L-citrulline Mitigates the Pro-inflammatory Response of Neonatal Alveolar Type 1 Cells Exposed to Lipopolysaccharide and Tumour Necrosis Factor-alpha

A. Mohammadi[1], R. Higazy[2], J. Ivanovska[3], H. Tran[1], N. Ivanovski[4], E. B. Gauda[5]; [1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [2]Institute of Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [3]The Hospital for Sick Children, Toronto, ON, Canada, [4]Department of Physiology, University of Toronto, Toronto, ON, Canada, [5]Division of Neonatology, The Hospital for Sick Children, Toronto, ON, Canada.

Corresponding author's email: atefeh.mohammadi@sickkids.ca

Rationale: Bronchopulmonary dysplasia (BPD) is the most common lung disease in premature infants. Inflammation during the early stages of lung development initiates BPD pathogenesis and damages alveolar Type 1 (AT1) cells, causing arrested alveolarization. The role of AT1 cells in inflammation is not understood. Moreover, current BPD therapies have adverse side effects, posing an unmet need for safe and effective treatments. L-citrulline is a naturally occurring amino acid that preserves alveolarization in various pre-clinical BPD models. Thus, we hypothesize that L-citrulline treatment will decrease the expression of pro-inflammatory mediators in neonatal AT1 cells exposed to lipopolysaccharide (LPS) and tumour necrosis factor-alpha (TNF-α) in vitro. Methods: AT1 cells were isolated from neonatal rats on postnatal day 4 using fluorescence activated cell sorting. Cells were serum starved for 24h and exposed to LPS (5, 10, 25µg/mL), TNF-α (5, 10, 25ng/mL), or a combination of LPS and TNF-α (10 µg/mL and 5ng/mL) for 18h in vitro. L-citrulline (0.5, 2, 3, 4, 5, 7.5, 10mM) treatment was added 1h prior to LPS and TNF-α exposure. Conditioned media was collected for cytokine and chemokine analysis by multiplex ELISA (Millipore RECYTMAG-65K). Results: AT1 cells exposed to both LPS and TNF-α produced a greater inflammatory response than those exposed to LPS or TNF-α alone. After 18h, AT1 cells from the LPS and TNF-α group increased the production of IL-6 by 243-fold, CCL2 by 34-fold, and CCL3 by 9-fold compared to control (all $p<0.05$, $n=4$). IL-1β and IFN-γ, and the anti-inflammatory cytokines, IL-4 and IL-10, were not detected in any experimental groups at this time point. In the presence of L-citrulline, cytokine and chemokine production were decreased in a dose-dependent manner. At 10mM, IL-6 was decreased by 5-fold, CCL2 by 1.5-fold, and CCL3 by 9-fold compared to the LPS and TNF-α group (all $p<0.05$, $n=4$). Conclusion: This is the first study to characterize the response of neonatal AT1 cells to potent inflammatory stimuli in the presence and absence of L-citrulline. We show that LPS and TNF-α induce the expression of the pro-inflammatory cytokines and chemokines IL-6, CCL2, and CCL3 in primary neonatal AT1 cells, which is markedly improved by L-citrulline treatment. These inflammatory mediators are strongly associated with the development of BPD and are predictors of adverse pulmonary outcomes in neonates. If our findings can be translated to human infants, L-citrulline is a safe and well-tolerated therapeutic that may attenuate alveolar damage induced by inflammation during early development.

This abstract is funded by: This work was funded by the Women's Auxiliary of The Hospital for Sick Children.