# Breaking B Cell Anergy: Exploring "Redemption" Cocktails

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## Introduction

- Autoimmunity affects over 24 million people in the United States.
- A possible culprit driving autoimmune diseases are anergic B cells ($B_{ND}$ cells).
  - $B_{ND}$ cells are autoreactive B cells that have escaped central tolerance and are hyporesponsive in the periphery.
- Recently, autoantibodies (aAbs) have been identified in severe COVID-19 disease.
  - These aAbs may drive pathology in severe disease.

HYPOTHESIS

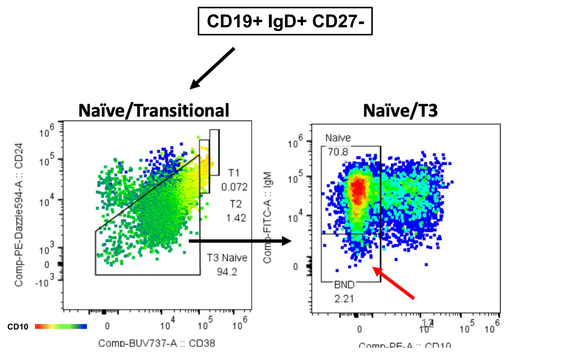
➢ **Strong inflammation in disease relaxes peripheral immunological tolerance, breaking anergy in $B_{ND}$ cells and producing pathogenic aAbs.**

### $B_{ND}$ Cells are Naïve-like Autoreactive B Cells

- The $B_{ND}$ cell population is enriched in autoreactive and polyreactive clones.
  - Enriched reactivity includes (but not limited to):
    - dsDNA
    - ANA
    - Cardiolipin
    - Chromatin
    - Smith
    - Insulin
    - HEp-2

Anergic B cells differ from naïve B cells in their expression of surface IgM (below) and their autoreactivity. $B_{ND}$ cells more frequently bind a wide variety of autoantigens (described above) when compared with naïve B cells.

### Defining $B_{ND}$ Cells from Peripheral Blood



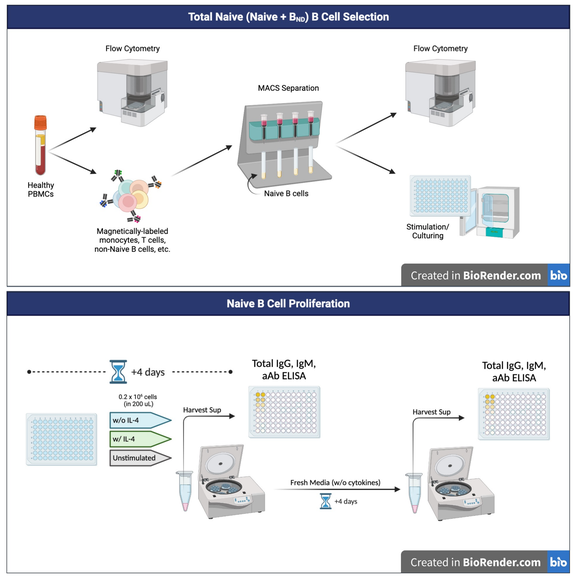

Castleman et al. *in prep.*

$B_{ND}$ cells are characterized by (in healthy donors):
- ↓ BCR Signaling cascade
- ↓ Ca2+ flux
- Minimal antibody production
- ↑ Inhibitory receptors

In disease (SLE, diabetes, Graves, Hashimoto, etc.), $B_{ND}$ cells have:
- ↓ Presence in periphery (activation?)
- ↑ BCR Signaling cascade
- ↑ Ca2+ flux
- ↓ Inhibitory receptors

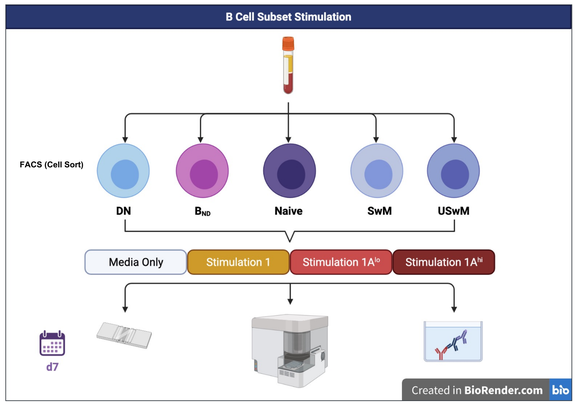
## Methodology

### Isolating & Culturing Total Naïve B Cells from Healthy PBMCs




Standard stimulation conditions include IL-2, CpG, anti-Ig, and depending on the condition, IL-4 (to attempt to rescue $B_{ND}$ antibody production). All cell supernatants were collected and assayed by ELISA. ELISA antigens included total IgG, total IgM, cardiolipin IgM, and various other autoantigens (when sufficient supernatant was available). All cell pellets were harvested and stained for cell markers and assayed using a Cytek Aurora and results analyzed using FlowJo and GraphPad Prism.

