# LOSS OF TOLERANCE AND ANERGY IN HUMAN PERIPHERAL B CELLS DURING SARS-CoV-2 INFECTION

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**Moriah. J Castleman,** Megan M. Stumpf, Nicholas Therrien, Roberta Pelanda, and Raul M. Torres
University of Colorado, Anschutz Medical Campus, Department of Immunology and Microbiology, Aurora, CO, U.S.A.

## BACKGROUND

- SARS-CoV-2 infection promotes production of autoreactive antibodies with specificity to varying targets (Type I IFN, phospholipids, nuclear or tissue specific targets).
- The source of autoreactive antibodies is likely a heterogenous subset of B cells.
- $B_{ND}$ cells are mature naïve B cells that do not express IgM but do express IgD and are enriched in autoreactive specificities.
- In healthy humans $B_{ND}$ cells are held in an anergic state (high expression of inhibitory receptors and minimal response to BCR stimulation).
- Strong stimuli *in vitro* can break anergy in $B_{ND}$ cell.
- *We hypothesized that robust inflammation associated with ongoing viral infection from SARS-CoV2 may relax peripheral B cell tolerance and promote breakage of anergy in $B_{ND}$ cells.*

## METHODS

- Plasma and PBMCs were collected from healthy controls (N=10), subjects immunized with Pfizer BNT162b2-mRNA/ Moderna mRNA-1273 (N=10), subjects with mild (N=11) or severe SARS-CoV-2 infection (N=14).
- Flow cytometry was used to phenotype $B_{ND}$ cells *ex vivo* and measure the functional response (phosphorylation of signaling proteins) *in vitro* with BCR crosslinking.
- Inflammatory cytokines and levels of autoreactive antibodies were measured in plasma by multiplex.

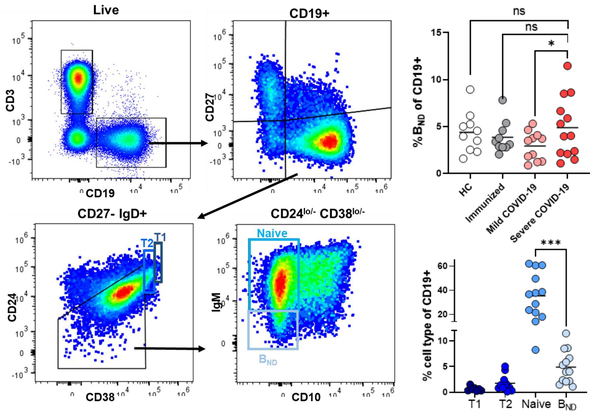


Figure 1. Identification of $B_{ND}$ cells by flow cytometry and enumeration of $B_{ND}$ cells in PBMCs.

**SARS-CoV-2 infection phenotypically and functionally changes $B_{ND}$ cells promoting relaxation of peripheral tolerance. This change is correlated with systemic inflammation and production of autoreactive antibodies.**

## RESULTS

- $B_{ND}$ cells from severe SARS-CoV-2 infection have higher expression of activation markers CD68 and CD86 with lower expression of inhibitory receptors CD22 and CD72 compared to healthy controls.

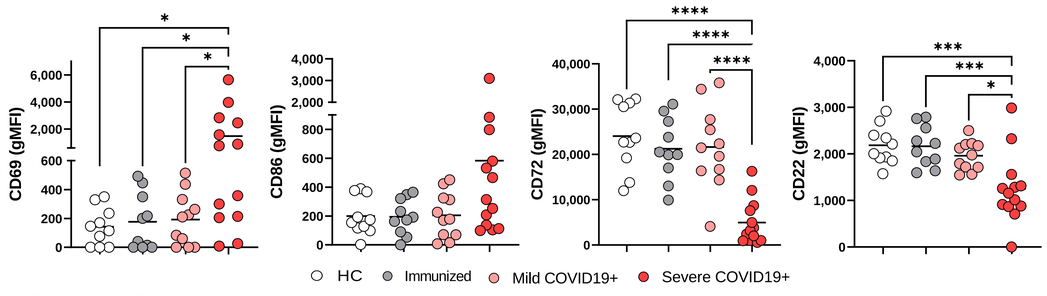


Figure 2. Expression (gMFI) of surface markers on $B_{ND}$ cells.

