

## **Breaking B Cell Anergy: Exploring “Redemption” Cocktails**

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Autoimmunity affects over 24 million people in the United States. A possible culprit driving certain autoimmunities including systemic lupus erythematosus (SLE) and type 1 diabetes (T1D) are anergic B cells ( $B_{ND}$ ), which are autoreactive B cells that have escaped central tolerance. Recently, autoantibodies (aAbs) against self-antigens were identified in severe COVID-19 and may drive pathology in severe disease. Here, we propose that strong inflammation in disease relaxes peripheral immunological tolerance, thus, breaking anergy in  $B_{ND}$  cells and producing aAbs.

To study whether  $B_{ND}$  cells activate with strong inflammation, naïve B cells or sorted B cell subsets were isolated from peripheral blood mononuclear cells of 5 healthy donors and cultured for up to eight days with stimuli combinations, including IL-2, CpG, CD40 ligand, anti-Ig, IL-4, IL-6, IL-1 $\beta$ , and TNF- $\alpha$ . Cell supernatants were tested using cardiolipin and total antibody (Ab) enzyme-linked immunosorbent assays (ELISAs). Cells were stained for cell surface marker expression and measured using flow cytometry.

Of all cocktails tested, the presence of IL-4 limited total Ab and cardiolipin Ab production. IL-6, TNF- $\alpha$ , and IL-1 $\beta$  increased total IgG production and anti-cardiolipin Abs. Activation markers were upregulated on  $B_{ND}$  cells suggesting successful activation in response to inflammation. However, when B cell subsets were separately stimulated from one donor, unswitched memory cells were the only subset that produced cardiolipin Abs.

$B_{ND}$  cells can be activated using inflammatory stimuli mimicking those found in severe COVID-19 to upregulate surface activation markers and elicit aAb production. Individuals may differ in the B cell subtype of their autoreactive B cells or depending on past exposures. Further studies should be done to determine whether the autoreactive unswitched memory cells originate as  $B_{ND}$  cells in the periphery prior to differentiation.