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 (BAn cells).
• BAn 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 relieves peripheral immunological tolerance, breaking energy in BAn cells and producing pathogenic aAbs.

BAn Cells are Naïve-like Autoreactive B Cells

• The BAn 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 naive B cells in their expression of surface IgM (below) and their autoreactivity. BAn cells more frequently bind a wide variety of autoantigens (described above) when compared with naive B cells.

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 BAn 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.

Results

Total Naïve B Cell Antibody Production and Cell Surface Markers

Figure 1. IL-4 limits autoantibody production but activates BAn cells (n=5).

B Cell Subset Total Antibody and Autoantibody Production

Figure 2. All five B cell subsets from donor HD01 make IgM antibody with the standard stimulation condition but switched memory B cells were the only subset that produced detectable anti-cardiolipin IgM.

Future Directions

• Repeat B cell subset using a new donor
• Try IL-4 (Sim 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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