- Upon BCR crosslinking, $B_{ND}$ cells have higher levels of downstream signaling components of the BCR (pPLCγ2, pBlnk, and pSyk) when compared to healthy controls.

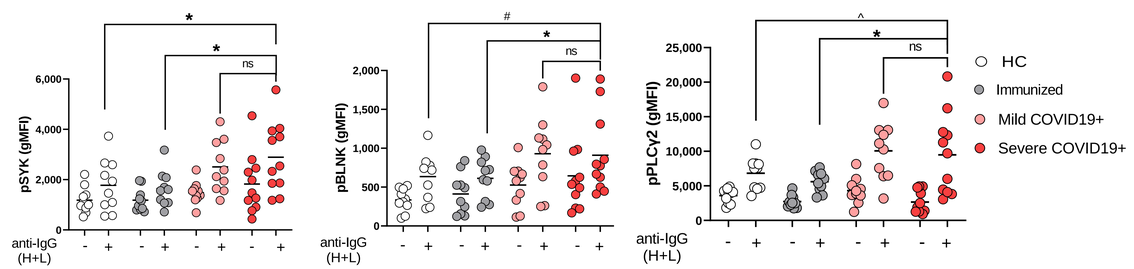


Figure 3. Expression (gMFI) of pSYK, pBLNK and pPLC2 with and without 5 minute stimulation by anti-human Ig F(ab')2 in $B_{ND}$ cells.

## RESULTS

- Severe SARS-CoV-2 infection induces higher levels of inflammatory cytokines (IFNγ, TNFα, IL-6 and CRP) and autoreactive antibodies
- Inflammatory levels correlated with changes in $B_{ND}$ phenotype and function.

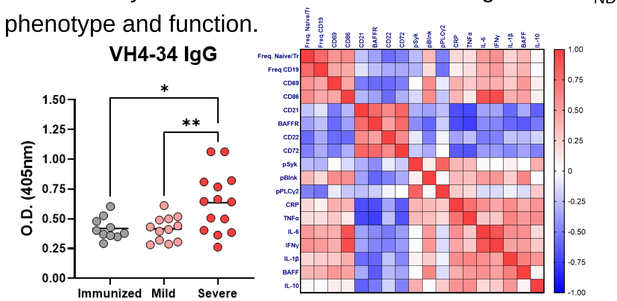


Figure 4. Levels of autoreactive antibodies VH4-34 in plasma. Correlation matrix of phenotype and function of $B_{ND}$ with levels of inflammatory cytokines.

## CONCLUSIONS

- $B_{ND}$ cells from severe SARS-CoV-2 have a phenotypical breach in anergy (higher activation markers and less inhibitory receptors).
- $B_{ND}$ cells from severe SARS-CoV-2 have a functional breach in anergy (higher phosphorylation of signaling molecules after BCR stimulation).
- Changes in $B_{ND}$ cells correlate with increased levels of systemic inflammation and autoreactive antibodies.

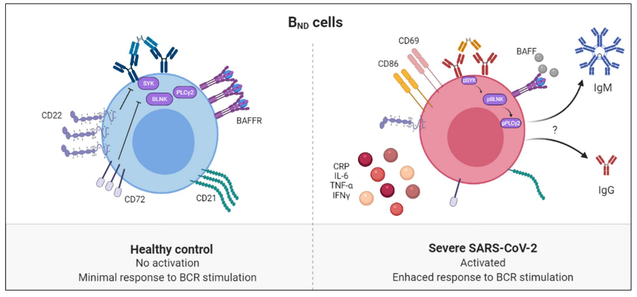


Figure 5. Differences in $B_{ND}$ cells in healthy controls compared to virally infected subjects.

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**Author Contact Information:** Moriah.Castleman@CUAnschutz.edu and Raul.Torres@CUAnschutz.edu
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