Anabasum Reduces Excessive Inflammatory Responses in Cystic Fibrosis Patient-Derived Lung Macrophages

Poster # 104

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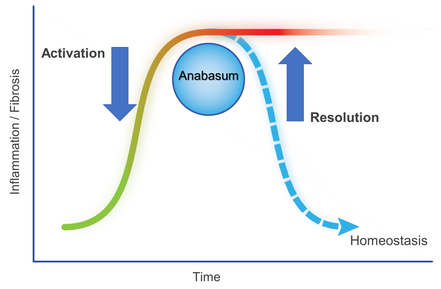
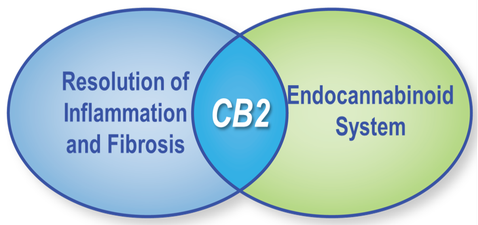
Background

Cystic Fibrosis (CF) is a genetic disease characterized, in part, by an excessive and persistent airway inflammatory response to infection with macrophages playing an important role in this harmful hyper-inflammatory response in the lungs. Anabasum (fka JBT-101) is a synthetic selective cannabinoid receptor type 2 (CB2) agonist that activates resolution of innate immune responses by stimulating the production of specialized pro-resolving lipid mediators which in turn reduce production of pro-inflammatory cytokines that are thought to play an important role in CF lung inflammation, such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and IL-6. In a recent Phase 2 study in adults with CF, anabasum had an acceptable safety profile and reduced pulmonary exacerbations, concomitant with a reduction in sputum inflammatory cells and mediators. To further test the potential of anabasum to resolve lung inflammation in CF, this study tested the direct effects of anabasum on alveolar macrophages (AMs) isolated from CF lungs.

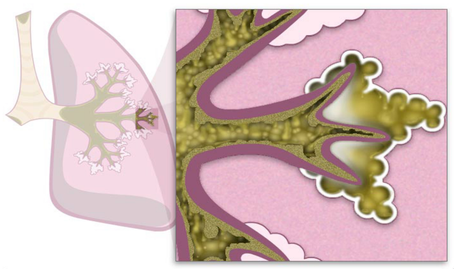
Resolution of Chronic Inflammation and Fibrosis

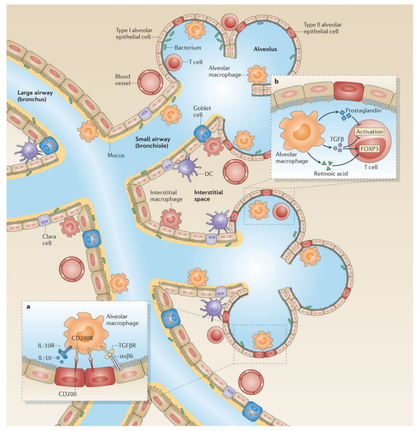




Objective

To determine the effect of anabasum on production and secretion of inflammatory cytokines by CF alveolar macrophages.





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Methods

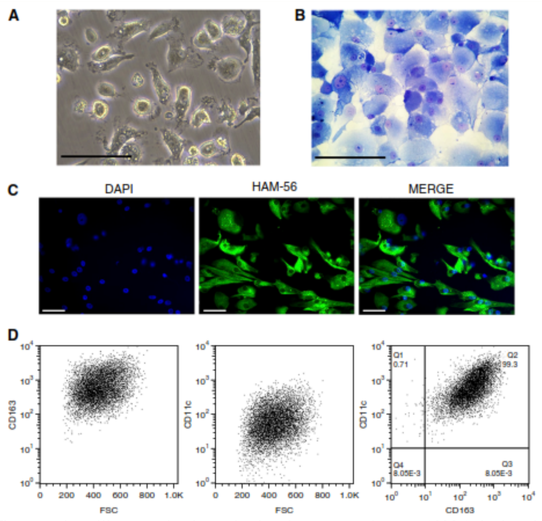


Figure 1. Characterization of alveolar macrophages (AMs) isolated from resected human lungs. (A) Adherent cells were isolated as described in the methods. Examination of cell morphology by phase-contrast inverted light microscopy. (B) Morphology of sedimented AMs. Cells were sedimented onto slides (cytospin), stained (Diff-Quik), and examined by light microscopy. (C) Immunofluorescence microscopy of AMs. Green = macrophage migration inhibitor factor HAM-56 (human macrophage marker). Blue = nuclei stain with 4'6-diamidino-2-phenylindole (DAPI). (D) Flow cytometric analysis of AMs. The preparations were analyzed according to their forward scatter characteristics (FSC), CD163 (macrophage marker), and CD11c (macrophage marker) expression in human AMs. Number in right panel indicates the percentage of cells positive for the macrophage markers. Scale bars = 100 mm. Data from [1]Bob A. Lubamba, Lisa C. Jones, Wanda K. O'Neal, Richard C. Boucher, and Carla M. P. Ribeiro. 2015 Am J Respir Crit Care Med Vol 192, Issue 12, pp 1449–1461.

