Revealing the Role of BCAAs in Macrophage Immune Response

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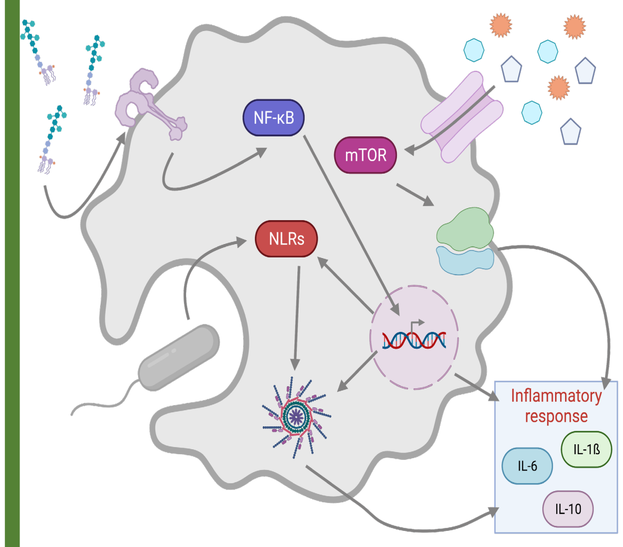
Abstract



Macrophages take inputs from inflammatory and metabolic sources to respond to various disease states through the release of inflammatory cytokines. The inflammasome is a multiprotein complex that regulates these inflammatory responses. It facilitates cytokine release through the induction of pyroptosis, a mode of inflammatory cell death. Recent work has begun to reveal how the presence or absence of certain metabolites mediates inflammatory responses. However, the specific metabolites and pathways responsible remain poorly understood. We demonstrated that BCAAs regulate cytokine secretion and therefore inflammasome activity in macrophages, likely through both mTOR dependent and independent mechanisms. Future work will expand upon these findings to fully characterizing how BCAAs regulate macrophage activity and introduce disease relevant models with deficiencies in BCAA metabolism.

Branched Chain Amino Acids (BCAAs) have multiple important functions within cells

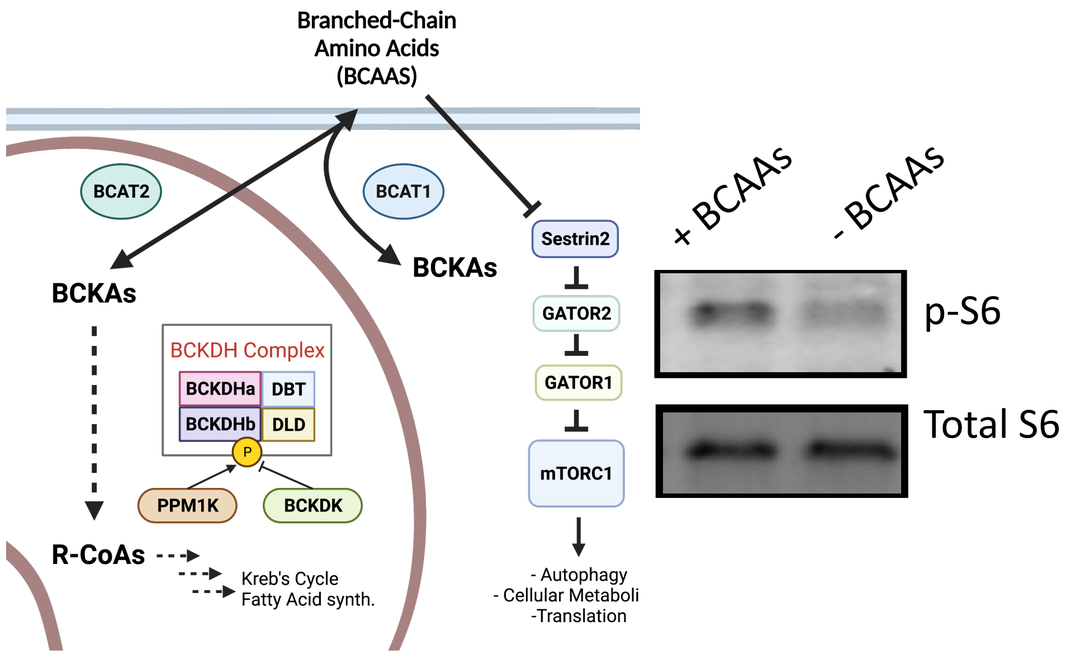


Figure 1: Branched Chain Amino Acids (BCAAs), including leucine, isoleucine, and valine, act in more than just protein synthesis in a cell. These metabolites can activate mTORC1 through Sestrin2, including in macrophages as shown by western blot. They can also be completely metabolized in the mitochondria to generate metabolites that feed into central metabolism like the TCA Cycle.

