THE CITRULLINE RECYCLING PATHWAY IS INCAPABLE OF SUPPLYING ARGININE FOR NO PRODUCTION IN RAW 264.7 MACROPHAGES

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Background: Arginine (ARG) is conditionally essential in sepsis and its depletion is thought to contribute to multiorgan injury. Nitric oxide synthase (NOS) catalyzes ARG into nitric oxide (NO), an important vasodilator and immunomodulator, and citrulline (CIT). Argininosuccinate synthase (ASS) and lyase (ASL), key enzymes of the CIT recycling pathway, can potentially regenerate the intracellular pool of ARG from CIT. Supplementation of ARG in sepsis remains controversial, though CIT supplementation remains a therapeutic alternative to restore ARG concentrations. NOS in activated macrophages is highly active, therefore it is not clear to what extent the CIT recycling pathway can sustain NO production. We aimed to determine the capability of ASS and ASL to restore NO production in an activated, widely used macrophage cell line.

Materials/Methods: RAW 264.7 murine macrophages grown in complete media were activated with LPS (0.1-1.0 ug/mL) for 6 to 24 hours and total protein extracts were analyzed by Western blotting for inducible NOS, ASS, ASL and arginase. Cells were deprived of ARG and supplemented with various concentrations of ARG (0-1000 uM) or CIT (0-2000 uM) and subjected to 24 hours of LPS (1 ug/mL) treatment. The effect on NO release was assessed by analyzing the nitrite content of macrophage culture supernatants (Griess assay). Data was analyzed by Student's t-test with significance at $p<0.05$.

Results: NO production by unstimulated macrophages decreased slightly with ARG supplementation (Con vs ARG, fold change 0.35-0.74 [62.5-1000uM ARG], $p<0.05$ except 250 uM ARG). LPS-stimulated macrophages were unable to increase NO production in the absence of ARG (Con vs LPS, fold change 0.75, $p=0.20$). With increasing ARG supplementation, LPS-stimulated macrophages produced significantly more NO compared to unstimulated macrophages (Con vs ARG, fold change 4.5-10.5 [62.5 uM-1000uM ARG], $p<0.001$). Despite expression of ASS and ASL proteins, CIT supplementation after ARG deprivation did not permit NO production in unstimulated or LPS-stimulated macrophages (Con vs CIT, fold change 0.69-1.05, $p>0.20$).

Conclusions: CIT supplementation was inadequate to restore NO production in activated RAW 264.7 macrophages despite intact protein expression of ASS and ASL, suggesting an apparent dependency of these cells on exogenous ARG. Our findings highlight cell-specific regulation of CIT recycling and potential post-translational regulation of ASS and ASL in macrophages.

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