AMs were isolated from lungs excised from two CF patients undergoing lung transplantation[1]. To simulate their response to infection, the macrophages were stimulated with *Pseudomonas aeruginosa* lipopolysaccharide (LPS, 100 ng/mL) for 6 hours. The AMs were exposed to anabasum (1, 3, 10 μM) 6 hours prior to, concomitantly, or 2 hours after LPS stimulation. Following 6 hr LPS treatment, the following biomarkers were evaluated: 1) TNF-α, IL-1β, IL-6, and IL-8 secretion, measured in culture supernatants using ELISA assays, and 2) the mRNA levels of X-box binding protein-1 (XBP-1), a key transcription factor with a role in LPS-induced ER stress and inflammatory responses in CF AMs, measured by quantitative reverse transcriptase polymerase chain reaction.

Results

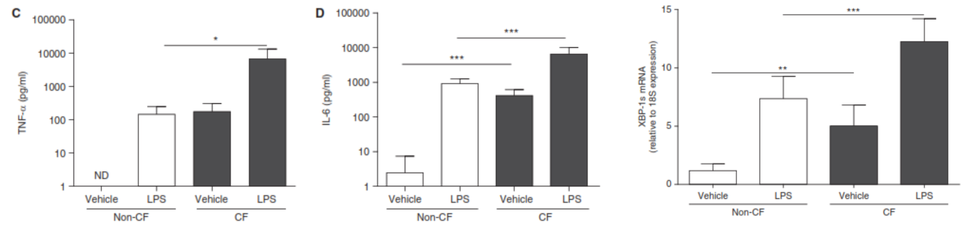
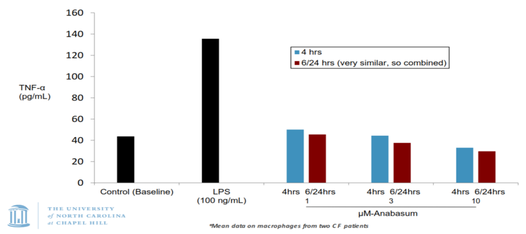
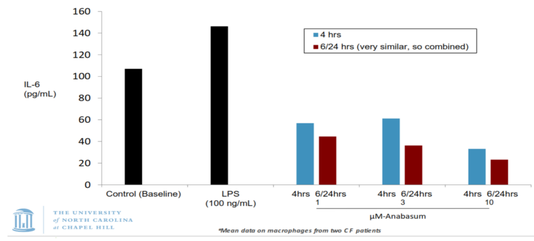


Figure 2. Primary cultures of CF AMs exhibit robust basal and LPS-induced TNF-α and IL-6 production. Primary cultures of non-CF and CF AMs were stimulated for 6 h with 0.1% dimethyl sulfoxide (vehicle) or 100 ng/ml LPS from *P. aeruginosa*. Levels of TNF-α (A) and IL-6 (B) protein secretion into the culture media were determined by ELISA. (C) Quantitative reverse transcriptase polymerase chain reaction was used to determine the mRNA levels of XBP-1s. Data are expressed as fold change relative to 18S mRNA. Open bars and solid bars correspond to non-CF and CF AMs, respectively. The y-axis uses a logarithmic scale. Data are from six independent experiments and represent mean ± SD. Unpaired t test was used to compare non-CF with CF AMs and paired t test was used to compare vehicle with LPS treatment. *P<0.05, **P <0.01, ***P<0.001. ND = not detected. Data from Bob A. Lubamba, Lisa C. Jones, Wanda K. O'Neal, Richard C. Boucher, and Carla M. P. Ribeiro. 2015 Am J Respir Crit Care Med Vol 192, Issue 12, pp 1449–1461.

EFFECT OF ANABASUM ON TNF-α PRODUCTION BY LPS-TREATED CF PULMONARY MACROPHAGES*

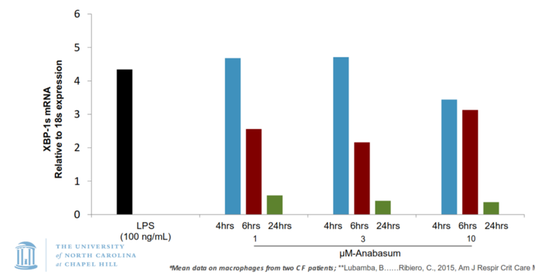


EFFECT OF ANABASUM ON IL-6 PRODUCTION BY LPS-TREATED CF PULMONARY MACROPHAGES*



Anabasum reduced LPS-stimulated production of TNF-α and IL-6 by CF AMs in a dose-dependent fashion, with up to >75% inhibition at 10 μM anabasum with only a minor inhibition of IL-8 levels (data not shown).

EFFECT OF CB2 RECEPTOR AGONIST (ANABASUM) ON EXPRESSION OF XBP-1s IN CF PULMONARY MACROPHAGES*: A MARKER OF INFLAMMATORY RESPONSE**



Furthermore, Anabasum inhibited the expression levels of the spliced (active) form of XBP-1 in CF AMs by up to ~85% in a time-dependent manner with 24 hr treatment being maximal. XBP-1s is a ER stress-induced transcription factor that plays a key role in LPS-triggered inflammatory responses.

Conclusion

The inhibition by anabasum of TNF-α and IL-6 production by CF AMs provides evidence for direct effects of anabasum on an important cell type and inflammatory mediators in the pathogenesis of CF lung disease. These results provide further support for testing the therapeutic benefit of anabasum in CF patients.

Acknowledgements

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