Leucine and Leucine + Valine Restore Expression of Several Genes

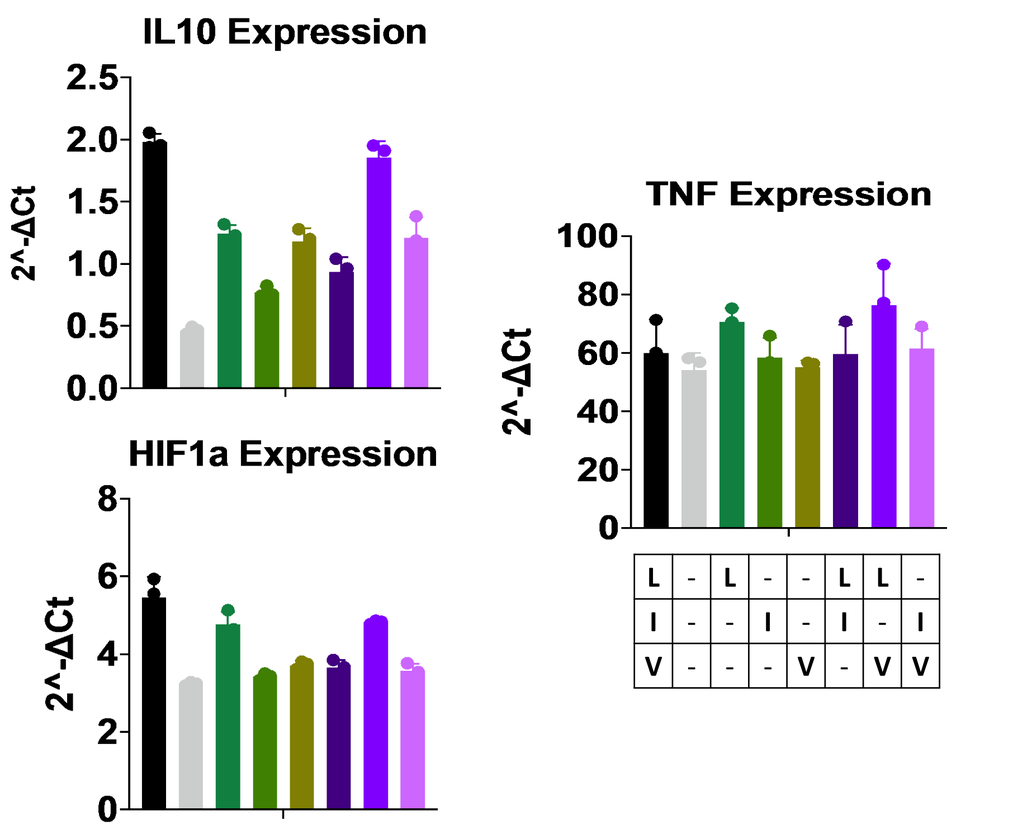


Figure 2: Macrophages were cultured in the presence or absence of individual BCAAs, alone or in combination. Cells were treated with LPS for 4 hours before RNA isolation and quantification by RT-qPCR. Values plotted in reference to Pol2A. (From left to right: + BCAAs, - BCAAs, L, I, V, LI, LV, IV)

