**Calibration Targets for B-Cell ABM**

General Notes:

* Each run of the ABM consists of 2 exposures
  + The first exposure was given to establish a pre-circulating population of B-cells
  + The second exposure was given to measure the response either to another normal exposure or a septic-level exposure
* 1st exposure of antigen was given at 480 ticks
  + For both normal and sepsis cases, a normal level (30 antigen turtles) of antigen was given as 1st exposure
* 2nd exposure of antigen was given at 2900 ticks
  + For normal cases, a normal level (30 antigen turtles) of antigen was given as 2nd exposure
  + For sepsis cases, a high level (300 antigen turtles) of antigen was given as 2nd exposure

Overall Calibration Goals of the Normal (2nd exposure) cases

* To see a larger overall B-cell population increase upon 2nd normal exposure to represent a stronger 2nd response to a previously seen antigen

Overall Calibration Goals of Septic (2nd exposure) cases

* To see lower B-cell activation (CD-21 expression) on septic 2nd exposure compared to a normal 2nd exposure
  + Source: <https://www.jimmunol.org/content/200/7/2418>
* To see increased B-cell apoptosis
  + Source: <https://www.jimmunol.org/content/200/7/2418>
* To see increased B-reg cell populations
  + Source: <https://www.jimmunol.org/content/200/7/2418>
* To see increased IL-10 levels, representing immunosuppresion
  + Source: <https://www.jimmunol.org/content/200/7/2418>
* To see a roughly 30-days delayed response in overall B-cell populations after septic antigen exposure
  + Source: <https://www.frontiersin.org/articles/10.3389/fimmu.2018.02532/full>

**Breakdown of Individual Cell Types in Normal 2nd Exposure Calibration**

Short-Lived Plasma Cells

* Goal: To see a larger SLPC response on 2nd exposure than on 1st exposure
* Results:
* Notes: We see that the average # of SLPCs produced on 2nd exposure is greater than the average # of SLPCs produced on the 1st exposure

Long-Lived Plasma Cells

* Goal: To see a larger LLPC response on 2nd exposure than on 1st exposure
* Results:
* Notes: We see an increase in the total number of LLPCs after 2nd exposure. I think the magnitude of increase appears smaller than the 1st exposure because the LLPCs that were made during the 1st exposure are dying off at the same time new LLPCs from the 2nd exposure are being produced.

Memory B Cells

* Goal: To see a larger memory B cell response on 2nd exposure than on 1st exposure
* Results:
* Notes: Here, the calibration goal isn’t fully met. The memory B-cell population doesn’t surpass the 1st exposure cell counts. I haven’t figured out exactly why this is happening, but for some reason, the cytokines that are produced during the 2nd exposure push the B-cell differentiation away from memory B-cells and towards plasma B-cells (LLPCs or SLPCs), which I think is why we see a decreased memory B-cell response on 2nd exposure.

**Breakdown of Individual Cell Types and Other Metrics in Septic 2nd Exposure Calibration**

SLPCs

* Goal: To see a delay in SLPC response after the septic 2nd exposure compared to the normal 2nd exposure
* Results:
* Notes: In the normal 2nd exposure, the SLPC response starts immediately after the 2nd exposure at 2900 ticks. In the septic 2nd exposure, we don’t see a significant SLPC response start until around the 3400 tick mark, corresponding to a roughly 11-day delay in SLPC response.

LLPC

* Goals:
  + To see a delay in LLPC response after the septic 2nd exposure compared to the normal 2nd exposure
  + To see a drop in LLPC count after septic 2nd exposure, representing apoptosis and differentiation into B-reg cells.
* Results:
* Notes: We see an immediate, significant decrease on LLPC population after septic 2nd exposure at 2900 ticks. Then, in the septic runs, the LLPC population does not begin to significantly increase until the 3600 tick mark, corresponding to a roughly 15 day delay in LLPC response.

Memory B-Cells

* Goals:
  + To see a delay in Memory B-cell response after the septic 2nd exposure compared to the normal 2nd exposure
  + To see a drop in Memory B-cell count after septic 2nd exposure, representing apoptosis and differentiation into B-reg cells.
* Notes: We see a significant decline in Memory B cell counts at septic 2nd exposure at 2900 ticks, due to increased apoptosis as well as increased differentiation into Breg cells. We also see a prolonged period of low Memory B Cell numbers lasting roughly 35 days, representing a 35-day delay in Memory B cell response.

Breg Cells

* Goals: To see an increased Breg cell response on septic 2nd exposure compared to a normal 2nd exposure
* Results:
* Notes: We see that on septic 2nd exposure, there is a much larger population of Breg cells (roughly 120 Breg cells) that are produced compared to the normal 2nd exposure (roughly 80 Breg cells).
* Breg populations were not directly calibrated using the literature data. Instead, I used the data that showed increased IL-10 production during sepsis, and extrapolated that to mean increased Breg cell counts during sepsis since Breg cells are the predominant source of IL-10.

IL-10 Production

* Goals : Similar to the goals of the Breg responses, we want to see an increased level of IL-10 produced on septic 2nd exposure compared to a normal 2nd exposure
* Results:
* Notes: We see a much larger level of IL-10 produced on septic 2nd exposure (roughly 6000) compared to a normal 2nd exposure (roughly 3800). This increased IL-10 is the predominant factor in the decreased CD-21 expression, and therefore B-cell activation level, seen below in a septic 2nd exposure

Average CD-21 Expression

* Goals : To see decreased CD-21 expression levels on B-cells on a septic 2nd exposure compared to a normal 2nd exposure. This represents decreased B-cell activation, or B-cell anergy
* Results:
* Notes : We see a significant drop in CD-21 expression after a septic 2nd exposure, whereas there is no noticeable drop in CD-21 expression after a normal 2nd exposure.

Level of Apoptosis

* Goals : To see increased number of cells apoptosing after a septic 2nd exposure compared to a normal 2nd exposure
* Results:
* Notes : We see a significant increase in the number of cells that apoptose after a septic 2nd exposure, whereas there is no noticeable increase in cell apoptosis after a normal 2nd exposure.