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To whom it may concern,

Please find below the final report for the CFWA Hardie Foundation Scholarship-funded project titled "The role of interleukin and necrosis leading to neutrophilic inflammation in children with cystic fibrosis", and an attached list of outcomes from this project.

The first aim of this project was to identify interleukin-1 driven inflammation in the airways of young children with cystic fibrosis. First, a comprehensive review of the existing literature was undertaken, resulting in a publication of a review article in the European Respiratory Journal (Montgomery *et al*., 2017; European Respiratory Journal, 49(1)). Utilising samples from the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) cohort, we were able to measure interleukin-1 cytokines in bronchoalveolar lavage fluid from children between 3 months and 6 years of age, which was elevated in children with active respiratory infection. Additionally, the levels of IL-1 were associated with both neutrophilic inflammation in the airway, as well as lung disease as measured by computed tomography. These findings were published in the Journal of Cystic Fibrosis (Montgomery *et al*., 2018; Journal of Cystic Fibrosis, S1569-1993(18)30588-5).

The second part of this project was to investigate the effect of lack of oxygen and rhinovirus infection on airway epithelial cells from children with and without cystic fibrosis, to determine whether necrosis of airway epithelial cells leads to neutrophilic inflammation in the airway. This was achieved by utilising bronchial brushings obtained from both children with and without cystic fibrosis, which were cultured in the laboratory and placed in an anaerobic environment over 48 hours or infected with rhinovirus over 48 hours. To identify the types of cell death occurring following injury, the cells were collected and analysed via flow cytometry using an adapted

methodology optimised for this study. This methodology was published in the Journal of Biological Methods (Montgomery *et al*., 2020; Journal of Biological Methods, *In Press*). Utilising this method, exposure to a lack of oxygen increased necrosis of airway epithelial cells from children with and without CF but had no effect on inflammatory responses. However, rhinovirus infection increased necrosis of airway epithelial cells from children with and without CF, but also increased apoptosis in children without CF. Rhinovirus infection induced a broad inflammatory response of airway epithelial cells, increasing levels of interleukin-8, the main neutrophil chemoattractant and several viral-associated cytokines. Most importantly, levels of interleukin-1 were associated with necrosis of airway epithelial cells from children with CF but not cells from children without CF, suggesting a CF-specific response to rhinovirus infection drives epithelial cell necrosis and leads to interleukin-1 release. These findings were published in Frontiers of Immunology (Montgomery *et al*., 2020; Frontiers Immunology, 11, 596).

In conclusion, the project funded by the CFWA Hardie Foundation scholarship suggests an important role for interleukin-1 signalling in early lung disease in CF, and potentially other lung diseases characterised by airway mucus obstruction, rhinovirus infection, and neutrophilic inflammation. As interleukin-1 signalling can be targeted therapeutically, this study supports new anti-inflammatory therapeutic development that targets the downstream effects of interleukin-1 signalling to mitigate inflammatory triggers of early lung disease in young children with cystic fibrosis.

Many thanks for the generous support throughout my PhD candidature.

Yours sincerely,

Dr. Samuel Montgomery

List of outcomes

Publications

Montgomery ST, Mall MA, Kicic A, Stick ST, on behalf of AREST CF (2017). Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies. Eur. Respir. J., 2017; 49(1). Role: First Author

Montgomery ST, Dittrich AS, Garratt LW, Turkovic L, Frey DL, Stick SM, Mall MA, Kicic A on behalf of AREST CF (2018). Interleukin-1 drives inflammation and structural lung disease in young children with cystic fibrosis. Journal of Cystic Fibrosis, 2018; S1569-1993(18)30588-5. Role: First Author

Montgomery ST, Frey DL, Mall MA, Stick SM, Kicic A, on behalf of AREST CF (2020). Rhinovirus infection is associated with airway epithelial cell necrosis and inflammation via interleukin-1 in young children with cystic fibrosis. Front Immunol, 2020, 11, 596. Role: First Author

Montgomery ST, Stick SM, Kicic A, on behalf of WAERP, AREST CF (2020). An adapted novel flow cytometry methodology to delineate types of cell death in airway epithelial cells. Journal of Biological Methods, *In Press*. Role: First Author

Published conference abstracts

Montgomery ST, Garratt LW, Berry LJ, Rosenow T, Mok C, Mall M, Stick SM, Kicic A on behalf of AREST CF (2017). Elevated IL-1α in paediatric CF airways is associated with neutrophilia, inflammation, and structural lung changes. Respirology, 2017, 22(Suppl 2), 150

Montgomery ST, Stick SM, Kicic A (2018). Differentiation between apoptotic and necrotic cell death in airway epithelial cells in response to viral infection and anaerobic conditions using flow cytometry. Respirology, 2018, 23(Suppl 1), TO049.

Montgomery ST, Dittrich AS, Garratt LW, Turkovic L, Frey DL, Stick SM, Mall MA, Kicic A on behalf of AREST CF (2018). IL-1 is associated with structural lung disease in children with cystic fibrosis. Pediatric Pulmonology, 53, 182.

Montgomery ST, Dittrich AS, Garratt LW, Turkovic L, Frey DL, Rosenow T, Stick SM, Mall MA, Kicic A on behalf of AREST CF (2019). IL-1 is associated with airway epithelial cell necrosis in children with cystic fibrosis. Respirology, 24(Suppl 1), TP034.

Montgomery ST, Dittrich AS, Garratt LW, Turkovic L, Frey DL, Rosenow T, Stick SM, Mall MA, Kicic A on behalf of AREST CF (2019). IL-1 is associated with airway epithelial cell necrosis in children with cystic fibrosis. Pediatric Pulmonology