MZp's NR4A1, Nr4A3, CD39, CD73 and CD83 and protein expression levels (GeoMFI) by flow cytometry.

Results

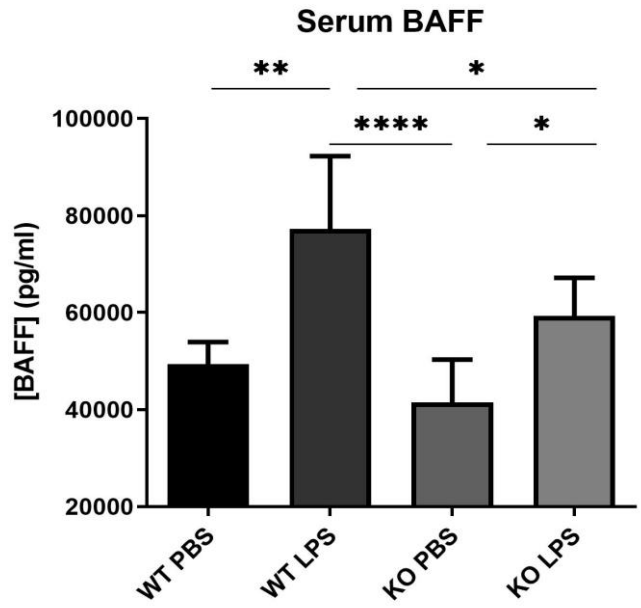


Fig. 1: Lower serum BAFF levels on $NR4A3^{-/-}$ mice (**KO**) following an immune challenge. Mice were either treated with PBS (negative control) or LPS (18 hours) before being sacrificed.

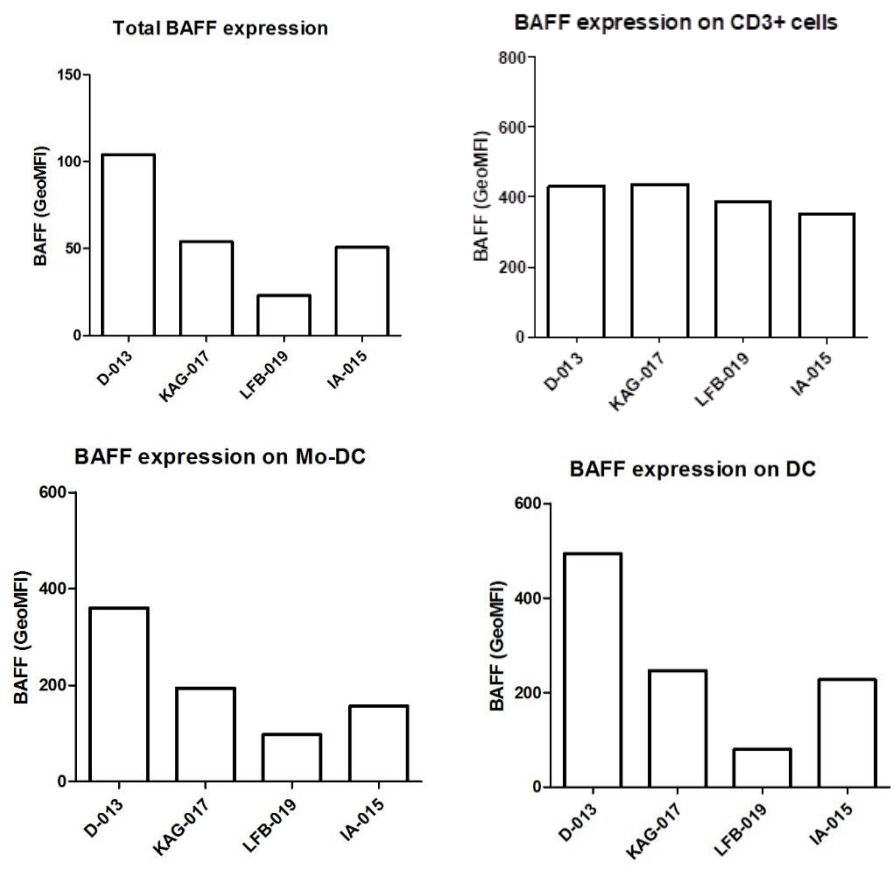


Fig. 2: Total BAFF expression in tonsils varies between donors, and this variation is DC and Mo-DC dependant, as BAFF expression on $CD3^+$ is similar between the 4 donors.

