Glutamine is required for M1-like polarization in response to *Mycobacterium tuberculosis* infection

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Abstract

In response to *Mycobacterium tuberculosis* infection, macrophages mount early proinflammatory and antimicrobial responses similar to those observed in M1 macrophages classically activated by LPS and IFN-γ. A metabolic reprogramming to HIF-1-mediated uptake of glucose and its metabolism by glycolysis is required for M1-like polarization, but little is known about other metabolic programs driving the M1-like polarization during *M. tuberculosis* infection. We report that glutamine also serves as important carbon and nitrogen source for the metabolic reprogramming of M1-like macrophages. Using widely targeted metabolite screening, we first identified association of glutamine and/or glutamate with highly impacted metabolic pathways of M1-like macrophages. We then demonstrated by stable isotope-assisted metabolomics of $U^{13}C$ glutamine and $U^{13}C$ glucose that glutamine, rather than glucose, is catabolized in both the oxidative and reductive TCA cycle of M1-like macrophages, thereby generating signaling molecules that include succinate, biosynthetic precursors such as aspartate, and the antimicrobial metabolite itaconate. $U^{15}N$ glutamine tracing metabolomics further revealed participation of glutamine nitrogen into synthesis of intermediates of purine and pyrimidine metabolism plus amino acids including aspartate. The findings are corroborated by diminished M1 polarization via chemical inhibition of glutaminase (GLS), the key enzyme of the glutaminolysis pathway, and by genetic deletion of *GLS* in infected macrophages. Thus, the catabolism of glutamine, as an integral component of metabolic reprogramming in activating macrophages, will along with elevated glucose metabolism by glycolysis fulfill cellular demand for bioenergetic and biosynthetic precursors of M1-like macrophages. Knowledge of these new immunometabolic features of M1-like macrophages is expected to advance the development of host-directed therapies for better treatment outcome of tuberculosis.