### B Cell Subset Sorting and Culturing

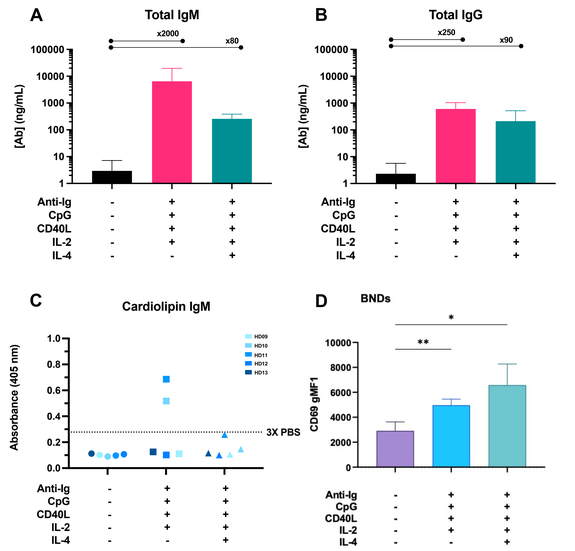



A fresh LRS chamber was obtained from a healthy donor (Vitalant) and PBMCs were isolated. The PBMCs were first magnetically labeled for non-B cells and passed over MACS columns (see above). The enriched B cells were further sorted into subsets and then plated and stimulated with either media only, standard stimulation (above), or standard stimulation and varying concentrations of IL-6, IL-1β, and TNF-α ($1A^{lo/hi}$).

## Results

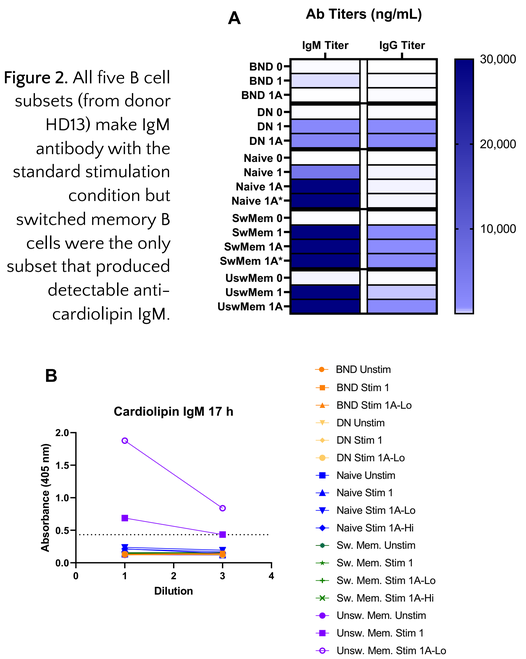
### Total Naive B Cell Antibody Production and Cell Surface Markers

**Figure 1.** IL-4 limits autoantibody production but activates $B_{ND}$ cells (n=5).




### B Cell Subset Total Antibody and Autoantibody Production

**Figure 2.** All five B cell subsets (from donor HD13) make IgM antibody with the standard stimulation condition but switched memory B cells were the only subset that produced detectable anti-cardiolipin IgM.




## Conclusions

- Anergic B cells can be activated using inflammatory stimuli.
- IL-4 limits autoantibody and total antibody production.
- IL-4 upregulates activation markers on the surface of $B_{ND}$ cells.
- 2/5 (40%) of healthy donors' naïve B cells were stimulated to produce anti-cardiolipin IgM.
- We are unable to rule out $B_{ND}$ cells for autoantibody production due to lack of total antibody production.
- However, unswitched memory B cells do produce anti-cardiolipin IgM after stimulation.
- Addition of IL-6, IL-1β, and TNF-α increased the anti-cardiolipin antibody response in the unswitched memory B cells.

Despite being unable to rule out $B_{ND}$ autoantibody production in donor HD13, it is still possible that this individual had a prior COVID-19 infection we are unaware of, potentially causing activation and differentiation of relevant $B_{ND}$ cells to memory cells. For this reason, further studies should be conducted with a variety of donors with and without SARS-CoV-2 exposure(s).

## Future Directions

- ➢ Repeat B cell subset using a new donor
- ➢ Try IL-4 (Stim 2) Cocktail in B cell subset culture experiment
- ➢ Try other TLR ligands (TLR7? TLR4?)
- ➢ Remove cocktail components individually and retest
- ➢ Find cardiolipin IgM standard for cardiolipin ELISAs
- ➢ Assay more autoantigens (ACE-2, ANA, HEp-2, etc.)
- ➢ Investigate potential mechanisms for anergy breaking (REL/NFκB)
- ➢ *In vivo* studies using humanized mice model

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