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# CPG-STIMULATED MOUSE B CELLS REQUIRE GLUTAMINE METABOLISM AND mTORC1 ACTIVATION FOR CYTOKINE PRODUCTION

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Effector functions of immune cells have demonstrated a dependence on specific metabolic pathway utilization. The metabolic pathways B cells need to acquire effector functions are unknown, but may uncover how B cells acquire pathogenic features associated with autoimmune diseases. Thus, we sought to identify the metabolic requirements of B cell effector function in mouse B cells.

B cells were isolated from the spleens of C57Bl/6J mice using negative selection magnetic beads. B cells were then activated overnight with CpG, which activates B cells through TLR9. B cells were incubated overnight with pharmacological inhibitors, which was used to prevent utilization of one of three main carbon sources: UK5099 prevented pyruvate transport into the mitochondria, etomoxir inhibited fatty acid oxidation, and BPTES inhibited glutaminolysis. To control for off target effects, glutamine titration and galactose replacement experiments were performed. The XF Seahorse Analyzer was used to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) as surrogates for mitochondrial respiration and aerobic glycolysis, respectively. ELISA was used to measure cytokine secretion. We also interrogated the role of mTORC1 activation in cytokine secretion; Western blots for phosphorylation of Akt and S6K, upstream and downstream proteins of mTORC1 respectively, were used to examine mTOR activation.

We found that CpG activation of B cells was reliant on glutamine and drove mTOR activation, both of which were capable of driving glycolysis and mitochondrial activity. Glutamine and mTOR activation were also required to produce the inflammatory cytokine IL-6 and immunosuppressive cytokine IL-10, although differentially: while IL-6 required mTOR and glutaminolysis, IL-10 only required glycolysis. Finally, mTORC1 activation was independent of glutamine, indicating that mTORC1 activation is likely driven directly by TLR activation.

B cell activation and cytokine secretion both exhibit a dependence on glutamine utilization and mTOR activation, which drives glycolytic and mitochondrial activity. Production of cytokines also is dependent on these processes, but in a complicated fashion. These data suggest that inflammatory responses may be dependent on glutamine and mTOR activation, while immunoregulatory responses are dependent on glycolysis. Future steps will be to perform metabolic flux studies to determine the metabolic pathways necessary for B cell cytokine production. These results may result in more specific immunomodulatory therapies for autoimmune diseases.