Individual BCAAs are partially sufficient to restore cytokine secretion

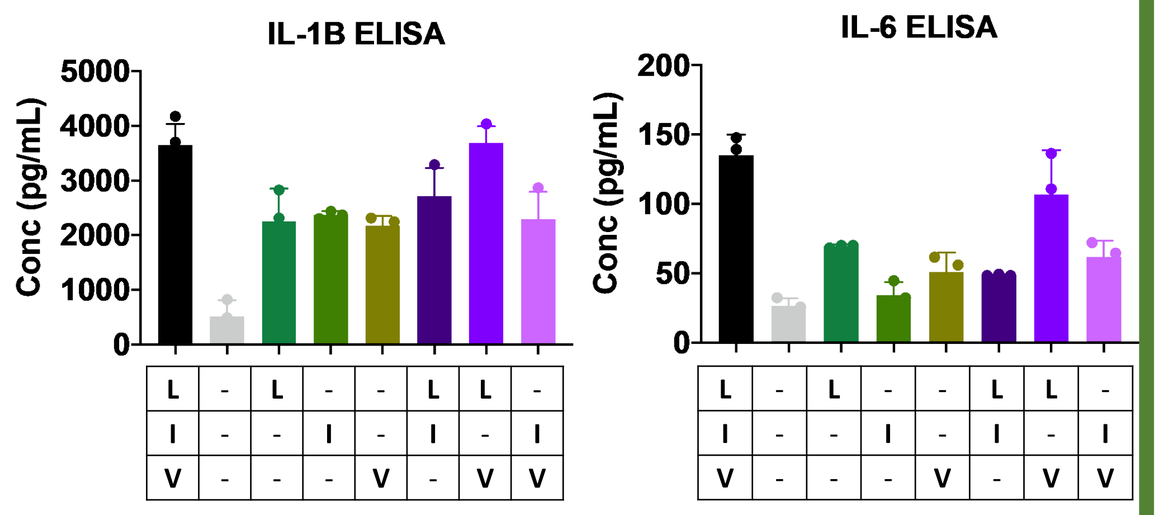


Figure 3: BMDMs were switched from complete media to media that contained .4 mM of the individual BCAAs alone or in combination. Cells were stimulated with LPS (10 ng/mL, 4hrs) and ATP (5 mM) to induce pyroptosis. Cytokine secretion was analyzed by ELISA from cellular supernatants.

Differences in S6K Activity Suggest mTOR Independent Mechanisms

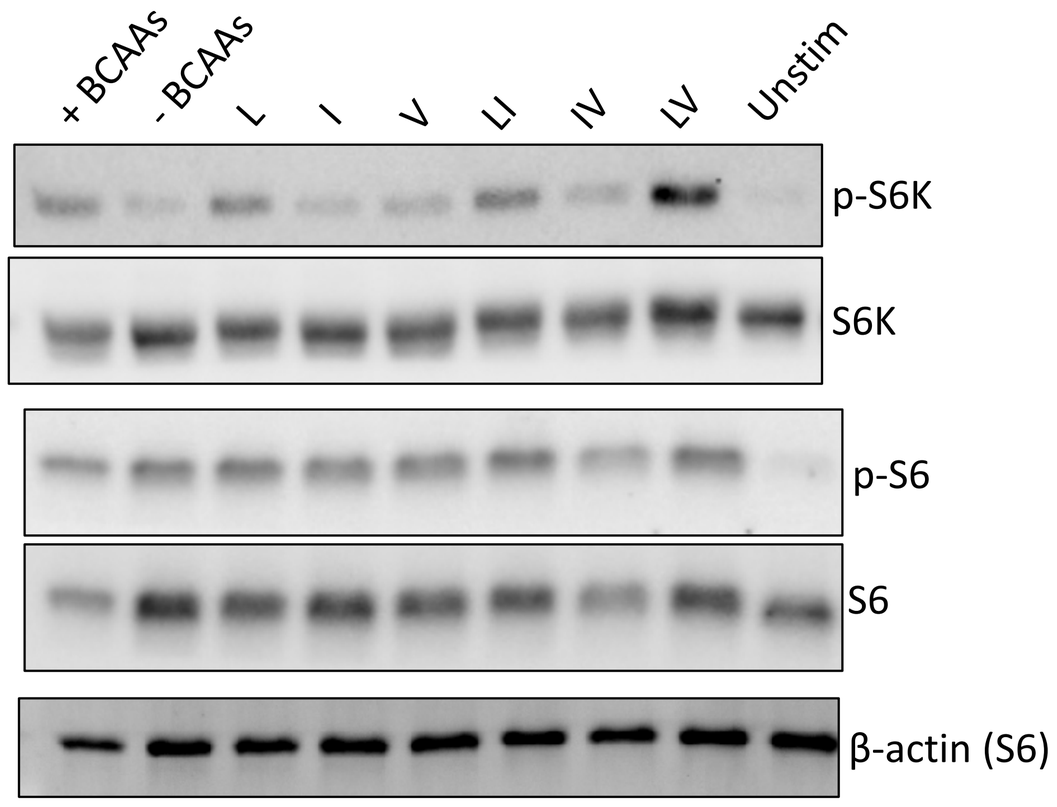


Figure 4: BMDMs were cultured in media with BCAAs alone or in combination. The cells were stimmed with LPS 1 hour before protein isolation. Note that L alone only slightly raises S6K activity, far less than L + V, while I and V do not.

Conclusions and Future Directions

Conclusions

- BCAAs regulate mTORC1 activity within macrophages.
- Macrophages are dependent on all three BCAAs to secrete a maximum amount of IL-1B and IL-6, but individual BCAAs can partially restore secretion of these cytokines.
- L influences mTOR while I and V do not (Fig. 4), but cytokine secretion is still recovered for all three individual BCAAs (Fig. 3)
- This implies an mTOR independent mechanism for recovery of cytokine secretion

Future Directions

- Define whether BCAAs and related metabolites alter NF-kB signaling
- Explore cell death dynamics during DAMP activation of pyroptosis through Western blots and live dye assays.
- Define translational alterations to macrophages upon BCAA and other metabolite deprivation

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