Results

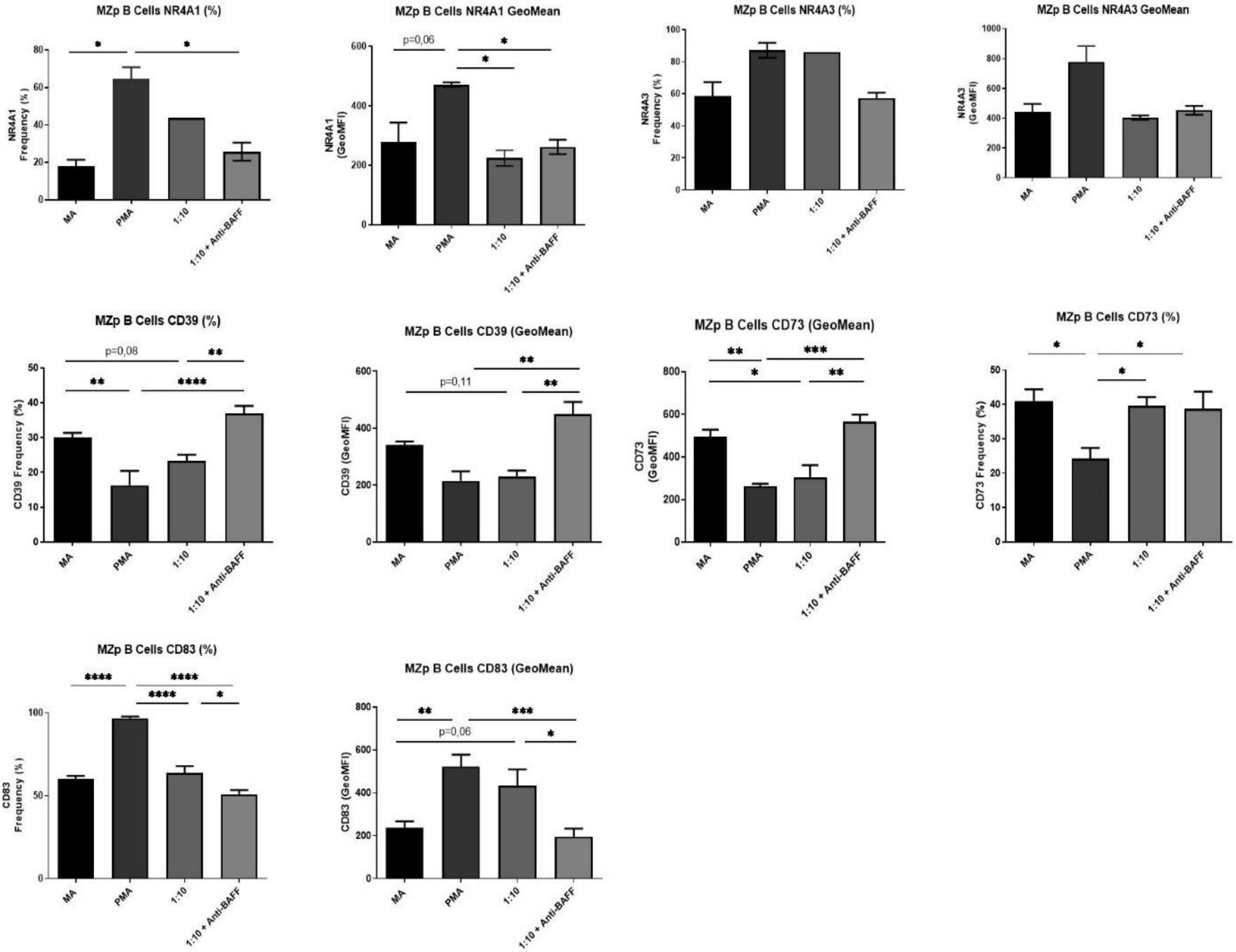


Fig. 3: DCs upregulate NR4A1, Nr4A3 and CD83 expression of MZp in a BAFF-dependant manner. CD39 and CD73 are downregulated, also in a BAFF-dependant manner. The experience was done with a single donor (KAG-017) twice. **MA** = Medium Alone; **PMA** = PMA/Ionomycin **1:10** = Coculture of DC and B cells; **1:10 + Anti-BAFF**: Coculture with the Anti-BAFF antibody.

Conclusion

- DCs and Mo-DCs are important BAFF-providing cells and contribute to the BAFF pool in the blood and in the tonsils.
  - Total serum BAFF was lower in Mo-DC-deprived mice following an immune-challenge.
  - Total BAFF in tonsils varies between different donors, and the same variation is seen between the DC and Mo-DC.
- In a physiological context, DCs modulate the regulatory markers of MZp B cells by increasing their expression and the frequency of cells expressing them, and this modulation is BAFF-dependant.
  - The addition of the Anti-BAFF antibody causes their levels to stay at their baseline.
- In the HIV context, DC and Mo-DC overexpress BAFF, and blood MZp frequencies are increased, early on and beyond therapy. It's possible that the excess of BAFF in this context contributes for the MZp dysregulations and their Breg capacities. Further studies are required to address these issues.
- We also believe that the higher levels of the MZP B-cells impede on an adequate immune response and clearance of the disease.

Conflicts of Interest

- **Conflict of Interest Disclosure**: We have no conflicts of interest

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